Effect of the urinary pH in urinary tract infections in kidney transplant recipients

Doctoral Thesis

PhD student: Sara Fontserè Recuenco

Directors: María Eugenia Pachón Ibáñez and María Elisa Cordero Matía

Tutor: María Elisa Cordero Matía

Department of Medicine

University of Seville

Seville 2023

Dr María Eugenia Pachón Ibáñez, "Nicolás Monardes" researcher in the University Hospital Virgen del Rocío / Biomedicine Research Institute of Seville (IBiS), as director of the doctoral thesis and Prof. María Elisa Cordero Matía, professor of Medicine in the University of Seville, as director and tutor of the Doctoral Thesis,

CERTIFY:

The Thesis entitled "Effect of the urinary pH in urinary tract infections in kidney transplant recipients", submitted for the degree of Doctor in Biomedical Research in the University of Seville by the student Sara Fontserè Recuenco under our supervision, meets the requirements to be presented, and to opt for the International Doctor mention.

For all intents and purposes, the following certification is signed in Seville, on the 5th of September of 2023.

María Eugenia Pachón Ibáñez
Director of the Doctoral Thesis

María Elisa Cordero Matía
Director and tutor of the Doctoral Thesis

FUNDING

This work was supported by the Instituto de Salud Carlos III, Subdirección General de Evaluación y Fomento de la Investigación, Ministerio de Economía, Industria y Competitividad (PI17-01405) and by Plan Nacional de I + D + i 2013–2016 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Ciencia, Innovación y Universidades, Spanish Network for Research in Infectious Diseases (REIPI RD16/0016/0009) - cofinanced by European Development Regional Fund "A way to achieve Europe", Operative program Intelligent Growth 2014–2020.

THANK YOU NOTE

A todos aquellos que me han acompañado en este camino.

Por la producción y difusión del conocimiento.

“El corazón del prudente adquiere sabiduría;

y el oído de los sabios busca la ciencia.”

- Proverbios 18:15 -

To whoever has been accompanying me in this journey.

To promote the production and divulgation of scientific knowledge.

‘The heart of the discerning acquires knowledge,

for the ears of the wise seek it out.’

- Proverbs 18:15 -

SCIENTIFIC PRODUCTION derived from this PhD thesis

Publication:

Fontserè S, Infante-Domínguez C, Suárez-Benjumea A, Suñer-Poblet M, González-Corvillo C, Martín-Gutiérrez G, *et al*. Impact of treating asymptomatic bacteriuria in kidney transplant recipients: A prospective cohort study. Antibiotics (Basel). 2021;10 (2):218. PMID: 33671718. DOI: 10.3390/antibiotics10020218 (Annex 10.1).

Congresses:

- **Fontserè S,** Infante-Domínguez C, Suárez A, M. Suñer, González C, Martín-Gutiérrez G, *et al*. Infecciones urinarias bajas en trasplantados renales: características clínicas y evolutivas. XXIII Congreso de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC). Madrid, 23-25th of May 2019. Poster.
- Infante-Domínguez C, **Fontserè S**, Suárez A, Suñer M, González C, Martín-Gandul C, *et al*. Características epidemiológicas, clínicas y microbiológicas de bacteriurias asintomáticas del tracto urinario en trasplantados renales. Factores de riesgo y pronóstico. XXIII Congreso de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC). Madrid, 23-25th of May 2019. Poster.
- **Fontserè S**, Infante C, Suárez A, Suñer M, González C, Martín G, *et al*. Infecciones del tracto urinario recurrentes en trasplantados renales. Características, factores de riesgo y factores pronósticos asociados. XIX Congreso de la Sociedad Andaluza de Enfermedades Infecciosas (SAEI). Sevilla, 21-23th of November 2019. Poster.

- **Fontserè S**, Infante-Domínguez C, Suárez-Benjumea A, Suñer-Poblet M, González-Corvillo C, Rodríguez A, *et al*. Fosfomicina *vs*. ciprofloxacino en el tratamiento de la infección urinaria baja no complicada del trasplantado renal: estudio prospectivo. XIV Congreso Nacional de la Sociedad Española de Enfermedades Infecciosas y Microbiología Clínica (SEIMC). Zaragoza, 4-6 de junio de 2020. [Event canceled due to COVID-19 pandemia, accepted as publication in the virtual abstract book of SEIMC in 2020 (ISBN - 978-84-09-22864-5)].
- **Fontserè-Recuenco S**, Suárez-Benjumea A, González-Corvillo C, Infante-Domínguez C, Herrera-Espejo S, Rodríguez-Villodres A, *et al*. Acidic urine pH worsens clinical outcomes of *Escherichia coli* bacteriurias. 32nd ECCMID 2022 Congress. Lisbon, 23-26th of April 2022. Oral presentation.

OTHER SCIENTIFIC PRODUCTION related to this PhD thesis

Publications:

- **Fontserè S**, Chacón N, Cordero E. Epidemiology, risk factors and impact of urinary tract infection in kidney transplant recipients En: Luis Martín y Juan José Villén. Actualización en el proceso de donación y trasplantes. 2016. P: 378-83. ISBN: 978-84-617-8139-3.
- **Fontserè S**, Chacón-Mora N, Cordero E. Review of urinary tract infection in kidney transplant recipients: incidence, burden of disease, risk factors and impact on the graft survival. Int. J. Transplant Res Med. 2017; 3:026. doi.org/10.23937/2572-4045.1510026. ISSN: 2572-4045.
- **Fontserè S,** Cordero E. Urinary tract infection in kidney transplant recipients: burden of this disease and participation of multi resistant microorganisms. En: Zaida Ruiz de Azúa López y Luis Martín Villén. Actualización en el proceso de donación y trasplantes. 2018. P: 338-44. ISBN: 978-84-09-13134-1.
- **Fontserè S,** Cordero E. Urinary tract infection in kidney transplant recipients. Management of urinary infections and asymptomatic bacteriuria. En: Zaida Ruiz de Azúa López y Luis Martín Villén. Actualización en el proceso de donación y trasplantes. 2018. P: 300-09. ISBN: 978-84-09- 13134-1.

Congresses:

- Carretero M, Cebrero T, Labrador G, Smani Y, Cisneros J, Pachon-Diaz J, Blazquez J, Cordero E, Pachon-Ibáñez M. Urine alkaline pH effect on ciprofloxacin and fosfomycin efficacy in a murine urinary tract infection model by *Escherichia coli*. ESCMID 2020, Paris. [Event canceled due to COVID-19 pandemia, accepted

as publication in the abstract book of 30th ECCMID in 2020 (no ISBN proportionated)].

- Carretero M, Herrera E, Bahamonde MA, Guisado AB, Cebrero T, Martín-Gutiérrez G, Blazquez J, Pachon-Diaz J, Cordero E, Pachon-Ibáñez M. Acidic urine pH effect in the kidney infection by uropathogenic *Escherichia coli* strains in a murine urinary tract infection model. ECCMID 2021, Vienna. E-poster.

INDEX

ABREVIATIONS

1. INTRODUCTION

1.1. *Escherichia coli* (*E. coli*):

1.1.1. Epidemiology of *E. coli*

1.1.2. Intestinal and other types of *E. coli*, and its clinical spectrum

1.1.3. Virulence factors in uropathogenic *E. coli* (UPEC)

1.1.4. Prevalence of *E. coli* infections

1.1.5. Treatment of *E. coli* infections and its resistance mechanisms

1.1.5.1. Ceftriaxone and β-lactamases

1.1.5.2. Amoxicillin-clavulanic acid (A/C)

1.1.5.3. Piperacillin-tazobactam (P/T)

1.1.5.4. Nitrofurantoin

1.1.5.5. Cotrimoxazole

1.1.5.6. Aminoglycosides

1.2. Low-level resistance (LLR) in *E. coli* and urine physiological conditions

1.3. Ciprofloxacin role in UPEC:

1.3.1. Resistance mechanisms

1.3.2. Effect of urinary tract physiological conditions on ciprofloxacin activity and resistance

1.3.3. Low level quinolone resistance (LLQR)

1.4. Fosfomycin role in UPEC:

1.4.1. Resistance mechanisms

1.4.2. Effect of urinary tract physiological conditions on fosfomycin activity and resistance

1.4.3. Low level fosfomycin resistance (LLFR)

1.5. Urinary tract infections in the kidney transplant recipients (KTR):

1.5.1. Incidence of UTI in solid organ transplant (SOT) and KTR

1.5.2. Burden of UTI in KTR

1.5.3. Risk factors of UTI in KTR

1.5.4. Impact of UTI on graft survival, graft dysfunction and mortality

1.5.5. Resistant and multidrug resistant microorganisms in KTR with UTIs

1.5.6. Recurrent UTIs in KTR

1.5.7. Management of UTI in KTR

1.5.8. Antimicrobial therapy used for UTI in KTR

1.5.9. Management of asymptomatic bacteriuria

1.5.10. Urinary tract infections antibiotic prophylaxis in KTR

1.5.11. Non antimicrobial strategies to prevent recurrent UTI

2. JUSTIFICATION

3. HYPOTHESIS

4. OBJECTIVES

5. METHODS

5.1. Study design, population, and enrolment period

5.2. Inclusion and exclusion criteria

5.3. Interventions

5.4. Sample size estimation

5.5. Definitions

5.6. Follow-up

5.7. Variables

5.8. Urine studies

5.9. Primary and secondary outcomes

5.10. Ethic considerations

5.11. Statistical analysis

6. RESULTS

6.1. Objective 1: To determine the impact of antimicrobial therapy in the outcome of asymptomatic bacteriuria in a cohort of KTR.

6.1.1. Baseline characteristics

6.1.2. Etiology and antimicrobial susceptibities

6.1.3. Antimicrobial therapy

6.1.4. Outcome

6.1.4.1. Microbiological outcome

6.1.4.2. Clinical outcome

6.1.5. Impact of antibiotic treatment in asymptomatic bacteriuria outcome

6.1.6. Risk factors and prognosis factors of recurrent UTI

6.2. Objective 2: To compare the outcome of lower UTI caused by *E. coli* in KTR treated with ciprofloxacin and fosfomycin, according to the urinary pH.

6.2.1. Baseline and clinical characteristics of lower UTI infections caused by *E. coli*

6.2.2. Microbiological and clinical outcomes in *E. coli* UTI in KTR.

6.2.3. Clinical and microbiology effectiveness of fosfomycin therapy in *E. coli* bacteriuria in KTR

6.2.4. Impact of urine pH on microbiological and clinical outcome

6.2.5. Impact of urine pH on ciprofloxacin and fosfomycin efficacy

6.2.5.1. Multivariate analyses of the effect of fosfomycin and pH on microbiological and clinical outcome

6.2.5.1.1. Microbiological failure

6.2.5.1.2. Clinical outcome

6.3. Objective 3: To analyse the clinical and microbiological outcomes in lower UTI caused by different uropathogens in KTR treated with ciprofloxacin and fosfomycin, according to the urine pH.

6.3.1. Baseline and clinical characteristics of lower UTI infections according to the antibiotic used.

6.3.2. Outcomes of lower UTI infections with different uropathogens

6.3.2.1. Clinical and microbiology effectiveness of fosfomycin therapy

6.3.2.2. Impact of urine pH on microbiological and clinical outcome

6.3.2.3. Impact of urine pH on ciprofloxacin and fosfomycin efficacy

6.3.3. Multivariate analyses of the effect of fosfomycin and pH on microbiological and clinical outcome

6.3.3.1. Microbiological failure

6.3.3.2. Clinical outcome

6.4. Objective 4: To study the prevalence, phenotypic characteristics and clinical impact and outcomes of *E. coli* strains with LLQR and/or LLFR, treated with ciprofloxacin and fosfomycin, according to the urine pH.

6.4.1. Baseline, clinical characteristics and prevalence of LLQR and LLFR strains in lower UTI caused by *E. coli* in KTR

6.4.2. Microbiological and clinical outcomes of LLR UTIs treated with ciprofloxacin and fosfomycin, according to the urinary pH

6.4.2.1. Ciprofloxacin therapy

6.4.2.2. Fosfomycin therapy

6.4.2.3. Impact of urine pH on ciprofloxacin and fosfomycin efficacy in LLR strains

6.4.3. Risk of antimicrobial resistance after ciprofloxacin and fosfomycin therapy. Impact of LLR

7. GENERAL DISCUSION

8. CONCLUSIONS

9. BIBLIOGRAPHY

10. ANNEXES

10.1. Publications derived from this PhD thesis

10.2. Data base form

10.3. Informed consent

10.4. Approval of the local ethics committee

ABREVIATIONS

AB: asymptomatic bacteriuria

A/C: amoxicillin-clavulanic acid

APN: acute pyelonephritis

A/S: ampicillin-sulbactam

BL: β-lactamase

BL/BLI: β-lactam/ β-lactamase inhibitor

cAMP: cyclic AMP

CFU: colony-forming units

CI: confidence interval

CIP: ciprofloxacin

CMV: cytomegalovirus

CNF-1: cytotoxic necrotizing factor-1

DALYs: disability-adjusted life years

DNA: deoxyribonucleic acid

EPINE: *Estudio de Prevalencia de Infecciones Nosocomiales en España* (prevalence study about nosocomial infections in Spain)

ESBL: extended-spectrum β-lactamases

ESRI: extended-spectrum resistance to β-lactams/ β-lactamase inhibitors

EUCAST: European Committee on Antimicrobial Susceptibility Testing

ExPEC: extraintestinal pathogenic *E. coli*

FNR: fumarate and nitrate reduction

FOS: fosfomycin

GNB: gram-negative bacilli

HAI: health-care associated infections

IBC: intracellular bacterial communities

IQR: interquartile range

KTR: kidney transplant recipient

LLFR: low-level fosfomycin resistant

LLQR: low-level quinolone resistant

LLR: low-level resistance

MBC: minimum bactericidal concentration

MDR: multi drug resistant/resistance

MIC: minimum inhibitory concentration

MMF: mycophenolate mofetil

MPA: mycophenolic acid

m-TOR inhibitor: inhibitors of the mechanistic target of rapamycin (sirolimus, everolimus)

OR: odds ratio

PBP: penicillin-binding protein

P/T: piperacillin-tazobactam

QALYs: quality-adjusted life years

RNA: ribonucleic acid

RUTI: recurrent UTI

SOT: solid organ transplant

ST: sequencetype

UTI: urinary tract infection

UPEC: uropathogenic *E. coli*

1. INTRODUCTION

1.1. *Escherichia coli*

1.1.1. Epidemiology of *E. coli*

The *Enterobacteriaceae* microorganisms' family, from the *Enterobacterales* order, is one of the most heterogeneous and biggest groups of gram-negative bacilli (GNB) with clinical relevance. Most of them are environmental germs, but also colonize diverse animals (1). The greatest member from this gender is *E. coli* (2), which plays an important clinical role for the human pathology, and will be the main character in this thesis.

Escherichia coli, as many other enterobacteria such as *Klebsiella pneumoniae*, are part of the habitual flora in human guts and in other mammals, where they maintain a symbiotic relationship, such as commensal and mutual type, with their host. In fact, *E. coli* is the predominant facultative anaerobic bacteria in the colon microbiota (1).

Escherichia coli is oxidase-negative, ferments lactose and can contain mobile elements, among other features (3). Most of the strains do not cause disease in the human being, colonizing newborns just after the delivery through the maternal intestinal and vaginal flora (1). However, some strains can behave as opportunistic pathogens in certain circumstances as: immunosuppression, anatomic-functional abnormalities or deep tissue invasion after injury (trauma or surgery) (3,4). It is transmitted typically by contaminated food or water intake, animal-to-human contact, person-to-person contact, or faecal contaminated fomites (3,5).

1.1.2. Intestinal and other types of *E. coli,* and its clinical spectrum

Escherichia coli can be differentiated by the infection location in intestinal (also named diarrheagenic, with diverse clinical presentation) (6), and extra-intestinal (3), also called extraintestinal pathogenic *E. coli* (ExPEC) (7). The ExPEC group can produce many different infections as: urinary tract infections (UTI) –the majority-, bacteraemia, or intra-abdominal infections (8). Its extraintestinal pathogenicity is linked to diverse virulence factors (9), that determines a higher sepsis and bloodstream infections risk. The most predominant ExPEC clone (sequencetype (ST) 131), has been linked to fluoroquinolones resistance and extended-spectrum β-lactamases (ESBL) production, making more difficult an optimal treatment (10). Some reports suggest that the most frequent origin of ExPEC infections is the urinary tract (50-67%) (11,12). Besides, the most common cause of UTI in all populations is *E. coli* itself (13), also called uropathogenic *E. coli* (UPEC). This is a heterogeneous group within the classification of ExPEC (14). Uropathogenic *E. coli* is the main cause of community-acquired (>80%) and nosocomial UTI (>50%), and its infection is associated with substantial medical costs and morbidity worldwide (15). Regarding virulence factors, UPEC differ from intestinal *E. coli* in the content of extra-genetic material, grouped in pathogenicity associated areas, that contribute to its ability to cause disease (16), like fimbriae, adhesins, biofilms, flagella, capsule, autotransporter proteins, iron-acquisition systems, porins (like ompA), or toxins (17).

1.1.3. Virulence factors in uropathogenic *E. coli* (UPEC)

The pathogenic cycle of UPEC infection within the bladder needs some of its virulence factors. UPEC strains ascend from the colonized urethra or perineal area to the bladder through the action of flagella, propelling up. Once there, UPEC binds and invades the superficial bladder cells through: 1) filamentous adhesive organelles (like type 1 pili or

P fimbriae), which get adhered to a variety of mannose-containing glyco-protein receptors in the urothelial cells (18); 2) Dr-adhesins family, -afimbrial proteins on their surface- which also helps in adhesion to the urinary tract (19); and/or 3) autotransporter proteins, which activate signal pathways to control the structural and functional organization of the host cell (20).

Once in, UPEC are transferred into membrane-bound compartments, similar to endosomes (21), and replicate to form a biofilm-like known as intracellular bacterial communities (IBC). The IBC protect themselves from host immune system, antibiotics and other noxa (22). While some UPEC are kept in a quiescent state, being a reservoir, a subpopulation progresses into a distinct developmental phase (23). Then UPEC compromises the integrity of the infected cells, and begin to spill out into urine and/or to infect adjacent cells (24).

Most of these virulence factors are plasmid codified (25), so they are transmissible in high combination ways, allowing *E. coli* to diversify its variety of infections (26).

The virulence factors for UPEC are also known as part of the urofitness determinants (27). So, they fit the bacteria out with the proper features and skills to be the most capable to hand its genes to its progeny. Naturally, most of mutations are linked to bacteria fitness cost, often manifested as reduction in growth rate. The reasons why mutations are kept if they reduce the survival rate, or other fitness costs, are diverse. Some mutations are cost free, or additional compensatory mutations can be acquired, so the accumulative resistances at the end have a low fitness cost, or different mechanisms are co-inherited together (co-resistance acquisition), or even a third gene expression is obtained for the same cost (28).

Other urofitness determinants have been described:

Limitation in iron acquisition (and other metals like zinc or manganese) from the bacteria is a defence mechanism from the host against the survival of bacteria (29,30). So UPEC can upregulate the expression of genes involved in its own iron resources. This uptake is done by siderophores, iron transporters, outer-membrane heme receptors and the *TonB-ExbB-ExbD* complex, among others (27). Mutations in this complex, or in *Fur "*box genes", which regulate siderophores and heme-uptake systems (31), lead to resistant strains. "*Fur* boxes" also up-regulate other genes like *acnA, ftnA, fumA, sdhCDAB,* and down-regulate *ryhB,* or small RNA elements with its own function in the iron balance (32). So, all of them can be a mutation target leading to an increased virulence. In addition, heme receptors can also be regulated by *chuA* and *hma* genes. *ChuA* also up-regulates IBC formation within bladder (33). Other genes like *fepA, iha*, and *iroN*, or even those encoding for aerobactin and yersiniabactin siderophores receptors (like *fyuA*) contribute to its regulation, being also good targets for determinant mutations (34).

In-cytoplasm manganese and ferrous iron transporters systems are encrypted by *SitA, SitB, SitC,* and *SitD* genes, which are regulated as well by *Fur* and *MntR* genes (35). While zinc transporters are encoded by *znuACB* and *zupT* genes, which are connected as well with the manganese and ferrous system. E.g.: mutants lacking *znuACB* show *Fur*-regulated genes derepression and are highly damaged by oxidative stress (36). So, iron homeostasis seems to be a complex multichannel, overlapping layers, regulatory mechanism. And its mutations lead somehow, even when redundancy regulation exists, to play a key role for bacteria survival and clinical resistances (27).

Mutations in flagella genes, which help in UPEC motility, favours cystitis by promoting IBC formation -through *Auf* or *Yad* genes (37)-, and renal epithelial invasion -through the overexpression of *fliC* gene, among others- (38–40). Also *papX* gene overexpression

(encoding for P fimbriae) leads to a reduction, through *flhDC* master regulator via, of the flagellar biosynthesis and chemotaxis (41–43). So, UPEC motility mechanisms -including adhesion- are as well interconnected.

Regarding toxins, UPEC produce at least four types, but the ones with registered mutations are α-hemolysin and cytotoxic necrotizing factor-1 (CNF-1) (27). Hemolysin A is the better characterized. It is encrypted by *hlyCABD* genes. Besides its cytolitic activity against diverse leukocytes cells, its overexpression dampens host immune response to the infection, conferring advantage when UTI is initiated (44,45). The up-regulation of CNF-1 also gives advantage to the strain when UTI is starting, via *RhoA* and *Rac1* genes, with activity against neutrophils (46,47).

RfaH gene encodes a transcriptional protein that interferes in lipopolysaccharide biosynthesis, capsule production, hemolysin A and/or CNF-1 activity. Its loss causes an increased IBC formation, and also up-regulates *flu* gene which encodes for an auto aggregation and IBC biofilm protein (48).

So, it seems that different virulence factors are linked when only one is modified. E.g.: curli proteins, which are amyloid-like proteins forming part of the extracellular matrix UPEC biofilm, are encrypted by *CsgA*. When knocked out, the mutant blocks adherence and biofilm *in vitro* formation (49). *UpaB* gene lacking, which encodes for an urokinase-type autotransporter protein participating in adherence, displays a high urofitness (50). *Ag43a* and *ag43b* -subtypes of *flu* genes- knock out, have high persistence rates in urinary tract (51).

Also it has been reported rapid growth strains as virulence determinants, like asymptomatic bacteriuria strains compared to invasive UPEC ones, which exhibit faster growth in urine and outcompetes to UPEC (52). So the asymptomatic bacteriuria strains

might compensate the lack of specific virulence determinants (like adherence organelles) and ensure their persistence in the host (27).

Another noteworthy way to modify the virulence factors expression is antibiotics exposition: sub-inhibitory concentrations of nitrofurantoin or ciprofloxacin can upregulate genes encoding S fimbrial adhesion (*sfa*) and FiC fimbriae (*foc*) proteins in UPEC (53).

There are plenty of defined modified gens (like *fmB, fmE* in fimbrial expression; *hns* in motility encrypting (54); *DegS, DegP* involved in combating envelope stress (55,56); *hfq, rpoE* in riboregulation and membrane-stress response (57); *QseBC* system in infective mechanisms (111), among many others) which increase UPEC *in vitro* or in murine models virulence and fitness (27). Some authors already pointed out the importance when extrapolating information obtained from laboratory and animal models to human health field, as environmental conditions could determine which genes and resistance mechanisms are expressed *in vitro*, and *in vivo* (27). For example: *glnA* and *glnPQ* are up-regulated *in vivo* in UPEC when experience nitrogen limitation, *frd*ABCD (encoding FNR, fumarate and nitrate reduction protein) is down-regulated *in vivo* when UPEC experience aerobic conditions -both regulations reported *in vitro* environments as well-, but *fimH* which is involved in biogenesis of type 1 fimbria in the murine model is poorly expressed in human samples (14).

1.1.4. Prevalence of *E. coli* infections

The *Estudio de Prevalencia de Infecciones Nosocomiales en España* (EPINE) identifies the enterobacteria group as the most frequent etiology bacteria group (28.1%), in both community-acquired and health-care associated infections (HAI) (58). *Escherichia coli*

is the most common bacteria causing 13.8% of the total of infections and 14.4% of those communit-acquired. *Klebsiella pneumonia* was responsible for 4.7% of the total of infections and 6.5% of those in the HAI setting. Both bacteria have special role in UTI, being this site of infection the third most common in the HAI setting and the fourth in the community one. From another point of view, UTI leads to the consumption of almost 16% of the total of antibiotics (27% when only considering long-term care facilities centres): lower UTIs reach 12.3% and upper UTIs reach 3.2% of the total antibiotic consumption. Other *E. coli* infection sites are surgical site infections in HAI, and bacteraemia in community-acquired infections. Althoug *K. pneumoniae* presents the same scenario, respiratory tract infections are most frequent in the HAI setting (58).

As previously stated, UTIs are classified as lower or upper (pyelonephritis), and uncomplicated or complicated (13). Complicated UTIs affect hosts with structural or functional urinary tract abnormalities, like pregnancy, obstruction, catheters or drainage devices, renal failure, renal transplantation or other kind of immunosuppression (22). These distinctions have been used to guide the antibiotic and length of therapy, usually choosing broader-spectrum agents and longer courses (59).

1.1.5. Treatment of *E. coli* infections and its resistance mechanisms

Therapy for *E. coli* infections depends on the type and severity of the infection, its location, the host features, previous infections or colonization, the use of previous antibiotics, and the resistances profiles in the local area. The most commonly used antibiotics for *E. coli* infections (and other enterobacteria) are β-lactams, β-lactams/β-lactamase inhibitors (B/BLIs), fluoroquinolones, cotrimoxazole, fosfomycin and aminoglycosides, among others (26). In fact, and in order, amoxicillin-clavulanic (A/C), ceftriaxone, piperacillin-tazobactam (P/T) and levofloxacin are the current main used

antibiotics in Spain for any human use. Specially, when talking about the most relevant antibiotics for this PhD, ciprofloxacin is used in 5% of overall indications, and fosfomycin in 0.61% (58).

Despite the wide therapeutic options available, the increasing resistances rates in *E. coli* and *K. pneumoniae* -like in other enterobacteria- to one or more antibiotic groups, has reduced their efficacy (60). Over half of the *E. coli* isolates in 2021, and over a third of *K. pneumoniae* ones, were resistant to at least one antimicrobial group on the last European Antimicrobial Resistance Report (EARS-Net) (61). In Spain, resistance to all antibiotics studied in *E. coli* has increased from 2001 to 2020, with about a quarter of strains isolated from blood showing resistance to three or more families of antibiotics. Resistance to third-generation cephalosporins in *K. pneumoniae* has increased from 10.2% in 2010 to 30% in 2020 (62) -and up to 38.5% in 2022 in HAI setting (58)-. This increase has been accompanied by a significant high resistance rate to other families of antibiotics such as fluoroquinolones (30% resistance to ciprofloxacin in 2020) and aminoglycosides (17% resistance to gentamicin in 2020) (62). Global European *E. coli* resistance rate to fluoroquinolones by 2021 was almost 22% (61), but this rate was higher in Spain, Italy and other Est-European countries (63).

It must be taken into account that after COVID-19 pandemic breakout, the rising rates have been maintained or even decreased, probably due to the lack of activities targeting these pathogens (61).

According to EPINE (58), and regarding carbapenems-resistance, *E. coli*'s remained rare (0.8%) but *K. pneumonia'*s has reached the highest resistances reported to date (11.3%) in 2022. In some countries in Europe it was ≥ 25% by 2021(63). Among *E. coli* third-generation cephalosporins resistance rate is 17.8% in HAI setting and 16% in

community-acquired one (58). By 2021 *E. coli* and *K. pneumoniae* third-generation cephalosporins resistance infections were reported as two of the three bacteria causing the largest burden of disease in Europe, generating >58% of the total burden measured in disability-adjusted life years (DALYs) (61).

The UPEC strains exhibit a high recurrence rate. More than 68% of recurring UTIs (even one year after the initial infection) are caused by the original *E. coli* strain in the general population (64,65). Some factors that might contribute to recurrence are: IBC films, sub-optimal antibiotic concentrations (secondarily to an improper dosage, low drug distribution or penetration/activity into the urinary tract), low primary efficacy of the treatment chosen, poor patient compliance and multi-drug resistance (MDR) (26,66–68).

Antimicrobial resistance increases the risk of length of treatment from the time of an initial diagnosis to an effective therapy, and toxicity related to use alternative antimicrobial agents (68).

By 2050 it is predicted that MDR infections will cause more deaths per year than cancer and will great expenses, confirming the current MDR crisis emergence (69).

Moreover, it must be remarked that MDR development and spread are driven by previous antibiotic use, where UTI recurrences can take their role (70,71). The increase in multi-resistance *E. coli* and *K. pneumoniae* strains has been specially high in fluoroquinolones and β-lactams antibiotic families (26). Antibiotic resistance to *E. coli* is one of the most important problems nowadays as *E. coli* itself functions as one of the world biggest reservoirs. Moreover, as virulence factors and antibiotic resistance genes can be plasmid codified; they can be highly transmitted between strains or even to other bacteria. Recently has been enumerated its rising number genes (72). This could explain

the apparition of resistances mechanisms to almost every family antibiotic group. Below, the most used antibiotics for UPEC infections and its resistance mechanisms are reviewed.

Prior to it, it is worth to mention that antibiotic-resistant bacteria can be determined by *de novo* mutation rate, and the horizontal gene transfer mechanisms. Most of them are focused on the antibiotic target (replacing, reducing or modifying it), target protection system, increasing or acquiring new drug efflux, gene amplification, or knocking out the gene, for example. A better understanding of how the horizontal gene transference works, would allow us to get a prediction of the resistance development mechanisms to come and how we can deal with the current ones. These horizontal transferences are, basically, conjugation (via plasmids and pilus), transposition (jumping DNA elements from one to other bacteria), transformation (uptake of foreign genetic material in the environment), and transduction (via bacteriophages) (28).

1.1.5.1. Ceftriaxone and β-lactamases

Ceftriaxone is the most world-wide used β-lactam antibiotic, due to its efficacy, high tolerance, and wide spectrum. Cephalosporins get adhesion to penicillin-binding-proteins (PBPs) at the bacterial cell wall, inhibiting its synthesis and leading to its own lysis. Third-generation cephalosporins like ceftriaxone, ceftazidime or cefotaxime have a high activity against enterobacteria in sepsis, blood-stream infections, or intrabdominal or respiratory infections, for example (73). Its usage has diminished since the rising rate of ESBL-producers enterobacteria like AmpC β-lactamases (74). *Escherichia coli* resistance mechanism towards ceftriaxone is basically the production of β-lactamases, which are bacterial enzymes that hydrolyses the betalactamic ring structure, inhibiting the union to PBPs (75). Apart from plasmids, β-lactamases can be

coded by bacterial chromosome, transposons or other mobile genetic elements. They can spread through a big group of gram-negative microorganisms, specially *Enterobacterales,* and also have the skill to expand its spectrum by using new substrates (76).

There are four types of β-lactamases, following Ambler classification based in their amino acid sequence. Class A β-lactamases are the biggest group and the most common *E. coli* β-lactamase. They are based in serine substrates and initially they inhibited penicillins and first-generation cephalosporins (TEM and SHV type), or third-generation cephalosporins like cefotaxime (named CTX-M) (76). Class B β-lactamases (known as well as metalloproteinases, because need zinc ions as substrates), inhibits penicillins, cephalosporins, and carbapenems, too; but not monobactamic antibiotics (77). Class C β-lactamases (named AmpC) can express resistance by inducing the derepression of its expression under some betalactamic pressure -among other conditions-, leading to its own hyperproduction (78). This β-lactamases inhibit third-generation cephalosporins specially, but also penicillins, cefoxitin, aztreonam, and other cephalosporins (79). *Escherichia coli* is one of the *Enterobacteriaceae* which expresses bla_{AmpC} gene in a constitutive way, so AmpC cannot be derepressed (80). Class D β-lactamases (called OXA), which are always plasmid codified, inhibit cephalosporins, even though they play a more important role against carbapenems (81).

Whenever an antibiotic appears its resistance does, too. During the late 80's ESBL were reported (82). These were, initially, class A β-lactamases, like TEM, CTX-M or SHV, which had mutated and could acquire a specific resistance profile, specifically to oximino-cephalosporins (cefotaxime, ceftazidime, ceftriaxone, cefuroxime and cefepime) and to monobactams (aztreonam), but kept their susceptibility to cephamycins (cefoxitin) and to carbapenems (imipenem, meropenem and ertapenem)

(82,83). Originally, these enzymes could be hydrolysed by ampicillin-sulbactam (A/S), amoxicillin-clavulanic acid (A/C) or piperacillin-tazobactam (P/T) (84). During the years, β-lactamases have evolved and mixed up rapidly, and currently exists carbapenemases, which confer resistance to almost all β-lactams due to their higher hydrolyse spectrum (85).

From the 2000's, ESBL CTX-M-producer *E. coli* has emerged as a common cause of community-acquired infections, especially in the urinary tract (86), being nowadays ESBL-producers *E. coli* a health worldwide threat (83,87). In Europe, third-generation cephalosporin's resistance rate in *E. coli* due to ESBL-production raised from 12% (in 2012) to 15% (in 2017), and was maintained below 14% (in 2021). This rate shows a north-to-south gradient, being higher in the south of Europe, as reported for other type of antibiotic resistances, like previously commented for fluoroquinolones (61).

1.1.5.2. Amoxicillin-clavulanic acid (A/C)

As a betalactam/betalactam inhibitor (B/BLI) antibiotic group, it appears secondarily to the need to combat β-lactamases. It was the first B/BLI commercialized for clinical use (88) and it inhibits some β-lactamases (penicillinase-like) found in most of *E. coli, K. pneumoniae, Proteus mirabilis, Haemophilus influenza, Neisseria gonorrhoeae, Moraxella catarrhalis* or *Bacteroides* spp (75). However it cannot inhibit classes B and C β-lactamases (77,79). On the other hand, it can partially hydrolyse carbapenemases (some from class A) (89). Nowadays, A/C is used as empirical treatment in non-severe, and mainly community-acquired, respiratory tract, soft tissue infections, UTI and/or intra-abdominal infections (88).

1.1.5.3. Piperacillin-tazobactam (P/T)

Piperacillin has activity against *Pseudomonas aeruginosa*, *Streptococcus pneumoniae*, *Enterococcus faecalis* and some anaerobes (90). When combined with tazobactam, it reduces the minimum inhibitory concentration (MIC) of piperacillin of *Enterobacterales, H. influenzae, N. gonorrhoeae* and *M. catarrhalis* with some type of betalactamase production (91). Previously, the combination was recommended as a first-choice empirical treatment for hospital acquired infections and certain severe community-acquired bacterial infections, including blood-stream and intra-abdominal infections, or complicated UTIs (92,93). In fact, was one of the most widely used B/BLI in the nosocomial setting due to its bigger spectrum (58), even though, it cannot inhibit class C β-lactamases (79). Secondarily to its excessive prescription, it has been also reported a rising rate of resistance to B/BLI (94). Specifically, resistance to P/T among *E. coli* strains increased from 7% to 10% in Spain by 2017 in intra-abdominal infections (95), and from 23-48% (in 2014-2016 period) in Turkey in bloodstream infections (96). Similar rising resistance rates in other *E. coli* infections have been reported in United Kingdom or USA (74,97).

Like other betalactamase, those with activity against B/BLI are originally basic β-lactamases that have been modified by: hiperproduction of TEM (called TEM-1) or AmpC, mutated TEM-1 (called IRT: inhibitor resistant TEM), TEM variants with higher hydrolytic capacities, modified porins mixed with some β-lactamases type, production of OXA-1 type or SHV type, etc. The most common mechanism in *E. coli* is the combination with TEM type β-lactamases (98–102).

Even if all B/BLI above mentioned could inhibit TEM β-lactamases, ampicillin/sulbactam (A/S) and A/C are more often affected, because P/T keeps major activity against β-lactamases (103). The β-lactamases that includes specifically activity against combined B/BLI, also named extended-spectrum resistance to β-lactams/ β-

lactamase inhibitors (ESRI) (103), can be divided into low-level ESRI (when affecting A/S or A/C), medium-level ESRI (when affecting A/S and A/C), and high-level ERSI when P/T is also ineffective (75,104,105).

The progressive way through which *E. coli* gets theses resistances seems to be in a consecutively method, but it is partially unknown. It has been reported that P/T concentration varies from sub- to supra-inhibitory levels during clinical treatment period (physiologically, for example between the intra-abdominal space and bloodstream, among other body compartments), which may promote the emergence of resistant *E. coli* isolates (106). So on, low and medium-level ERSI strains have the potential to acquire high-level ERSI. It has been proven lately that P/T exposure (*in vivo* and *in vitro*) drives to high-level *E. coli* clinical isolates which carry bla_{TEM} gen (codifying TEM type β-lactamases). This acquisition takes place through a higher copy number of bla_{TEM} gen, which confers a higher β-lactamase activity and a higher MIC to P/T. So, these low P/T concentrations could drive to the hyperproduction of TEM, promoting ESRI development, and consequently heading to therapeutic failures (103).

In conclusion, current MIC cut-off for P/T could be below the optimal dosage to treat some *E. coli* infections when drug concentrations cannot be guaranteed to reach proper levels at the infection site. This study also reflected that after *in vitro* P/T exposure, cephalosporin resistance was also developed, including ceftolozane/tazobactam. Some of those strains were carrying bla_{TEM} genes, as well (103).

1.1.5.4. Nitrofurantoin

This antibiotic is recommended as a first-line empiric regimen for non-complicated UTI in young women (107), and has persisted in the community setting during the years with a very low resistance rate: around 0.8% in the USA before 2012 (108), and below 1.5%

in some European countries after it (109,110). In the hospital setting, the proportion of resistances ranges from 2.3% in Argentina (111), 3-5% in diverse European countries, and up to 13% in some Asian countries (112–115). Its efficiency, cost-effectiveness and low adverse-effects ratio, added to its low environmental impact explain this antibiotic as a first-choice option (116). Nitrofurantoin interferes in a bacteriostatic and bactericidal way affecting different enzymes, involved in citric acid cycle, DNA, RNA and proteins synthesis (117).

Nitrofurantoin resistance can be induced by chromosomal nitroreductase genes (*nfsA* and *nfsB*) which take part in the antibiotic processing (118) or by the deletion in *rib*E (involved in the synthesis of the cofactor for the previous mentioned genes) (119). But can also be induced by plasmid encoded genes like *OqxAB*, which produce an efflux pump with broad substrate specificity. Therefore, it also carries other co-resistances like chloramphenicol, ciprofloxacin, nalidixic acid or trimethoprim resistance. In fact, it can be found in MDR *E. coli* isolates and other enterobacteria strains (120).

1.1.5.5. Cotrimoxazole

Unlike nitrofurantoin, trimethoprim-sulfamethoxazole's UPEC resistance world-wide is higher than 20%. In some countries as East-European ones, India, or Brazil and certain conditions as the past use for prophylaxis or treatments, the proportion of resistant strains is higher than 60% (116,121–123). Therefore, this antimicrobial is no longer recommended as empiric therapy of uncomplicated UTI (124,125). Cotrimoxazole has a bactericidal effect by interfering with the nucleic acids biosynthesis: trimethoprim's target is the inhibition of dyhydrofolate metabolism and sulfamethoxazole's target is the inhibition of dihydropteroate one.

Some mechanisms for trimethoprim resistance are the overproduction of dihydrofolate reductase, encoded by *dhfrAB* genes, or acquiring low-affinity target genes (so cotrimoxazole cannot bind to) (126). But like in other cases, the most important mechanisms are plasmid-mediated through *dhfrI* and *dhfrII* variants (127).

Resistance to sulphonamides are plasmid-mediated through *sulI* and *sulII* genes, which encrypt dihydropteroate synthase, but also appear spontaneously (128).

1.1.5.6. Aminoglycosides

These antibiotics bind to the bacterial ribosome subunits inhibiting RNA synthesis (129). Despite their efficacy, these antibiotics are seldom used because of their related adverse events and the absence of oral presentations. However, they have to be considered for the treatment of MDR or complicated UTIs (107).

Another aspect that may favour the use of aminoglycosides is that despite they might induce resistance to other antibiotics, their impact is lower than that observed with other antibiotics as fluorquinolones (129,130). Some resistance mechanisms involved are drug efflux pumps, the ribosomal target sites modification, and enzymatic modifications of antibiotics (129). Being the last one the major mechanism (128).

It has been described that *AcrAB* efflux pump plays a major role in *E. coli* resistances as a multiple-antibiotic-resistance mechanism (131). When this efflux pump (bind to *TolC)* is overexpressed leads to fluoroquinolones, A/C and trimethoprim cross-resistances emerging among the UPEC strains. But also that in case of aminoglycosides resistance *AcrAB-TolC* expression is reduced leading to ciprofloxacin susceptibility and the other way round, presenting an opposite dependence (130).

1.2. Low-level resistance (LLR) in *E. coli* and urine physiological conditions:

In natural conditions, strains with slightly higher antibiotic resistance than the common susceptible population exist. This concept is named low-level resistant (LLR). Low-level strains' potential to acquire new resistances was previously reported in the 2000s (132). This population seem to promote the selection of fully resistant strains, being considered as an intermediary stage in the development of high-resistant strains. Even if LLR existence allows bacterial growth at clinically relevant concentrations, they may not be detectable by standard susceptibility testing procedures. So, they are categorized as "susceptible" although 1) probably carrying genetic determinants that reduce the antibiotics efficacy, and 2) might show folded up MIC levels (but in between the susceptible range). In fact, it is proposed that LLR may be the responsible, under sub-inhibitory antibiotic levels, for the evolution and dissemination of resistant strains, serving as a getaway for clinical resistance, and influencing the outcomes of antibiotic treatment in clinical setting (132).

Specifically in the UTI setting, there are other urinary tract physiological conditions, apart from sub-inhibitory antibiotic concentrations, which could active LLR. Some of them are also related to physicochemical, pharmacokinetics and pharmacological properties of the antibiotics. So, even if the microorganism is susceptible to the antibiotic, or despite the optimal antibiotic concentration in bladder, these conditions could lead to the survival of a relevant number of microorganisms, and therefore an unsuccessful therapy, due to the reduction of the antibiotic activity. The selection of the survival microbes could boost the emergence of a broader range of mutant variants -with a low fitness cost and diverse phenotypic effects-, causing a new infection by these LLR microorganisms' populations (66,133). Apart from that, one collateral antibiotic side-effect is to modify the composition of the microbiota, acting as a selective pressure

for bacteria and fungi selection which can lead to the development of resistances, as well (22).

Some of these physiological determinants are below explained.

Urine, composed by a complex mix liquid of water -majorly-, ions, proteins, and others; was thought to be part of the primary defence mechanism because of its high osmolality, low pH values, or high concentrations of urea, which could inhibit bacterial growth. But UPEC strains can grow under all these conditions adapting themselves to an efficient use of the available resources (16). E.g.: calcium and magnesium ions concentrations have been previously reported to affect the outcome of antibiotic susceptibility testing results, as well as some intracellular transporters substrates (like glucose-6-phospate for fosfomycin). Urinary pH levels also interfere in the antibacterial activity of some therapeutic agents used for UTI (134–136).

Another noteworthy condition is how the urine varies itself (137), like in case of an infection. E.g.: different amino acids proportions found in urine can determine an optimal or a reduced bacterial growth (138), and the genes encoding these amino acid and carbohydrate metabolism systems vary in a significative way during UTI (139). An infected bladder urine turns into anaerobic field due to the oxygen consumption by the infecting microbes (140). Most of the bactericidal lethality is partially induced by damaging reactive oxygen species. So it has been reported that the killing efficacy of aminoglycosides, fluoroquinolones and β-lactams antibiotics is decreased under strict anaerobic conditions (141). Also, most urine samples from patients with UTI have a pH≤6.5, so the prescribed antibiotic for UTI must remain active under acidic environment. A feature that ciprofloxacin does not accomplish *in vitro,* for example (142,143), while fosfomycin does (144).

1.3. Ciprofloxacin role in UPEC:

Quinolones were discovered in 1962 by distillation of quinine, and all current derivatives have a dual ring structure, but were used in clinical field since 1983 (145,146). The potency of quinolones was improved against GNB with the development of fluoroquinolones by adding a 6-fluoro group which enhances a broader spectrum and their pharmacokinetic properties (146). Ciprofloxacin is a second-generation fluoroquinolone antibiotic, while levofloxacin or moxifloxacin are third-generation ones (147). Its clinical and medical importance fall to be an active pharmacophore, so structurally diverse ligands can bind to a common receptor site, leading it to have various activities: antibacterial, anticancer, antitumor, antitubercular, antiviral, antimalarial, among others. Focusing on its antibacterial role, ciprofloxacin acts by forming a complex with DNA gyrase in gram-negative bacteria and topoisomerase IV in gram-positive bacteria affecting the synthesis of bacterial mRNAs (transcription) and DNA replication (supercoiling DNA) (148). When ciprofloxacin binds them, the DNA breaks (149). It does not affect the human DNA because DNA gyrases are not present in eukaryotic cells, and the equivalent topoisomerases (type II DNA topoisomerase) are not sensitive to fluoroquinolone inhibition (150).

Some of the ciprofloxacin approved indications are UTI, some sexually transmitted infections, skin, bone and joint infections, prostatitis, typhoid fever, gastrointestinal infections, anthrax, or salmonellosis; but it can also be used as alternative regimen in lower respiratory tract infections, tuberculosis; polymicrobial infections or in patients with predisposing factors for resistant gram-negative infections (151,152).

Its high bioavailability (≥70% of the oral dose) (153), makes this antibiotic a good option for ambulatory-management of infections (154). Moreover, during oral treatment

of UTIs the ciprofloxacin concentration in urine is very high (>900 mg/L), much higher than the minimal bactericidal concentration (MBC) (defined in 0.008 mg/L) for *E. coli*. The urine levels keep over this level up to 72 h after a single dose of ciprofloxacin (155), making ciprofloxacin one of the most commonly used antibiotics for UTI treatment (156,157).

1.3.1. Ciprofloxacin resistance mechanisms

In the past decades, research has been trying to go beyond the qualitative phenotypic characterization (sensible or resistant), but to achieve the genotypic or even the quantitative phenotypic categorization ("how much sensible or resistant is") in ciprofloxacin resistance mechanisms to *E. coli* (149). Further than that, some authors point out the need to obtain alternative antimicrobial susceptibility testing, as phenotype techniques sometimes may not match MIC levels (158), and most of the genotype testing (such as PCR or microarray techniques) do not exhibit MIC levels but the carriage of certain mutations or enzymes which do not necessarily match with the resistance mechanisms, some of them are not fast tests, neither present the optimal negative and/or positive predictive value, or are not widely accessible (128).

Four main mechanisms have been reported for ciprofloxacin resistance in *E. coli*: target alterations can be found in DNA gyrase and topoisomerase IV, efflux pumps which can decrease the drug accumulation and enzymes that block drug targets or modify the drug itself. Combinations of these mechanisms might be observed (149,159,160).

Gyrase and topoisomerase IV are composed of two subunits encoded by genes *gyrA* and *gyrB*, and *parC* and *parE*, respectively. Ciprofloxacin resistance can be acquired through mutations in all these genes (161). Most of them accumulate consecutively,

while ciprofloxacin MIC increases steeply, but some specific mutations, like in *parE* case, MIC values are not affected (149).

Mutations can also affect efflux or permeability target points of the bacteria such as *marR, soxS* or *acrR* (regulators of *acrAB* and *tolC* genes which encode transmembrane efflux pumps) (161–165). Other genes, like *qnr* family, located on transmissible plasmids have been detected (166): *qnrA, qnrB, qnrC, qnrD, qnrE, qnrS* and *qnrVC* (166,167), along with other mutations like *aac(6')Ib-cr* and *crpP* genes (168,169).

Other genes or some operons related to efflux pumps have not shown any effect in ciprofloxacin MIC, like *qnrVC* (163). Accumulative and mixed step-by-step modifications have been identified, e.g. mutation in *rpoB* gene needs previous ones to arise (170), or the most prevalence combination found *gyrA* (Ser83, Asp87) and *parC* (Ser80) probably due to their low fitness costs and an exponential effect in MIC levels (164,171). Mutation in *acrB* can increase susceptibility to other antimicrobials, as previously commented (149).

In fact, an isolate can possess multiple resistance determinants (plasmid-mediated or chromosomal-mediated genes) encoding for multiple resistance mechanisms. In addition, multiple resistance determinants can all encode for a single resistance mechanism. For example, the presence of any of the mutations in *Ser83Phe, Ser83Thr (gyrA), Ser80Arg (parC)*, combined with any *qnr* gene alterations, are the most important resistance (*in vitro* and *in vivo*) determinants reported to ciprofloxacin MIC in *E. coli* (149).

Another specific mutagenesis mechanism is SOS response. It is activated by the DNA damaged after being under exogenous substances, like ciprofloxacin. It works via *LexA/RecA* pathway, which derepresses SOS promoters. The DNA damage repair system with its error-prone DNA polymerase activity itself, induces mutations as well

(172–175). The SOS response also activates *qnr* genes (176), and promotes the horizontal elements gene transference of "jumping genes", that in the presence of ciprofloxacin can confer resistance to other antibiotics like chloramphenicol, sulphonamides, streptomycin and trimethoprim (177).

1.3.2. Effect of urinary tract physiological conditions on ciprofloxacin activity and resistance

As for β-lactams occurred, the physicochemical proprieties of quinolones have relevant consequences for their pharmacokinetics and pharmacodynamics (134). Ciprofloxacin is an ampholyte that can exist in four different pH-dependent protonation forms: cation, zwitterion, neutral, and anion (178). The ionization status of ciprofloxacin increases its bioavailability, depending on the pH. At the isoelectric point of quinolones in water (about pH 7), zwitterionic and neutral species are the most common found (179,180). The zwitterionic form rises bacterial permeability through porines (favouring increased bacterial accumulation) (180,181), and neutral form improves its intestinal absorption (182), modifying consequently its antimicrobial activity.

But for most of urine samples from *E. coli* UTIs, pH values are lower than 6.5, as previously said (142). At that pH ciprofloxacin it is in cationic status, decreasing its penetration into bacteria and so, reducing its activity (183,184).

Divalent cations, as well, reduce ciprofloxacin activity by forming complexes with ciprofloxacin molecules (in magnesium case) (185) or by not allowing the ciprofloxacin uptake by bacteria (in calcium case) (186). Anaerobic conditions can also raise MIC values (162), lowering ciprofloxacin lethally activity (187), as previously commented.

To sum up, these urinary tract physiological conditions can modify ciprofloxacin activity –among others antibiotics-, which in combination with de mutagenesis induced by ciprofloxacin itself previously explained could justify the generation of diverse resistance mechanisms. This situation can lead low-level quinolone resistant (LLQR) strains to survive and produce recurrence UTI by UPEC.

So, in *in vitro* scenarios, acidic urine pH is proposed to reduce ciprofloxacin activity which could generate an ideal environment for the selection of strains harbouring LLQR determinants, decreasing their susceptibility to ciprofloxacin, allowing for the survival of microorganisms traditionally considered as susceptible (143,188).

1.3.3. Low level quinolone resistance (LLQR)

The European Committee on Antimicrobial Susceptibility Testing (EUCAST) of *E. coli* clinical breakpoints established for ciprofloxacin a MIC ≤0.25 µg/mL as the susceptible cut-off point and >0.5 µg/mL as non-susceptible cut-off point (189,190). So, LLQR have higher MICs than the settled cut-off value, but remaining below the resistance breakpoint (161,191). Lately, the definition of susceptibility categories has changed, especially the old intermediate category, now re-named as "susceptible increased exposure category". It suggests a higher-than-normal drug dosage to ensure efficacy, and also it helps in not boosting resistances in this buffer zone (189,192). So, based on epidemiological cut-off (which could distinguish bacteria with and without acquired resistance mechanisms and help predicting their clinical susceptibility) (189), LLQR are found in the range from 0.06-0.5 µg/mL (158,193).

The prevalence of *E. coli* LLQR, range from 18 to 71% and it has been described in varies studies which presented single or mixed mutations genes (161,191). The prevalence LLQR rates varies depending on the local microbiological resistance rate, patient features (higher in inpatients), and other antibiotic susceptibility patterns (194–196). Although LLQR isolated from UPEC infections carry plasmid-mediated (or others) resistance determinants that can lead to their survival and justify UTI treatment failure and even recurrent UTIs, the key point leading to a survival under the extremely high ciprofloxacin concentrations in the urinary tract has not been established. Physiological conditions as urine pH play a role in this situation (143) and might be considered when performing ciprofloxacin susceptibility and in the selection of the antimicrobial in lower UTI caused by UPEC (188).

1.4. Fosfomycin role in UPEC:

Fosfomycin inhibits the bacterial cell wall formation by interfering with the peptidoglycan biosynthesis, in earlier steps than β-lactams or glycopeptides (197,198), which makes less probable the induction of cross-over resistance with other antibiotics (199). In fact, it is a class by its own, being unrelated to any other antibiotic family (200).

Fosfomycin is mainly excreted in the urine, obtaining very high concentrations (1053-4415 mg/L) within 4 hours after a single oral 3 g dose, enough to eradicate any susceptible bacteria, maintaining urine concentration >128 mg/L for more than 36 hours thereafter (201,202), being fosfomycin MBC for *E. coli* strains defined as <0.5 mg/L (203). The excretion of fosfomycin decreases with renal impairment but also raises its elimination half-life, resulting in higher urinary concentrations after 48 hours after

administration. Its use is approved in end stage of chronic kidney disease (<20 ml/min) (204) an dose adjustment in patients with renal insufficiency is not required, contrary to nitrofurantoin (and other antibiotics active against UTI) that is also contraindicated in advanced stages (<40 ml/min) (205). Oral fosfomycin-trometamol and intravenous fosfomycin (up to 18-24 g per day) are both well tolerated (206,207). All these properties makes from fosfomycin-trometamol the preferred formulation in single dosage or *"every other day"* regimen for the treatment of uncomplicated lower tract UTI (208,209).

Fosfomycin has a wide spectrum bactericidal activity against gram-positive and gram-negative bacteria. In the context of MDR bacteria emergency, fosfomycin has been revaluated as a potential option in diverse clinical settings (210). Fosfomycin, also, has an excellent capacity for diffusion and penetration in different tissues as soft-tissue, bone, lung, gastrointestinal tract, or even the central nervous system. It has also been proposed as combination therapy to treat sepsis and intra-abdominal infections caused by MDR bacteria -such as ESBL-producing bacteria and carbapenem-resistant bacteria- with susceptibility to fosfomycin (199,207,211). Its oral administration is approved for urological prophylaxis procedures, non-complicated, complicated, and recurrent or even MDR UTI (207,212–214).

It must be considered that fosfomycin efficacy in UPEC strains is different to the efficacy observed with other *Enterobaterales*, specially *K. pneumonia* isolates, which show a higher MIC and MBC, probably due to its larger and widely spread fosfomycin and other antibiotic-resistant mechanisms (215). Moreover, it is important to highlight that fosfomycin penetrates poorly into the renal parenchyma and classically is not

recommended in cases of pyelonephritis as it occurs with nitrofurantoin (216), but the trend is changing towards its use in general population (217,218).

1.4.1. Fosfomycin resistance mechanisms

Resistance mechanisms to fosfomycin can be acquired as linked to its transporters, encoding system, or fosfomycin-modifiers enzymes and intrinsic, like modifying its binding target (211,219).

To be actively transported into the *E. coli* bacterial cytoplasm, fosfomycin needs efflux pumps family transporters: GlpT and UhpT. The first exchanges glycerol-3-phosphate and the second glucose-6-phosphate. UhpT for its own transcriptional activation have UhpA and UhpB regulators. Few genes linked to the encrypting of its transporters have been reported (220,221), which decrease fosfomycin uptake by the bacterial cells, and also to the codification of its transport system regulators (202): *glpT*, *uhpT*, *uhpABC* genes.

For the full expression of the transporters, cyclic AMP (cAMP) is required. The cAMP system can be affected in multiple ways: mutations in *cyaA* or *ptsI* genes produce a decrease in the intracellular cAMP levels leading to a reduced expression of them (200,222). The inactivation of the cAMP receptor protein (named CRP and encoded by *crp* gene) could be another via of resistance induction through the inactivation of intracellular pathways in response to low-glucose situations, or other stresses (223), but it has not been well defined. Also, alterations in the binding complex cAMP-CRP, which participates in the upregulation of *glpTQ* operon expression (224,225) or the *uhpT* transcription (226,227), could lead to resistance.

Once in the cytoplasm, fosfomycin binds covalently and inhibits UDP-GlcNAc enolpyruvyl transferase (*murA* gene), an essential enzyme for peptidoglycan

biosynthesis. Its own mutation also leads to fosfomycin resistance by its inactivation (228). Also, *MurA* overexpression implies higher MIC levels (229), reaching clinical resistance levels at a low fitness cost (230).

Another plasmid-mediated fosfomycin resistance mechanism could be through *Fos* family genes, which codify enzymes responsible for fosfomycin modification (219). Fosfomycin is a phosphonic acid derivative (201) and it can be processed by phosphorylation of the phosphonate group (*FomA* and *FomB* driven) (231), or by cleaving the epoxide ring in the fosfomycin structure. This last one may be catalysed by glutathione transferase (determined by *FosA* gene), L-cysteine thiol transferase (*Fos B*) or fosfomycin-specific epoxide hydrolase (*FosX*) (232). But there are plenty involved: *fomA, fomB, fosA, fosA2, fosA4, fosA5, fosB, fosB1, fosB2, fosC, fosD, fosE, fosF, fosG, fosK, and fosX* (202,212). Alterations in each gene could lead to fosfomycin modification and a path to resistance.

Commonly, the plasmids carrying *Fos* also carry additional resistance genes (233) increasing the risk for co-selection of other fosfomycin-resistant mechanisms under the selective pressure by other antimicrobial agents (72). Also, these plasmids can confer co-resistance to other antibiotics like β-lactams, aminoglycosides, or fluoroquinolones (234). In some Asian areas *fosA3* gene is frequently found in plasmids together with ESBL genes like $bla_{CTX\text{-}M\text{-}55}$, $bla_{CTX\text{-}M\text{-}15}$, *or* $bla_{CTX\text{-}M\text{-}123}$ in different animals (235,236), which also co-harbour *cfr* (a MDR gene) (237).

Contrary to the general belief of the difficulty to have fosfomycin resistances due to its unique features and to its narrow use compared with other antibiotics (212), fosfomycin resistances have been detected in *Enterobacterales* since 1980s (238). They have been spread mainly in *P. aeruginosa* isolates (212). The plasmid-mediated genes seem to be the responsible for its rising current resistance rate (211), mostly related with an

increase of fosfomycin-resistant and ESBL-producing *E. coli* strains (from 2.2% in 2003 to 21.7% in 2008, in Asian studies) (239–241). The Spanish rate of fosfomycin resistance in non-MDR *E. coli* strains, was in the range of 2.8-4.7% from 2006 to 2012, being 1.3% for other European countries for the same period, and the rate for ESBL-producing *E. coli* strains around 7% (109). Another recent study has shown a trend to decrease the fosfomycin resistant rate in UPEC strains (from 5 to 1.3% from 2014 to 2019 in United Kingdom) (110).

1.4.2. Effect of urinary tract physiological conditions on fosfomycin activity and resistance

Contrary to ciprofloxacin, the UTI-like environments such as acidic pH values (242) and anaerobiosis can convert most of the strains categorized as resistant to fosfomycin to susceptible status, leading to a better fosfomycin activity against UPEC and it LLFR strains (144). The molecular bases for these changes are uncertain. But acidic pH values protonate fosfomycin partially in a closer lipophilic-like state, easing its penetration into the bacteria and increasing its effect (243). Moreover, anaerobic environment activates the expression of FNR protein, the binding of *glpT, uhpT* efflux pumps (244), and the expression of *crp* and *cyaA* inner cell transporters (245), increasing fosfomycin susceptibility among UPEC.

1.4.3. Low level fosfomycin resistance (LLFR)

Some studies have described the prevalence of mutations in UPEC and their contribution of these mutations to fosfomycin resistance. However, it is not well known how the mutants arise and spread during UTI. High resistant mutants may appear depending on other unperceived pre-existing mutations. The presence of certain

mutations as *glpT* or *ptsI* could have no impact on MIC levels (246), being difficult to detect the pre-existing mutations by standard tests.

As previously stated with ciprofloxacin, low-level resistant to fosfomycin (LLFR) has been described. Following EUCAST guidelines in 2020, susceptible strains were MIC ≤ 32 µg/mL, and resistant ones were > 32 µg/mL (189). The LLFR range of action could be defined from MIC 2 to 32 µg/mL, as 2 µg/mL was reported as the high original MIC where no UPEC strains (neither LLFR or resistant ones) could survive after a single dose of fosfomycin in an *in vitro* model (247). By the time the results were written EUCAST updated the fosfomycin cut-off to 8 µg/mL for enterobacteria, so LLFR was re-categorized as MIC from 2 to 8 µg/mL (190).

The LLFR strains are involved, as well, in the heteroresistance concept, where small subpopulations of seemingly susceptible bacteria grow after the exposure to an antibiotic, due to its resistances (212). Heteroresistance has been reported to be multifactorial for *E. coli* after fosfomycin exposure (222,231,248,249).

It was thought that a reason why fosfomycin is still effective for UTI with a low rate of fosfomycin resistance during UPEC treatment, was the high fitness cost of mutants -contrary to ciprofloxacin-, and the high urinary concentrations of the antibiotic (211,244,250). However, this hypothesis, has lately been rejected (249).

In spite of that, it is worthy to understand how LLFR mutants contribute to fosfomycin-resistance and bacterial fitness under UTI conditions (144), as it is a population being either a step towards the evolution of resistance. The main questions are whether resistance to fosfomycin will compromise its non-UTI and UTI indications use (211), and if LLFR population triggers the development to full-resistant-strains according to the treatment used and the urinary conditions.

1.5. Urinary tract infections in the kidney transplant recipients (KTR):

Infectious are the major cause of morbidity and mortality among transplant recipients. Urinary tract infections are the most common infectious complication in KTR with a very wide reported incidence that ranges from 25% to 75% (251–254). This large interval can be explained due to differences in definition, diagnostic criteria, study design, and length of observation.

1.5.1. Incidence of UTI in solid organ transplant (SOT) and KTR

Despite improvements in surgical techniques, antimicrobial prophylaxis, new immunosuppressive therapies and hygiene measures, infectious complications remain a major cause of morbidity and mortality in SOT recipients (251,253–256). In fact, infections are reported as the first cause of post-transplant complications, followed by cancer and major cardiovascular events, but the second cause of mortality in SOT patients (257). Urinary tract infections are one of the most common infectious complications among them and a main use of antibiotics therapy (258).

One of the largest prospective series reported that 4.4% of SOT recipients developed UTI with an overall incidence of 0.23 episodes per 1000 days of transplant. This incidence varies significantly depending on the type of transplanted organ. Kidney transplant recipients have the highest risk of UTI, with an incidence of 0.45 episodes per 1000 days of transplant and a frequency of 7.3%, followed by kidney-pancreas (5%), heart (2.2%), liver (1.6%) and lung recipients (0.7%) (255). Other authors described an incidence ranging from 4% to 75% in renal allograft recipients (251,253,254,256,259–262). Moreover, it has been described that 25-47% of renal recipients have at least one symptomatic UTI during follow-up (252,259). The incidence of exclusively pyelonephritis in KTR is 2.8 episodes per 100 patient-year

(263). This wide interval might be related to the heterogeneity at establishing the definition and classification of UTI -asymptomatic bacteriuria, acute pyelonephritis, lower UTI, urosepsis, etc.-, frequency of routine urine culture testing, diagnosis bias, follow-up times, surgical techniques strategies, antimicrobial prophylaxis, and immunosuppression regimen, and the retrospective design of most of the studies here reviewed.

1.5.2. Burden of UTI in KTR

Suffering UTI during the first year after transplantation increases the mortality and the costs (extra 1800$) per event, as well as the risk of graft loss. The extra cost and clinical consequences persist during the second and third year after transplantation. Cost and clinical implications of suffering sepsis or pneumonia are higher than in UTI, but globally, UTIs has more impact cause as its incidence is much higher (264). Lentine *et al.* studied the impact of acute rejection. The cost of its treatment during the first year after the transplantation was similar to the cost of the UTI in the same period (265). Although treatment of an episode of acute rejection is more expensive than treating ITU, the prevalence of UTI is higher, and makes this entity the first cause of expenses in KTR (266).

1.5.3. Risk factors of UTI in KTR

Most episodes of UTI occur during the first 6 months after the transplant (251), being the first month the main period of events (255,258). During the first month, asymptomatic bacteriuria occurs in 22-71% of the patients (260,267–269), and symptomatic UTI in 12-34% (252,268). The study with the longer follow-up time, 36 months, recorded an incidence of acute pyelonephritis during the first 6 months of

6.4%, with an incidence of 10% at the end of follow up. The rate of urologic-related sepsis in the first six months and 36 months from transplantation were 0.6% and 5%, respectively (267). Apart from that, very few authors stratify the incidence by type of UTI. In the RESITRA cohort the distribution of UTI during the first three months of the transplant were 82% cystitis and 18% acute pyelonephritis (255). Other studies, have reported that during the first 6 years after transplantation there were 18-38% asymptomatic bacteriuria, 7.6-25% cystitis, 12.5-22% acute pyelonephritis, and 4% sepsis (270,271).

The following risk factors of pyelonephritis have been described: female sex, acute rejection, use of mycophenolate (MMF) as immunosuppressor agent, age, days of bladder catheterization, presence of urological catheter, genitourinary structural or functional abnormalities, UTI during the month prior to the transplant, ureteral stent, frequent episodes of acute rejection, cytomegalovirus (CMV) disease, illness of the native kidney (e.g. polycystic kidneys or hydronephrosis), more than two asymptomatic bacteriuria episodes; and other features in the donor as cadaveric donor graft, advanced age or hypertension (266,270–273).

Reported risk factors of acute cystitis are: female sex (not confirmed in other studies (264,274)), bladder catheterization ≥ one week, no preoperative prophylactic antibiotic, immunosuppressant induction therapy, recurrent UTI before transplantation, acute rejection, CMV disease, asymptomatic bacteriuria, age, haemodialysis just after transplant (reflecting delayed graft function), obesity, neurogenic bladder, polycystic kidney, renal lithiasis (261,270–272,274) and other features in the donor as cadaveric donor graft, advanced age or hypertension (264,266,275).

Risk factors of asymptomatic bacteriuria are: female sex, immunosuppressant induction agent, morbidity by Charlson index, past acute rejection episodes, CMV disease, acute glomerulonephritis, double transplant, and advanced age of the donor (267,270,276).
Elderly patients and those with long bladder catheterization are at higher risk of UTI-related bacteraemia (272).

1.5.4. Impact of UTI on graft survival, graft dysfunction and mortality

The effect of UTI on the graft and organ recipient remains controversial. So far, a consensus has not been established whether the development of UTI in KTR carries a higher mortality or graft loss, although a tendency to graft dysfunction has been suggested. E.g.: some authors described that UTI raises mortality but not acute rejection (277), while others affirms that early symptomatic UTI also increases the risk of acute rejection (278). Previously to 2008, UTI (including asymptomatic bacteriuria) was reported to be associated to chronic rejection, papillary necrosis, mortality and graft dysfunction (253,279,280). Since then, and despite several studies carried out (259,261,281–286), no definitive conclusions have been reached out.

Graft acute pyelonephritis has been pointed as an independent risk factor for impaired renal function during the first year after transplantation (253,287), while others found only early pyelonephritis as a predictor of renal impairment, but at long-term and accompanied by renal graft loss and recipient mortality (263). A third study could not find these associations by the third year of follow-up (267).
Symptomatic UTI in the first year has been related to graft rejection (288), while in another study any kind of UTI in the same period has had no relevant outcomes (282).
Early graft pyelonephritis and UTI have been associated with graft loss in some studies

(279,280), while any type of UTI during the first six months after transplant was associated with death in another study (280). In latter studies no impact have been related to any type of UTI even in the immediate transplantation time (259,261). Still in UTI-related bacteraemia cases no associations were seen (255,285).

Other authors stood out the importance of the microorganism responsible for the UTI, and not the type of UTI itself. E.g.: the production of P fimbriae by *E. coli* has been related to renal injury after UTI (289).

It has been suggested that UTI can be both cause and consequence of acute rejection: early UTI could cause renal graft scares deteriorating the renal function (measured by radiotracers) (282) and trigger acute graft rejection, and the rejection may promote UTIs by increasing the immunosuppression net (271).

Most bacteriuria episodes after transplantation are asymptomatic. Recurrent asymptomatic bacteriuria had been linked to acute rejection (286), to chronic rejection (280) and to subclinical damage, probably due to pro-inflammatory pathways (with an over-expression of TNF-α, Il-6 and IF- δ) (290,291).

Untreated (or even treated) persistent bacteriuria, which can also be categorized as asymptomatic bacteriuria, was postulated as a harming situation (276,292).

Previous studies have reported an association between asymptomatic bacteriuria and the risk for developing allograft pyelonephritis (267,292) and blood stream infection (285) that increases the hazard ratio for renal allograft failure three times and the risk for all-cause mortality two times (293). Its presence has been associated to acute rejection in two retrospective studies (285,292). However, given the retrospective design, it could not be concluded a causality relationship (294).

A small clinical trial reported that asymptomatic bacteriuria are not followed by symptomatic UTI (259). On the other hand, other studies did not observed any

association between asymptomatic bacteriuria and acute rejection (287), renal impairment (283), and/or all-cause short-term mortality (259). There is a theory called the bacterial interference, which sustains that asymptomatic bacteriurias are caused by low-virulence microorganisms. Therefore, keeping them untreated allows competition with those strains that might cause symptomatic episodes (258). At the time of the design and initiation of this PhD thesis, recommendations about treating asymptomatic bacteriuria were not established. On one hand it was thought that its treatment could avoid pyelonephritis or even rejection (295,296). Prospective studies and trials affirm that these events might act as confusion variables for treated asymptomatic bacteriuria cases (294), because we trend to treat more the most disadvantaged cases. Current practice standards suggest follow-up of asymptomatic bacteriuria, and only treat them when the serum creatinine increases, rejection occurs, or in the first two months after the transplant (256,268,287,297). However, some authors promote the idea of "non-innocent UTI" (294,298–300).

In summary, definitive effects of UTI in KTR are controversial. Early events seem to carry a higher risk, while others demonstrate no risk at all, at least for asymptomatic bacteriuria and acute cystitis. Regarding graft pyelonephritis occurring early after the transplant might be more dangerous than those occurring later (301). More prospective and trial studies are needed to clarify this issue.

1.5.5. Resistant and multidrug resistant microorganisms in KTR with UTIs

Epidemiologically, the most frequent microorganisms causing UTI in the SOT setting are, as in the general population, GNB, mainly *E. coli*, followed by *Klebsiella* spp., *P. aeruginosa* and *Enterococcus* spp. (302). Some authors propose enterobacteria from the donor and nosocomial *Pseudomonas* spp., and other *Enterobacteriaceae*, as the major

source of post-transplant infections (257). There is an increasing rate of MDR strains of uropathogens worldwide (302,303), as it is in UPEC strains (116). Specifically in general European population, the prevalence of MDR UPEC strains in 2018 were >20%, while ESBL-producing UPEC strains increased to >60%. The Spanish RESITRA cohort reported among KTR included a 25% UTI caused by ESBL-producing UPEC (255), similar to older Spanish rates (304), but lower than later studies (38.4%) (259). In a Spanish study comparing two cohorts of UTI (over 1000 samples) in KTR in 2002-2004 *vs.* 2011-2013, a rising of non-susceptibility rates among *Enterobacteriaceae* for every class of antibiotics were observed (40% in general, 55% to A/C and 33% to P/T), but not for fosfomycin (303).

As previously commented, MDRs rates in UTI in KTR depends on multiple factors. Local epidemiology is an important one: ESBL-producing *Enterobacterales* rate in UTI was 52.5% in Poland (305), 18.6% in Brazil (306), and 3.4% in the US (302). In a Turkish study, ESBL-producing UPEC and *Klebsiella* spp. caused 53% of the UTIs among all etiologies in KTR (307).

Quinolone resistance *Enterobacterales* are common in UTI in KTR and it has been reported in 38-50% in *E. coli*, 25-31% in *Klebsiella* spp., and 21-25% in *P. aeruginosa* (255,308). This high rate could diminish afterwards. E.g.: the 50% of ciprofloxacin-resistant UPEC rate during the first month post-transplant was dropped to 32.4 % after the sixth month (309).

In the general population, ESBL-producing enterobacteria UTI risk are associated to contact with health care services or nursing home residencies, prolonged hospital stay, intensive care unit admission, prior antimicrobial therapy and recurrent UTI, among

others (310–312). Solid organ transplant recipients share many of these factors that predispose to MDR infections (310–312).

In transplant recipients, UTIs caused by the ESBL-producing *Enterobacterales'* risk factors are: renal surgery, need for a second surgery, diabetes, bladder catheterization, urethral stent, urinary obstruction, prior UTI episodes, recurrent UTI, prior ESBL-producing bacterial infections, previous use of cephalosporins, carbapenems or glycopeptides, or even the use of cotrimoxazole as postoperative prophylaxis (302,305,306,313).

Other classic factors related to UTI have not been clearly associated with ESBL-producing *Enterobacterales* in the general population, as gender (306), the long urethra male barrier (314), or receiving previous antimicrobial therapy (259). Regarding age, one study did not observe any differences in the rate of MDR UTI in women over 65 years old compared to younger women (315), while a Spanish study reported an increased risk in women older than 60 years (316).

Multidrug resistant infections in KTR (and other SOT recipients) are associated with increased mortality and graft failure (302,317,318) and favours the recurrence of UTI (288).

In a large Spanish cohort study, it was reported that *K. pneumoniae* UTI increased the odds of microbiological failure and ulterior pyelonephritis after receiving treatment in KTR (303). Brizendine *et al*. reported that carbapenem-resistant *K. pneumoniae* bacteriuria (asymptomatic and symptomatic) were associated to higher mortality (310,319). As most of the risk factors for MDR are not modifiable, it is plausible that in the coming years sepsis and allograft dysfunction secondary to infections would increase the mortality in the SOT setting (302).

1.5.6. Recurrent UTIs in KTR

Almost half of KTR will develop at least one symptomatic UTI (252,259), but only in a third the infection will recur (277,290,320–322).

Recurrent UTI is defined as the presence of three or more episodes of UTI (symptomatic and confirmed by urine culture) over a 12-month period, or two episodes in the previous 6 months (323). Most authors include asymptomatic bacteriuria in this definition (or at least they do not differentiate). Recurrent UTI, is common in KTR, with an incidence rate of 3-27% (277,290,320–322), while it is uncommon in other types of SOT recipients (2.7%) (255). These infections worsen the quality of life and increase the cost of kidney transplantation (253,324). The long-term effect on graft function or survival has not been conclusively established. Evidence to identify those patients at high risk of recurrent UTI is also deficient.

Risk factors for recurrent UTI are not well defined. Many of them are also predisposing factors to UTI and include: advanced age, female gender, Afro-American ethnicity, diabetes mellitus, UTI immediate before the transplant, chronic hydronephrosis, vesico-ureteric reflux or polycystic kidneys as underlying disease, native kidney pyelonephritis, previous pyelonephritis, dead donor or expanded criteria donor, ureteral stent beyond 30 days after transplantation, UTI in the first month post-transplant, concomitant cytomegalovirus disease, viral hepatitis, re-transplantation, use of thymoglobulin, and prolonged time in haemodialysis (262,271,272,289,320,321,325–328).

Regarding aetiology, MDR UTIs were associated with recurrent UTI (306,329), along with a longer hospital stay and higher mortality even in asymptomatic patients (303); with a progressively increasing incidence in every later event (306). Other associated conditions to MDR recurrent UTIs were persisting bacteriuria after 48 hours of proper

antibiotic (330), P fimbriae-producing UPEC strains (289), or carbapenemase-resistant *K. pneumoniae*.

Recurrent UTI must be promptly investigated in KTR to rule out the existence of anatomical or functional abnormalities of the urinary tract such as urinary tract obstruction, strictures, stenosis, new vesico-ureteric reflux, renal calculi, neurogenic bladder, or complex cysts. A meticulous exam including imaging studies of the urinary tract, cystoscopy, cystogram, uroflowmetry, and other urodynamic techniques, radiologic or even nuclear techniques, should be carried out if necessary (301,331–333). Other physio pathological mechanisms explaining recurrent UTI are shared with those described in non-transplant population as urethral or introitus colonization, prostatitis or other kind of urologic parenchymal involvement (orchitis, epididymitis, etc.), and benign prostate hyperplasia (324).

Once anatomical or functional abnormalities are discarded, other predisposing factors should be considered. Excessive immunosuppression should be evaluated, as well as infection by specific microorganisms such as *Mycobacterium tuberculosis*, polyomavirus BK virus (260), *Corynebacterium urealyticum* when there is obstructive uropathy, or *Microsporidia* (334,335), as these infections need special techniques for their detection. Recurrent UTI might be due to an unidentified infectious focus, too. E.g: a persistent infection of the native kidneys, perpetuated by the reduced incoming blood and the difficult accessing antibiotics (273).

But the reality is that most patients with post-transplant recurrent UTI do not have identifiable abnormalities to justify the recurrences (324). A later study shows that patients with recurrent UTI, have an increased activation threshold of mucosal-associated T-cells (CD8+), showing a shared functional impairment of these cells which are proposed to be involved in the pathophysiology of recurrent UTIs (336).

Results of the studies focusing on the impact of recurrent UTI on the KTR outcome are diverse. An American retrospective cohort of 2500 patients stated that recurrent UTI reduced the graft function, graft survival and patient survival (326). An Arabic retrospective cohort study of 1000 patients showed no differences in allograft function and survival rates between recurrent *vs.* non-recurrent UTIs (327). About recurrent asymptomatic bacteriuria, they were associated with pyelonephritis but not with allograft dysfunction in a small prospective study (267). Other reports addressing MDR recurrent UTIs found no effect in graft function (329). Considering these data, it can be concluded that recurrent UTIs have a real and high economical cost but no clear impact in graft function, rejection, or recipient´s survival.

Stopping the screening of asymptomatic bacteriuria would decrease the number of recurrent UTI (as sometimes are misclassified with symptomatic events and treated like that), lowering consequently the exposure to antimicrobials in KTR, its costs, and its consequences. Parallel to the increasing rate of MDR strains, recurrent UTI might force us to use alternative antimicrobial treatment regimes, which are generally more toxic, less effective, and more expensive (68). Given the fragility of KTR, a reduction in the inappropriate use of antimicrobial agents would decrease costs and reduce adverse outcomes (298).

The treatment of recurrent UTI should be guided by previous UTI aetiology and previous antibiotics used, as well as local epidemiology (313). The development of specific antimicrobial stewardship programmes is warranted in this sub-population (337).

The duration of antimicrobial therapy is not established as there are not controlled studies that address recurrent UTI therapy, and all recommendations are based on

observational studies or expert opinion. Some old studies propose a 6-week treatment period for recurrent UTI (260,301), while others suggest prolonging it for at least three months, or even indefinitely (332). Spanish guidelines, among others, recommend cotrimoxazole as a good option for recurrent UTI in SOT population at least for six weeks, avoiding the use of quinolones (268,313), while other groups prolonged from six weeks to three months (270,332). The new American guidelines recommend the same length of treatment as simple cystitis or complicated UTI (maximum 21 days) without defining specific antibiotics (338).

Apart from antibiotic, another important strategy is to remove indwelling catheters, if present and unneeded (339,340).

1.5.7. Management of UTI in KTR

Urinary tract infections in KTR recipient were considered and managed as complicated UTI (219). Empirical antibiotic therapy of UTI in SOT patients, as well as their optimal duration, is based on the established recommendations for the general population due to the lack of randomized clinical trials in this population. To guide empirical therapy, as previously said, it is necessary to consider host clinical characteristics, including infection severity, local epidemiological data, patient´s history of resistant microorganisms and prior antibiotic therapies. Removal or replacement of urinary tract devices by the time of UTI diagnosis such as urethral catheters and urologic stents is recommended, too (332). Once susceptibility data is available, the most narrow-spectrum antibiotic should be used to complete the length of the therapy.

The current American Society of Transplantation guidelines recommends for lower-UTI in SOT setting: 14 to 21 days for complicated UTI with narrow-spectrum antibiotic based on susceptibility, 5 to 10 days for cystitis (recommending against single-dose or

3-day treatment course), and similar management for recurrent UTIs according to the urological symptoms, even though non-antimicrobial prevention strategies are preferred. In case of severe UTI event lower dose prophylaxis antibiotic could be considered in the first post-transplant month avoiding cotrimoxazole. No other specific antibiotic recommendation is given (338).

The European urological infections guidelines for general population recommends avoiding A/C use for complicated UTIs, suggesting in cystitis cases, the use of cotrimoxazole only in low MDR rates areas or in men cases. Fluoroquinolones should be avoided, as well (107).

The last Spanish guidelines in transplant recipients recommend treating cystitis with fosfomycin, A/C, or a second/third-generation oral cephalosporin (e.g. cefixime or cefadroxil), avoiding nitrofurantoin due to high resistances rates, and suggesting fosfomycin as first-option when MDR risk factors are present (313). In case of asymptomatic bacteriuria episodes, these guidelines keeps recommending to use the antimicrobial agent, route, and length, oral via, as the one used in cystitis (313).

Short courses (single dose-three day's regimen) in asymptomatic bacteriuria or cystitis, or 7 days regimen for acute pyelonephritis have not been properly studied in SOT recipients. Therefore, short regimens are not recommended in this setting (313,338). However, in the general population, short courses (7 days) are recommended in pyelonephritis (341), as well as single or double doses regimens are for cystitis (342), even in complicated UTI cases –including pyelonephritis- treated with fosfomycin-trometamol (343–346). Progressively, more retrospective studies regarding this issue are being published in KTR (347–351), however prospective studies are lacking.

1.5.8. Antimicrobial therapy used for UTI in KTR

At the design of this PhD thesis ciprofloxacin used to be recommended for the treatment of UTI (154,313,338). However, the increase resistance to fluoroquinolones (298,303,352,353), in UPEC precluded its use as empirical therapy in pyelonephritis. In 2018 fluoroquinolones use was restricted by the European and American Drug Agencies due to severe and permanent side-effects. It was recommended to avoid fluoroquinolones and quinolones in elderly, patients with kidney disease, SOT population, or patients under corticosteroid treatment because of a higher risk of tendon injury (354) and aorta injury (355). Moreover, they should not be prescribed for mild bacterial infections (as could be asymptomatic bacteriuria and uncomplicated cystitis) and specifically for recurrent lower UTI, if other antibiotics can be used (356).

Fosfomycin is an antibiotic classically used for the treatment of uncomplicated UTIs in women (214). It is active against most MDR uropathogens (excluding non-fermenting GNB) (357–359) –even in the KTR scenario (302,303,360,361)-, and has big pharmacokinetic and pharmacodynamic advantages as already mentioned (219). As far as the rate of resistances bacteria is increasing and leaving few options for oral therapy, fosfomycin seems to be a proper alternative.

In the general population a meta-analysis of 27 trials compared a single 3 g oral dose of fosfomycin-trometamol with regimens of 3-7 days of fluoroquinolones (norfloxacin, ciprofloxacin, ofloxacin, pefloxacin), pipemidic acid, trimethoprim, cotrimoxazole, β-lactams (cephalexin, amoxicillin) and nitrofurantoin for the treatment of cystitis. No differences in microbiological success between these regimens were found, but fosfomycin was associated with fewer adverse effects in comparison with longer regimens in pregnant women (362). Results in other populations are less clear (214).

Regarding MDR UTIs scenario, fosfomycin activity (over 90%) and efficacy against ESBL-producing UPEC, *K. pneumoniae* and other enterobacteria have been proven in

retrospective (213,363) and prospective (364,365) studies, showing that the dosage used in them -a single 3 g oral dose or repeated "every other day" regimens- of fosfomycin-trometamol may be a good option in uncomplicated cystitis. However, very few data were available regarding its use in KTR at the time of this PhD thesis design.

Reit *et al*. evaluated clinical and microbiological outcomes after fosfomycin treatment (both dosages previously mentioned) of 14 events (including asymptomatic bacteriuria, cystitis and pyelonephritis) in KTR, with 11 MDR cases. Microbiological recurrence, persistence and cure rates were 54%, 21% and 31%, respectively, in a 3 months' period. Future bacteriuria did not present less susceptibility to fosfomycin. Neither renal impairment nor systemic infection was developed. So, apart from its low effectivity, fosfomycin appeared to be safe (366). Neuner *et al.* (360) administered fosfomycin in 41 MDR UTI events (asymptomatic bacteriuria and cystitis), including 15 SOT recipients (10 KTR). The overall microbiologic cure rate was 59% but dropped to 21% in SOT recipients. Non-cured group counted for a higher stent rate and, 46% in carbapenem-resistant *K. pneumoniae* strains could justify it. This MDR bacteria seems to be associated with worse outcomes as previously referred (303,310,317,319). No other rates were provided. Some limitations of this study were the heterogenicity, the frequency and number of doses of fosfomycin.

New studies appeared showing better results. Loethen *et al.* include retrospectively 76 cystitis of 63 KTR with a 85% of clinical cure (defined as no need to retreat) after treating them with different multiple 3g doses of fosfomycin (350). Kerstenetzky *et al*. retrospectively recruited 76 episodes treated with 3g doses of fosfomycin in 64 patients (36 of them KTR) with cystitis and renal dysfunction. They presented a 34% of microbiological cure (only test-of-cure in 45% of the cases), and 86% of clinical cure.

Only 11% needed to be retreated. Renal impairment did not affect the efficacy, even with glomerular filtration rates below 20 mL/min (38% of the cases). Some limitations of this study were the absence of fosfomycin susceptibility, and that some cases of cystitis did not have a microbiological isolation (pyuria and symptoms were enough to define cystitis) (349). Ten Doesschaet *et al.* conducted a retrospective study in KTR in two transplant hospitals reporting ≤30% microbiological cure. Nonetheless, clinical cure was observed in 70% of lower-UTIs and 80% of pyelonephritis, with 30% of global recurrences. It included 53 events, and 5 step-down treatments for pyelonephritis cases. They recommended to use fosfomycin only as a last-resort for oral treatment (348).

The largest retrospective multi-centre cohort addressing the use of fosfomycin in KTR was a Spanish study that included only cystitis events (55% of MDR cases). The authors concluded that oral fosfomycin is an alternative option for the treatment of cystitis in KTR given its clinical efficacy and safety, low potential adverse events, and low ecological impact. Risk factors of fosfomycin failure were male gender and the presence of percutaneous nephrostomy (347). Recently, the same group reported a retrospective cohort of 133 KTR with only asymptomatic bacteriuria, including 57% of ESBL-producing *Enterobacterales,* treated with different fosfomycin presentations. Previous UTI and the use of fosfomycin as salvage therapy were predictors of microbiological failure, whose rate was 40% (351).

Intravenous fosfomycin has also been tested in KTR with UTI, but its evidence is minimum (367). This retrospective communication included 11 carbapenem-resistance enterobacteria UTI treated with 3-6g daily fosfomycin for 7-14 days, reporting a microbiological cure of 43% and clinical cure rate of 91%.

In a retrospective study carried out in our centre, we observed that resistance to ciprofloxacin was more common in asymptomatic bacteriuria isolates compared to

those causing cystitis (73% *vs.* 45%) and that patients with cystitis treated with fosfomycin had a better microbiological response than asymptomatic bacteriuria. Considering this facts, it might be suggested that asymptomatic bacteriuria strains could differ from isolates in symptomatic lower UTI in terms of ciprofloxacin and/or fosfomycin resistant mechanisms and other virulence factors (368). But, another study reported that asymptomatic bacteriuria strains contain similar virulence factors as symptomatic ones, with their capability to harm (300).

Summing up, fosfomycin seems to be a good antibiotic option in SOT UTI cases with a microbiological cure rate ranging from 20 to 85% (347,349–351,360,366).

However, the evidence available supporting its use is not strong. The retrospective design, small size and single-centre series, the use of fosfomycin as non-first-line therapy, in combination with other antibiotics or after previous treatment failure, non-differentiating asymptomatic bacteriuria, cystitis and pyelonephritis episodes, the absence of fosfomycin susceptibility and test-of-cure, and the heterogeneous and/or unknown fosfomycin dosage, doses or type of presentation (calcium or trometamol) are important limitations. Therefore, prospective studies are warranted to evaluate the use of fosfomycin in UTI in KTR.

1.5.9. Management of asymptomatic bacteriuria

It was a common belief that asymptomatic bacteriuria in renal recipients need to be treated (258,273). Asymptomatic bacteriuria was managed as symptomatic events because of the fear that immunosuppressant therapies and comorbidities as diabetes, neuropathy, or allograft denervation, may mask clinical signs of UTI and secondary cause rapid and severe complications as bacteriaemia, allograft impairment, graft loss, or mortality (259,273,291).

Most theories of how asymptomatic bacteriuria and/or recurrent bacteriuria events could affect KTR are unspecific and *in vitro*-postulated. Some of these theories include one of more of the following: bacteriuria might activate the immune system leading to rejection by pro-inflamatory cytokines activity; virulent UPEC graft infections might cause interstitial scars with impact on glomerular function; recurrent bacteriuria might induce the reduction of immunosuppressant therapy which could also lead to rejection; asymptomatic bacteriuria could also activate cytokines leading to chronic inflammation, and renal damage (289–291,369).

Green *et al*. reported in a cohort of 112 KTR that asymptomatic bacteriuria treatment of KTR was associated with a higher risk of symptomatic UTI during the first year after transplantation and length of hospitalization (286). El Amari *et al.*, in a cohort of 334, reported no association between asymptomatic bacteriuria treatment and ulterior pyelonephritis or symptomatic UTI. Each patient had at least two asymptomatic bacteriuria 2.3 months apart from each other, without relation to the prior asymptomatic event, or the previous therapy. But in the second event, 78% of the cases were presenting MDR strains. Treatment did not affect the rate of microbiological cure (similar to the spontaneous clearance, that was <60%) (287).

Boskabadi *et al*. in a case-control study, compared 43 treated asymptomatic bacteriuria events and 45 untreated in KTR, all of them considered late UTIs. No differences in the incidence of symptomatic UTI, further asymptomatic bacteriuria episodes or renal impairment were found during one year of follow-up. The asymptomatic bacteriuria recurrence rate was 58-73% (283).

Two Spanish trials address the impact of treating asymptomatic bacteriuria in KTR. The first one included asymptomatic bacteriuria beyond the first-month transplant with 2 years of follow-up. This study reported no association of asymptomatic bacteriuria

therapy with pyelonephritis or cystitis, *Clostridioides difficile* infections, and colonization or infection by MDR bacteria during follow-up (259). The microbiological persistence in the treated group was 66%, and 53% in those untreated. When *K. pneumoniae* or MDR bacteria were involved, microbiological persistence raised to 70%. Only 3.6% of total asymptomatic bacteriuria progressed to symptomatic UTI, and only one third of them -3 events- to pyelonephritis. The second study evaluated the development of pyelonephritis, bacteraemia, cystitis, graft rejection, graft function, graft loss, opportunistic infections, need for hospitalization, and mortality according to the treatment of asymptomatic bacteriuria. Although no differences were found between both arms, more MDR isolates were obtained after treating asymptomatic bacteriuria, including isolates resistant to fosfomycin (370).

In 2019 two meta-analysis and reviews were published summing up all the previous studios on this topic, with similar conclusions. This studies highlighted that the evidence regarding treatment of asymptomatic bacteriuria in KTR is scarce (371,372). There is still one clinical trial ongoing in this same issue (373), and in 2020 was published a clinical trial assessing the impact of treating 200 asymptomatic bacteriuria events beyond the second month after transplant (374): antibiotic use was associated with fewer subsequent cases of bacteriuria, without significant clinical benefit over the 1-year study period but more cases of resistant bacteria in the ulterior events. Similar results are shown in a recent retrospective Spanish cohort (351).

The treatment of asymptomatic bacteriuria has been also questioned in other populations such as pregnant women, diabetic patients, those with medullar injury, institutionalized people, or elderly (272,375–379). No clinical benefits and higher risk of bacterial resistance and ulterior symptomatic UTI have been described. Nowadays,

benefit for treating asymptomatic bacteriuria has only been proved in pregnant women and before urologic procedures with bleeding risk (296,297).

After the design and beginning of this PhD thesis, international guidelines made the recommendation against the treatment or even the screening of asymptomatic bacteriuria after the first-second month of the transplant, and the need of prospective data and subpopulation studies with a higher risk of adverse outcomes (297,338).

On the other hand, and despite of the commented studies, some groups suggest that the decision of treating asymptomatic bacteriuria in KTR should be up to every physician, in every event. Mainly, secondarily to the lack of robust evidence (380), and taking in account that asymptomatic bacteriuria has been defined as a surrogate variable for bad prognosis in KTR. So, asymptomatic bacteriuria is not a risk factor itself, but is a marker of increased susceptibility to worse outcomes (331,381). That might be the reason for some physicians to elect treating asymptomatic bacteriuria when accompanied by viral coinfection, renal dysfunction, any kind of rejection activity, urinary devices, recent history of symptomatic UTI, elevated count of urine leucocyte, increased serum acute phase reactants, or occurrence within the few months after transplantation (382). Most of these conditions are out of the evidence-based practice, as stated before.

1.5.10. Urinary tract infections antibiotic prophylaxis in KTR

Cotrimoxazole is commonly used as prophylaxis in the transplant setting. This drug had been previously used to prevent *Pneumocystis jiroveci, Toxoplasma gondii or Listeria* spp. infections, among others. Spanish and other UTI management guidelines recommend the use of cotrimoxazole prophylaxis for 3–6 months after the transplant (268,305,313). Other tested antibiotics are one-month ciprofloxacin after transplant,

being similar (and better in some reports) to cotrimoxazole for reducing the risk of UTI in KTR (383–385), but as previously stated it is not recommended for safety reasons (386). Fosfomycin seems to be useless for this purpose (387).

Some authors, state that cotrimoxazole prophylaxis after the transplant could induce bacterial resistance and fail to prevent early post-transplant infections (260), usually acquired in the hospital. Indeed, *E. coli* resistance rate to cotrimoxazole in the Spanish RESITRA cohort was high (77% of the cases) (255). This rate was even higher for *E. coli* isolates (up to 89%) in other studies (270,309), and for other pathogens, such as *K. pneumoniae*, with a resistance to cotrimoxazole rate observed in nearly 80% of the isolates. The rate of resistance to cotrimoxazole in other GNB-UTI may reach 70% of the isolates (388).

This resistance to cotrimoxazole can be explained by its use as *P. jiroveci* prophylaxis pneumonia in KTR and other immunocompromised hosts, and the great potency of GNB for developing resistance after exposure to this antibiotic (256,389). Cotrimoxazole-resistant *E. coli* rate has been reported after just one month of its prophylaxis administration (390). Alangarden *et al.* reported that 75% of patients with prior cotrimoxazole exposure were resistant in a posterior UTI episode (256). This resistance could appear during the subsequent six months of prophylaxis and might persist for a long period after discontinuation (385).

Despite this high rate of resistance, some authors reported that cotrimoxazole in KTR decreased the incidence of all cause infections after transplantation, especially UTI and bacteraemia. It also reduced graft loss, time to apyrexia and days of hospital stay (255,286). Conversely, other studies have reported no impact of prophylaxis in the incidence of UTI (270,309). In a retrospective single-centre cohort study reported that

cotrimoxazole prophylaxis did not reduce the risk of pyelonephritis, even with increased doses and duration (389). In a retrospective KTR study, cotrimoxazole did not reduce the incidence of UTI, while increased both amoxicillin and cotrimoxazole resistance rate (270). Co-resistance of amoxicillin and cotrimoxazole might be related to the fact that resistance genes for these antimicrobials are located on the same plasmid (391,392), as previously commented. The differences reported here might be explained by different dosage and duration of antibiotics, immunosuppression regimens, or population characteristics (270,309).

Opposite to the common knowledge, lowering the wide human clinical use of sulphonamides have no effect on its *E. coli*-resistant rate, probably because the other antibiotic co-resistances inheritance are persistently used (393); its use in food-producing and animals companies use continues (72); or presumably its low fitness cost (394).

Secondary antimicrobial prophylaxis for prevention of recurrent UTI in SOT seems to be anecdotic. A retrospective Turkish study with 136 KTR, 15 out of 34 patients with recurrent UTI received nitrofurantoin for 2.5-3 months and had similar rates of recurrence as the non-intervention group (321). Some meta-analyses in non-KTR address this issue in young women (395,396), and in old adults (397): the use of nitrofurantoin, norfloxacin, or cotrimoxazole had favourable results. They highlight the many clinical uncertainties which remain unaddressed at using long-term antibiotics for prevention of recurrent UTI. The American guidelines suggests secondary prophylaxis for recurrent UTI only in selected patients who had severe recurrent events, such as pyelonephritis, preferring non-antimicrobial prevention strategies (338).

In the general population, weekly 3 g of fosfomycin has demonstrated non-inferiority in prevention of recurrent UTI compared with a fluoroquinolone agent (398). Another

study that included 9 KTR using the same fosfomycin dosage for 6 months in secondary prophylaxis of recurrent UTI after severe MDR enterobacteria urosepsis, proved to be safe but without high efficacy rates (399).

Considering all this and given the increasing resistance of the main uropathogens to cotrimoxazole or fluoroquinolones, studies addressing the role of fosfomycin in the treatment of UTI, and recurrent UTI are needed.

1.5.11. Non antimicrobial strategies to prevent recurrent UTI

There is little information about non-pharmacological strategies for preventing the development of recurrent UTI in SOT recipients.

In non-immunocompromised woman, daily antibiotic prophylaxis (nitrofurantoin 100 mg) compared to cranberries, hormone therapy, self-initiated treatment or acupuncture was more effective but also more expensive (396). Self-initiated treatment does not prevent future UTIs but it is cheaper and improves quality of life (QALYs). The cons of cranberries and acupuncture strategies are that they are not commonly included in health insurance. Estrogens in post-menopausal women with lowered hormone levels appear to reinforce the vesical trigone, but dosage or administration mode have not been defined (332).

Pagonas *et al.* retrospectively studied the prophylactic use of fixed-dosage cranberry extract and L-methionine in KTR after 3 years of the transplant and found that using them led to an overall reduction in the incidence of recurrent UTI by 50%. Cranberries seems to have a dependent doses effect, and L-methionine is not recommended when renal function is <30 ml/min because it facilitates metabolic acidosis (400). Their mechanism are not well defined, but it is speculated that both interfere with the adhesion of uropathogenic bacteria, primarily UPEC (401).

Other successful strategies in women are topical application of *Lactobacillus*, which diminishes UTI by reducing the vaginal pH and lowering chances of bacterial overgrowth (402,403). No studies in SOT patients have been made with probiotics, but interventional studies were done in young women with good results (404), while failed in old women (390).

In KTR, some non-pharmacological but alternative options that can be proposed in recurrent UTI are surgical as bilateral nephrectomy in polycystic renal disease (with a high surgery risk), and laparoscopic native nephrectomy in cases of hydronephrosis or vesico-ureteral reflux (405–407).

Some new strategies have been reported as vaccines or faecal microbiota transplantation. A clinical trial carried out in healthy women, evaluated the impact of an immunogenic bio conjugate vaccine against ExPEC on preventing symptomatic UTIs, bacteraemia and other invasive UPEC infections, possibly by a cross-reactivity between the different pathogenic serotypes (408). It was safe, but it is still pending on phase 2 studies results. Faecal microbiota transplantation has been used in selected KTR cases with recurrent *Clostridioides difficile* infections. It decreases future UTI and improves their susceptibility, by decolonizing MDR microorganism in faeces, and re-establishing the enteric commensals (409). Future research is also focused on anti-virulence factors UPEC mechanisms, such as pilicides and manosides. But most of them are in pre-developing stages (19,324,410).

There are not many robust studies in the non-pharmacological area for preventing recurrent UTI, and very few include KTR. Most of these strategies appear to reduce UTI, particularly in women, but not all the studies are conclusive. Adherence, long time tolerance, toxicity, and rare adverse events must also be considered. However, in the

MDR era, this approach needs to be studied. Proving its effectiveness might be a challenge, but this could lead to use them as chronic treatments, reducing the side effects of antibiotics, especially in susceptible populations, like KTR.

The conclusion is to highlight the uncertainty that still exists in the topics commented above because of the lack of prospective data and large clinical trials.

2. JUSTIFICATION

Urinary tract infections (ITU) management is challenging in KTR given its high incidence, adverse outcome and increasing multiresistance. Despite being the most common infection in these patients, available evidence is not strong. The alarming rise in ESBL- and carbapenemase-producing strains in this population might compromise the feasibility of programs of kidney and other solid organ transplantation. At the time of this PhD thesis design, it was not established if asymptomatic bacteriuria needed to be treated.

In this context, fosfomycin could become the first option for treating UTI in KTR. However, the available experience on the usefulness and safety of oral fosfomycin for the treatment of these infections is very scarce and is based in retrospective studies with heterogenous doses.

Although there are *in vitro* data, the effect of urinary pH within the physiological range on the clinical and microbiological effectiveness of fosfomycin in the KTR with UTI caused by *E. coli* is unknown. Besides, the real incidence of LLQR and LLFR among *E. coli* and its impact on clinical efficacy and further development of high-level resistance is unknown, too.

Currently, the use of quinolones is restricted for the treatment of uncomplicated UTI in which there is no other therapeutic alternative. In this context, it is essential to optimize the use of commonly used first-rate antimicrobials, such as fosfomycin, to improve their effectiveness and preserve their activity reducing the risk of resistance development. Data obtained from a clinical cohort of UTI with LLFR would provide essential and novel information that would improve clinical practice when evaluating the effectiveness of fosfomycin. In addition, if the hypothesis of the present study is true,

the choice of the most appropriate antimicrobial, based not only on the sensitivity pattern but also on the physiological conditions of the patient at the time of diagnosis, could help achieve optimal and personalized use of antibiotics in UTI in KTR and, consequently, improve cure rates, reduce recurrences and avoid the risk of developing microbial resistance of a highly prevalent disease in a high-risk population. Furthermore, it will also provide prospective data about fosfomycin use in the immunosuppressed population with urinary infections.

3. HYPOTHESIS

1) Treatment of asymptomatic bacteriuria is not necessary in KTR.

2) The acidic urinary pH reduces the clinical and microbiological response to ciprofloxacin in lower UTI in KTR, but not to fosfomycin.

3) There are LLQR and LLFR *E. coli* strains causing UTI in KTR.

4) Low level quinolone resistant and LLFR *E. coli* strains can develop high-level resistance mutations during antibiotic treatment with ciprofloxacin and fosfomycin.

4. OBJECTIVES

1) To determine the impact of antimicrobial therapy in the outcome of asymptomatic bacteriuria in a cohort of KTR.

2) To compare the outcome of lower UTI caused by *E. coli* in KTR treated with ciprofloxacin and fosfomycin, according to the urinary pH.

3) To analyse the clinical and microbiological outcomes in lower UTI caused by different uropathogens in KTR treated with ciprofloxacin and fosfomycin, according to the urine pH.

4) To study the prevalence, phenotypic characteristics, clinical impact, and outcomes of *E. coli* strains with LLQR and/or LLFR, treated with ciprofloxacin and fosfomycin, according to the urinary pH.

5. METHODS

5.1. Study design, population, and enrolment period

Design: Prospective observational cohort of consecutive cases attended at the University Hospital Virgen del Rocío. Two cohorts will be included:

- Cohort A: consecutive cases of uncomplicated-cystitis and asymptomatic bacteriuria in KTR that attended the outpatient clinic from January 2017 to June 2017.
- Cohort B: consecutive cases of all recruitable uncomplicated-cystitis and asymptomatic bacteriuria cases by *E. coli*, treated with ciprofloxacin or fosfomycin, in KTRs that attended the outpatient clinic from January 2017 to January 2020.

Patients with *E. coli* ITU infection occurring from January 2017 to June 2017 can be included in both cohorts when inclusion and exclusion criteria were accomplished.

5.2. Inclusion and exclusion criteria

Cohort A:

The inclusion criteria for the study were: a) adult patients (≥16 years); b) kidney recipients with follow-up at University Hospital Virgen del Rocío; c) survival after the transplant longer than 7 days; and d) presence of a bacterial count of $\geq 10^5$ CFU/ml in urine.

The exclusion criteria were: a) absence of informed consent after information regarding the study had been given to the patient; b) impossibility to attend regular outpatient follow-up in our centre; c) presence of pyelonephritis and/or prostatitis, d) anatomic-functional urological anomalies; and e) ciprofloxacin and/or fosfomycin allergy.

To elucidate objective 1 (to determine the impact of antimicrobial therapy in the outcome of asymptomatic bacteriuria in a cohort of KTR) only an episode per patient was included.

Cohort B:

The inclusion criteria for the study were: a) adult patients (≥16 years); b) kidney recipients conducting their monitoring visits at the study center during the period of inclusion; c) survival after the transplant longer than 7 days; d) urine isolation of *E. coli* with $\geq 10^5$ CFU/ml susceptible to ciprofloxacin and/or fosfomycin; and e) bacteriuria was treatable with ciprofloxacin or fosfomycin.

The exclusion criteria were a) absence of informed consent after information regarding the study had been given to the patient; b) impossibility to attend regular outpatient follow-up in our centre; c) presence of pyelonephritis and/or prostatitis, d) indwelling catheters or vesiculo-ureteral reflux; and e) ciprofloxacin and/or fosfomycin allergy.

To elucidate objective 2 (to compare the outcome of lower UTI caused by *E. coli* in KTR treated with fosfomycin and ciprofloxacin, according to the urinary pH) two episodes of a unique patient could be included if all the following conditions were accomplished: a) more than six months since the first episode included and b) sterile culture after six months of the first episode follow-up.

To elucidate objective 3 (to analyze, in lower UTI caused by different uropathogens, the impact of pH in the clinical and microbiological outcome after treatment with ciprofloxacin and fosfomycin) the inclusion criteria *"d"* was modified to the presence of any uropathogen count of $\geq 10^5$ CFU/ml in urine. Two episodes of the same patient could be included when having a negative urine culture between episodes and had passed more than six months since the first episode.

To elucidate objective 4 (to study the prevalence, phenotypic characteristics and clinical impact and outcomes of *E. coli* strains with LLQR and/or LLFR, treated with ciprofloxacin and fosfomycin, according to the urinary pH), events without pH, follow-up urine culture, or strains not well processed were dismissed. Missing data corresponded to 38 events (24.2%) from cohort B. Only one episode per patient was included.

5.3. Interventions

There was not any intervention different to the standard clinical practice.

In cohort A the decision to treat the asymptomatic bacteriuria and cystitis episodes and the antimicrobial therapy prescribed was at the choice of the physicians in charge of the patients.

In cohort B, patients were treated with ciprofloxacin 250 mg twice daily for 5 days (adjusted to renal function) and fosfomycin was given 3g x 2 doses 48 hours apart according to the decision of the physician in charge of the patient.

5.4. Sample size estimation

The cohort A had no defined minimum-sample as it was initially designed as a pivot study.

Regarding cohort B, at Hospital Universitario Virgen del Rocío, a cohort of more than 1500 KTR is followed, with an average of 110 new kidney transplants per year. Over 2016 the Microbiology Service isolated UPEC from 304 urine samples of 180 KTR, 60% were susceptible to ciprofloxacin and fosfomycin. Therefore, for cohort B it was estimated to recruit 200 bacteriurias during the first 2 years of the study. This sample would allow accomplishing the objectives proposed.

5.5. Definitions

The GESITRA/REIPI UTI guidelines (313) were used:

Bacteriuria: urine specimens isolated in quantitative counts ≥ 10^5 colony-forming units (CFU)/mL.

Asymptomatic bacteriuria: the presence of bacteriuria in the absence of any symptoms of a UTI.

Cystitis: bacteriuria and clinical manifestations such as dysuria, pollakiuria, urinary urgency, suprapubic pain, and/or hematuria, in the absence of pyelonephritis symptoms.

Acute pyelonephritis: the simultaneous presence of bacteriuria and/or bacteremia and fever, with one or more of the following: lumbar pain (if native kidney involved), renal allograft tenderness (if transplanted kidney involved), chills, or cystitis symptoms.

Clinical cure: the resolution of symptoms one month after inclusion in case of cystitis. In cohort B it also included the absence of developing urological symptoms during the follow-up.

Microbiological cure (eradication): negative urine culture at follow-up.

Microbiological re-infection: new episode of infection by a different pathogen than the initially isolated.

Microbiological relapse: detection of the same initial pathogen during the follow-up, with an intermediate sterile culture.

Microbiological persistence: detection of the same initial pathogen during the follow-up, without intermediate sterile culture.

Mortality: death during the six months prior to the follow-up.

Impairment of renal function: elevation of creatinine ≥ 0.5 mg/dl during follow-up.

Rejection: histological immune activity on kidney tissue or suspected if biopsy cannot take place.

Graft loss: need to start hemodialysis or proceed to a new kidney transplant.

Adverse events: any event that leads to antibiotic withdrawal, including *Clostridioides difficile* infection.

MDR: the uropathogen was categorized as MDR if it was resistant to at least 3 different antimicrobial categories according to international criteria (60).

Acidic urine pH was defined by 6 or less (143).

Basic urine pH was defined by more than 7.5 (143,188).

5.6. Follow-up

In both cohorts, patients were followed-up for six months after inclusion. Urine cultures were performed one and six months after inclusion. In cohort B, and in case of *E. coli* infection, MIC determinations were performed for fosfomycin and ciprofloxacin.

In patients with urinary symptoms during the follow-up, urine cultures were also performed. After the kidney transplant, patients were visited at least every 15 days during the first 3 months after the surgery, monthly until the first year, and every 3-6 months thereafter. Urine cultures were systematically performed at each visit and, whenever necessary, if UTI symptoms were observed.

5.7. Variables

Demographics, chronic underlying diseases, time from the kidney transplantation, immunosuppressant regimens, clinical data, antimicrobial therapy, and outcome from electronic medical record of the patients included were recorded in a standardized database (see details in Annex 10.2).

5.8. Urine studies

Urine samples were processed within 4–8 h after collection. The pH, leukocyte count and nitrites were measured in the urine. The Microbiology Service identified the bacterial isolates and performed susceptibility testing by conventional biochemical tests (biochemical testing, pigment production, growth, and colony characteristics). The causative organism and antibiogram were identified using the MicroScan WalkAway® plus System (Beckman Coulter, Switzerland). When the identification was uncertain, it was confirmed by the Bruker Biotyper MALDI-TOF MS system (Bruker Daltonik GmbH, Leipzig, Germany). The EUCAST criteria for categorizing susceptibility and resistance patterns were used (189), and renewed by following up-dates (190).

To elucidate objective 4, all isolates were cryopreserved in glycerol solution (15%) at -80ºC. Plates were categorized, also, according to the previous definitions. Ciprofloxacin MIC values between 0.06-0.5µg/mL were considered as LLQR (187, 188), while fosfomycin MIC values between 2-8 µg/mL were considered as LLFR according to previous studies and modified by actual resistance EUCAST criteria (240, 244, 245, 395). For this, MIC studies were carried out by broth microdilution for ciprofloxacin, and by agar dilution method for fosfomycin. These techniques were used as previously described in other reports (412,413).

Clonal studies and MIC determinations (as well) were performed for UPEC isolates from relapsed samples at 14 days. The whole genome sequencing method (414) was used to characterize the clonal relationship between the clinical isolated of UPEC sensitive to ciprofloxacin or fosfomycin, and the resistant strain.

5.9. Primary and secondary outcomes

Primary outcome: microbiological eradication at one month after the onset of antibiotic therapy.

Secondary outcomes: microbiological cure at six months, symptomatic UTI one month and six months after UTI diagnosis.

Other secondary outcomes evaluated at six months were: relapses and re-infections, graft-rejection, renal impairment, graft loss, mortality, and safety -defined as the presence of adverse events that lead to antibiotic withdrawal-.

5.10. Ethical considerations

Informed consent was obtained in every bacteriuria recruited (Annex 10.3).

The study was approved by the Ethics Committee of the University Hospitals Virgen del Rocío and Virgen Macarena (FIS-CIP-2016-01), Seville, Spain (Annex 10.4).

5.11. Statistical analysis

A descriptive statistical analysis was performed. Continuous variables were expressed as median and interquartile range or mean and standard deviation if adjusted to a normal distribution and evaluated by Shapiro–Wilk or Kolmogorov–Smirnov tests when appropriate. For bivariate analysis, the chi-square test or the Fisher exact test was used for categorical variables; Bonferroni correction was applied when appropriate. For quantitative variables, the Mann–Whitney test or Student's t-test were used based on their distribution. If the variance was not homogeneous (Levene test), an ANOVA test was applied. The relative risks were expressed as odds ratios (ORs) and 95% confidence intervals (CI). Multivariate models were used to adjust for possible confounding variables. The clinically relevant and statistically significant variables found in the bivariate analysis were included in a matrix analysis (checked by chi-square test for

categorical variables and Student's t-test for quantitative variables). Only the independent variables were finally included, which was the multivariate model that described the outcome better. Significance was established at $p<0.05$. All reported *p*-values are based on two-tailed tests. Statistical analyses were performed using SPSS version 24.0 software (SPSS, Chicago, IL, USA).

6. RESULTS

6.1. Objective 1: To determine the impact of antimicrobial therapy in the outcome of asymptomatic bacteriuria in a cohort of KTR.

6.1.1. Baseline characteristics

The study included a total of 197 patients, 175 (88.8%) with asymptomatic bacteriuria and 22 (11.2%) with cystitis. The median age was 59 years (IQR: 48–69) and 104 (52.8%) were women. The median time after transplantation was 3.8 years (IQR: 0.8–10). Our study included 58 (29.4%) and 10 (6.9%) KTR in the previous year and month, respectively. The most common immunosuppressive drug combination was mycophenolate, prednisone, and tacrolimus (124, 62.9%). Induction therapy was used in 91 (46.1%) patients: 67 (34.0%) with basiliximab or daclizumab and 24 (12.1%) with thymoglobulin. Instrumentation of the urinary tract took place in 40 (20.3%) patients, 24 (12.2%) within the previous six months of the UTI diagnosis.

In the previous six months, 80 patients were diagnosed with at least one episode of bacteriuria (40.6%), 15 with pyelonephritis (7.6%), and 10 with cystitis (5.1%).

At inclusion, 70 (35.5%) patients had viral coinfections: Cytomegalovirus infection in 45 (22.8%) cases and BK virus infection in 19 (9.6%). Patients with cystitis were more frequently co-infected with the hepatitis C virus (33.3% *vs.* 3.3%, p=0.01, OR=2.3, 95% CI 2.3–18.5). No differences were found in other demographics and transplant-related variables (Table 1).

Patients with cystitis had more frequently detectable urinary nitrites than patients with asymptomatic bacteriuria (35.7% *vs.* 9.1%, p=0.01, OR= 3.4, 95% CI 1.3–9.1).

Table 1. Baseline, clinical and microbiological features of kidney recipients with bacteriuria.

Variables	**All cases (N=197)**	**AB (N=175)**	**Cystitis (N=22)**	***p*-value**
Time from transplant to inclusion (years) - median (IQR)	3.76 (0.78-10.3)	3.85 (0.77-9.92)	2.35 (0.63-11.4)	0.48
Diabetes mellitus - n (%)	46 (23.4)	42 (24.0)	4 (18.2)	0.79
Transplant indication: - n (%)				0.97
- Tubulointerstitial	40 (20.3)	35 (20.0)	5 (22.7)	-
- Glomerulonephritis	40 (20.3)	36 (20.6)	4 (18.2)	-
- Polycystic kidney disease	36 (18.3)	32 (18.3)	4 (18.2)	-
- Diabetic nephropathy	11 (5.6)	9 (5.1)	2 (9.1)	-
- Hypertension/renovascular	16 (8.1)	15 (8.6)	1 (4.5)	-
- Tumoral	4 (2.0)	4 (2.3)	0 (0)	-
-Etiology uncertain /unknown	49 (24.9)	43 (24.6)	6 (27.3)	-
Charlson index - median (IQR)	3 (2-5)	3(2-5)	4(2-5)	-
Induction drug: - n (%)				
- None	99 (50.3)	87 (49.7)	12 (54.5)	-
- Basiliximab	56 (28.4)	49 (28.0)	7 (31.8)	-
- Daclizumab	11 (5.6)	10 (5.7)	1 (4.5)	-
- Thymoglobulin	24 (12.2)	23 (13.1)	1 (4.5)	-
Current immunosuppression: - n (%)				

MMF	142 (72.1)	126 (72.0)	16 (72.7)	-
Corticosteroids	180 (91.4)	161 (92.0)	19 (86.4)	-
Tacrolimus	174 (88.3)	155 (88.6)	19 (86.4)	-
M-TOR inhibitors	10 (5.1)	9 (5.1)	1 (4.5)	-
Cyclosporine	12 (6.1)	10 (5.7)	2 (9.1)	-
Urinary instrumentation: - n (%)	40 (20.3)	35 (20.0)	5 (22.7)	0.84
- Double J stent	34 (17.3)	29 (16.6)	5 (22.7)	-
- Urethral catheter	3 (1.5)	3 (1.7)	0 (0)	-
- Nephrostomy	3 (1.5)	3 (1.7)	0 (0)	-
Length of instrumentation (days) - median (IQR)	0 (0-26)	0 (0-26)	0 (0-43.5)	0.52

AB: asymptomatic bacteriuria, IQR: interquartile range; MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), m-TOR inhibitor: inhibitors of rapamycin (sirolimus, everolimus).

6.1.2. Etiology and antimicrobial susceptibilities

Most frequent etiologies were *E. coli* (89, 45.2%), *K. pneumoniae* (30, 15.2%), *E. faecalis* (23, 11.6%), and *P. aeruginosa* (13, 6.6%) (Table 2). Etiologies were similar in patients with cystitis and asymptomatic bacteriuria. There were 60 (30.4%) isolates resistant to cotrimoxazole, 55 (27.9%) to ciprofloxacin, 38 (19.2%) to amoxicillin-clavulanate, 21 (10.6%) to third- and/or fourth generation cephalosporins, and 19 (9.6%) to fosfomycin. No differences were observed in antimicrobial susceptibility between isolates from patients with cystitis and asymptomatic bacteriuria (Table 2).

Table 2. Aetiology and antibiotic resistances of the episodes included.

Variables, n (%)	**All cases (N=197)**	**AB (N=175)**	**Cystitis (N=22)**	***p*-value**
Etiology: - *Escherichia coli*	89 (45.2)	79 (45.1)	10 (45.5)	0.93
- *E. coli* ESBL-producers	5 (2.5)	5 (2.9)	0 (0)	0.55
- *Klebsiella pneumoniae*	30 (15.2)	28 (16.0)	1 (4.5)	0.15
- *K. pneumoniae* ESBL-producers	5 (2.5)	4 (2.3)	1 (4.5)	0.54
- *Enterococcus faecalis*	23 (11.6)	20 (11.4)	3 (13.6)	0.76
- *Pseudomonas aeruginosa*	13 (6.6)	11 (6.3)	2 (9.1)	0.62
- *Klebsiella oxytoca*	8 (4.0)	6 (3.4)	2 (9.1)	0.27
- *Proteus mirabilis*	7 (3.6)	6 (3.4)	1(4.5)	0.75
- *Morganella morganii*	4 (2.0)	4 (2.3)	0 (0)	0.62
- *Enterobacter aerogenes*	4 (2.0)	3 (1.7)	1(4.5)	0.44
- *Enterobacter cloacae*	3 (1.5)	2 (1.4)	1 (0.4)	0.33
Antibiotic resistance: - Ciprofloxacin	55 (27.9)	45 (25.7)	10 (45.5)	0.16
- Fosfomycin	19 (9.6)	17 (9.7)	2 (9)	0.94
- Amoxicillin-clavulanate	38 (19.2)	31 (17.7)	7 (31.8)	0.12
- Cephalosporins	21 (10.6)	16 (9.1)	5 (22.7)	0.08
- Cotrimoxazole	60 (30.4)	53 (30.5)	7 (31.8)	0.73

AB: asymptomatic bacteriuria, ESBL-producers: extended spectrum beta-lactamases-producers.

6.1.3. Antimicrobial therapy

Seventy-five (38.1%) patients received antimicrobial treatment. Patients with cystitis received more frequently antibiotics than those with asymptomatic bacteriuria (21

(95.4%) *vs.* 54 (30.8%), $p<0.01$, OR=34, 95% CI 4.69-248.7). The most common antibiotics prescribed were fosfomycin, ciprofloxacin, and amoxicillin-clavulanate, without differences between cystitis and asymptomatic bacteriuria (Table 3).

Table 3. Antimicrobial therapy.

Variables, n (%)	All cases (N=197)	AB (N=175)	Cystitis (N=22)	*p*-value
Treatment:	75 (38.1)	54 (30.9)	21 (95.5)	<0.01
- Ciprofloxacin	22 (11.2)	16 (9.1)	6 (27.3)	0.01
- Fosfomycin	29 (14.7)	19 (10.9)	10 (45.5)	<0.01
- Amoxicillin-clavulanate	16 (8.1)	12 (6.9)	4 (18.2)	0.07
- Cephalosporins	5 (2.5)	4 (2.3)	1 (4.5)	0.53
- Cotrimoxazole	3 (1.5)	3 (1.7)	0 (0)	0.54

AB: asymptomatic bacteriuria.

6.1.4. Outcome

6.1.4.1. Microbiological outcome

Regarding the microbiological outcome, at one-month of follow-up, 111 (56.3%) patients were microbiologically cured. In 40 (20.3%) patients, the bacteriuria persisted, 11 (5.5%) patients relapsed, and 14 (7.1%) patients were re-infected; 21 (10.6%) patients had no urine cultures at this time.

At six-month of follow-up, 53 (26.9%) were cured, and 34 (17.2%), 27 (13.7%), and 37 (18.7%) had persistence, relapse, and re-infection, respectively. No differences were found in microbiological cure at any follow-up time-points regarding asymptomatic bacteriuria or cystitis diagnosis at inclusion (Table 4).

6.1.4.2. Clinical outcome

At one-month of follow-up, 191 (96.9%) out of the 197 patients were cured. Four patients reported cystitis (2.0%) and two pyelonephritis (1.0%), without differences between patients with asymptomatic bacteriuria or cystitis at inclusion.

At six-month of follow-up, 181 (91.8%) patients were cured. During these six months and eight patients reported cystitis (4.0%) and eight pyelonephritis (4.0%), without differences between patients with asymptomatic bacteriuria or cystitis at inclusion (Table 4).

The most frequent etiologies of symptomatic UTIs during the follow-up were *E. coli* (64.7%) and *E. faecalis* (29.2%).

At six months of follow-up, renal function worsened in 10 (5.1%) patients, four (2.0%) had graft rejection, and one (0.5%) lost the graft. Six (3.0%) patients died during the six months of follow-up (five asymptomatic bacteriuria and one cystitis), none because of the UTI. The graft and survival outcomes of patients with asymptomatic bacteriuria and cystitis were similar (Table 4).

Table 4. Microbiological and clinical outcomes of the total events, asymptomatic bacteriuria and cystitis.

Variables, n (%)	**All cases (N=197)**	**AB (N=175)**	**Cystitis (N=22)**	***p*-value**
One month's - Microbiological:				
- Cure	111 (56.3)	99 (56.7)	12 (54.5)	0.51
- Persistence	40 (20.3)	35 (20.0)	5 (22.7)	0.75
- Relapse	11 (5.5)	11 (6.3)	0 (0)	0.26

- Re-infection	14 (7.1)	10 (5.7)	4 (18.2)	0.07
- Without follow up data	21 (10.6)	20 (11.4)	1 (4.5)	0.35
- Clinical: - Asymptomatic	191 (96.9)	170 (97.1)	21 (95.4)	0.5
- Cystitis	4 (2.0)	3 (2)	1 (4.5)	0.6
- Pyelonephritis	2 (1.0)	2 (1)	0 (0)	0.6
Six months' - Microbiological: - Cure	53 (26.9)	48 (27.4)	5 (22.7)	0.45
- Persistence	34 (17.2)	31 (17.7)	3 (13.6)	0.68
- Relapse	27 (13.7)	24 (13.7)	3 (13.6)	0.96
- Re-infection	37 (18.7)	31 (17.7)	6 (27.2)	0.29
- Without follow up data	58 (29.4)	49 (28.0)	9 (40.9)	0.3
- Clinical: - Asymptomatic	181 (91.8)	163 (93.1)	19 (86.3)	0.22
- Cystitis	8 (4.0)	6 (3.4)	1 (4.5)	0.26
- Pyelonephritis	8 (4.0)	6 (3.4)	2 (9)	0.26
- Graft outcome: - Graft rejection	4 (2.0)	4 (2.3)	0 (0)	0.17
- Graft dysfunction	10 (5.1)	8 (4.6)	2 (9.1)	0.29
- Graft loss	1 (0.5)	1 (0.6)	0 (0)	0.17
- Mortality	6 (3.0)	5 (2.8)	1 (4.5)	0.64

AB: asymptomatic bacteriuria. Data at sixth month includes data at first month.

6.1.5. Impact of antibiotic treatment in asymptomatic bacteriuria outcome

Among patients with asymptomatic bacteriuria, 54 (30.8%) received antimicrobial therapy; most common treatments were fosfomycin (19, 35.2%), ciprofloxacin (16, 29.6%), and amoxicillin-clavulanate (12, 22.2%). A higher proportion of treated asymptomatic bacteriuria patients, when compared to those untreated, received the transplant in the six months before inclusion (30.2% *vs.* 16.7%, p=0.04, OR= 2.16, 95% CI 1–4.6) and had isolates resistant to cotrimoxazole (48.1% *vs*. 21.5%, p<0.01, OR= 2.24, 95% CI 1.4–3.4). Moreover, there were trends to higher creatinine levels before and during the actual episode in treated *vs*. untreated asymptomatic bacteriuria patients: 1.78 *vs.* 1.54 mg/dL (p=0.07) and 1.77 *vs.* 1.57 mg/dL (p=0.08), respectively. No differences were observed among the rest of the analysed variables (Table 5).

Regarding the microbiological outcome, at one-month follow-up, patients with treated asymptomatic bacteriuria experienced a microbiological cure less frequently than those untreated (44.4% *vs.* 61.9%, p<0.01, OR= 0.49, 95% CI 0.25–0.94). These patients also had a higher number of relapses (12.9% *vs*. 3.3%, p<0.05, OR= 2.2, 95% CI 1.3–3.7), and re-infections (12.9% *vs*. 2.4%, p<0.01, OR= 2.4, 95% CI 1.5–3.9). At six-month of follow-up, microbiological outcomes were similar in treated and untreated asymptomatic bacteriuria (Table 6).

Table 5. Characteristics of treated and untreated patients with asymptomatic bacteriuria.

Variables	**Treated AB (N=54)**	**Untreated AB (N=121)**	***p*-value**	**OR (95% CI)**
Age (years) - median (IQR)	58.1 (45-71.1)	57.3 (44-70.7)	0.71	1.0 (0.98-1.03)
Sex (female) - n (%)	30 (55.6)	62 (51.2)	0.59	1.19 (0.62-2.2)
Time since transplant < 6 months - n (%)	16 (30.2)	20 (16.7)	0.04	2.16 (1.01-4.61)
Diabetes mellitus - n (%)	15 (27.8)	27 (22.3)	0.43	1.3 (0.64-2.7)
Transplant indication: - n (%)			0.37	-
- Tubulointerstitial	11 (20.4)	24 (20.6)	-	-
- Glomerulonephritis	10 (18.5)	26 (21.7)	-	-
- Polycystic kidney disease	6 (11.1)	26 (21.7)	-	-
- Hypertension/renovascular	4 (7.4)	11 (9.2)	-	-
- Diabetic nephropathy	2 (3.7)	7 (5.8)	-	-
- Tumoral	2 (3.7)	2 (1.7)	-	-
-Etiology uncertain /unknown	19 (35.2)	24 (20)	-	-
Induction drug: - n (%)			0.40	-
- None	30 (55.6)	57 (49.6)	-	-
- Basiliximab	13 (24.1)	36 (31.3)	-	-
- Thymoglobulin	7 (13.0)	16 (13.9)	-	-
- Daclizumab	4 (7.4)	6 (5.2)	-	-
Current immunosuppression: - n (%)			0.32	-
Corticosteroids	53 (98.1)	108 (89.2)	-	-
Tacrolimus	51 (94.4)	104 (85.9)	-	-
MMF	42 (77.8)	84 (69.4)	-	-
M-TOR inhibitors	4 (7.4)	5 (4.1)	-	-
Cyclosporine	1 (1.8)	9 (7.4)	-	-
Urinary instrumentation: - n (%)			0.23	-
- Double J stent	11 (20.4)	18 (15.1)	-	-
- Urethral catheter	2 (3.7)	1 (0.8)	-	-
- Nephrostomy	2 (3.7)	1 (0.8)	-	-
Length of instrumentation	24 (0-73.6)	11.1 (0-39)	0.15	1.0 (0.99-1.02)

(days) - median (IQR)				
Previous creatininemia (mg/dl) - median (IQR)	1.78 (0.82-2.75)	1.54 (0.86-2.22)	0.07	1.44 (0.78-2.64)
Creatininemia at the time of inclusion (mg/dl) - median (IQR)	1.77 (0.98-2.57)	1.57 (0.88-2.25)	0.08	1.06 (0.55-2.05)
Etiology: - n (%) - *E. coli*	22 (40.7)	57 (47.1)	0.27 -	- -
-- *E. coli* ESBL-producers	2 (3.7)	3 (2.5)	-	-
- *K. pneumoniae*	10 (18.5)	18 (14.8)	-	-
--*K. pneumoniae* ESBL-producers	2 (3.7)	2 (1.6)	-	-
- *E. faecalis*	4 (7.4)	16 (13.2)	-	-
- *P. aeruginosa*	3 (5.5)	8 (6.6)	-	-
- *K. oxytoca*	3 (5.5)	3 (2.5)	-	-
- *P. mirabilis*	5 (9.3)	1 (0.8)	-	-
- *M. morganii*	1 (1.9)	3 (2.5)	-	-
- *E. aerogenes*	0 (0)	3 (2.5)	-	-
- *E. cloacae*	1 (1.9)	1 (0.8)	-	-
Antibiotic resistance: - n (%) - Cotrimoxazole	26 (48.1)	26 (21.5)	<0.01	2.21 (1.4-3.4)
- Ciprofloxacin	13 (24.1)	32 (26.4)	0.88	-
- Amoxicillin-clavulanate	11 (20.4)	20 (16.5)	0.66	-
- Cephalosporins	8 (14.8)	8 (6.6)	0.10	-
- Fosfomycin	5 (9.3)	12 (9.9)	0.76	-
Treatment: - n (%) - Fosfomycin	19 (35.2)	-	-	-
- Ciprofloxacin	16 (29.6)	-	-	-
- Amoxicillin-clavulanate	12 (22.2)	-	-	-
- Cephalosporins	4 (7.4)	-	-	-
- Cotrimoxazole	3 (5.5)	-	-	-

AB: asymptomatic bacteriuria, IQR: interquartile range.

Table 6. Microbiological and clinical outcomes of treated and untreated asymptomatic bacteriuria.

Variables, n (%)	Treated AB (N=54)	Untreated AB (N=121)	*p*-value	OR (95% CI)
One month's				
- Microbiological:				
- Cure	24 (44.4)	75 (61.9)	<0.01	0.49 (0.25-0.94)
- Persistence	10 (18.5)	25 (20.6)	0.7	0.9 (0.51-1.6)
- Relapse	7 (12.9)	4 (3.3)	0.04	2.2 (1.3-3.69)
- Re-infection	7 (12.9)	3 (2.5)	<0.01	2.4 (1.5-3.9)
- Without follow up data	6 (11.1)	14 (11.6)	0.95	0.97 (0.48-1.9)
- Clinical:				
- Cystitis	2 (3.7)	1 (0.8)	0.25	2.2 (0.96-5,1)
- Pyelonephritis	2 (3.7)	0 (0.0)	0.09	3.3 (2.65-4.2)
Six months'				
- Microbiological:				
- Cure	13 (24.1)	37 (30.6)	0.3	0.8 (0.4-1.3)
- Persistence	12 (22.2)	25 (20.6)	0.8	1.06 (0.6-1.8)
- Relapse	12 (22.2)	14 (11.6)	0.06	1.6 (1.01-2.7)
- Re-infection	14 (25.9)	23 (19)	0.11	1.3 (0.80-2.12)
- Without follow up data	13 (24.1)	36 (29.8)	0.45	0.8 (0.48-1.38)
- Clinical:				
- Cystitis	2 (3.7)	3 (2.5)	0.66	1.3 (0.44-3.92)
- Pyelonephritis	4 (7.4)	1 (0.8)	0.03	2.8 (1.8-4.3)
- Graft outcome:				
- Graft rejection	1 (1.8)	3 (2.5)	0.8	0.8 (0.14-4.5)
- Graft dysfunction	2 (3.7)	6 (4.9)	0.7	0.7 (0.14-3.8)
- Graft loss	0 (0.0)	1 (0.8)	0.7	-
- Mortality	2 (3.7)	3 (2.5)	0.66	0.86 (0.41-1.78)

AB: asymptomatic bacteriuria. Data at sixth month includes data at first month.

The episodes of symptomatic UTIs in patients with initial asymptomatic bacteriuria during the follow-up are summarized in Table 6. After one month, four (7.4%) out of 54 treated asymptomatic bacteriuria patients presented with a symptomatic UTI (two cystitis and two pyelonephritis), whereas one (0.8%) out of 121 untreated asymptomatic bacteriuria patients had a cystitis episode (p=0.03, OR=2.72, 95% CI 1.65–4.46). After six months, 6 (11.1%) treated *vs.* 4 (3.3%) untreated asymptomatic bacteriuria patients had UTI episodes (p=0.07, OR=3.65, 95% CI 0.98–13.53).

A multivariate analysis was performed to evaluate possible confounding variables of the effect of treating bacteriuria at the risk of developing symptomatic UTIs in the six months after inclusion. The following variables were identified as independent risk factors: Use of thymoglobulin as the induction drug (p<0.01, OR=8, 95% CI 1.9–34.2), pyelonephritis previous to the inclusion (p<0.01, OR=12, 95%CI 2.7–53.5), antimicrobial treatment of bacteriuria (p=0.02, OR=5, 95%CI 1.2–20.6), and time since transplantation less than one year (p=0.01, OR=5.7, 95%CI 1.5–22.2) (Table 7).

When a multivariate sub-analysis was performed only in asymptomatic bacteriurias cases to evaluate the risk of developing symptomatic UTIs in the six months of follow-up, 11 symptomatic UTIs were found out of the 147 asymptomatic bacteriuria studied. The following variables were identified as independent risk factors: use of thymoglobulin as the induction drug (p=0.02, OR=10.4, 95% CI 2.2–47.4), nosocomial acquisition of the asymptomatic bacteriuria (p=0.03, OR=7.6, 95% CI 1.2–48.5), and antimicrobial treatment of the asymptomatic bacteriuria (p=0.01, OR=6.6, 95% CI 1.3–31.3).

Table 7. Risk factors of developing symptomatic UTI during the six months of follow-up.

Variables	Symptomatic UTI (N=15)	Non symptomatic UTI (N=182)	Crude OR (95% CI)	*p*-value	Adjusted OR (95% CI)
Urinary pH - median (IQR)	6.7 (6.2-7.2)	6.4 (5.9-6.9)	0.11 (0.01-0.2)	0.06	-
Previous creatininemia (mg/dl) - median (IQR)	2.07 (0.87-3.27)	1.55 (0.84-2.27)	0.06 (0.0-0.12)	0.02	-
Time since transplant <1 year - n (%)	9 (60)	49 (27.22)	4.01 (1.4-11.9)	0.01	5.7 (1.4-22.2)
Pre-transplant recurrent UTI - n (%)	4 (26.7)	28 (15.3)	-	0.3	-
Urinary reflux - n (%)	0 (0.0)	13 (7.1)	-	0.3	-
MMF doses (mg/day) - median (IQR)	700 (435-1045)	750 (329-1268)	-	0.6	-
Induction treatment: - n (%) - No drug	11 (73.3) 4 (26.7)	80 (43.9) 95 (52.2)	3.2 (1.1-9.7) 0.36 (0.12-1)	0.01 0.05	- -
- Basiliximab	4 (26.7)	52 (28.7)	-	1.0	-
- Daclizumab	1 (6.6)	10 (5.5)	-	1.0	-
- Thymoglobulin	6 (40)	18 (9.9)	4.6 (1.8-11.8)	<0.01	8 (1.9-34.2)
APN within the previous 6 months- n (%)	11 (73.3)	60 (32.9)	4.8 (1.6-14.7)	<0.01	12 (2.7-53.5)
UTI < 2 months after transplant - n (%)	13 (86.7)	93 (51.1)	4.6 (1.1-19.8)	0.03	-

Acute rejection previous 6 months - n (%)	2 (13.3)	12 (6.6)	-	0.49	-
Urinary instrumentation - n (%)	5 (33.3)	35 (19.2)	-	0.36	-
Obstructive uropathy post-transplant - n (%)	0 (0.0)	10 (5.5)	-	0.59	-
Hospital-acquired bacteriuria - n (%)	11 (73.3)	167 (91.7)	0.24 (0.06-0.9)	0.04	-
Antibiotic therapy of the present episode - n (%)	11 (73.3)	64 (35.2)	4.7 (1.5-13.5)	0.02	5 (1.2-20.6)
Microbiological cure at 1 month - n (%)	4 (26.6)	107 (58.8)	0.2 (0.09-0.85)	0.01	-

UTI: urinary tract infection, IQR: interquartile range, MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), APN: acute pyelonephritis.

6.1.6. Risk factors and prognosis factors of recurrent UTI

A sub-analysis in recurrent UTI was performed for 191 bacteriurias initially included: twenty-one (10.6%) events were found during the follow-up. Recurrent UTI was associated to previous pyelonephritis events, indwelling urinary catheters (double J stent), and worse initial renal function, and was not associated to the different aetiologies. The only independent variable link to present recurrent UTI was the worse initial renal function (p=0.01, OR=1.9, 95% CI 1.17-3.25) (Table 8).

Classic risk factors for recurrent UTI were not confirmed in this cohort (diabetes, anatomic-functional urological anomalies, gender, viral or bacterial coinfections, etc.). Recurrent UTI was associated with a higher risk of acute rejection during follow-up.

Table 8. Differential characteristics of the general cohort, and the subcohort with recurrent UTI and those with non-recurrent UTI during the follow-up, including classic risk factors associated to develop recurrent UTI.

Variables	**General cohort (N=197)**	**RUTI (N=21)**	**Non-RUTI (N=170)**	***p*-value**	**OR (95% CI)**
Age (years) - median (IQR)	59 (48-69)	57 (49-71)	59 (50-68)	0.41	-
Sex (female) - n (%)	104 (53)	12 (57)	87 (51)	0.61	-
AB as first event - n (%)	175 (88.8)	20 (95)	150 (88)	0.33	-
Time from transplant to inclusion (days) - median (IQR)	1374 (283-3764)	58 (39-528)	223 (66-1135)	0.07	0.54 (0.45-1.27)
Diabetes mellitus - n (%)	46 (23)	7 (33)	39 (24)	0.29	-
Transplant indication - n (%)	-	-	-	0.2	-
Over one year of haemodyalisis - n (%)	129 (65)	12 (57)	114 (67)	0.21	-
APN within the previous 6 months - n (%)	15 (8)	4 (19)	11 (6)	0.04	-
Previous creatininemia (mg/dL) - median (IQR)	1.5 (1.2-1.9)	1.8 (1.3-2.3)	1.5 (1.1-1.9)	0.01	1.9 (1.17-3.25)
Urological anomalies: - n (%)					-
-Vesicoureteral reflux	20 (10)	1 (5)	12 (7)	0.69	-
-Polycystic kidney disease	36 (13)	2 (10)	33 (20)	0.29	-
-Anatomic urological anomalies	20 (10)	1 (5)	19 (11)	0.37	-

-Post-transplant obstructive uropathy	10 (5)	0 (0)	10 (6)	0.11	-
Current immunesuppression - n (%)	-	-	-	0.96	-
Cadaveric donor - n (%)	174 (89)	19 (91)	150 (89)	0.82	-
Thymoglobulin induction therapy - n (%)	24 (12)	4 (20)	19 (11)	0.32	-
Over ten days of double J stent - n (%)	34 (17)	7 (33)	26 (15)	0.03	2.65 (1.2-5.9)
UTI < 2 months after transplant - n (%)	106 (59)	14 (67)	91 (58)	0.31	-
UTI by ESBL-producers - n (%)	11 (6)	2 (10)	8 (5)	0.3	-
CMV coinfection - n (%)	45 (64)	6 (29)	39 (23)	0.56	-
HCV coinfection - n (%)	5 (7)	0 (0)	5 (3)	0.55	-
BK virus coinfection - n (%)	19 (27)	3 (14)	16 (10)	0.48	-
TBC coinfection - n (%)	2 (1)	0 (0)	2 (1)	0.79	-
Graft rejection - n (%)	4 (2)	3 (14)	1 (0.6)	<0.01	7.7 (3.8-15.8)
Graft dysfunction - n (%)	10 (5.1)	1 (5)	9 (5.3)	0.93	-
Graft loss - n (%)	1 (0.5)	0 (0)	1 (0.6)	0.58	-
Mortality - n (%)	6 (3.0)	1 (5)	5 (2.9)	0.97	-

RUTI: recurrent urinary tract infection, IQR: interquartile range, AB: asymptomatic bacteriuria, APN: acute pyelonephritis, UTI: urinary tract infection, ESBL-producers: extended spectrum beta-lactamases producers, CMV: cytomegalovirus, HCV: hepatitis C virus, TBC: tuberculosis.

6.2. Objective 2: To compare the outcome of lower UTI caused by *E. coli* in KTR treated with ciprofloxacin and fosfomycin, according to the urinary pH.

6.2.1. Baseline and clinical characteristics of lower UTI infections caused by *E. coli*

One hundred fifty-seven *E. coli* non-complicated lower tract UTI episodes in 143 KTR were included, of whom, 27 (17.2%) had cystitis and 130 (82.8%) asymptomatic bacteriuria. The median age of patients included was 58 years (IQR: 51-67). Ninety patients (62.9%) were women.

At inclusion, the median creatinine and glomerular filtration were 1.51 mg/dL (IQR 1.26-1.96) and 78.3 (IQR 50.3-85.8) mL/min, respectively. The median time since transplantation was 17.8 months (IQR: 4-87). Sixty-three (44.1%) and 21 (14.7%) patients were transplanted for less than 12 and 2 months, respectively. Forty-seven patients were on cotrimoxazole prophylaxis (32.9%). The most common immunosuppressant combination drugs were MMF, prednisone and tacrolimus (n=102, 71.3%). One hundred and fifteen patients (80.4%) received MMF. Concerning the induction therapy, thymoglobulin was administrated in the last three months in eight cases and basiliximab in eleven (5.6% and 7.7%, respectively). Twenty-nine episodes (18.5%) were hospital-acquired.

In the previous six months of inclusion, 92 cases (58.6%) had at least one episode of bacteriuria: 58 asymptomatic bacteriuria (36.94%), 20 acute pyelonephritis (12.73%), and 14 cystitis (8.9%).

In the previous three months, 63 cases (40.1%) had received antibiotics. The antibiotics used were: third and/or fourth generation cephalosporins 18 cases (11.5%), fosfomycin 18 cases (11.5%), ciprofloxacin 13 cases (8.3%), and other antibiotics 14 cases (8.9%).

Table 9. Baseline characteristics of KTR with *E. coli* bacteriuria.

Variables	**N=143**
Age - median (IQR)	58 (51-67)
Sex (female) - n (%)	90 (62.9)
Months from transplantation - median (IQR)	17.76 (4-87)
Time since transplant <2 months - n (%)	21 (14.7)
Retransplantation - n (%)	15 (10.5)
Underlying end-stage renal disease: - n (%)	
- Glomerulonephritis	31 (21.7)
- Tubulointerstitial	25 (17.5)
- Polycystic kidney disease	13 (9.1)
- Diabetic nephropathy	12 (8.4)
- Hypertension / renovascular	11 (7.7)
- Tumoral	4 (2.8)
- Vesicoureteral reflux	1 (0.7)
- Mix or unknown aetiology	46 (32.2)
Living donor - n (%)	15 (10.5)
Induction therapy within previous 3 months: - n (%)	19/139 (13.6)
- Thymoglobulin	8 (5.6)
- Basiliximab	11 (7.7)
Current immunosuppression: - n (%)	
- Corticosteroids	135 (94.4)
- Tacrolimus	133 (93)
- MMF	115 (80.4)
- M-TOR inhibitors	8 (5.6)
- Cyclosporine	4 (2.8)
Cotrimoxazole prophylaxis - n (%)	47 (32.9)
Acute rejection within the previous 6 months - n (%)	13 (9.1)
Rejection treatment in the previous 6 months: - n (%)	
- Corticosteroid’s bolus	13 (9.1)
- Plasmapheresis	3 (2.1)
- Rituximab	3 (2.1)
- Thymoglobulin	2 (1.4)

IQR: interquartile range, MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), m-TOR inhibitor: inhibitors of the mechanistic target of rapamycin (sirolimus, everolimus).

Data on demographics, baseline characteristics at inclusion of patients with *E. coli* bacteriuria are detailed in Table 9; and antimicrobial susceptibilities of *E. coli'*s episodes are detailed in Table 10.

Table 10. Description of *E. coli* bacteriuria episodes included.

Variables	**N= 157**
Types of ITU episodes: - n (%)	
- Cystitis	27 (17.2)
- Asymptomatic bacteriuria	130 (82.8)
Bacteriuria within the previous 6 months - n (%)	92 (58.6)
Antibiotic use within the previous 3 months - n (%)	63 (40.1)
Hospital-acquired UTI- n (%)	29 (18.5)
Glomerular filtration at inclusion (mL/min) - median (IQR)	78.3 (50.3-85.8)
Urine pH - median (IQR)	6 (6-6.5)
Baseline *E. coli* antibiotic resistance: - n (%)	
- Cotrimoxazole	85 (54.1)
- Ciprofloxacin	50 (31.8)
- Amoxicillin-clavulanate	29 (18.5)
- Fosfomycin	11 (7)
- Cephalosporins	3 (1.9)
- ESBL-producers	3 (1.9)
Antibiotic therapy of the present episode: - n (%)	
- Ciprofloxacin	35 (22.3%)
- Fosfomycin	122 (77.7%)

IQR: interquartile range, UTI: urinary tract infection, ESBL: extended spectrum beta-lactamase producers.

No differences regarding cystitis and asymptomatic bacteriuria episodes were found in terms of demographics, transplantation characteristics, or other risk factors related to poor prognosis (Table 11).

Table 11. Baseline and clinical characteristics of UTI episodes according to presence of urological symptoms at inclusion (N=157).

Variables, n (%)	**AB (N= 130)**	**Cystitis (N= 27)**	***p*-value**	**OR (95% CI)**
Age ≥ 58 years	68 (52.3)	13 (48.1)	0.69	1.08 (0.71-1.66)
Sex (female)	83 (63.8)	21 (77.8)	0.16	0.82 (0.64-1.04)
Time since transplant < 2 months	19 (14.6)	2 (7.4)	0.53	1.97 (0.48-7.97)
Polycystic kidney disease	9 (6.9)	5 (18.5)	0.08	0.75 (0.51-1.13)
Diabetes mellitus	10 (7.7)	2 (7.4)	0.98	1 (0.77-1.3)
CMV coinfection	25 (19.2)	5 (18.5)	0.93	1.03 (0.43-2.46)
MMF	108 (83.1)	21 (77.8)	0.58	1.08 (0.86-1.3)
Induction therapy	81 (63.77)[+]	17 (65.3)[++]	0.89	0.98 (0.85-1.14)
Pre-transplant recurrent UTI	19 (17.8)[+++]	7 (33.3)[++++]	0.13	0.53 (0.25-1.10)
Symptomatic UTI <6 months	28 (21.5)	6 (22.2)	0.93	0.96 (0.44-2.11)
Acute rejection <6 months	11 (8.5)	4 (14.8)	0.29	0.57 (0.19-1.66)
UTI <2 months after transplant	53 (42.1)[+++++]	13 (48.1)	0.56	0.87 (0.56-1.35)
Hospital-acquired UTI	25 (19.2)	4 (14.8)	0.78	1.29 (0.49-3.4)
Glomerular filtration ≤ 78 mL/min	66 (50.8)	12 (44.4)	0.55	1.14 (0.72-1.79)
Acidic urine pH	64 (49.2)	17 (63)	0.19	0.78 (0.55- 1.09)
Fosfomycin therapy	104 (80)	18 (66.7)	0.13	1.2 (0.9-1.58)
MDR strain	12 (9.2)	5 (18.5)	0.17	0.49 (0.19-1.29)

CMV: cytomegalovirus, MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), AB: asymptomatic bacteriuria, UTI: urinary tract infection, MDR: multidrug resistant. [+] Data available in 127 episodes, [++] data available in 26 episodes, [+++] data available in 107 episodes, [++++]data available in 21 episodes, [++++] data available in 126 episodes.

Of the total of 157 episodes included, 122 were treated with fosfomycin (77.7%) and 35 with ciprofloxacin (22.3%).

Episodes treated with fosfomycin, compared to those treated with ciprofloxacin were more frequently hospital-acquired (23% *vs.* 2.9%, *p*=0.02, OR=10.12, 95% CI 1.32-77.33). No differences were observed in the rest of demographics, transplant-related, clinical and microbiological variables at inclusion (Table 12).

Table 12. Baseline, clinical and microbiological characteristics of *E. coli* bacteriurias episodes in renal recipients, according to ciprofloxacin or fosfomycin therapy.

Variables, n (%)	**Ciprofloxacin (N=35)**	**Fosfomycin (N=122)**	***p*-value**	**OR (95% CI)**
Age ≥ 58 years	17 (48.6)	64 (52.5)	0.68	1.12 (0.62-2.02)
Sex (female)	26 (74.3)	78 (63.9)	0.25	0.67 (0.34-1.3)
Retransplantation	3 (8.6)	14 (11.5)	0.76	1.29 (0.44-3.78)
Time since transplant <2 months	5 (14.3)	16 (13.1)	0.78	0.92 (0.40-2.12)
Time since transplant < 1 year	17 (48.6)	49 (40.2)	0.37	0.71 (0.33-1.51)
Polycystic kidney disease	3 (8.6)	11 (9)	0.13	1.88 (0.78-2.4)
CMV coinfection	5 (14.3)	25 (20.5)	0.41	1.54 (0.54-4.39)
MMF	29 (82.9)	100 (82)	0.9	0.95 (0.41-2.16)
M-TOR inhibitor	1 (2.9)	7 (5.7)	0.68	1.83 (0.28-11.69)
Pre-transplant recurrent UTI	2 (8.7) [+]	24 (22.9) [++]	0.16	2.67 (0.67-10.69)
Thymoglobulin	2 (25)	6 (31.6)	0.77	1.79 (0.68-1.11)

induction therapy <3 months				
Antibiotic use <3 months	15 (42.9)	48 (39.3)	0.7	0.94 (0.68-1.29)
Symptomatic UTI < 6 months	6 (17.1)	28 (23)	0.46	1.33 (0.60-2.95)
Acute rejection <6 months	2 (5.7)	13 (10.7)	0.52	1.9 (0.45-9.16)
UTI < 2 months after transplant	16 (47.1) [+++]	50 (42) [++++]	0.6	0.91 (0.64-1.29)
Cystitis at inclusion	9 (25.7)	18 (14.8)	0.13	0.5 (0.20-1.24)
Hospital-acquired UTI	1 (2.9)	28 (23)	0.02	10.12 (1.32-77.33)
Glomerular filtration ≤ 78 mL/min	14 (40)	64 (52.5)	0.19	1.65 (0.77-3.55)
Acidic pH	16 (45.7)	65 (53.3)	0.43	1.26 (0.70-2.27)
Basic pH	2 (5.7)	2 (1.6)	0.21	0.27 (0.03-2.02)
Neutral pH	17 (48.6)	55 (45.1)	0.71	0.86 (0.40-1.84)
Antibiotic resistance: - Ciprofloxacin	0 (0)	50 (41)	<0.01	3.2 (1.3-7.9)
- Fosfomycin	1 (2.9)	0 (0)	0.73	0.72 (0.11-4.64)
- Amoxicillin-clavulanate	10 (28.6)	19 (15.6)	0.08	0.84 (0.67-1.05)
- Cephalosporins	0 (0)	3 (2.5)	1	1.02 (0.99-1.05)
- Cotrimoxazole	16 (45.7)	69 (56.6)	0.25	1.54 (0.72-3.29)
- MDR	2 (5.7)	15 (12.3)	0.36	2.31 (0.5-10.64)
- ESBL-producers	0 (0)	3 (2.4)	0.46	1.13 (0.9-1.42)
- Carbapenem-resistance	0 (0)	0 (0)	-	-

CMV: cytomegalovirus, MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), UTI: urinary tract infection, MDR: multidrug resistance, ESBL: extended spectrum beta-lactamase-producers. [+] Data available in 23 episodes, [++]data available in 105 episodes, [+++]data available in 34 episodes, [++++] data available in 119 episodes.

Table 13. Baseline, clinical and microbiological characteristics of *E. coli* bacteriurias episodes in renal recipients, according to the urine pH at inclusion.

Variables, n (%)	**Acidic pH (N=81)**	**Neutral pH (N=72)**	**Basic pH (N=4)**	***p*-value**	***p*-value (acidic *vs*. non-acidic)**	**OR (95% CI)**
Age ≥ 58 years	33 (40.7)	45 (62.5)	3 (75)	0.01[μ]	<0.01	0.40 (0.21-0.76)
Sex (female)	56 (69.1)	45 (62.5)	3 (75)	0.64	0.42	1.3 (0.67-2.5)
Retransplantation	12 (14.8)	5 (6.9)	0 (0)	0.22	0.09	2.4 (0.82-7.37)
Time since transplant <2 months	14 (17.3)	6 (8.3)	1 (25)	0.21	0.13	2.06 (0.78-5.42)
Time since transplant <1 year	31 (38.3)	33 (45.8)	2 (50)	0.60	0.32	0.72 (0.38-1.37)
Polycystic kidney disease	8 (9.9)	6 (8.3)	0 (0)	0.73	0.66	1.27 (0.42-3.87)
CMV coinfection	11 (13.6)	19 (26.4)	0 (0)	0.08	0.06	0.47 (0.20-1.07)
MMF	69 (85.2)	57 (79.2)	3 (75)	0.58	0.30	1.53 (0.67-3.49)
M-TOR inhibitor	3 (3.7)	5 (6.9)	0 (0)	0.59	0.41	0.54 (0.12-2.36)
Pre-transplant recurrent UTI	15 (23.1) [+]	10 (16.9) [++]	1 (25)	0.67	0.43	1.41 (0.59-3.38)
Thymoglobulin induction therapy <3 months	5 (6.2)	3 (4.2)	0 (0)	0.77	0.72	1.60 (0.36-6.94)
Antibiotic use <3 months	35 (43.2)	27 (37.5)	1 (25)	0.63	0.41	1.30 (0.68-2.47)
Symptomatic UTI < 6 months	19 (23.5)	14 (19.4)	1 (25)	0.82	0.57	1.24 (0.58-2.67)

Acute rejection <6 months	5 (6.2)	10 (13.9)	0 (0)	0.21	0.13	0.43 (0.14-1.33)
UTI < 2 months after transplant	31 (39.7) [+++]	33 (46.5) [++++]	2/4 (50)	0.68	0.38	0.75 (0.39-1.43)
Cystitis at inclusion	17 (21)	10 (13.9)	0 (0)	0.33	0.19	1.75 (0.74-4.11)
Hospital-acquired UTI	17 (21)	12 (16.7)	0 (0)	0.49	0.40	0.75 (0.38-1.46)
Glomerular filtration ≤ 78 mL/min	34 (42)	42 (58.3)	2 (50)	0.13	0.04	0.52 (0.27-0.99)
Fosfomycin therapy	65 (80.2)	55 (76.4)	2 (50)	0.34	0.43	1.35 (0.63-2.87)
Antibiotic resistance: - Ciprofloxacin	27 (33.3)	23 (31.9)	0 (0)	0.12	0.68	1.15 (0.58-2.25)
- Fosfomycin	1 (1.2)	0 (0)	0 (0)	0.11	0.73	1.72 (0.11-4.64)
- Amoxicillin-clavulanate	15 (18.5)	14 (18.4)	0 (0)	0.62	0.98	1.01 (0.86-1.16)
- Cephalosporins	3 (3.7)	0 (0)	0 (0)	0.23	0.24	1.03 (0.99-1.08)
- Cotrimoxazole	42 (51.9)	40 (55.6)	3 (75)	0.62	0.55	0.9 (0.64-1.26)
- MDR	14 (17.3)	3 (4.2)	0 (0)	0.02 [µ]	<0.01	1.16 (1.04-1.29)
- ESBL-producers	3 (3.7)	0 (0)	0 (0)	0.23	0.24	1.03 (0.99-1.08)
- Carbapenem-resistance	0 (0)	0 (0)	0 (0)	-	-	-

CMV: cytomegalovirus, MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), UTI: urinary tract infection, MDR: multidrug resistance, ESBL: extended spectrum beta-lactamase-producers. [+] Data available in 65 episodes, [++]data available in 59 episodes, [+++]data

available in 78 episodes, ++++ data available in 71 episodes. µ: Significative *p*-value when stratified by acidic, neutral and basic urine pH.

In 81 episodes (51.6%) urine pH was acidic, in 72 (45.8%) was neutral and in 4 was basic (2.5%). Patients with acidic urine pH at inclusion were younger (40.7% *vs.* 63.1%, $p<0.01$, adjusted OR=0.29, 95% CI 0.12-0.72) and had more frequently MDR than those with non-acidic urine pH (17.3% *vs.* 3.9%, $p<0.01$, OR=7.30, 95% CI 1.66-32.0). No differences were observed in the rest of demographics, transplant-related, clinical and microbiological variables at inclusion (Table 13).

6.2.2. Microbiological and clinical outcomes of *E. coli* ITU in KTR

One month after the inclusion, 121/157 cases (77.1%) were microbiologically cured. In 32 cases (20.4%) the bacteriuria persisted, and 4 (2.5%) were re-infected.

Six months after inclusion, 58 of 147 cases were microbiologically cured (39.45%). In 10 patients, microbiological data at this point of follow-up were not available (Table 14).

Regarding clinical outcome, at the first month follow-up, 143 cases were asymptomatic (91.1%) and 14 (8.9%) had symptomatic UTI: thirteen cystitis (8.2%) and one (0.6%) pyelonephritis. Of those with symptoms at one month of follow-up up, five episodes (18.5%) were included as cystitis and eight cases (6.1%) were included as asymptomatic bacteriuria. The acute pyelonephritis at one month was initially an asymptomatic bacteriuria event.

During the six months of follow-up, 132 (84.07%) did not develop symptomatic UTI, and 18 (11.4%) had at least one episode of cystitis and seven (4.5%) had pyelonephritis (Table 14). Globally, five patients (19.1%) developed symptoms in different episodes.

After six-months of follow-up, the renal function worsened in 18 (11.5%) patients -in two cases accompanied with symptomatic UTI-, nine (5.7%) had a graft rejection, and one (0.6%) lost the graft (Table 14). During the follow-up 47 events (29.4%) received another antibiotic course, mainly due to a new symptomatic UTI, or asymptomatic bacteriuria with renal worsening.

One patient had mild diarrhoea (non-*Clostridioides difficile* infection) that recovered without fosfomycin withdrawal. One patient died (0.6%) at the fourth month of the follow-up. She was admitted due to renal failure in the context of an acute pyelonephritis caused by *E. coli* (urine pH=8). No other major side-effects related to the antibiotics prescribed were reported.

Microbiological cure at one month was associated to microbiological cure at 6 months (45% *vs*. 22.2%, *p*=0.01, OR=1.41, 95% CI 1.11-1.80). However, it was not associated to symptomatic UTI during one and six months of follow-up.

Patients with symptomatic UTI at one month, had more frequently symptomatic UTI (35.7% *vs.*13.3%, *p*=0.05, OR=2.68, 95% CI 1.18-6.09) and pyelonephritis (21.4% *vs.* 2.8%, *p*=0.01, OR=7.66, 95% CI 1.9-30.84) during the six months of follow-up.
Microbiological failure and symptomatic UTI during follow-up were not associated to graft rejection and graft dysfunction at the end of follow-up.

Table 14. Microbiological and clinical outcomes of the episodes of *E. coli* UTI episodes.

Variables, n (%)	**N=157**
One month follow-up outcome: - Microbiological: - Cure	121 (77.1)
- Persistence	32 (20.4)
- Relapse	0 (0)
- Re-infection	4 (2.5)
- Clinical: - Symptomatic UTI	14 (8.9)
- Cystitis	13 (8.2)
- Pyelonephritis	1 (0.6)
Six months follow-up outcome: - Microbiological: - Cure	58/147 (39.5)
- Persistence	39 (24.8) [+]
- Relapse	57 (36.3) [+]
- Re-infection	30 (19.1) [+]
- Without follow-up up data	10 (6.8)
- Clinical:	
- Symptomatic UTI	25 (15.9)
- Cystitis	18 (11.4)
- Pyelonephritis	7 (4.5)
- Graft outcome: - Graft dysfunction	18 (11.5)
- Graft rejection	9 (5.7)
- Graft loss	1 (0.6)
- Mortality	1 (0.6)

UTI: urinary tract infection. [+] Data do not sum 100% because each event could present diverse outcomes (persistence/relapses/re-infections) during the same follow-up. Data at sixth month includes data at first month.

Regarding other secondary outcomes, UTI re-infections at six months were associated to graft dysfunction at the end of follow-up (7/31 (22.6%) *vs.* 11/126 (8.7%), p=0.03,

OR=3.05, 95% CI 1.07-8.66) with a trend to more graft renal rejection (4/31 (12.9%) *vs.* 5/126 (3.9%), *p*=0.07, OR=1.1, 95% CI 0.95-1.26).

Clinical and microbiological outcomes were similar in patients with asymptomatic bacteriuria and cystitis at diagnosis. Although not statistically different, patients with cystitis, compared to patients with asymptomatic bacteriuria, had more symptomatic UTI at first month (18.5% *vs.* 6.9%, *p*=0.06, OR=2.32, 95% CI 1.04-5.17), and less microbiological failure (42.3% *vs.* 64.5%, *p*=0.07, OR=0.52, 95% CI 0.26-1.05) and less re-infections at 6 months (7.4% *vs*. 22.3%, *p*=0.07, OR=0.32, 95% CI 0.08-1.3) (Table 15).

Table 15. Microbiological and clinical outcome of *E. coli* bacteriuria according to the presence of urological symptoms at diagnosis.

Variables, n (%)	**AB (N=130)**	**Cystitis (N=27)**	***p*-value**	**OR (95% CI)**
One month follow-up:				
- Microbiological failure	29 (22.3)	7 (25.9)	0.68	0.86 (0.42-1.75)
- Symptomatic UTI	9 (6.9)	5 (18.5)	0.06	0.37 (0.13-1.02)
Six months follow-up:				
- Microbiological failure	78 (64.5) [+]	11 (42.3) [++]	0.07	1.52 (0.95-2.43)
- Re-infections	29 (22.3)	2 (7.4)	0.07	3.0 (0.76-11.87)
- Relapses	51 (39.2)	8 (29.6)	0.34	1.32 (0.71-2.46)
- Symptomatic UTI	26 (20)	4 (14.8)	0.78	1.03 (0.38-2.79)
- Pyelonephritis	6 (4.6)	1 (3.7)	1	1.24 (0.15-9.9)
- Graft dysfunction	16 (12.3)	2 (7.4)	0.74	1.66 (0.4-6.8)
- Graft rejection	7 (5.4)	2 (7.4)	0.65	0.72 (0.16-3.31)
- Mortality	1 (0.8)	0 (0)	1	1.20 (1.12-1.29)

UTI: urinary tract infection. [+] Data available in 121 episodes, [++] data available in 26 episodes.

6.2.3. Clinical and microbiology effectiveness of fosfomycin therapy in *E. coli* bacteriuria in KTR

A total of 31 episodes treated with fosfomycin at one month of follow-up experienced microbiological failure (25.4%) and 11 (9%) had symptomatic UTI.

During the six months of follow-up up, 76 episodes (65.5%) experienced microbiological failure and 24 patients (19.7%) had symptomatic UTI during follow-up.

At one-month of follow-up, clinical and microbiological effectiveness of fosfomycin was not different from that of ciprofloxacin. However, after 6 months of follow-up, fosfomycin was associated to more symptomatic UTI during follow-up (24 (19.7%) *vs.* 1 (2.9%), p=0.01, OR=1.20, 95% CI 1.08-1.34) and to more microbiological failure (76 (65.5%) *vs.* 13 (41.9%), p=0.01, OR=2.63, 95% CI 1.17-5.91) (Table 16). When these associations were analysed under the effect of the urine pH at inclusion and its interaction with the treatment received in a logistic regression model, to receive fosfomycin was not associated to more symptomatic events (p=0.22, OR=3.75, 95% CI 0.45-31.04) neither to more microbiological failure (p=0.62, OR=1.41, 95% CI 0.35-5.6) during follow-up.

As previously stated, the only difference in the baseline, clinical and microbiological characteristics of episodes treated with fosfomycin and those treated with ciprofloxacin was a higher rate of hospital-acquired infection in the fosfomycin group (Table 12).

Other relevant outcomes, as graft loss, graft dysfunction, mortality and rejection, were similar in patients treated with fosfomycin and ciprofloxacin (Table 16).

Table 16. Microbiological and clinical outcome of episodes treated with ciprofloxacin and fosfomycin.

Variables, n (%)	Ciprofloxacin (N=35)	Fosfomycin (N=122)	*p*-value	OR (95% CI)
One-month´s: - Microbiological failure	5 (14.3)	31 (25.4)	0.17	1.14 (0.96-1.35)
- Symptomatic UTI	3 (8.6)	11 (9)	0.93	1.01 (0.75-1.34)
Six-months': - Microbiological failure	13 (41.9) [+]	76 (65.5) [++]	0.01	2.63 (1.17-5.91)
- Symptomatic UTI	1 (2.9)	24 (19.7)	0.01	1.20 (1.08-1.34)
- Pyelonephritis	1 (2.9)	6 (4.9)	0.60	1.10 (0.80-1.51)
- Graft rejection	2 (2.9)	8 (6.6)	0.42	1.02 (0.74-1.41)
- Graft dysfunction	3 (8.6)	15 (12.3)	0.54	1.08 (0.86-1.35)
- Graft loss	0 (0)	1 (0.8)	0.99	1.0 (0.99-1.02)
- Mortality	0 (0)	1 (0.8)	0.99	1.0 (0.99-1.02)
- UTI relapses	10 (28.6)	49 (40.2)	0.21	1.11 (0.94-1.31)
- UTI re-infections	8 (22.9)	23 (18.9)	0.6	0.94 (0.75-1.18)

UTI: urinary tract infection. [+] Data available in 31 episodes, [++]data available in 116 episodes.

6.2.4. Impact of urine pH on microbiological and clinical outcome

The baseline pH was 6, IQR (6-6.5). In 81 episodes the urine pH was acidic (≤6) at the time of diagnosis, while only four were basic (pH>7.5). In the bivariate analysis, multidrug resistant *E. coli* was associated to acidic urine pH (14 (17.3%) *vs.* 3 (3.9%), $p<0.01$, OR=1.16, 95% CI 1.04-1.29).

Acidic compared to neutral-basic urine pH was not associated with one-month's microbiological failure (23.4% *vs.* 22.3%, $p>0.05$). However, it was associated with symptomatic UTI during the first month after inclusion (16% *vs.* 1.3%, $p<0.01$, OR=1.17, 95% CI 1.06-1.29). When this association was analysed under the effect of the treatment received and its interaction with the urine pH at inclusion in a logistic

regression model, acidic urine pH kept its effect (*p*=0.02, OR=10.18, 95% CI 1.26-82.23).

At six months of follow-up acidic urine was neither associated with microbiological failure (46/77 (59.7%) *vs*. 43/70 (61.4%), *p*=0.83, OR=0.93, 95% CI 0.48-1.80) nor symptomatic UTI during follow-up (14 (17.3%) *vs.* 11 (14.5%), *p*=0.47, OR=0.89, 95% CI 0.57-1.43) (Table 17).

Table 17. Microbiological and clinical outcomes therapy according to the initial urine pH.

Variables, n (%)	**Acidic pH (N=81)**	**Neutral pH (N=72)**	**Basic pH (N=4)**	***p*-value (acidic *vs*. non-acidic)**	**OR (95% CI)**
One-month´s:					
- Microbiological failure	19 (23.4)	17 (22.3)	0 (0)	0.87	1.01 (0.85-1.20)
- Symptomatic UTI	13 (16)	1 (1.4)	0 (0)	0.01	1.17 (1.06-1.29)
Six-months':					
- Microbiological failure	46 (59.7) $^{+}$	41 (60.3) $^{++}$	2 (100) $^{+++}$	0.83	0.93 (0.48-1.80)
- Symptomatic UTI	14 (17.3)	10 (13.9)	1 (25)	0.47	0.89 (0.57-1.43)
- Pyelonephritis	6 (7.4)	0 (0)	1 (25)	0.07	1.71 (1.21-2.41)
- Graft rejection	4 (4.9)	5 (6.9)	0 (0)	0.66	0.98 (0.90-1.06)
- Graft dysfunction	12 (14.8)	5 (6.9)	1 (25)	0.18	1.08 (0.96-1.21)
- Graft loss	0 (0)	1 (1.4)	0 (0)	0.99	0 (0.90-1.02)
- Mortality	0 (0)	0 (0)	1 (25)	0.99	0 (0.90-1.02)
- UTI relapses	33 (40.7)	26 (36.1)	0 (0)	0.39	1.32 (0.69-2.53)
- UTI re-infections	18 (22.2)	12 (16.7)	1 (25)	0.42	1.38 (0.62-3.06)

UTI: urinary tract infection. $^{+}$ Data available in 77 episodes, $^{++}$data available in 68 episodes, $^{+++}$ data available in 2 episodes. $^{\mu:}$ Significative *p*-value when stratified by acidic, neutral and basic urine pH.

A trend to a higher risk of developing pyelonephritis during the six months of follow-up up occurred in patients with acidic pH at the time of diagnosis: 6 (7.4%) *vs*. 1 (1.3%), *p*=0.07.

No differences were observed regarding graft loss, graft dysfunction, mortality and rejection according to urine pH (Table 17).

6.2.5. Impact of urine pH on ciprofloxacin and fosfomycin efficacy

Episodes treated with fosfomycin had similar one-month microbiological failure in cases of acidic urine pH compared to those with neutral or basic pH (24.6% *vs.* 26.3%, *p*=0.83, OR=0.95, 95% CI 0.64-1.41). The same was observed in episodes treated with ciprofloxacin (18.8% *vs.* 10.5%, *p*=0.64, OR=1.38, 95% CI 0.60-3.15).

Among patients treated with fosfomycin, those with acidic urine pH had a higher risk of symptomatic UTI during the first month of follow-up (15.4% *vs.* 1.8% *p*<0.01, OR=1.16, 95% CI 1.04-1.29). In patients treated with ciprofloxacin, only those with acidic urine pH had symptomatic UTI in the first month after inclusion (18.8% *vs.* 0%, *p*=0.08, OR=1.23, 95% CI 0.97-1.55) (Table 18).

Regarding other secondary outcomes, only a trend to a higher incidence of graft dysfunction was observed in those with acidic urine pH receiving ciprofloxacin (3 (18.8%) *vs.* 0 (0%); *p*=0.08; OR=1.19, 95% CI 0.83-1.7).

Table 18. Microbiological and clinical outcomes after ciprofloxacin or fosfomycin therapy, stratified by the initial urine pH.

Variables, n (%)	**Ciprofloxacin (N=35)**		**Fosfomycin (N=122)**		***p*-value**	**OR (95% CI)**
	Acidic pH (N=16)	**Non-acidic pH (N=19)**	**Acidic pH (N=65)**	**Non-acidic pH (N=57)**		
One month´s microbiological failure	5 (14.3)		31 (25.4)		0.16	1.14 (0.96-1.35)
	Acidic vs. non-acidic pH		***Acidic vs. non-acidic pH***			
	3 (18.8%) *vs.* 2 (10.5%), *p*=0.64		16 (24.6%) *vs.* 15 (26.3%), *p*=0.83		-	
One month´s symptomatic UTI	3 (8.6)		11 (9)		1	1.01 (0.75-1.34)
	Acidic vs. non-acidic pH		***Acidic vs. non-acidic pH***			
	3 (18.8%) *vs.* 0 (0%), *p*=0.08		10 (15.4%) *vs.* 1 (1.7%), *p*=0.01			
Six months´microbiological failure	13 (41.9) [+]		76 (65.5) [++]		0.01	1.26 (1.04-1.52)
	Acidic vs. non-acidic pH		***Acidic vs. non-acidic pH***			
	8/15 (53.3%) *vs.* 5/16 (31.3%), *p*=0.21		38/62 (61.3%) *vs.* 38/54 (70.4%), *p*=0.30		-	
Six months´ symptomatic UTI	1 (2.9)		24 (19.7)		0.02	1.29 (1.13-1.47)
	Acidic vs. non-acidic pH		***Acidic vs. non-acidic pH***			
	1 (6.3%) *vs.* 0 (0%), *p*=0.4		13 (20%) *vs.* 11 (19.3%), *p*=0.92		-	
Six months´ pyelonephritis	1 (2.9)		6 (4.9)		1	1.10 (0.80-1.51)
	Acidic vs. non-acidic pH		***Acidic vs. non-acidic pH***			
	1 (6.3%) *vs.* 0 (0%), *p*=0.45		5 (7.7%) *vs.* 1 (1.8%), *p*=0.21		-	

UTI: urinary tract infection. [+] Data available in 31 episodes, [++] data available in 116 episodes.

6.2.5.1. Multivariate analyses of the effect of fosfomycin and pH on microbiological and clinical outcome

Multivariate models were used to adjust for possible confounding variables of the effect of fosfomycin therapy and urine pH on microbiological and clinical outcomes (at one and six months of follow-up).

6.2.5.1.1. Microbiological failure

Fosfomycin was not associated to one-month microbiological failure while time since transplant >1 year (77.8% *vs*. 52.1%) and recurrent UTI before transplant (34.4% *vs.* 15.6%) were (Table 19).

Neither fosfomycin nor acidic urine pH was associated to microbiological failure when adjusted for other confounding variables (including antibiotic received and urine pH at inclusion interaction in a regression logistic analysis) (Table 20).

Table 19. Bivariate analysis of factors associated with microbiological failure at one month of follow-up.

Variables, n (%)	**Microbiological cure (N=121)**	**Microbiological failure (N=36)**	***p*-value**	**OR (95% CI)**
Age ≥ 58 years	60 (49.6)	21 (58.3)	0.35	1.17 (0.84-1.63)
Sex (female)	80 (66.1)	24 (66.7)	0.95	1.0 (0.77-1.31)
Retransplantation	14 (11.6)	3 (8.3)	0.76	0.72 (0.21-2.36)
Time since transplant <2 months	17 (14)	4 (11.1)	0.78	0.79 (0.28-2.2)
Time since	58 (47.9)	8 (22.2)	<0.01	0.31 (0.13-0.73)

transplant <1 year				
MMF	103 (85.1)	26 (72.2)	0.09	1.24 (0.93-1.65)
M-TOR inhibitor	4 (3.3)	4 (11.1)	0.08	3.36 (0.88-12.77)
Pre-transplant recurrent UTI	15 (15.6) +	11 (34.4) ++	0.02	2.2 (1.12-4.28)
Thymoglobulin induction therapy <3 months	6 (26.1) +++	2 (50) ++++	0.4	1.36 (0.75-2.48)
Antibiotic use <3 months	48 (39.7)	15 (41.7)	0.83	1.05 (0.67-1.63)
Symptomatic UTI < 6 months	29 (24)	5 (13.9)	0.19	0.96 (0.44-2.11)
Acute rejection previous 6 months	12 (9.9)	3 (8.3)	1	0.58 (0.24-1.38)
UTI < 2 months after transplant	52 (44.4) +++++	14 (38.9)	0.55	0.87 (0.55-1.38)
Cystitis at inclusion	20 (16.5)	7 (19.4)	0.68	1.17 (0.54-2.55)
Hospital-acquired UTI	20 (16.5)	9 (25)	0.25	1.5 (0.75-3.02)
Glomerular filtration ≤ 78 mL/min	61 (50.4)	17 (47.2)	0.73	0.93 (0.63-1.38)
Urine pH ≤ 6	62 (51.2)	19 (52.8)	0.87	1.03 (0.72- 1.46)
MDR *E. coli*	11 (9.1)	6 (16.7)	0.22	1.83 (0.72-4.61)
Fosfomycin	91 (75.2)	31 (86.1)	0.16	1.14 (0.97-1.35)

MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), m-TOR inhibitor: inhibitors of the mechanistic target of rapamycin (sirolimus, everolimus), UTI: urinary tract infection, MDR: multidrug resistance. $^{+}$ Data available in 96 episodes $^{++}$ data available in 32 episodes, $^{+++}$ data available in 23 episodes, $^{++++}$ data available in 4 episodes; $^{+++++}$ data available in 117 episodes.

Table 20. Multivariate analysis of factors associated with microbiological failure at one month of follow-up.

Variables, n (%)	Adjusted *p*-value	Adjusted OR (95% CI)
Age ≥ 58 years	0.21	1.70 (0.73-3.95)
Time since transplant < 1 year	<0.01	0.30 (0.12-0.74)
Glomerular filtration ≤ 78 mL/min	0.45	0.72 (0.31-1.67)
Urine pH ≤ 6	0.93	1.03 (0.46-2.28)
Ciprofloxacin	0.22	0.51 (0.17-1.49)

When the analysis was restricted to cases treated with fosfomycin, the same results were observed: time since transplant more than one year was independently associated to microbiological failure at one month (p=0.02) while acidic urine pH was not.

Fosfomycin therapy was associated with microbiological failure at six months of follow-up in the bivariate analysis. Acidic urine pH was not associated to this outcome. When stratified by acidic urine pH, fosfomycin was associated to microbiological failure only with non-acidic urine at baseline, while with acidic urine both antibiotics had similar microbiological failure (Table 21). Other factors associated to microbiological failure at six months were asymptomatic bacteriuria at inclusion (25.9% *vs.* 12.4%), and absence of microbiological cure at one month (79.3% *vs.* 50.6%) (Table 22).

Table 21. Microbiological outcome at six months after ciprofloxacin or fosfomycin therapy, stratified by the initial urine pH.

	Ciprofloxacin	Fosfomycin	*p*-value	OR (95% CI)
Microbiological failure in acidic pH - n (%)	8/15 (53.3%)	38/62 (61.3%)	0.58	-
Microbiological failure in non-acidic pH - n (%)	5/16 (31.3%)	38/54 (70.4%)	<0.01	1.49 (1.07-2.07)

Table 22. Bivariate analysis of factors associated with microbiological failure after six months of follow-up.

Variables, n (%)	Microbiological cure (N=58)	Microbiological failure (N=89)	*p*-value	OR (95%CI)
Age ≥ 58 years	33 (56.9)	43 (48.3)	0.3	0.85 (0.62-1.15)
Sex (female)	35 (60.3)	60 (67.4)	0.38	1.1 (0.86-1.44)
Retransplantation	6 (10.3)	9 (10.1)	0.96	0.97 (0.36-2.6)
Time since transplant <2 months	8 (13.8)	13 (14.6)	1	1.05 (0.46-2.39)
Time since transplant <1 year	26 (44.8)	39 (43.8)	0.9	0.97 (0.67-1.41)
MMF	47 (81)	73 (82)	0.88	1.01 (0.86-1.18)
M-TOR inhibitor	2 (3.4)	6 (6.7)	0.48	1.95 (0.4-9.35)
Pre-transplant recurrent UTI	7 (16.7) +	18 (23.7) ++	0.37	1.42 (0.64-3.12)
Thymoglobulin induction therapy <3 months	5 (50) +++	3 (17.6) ++++	0.11	0.36 (0.05-2.48)
Antibiotic use < 3 months	20 (34.5)	42 (47.2)	0.12	1.36 (0.9-2.07)
Symptomatic UTI < 6 months	9 (15.5)	22 (24.7)	0.18	1.59 (0.79-3.21)
Acute rejection previous 6 months	6 (10.3)	9 (10.1)	0.96	0.97 (0.36-2.6)
UTI < 2 months after transplant	24 (42.9) +++++	42 (48.3) ++++++	0.52	1.12 (0.77-1.63)
Cystitis at inclusion	15 (25.9)	11 (12.4)	0.03	0.47 (0.23-0.96)
Hospital-acquired UTI	10 (17.2)	18 (20.2)	0.65	1.17 (0.58-2.35)
Glomerular filtration ≤ 78 mL/min	34 (58.6)	42 (47.2)	0.17	0.8 (0.59-1.09)
Urine pH ≤ 6	31 (53.4)	46 (51.7)	0.83	0.96 (0.7- 1.32)
MDR *E. coli*	6 (10.3)	11 (12.4)	0.7	1.19 (0.46-3.05)
Fosfomycin	40 (69)	76 (85.4)	0.01	1.68 (1.14-1.14)
Microbiological cure	46 (79.3)	45 (50.6)	0.01	0.79 (0.66-0.94)

at 1 month				
Urological symptoms at 1 month	4 (28.6)	9 (10.1)	0.5	0.96 (0.87-1.06)

MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), m-TOR inhibitor: inhibitors of the mechanistic target of rapamycin (sirolimus, everolimus), UTI: urinary tract infection, MDR: multidrug resistant. $^{+}$ Data available in 42 episodes $^{++}$ data available in 76 episodes, $^{+++}$ data available in 10 episodes, $^{++++}$ data available in 71 episodes; $^{+++++}$ data available in 56 episodes; $^{++++++}$ data available in 87 episodes.

In the multivariate analysis, treatment with ciprofloxacin therapy protected from microbiological failure at six months. The urine pH was not associated to microbiological failure. The interaction of the antibiotic received and the urine pH at inclusion was also explored without significative results. Other relevant detected interactions were tested and excluded due to absence of influence: age ≥ 58 years, gender, glomerular filtration ≤ 78 mL/min, multidrug resistant strains, cytomegalovirus coinfection, use of MMF, use of m-TOR inhibitor, and polycystic kidney as baseline disease (Table 23).

Table 23. Multivariate analysis of factors associated with microbiological failure after six months of follow-up (N=147).

Variables, n (%)	**Adjusted *p*-value**	**Adjusted OR (95% CI)**
Time since transplant < 2 months	0.95	1.03 (0.37-2.86)
Cystitis at inclusion	0.07	0.43 (0.18-1.07)
Hospital-acquired UTI	0.92	1.04 (0.42-2.59)
Urinary pH ≤ 6	0.93	0.97 (0.48-1.94)
Ciprofloxacin	0.03	0.39 (0.17-0.92)

UTI: urinary tract infection.

When only cases treated with fosfomycin were analysed, asymptomatic bacteriuria at diagnosis trend to be associated to six-month's microbiological failure (*p*=0.09, OR=0.40, 95% CI 0.14-1.15). Acidic urine pH was not associated to this outcome.

6.2.5.1.2. Clinical outcome

Fosfomycin was not associated to symptomatic UTI at one month of follow-up while acidic urine pH was (Table 24). Other factors associated with symptomatic UTI at one month of follow-up were: use of antibiotic within the previous 3 months, symptomatic UTI within the previous 6 months, and multidrug resistant *E. coli*. Although not statistically significant, there was a trend towards a worse clinical outcome in patients with cystitis at diagnosis (Table 25).

Table 24. Clinical outcome at one month after ciprofloxacin or fosfomycin therapy, stratified by the initial urine pH.

	Ciprofloxacin	**Fosfomycin**	***p*-value**	**OR (95% CI)**
Symptomatic UTI in acidic pH - n (%)	3/16 (18.8%)	10/65 (15.4%)	0.72	-
Symptomatic UTI in non acidic pH - n (%)	1/57 (1.7%)	0/19 (0%)	0.75	-

Fosfomycin therapy was not associated with symptomatic UTI at one month when adjusted for other possible confounding variables. Acidic urine pH at inclusion was independently associated with symptomatic UTI at one month of follow-up in the regression logistic analyses performed. History of symptomatic UTI in the previous 6 months of the episode, and multidrug resistant *E. coli* were associated to symptomatic UTI within the first month of bacteriuria, too. The interaction of the antibiotic received

Table 25. Bivariate analysis of factors associated with symptomatic UTI after one month of follow-up.

Variables, n (%)	**Absence of symptomatic UTI (N=143)**	**Symptomatic UTI (N=14)**	***p*-value**	**OR (95% CI)**
Age ≥ 58 years	73 (51)	8 (57.1)	0.66	1.11 (0.69-1.8)
Sex (female)	93 (65)	11 (78.6)	0.38	1.2 (0.89-1.62)
Retransplantation	14 (9.8)	3 (21.4)	0.52	2.18 (0.71-6.7)
Time since transplant < 2 months	17 (11.9)	4 (28.6)	0.09	2.4 (0.93-6.15)
MMF	117 (81.8)	12 (85.7)	1	1.04 (0.83-1.31)
M-TOR inhibitor	8 (5.6)	0 (0)	1	0.36 (0.18-1.4)
Pre-transplant recurrent UTI	25 (21.4) [+]	1 (9.1) [++]	0.46	0.42 (0.06-2.84)
Thymoglobulin induction < 3 months	7 (30.4) [+++]	1 (25) [++++]	0.92	0.36 (0.15-1.48)
Antibiotic use < 3 months	52 (36.4)	11 (78.6)	<0.01	2.16 (1.52-3.06)
Symptomatic UTI < 6 months	27 (18.9)	7 (50)	0.01	2.64 (1.41-4.94)
Acute rejection previous 6 months	13 (9.1)	2 (14.3)	0.62	1.57 (0.39-6.27)
UTI < 2 months after transplant	59 (42.4) [+++++]	7 (50)	0.58	1.17 (0.67-2.05)
Cystitis at inclusion	22 (15.4)	5 (35.7)	0.06	2.32 (1.04-5.17)
Hospital-acquired UTI	25 (17.5)	4 (28.6)	0.29	1.63 (0.66-4.02)
Glomerular filtration at inclusion ≤ 78 ml/min	72 (50.3)	6 (42.9)	0.59	0.85 (0.45-1.59)
Urine pH ≤ 6	68 (47.6)	13 (92.9)	<0.01	1.95 (1.55-2.44)
MDR *E. coli*	11 (7.7)	6 (42.9)	<0.01	5.57 (2.43-12.77)
- Ciprofloxacin resistant *E. coli*	42 (29.4)	8 (57.1)	0.04	3.55 (1.93-7.15)
- Cephalosporin resistant *E. coli*	1 (0.7)	2 (14.3)	0.02	1.92 (1.12-2.9)
Fosfomycin	111 (77.6)	11 (78.6)	1	1.01 (0.75-1.34)

MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), m-TOR inhibitor: inhibitors of the mechanistic target of rapamycin (sirolimus, everolimus), UTI: urinary tract infection, MDR: multidrug resistant. [+] Data available in 117 episodes [++] data available in 11 episodes, [+++] data available in 23 episodes, [++++] data available in 4 episodes; [+++++] data available in 123 episodes.

Tables 26. Multiple logistic regression analyses of factors associated with symptomatic UTI after one month of follow-up.

	***p*-value**	**OR**	**95% CI**
Ciprofloxacin	0.84	1.15	0.28-4.67
Urine pH ≤ 6	0.01	13.53	1.71-107.17
Time since transplant < 2 months	0.20	2.36	0.63-8.88

	***p*-value**	**OR**	**95% CI**
Ciprofloxacin	0.77	1.23	0.29-5.25
Urine pH ≤ 6	0.01	14.79	1.84-118.4
Symptomatic UTI < 6 months	0.01	4.47	1.35-14.79

	***p*-value**	**OR**	**95% CI**
Ciprofloxacin	0.59	0.95	0.23-3.97
Urine pH ≤ 6	0.01	13.39	1.69-105.93
Cystitis at inclusion	0.13	2.60	0.74-9.08

	***p*-value**	**OR**	**95% CI**
Ciprofloxacin	0.72	1.3	0.30-5.64
Urine pH ≤ 6	0.02	10.73	1.32-86.67
Multidrug resistant *E. coli*	<0.01	6.05	1.67-21.93

	***p*-value**	**OR**	**95% CI**
Ciprofloxacin	0.93	1.05	0.25-4.34
Urine pH ≤ 6	0.01	14.38	1.81-113.67
Polycystic kidney disease	0.12	3.27	0.71-14.95

UTI: urinary tract infection.

and urine pH at inclusion was also explored with no influence in this outcome (Table 26).

When only cases treated with fosfomycin were analysed, acidic urine pH (p=0.02, OR=10.32, 95% CI 1.27-83.86) and multidrug resistant strains (p<0.01, OR=10.04, 95% CI 2.43-41.44) showed independent associations with the presence of symptomatic UTI within one month of inclusion.

Fosfomycin use leads to more symptoms at six months (19.3 *vs.* 0%). Acidic urine pH was not associated to this outcome. Fosfomycin and ciprofloxacin showed similar efficacy in both urine pH groups (Table 27).

When stratified by acidic urine pH, fosfomycin was associated to symptomatic UTI during the 6 months of follow-up only with non-acidic urine at baseline, while with acidic urine both antibiotics had similar outcome.

In the bivariate analysis, fosfomycin therapy of the current episode was associated to the presence of symptomatic UTI during the 6 months of follow-up (96% *vs.* 74%). Other factors associated with this endpoint were: retransplantation (28% *vs.* 7.6%) and symptomatic bacteriuria within the six months before the inclusion (36% *vs.* 18.8%).

A trend to more symptomatic UTI by first month of follow-up (6.8% *vs.* 20%), hospital-acquired events (32% *vs.* 15.8%) and pre-transplant recurrent UTI (34.8% *vs.* 17.1%) also were observed with this endpoint (Table 28).

Table 27. Clinical outcome at six months after ciprofloxacin or fosfomycin therapy, stratified by the initial urine pH.

	Ciprofloxacin	**Fosfomycin**	***p*-value**	**OR (95% CI)**

Symptomatic UTI in acidic pH - n (%)	1/16 (6.3%)	13/65 (20%)	0.21	-
Symptomatic UTI in non acidic pH - n (%)	0/19 (0%)	11/57 (19.3%)	0.03	1.41 (1.20-1.65)

When logistic regression analyses were performed, urine pH was not associated with symptomatic UTI at six months when adjusted for other possible confounding variables. While the use of ciprofloxacin protected to present this outcome, retransplantation was a risk. The interaction of the antibiotic received and urine pH at inclusion was also explored with no influence in this outcome. Other relevant detected interactions were tested and excluded due to absence of influence in the diverse regression logistic analyses performed: time since transplant <2 months, age ≥ 58 years, gender, glomerular filtration ≤ 78 mL/min, cystitis at inclusion, hospital acquired-UTI, cytomegalovirus coinfection, use of MMF, use of m-TOR inhibitor, and polycystic kidney as baseline disease (Tables 29).

When only cases treated with fosfomycin were analysed, retransplantation was the only variable associated to this outcome (p<0.01, OR=5.53, 95% CI 1.68-18.18). To present a symptomatic event 6 months before the inclusion trend to be associated to more symptomatic UTI at sixth month of follow-up (p=0.06, OR=2.49, 95% CI 0.94-6.56).

Although not statistically different, in the bivariate analysis, patients with acidic urine pH at inclusion had more frequently pyelonephritis during follow-up than those with non-acidic pH (85.7% *vs.* 50%). Fosfomycin was not associated to the presence of pyelonephritis during the 6 months of follow-up. Those patients with symptomatic UTI one month after inclusion had more frequently pyelonephritis than those who did not presented UTI symptoms (Table 30).

Table 28. Bivariate analysis of factors associated with symptomatic UTI after six months of follow-up.

Variables, n (%)	**Absence of symptomatic UTI (N=132)**	**Symptomatic UTI (N=25)**	***p*-value**	**OR (95% CI)**
Age ≥ 58 years	70 (53)	11 (44)	0.66	0.73 (0.35-1.52)
Sex (female)	86 (65.2)	18 (72)	0.60	1.08 (0.81-1.44)
Retransplantation	10 (7.6)	7 (28)	<0.01	3.87 (1.63-9.1)
Time since transplant <2 months	18 (13.5)	3 (12)	1	0.88 (0.28-2.69)
MMF	108 (81.8)	21 (84)	0.57	3.32 (0.85-13.0)
M-TOR inhibitors	5 (3.8)	3 (12)	0.10	1.09 (0.94-1.26)
Pre-transplant recurrent UTI	18 (17.1) [+]	8 (34.8) [++]	0.08	2.57 (0.95-6.98)
Thymoglobulin induction therapy <3 months	7 (30.4) [+++]	1 (25) [++++]	0.6	0.36 (0.15-1.48)
Antibiotic use < 3 months	49 (37.1)	14 (56)	0.04	1.58 (1.05-2.37)
Symptomatic UTI < 6 months	25 (18.8)	9 (36)	0.04	1.99 (1.06-3.73)
Acute rejection previous 6 months	12 (9.1)	3 (12)	0.70	1.38 (0.42-4.54)
UTI < 2 months after transplant	57 (44.5) [+++++]	9 (36)	0.54	0.84 (0.48-1.47)
Cystitis at inclusion	23 (17.3)	4 (16)	1	0.96 (0.36-2.53)
Hospital-acquired UTI	21 (15.8)	8 (32)	0.07	2.07 (0.99-4.34)
Glomerular filtration ≤ 78 ml/min	68 (51.5)	10 (40)	0.19	0.72 (0.42-1.24)
Urine pH ≤ 6	67 (50.4)	14 (58.3)	0.47	1.15 (0.79-1.69)
MDR *E. coli*	13 (9.8)	4 (16)	0.29	1.70 (0.6-4.79)
Fosfomycin therapy	98 (74.2)	24 (96)	0.02	1.28 (1.13-1.46)
Microbiological cure at 1 month	101 (76.5)	20 (80)	1	1.03 (0.82-1.29)
Urological symptoms at 1 month	9 (6.8)	5 (20)	0.06	2.55 (1.13-5.75)

MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), m-TOR inhibitor: inhibitors of the mechanistic target of rapamycin (sirolimus, everolimus), UTI: urinary tract infection, MDR: multidrug resistance. $^{+}$ Data available in 105 episodes $^{++}$ data available in 23 episodes, $^{+++}$ data available in 23 episodes, $^{++++}$ data available in 4 episodes; $^{+++++}$ data available in 128 episodes.

Tables 29. Multiple logistic regression analyses of factors associated with symptomatic UTI after six months of follow-up.

	***p*-value**	**OR**	**OR (95% CI)**
Ciprofloxacin	0.04	0.11	0.01-0.92
Urine pH ≤ 6	0.96	0.97	0.39-2.44
Retransplantation	<0.01	4.84	0.63-8.88

	***p*-value**	**OR**	**OR (95% CI)**
Ciprofloxacin	0.04	0.12	0.01-0.96
Urine pH ≤ 6	0.79	1.12	0.46-2.74
Symptomatic UTI < 6 months	0.08	2.29	0.89-5.92

	***p*-value**	**OR**	**OR (95% CI)**
Ciprofloxacin	0.04	0.12	0.01-0.95
Urine pH ≤ 6	0.83	1.09	0.44-2.70
MDR *E. coli*	0.53	1.48	0.42-5.24

UTI: urinary tract infection, MDR: multidrug resistant.

Regarding relapses and persistant events during the follow-up, in 30 cases new resistances were observed (19.1%): 12 to fosfomycin (7.6%) and 18 (11.46%) to ciprofloxacin, with no association according to the treatment received. In some of these strains, the ST could be performed as detailed in objective 4.

Table 30. Bivariate analysis of factors associated to the development of acute pyelonephritis during six months of follow-up.

Variables, n (%)	**No APN at six months (N=150)**	**APN at six months (N=7)**	***p-value***	**OR (95% CI)**
Age ≥ 58 years	78 (52)	3 (42.9)	0.71	0.84 (0.43-1.63)
Sex (female)	100 (66.7)	4 (57.1)	0.69	0.66 (0.14-3.09)
Retransplantation	16 (10.7)	1 (14.3)	0.55	1.39 (0.16-12.34)
Time since transplant < 2 months	20 (13.3)	1 (14.3)	1	1.08 (0.12-9.47)
Polycystic kidney disease	12 (8)	2 (28.6)	0.13	1.88 (0.78-2.4)
Symptomatic UTI < 6 months	33 (22)	1 (14.3)	1	0.91 (0.66-1.24)
Acute rejection previous 6 months	15 (10)	0 (0)	1	0.9 (0.85-0.94)
Cystitis at inclusion	26 (17.3)	1 (14.3)	1	0.96 (0.7-1.31)
Hospital-acquired UTI	27 (18)	2 (28.6)	0.61	1.15 (0.71-1.84)
Glomerular filtration ≤ 78 mL/min	74 (49.3)	4 (57.1)	0.7	1.18 (0.49-2.82)
Urine pH ≤ 6	75 (50)	6 (85.7)	0.07	6 (0.7- 51.04)
MDR E. coli	15 (10)	2 (28.6)	0.16	1.26 (0.78-2.01)
Fosfomycin therapy	116 (77.3)	6 (85.7)	1	1.75 (0.2-15.11)
Microbiological cure at 1 month	116 (77.3)	5 (71.4)	0.66	0.79 (0.23-2.65)
Urological symptoms at 1 month	11 (7.3)	3 (42.9)	0.01	7.66 (1.90-30.8)

APN: pyelonephritis, UTI: urinary tract infection, MDR: multidrug resistant.

6.3. Objective 3: To analyse the clinical and microbiological outcomes in lower UTI caused by different uropathogens in KTR treated with ciprofloxacin and fosfomycin, according to the urine pH.

After the recommendation of restriction of fluoroquinolones (354,355), including KTR population, the treatment of these patients stopped. This affected the size of the cohort B that was planned at the beginning of the study. In order to increase the number of episodes treated with ciprofloxacin and to evaluate if these results were not restricted to *E. coli* UTI, all cases of bacteriuria from cohort B treated with ciprofloxacin or fosfomycin that were initially recruited during the pilot study time were included. In 12 patients, a second or third episode was included, after confirming at least one negative urine culture between episodes.

6.3.1. Baseline and clinical characteristics of lower UTI infections according to the antibiotic used

A total of 197 bacteriuria episodes, 165 asymptomatic bacteriuria (83.8%) and 32 cystitis (16.2%), in 174 renal recipients were included, of whom 138 (70.1%) received fosfomycin and 59 (29.9%) ciprofloxacin. Demographics, transplantation characteristics, data on underlying end-stage renal disease, previous allograft rejection, basal glomerular filtration rates, and antimicrobial susceptibilities are detailed in Table 31. Episodes treated with fosfomycin had less frequently received a transplant in the previous two months, (16 (13.2%) *vs*. 14 (26.4%), p=0.04, OR=0.58, 95% CI 0.36-0.92), and basiliximab as induction therapy (65 (4.3%) *vs.* 8 (15.6%), p=0.02, OR=0.53, 95% CI 0.26-0.87).

Table 31. Baseline characteristics of KTR included with different uropathogens.

Variables	**Total (N=174)**	**Ciprofloxacin (N=53)**	**Fosfomycin (N=121)**	***p-value***
Age - median (IQR)	58 (51-67)	57 (47.5-66.5)	59 (52.5-67)	0.16
Sex (female) - n (%)	108 (62.1)	34 (64.2)	74 (61.2)	0.70
Months from transplantation - median (IQR)	16 (3.6-77.7)	10.2 (1.96-55.5)	17.76 (4.6-82.8)	0.29
Time since transplant <2 months -n (%)	30 (17.2)	14 (26.4)	16 (13.2)	0.04
Time since transplant<1 year -n (%)	80 (45.9)	28 (52.8)	52 (42.9)	0.23
Retransplantation - n (%)	17 (9.8)	4 (7.5)	13 (10.7)	0.51
Underlying end-stage renal disease - n (%) - Glomerulonephritis - Tubulointerstitial - Polycystic kidney disease - Hypertension / renovascular - Diabetic nephropathy - Tumoral - Vesicoureteral reflux - Mix or unknown aetiology	 41 (23.6) 29 (16.7) 16 (9.2) 14 (8) 12 (6.9) 5 (2.9) 1 (0.05) 56 (32.2)	 14 (26.4) 11 (20.8) 5 (9.4) 1 (1.9) 3 (5.7) 2 (3.8) 0 (0) 17 (32.1)	 27 (22.3) 18 (14.9) 11 (9.1) 13 (10.8) 9 (8.3) 3 (2.5) 1 (0.8) 39 (32.2)	 0.55 0.34 0.92 0.04 0.70 0.64 0.69 0.99
Living donor - n (%)	15 (8.6)	4 (7.5)	11 (9.1)	1
Induction therapy within previous 3 months - n (%)	23/166 (13.8)	13/51 (25.4)	10/115 (8.6)	<0.01

- Thymoglobulin - Basiliximab	10 (6) 13 (7.8)	5 (9.8) 8 (15.6)	5 (4.3) 5 (4.3)	0.28 0.02
Need for new surgery within the first month of transplantation - n (%)	1 (0.05)	1 (1.8)	0 (0)	0.23
Surgical site complication	18 (10.3)	5 (9.4)	13 (10.7)	0.31
Haemodialysis due to delayed graft function	27 (15.5)	8 (15.09)	19 (15.7)	0.91
Current immunosuppression - n (%) - Corticosteroids - Tacrolimus - MMF - M-TOR inhibitors - Cyclosporine	166 (95.4) 164 (94.3) 139 (79.9) 13 (7.5) 4 (2.2)	53 (100) 50 (94.3) 46 (86.8) 2 (3.8) 1 (1.9)	113 (93.4) 114 (94.2) 93 (76.9) 11 (9.1) 3 (2.5)	0.49 0.10 0.13 0.34 0.87
Pre-transplant recurrent UTI - n (%)	22 (15.1) $^{+}$	4 (9.7) $^{++}$	18 (17.3) $^{+++}$	0.25
UTI < 2 months after transplant - n (%)	75 (45.1)$^{++++}$	27 (52.9)$^{+++++}$	48 (41.7)$^{++++++}$	0.18
Cotrimoxazole prophylaxis - n (%)	65 (37.4)	22 (41.5)	43 (35.5)	0.45
Acute rejection within the previous 6 months - n (%)	13 (9.1)	4 (7.5)	12 (9.9)	0.77
Rejection treatment in the previous 6 months - n (%) - Corticosteroid's bolus - Thymoglobulin - Plasmapheresis - Rituximab	16 (9.2) 5 (2.8) 3 (1.8) 4 (2.4)	3 (5.7) 2 (1.9) 2 (3.8) 2 (3.8)	13 (10.7) 3 (2.5) 1 (0.8) 2 (1.7)	0.39 0.33 0.6 0.44

IQR: interquartile range, MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), m-TOR inhibitor: inhibitors of the mechanistic target of rapamycin (sirolimus, everolimus), UTI: urinary tract infection. [+] Data available in 145 episodes [++] data available in 41 episodes, [+++] data available in 104 episodes, [++++] data available in 166 episodes; [+++++] data available in 51 episodes; [++++++] data available in 115 episodes.

The proportion of cystitis was similar in both treatment groups (21 (15.2%) *vs.* 11 (18.6%), p=0.55). The main aetiologies of the episodes included were *E. coli* (n=155, 78.7%, including 3 ESBL-producers) and *K. pneumoniae* (n=29, 14.7%, including 2 ESBL-producers).

Fifty-three percent (n=105) of the microorganisms causing bacteriuria were resistant to cotrimoxazole, 27.9% (n=55) to ciprofloxacin, 18.9% (n=37) to amoxicillin-clavulanate, 6.6% (n=13) to third and/or fourth generation cephalosporin's, and 4.1% (n=8) to fosfomycin (Table 32).

Compared with ciprofloxacin treated episodes, those treated with fosfomycin were more frequently caused by *E. coli* (120 (87%) *vs.* 34 (57.6%), p<0.01, OR=1.86, 95% CI 1.29-2.67) and hospital-acquired (28 (20.3%) *vs.* 2 (3.4%), p<0.01, OR=1.41, 95% CI (1.22-1.63). On the other hand, fosfomycin treated episodes were less frequently caused by *K. pneumoniae* (14 (10.1%) *vs.* 15 (25.4%), p<0.01, OR=0.65, 95% CI 0.44-0.96), and had received less previous antibiotic courses (65 (47.1%) *vs.* 39 (66.1%), p<0.01, OR=0.79, 95% CI 0.66-0.95) (Table 32).

No differences regarding cystitis and asymptomatic bacteriuria episodes were found in terms of demographics, transplantation characteristics, or other risk factors related to poor prognosis, except that in the cystitis group trend to present more polycystic kidney as baseline disease (20% *vs.* 7.4%, p=0.03, OR=3.23, 95% CI 1.09-9.49).

Table 32. Baseline characteristics of the episodes included by different uropathogens.

Variables	**Total (N= 197)**	**Ciprofloxacin (N=59)**	**Fosfomycin (N=138)**	***p*-value**
Types of UTI episodes - n (%) - Cystitis - Asymptomatic bacteriuria	32 (16.2) 165 (83.8)	11 (18.6) 48 (81.4)	21 (15.2) 117 (84.2)	0.55
Bacteriuria within the previous 6 months - n (%) - Symptomatic - n (%) - Cystitis - n (%) - Pyelonephritis - n (%) - Asymptomatic - n (%)	122 (61.9) 51 (25.9) 23 (11.6) 28 (14.2) 71 (36.04)	31 (52.5) 13 (22.03) 6 (10.1) 7 (11.8) 18 (30.5)	91 (65.9) 38 (27.5) 17 (12.3) 21 (15.2) 53 (38.4)	0.08 0.41 0.69 0.52 0.29
Antibiotic use within the previous 3 months - n (%) - Quinolones - Fosfomycin - Cephalosporines	104 (52.8) 38 (36.5) 35 (33.6) 17 (16.3)	39 (66.1) 26 (66.7) 3 (7.7) 2 (5.1)	65 (47.1) 12 (18.4) 32 (49.2) 15 (23.07)	0.01 <0.01 <0.01 0.01
Hospital-acquired UTI - n (%)	30 (15.2)	2 (3.4)	28 (20.3)	<0.01
Glomerular filtration at inclusion (mL/min) - median (IQR)	78 (52-85)	79.5 (53.4-84.7)	76.7 (50.2-85.4)	0.76
Urine pH - median (IQR)	6 (6-6.5)	6 (6-6.5)	6 (6-6.5)	0.91
Etiology of the episodes - n (%) - *E. coli* - *K. pneumoniae* - *P. mirabilis* - *K. oxytoca* - *M. morganii*	155 (78.7) 29 (14.7) 4 (2) 3 (1.5) 2 (1) 2 (1)	34 (57.6) 15 (25.4) 1 (1.7) 0 (0) 2 (3.4) 2 (3.4)	121 (87.7) 14 (10.1) 3 (2.2) 3 (5.1) 0 (0) 0 (0)	<0.01 <0.01 0.89 0.34 0.08

- *C. Koseri*	1 (0.5)	1 (1.7)	0 (0)	0.08
- *E. faecalis*	1 (0.5)	1 (1.7)	0 (0)	0.29
- *E. cloacae*				0.29
Baseline antibiotic resistance - n (%)				
- Cotrimoxazole	105 (53.6)	29 (50)	76 (55.1)	0.51
- Ciprofloxacin	53 (26.9)	0 (0)	53 (38.4)	<0.01
- Amoxicillin-clavulanate	37 (18.9)	13 (22.4)	24 (17.4)	0.41
- Fosfomycin	7 (3.5)	7 (12.1)	0 (0)	<0.01
- Cephalosporins	13 (6.6)	6 (10.3)	7 (5.1)	0.21
- ESBL-producers	5 (2.5)	0 (0)	5 (3.6)	0.32
- Carbapenem-resistance	0 (0)	0 (0)	0 (0)	-
- MDR strain	18 (9.1)	3 (5.1)	15 (10.9)	0.19
Concomitant infection (CMV, HCV, BK virus)	67 (34)	15 (25.4)	52 (37.6)	0.09

IQR: interquartile range, UTI: urinary tract infection, ESBL: extended spectrum beta-lactamase producers, MDR: multidrug resistance, CMV: cytomegalovirus, HCV: hepatitis C virus.

Patients with acidic urine pH at inclusion were younger (54.4% *vs.* 36.2%, *p*=0.01, OR=1.75, 95% CI 1.29-2.36) and trend to had more frequently multidrug resistant strains than those with non-acidic urine pH (12.6% *vs.* 5.3%, *p*=0.07, OR=2.57, 95% CI 0.88-7.51). No other differences were observed in the rest of demographics, transplant-related, clinical, and microbiological variables at inclusion.

6.3.2. Outcomes of lower UTI infections with different uropathogens

One month after the inclusion, 136/197 cases (69.03%) were microbiologically cured. In 24 cases (12.2%) the bacteriuria persisted, in 13 (6.6%) the bacteriuria relapsed, and 7 (3.5%) were re-infected.

Six months after inclusion, 84 of 173 cases were microbiologically cured (48.5%). In 24 patients, microbiological data at this point of follow-up were not available (Table 33).

Regarding clinical outcome, at the first month of follow-up, 181 cases were asymptomatic (92%) and 16 (8.1%) had symptomatic UTI: 14 cystitis (7.4%) and 2 (1%) pyelonephritis. Of those with symptoms at one month of follow-up up, 6 episodes (18.8%) were included as cystitis and 10 cases (6.1%) were included as asymptomatic bacteriuria. The two acute pyelonephritis at one month were initially one cystitis and one asymptomatic bacteriuria event.

During the six months of follow-up, 160 (81.2%) did not develop symptomatic UTI, and 28 (14.2%) had at least one episode of cystitis and nine (4.5%) had pyelonephritis (Table 33).

After six-months of follow-up, the renal function worsened in 17 (8.6%) patients -in two cases accompanied with symptomatic UTI-, nine (4.5%) had a graft rejection, and three (1.5%) lost the graft (Table 33). During the follow-up 59 events (29.9%) received another antibiotic course, due to new symptomatic UTI or asymptomatic bacteriuria events.

One patient had mild diarrhoea (non-*Clostridioides difficile* infection) that recovered without fosfomycin withdrawal, and another patient died (0.5%) at the fourth month of the follow-up. She was admitted due to renal failure in the context of an acute pyelonephritis caused by *E. coli* (urine pH=8). No other major side-effects related to the antibiotics prescribed were reported.

Microbiological failure at one month presented a trend to more microbiological failure at 6 months (61.8% *vs.* 38.2%, p=0.06, OR=1.32, 95% CI 0.99-1.76). However, it was not associated to symptomatic UTI during one and six months of follow-up.

Patients with symptomatic UTI at one month, had more frequently pyelonephritis (25% *vs.* 2.8%, $p<0.01$, OR= 1.29, 95% CI 0.97-1.72), during the six months of follow-up.

Microbiological failure and symptomatic UTI during follow-up were not associated to graft rejection, graft dysfunction or graft loss at the end of follow-up.

No differences regarding cystitis and asymptomatic bacteriuria episodes were found in the diverse outcomes during the follow-up. Although not statistically different, patients with cystitis, compared to patients with asymptomatic bacteriuria, had more symptomatic UTI at first month (15.6% *vs.* 6.1%, $p=0.07$, OR=2.87, 95% CI 0.91-9.05) (Table 33).

Table 33. Microbiological and clinical outcome of *E. coli* bacteriuria according to the presence of urological symptoms at diagnosis.

Variables, n (%)	**Total (N= 197)**	**AB (N=165)**	**Cystitis (N=32)**	***p*-value**
One-month´s: - Microbiological failure	61 (31)	52 (31.5)	9 (28.1)	0.70
- Symptomatic UTI	16 (8.1)	10 (6.1)	5 (15.6)	0.07
Six-months': - Microbiological failure	89 (51.4) [+]	77 (53.8) [++]	12 (40) [+++]	0.22
- Symptomatic UTI	37 (18.8)	30 (18.2)	7 (21.9)	0.62
- Pyelonephritis	9 (4.5)	7 (4.2)	2 (6.2)	1
- Graft rejection	9 (4.6)	8 (4.8)	1 (3.1)	1
- Graft dysfunction	17 (8.6)	16 (9.7)	1 (3.1)	0.31
- Graft loss	3 (1.5)	2 (1.2)	1 (3.1)	0.48
- Mortality	1 (0.5)	1 (0.6)	0 (0)	1
- UTI relapses	71 (36)	62 (37.6)	9 (28.1)	0.30
- UTI re-infections	47 (23.9)	42 (25.5)	5 (15.6)	0.26

UTI: urinary tract infection. [+] Data available in 173 episodes, [++] data available in 143 episodes, [+++] data available in 30 episodes.

6.3.2.1. Clinical and microbiology effectiveness of fosfomycin therapy

One-month's and six-months' microbiological and clinical outcomes were similar in episodes treated with fosfomycin compared to those treated with ciprofloxacin, except for a higher risk of one-month's microbiological failure (12 (20.3%), *vs.* 49 (35.5%) *p*=0.03, OR=1.75, 95% CI 1.00-3.06) and a lower risk of urinary tract re-infections during the six months of follow-up in the group receiving fosfomycin (22 (37.3%) *vs.* 25 (18.1%), *p*<0.01, OR=0.70, 95% CI 0.53-0.93) (Table 34).

Table 34. Microbiological and clinical outcomes of bacteriurias according to the treatment received.

Variables, n (%)	**Ciprofloxacin (N=59)**	**Fosfomycin (N=138)**	***p*-value**	**OR (95% CI)**
One-month´s: - Microbiological failure	12 (20.3)	49 (35.5)	0.03	1.75 (1.00-3.06)
- Symptomatic UTI	5 (8.5)	11 (8)	1	0.99 (0.90-1.09)
Six-months': - Microbiological failure	21 (44.7) +	68 (54) ++	0.27	1.31 (0.80-2.14)
- Symptomatic UTI	7 (11.9)	30 (21.7)	0.10	1.71 (0.85-3.47)
- Pyelonephritis	3 (5.1)	6 (4.3)	1	0.84 (0.20-3.51)
- Graft rejection	0 (0)	9 (6.5)	0.06	1.45 (0.93-1.60)
- Graft dysfunction	2 (3.4)	15 (10.9)	0.08	2.69 (0.71-10.07)
- Graft loss	1 (1.7)	2 (1.4)	1	0.89 (0.17-4.50)
- Mortality	0 (0)	1 (0.7)	1	1.43 (1.30-1.56)
- UTI relapses	16 (27.1)	55 (39.9)	0.08	1.51 (0.92-2.48)
- UTI re-infections	22 (37.3)	25 (18.1)	<0.01	0.70 (0.53-0.93)

UTI: urinary tract infection. + Data available only in 47 episodes, ++ data available only in 126 episodes.

No differences in secondary outcomes, as graft rejection, graft dysfunction, or relapses, during the follow-up were seen according to the treatment received (Table 34).

6.3.2.2. Impact of urine pH on microbiological and clinical outcome

At inclusion, median urine pH was 6 (IQR 6-6.5). In 103 (52.28%) episodes the pH was acidic (≤6), in 88 (44.67%) neutral, and in 6 (3%) episodes was basic (pH>7.5).

Acidic urine, compared to neutral or basic urine, was not associated with microbiological failure (Table 35).

Table 35. Microbiological and clinical outcomes of bacteriurias according to the initial urine pH.

Variables, n (%)	**Acidic pH (N=103)**	**Neutral pH (N=88)**	**Basic pH (N=6)**	***p*-value (acidic *vs*. non-acidic)**
One-month´s: - Microbiological failure	32 (31.1)	17 (22.3)	0 (0)	0.97
- Symptomatic UTI	15 (14.6)	1 (1.1)	0 (0)	0.01
Six-months': - Microbiological failure	48 (52.2) [+]	40 (51.3) [++]	1 (33.3) [+++]	0.83
- Symptomatic UTI	23 (22.3)	14 (15.9)	0 (0)	0.18
- Pyelonephritis	8 (7.8)	0 (0)	1 (16.7)	0.06
- Graft rejection	4 (3.9)	5 (5.7)	0 (0)	0.73
- Graft dysfunction	11 (10.7)	5 (5.7)	1 (16.7)	0.28
- Graft loss	2 (1.9)	1 (1.1)	0 (0)	0.86
- Mortality	0 (0)	0 (0)	1 (16.7)	0.47
- UTI relapses	39 (37.9)	31 (35.2)	1 (16.7)	0.57
- UTI re-infections	28 (27.2)	17 (19.3)	2 (33.3)	0.38

UTI: urinary tract infection. [+] Data available in 92 episodes, [++]data available in 78 episodes, [+++] data available in 3 episodes.

Regarding clinical outcome, acidic urine at inclusion, compared with neutral or basic urine, was associated with more symptomatic UTI events at one month of follow-up in the bivariate analyses (15/103 (14.6%) *vs.* 1/94 (1.06%), $p<0.01$, OR=1.9, 95% CI 1.56-2.33), but not in a further multivariate analysis when controlled by the antibiotic received ($p=0.37$, OR=1.21, 95% CI 0.37-3.89).

Symptomatic UTI during the 6 months of follow-up occurred in 22.3% of patients with baseline acidic urine pH and in 15.9% of patients with neutral-basic urine pH ($p=0.18$). A trend towards a higher risk of pyelonephritis during the six months follow-up was observed in episodes with acidic urine pH (8/103 (7.8%) *vs.* 1/94 (1.06%), $p=0.06$, OR=3.9, 95% CI 0.62-24.76) (Table 35).

6.3.2.3. Impact of urine pH on ciprofloxacin and fosfomycin efficacy

Episodes treated with fosfomycin had similar one-month microbiological failure in cases of acidic urine pH compared to those with neutral or basic pH (33.3% *vs.* 37.9%, $p=0.57$, OR=0.90, 95% CI 0.64-1.28). The same was observed in episodes treated with ciprofloxacin (25.8% *vs.* 14.3%, $p=0.27$, OR=1.15, 95% CI 0.89-1.49). Similar results at six months of follow-up were observed (Table 36).

Among patients treated with fosfomycin, those with acidic urine pH had a higher risk of symptomatic UTI during the first month of follow-up (15.3% *vs.* 0%, $p=0.01$, OR=1.18, 95% CI 1.07-1.30).

Table 36. Microbiological and clinical outcomes after ciprofloxacin or fosfomycin therapy, stratified by the initial urine pH.

Variables, n (%)	**Ciprofloxacin (N=95)**		**Fosfomycin (N=138)**		***p*-value**	**OR (95% CI)**
	Acidic pH (N=31)	**Non-acidic pH (N=28)**	**Acidic pH (N=72)**	**Non-acidic pH (N=66)**		
One month´s microbiological failure	12 (20.3)		49 (35.5)		0.03	1.75 (1.00-3.06)
	Acidic vs. non-acidic pH		***Acidic vs. non-acidic pH***			
	8 (25.8%) *vs.* 4 (14.3%), *p*=0.27		24 (33.3%) *vs.* 25 (37.9%), *p*=0.57		-	
One month's symptomatic UTI	5 (8.5)		11 (8)		1	0.99 (0.90-1.09)
	Acidic vs. non-acidic pH		***Acidic vs. non-acidic pH***			
	4 (12.9%) *vs.* 1 (3.6%), *p*=0.35		11 (15.3%) *vs.* 0 (0%), *p*=0.01		-	
Six months' microbiological failure	21 (44.7) +		68 (54) ++		0.27	1.31 (0.80-2.14)
	Acidic vs. non-acidic pH		***Acidic vs. non-acidic pH***			
	14/26 (53.8%) *vs.* 7/21 (33.3%), *p*=0.16		34/66 (51.5%) *vs.* 34/60 (56.7%), *p*=0.56		-	
Six months´ symptomatic UTI	7 (11.9)		30 (21.7)		0.10	1.71 (0.85-3.47)
	Acidic vs. non-acidic pH		***Acidic vs. non-acidic pH***			
	6 (19.4%) *vs.* 1 (3.6%), *p*=0.10		17 (23.6%) *vs.* 13 (19.7%), *p*=0.57		-	
Six months´ pyelonephritis	3 (5.1)		6 (4.3)		1	0.84 (0.20-3.51)
	Acidic vs. non-acidic pH		***Acidic vs. non-acidic pH***			
	3 (9.7%) *vs.* 0 (0%), *p*=0.23		6 (6.9%) *vs.* 1 (1.5%), *p*=0.21		-	

UTI: urinary tract infection. [+] Data available in 47 episodes, [++] data available in 126 episodes.

The proportion of patients treated with ciprofloxacin, with acidic urine compared to those with neutral-basic urine who had symptomatic UTI in the first month of follow-up were not statistically different (12.9% *vs.* 3.6%, *p*=0.35, OR=1.10, 95% CI 0.95-1.29) (Table 36).

The clinical outcome after 6 months of follow-up according to the treatment used and the baseline urine pH is detailed in Table 36. There were not statistically differences among urine pH when receiving fosfomycin or ciprofloxacin at six months' symptomatic UTI and six months' pyelonephritis. No differences were found either regarding other secondary outcomes such as graft rejection, graft dysfunction or graft loss.

6.3.3. Multivariate analyses of the effect of fosfomycin and pH on microbiological and clinical outcome

Multivariate models were used to adjust for possible confounding variables of the effect of fosfomycin therapy and urine pH on microbiological and clinical outcomes (at one and six months of follow-up).

6.3.3.1. Microbiological failure

Fosfomycin was associate to one-month microbiological failure as stated before (Table 34), but when stratified by the urine pH at inclusion this association was only persistent in non-acidic pH (37.9% *vs.* 14.3%, *p*=0.02) (Table 37).

Time since transplant >1 year (68.9% *vs*. 49.3%) was associated to microbiological failure (Table 38).

Possible confounding factors of the effect of fosfomycin and urine pH in the microbiological failure at one month of follow-up were explored previously in different logistic regression multivariate analysis.

Table 37. Microbiological failure at one month after ciprofloxacin or fosfomycin therapy, stratified by the initial urine pH.

	Ciprofloxacin	**Fosfomycin**	***p*-value**	**OR (95% CI)**
Microbiological failure in acidic pH -n (%)	8/31 (25.8%)	24/72 (33.3%)	0.44	-
Microbiological failure in non-acidic pH -n (%)	4/28 (14.3%)	25/66 (37.9%)	0.02	2.67 (1.02-7.01)

Table 38. Bivariate analysis of factors associated with microbiological failure at one month of follow-up.

Variables, n (%)	**Microbiological cure (N=136)**	**Microbiological failure (N=61)**	***p*-value**	**OR (95% CI)**
Age ≥ 58 years	67 (49.3)	40 (65.6)	0.03	1.47 (1.04-3.66)
Sex (female)	88 (64.7)	41 (67.2)	0.73	1.07 (0.70-1.64)
Retransplantation	16 (11.8)	4 (6.6)	0.26	0.52 (0.161-1.46)
Time since transplant <2 months	23 (16.9)	8 (13.1)	0.49	0.74 (0.21-1.76)
Time since transplant <1 year	69 (50.7)	19 (31.1)	0.01	0.43 (0.23-0.83)
MMF	111 (81.6)	47 (77)	0.45	0.75 (0.63-1.58)
M-TOR inhibitor	8 (5.9)	6 (9.8)	0.37	1.74 (0.57-5.26)
Pre-transplant recurrent UTI	17 (15.2) $^{+}$	12 (21.8) $^{++}$	0.82	1.08 (0.92-1.27)
Thymoglobulin induction <3 months	8 (42.1) $^{+++}$	3 (60) $^{++++}$	0.82	1.05 (0.72-1.53)
Antibiotic use <3 months	68 (50)	36 (59)	0.24	1.22 (0.86-2.65)

Symptomatic UTI < 6 months	34 (25)	17 (27.9)	0.67	1.04 (0.86-1.24)
Acute rejection previous 6 months	13 (9.6)	7 (11.5)	0.68	1.02 (0.91-1.13)
UTI < 2 months after transplant	55 (42) $^{+++++}$	28 (48.3) $^{++++++}$	0.42	1.12 (0.84-1.49)
Cystitis at inclusion	23 (16.9)	9 (14.8)	0.70	0.97 (0.85-1.10)
Hospital-acquired UTI	18 (13.2)	12 (19.7)	0.24	1.08 (0.93-1.24)
Glomerular filtration ≤ 78 mL/min	69 (50.7)	30 (49.2)	0.84	0.96 (0.71-1.30)
Urine pH ≤ 6	71 (52.2)	32 (52.5)	0.97	1.01 (0.55- 1.84)
MDR *E. coli*	10 (7.4)	8 (13.1)	0.19	1.06 (0.95-1.18)
Fosfomycin	89 (65.4)	49 (80.3)	0.03	1.75 (1.00-3.06)

MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), m-TOR inhibitor: inhibitors of the mechanistic target of rapamycin (sirolimus, everolimus), UTI: urinary tract infection, MDR: multidrug resistant. $^{+}$ Data available in 112 episodes $^{++}$ data available in 55 episodes, $^{+++}$ data available in 19 episodes, $^{++++}$ data available in 5 episodes; $^{+++++}$ data available in 131 episodes, $^{++++++}$ data available in 58 episodes.

Variables with a *p*-value <0.10 in univariate comparisons and those considered clinically relevant were included. Interaction, confusion, and collinearity were thoroughly explored. The following variables were included: urine pH ≤ 6, time from transplant to bacteriuria <1 year, age ≥ 58 years, gender, glomerular filtration ≤ 78 mL/min, treatment received, multidrug resistant strains, hospital-acquired UTI, cytomegalovirus coinfection, urological symptoms at inclusion, use of MMF, use of m-TOR inhibitor, recurrent UTI previous to transplant, and polycystic kidney as baseline disease.

In the most suitable analysis, time since transplant more than one year (*p*=0.02, OR=2.13, 95% CI 1.10-4.11) and older recipients (*p*=0.02, OR=2.14, 95% CI 1.08-

4.24) were independently associated to microbiological failure at one month of follow-up. Acidic urine pH was not associated to microbiological failure when adjusted for other confounding variables (including antibiotic received and urine pH at inclusion interaction). Although not statically different, episodes treated with fosfomycin trend to be associated with microbiological failure (*p*=0.07, OR=1.97, 95% CI 0.93-4.16) (Table 39).

When the analysis was restricted to cases treated with fosfomycin, the same results were observed: time since transplant more than one year (*p*=0.04) and older recipients (*p*=0.01) were independently associated to microbiological failure at one month, while acidic urine pH was not.

Table 39. Multivariate analysis of factors associated with microbiological failure at one month of follow-up.

Variables, n (%)	**Adjusted *p*-value**	**Adjusted OR (95% CI)**
Age ≥ 58 years	0.02	2.14 (1.08-4.24)
Time since transplant < 1 year	0.02	0.46 (0.24-0.90)
Glomerular filtration ≤ 78 mL/min	0.45	0.77 (0.40-1.50)
Urine pH ≤ 6	0.79	1.08 (0.57-2.06)
Ciprofloxacin	0.07	0.50 (0.24-1.06)

Table 40. Microbiological failure at six months after ciprofloxacin or fosfomycin therapy, stratified by the initial urine pH.

	Ciprofloxacin	**Fosfomycin**	***p*-value**	**OR (95% CI)**
Microbiological failure in acidic pH -n (%)	14/26 (53.8%)	44/66 (66.6%)	0.84	-
Microbiological failure in non-acidic pH -n (%)	7/21 (33.3%)	34/60 (56.6%)	0.06	2.67 (0.97-7.01)

Table 41. Bivariate analysis of factors associated with microbiological failure after six months of follow-up.

Variables, n (%)	**Microbiological cure (N=84)**	**Microbiological failure (N=89)**	***p*-value**	**OR (95% CI)**
Age ≥ 58 years	43 (51.2)	46 (51.7)	0.94	1.01 (0.74-1.37)
Sex (female)	52 (61.9)	59 (66.3)	0.54	1.21 (0.65-2.25)
Retransplantation	9 (10.7)	9 (10.1)	0.89	0.93 (0.35-2.48)
Time since transplant <2 months	16 (19)	13 (14.6)	0.43	0.72 (0.32-1.62)
Time since transplant <1 year	42 (50)	37 (41.6)	0.26	0.72 (0.39-1.29)
MMF	72 (85.7)	68 (76.4)	0.11	0.54 (0.24-1.18)
M-TOR inhibitor	3 (3.6)	9 (10.1)	0.09	3.03 (0.79-11.63)
Pre-transplant recurrent UTI	10 (15.2) +	18 (23.1) ++	0.23	1.10 (0.94-1.29)
Thymoglobulin induction <3 months	9 (56) +++	2 (28.5) ++++	0.26	0.43 (0.15-1.80)
Antibiotic use < 3 months	41 (48.8)	48 (53.9)	0.50	1.11 (0.819-2.23)
Symptomatic UTI < 6 months	16 (19)	28 (31.5)	0.06	1.18 (0.99-1.40)
Acute rejection previous 6 months	10 (11.9)	9 (10.1)	0.70	0.98 (0.88-1.08)
UTI < 2 months after transplant	40 (48.8) +++++	39 (45.9) ++++++	0.70	0.94 (0.71-1.26)
Cystitis at inclusion	18 (21.4)	12 (13.5)	0.16	0.90 (0.79-1.04)
Hospital-acquired UTI	16 (19)	13 (14.6)	0.43	0.94 (0.82-1.08)
Glomerular filtration ≤ 78 ml/min	45 (53.6)	43 (48.3)	0.48	0.89 (0.66-1.21)
Urine pH ≤ 6	44 (52.4)	48 (53.9)	0.83	1.06 (0.58-1.93)
MDR *E. coli*	6 (7.1)	11 (12.4)	0.24	1.06 (0.96-1.16)
Fosfomycin	58 (69)	68 (76.4)	0.27	1.31 (0.80-2.14)
Microbiological failure at 1 month	21 (25)	34 (38.2)	0.06	1.21 (0.98-1.48)
Urological symptoms (1 month)	7 (8.3)	7 (7.9)	0.91	0.93 (0.31-2.80)

MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), m-TOR inhibitor: inhibitors of the mechanistic target of rapamycin (sirolimus, everolimus), UTI: urinary tract infection; MDR: multidrug resistance. $^{+}$ Data available in 66 episodes $^{++}$ data available in 78 episodes, $^{+++}$ data available in 16 episodes, $^{++++}$ data available in 7 episodes; $^{+++++}$ data available in 82 episodes; $^{++++++}$ data available in 85 episodes.

Neither fosfomycin therapy nor acidic urine pH was associated to microbiological failure at six months of follow-up in the bivariate analysis. However, although not statistically different, in patients with non-acidic urine pH, microbiological failure by sixth month trend to be more frequent in patients treated with fosfomycin (56.6%) than in those with ciprofloxacin (33.3%). On the other hand, patients treated with ciprofloxacin trend to have worse microbiological outcome when the urine pH was acidic than when it was neutral or non-acidic (53.8% *vs.* 33.3%, p=0.06, OR=2.67, 95% CI 1.02-7.01) (Table 40). The bivariate analysis of risk factors of microbiological failure at 6 month is detailed in Table 41.

In the multivariate analysis, treatment received, and urine pH were not associated to microbiological failure. Other relevant detected interactions were tested and excluded due to absence of influence: age $\geq$ 58 years, gender, time since transplant < 2 months, glomerular filtration $\leq$ 78 mL/min, multidrug resistant strains, cytomegalovirus coinfection, use of MMF, cystitis at inclusion and polycystic kidney as baseline disease. Only symptomatic UTI within the previous six months of the episode and to present microbiological failure at one month showed a tendency towards microbiological failure at sixth month of follow-up (Table 44).

Table 44. Multivariate analysis of factors associated with microbiological failure after six months of follow-up (N=173).

Variables, n (%)	Adjusted *p*-value	Adjusted OR (95% CI)
M-TOR inhibitor	0.13	2.83 (0.71-11.23)
Symptomatic UTI < 6 months	0.06	1.96 (0.95-4.03)
Microbiological failure at 1 month	0.08	1.82 (0.93-3.57)
Urine pH ≤ 6	0.71	1.12 (0.60-2.08)
Ciprofloxacin	0.50	0.79 (0.39-1.58)

M-TOR inhibitor: inhibitors of the mechanistic target of rapamycin (sirolimus, everolimus), UTI: urinary tract infection.

When only cases treated with fosfomycin were analysed, no significant differences were found.

6.3.3.2. Clinical outcome

Regarding one-month's clinical outcome, when the episodes treated were stratified according to the urine pH, no difference was found between fosfomycin and ciprofloxacin treated episodes (Table 45). In the bivariate analysis, acidic urine pH was associated to the presence of symptomatic UTI during the first month after inclusion. Other factors associated were symptomatic UTI at diagnosis and multidrug resistant *E. coli* (Table 46).

Table 45. Clinical outcome at one month after fosfomycin or ciprofloxacin therapy, stratified by the initial urine pH.

	Ciprofloxacin	Fosfomycin	*p*-value	OR (95% CI)
Symptomatic UTI in acidic pH -n (%)	3/31 (9.6%)	11/72 (15.2%)	0.54	-
Symptomatic UTI in non acidic pH -n (%)	1/28 (3.5%)	0/66 (0%)	0.29	-

UTI: urinary tract infection.

Table 46. Bivariate analysis of factors associated with symptomatic UTI after one month of follow-up.

Variables, n (%)	Absence of symptomatic UTI (N=181)	Symptomatic UTI (N=16)	*p*-value	OR (95% CI)
Age ≥ 58 years	98 (54.1)	9 (56.9)	0.87	1.04 (0.58-1.86)
Sex (female)	117 (64.6)	12 (75)	0.40	1.64 (0.50-5.29)
Polycystic kidney disease	14 (7.7)	3 (18.8)	0.14	2.75 (0.70-10.81)
Retransplantation	17 (9.4)	3 (18.8)	0.21	2.22 (0.57-8.5)
Time since transplant < 2 months	26 (14.4)	5 (31.1)	0.14	2.7 (0.87-8.43)
MMF	145 (80.1)	13 (81.3)	1	1.07 (0.29-3.97)
M-TOR inhibitor	14 (7.7)	0 (0)	0.61	0.92 (0.88-0.96)
Pre-transplant recurrent UTI	25 (18.1) [+]	1 (8.3) [++]	0.69	0.89 (0.74-1.07)
Thymoglobulin induction < 3 months	9 (45) [+++]	2 (50) [++++]	0.86	1.18 (0.19-7.06)
Antibiotic use < 3 months	92 (50.8)	12 (75)	0.06	1.96 (0.83-4.65)
Symptomatic UTI < 6 months	44 (24.3)	7 (43.8)	0.13	1.34 (0.86-2.08)
Acute rejection previous 6 months	18 (9.9)	2 (12.5)	0.66	1.09 (0.85-1.24)
UTI < 2 months after transplant	75 (43.1) [+++++]	8 (53.3) [++++++]	0.44	1.21 (0.69-2.12)
Cystitis at inclusion	26 (14.4)	6 (37.5)	0.02	3.09 (1.21-7.90)
Hospital-acquired UTI	27 (14.9)	3 (18.8)	0.71	1.04 (0.82-1.33)
Glomerular filtration ≤ 78 ml/min	93 (51.4)	6 (37.5)	0.28	0.77 (0.51-1.17)
Urine pH ≤ 6	88 (48.6)	15 (93.8)	0.01	8.22 (1.22-55.13)

MDR *E. coli*	14 (7.7)	4 (25)	0.04	1.23 (1.19-9.21)
- Ciprofloxacin resistant *E. coli*	46 (25.4)	7 (43.8)	0.24	2.11 (0.82-5.38)
- Cephalosporin resistant *E. coli*	11 (6.1)	2 (12.5)	0.28	1.07 (0.88-1.29)
Fosfomycin	127 (70.2)	11 (68.8)	1	0.94 (0.34-2.58)

MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), m-TOR inhibitor: inhibitors of the mechanistic target of rapamycin (sirolimus, everolimus), UTI: urinary tract infection, MDR: multidrug resistance. $^{+}$ Data available in 155 episodes $^{++}$ data available in 12 episodes, $^{+++}$ data available in 20 episodes, $^{++++}$ data available in 4 episodes; $^{+++++}$ data available in 174 episodes, $^{++++++}$ data available in 15 episodes.

Fosfomycin therapy was not associated with symptomatic UTI at one month when adjusted for other possible confounding variables. Acidic urine pH and cystitis at inclusion were independently associated with symptomatic UTI at one month of follow-up in the regression logistic analyses performed. The interaction of the antibiotic received and urine pH at inclusion was also explored with no influence in this outcome (Tables 47).

Tables 47. Multiple logistic regression analyses of factors associated with symptomatic UTI after one month of follow-up.

	***p*-value**	**OR**	**95% CI**
Ciprofloxacin	0.83	0.88	0.27-2.84
Acidic pH	<0.01	15.23	1.96-118.28
Antibiotic use < 3 months	0.10	2.74	0.81-9.23

	***p*-value**	**OR**	**95% CI**
Ciprofloxacin	0.97	1.01	0.31-3.27
Acidic pH	<0.01	16.02	2.05-125.07
Cystitis at inclusion	0.02	3.65	1.14-11.61

	***p*-value**	**OR**	**95% CI**
Ciprofloxacin	0.74	1.21	0.37-3.89
Acidic pH	0.01	14.49	1.86-112.72
MDR *E. coli*	0.09	3.09	0.81-11.65

MDR: Multidrug resistant.

When only cases treated with fosfomycin were analysed, antibiotic use within the previous 3 months *p*=0.03, OR=5.66, 95% CI 1.19-28.44), acidic urine pH (*p*=0.02, OR=10.32, 95% CI 1.27-83.86), and multidrug resistant strains (*p*=0.04, OR=4.40, 95% CI 1.02-18.96) showed independent associations with the presence of symptomatic UTI within one month of follow-up.

Neither fosfomycin use nor acidic urine pH were associated to symptomatic UTI at six months of follow-up. When the episodes treated were stratified according to the urine pH, it was observed that in episodes with acidic pH, the presence of symptomatic UTI during the 6 months did no differ according to the antibiotic used, while in those with non-acidic pH, patients receiving ciprofloxacin had less frequently UTI symptoms during 6 months of follow-up. But, when comparing ciprofloxacin efficacy, there was a trend to present more symptomatic UTI in acidic pH when compared to non-acidic pH (*p*=0.07, OR=1.78, 95% CI 1-17-2.69) (Table 48).

Table 48. Clinical outcome at six months after fosfomycin or ciprofloxacin therapy, stratified by the initial urine pH.

	Ciprofloxacin	**Fosfomycin**	***p*-value**	**OR (95% CI)**
Symptomatic UTI in acidic pH –n (%)	6/31 (19.4%)	17/72 (23.6%)	0.63	-
Symptomatic UTI in non acidic pH -n (%)	1/28 (3.6%)	13/66 (19.7%)	0.05	4.72 (0.69-32.01)

Table 49. Bivariate analysis of factors associated with symptomatic UTI after six months of follow-up.

Variables, n (%)	**Absence of symptomatic UTI (N=160)**	**Symptomatic UTI (N=37)**	***p-value***	**OR (95% CI)**
Age ≥ 58 years	90 (56.3)	17 (45.9)	025	0.80 (0.57-1.14)
Sex (female)	102 (63.8)	27 (73)	0.28	1.53 (0.76-2.36)
Retransplantation	12 (7.5)	8 (21.6)	0.02	3.40 (1.27-9.05)
Time since transplant <2 months	23 (14.4)	8 (21.6)	0.27	1.64 (0.66-4.03)
MMF	126 (78.8)	32 (86.5)	0.28	1.72 (0.65-4.76)
M-TOR inhibitors	10 (6.3)	4 (10.8)	0.30	1.81 (0.53-6.15)
Pre-transplant recurrent UTI	19 (14.3) $^{+}$	10 (29.4) $^{++}$	0.03	1.21 (0.96-1.52)
Thymoglobulin induction <3 months	8 (40) $^{+++}$	3 (75) $^{++++}$	0.26	3.54 (0.42-29.41)
Antibiotic use < 3 months	78 (48.8)	26 (70.3)	0.01	1.72 (1.02-2.89)
Symptomatic UTI < 6 months	37 (23.1)	14 (37.8)	0.06	1.23 (0.94-1.61)
Acute rejection previous 6 months	14 (8.8)	6 (16.2)	0.22	1.08 (0.93-1.26)
UTI < 2 months after transplant	67 (44.1) $^{+++++}$	16 (43.2)	0.92	0.98 (0.71-1.35)
Cystitis at inclusion	25 (15.6)	7 (18.9)	0.62	1.04 (0.87-1.23)
Hospital-acquired UTI	19 (11.9)	11 (29.7)	<0.01	1.25 (1.00-1.55)
Glomerular filtration ≤ 78 ml/min	83 (51.9)	16 (43.2)	0.34	0.84 (0.61-1.17)
Urine pH ≤ 6	80 (50)	23 (62.2)	0.18	1.32 (0.85-2.05)
MDR *E. coli*	12 (7.5)	6 (16.2)	0.11	1.10 (0.95-1.28)
Fosfomycin therapy	108 (67.5)	30 (81.1)	0.10	1.71 (0.85-3.47)
Microbiological failure at 1 month	47 (29.4)	14 (37.8)	0.31	1.13 (0.86-1.48)
Urological symptoms at 1 month	11 (6.9)	5 (13.5)	0.18	2.11 (0.68-6.51)

MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), m-TOR inhibitor: inhibitors of the mechanistic target of rapamycin (sirolimus, everolimus), UTI: urinary tract

infection, MDR: multidrug resistance. [+] Data available in 133 episodes [++] data available in 43 episodes, [+++] data available in 20 episodes, [++++] data available in 4 episodes; [+++++] data available in 152 episodes.

Other factors associated with symptomatic UTI at six months of follow-up were: retransplantation (21.6% *vs*. 7.5%), recurrent UTI before transplant (29.4% *vs*. 14.3%), antibiotic use in the previous three months (70.3% *vs*. 48.8%), more hospital-acquired events (29.7% *vs.* 11.9%) (Table 49).

Neither urine pH nor fosfomycin were associated with symptomatic UTI at six months of follow-up when adjusted for other possible confounding variables. Retransplantation and hospital-acquired events were independent risks for this outcome. The interaction of the antibiotic received and urine pH at inclusion was also explored with no influence in this outcome.

Table 50. Multivariate analysis of factors associated with symptomatic UTI after six months of follow-up.

Variables, n (%)	**Adjusted *p*-value**	**Adjusted OR (95% CI)**
Retransplantation	0.04	2.95 (1.05-8.27)
Symptomatic UTI < 6 months	0.07	2.06 (0.93-4.59)
Hospital-acquired UTI	0.03	2.68 (1.07-6.69)
Urine pH ≤ 6	0.33	1.46 (0.68-3.14)
Ciprofloxacin therapy	0.31	0.61 (0.24-1.56)

UTI: urinary tract infection.

Other relevant detected interactions were tested and excluded due to absence of influence in the multivariate analysis: time since transplant < 2 months, age ≥ 58 years, gender, glomerular filtration ≤ 78 mL/min, pre-transplant recurrent UTI,

antibiotic use < 3 months, cystitis at inclusion, cytomegalovirus coinfection, use of MMF, use of m-TOR inhibitor, and polycystic kidney as baseline disease (Table 50).

Table 51. Bivariate analysis of factors associated to the development of acute pyelonephritis during six months of follow-up.

Variables, n (%)	**No APN at six months (N=188)**	**APN at six months (N=9)**	***p*-value**	**OR (95% CI)**
Age ≥ 58 years	103 (54.8)	4 (54.8)	0.73	0.81 (0.44-1.49)
Sex (female)	124 (66)	5 (55.6)	0.49	0.64 (0.16-2.48)
Retransplantation	19 (10.1)	1 (11.1)	1	1.11 (0.13-9.37)
Time since transplant < 2 months	30 (16)	1 (11.1)	1	0.65 (0.79-5.45)
Polycystic kidney disease	15 (8)	2 (22.2)	0.17	3.29 (0.62-17.29
Symptomatic UTI < 6 months	50 (26.6)	1 (11.1)	0.45	0.82 (0.64-1.05)
Acute rejection previous 6 months	19 (10.1)	1 (11.1)	1	1.01 (0.79-1.28)
Cystitis at inclusion	30 (16)	2 (22.2)	0.64	1.08 (0.75-1.54)
Hospital-acquired UTI	28 (14.9)	2 (22.2)	0.62	1.09 (0.76-1.55)
Glomerular filtration ≤ 78 mL/min	95 (50.5)	4 (44.4)	0.74	0.89 (0.48-1.62)
Urine pH ≤ 6	95 (50.5)	8 (88.9)	0.03	4.45 (0.69-28.41)
MDR *E. coli*	17 (9)	1 (11.1)	0.58	1.02 (0.80-1.29)
Fosfomycin therapy	132 (70.2)	6 (66.7)	1	0.89 (0.34-2.31)
Microbiological failure at 1 month	58 (30.9)	3 (33.3)	1	1.03 (0.64-1.66)
Urological symptoms at 1 month	12 (6.4)	4 (44.4)	<0.01	11.73 (2.78-49.47)

APN: pyelonephritis, UTI: urinary tract infection, MDR: multidrug resistant.

When only cases treated with fosfomycin were analysed, retransplantation was the only variable associated to the presence of symptomatic UTI during the six months of follow-up (p<0.01, OR=5.12, 95% CI 1.59-16.40).

Factors associated to pyelonephritis during follow-up in the bivariate analysis were: acidic urine (88.9% *vs.* 50.5%) and symptomatic UTI one-month of follow-up (Table 51).

Regarding relapses and persistant events during the follow-up, 51 cases of new resistance were observed (25.9%): 24 to fosfomycin (12.1%) and 30 (15.2%) to ciprofloxacin (three cases to both antibiotics).

6.4. Objective 4: To study the prevalence, phenotypic characteristics and clinical impact and outcomes of *E. coli* strains with LLQR and/or LLFR, treated with ciprofloxacin and fosfomycin, according to the urine pH.

6.4.1. Baseline, clinical characteristics and prevalence of LLQR and LLFR strains in lower UTI caused by *E. coli* in KTR

From the 157 episodes included in cohort B (*E. coli* bacteriuria treated with ciprofloxacin and fosfomycin), 38 (24.2%) were discarded because follow-up urine samples were lacking. Therefore, 119 episodes in 119 KTR are included in the present analysis: 92 (77.3%) treated with fosfomycin and 27 (22.7%) with ciprofloxacin. To assure that this subpopulation was like the original one, bivariate analyses including baseline and outcome variables were performed. No differences were observed between both cohorts.

According to the research laboratory MIC determinations, four episodes (14.8%), were treated with ciprofloxacin being initially resistant to it, and eleven (12%) with fosfomycin being initially resistant to it.

So on, globally, and according to research MIC determinations and the current EUCAST breakpoints (>0.5 µg/mL for ciprofloxacin, and for >8 µg/mL fosfomycin), at inclusion 44 (37%) were resistant cases; 35 episodes were resistant to ciprofloxacin (29.4%) and 14 episodes were (11.8%) resistant to fosfomycin. Five events (4.2%) were resistant to both antibiotics. Low-level resistance was detected in 24 episodes (20.2%): 20 (16.8%) were LLQR (range 0.06-0.5 µg/mL), and six (5%) LLFR (range 2-8 µg/mL). In two episodes, LLQR and LLFR, were both detected. Fifty-seven events (47.9%) were fully-susceptible to both antibiotics (excluding LLR strains). There were six events which present a resistance and a low-level resistance strain at the same time: two ciprofloxacin resistant strains with LLFR and four fosfomycin

resistant strains with LLQR, which were considered resistant for bivariate analyses, but LLR (as well) for descriptive purposes (Table 52).

Among patients treated with ciprofloxacin, four were resistant (14.8%) to ciprofloxacin, three (11.1%) were resistant to fosfomycin, seven (25.9%) were LLQR, one (3.7%) were LLFR and 14 (51.9%) were fully susceptible to ciprofloxacin/fosfomycin (Table 53). From them, one LLR strain was both LLFR and LLQR strains, and one fosfomycin resistant strain was also LLQR strain.

In patients treated with fosfomycin, 11 were resistant to fosfomycin (12%), 31 were resistant to ciprofloxacin (33.7%), five (5.4%) were LLFR, 13 (14.1%) were LLQR and 43 (46.7%) were fully susceptible to ciprofloxacin/fosfomycin (Table 54). From them, five LLR strains were also resistant strains: three LLQR were fosfomycin resistant and two LLFR were ciprofloxacin resistant, one LLR strain was both LLFR and LLQR strains, and five were resistant to both antibiotics.

Risk factors of low-level resistant strains, when compared to fully-susceptible strains, were younger age (p<0.01, OR=0.24, 95% CI 0.08-0.70), use of antibiotic within the previous 3 months (p<0.01, OR=3.41, 95% CI 1.93-6.05), specifically more in LLFR than in LLQR strains (5/5 (100%) *vs.* 5/19 (26.3%), p<0.01). There was also a trend to have more previous kidney transplantation in the LLR group (p=0.06, OR=1.15, 95% CI 0.96-1.39) (Table 52).

Resistant strains, when compared to fully-susceptible strains, occurred more frequently in recent transplant (<2 months) (p=0.03, OR=1.77, 95% CI 1.15-2.70), and with antibiotic used within the previous 3 months (p<0.01, OR=3, 95% CI 2.02-4.45).

Low-level resistant strains, when compared to resistant strains, presented less symptomatic UTI within 6 months before inclusion (p=0.04, OR=0.18, 95% CI 0.27-1.33).

Table 52. Baseline characteristics, resistances' patterns, and description of LLR strains, resistant strains and fully-susceptible strains in *E. coli* bacteriurias' cohort with confirmed MICs.

Variables, n (%)	**Global (N=119)**	**Fully S strains (N=57)**	**LLR strains (N=24)**	**R strains (N=44)**
Age ≥ 58 years	56 (47.1)	33 (57.9) *	6 (25) *	19 (43.2)
Sex (female)	75 (63)	32 (56.1)	18 (75)	29 (65.9)
Retransplantation	10 (8.4)	2 (3.5) *	4 (16.7) *	5 (11.4)
Time since transplant < 2 months	18 (15.1)	5 (8.8) **	3 (12.5)	11 (25) **
Time since transplant < 1 years	55 (46.2)	28 (49.1)	9 (37.5)	20 (45.5)
Underlying end-stage renal disease:				
- Glomerulonephritis	26 (21.8)	13 (22.8)	7 (29.2)	7 (15.9)
- Tubulointerstitial	21 (17.6)	8 (14)	4 (16.7)	9 (20.5)
- Hypertension/ renovascular	11 (9.2)	8 (14)	2 (8.3)	4 (9.1)
- Polycystic kidney disease	11 (9.2)	5 (8.8)	1 (4.2)	5 (11.4)
- Diabetic nephropathy	11 (9.2)	9 (15.8)	0 (0)	2 (4.5)
- Tumoral	4 (3.4)	1 (1.8)	2 (8.3)	1 (2.3)
- Vesicoureteral reflux	1 (1.1)	0 (0)	0 (0)	1 (2.3)
- Mix or unknown aetiology	34 (28.6)	14 (24.6)	8 (33.3)	15 (34.1)
Living donor	12 (10.1)	6 (10.5)	2 (8.3)	4 (9.1)
Induction therapy within 3 previous months:	16 (16.5) [1]	6 (12.7) [2]	2 (11.8) [3]	9 (23.7) [4]
- Thymoglobulin	7 (6)	3 (12.7)	1 (5.9)	4 (10.5)
- Basiliximab	9 (7.5)	3 (12.7)	1 (5.9)	5 (13.1)

Current immunosuppression: - Tacrolimus - Corticosteroids - MMF - M-TOR inhibitors - Cyclosporine	 110 (92.4) 110 (92.4) 94 (79) 8 (6.7) 4 (3.3)	 52 (91.2) 53 (93) 48 (84.2) 2 (3.5) 1 (1.7)	 22 (91.7) 21 (87.5) 18 (75) 2 (8.3) 2 (8.4)	 41 (95.5) 41 (93.2) 34 (77.3) 4 (9.1) 1 (2.3)
Pre-transplant recurrent UTI	19 (20.7) [5]	9 (18.8) [6]	6 (35.3) [7]	5 (16.1) [8]
Antibiotic use within the previous 3 months	54 (45.4)	4 (7) */*	10 (41.7) *	23 (52.2) **
Symptomatic UTI within the previous 6 months	20 (16.8)	13 (22.8)	3 (12.5) ***	13 (29.5) ***
Acute rejection within the previous 6 months	13 (10.9)	8 (14)	2 (8.3)	3 (6.8)
UTI <2 months after transplant	51 (44) [9]	23 (41.8) [10]	8 (33.3) [11]	21 (48.8) [12]
Cystitis at inclusion	20 (16.8)	12 (21.1)	4 (16.7)	4 (9.1)
Hospital-acquired UTI	24 (20.2)	12 (21.1)	5 (20.8)	9 (20.5)
Glomerular filtration ≤ 78 mL/min	60 (50.4)	31 (54.4)	8 (33.3) ***	24 (54.5) ***
Acidic urinary pH	61 (51.3)	28 (49.1)	12 (50)	25 (56.8)
Fosfomycin therapy	92 (77.3)	43 (75.4)	17 (70.8)	37 (84.1)
Baseline *E. coli* antibiotic resistance: - Cotrimoxazole - Ciprofloxacin -Amoxicillin-clavulanate - Fosfomycin - Cephalosporins - ESBL-production -Carbapenem-resistance	 69 (58) 35 (29.4) 25 (21) 14 (11.7) 2 (1.7) 2 (1.7) 0 (0)	 30 (52.6) 0 (0) 9 (15.8) 0 (0) 1 (1.8) 1 (1.8) 0 (0)	 13 (54.2) 2 (8.3) 7 (29.2) 4 (16.7) 1 (4.2) 1 (4.2) 0 (0)	 29 (65.9) 35 (79.5) 10 (22.7) 14 (31.8) 0 (0) 0 (0) 0 (0)
MDR strain	12 (10.1)	4 (7)	2 (8.3)	6 (13.6)

Fully S: sensible strains excluding low-level resistances, LLR: low-level resistance strains, R: resistant strains, MMF: mycophenolate mofetil (or equivalent MPA: mycophenolic acid), m-

TOR inhibitor: inhibitors of rapamycin (sirolimus, everolimus), UTI: urinary tract infection, ESBL: extended spectrum beta-lactamases-producers, MDR: multidrug resistance. [1] Data available in 97 episodes, [2] data available in 47 episodes, [3] data available in 17 episodes, [4] data available in 38 episodes, [5] data available in 92 episodes, [6] data available in 48 episodes, [7] data available in 17 episodes, [8] data available in 31 episodes, [9] data available in 116 episodes, [10] data available in 55 episodes, [11] data available in 24 episodes, [12] data available in 43 episodes. * Significant *p*-value among LLR strains and fully-susceptible strains, ** significant *p*-value among fully-susceptible strains and resistant strains, *** significant *p*-value among LLR strains and resistant strains.

When sub-analyzing the resistant group, there were more previous symptomatic UTI in the baseline ciprofloxacin resistant group (12/35 (34.3%) *vs.* 1/14 (7.1%), *p*=0.05, OR=1.14, 95% CI 1.08-1.93) when compared with the baseline fosfomycin group. There was also a trend to show better kidney function in the LLR group (5/18 (27.8%) *vs.* 24/44 (54.5%), *p*=0.05, OR=0.50, 95% CI 0.23-1.12) (Table 52).

No baseline differences were found among the diverse strains according to the treatment received.

6.4.2. Microbiological and clinical outcomes of LLR UTIs treated with ciprofloxacin and fosfomycin, according to the urine pH

Globally, there were no differences among microbiological and clinical outcomes in fully susceptible, LLR and resistant strains.

When exploring secondary outcomes, a higher rate of relapses during follow-up was observed when presenting initial resistance to ciprofloxacin/fosfomycin compared to fully-susceptible strains (21/44 (47.7%) *vs.* 11/57 (19.3%), *p*<0.01, OR=1.96, 95% CI 1.29-2.98). A trend to more six-months´microbiological failure was also observed (28/44 (63.6%) *vs.* 24/55 (43.6%), *p*=0.05, OR=1.58, 95% CI 0.98-2.53).

6.4.2.1. Ciprofloxacin therapy

One-month's microbiological cure and six-month's microbiological failure were similar in patients treated with ciprofloxacin with LLR (LLQR/LLFR as well) and fully susceptible *E.coli* UTI.

Table 53. Microbiological and clinical outcomes of episodes treated with ciprofloxacin according to susceptibilities.

Variables, n (%)	**Fully S (N=14)[1]**	**LLR (N=7)[2]**	**LLQR (N=7)[3]**	**LLFR (N=1)[4]**	**CIP R (N=4)[5]**	**FOS R (N=3)[6]**
One-month´s:						
- Microbiological failure	4 (28.6)	3 (42.9)	3 (42.9)	1 (100)	0 (0)	0 (0)
- Acidic pH:	2 (50)	2 (40)	2 (40)	0 (0)	-	-
- Non-acidic pH:	2 (20)	1 (50)	1 (50)	1 (100)	-	-
- Symptomatic UTI	1 (7.1)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Six-months':						
- Microbiological failure	5 (35.7)	4 (57.1)	4 (57.1)	1 (100)	2 (50)	0 (0)
- Acidic pH:	2 (50)	3 (60)	3 (60)	0 (0)	2 (100)	-
- Non-acidic pH:	3 (30)	1 (50)	1 (50)	1 (100)	0 (0)	-
- Symptomatic UTI	0 (0)	1 (14.3)	1 (14.3)	0 (0)	0 (0)	0 (0)
- Pyelonephritis	0 (0)	1 (14.3)	1 (14.3)	0 (0)	0 (0)	0 (0)
- Graft rejection	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
- Graft dysfunction	0 (0)	2 (28.6)	2 (28.6)	0 (0)	0 (0)	0 (0)
- Acidic pH:	-	2 (40)	2 (40)	-	-	-
- Non-acidic pH:	-	0 (0)	0 (0)	-	-	-
- Graft loss	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
- Mortality	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
- UTI relapses	5 (35.7)	2 (28.6)	2 (28.6)	0 (0)	1 (25)	0 (0)
- Acidic pH:	2 (50)	2 (40)	2 (40)	-	1 (50)	-
- Non-acidic pH:	3 (30)	0 (0)	0 (0)	-	0 (0)	-
- UTI re-infections	3 (21.4)	2 (42.9)	3 (42.9)	1 (100)	1 (25)	0 (0)
- Acidic pH:	1 (25)	1 (20)	2 (20)	0 (0)	1 (50)	-
- Non-acidic pH:	2 (20)	1 (50)	1 (50)	1 (100)	0 (0)	-

Fully S: fully-susceptible strain to ciprofloxacin/fosfomycin (does not includes LLRs), LLR: low-level resistance strain, LLQR: low-level ciprofloxacin resistance strain, LLFR: low-level fosfomycin resistance strain, CIP R: ciprofloxacin resistant strain, FOS R: fosfomycin resistant strain, UTI: urinary tract infection. Columns data do not sum the total amount of cases because one LLR strain is both LLFR and LLQR strain, and one fosfomycin resistant strain is also a LLQR strain. [1] Fully S: acidic pH n=4// non-acidic n=10; [2] LLR: acidic pH n=5// non-acidic pH n=2; [3] LLQR: acidic pH n=5// non-acidic pH n=2; [4] LLFR: acidic pH n=0// non-acidic n=1; [5] CIP R: acidic pH n=2// non-acidic pH n=0; [6] FOS R: acidic pH n=2// non-acidic pH n=2.

Regarding clinical outcomes, LLQR/LLFR infections treated with ciprofloxacin were not associated with the presence of more symptomatic UTI at one month and six months of follow-up.

No relevant differences were found among secondary outcomes (Table 53).

6.4.2.2. Fosfomycin therapy

In patients treated with fosfomycin when compared to fully-susceptible strains, baseline ciprofloxacin-resistance was associated to worse microbiological outcomes: more one-month's microbiological failure (p=0.03, OR=1.82, 95% CI 1.09-3.03), six-month's microbiological failure (p=0.02, OR=1.96, 95% CI 1.05-3.67), and relapses (p<0.01, OR=2.69, 95% CI 1.62-4.47) (Table 54).

Fosfomycin resistant strains treated with fosfomycin, compared to fully sensible strains, were associated with more relapses during follow-up (p=0.03, OR=3.2, 95% CI 1.21-8.72).

One-month's and six-months' microbiological and clinical outcomes were similar in patients treated with fosfomycin with LLR strains compared to those fully-susceptible strains.

No differences could be found when compared LLR strains (LLQR and LLFR) with resistant strains (Table 54).

Table 54. Microbiological and clinical outcomes of episodes treated with fosfomycin according to susceptibilities.

Variables, n (%)	**Fully S (N=43)[1]**	**LLR (N=17)[2]**	**LLQR (N=13)[3]**	**LLFR (N=5)[4]**	**CIP R (N=31)[5]**	**FOS R (N=11)[6]**
One-month´s:						
- Microbiological failure	9 (20.9)	5 (29.4)	4 (30.8)	1 (20)	14 (45.2)	5 (45.5)
- Acidic pH	5 (20.8)	1 (14.2)	0 (0)	1 (33.3)	8 (50)	3 (33.3)
- Non-acidic pH	4 (21)	4 (40)	4 (44.4)	0 (0)	6 (40)	2 (100)
- Symptomatic UTI	4 (9.3)	0 (0)	0 (0)	0 (0)	2 (6.5)	3 (27.3)
- Acidic pH	4 (16.6)	-	-	-	2 (12.5)	3 (33.3)
- Non-acidic pH	0 (0)	-	-	-	0 (0)	0 (0)
Six-months':						
- Microbiological failure	19 (46.3)[+]	8 (47.1)	6 (46.2)	2 (40)	22 (71)	7 (63.6)
- Acidic pH	9 (37.5)	2 (28.5)	1 (25)	1(33.3)	10 (62.5)	5 (55.5)
- Non-acidic pH	10 (52.6)	6 (60)	5 (55.5)	1 (50)	12 (80)	2 (100)
- Symptomatic UTI	8 (18.6)	3 (17.6)	2 (15.4)	1 (20)	8 (25.8)	3 (27.3)
- Acidic pH	5 (20.8)	2 (28.5)	1 (25)	1 (33.3)	3 (18.7)	3 (33.3)
- Non-acidic pH	3 (15.8)	1 (10)	1 (11.1)	0 (0)	5 (33.3)	0 (0)
- Pyelonephritis	2 (4.7)	2 (11.8)	1 (7.7)	1 (20)	1 (3.2)	2 (18.2)
- Acidic pH	1 (4.1)	1 (14.2)	0 (0)	1 (33.3)	1 (6.2)	1 (11.1)
- Non-acidic pH	1 (5.2)	1 (10)	1 (11.1)	0 (0)	0 (0)	1 (50)
- Graft rejection	3 (7)	1 (5.9)	0 (0)	1 (20)	4 (12.9)	0 (0)
- Acidic pH	1 (4.1)	0 (0)	-	-	1 (6.2)	-
- Non-acidic pH	2 (10.5)	1 (10)	-	1 (50)	3 (20)	-
- Graft dysfunction	6 (14)	2 (11.8)	0 (0)	2 (40)	7 (22.6)	2 (18.2)
- Acidic pH	3 (12.5)	1 (14.2)	-	1 (33.3)	4 (25)	2 (22.2)
- Non-acidic pH	3 (15.7)	1 (10)	-	1 (50)	3 (20)	0 (0)

- Graft loss	0 (0)	1 (5.9)	1 (7.7)	1 (20)	0 (0)	0 (0)
- Mortality	1 (2.3)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
- UTI relapses	6 (14)	6 (35.3)	4 (30.8)	2 (40)	17 (54.8)	5 (45.5)
- Acidic pH	3 (12.5)	2 (28.5)	1 (11.1)	1 (33.3)	7 (44.7)	3 (33.3)
- Non-acidic pH	3 (15.7)	4 (40)	3 (33.3)	1 (50)	10 (66.6)	2 (100)
- UTI re-infections	8 (18.6)	1 (5.9)	0 (0)	1 (20)	5 (14.1)	0 (0)
- Acidic pH	5 (20.8)	1 (14.2)	-	1 (33.3)	3 (18.7)	-
- Non-acidic pH	3 (15.8)	0 (0)	-	0 (0)	2 (13.3)	-

Fully S: fully-susceptible strain to ciprofloxacin/fosfomycin (does not includes LLRs), LLR: low-level resistance strain, LLQR: low-level ciprofloxacin resistance strain, LLFR: low-level fosfomycin resistance strain, CIP R: ciprofloxacin resistant strain, FOS R: fosfomycin resistant strain, UTI: urinary tract infection. [+] Data available in 41 episodes: 23 with acidic urine and 28 with non-acidic urine. * Significant *p*-value. Columns data do not sum the total amount of cases because five LLR are also resistant strains: three LLQR are fosfomycin resistant and two LLFR are ciprofloxacin resistant strains, one LLR strain is both LLFR and LLQR, and five are resistant to both antibiotics. [1] Fully S: acidic pH n=24// non-acidic n=19; [2] LLR: acidic pH n=7// non-acidic pH n=10; [3] LLQR: acidic pH n=4// non-acidic pH n=9; [4] LLFR: acidic pH n=3// non-acidic n=2; [5] CIP R: acidic pH n=16// non-acidic pH n=15; [6] FOS R: acidic pH n=9// non-acidic pH n=2.

6.4.2.3. Impact of urine pH on ciprofloxacin and fosfomycin efficacy in LLR strains

In acidic urine, the primary and secondary outcomes were not significantly different when stratifying by the susceptibility of strains. The same was observed with non-acidic urine. Episodes caused by LLR strains had similar outcomes regardless of the urine pH.

It must be noted that among full fosfomycin resistant episodes treated with fosfomycin, one-month microbiological cure was observed in 66% of those with acidic urine pH and none of the two that had non-acidic pH.

When the primary outcomes were analysed under the effect of urine pH, antibiotic received and the presence of LLR strains (compared to resistant strains) through logistic regression models, no interactions could be detected.

The urine pH did neither affect the clinical and microbiological efficacy of ciprofloxacin in LLQR infections nor the clinical and microbiological efficacy of fosfomycin in LLFR infections.

6.4.3. Risk of antimicrobial resistance after ciprofloxacin and fosfomycin therapy. Impact of LLR.

At 14 days of follow-up, 27 episodes had persistent *E. coli* infection, of whom 24 had been treated with fosfomycin (26% of the total treated with fosfomycin) and three with ciprofloxacin (11.1% of those treated with ciprofloxacin) (Table 55).

None of the persistant episodes previously treated with ciprofloxacin developed new resistance to fosfomycin or ciprofloxacin, none of them were LLQR at baseline. Five patients (20%) treated with fosfomycin developed fosfomycin resistance, being one initially LLQR and 2 (8.3%) developed LLFR.

Seven out of 11 resistant strains isolated after fosfomycin therapy were sequenced, of 5 developed resistance to ciprofloxacin (5/7, 71%) and 5/7 (71%) to fosfomycin after 14 days follow-up. We sequenced these isolates to confirm the ST of these strains and that the isolate recovered at the follow-up (14 days) was the same as the one analysed at the basal episode. In the ciprofloxacin analysis, 2 (40%) of the isolates sequenced at both time points had the same ST (Pasteur scheme), considering them the same clone and therefore persistence of the infection by the same strain. When analysing the development of resistance to fosfomycin, 3 (60%) of the isolates sequenced at

both time points had the same ST (Pasteur scheme) and developed full fosfomycin resistance, and one (20%) LLFR (Table 56).

Table 55. Baseline and 14 days resistance pattern after ciprofloxacin and fosfomycin therapy.

Variables, n (%)	**Ciprofloxacin therapy**		**Fosfomycin therapy**	
	At inclusion (N=27)	At 14 days (N=3)	At inclusion (N=92)	At 14 days (N=24)
Ciprofloxacin:				
- Fully-susceptible	16 (59.3)	1 (33.3)	48 (52.2)	6 (25)
- LLQR	7 (25.9)	1 (33.3)	13 (14.1)	1 (4.2)
-- New LLQR	-	0 (0)	-	0 (0)
- Resistant	4 (14.8)	1 (33.3)	31 (33.7)	17 (70.8)
-- New resistant	-	0 (0)	-	6 (25)
Fosfomycin:				
- Fully-susceptible	23 (85.2)	3 (100)	76 (82.6)	16 (66.7)
- LLFR	1 (3.7)	0 (0)	5 (5.4)	2 (8.3)
-- New LLFR	-	-	-	2 (8.3)
- Resistant	3 (11.1)	0 (0)	11 (12)	6 (25)
-- New resistant	-	-	-	5 (20.8)

LLQR: low level ciprofloxacin resistance, and LLFR: low level fosfomycin resistance.
Note: Susceptible strains do not include LLR strains. New resistant and LLR strains are included in resistant and LLR strains at 14 days' rows.

No association between basal urine pH and the persistence of clonal strains were observed.

The 15 events treated with the antibiotic that were initially resistant had a favourable outcome (Tables 53 and 54), and no further resistances or LLR strains developed

rather than one ciprofloxacin-resistant strain after receiving fosfomycin, when initially resistant to it.

Table 56. Development of resistance to ciprofloxacin and/or fosfomycin after fosfomycin therapy.

Episodes	**Resistance to ciprofloxacin**		**Resistance to fosfomycin**	
	Basal	14-day follow-up	Basal	14-day follow-up
A	**ST3**	**ST3**	**ST3**	**ST3**
B	ST48	ST43	ST48	ST43
C	ST74	ST355		
D (LLFR)	**ST Nearest 518**	**ST Nearest 518**	**ST Nearest 518**	**ST Nearest 518**
E	ST7	ST649		
F			**ST7**	**ST7**
G			**ST3**	**ST3**

ST: sequencetype (Pasteur scheme). Episode D developed a LLFR strain and resistance to ciprofloxacin.

Globally, there was no association between the presence of LLR at diagnosis and the development of full resistance during follow-up. From the 24 baseline LLQR or LLFR *E. coli* infections, two (8.3%) developed new resistances: one initial LLFR treated with fosfomycin which acquired ciprofloxacin resistance and had LLFR clearance, and one initial LLQR treated with fosfomycin which acquired fosfomycin resistance and had LLQR clearance (Table 55).

7. GENERAL DISCUSION

7.1. Objective 1: To determine the impact of antimicrobial therapy in the outcome of asymptomatic bacteriuria in a cohort of KTR.

The objective 1 study shows that antimicrobial resistance is a major issue in kidney recipients with a UTI and that treating asymptomatic bacteriuria in kidney recipients diminishes the microbiological cure and increases the rates of microbiologic relapses and re-infections. In addition, treated asymptomatic bacteriuria patients were at higher risk of developing symptomatic UTIs in the following six months (including pyelonephritis events).

To our knowledge, this is the largest study to examine the epidemiology, clinical manifestation and impact of antimicrobial therapy on non-complicated UTI in kidney recipients, prospectively (299), as the BIRT trial addresses only the impact of antibiotics versus no therapy in KTR with 199 asymptomatic bacteriurias (374), and as Brune *et al.* retrospective multi-centre cohort does not focus on the impact of treating 241 out of the 1067 asymptomatic bacteriuria cases, but the impact of recurrent UTI (415).

The most common etiology of non-complicated UTI was *E. coli*, as described for the general population (297,416); however, the spectrum of etiologies was more diverse tan in non-immunocompromised hosts, with a higher frequency of *Enterococcus* spp. and *P. aeruginosa* infections (253,254). The high proportion of antimicrobial resistance found must also be highlighted; it is in the range of the proportion described in other studies of kidney recipients (44-77%) (253,302,417), and clearly higher than the incidence described in the general population (18-25%) (416,418).

One-third of the episodes included occurred during the first year after the transplant. It has been described that most episodes of bacteriuria occur early after transplantation (297). Several reasons have been hypothesized to explain these findings, including immunological net status or urinary instrumentation. The close follow-up of new kidney recipients could also have contributed to this finding.

No differences were found regarding the etiology and antimicrobial susceptibility of bacteriuria according to the presence of symptoms; however, in patients with bacteriuria, the presence of nitrites was associated with urinary symptoms. This finding has not been previously described in kidney recipients; however, a higher sensitivity of urinary nitrites at diagnosing cystitis when screening for bacteriuria in pregnant women, rather than asymptomatic bacteriuria, has been reported (419). Hepatitis C virus coinfection also occurred more frequently in patients with cystitis. In the RESITRA cohort, an association is reported between hepatitis C virus serostatus and receiving thymoglobulin or experiencing an upper UTI, which are, at the same time, risk factors for developing a symptomatic UTI in our cohort (420).

Treating asymptomatic bacteriuria did not improve one-month and six-month microbiological outcomes. It did not have any impact on the survival of either patients or grafts. This is in accordance with what has been previously described: the persistence of bacteriuria, relapse, or re-infection did not affect the survival, renal function, or allograft function (252,258,260,266,280,284,292). Some randomized trials assessing the impact of treatment asymptomatic bacteriuria on kidney recipients have already been reported, with results against treating, within small samples (258,421), within bigger samples after the immediate period post-transplant (374).

Preventive measures to reduce UTIs in transplant recipients, as antimicrobial prophylaxis, had not been reported to affect either the graft's or patient's survival;

however, antimicrobial prophylaxis reduced the incidence of bacteriuria and sepsis in a meta-analysis study (286). Fosfomycin has been studied in primary prophylaxis (in addition to the standard cotrimoxazole during the first seven weeks after transplantation when manipulating urinary catheters) and results are good: fosfomycin is safe and effective at reducing the number of symptomatic UTI (422). A secondary prophylaxis study with 3g of fosfomycin every 10 days during six months (following previous authors' methods (387)) after MDR recurrent UTI and urosepsis event also shows that fosfomycin is safe and effective (delaying time to recurrence) (399). Both were small sample sized studies.

In the present study, treating asymptomatic bacteriuria did not prevent the development of symptomatic UTI in the follow-up, as other studies had already reported (254,339,404). On the contrary, independently treating asymptomatic bacteriuria increased the risk of symptomatic UTIs in the following six months. Some factors were also identified to independently increase the risk of developing a symptomatic UTI during the six months of follow-up as induction therapy with thymoglobulin, time since transplantation less than one year, and acute pyelonephritis before the inclusion. These factors might be, as previously stated, surrogate markers of the global net-state of immunosuppression and urinary predisposing factors, which might have contributed to a higher risk of symptomatic infection (253,256,264).

In the current study asymptomatic bacteriuria (mainly) and cystitis events were included.

The open design of the study is also a limitation to be considered. It might explain the increased risk of symptomatic UTI in the follow-up in treated patients with asymptomatic bacteriuria: the physician in charge of the patient decided to treat more frequently patients who had asymptomatic bacteriuria and were recently transplanted,

the event was a hospital-acquired UTI, or received thymoglobulin as induction drug. All these are factors associated with a higher risk of symptomatic UTI in the follow-up, as previously stated (261,272,274). Although receiving treatment for asymptomatic bacteriuria was an independent risk factor of developing a symptomatic UTI within six months, the presence of other confounding factors not considered in the present analysis could not be ruled out.

Recurrent UTI is a frequent situation in our cohort (10%), as it has been described in other KTRs's cohort that reported an incidence ranging 6-46% (288,324,424). A worse initial renal function was independently associated to develop recurrent UTI during the follow-up. In our cohort, recurrent UTI was associated with a higher risk of acute rejection, without impacting in other final outcomes as mortality or graft loss. This risk and prognostic factors have been already reported (324,425). A recent study concludes that recurrent symptomatic UTIs are associated with lower one-year renal function and death-censored allograft survival (excluding mortality causes non-related to graft dysfunction) when compared to non-UTI events, occasional UTI or asymptomatic bacteriuria the first year after transplantation (415).

The high rate of resistant UTIs in KTR and the lack of clinical benefits of treating asymptomatic bacteriuria in the present study, together with no impact onto final outcomes during six months of follow-up, and an increased risk to develop resistances and future symptomatic events; support the guidelines and the last trial results recommendations to stop screening and treating asymptomatic bacteriuria.

The risk factors for developing a symptomatic UTI and recurrent UTI here described might help define a subpopulation that could benefit from specific strategies, such as close follow-up, antimicrobial prophylaxis, or self-antibiotic initiation once symptoms are present.

Moreover, establishing this protocol in our area has let the possibility to implement antibiotics stewardship strategies in the KTR setting narrowing the options for treating UTI events to more appropriate antibiotics, diminishing the length of antimicrobial courses, highly recommending not treating asymptomatic bacteriuria events after the immediate transplant, and reinforcing the multidisciplinary efforts towards a common area.

7.2. Objective 2: To compare the outcome of lower UTI caused by *E. coli* in KTR treated with ciprofloxacin and fosfomycin, according to the urinary pH.

Since the design and first results of this doctoral thesis, several studies focusing on the use of fosfomycin in different UTI scenarios have been published supporting its use in non-transplant recipients (217,218,343,344,426). Fosfomycin sensibility patterns in lower UTI are still preserved in general population, even in high-resistant rates areas like India with a clinical cure rate over 80% (345,427)**.**

The cohort of patients with *E. coli* bacteriuria could be comparable to the first objective´s cohort and to others previously published (347,351,370,374).

Fosfomycin use was independently associated to higher rates of microbiological failure and symptomatic UTI by sixth month, compared to ciprofloxacin, but not to one-month clinical and microbiological outcome. It must also be mentioned that more hospital-acquired UTI and baseline resistances to ciprofloxacin and to amoxicillin-clavulanate were observed in the fosfomycin group, which may be associated to present and/or develop symptomatic UTI in the KTR setting as previously mentioned.

In the present study, the microbiological cure by first month was 74.6%. This proportion is slightly higher to those described in other retrospective studies

addressing fosfomycin therapy in KTR: 59.9% in asymptomatic bacteriuria (351) or 70.2% in cystitis (347).

This is the first prospective study showing fosfomycin as a reliable option for UTI in KTR, given its safety with no withdrawals related to fosfomycin use and its similar *in vivo* 1 month-efficacy compared to ciprofloxacin. Fosfomycin has other advantages that can support its use in this setting as short courses, salvage therapy indication, outpatient use, active treatment against MDR UTI, replacement for quinolones and its risk to develop resistances and appropriate treatment for recurrent UTI, among others, as previously suggested (324,347,351).

Although no impact on the graft or the patient survival was observed, the association between fosfomycin use and more sixth months' microbiological failure and symptomatic UTI should be explored in more robust studies designed for this purpose.

To our knowledge, this work is the first that studies *in vivo* the effects of urine pH on the pathogenicity of *E. coli* infections as well as its interaction with the effectiveness of ciprofloxacin and fosfomycin in the treatment of UTI in KTR.

Patients with acidic urine pH and bacteriuria showed a higher risk of developing symptomatic bacteriuria during follow-up (and a trend towards more frequent APN), but not microbiological failure, compared to those with neutral or basic pH, independently of the treatment. A murine model showed similar clinical results in acidosis, increasing kidney bacterial burden and decreasing bacterial renal clearance (428).

In the stratified analysis, fosfomycin compared to ciprofloxacin with non-acidic urine was associated to more symptomatic and microbiological failure at six months but not

at one month. However, urinary pH had no impact on the efficacy of ciprofloxacin and fosfomycin on multivariate analyses.

The present study was not able to confirm *in vivo* the correlation previously observed *in vitro* studies, suggesting that acidic pH decreases the activity of ciprofloxacin (143,188), and that fosfomycin increases activity in non-alkaline pH (144).

It must be noted that non-significant higher rates of microbiological failure and symptomatic UTI by first and sixth month (as well as relapses) were found in the group receiving ciprofloxacin and baseline acidic urine pH. Further studies would need to be done with larger sample size to confirm these results.

Most of the variables associated to worse clinical outcomes by first and sixth month in this cohort such as recent transplants, MDR strains, previous use of antibiotics, more previous and current symptomatic UTI events, previous renal transplant, pre-transplant recurrent UTI, hospital-acquired events, have been described in other studies (267,298,324,367). Microbiological failure by first month was associated to microbiological failure by sixth month, but not to symptomatic UTIs during follow-up contrary to what has been previously reported (280,284).

One-month´s microbiological failure was independently associated to transplantation beyond 1 year. Although the incidence of UTI decreases as time passes since the transplant, (280,313), in those who suffer UTI, microbiological failure and recurrent UTI are more common (429).

Symptomatic UTI by first month was associated to symptomatic UTI during 6 months of follow-up, Developing a symptomatic UTI is a risk factor itself for future microbiological failure, too (351).

In this cohort, re-infections events were associated with graft dysfunction, and a trend to present graft rejection in bivariate analyses, as documented in old reports

(288,423). These findings, in need for deeper and proper studies, could diminish the innocent role of asymptomatic bacteriuria event after the immediate transplant period as previous authors already confronted (294,299,425).

Some limitations should be mentioned, first, the number of patients treated with ciprofloxacin is limited. In November 2018, the European Medicines Agency recommended to avoid the quinolones to treat mild/moderate bacterial infections, especially in patients with organ transplantation (354,355) and that preclude the inclusion of new cases. Besides, in 2019, the new American Guidelines for Asymptomatic Bacteriuria Management strongly recommended neither treating nor screening for asymptomatic bacteriuria after the immediate transplant (297). These circumstances emphasize the need to create evidence of the use of other antibiotic alternatives for UTI therapy in KTR, as the non-updated guidelines still recommend ciprofloxacin as one of the first-line options for UTI in SOT population (313,338).

Second, the small number of patients with acute pyelonephritis precluded us from exploring possible confounding variables.

Finally, this cohort included a small sample of cystitis cases that reduces the statistical power for comparative analyses. However, interactions, confounding variables and colinearity were explored thoroughly in multiple multivariate (and/or adjusted analyses) in order to avoid other biases or statistical errors.

This is the first cohort study that uses fosfomycin as first-line treatment in renal recipients with non-complicated UTI, in a medium prospective cohort size, with 6 months of follow-up, and comparing it with the *gold-standard* (ciprofloxacin), with a fixed dose and length antibiotic in both groups. The results of this thesis may help establishing the clinical and microbiological efficacy of fosfomycin, in ITU in KTR and improving our stewardship antibiotic program in the KTR setting area: shortening

treatment length and choosing antibiotics with less ecological impact, but active at the same time against multidrug resistant infections.

7.3. Objective 3: To analyse the clinical and microbiological outcomes in lower UTI caused by different uropathogens in KTR treated with ciprofloxacin and fosfomycin, according to the urine pH.

Given the limitations previously explained regarding the inclusion of episodes treated with ciprofloxacin, we considered evaluating the impact of pH in treating bacteriuria caused by other enterobacteria in a prospective cohort of KTR, treated with ciprofloxacin and fosfomycin. This cohort could help to validate our internal results in UTI in the daily practice and to increase the episodes treated with ciprofloxacin.

As previously referred, baseline characteristics were similar in both cohorts. *E. coli* and *K. pneumoniae* were the most common pathogens included. Clinical and microbiological outcome were similar, except for a higher six-months' microbiological cure in the later cohort without differences according to the antibiotic used.

In this cohort, microbiological failure at first month was independently associated to elder recipients, as other reports already pointed out (285).

Acidic urine pH was associated with worse clinical cure rates by first month (including a trend to more pyelonephritis events by sixth month in the bivariate analysis), as occurred in objective 2, the pathogenic of this finding is unknown.

Both treatments showed similar efficacy during the follow-up, even when stratifying results by urinary pH or initial cystitis. At one-month of follow-up the efficacy of fosfomycin was similar to those reported by recent studies treating diverse

uropathogens with fosfomycin: 59.9% in asymptomatic bacteriuria and 70.2% in cystitis cases (347,351). However, the efficacy of fosfomycin by first month in this cohort was lower than the one observed in objective 2 (64.5% *vs.*74.6%). This difference could be related to a lower efficacy of fosfomycin as therapy of fosfomycin-susceptible UTI. This has been reported in an *in vitro* bladder infection model after a single dose of 3 g (247,430).

Urine pH seems to have low impact in *in vitro* fosfomycin activity against *K. pneumoniae* (430). Besides, an ongoing study carried out in our group confirms no effect of urine pH in the outcome of *K. pneumoniae* bacteriuria treated with fosfomycin and ciprofloxacin.

Noteworthy, the use of ciprofloxacin raised the risk for re-infections. Similar results were found in a *post-hoc* analysis from cohort A, where treating bacteriuria led to more re-infections if receiving ciprofloxacin. No other reports have been found supporting this result in the bibliography reviewed. Ciprofloxacin group did not include more percentage of surrogate variables favouring this outcome.

Considering these results fosfomycin could be an option for the empirical therapy of cystitis in KTR, with no impact of urine pH in its expected efficacy.

7.4. Objective 4. To study the prevalence, phenotypic characteristics and clinical impact and outcomes of *E. coli* strains with LLQR and/or LLFR, treated with ciprofloxacin and fosfomycin, according to the urine pH.

As far as we know, this is the first study that analyze the prevalence of LLQR and LLFR in *E. coli* causing UTI in KTRs, showing higher rate of LLQR strains (20%) than that observed in the general population (161,191). The proportion of LLFR has

not been previously determined, and affected 5% of the episodes included, while the proportion of fully ciprofloxacin and fosfomycin resistant strains were similar to those previously reported in KTRs (303,309).

It must be noted that 15 episodes were resistant to the antibiotic received (12.6%), according to the research laboratory MIC determinations. This occurred as techniques for MIC determinations used in the Hospital Microbiology Service and the research laboratory were different, as previously reported (431), and due to changes in the EUCAST breakpoints from 2020 to 2021. The final outcomes were similar to those fully susceptible, probably due the high antibiotic concentration obtained in the bladder.

More relapses were observed in fosfomycin resistant episodes treated with fosfomycin. A higher frequency of acidic urine pH could have act as a possible confounder.

The presence of LLR strains was not related to present other resistance pattern, neither to develop specific resistances. But, it was associated to the use of antibiotic within the previous three months (especially in LLFR strains) and younger age of the recipients, compared to fully-susceptible strains.

There were no differences in primary outcomes when sub-analysing by subtype of strains (fully-susceptible, LLR and resistant strains). Among secondary outcomes, no relevant differences were detected, either.

Presenting LLR strains seems to be a dynamic process (appearance, clearance) without a defined impact in the outcome. Urine pH did not affect the clinical or microbiological outcome of LLR infections.

No other relevant findings when sub-analysing LLR subtypes according to the treatment and urinary pH were observed.

After sequencing, three of the seven strains treated with fosfomycin that developed resistance after treatment presented a different ST, therefore in these cases we considered the follow-up isolate to be a superinfection.

However, in the other four strains that developed resistance after fosfomycin therapy, three new resistance to fosfomycin and two to ciprofloxacin (one of them developed resistance to both antibiotics), the same ST was confirmed. Three sequence types were found: ST3, ST7, and ST518; but they were not carrying any of the major cromosomic or plasmidic antimicrobial resistance genes associated to fosfomycin or ciprofloxacin resistance already described (116). The ST3 (also named ST69 in Warwick scheme), found in two of the persistent strains that developed ciprofloxacin-fosfomycin-resistance, respectively, was highly associated to carry *chuA* and *fyuA* genes which encode virulent factors involved in the IBC formation in UPEC strains, as commented initially (440). Carrying those virulent factors could ultimately lead to resistant strains development, and to express mutagenic genes during UTI process (300,441,442), as well as to develop recurrent UTI (443). This ST has also been related to prolonged in-hospital stay in *E. coli*-bloodstream infections when compared to others ST (444), and to exhibit robust MDR profiles and carrying plasmatic virulent factors in Australian UPEC strains (445).

New fosfomycin resistance could be justified by its high rate of heteroresistances, with the consequent selection of minority initial strains which may re-grow as immediate re-infections (by same clonal or another *E. coli* strain) and express their own resistances-associated traits as permutant subpopulations (432–434).

About the over-crossing resistance development (ciprofloxacin resistance after fosfomycin treatment), we speculate that unknown resistant mechanisms are suspected to be involved, as mutagenesis frequency to ciprofloxacin is high, complex,

feasible and very diverse as in general population, as previously explained. Another explanation could be the result of general resistances expansion due to ciprofloxacin (and other antibiotics) overuse or missuse, independently of the treatment prescribed in the current event (435,436). However, no studies regarding this issue (crossed-over resistances, collateral resistances, associated resistances, or co-resistances) could be found, neither any shared resistance mechanism is known; rather than inverse collateral susceptibility –ciprofloxacin resistance confers fosfomycin susceptibility- reported in an *in vitro* study (434).

Among the cohort B and its modifications to elucidate objectives 3 and 4, the persistent/relapse rate after during all the follow-up period was similar (16.8-25.9%), while other cohorts reported 13-21% (348) or up to 40% (351) within the first month. More ciprofloxacin resistances after receiving ciprofloxacin in the cohort to elucidate objective 3 were found, which could be due to the higher percentage of *K. pneumoniae* included, with greater capacity to acquire and spread resistances when compared to *E. coli* UTI (303).

8. CONCLUSIONS

1) This study supports the recommendation of stopping screening and treating asymptomatic bacteriuria.

2) Fosfomycin is safe, effective and valid for non-complicated *E. coli* UTI in KTR. Fosfomycin would avoid the use of broad-spectrum antibiotic and with high-risk of side effects, such as quinolones. The worse microbiological and clinical outcomes by sixth month when receiving fosfomycin, with no impact on the graft or the patient survival, should be explored in specific studies.

3) Fosfomycin has also been validated for non-complicated bacteriuria in KTR by other frequent uropathogens. Two doses of 3g of fosfomycin within 72h could be a good option in our current clinical practice.

4) Acidic urine increases the risk of symptomatic UTI (including pyelonephritis events) but does not affect the microbiological efficacy of ciprofloxacin and fosfomycin in KTR bacteriuria. Further studies should be focused in the pathogenic of this finding.
   In *E. coli* bacteriurias, fosfomycin use in non-acidic infected urine seems to increase the risk of symptomatic and microbiological failure at six months.

5) The presence of resistances and low-level resistances in KTR bacteriuria is high.

6) Low level resistance strains do not lead to resistances development or worse clinical-microbiological outcomes. Acidic urinary pH has no impact either.

9. BIBLIOGRAPHY

1. Brenner D, Farmer J, Family I. *Enterobacteriaceae*. In: Bergey's Manual of Systematic Bacteriology. New York, USA: Springer; 2005. p. 587–607.
2. Adeolu M, Alnajar S, Naushad S, Gupta RS. Genome-based phylogeny and taxonomy of the *'Enterobacteriales'*: proposal for enterobacterales ord. nov. divided into the families *Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morgane.* Int J Syst Evol Microbiol. 2016;66(12):5575–99.
3. Strockbine N, Bopp C, Fields P, Kaper J, Nataro J. *Escherichia, Shigella, and Salmonella*. In: Jorgensen J, editor. Manual of Clinical Microbiology. 11th ed. USA: ASM Press; 2015. p. 685–712.
4. Paczosa M, Mecsas J. *Klebsiella pneumoniae*: going on the offense with a strong defense. Microbiol Mol Biol Rev. 2016;80(3):629–61.
5. Forsythe S, Abbott S, Pitout J. *Klebsiella, Enterobacter, Citrobacter, Cronobacter, Serratia, Plesiomonas*, and other *Enterobacteriaceae*. In: Manual of Clinical Microbiology. USA: ASM Press; 2015. p. 714–37.
6. Nataro J, Kaper J. Diarrheagenic *Escherichia coli*. Clin Microbiol Rev. 1998;11:142–201.
7. Russo TA, Johnson JR. Proposal for a new inclusive designation for extraintestinal pathogenic isolates of *Escherichia coli*: ExPEC. J Infect Dis. 2000;181(5):1753–4.
8. Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout J. Global extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. Clin Microbiol Rev. 2019;32(3).
9. Dale AP, Woodford N. Extra-intestinal pathogenic *Escherichia coli* (ExPEC): Disease, carriage and clones. J Infect. 2015;71:615–26.
10. Shaik S, Ranjan A, Tiwari SK, Hussain A, Nandanwar N, Kumar N, et al. Comparative genomic analysis of globally dominant ST131 clone with other epidemiologically successful extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. MBio. 2017;8(5):e01596-17.
11. Daga AP, Koga VL, Soncini JGM, De Matos CM, Perugini MRE, Pelisson M, et al. *Escherichia coli* bloodstream infections in patients at a university hospital: virulence factors and clinical characteristics. Front Cell Infect Microbiol. 2019;9(191).
12. Banu A, Kabbin JS, Anand M. Extraintestinal infections due to *Escherichia coli*: an emerging issue. J Clin Diagnostic Res. 2011;5(3):486–90.
13. Foxman B. The epidemiology of urinary tract infection. Nat Rev Urol. 2010;7:653–60.
14. Spurbeck R, Mobley H. Uropathogenic *Escherichia coli*. In: Donnenberg MS, editor. *Escherichia coli*. Second Ed. Boston: Academic Press; 2013. p. 275–304.
15. Foxman B. Epidemiology of urinary tract infections: incidence, morbidity, and economic costs. Am J Med. 2002;113(1):5–13.
16. Mobley H, Donnenberg M, Hagan E. Uropathogenic *Escherichia coli*. EcoSal Plus. 2009;3(2).
17. Ulett GC, Totsika M, Schaale K, Carey AJ, Sweet MJ, Schembri MA.

Uropathogenic *Escherichia coli* virulence and innate immune responses during urinary tract infection. Curr Opin Microbiol. 2013;16(1):100–7.
18. Lewis AJ, Richards AC, Mulvey MA. Invasion of host cells and tissues by uropathogenic bacteria. Microbiol Spectr. 2016;4(6):UTI-0026-2016.
19. Cegelski L, Marshall G, Eldridge G, Hultgren S. The biology and future prospects of antivirulence therapies. Nat Rev Microbiol. 2008;6:17–27.
20. Servin AL. Pathogenesis of human diffusely adhering *Escherichia coli* expressing Afa/Dr adhesins (Afa/Dr DAEC): current insights and future challenges. Clin Microbiol Rev. 2014;27(4):823–69.
21. Eto DS, Sundsbak JL, Mulvey MA. Actin-gated intracellular growth and resurgence of uropathogenic *Escherichia coli*. Cell Microbiol. 2006;8(4):704–17.
22. Flores-Mireles AL, Walker JN, Caparon M, Hultgren SJ. Urinary tract infections: epidemiology, mechanisms of infection and treatment options. Nat Rev Microbiol. 2015;13(5):269–84.
23. Justice SS, Hunstad DA, Cegelski L, Hultgren SJ. Morphological plasticity as a bacterial survival strategy. Nat Rev Microbiol. 2008;6(2):162–8.
24. Barber AE, Norton JP, Wiles TJ, Mulvey MA. Strengths and limitations of model systems for the study of urinary tract infections and related pathologies. Microbiol Mol Biol Rev. 2016;80(2):351–67.
25. Hacker J, Carniel E. Ecological fitness, genomic islands and bacterial pathogenicity. A Darwinian view of the evolution of microbes. EMBO Rep. 2001;2(5):376–81.
26. Vila J, Sáez-López E, Johnson J, Römling U, Dobrindt U, Cantón R, et al. *Escherichia coli*: an old friend with new tidings. FEMS Microbiol Rev. 2016;40(4):437–63.
27. Subashchandrabose S, Mobley H. Virulence and fitness determinants of Uropathogenic *Escherichia coli*. Microbiol Spectr. 2015;3(4):UTI-0015-2012.
28. Andersson DI, Hughes D. Antibiotic resistance and its cost: is it possible to reverse resistance? Vol. 8, Nature Reviews Microbiology. Nature Publishing Group; 2010. p. 260–71.
29. Corbin BD, Seeley EH, Raab A, Feldmann J, Miller MR, Torres VJ, et al. Metal chelation and inhibition of bacterial growth in tissue abscesses. Science. 2008;319(5865):962–5.
30. Haley KP, Skaar EP. A battle for iron: host sequestration and *Staphylococcus aureus* acquisition. Microbes Infect. 2012;14(3):217–27.
31. Lee J-W, Helmann JD. Functional specialization within the Fur family of metalloregulators. BioMetals. 2007;20(3):485.
32. Massé E, Gottesman S. A small RNA regulates the expression of genes involved in iron metabolism in *Escherichia coli*. Proc Natl Acad Sci U S A. 2002;99(7):4620–5.
33. Reigstad CS, Hultgren SJ, Gordon JI. Functional genomic studies of uropathogenic *Escherichia coli* and host urothelial cells when intracellular bacterial communities are assembled. J Biol Chem. 2007;282(29):21259–67.
34. Garcia EC, Brumbaugh AR, Mobley HLT. Redundancy and specificity of *Escherichia coli* iron acquisition systems during urinary tract infection. Infect Immun. 2011;79(3):1225–35.
35. Ikeda JS, Janakiraman A, Kehres DG, Maguire ME, Slauch JM. Transcriptional regulation of sitABCD of *Salmonella* enterica serovar

Typhimurium by MntR and Fur. J Bacteriol. 2005;187(3):912–22.
36. Sabri M, Houle S, Dozois CM. Roles of the extraintestinal pathogenic *Escherichia coli* ZnuACB and ZupT zinc transporters during urinary tract infection. Infect Immun. 2009;77(3):1155–64.
37. Hancock V, Vejborg RM, Klemm P. Functional genomics of probiotic *Escherichia coli* Nissle 1917 and 83972, and UPEC strain CFT073: comparison of transcriptomes, growth and biofilm formation. Mol Genet genomics. 2010;284(6):437–54.
38. Wright KJ, Seed PC, Hultgren SJ. Uropathogenic *Escherichia coli* flagella aid in efficient urinary tract colonization. Infect Immun. 2005;73(11):7657–68.
39. Pichon C, Héchard C, du Merle L, Chaudray C, Bonne I, Guadagnini S, et al. Uropathogenic *Escherichia coli* AL511 requires flagellum to enter renal collecting duct cells. Cell Microbiol. 2009;11(4):616–28.
40. Lane MC, Alteri CJ, Smith SN, Mobley HLT. Expression of flagella is coincident with uropathogenic *Escherichia coli* ascension to the upper urinary tract. Proc Natl Acad Sci U S A. 2007;104(42):16669–74.
41. Reiss DJ, Mobley HLT. Determination of target sequence bound by PapX, repressor of bacterial motility, in flhD promoter using systematic evolution of ligands by exponential enrichment (SELEX) and high throughput sequencing. J Biol Chem. 2011;286(52):44726–38.
42. Li X, Rasko DA, Lockatell C V, Johnson DE, Mobley HL. Repression of bacterial motility by a novel fimbrial gene product. EMBO J. 2001;20(17):4854–62.
43. Simms AN, Mobley HLT. Multiple genes repress motility in uropathogenic *Escherichia coli* constitutively expressing type 1 fimbriae. J Bacteriol. 2008;190(10):3747–56.
44. Nagy G, Altenhoefer A, Knapp O, Maier E, Dobrindt U, Blum-Oehler G, et al. Both alpha-haemolysin determinants contribute to full virulence of uropathogenic *Escherichia coli* strain 536. Microbes Infect. 2006;8(8):2006–12.
45. Dhakal BK, Mulvey MA. The UPEC pore-forming toxin α-hemolysin triggers proteolysis of host proteins to disrupt cell adhesion, inflammatory, and survival pathways. Cell Host Microbe. 2012;11(1):58–69.
46. Davis JM, Rasmussen SB, O'Brien AD. Cytotoxic necrotizing factor type 1 production by uropathogenic *Escherichia coli* modulates polymorphonuclear leukocyte function. Infect Immun. 2005;73(9):5301–10.
47. Rippere-Lampe KE, O'Brien AD, Conran R, Lockman HA. Mutation of the gene encoding cytotoxic necrotizing factor type 1 (cnf(1)) attenuates the virulence of uropathogenic *Escherichia coli*. Infect Immun. 2001;69(6):3954–64.
48. Beloin C, Michaelis K, Lindner K, Landini P, Hacker J, Ghigo J-M, et al. The transcriptional antiterminator RfaH represses biofilm formation in *Escherichia coli*. J Bacteriol. 2006;188(4):1316–31.
49. Kai-Larsen Y, Lüthje P, Chromek M, Peters V, Wang X, Holm A, et al. Uropathogenic *Escherichia coli* modulates immune responses and its curli fimbriae interact with the antimicrobial peptide LL-37. PLoS Pathog. 2010;6(7):e1001010.
50. Valle J, Mabbett AN, Ulett GC, Toledo-Arana A, Wecker K, Totsika M, et al. UpaG, a new member of the trimeric autotransporter family of adhesins in

uropathogenic *Escherichia coli*. J Bacteriol. 2008;190(12):4147–61.
51. Ulett GC, Valle J, Beloin C, Sherlock O, Ghigo J-M, Schembri MA. Functional analysis of antigen 43 in uropathogenic *Escherichia coli* reveals a role in long-term persistence in the urinary tract. Infect Immun. 2007;75(7):3233–44.
52. Roos V, Ulett GC, Schembri MA, Klemm P. The asymptomatic bacteriuria *Escherichia coli* strain 83972 outcompetes uropathogenic *E. coli* strains in human urine. Infect Immun. 2006;74(1):615–24.
53. Gümüş D, Kalaycı-Yüksek F, Yörük E, Uz G, Çelik E, Arslan C, et al. Alterations of growth rate and gene expression levels of UPEC by antibiotics at sub-MIC. Folia Microbiol. 2018;63:451–457.
54. Müller CM, Dobrindt U, Nagy G, Emödy L, Uhlin BE, Hacker J. Role of histone-like proteins H-NS and StpA in expression of virulence determinants of uropathogenic *Escherichia coli*. J Bacteriol. 2006;188(15):5428–38.
55. Redford P, Welch RA. Role of sigma E-regulated genes in *Escherichia coli* uropathogenesis. Infect Immun. 2006;74(7):4030–8.
56. Redford P, Roesch PL, Welch RA. DegS is necessary for virulence and is among extraintestinal *Escherichia coli* genes induced in murine peritonitis. Infect Immun. 2003;71(6):3088–96.
57. Kulesus RR, Diaz-Perez K, Slechta ES, Eto DS, Mulvey MA. Impact of the RNA chaperone Hfq on the fitness and virulence potential of uropathogenic *Escherichia coli*. Infect Immun. 2008;76(7):3019–26.
58. Grupo de trabajo EPINE. Prevalencia de infecciones relacionadas con la asistencia sanitaria y uso de antimicrobianos en hospitales de agudos en 2021. Madrid; 2022. [Internet]. Available from: https://epine.es/api/documento-publico/2022%20EPINE%20Informe%20Espa%C3%B1a%2020221201.
59. Mulvey K and S. Urinary tract infections: molecular pathogenesis and clinical management. Washington (DC): 2th Ed. ASM Pres; 2017. p. 450-497.
60. Magiorakos A, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268–81.
61. European Centre for Disease Prevention and Control. Antimicrobial resistance in the EU/EEA- Annual Epidemiological Report of the European Antimicrobial Resistance Suverillance Network (EARS-Net) 2021. Stockholm: ECDC; 2022. [Internet]. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/AER-EARS-Net-2021_2022-final.pdf
62. Equipo EARS-Net España. Memoria de la red de vigilancia de la resistencia a antibióticos 2018 y 2019. Madrid: EARS-Net España; 2020. [Internet]. Available from: https://www.isciii.es/QuienesSomos/CentrosPropios/CNM/ResistenciasAntibacterianas/Investigaci.
63. European Centre for Disease Prevention and Control and World Health Organitzation. Antimicrobial resistance surveillance in Europe - 2021 data. Stockholm: ECDC; 2023. [Internet]. Available from: https://www.ecdc.europa.eu/sites/default/files/documents/Antimicrobial%20resistance%20surveillance%20in%20Europe%202023%20-%202021%20data.pdf.
64. Russo TA, Stapleton A, Wenderoth S, Hooton TM, Stamm WE. Chromosomal

restriction fragment length polymorphism analysis of *Escherichia coli* strains causing recurrent urinary tract infections in young women. J Infect Dis. 1995;172(2):440–5.
65. Ikäheimo R, Siitonen A, Heiskanen T, Kärkkäinen U, Kuosmanen P, Lipponen P, et al. Recurrence of urinary tract infection in a primary care setting: analysis of a 1-year follow-up of 179 women. Clin Infect Dis. 1996;22(1):91–9.
66. Andersson DI, Hughes D. Microbiological effects of sublethal levels of antibiotics. Nat Rev Microbiol. 2014;12(7):465–78.
67. Rosen DA, Hooton TM, Stamm WE, Humphrey PA, Hultgren SJ. Detection of intracellular bacterial communities in human urinary tract infection. PLoS Med. 2007;4(12):e329.
68. Hughes D. Selection and evolution of resistance to antimicrobial drugs. IUBMB Life. 2014;66(8):521–9.
69. O'Neill J. Review on antimicrobial resistance. Antimicrobial resistance: tackling a crisis for the health and wealth of nations. [Internet]. London. 2014. Available from: https://www.oecd.org/health/health-systems/AMR-Tackling-the-Burden-in-the-EU-OECD-ECDC-Briefing-Note-2019.pdf
70. Austin DJ, Kristinsson KG, Anderson RM. The relationship between the volume of antimicrobial consumption in human communities and the frequency of resistance. Proc Natl Acad Sci U S A. 1999;96(3):1152–6.
71. Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. Nat Med. 2004;10(12):S122-9.
72. Poirel L, Madec J, Lupo A, Schink A, Kieffer N, Nordmann P, et al. Antimicrobial resistance in *Escherichia coli*. Microbiol Spectr. 2018;6(4):1–27.
73. Kalman D, Barriere SL. Review of the pharmacology, pharmacokinetics, and clinical use of cephalosporins. Texas Hear Inst J. 1990;17(3):203–15.
74. McNulty CAM, Wilson APR, Hawkey PM, Otter JA, Enoch DA, Livermore DM, et al. Treatment of infections caused by multidrug-resistant Gram-negative bacteria: report of the British Society for Antimicrobial Chemotherapy/Healthcare Infection Society/British Infection Association Joint Working Party. J Antimicrob Chemother. 2018;73(S3):iii2–78.
75. Drawz SM, Bonomo RA. Three decades of beta-lactamase inhibitors. Clin Microbiol Rev. 2010;23(1):160–201.
76. Tooke CL, Hinchliffe P, Bragginton EC, Colenso CK, Hirvonen VHA, Takebayashi Y, et al. β-lactamases and β-lactamase inhibitors in the 21st century. J Mol Biol. 2019;431(18):3472–500.
77. Bebrone C. Metallo-beta-lactamases (classification, activity, genetic organization, structure, zinc coordination) and their superfamily. Biochem Pharmacol. 2007;74(12):1686–701.
78. Juan C, Torrens G, González-Nicolau M, Oliver A. Diversity and regulation of intrinsic β-lactamases from non-fermenting and other Gram-negative opportunistic pathogens. FEMS Microbiol Rev. 2017;41(6):781–815.
79. Jacoby GA. AmpC beta-lactamases. Clin Microbiol Rev. 2009;22(1):161–82.
80. Jaurin B, Grundström T, Normark S. Sequence elements determining ampC promoter strength in *E. coli*. EMBO J. 1982;1(7):875–81.
81. Evans BA, Amyes SGB. OXA β-lactamases. Clin Microbiol Rev. 2014;27(2):241–63.
82. Philippon A, Labia R, Jacoby G. Extended-spectrum beta-lactamases. Antimicrob Agents Chemother. 1989;33(8):1131–6.

83. Pitout J, Laupland KB. Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. Lancet Infect Dis. 2008;8(3):159–66.
84. Peirano G, Pitout J. Extended-spectrum β-lactamase-producing *Enterobacteriaceae*: update on molecular epidemiology and treatment options. Drugs. 2019;79(14):1529–41.
85. Livermore DM, Woodford N. The beta-lactamase threat in *Enterobacteriaceae, Pseudomonas and Acinetobacter*. Trends Microbiol. 2006;14(9):413–20.
86. Pitout J, Nordmann P, Laupland KB, Poirel L. Emergence of *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBLs) in the community. J Antimicrob Chemother. 2005;56(1):52–9.
87. Rogers BA, Sidjabat HE, Paterson DL. *Escherichia coli* O25b-ST131: a pandemic, multiresistant, community-associated strain. J Antimicrob Chemother. 2011;66(1):1–14.
88. White AR, Kaye C, Poupard J, Pypstra R, Woodnutt G, Wynne B. Augmentin (amoxicillin/clavulanate) in the treatment of community-acquired respiratory tract infection: a review of the continuing development of an innovative antimicrobial agent. J Antimicrob Chemother. 2004;53(S1):i3-20.
89. Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing *Enterobacteriaceae*. Emerg Infect Dis. 2011;17(10):1791–8.
90. Livermore D. Beta-lactamase-mediated resistance and opportunities for its control. J Antimicrob Chemother. 1998;41(Suppl D):25–41.
91. Jones RN, Pfaller MA, Fuchs PC, Aldridge K, Allen SD, Gerlach EH. Piperacillin/tazobactam (YTR 830) combination. Comparative antimicrobial activity against 5889 recent aerobic clinical isolates and 60 *Bacteroides fragilis* group strains. Diagn Microbiol Infect Dis. 1989;12(6):489–94.
92. Gin A, Dilay L, Karlowsky JA, Walkty A, Rubinstein E, Zhanel GG. Piperacillin-tazobactam: a beta-lactam/beta-lactamase inhibitor combination. Expert Rev Anti Infect Ther. 2007;5(3):365–83.
93. Vazquez-Guillamet MC, Vazquez R, Micek ST, Kollef MH. Predicting resistance to piperacillin-tazobactam, cefepime and meropenem in septic patients with bloodstream infection due to Gram-negative bacteria. Clin Infect Dis. 2017;65(10):1607–14.
94. Rodríguez-Villodres Á, Gutiérrez Linares A, Gálvez-Benitez L, Pachón J, Lepe JA, Smani Y. Semirapid detection of piperacillin/tazobactam tesistance and extended-spectrum resistance to β-lactams/β-Lactamase inhibitors in clinical isolates of *Escherichia coli*. Microbiol Spectr. 2021;9(2).
95. Cantón R, Pérez Sáenz JL, Calvo J, Castillo FJ, Díaz-Regañón J, López-Hontangas JL, et al. Monitoring the antimicrobial susceptibility of Gram-negative organisms involved in intraabdominal and urinary tract infections recovered during the SMART study (Spain, 2016 and 2017). Rev esp Quim. 2019;32(2):145–55.
96. Bayraktar, B; Süleyman, P; Mehmet EB AE. Antibiotic resistance trends of extended spectrum Beta lactamase producing *Escherichia coli and Klebsiella pneumoniae* bloodstream infections over the years. MedBull Sisli Etfal Hosp. 2018;53(1):70–5.
97. Public Health England. English Surveillance Programme for Antimicrobial Utilisation and Resistance (ESPAUR). Report 2018 – 2019. [Internet]. Available from:

https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/843129/English_Surveillance_Programme_for_Antimicrobial_Utilisation_and_Resistance_2019.pdf
98. Wu PJ, Shannon K, Phillips I. Mechanisms of hyperproduction of TEM-1 beta-lactamase by clinical isolates of *Escherichia coli*. J Antimicrob Chemother. 1995;36(6):927–39.
99. Blazquez J, Baquero MR, Canton R, Alos I, Baquero F. Characterization of a new TEM-type beta-lactamase resistant to clavulanate, sulbactam, and tazobactam in a clinical isolate of *Escherichia coli*. Antimicrob Agents Chemother. 1993;37(10):2059–63.
100. Helfand MS, Bethel CR, Hujer AM, Hujer KM, Anderson VE, Bonomo RA. Understanding resistance to beta-lactams and beta-lactamase inhibitors in the SHV beta-lactamase: lessons from the mutagenesis of SER-130. J Biol Chem. 2003;278(52):52724–9.
101. Philippon A, Arlet G, Jacoby GA. Plasmid-determined AmpC-type beta-lactamases. Antimicrob Agents Chemother. 2002;46(1):1–11.
102. Livermore DM, Day M, Cleary P, Hopkins KL, Toleman MA, Wareham DW, et al. OXA-1 β-lactamase and non-susceptibility to penicillin/β-lactamase inhibitor combinations among ESBL-producing *Escherichia coli*. J Antimicrob Chemother. 2019;74(2):326–33.
103. Rodríguez-Villodres Á, Gil-Marqués ML, Álvarez-Marín R, Bonnin RA, Pachón-Ibáñez ME, Aguilar-Guisado M, et al. Extended-spectrum resistance to β-lactams/β-lactamase inhibitors (ESRI) evolved from low-level resistant Escherichia coli. J Antimicrob Chemother. 2020;75(1):77–85.
104. Chaïbi EB, Sirot D, Paul G, Labia R. Inhibitor-resistant TEM beta-lactamases: phenotypic, genetic and biochemical characteristics. J Antimicrob Chemother. 1999;43(4):447–58.
105. Cantón R, Morosini M, de la Maza O, de la Pedrosa E. IRT and CMT beta-lactamases and inhibitor resistance. Clin Microbiol Infect. 2008;14 Suppl 1:53–62.
106. Murao N, Ohge H, Ikawa K, Watadani Y, Uegami S, Shigemoto N, et al. Pharmacokinetics of piperacillin-tazobactam in plasma, peritoneal fluid and peritoneum of surgery patients, and dosing considerations based on site-specific pharmacodynamic target attainment. Int J Antimicrob Agents. 2017;50(3):393–8.
107. Bonkat G, Pickard R, Bartoletti R, Bruyère F, Geerlings SE, Wagenlehner F et al. Guidelines on urological infections from European Association of Urology [Internet]. 2017. p. 247–69. Available from: http://www.uroweb.org/guidelines
108. Sanchez G V, Babiker A, Master RN, Luu T, Mathur A, Bordon J. Antibiotic resistance among urinary isolates from female outpatients in the United States in 2003 and 2012. Antimicrob Agents Chemother. 2016;60(5):2680–3.
109. Kresken M, Körber-Irrgang B, Biedenbach DJ et al. Comparative *in vitro* activity of oral antimicrobial agents against *Enterobacteriaceae* from patients with community-acquired urinary tract infections in three European countries UTI in each country was examined as well as the association between lab. Clin Microbiol Infect. 2016;22(1):63.e1-63.e5.
110. Ong A, Mahobia N, Browning D, Schembri M, Somani BK. Trends in antibiotic resistance for over 700,000 *Escherichia coli* positive urinary tract infections over six years (2014–2019) from a university teaching hospital. Cent

Eur J Urol. 2021;74(2):249–54.
111. Delpech G, García Allende N, Lissarrague S, Sparo M. Antimicrobial resistance of uropathogenic *Escherichia coli* from elderly patients at a general hospital, Argentina. Open Infect Dis J. 2018;10:79–87.
112. Ciontea AS, Cristea D, Andrei MM, Popa A UC. *In vitro* antimicrobial resistance of urinary *Escherichia coli* isolates from outpatients collected in a laboratory during two years, 2015–2017. Roum Arch Microbiol Immunol. 2018;77(1):28–32.
113. Lavigne J-P, Bruyère F, Bernard L, Combescure C, Ronco E, Lanotte P, et al. Resistance and virulence potential of uropathogenic *Escherichia coli* strains isolated from patients hospitalized in urology departments: a French prospective multicentre study. J Med Microbiol. 2016;65(6):530–7.
114. Kot B, Wicha J, Grużewska A, Piechota M, Wolska K, Obrębska M. Virulence factors, biofilm-forming ability, and antimicrobial resistance of urinary *Escherichia coli* strains isolated from hospitalized patients. Turkish J Med Sci. 2016;46(6):1908–14.
115. van der Donk C, van de Bovenkamp J, De Brauwer E, De Mol P, Feldhoff K, Kalka-Moll W, et al. Antimicrobial resistance and spread of multi drug resistant *Escherichia coli* isolates collected from nine urology services in the Euregion Meuse-Rhine. PLoS One. 2012;7(10):e47707.
116. Kot B. Antibiotic resistance among uropathogenic *Escherichia coli*. Polish J Microbiol. 2019;68(4):403–15.
117. McOsker CC, Fitzpatrick PM. Nitrofurantoin: mechanism of action and implications for resistance development in common uropathogens. J Antimicrob Chemother. 1994;33 Suppl A:23–30.
118. Sandegren L, Lindqvist A, Kahlmeter G, Andersson DI. Nitrofurantoin resistance mechanism and fitness cost in *Escherichia coli*. J Antimicrob Chemother. 2008;62(3):495–503.
119. Vervoort J, Xavier BB, Stewardson A, Coenen S, Godycki-Cwirko M, Adriaenssens N, et al. An *in vitro* deletion in ribE encoding lumazine synthase contributes to nitrofurantoin resistance in *Escherichia coli.* Antimicrob Agents Chemother. 2014;58(12):7225–33.
120. Ho P-L, Ng K-Y, Lo W-U, Law PY, Lai EL-Y, Wang Y, et al. Plasmid-mediated OqxAB is an important mechanism for nitrofurantoin resistance in *Escherichia coli*. Antimicrob Agents Chemother. 2016;60(1):537–43.
121. Cunha MA, Assunção GLM, Medeiros IM, Freitas MR. Antibiotic resistance patterns of urinary tract infections in a Northeastern Brazilian capital. Rev Inst Med Trop Sao Paulo. 2016;58:2.
122. Sanchez G V, Master RN, Karlowsky JA, Bordon JM. *In vitro* antimicrobial resistance of urinary Escherichia coli isolates among U.S. outpatients from 2000 to 2010. Antimicrob Agents Chemother. 2012;56(4):2181–3.
123. Prasada S, Bhat A, Bhat S, Shenoy Mulki S, Tulasidas S. Changing antibiotic susceptibility pattern in uropathogenic *Escherichia coli* over a period of 5 years in a tertiary care center. Infect Drug Resist. 2019;12:1439–43.
124. Bartoletti R, Cai T, Wagenlehner FM, Naber K, Bjerklund Johansen TE. Treatment of urinary tract infections and antibiotic stewardship. Eur Urol Suppl. 2016;15(4):81–7.
125. Erb S, Frei R, Tschudin Sutter S, Egli A, Dangel M, Bonkat G, et al. Basic patient characteristics predict antimicrobial resistance in *E. coli* from urinary

tract specimens: a retrospective cohort analysis of 5246 urine samples. Swiss Med Wkly. 2018;148:w14660.
126. Sköld O. Resistance to trimethoprim and sulfonamides. Vet Res. 2001;32(3–4):261–73.
127. Huovinen P. Resistance to trimethoprim-sulfamethoxazole. Clin Infect Dis. 2001;32(11):1608–14.
128. Fleece ME, Pholwat S, Mathers AJ, Houpt ER, Fleece ME, Pholwat S, et al. Expert review of molecular diagnostics of antimicrobial resistance in *Escherichia coli*. Expert Rev Mol Diagn. 2018;18(3):207–17.
129. Song W, Kim Y-H, Sim S-H, Hwang S, Lee J-H, Lee Y, et al. Antibiotic stress-induced modulation of the endoribonucleolytic activity of RNase III and RNase G confers resistance to aminoglycoside antibiotics in *Escherichia coli*. Nucleic Acids Res. 2014;42(7):4669–81.
130. Adamus-Białek W, Wawszczak M, Arabski M, Majchrzak M, Gulba M, Jarych D, et al. Ciprofloxacin, amoxicillin, and aminoglycosides stimulate genetic and phenotypic changes in uropathogenic *Escherichia coli* strains. Virulence. 2019;10(1):260–76.
131. Suzuki S, Horinouchi T, Furusawa C. Prediction of antibiotic resistance by gene expression profiles. Nat Commun. 2014;5:5792.
132. Baquero F. Low-level antibacterial resistance: a gateway to clinical resistance. Drug Resist Updat. 2001;4(2):93–105.
133. Andersson DI, Hughes D. Evolution of antibiotic resistance at non-lethal drug concentrations. Drug Resist Updat. 2012;15(3):162–72.
134. Kamberi M, Tsutsumi K, Kotegawa T, Kawano K, Nakamura K, Niki Y, et al. Influences of urinary pH on ciprofloxacin pharmacokinetics in humans and antimicrobial activity *in vitro versus* those of sparfloxacin. Antimicrob Agents Chemother. 1999;43(3):525–9.
135. Ozdemir M, Crewe KH, Tucker GT, Rostami-Hodjegan A. Assessment of *in vivo* CYP2D6 activity: differential sensitivity of commonly used probes to urine pH. J Clin Pharmacol. 2004;44(12):1398–404.
136. Martín-Gutiérrez G, Docobo-Pérez F, Rodríguez-Martínez JM, Pascual A, Blázquez J, Rodriguez-Beltrán J. Detection of low-level fosfomycin-resistant variants by decreasing glucose-6-phosphate concentration in fosfomycin susceptibility determination. Antibiotics. 2020;9(11):1–7.
137. Cahill DJ, Fry CH, Foxall PJ. Variation in urine composition in the human urinary tract: evidence of urothelial function in situ? J Urol. 2003;169(3):871–4.
138. Hull RA, Hull SI. Nutritional requirements for growth of uropathogenic *Escherichia coli* in human urine. Infect Immun. 1997;65(5):1960–1.
139. Snyder JA, Haugen BJ, Buckles EL, Lockatell CV, Johnson DE, Donnenberg MS, et al. Transcriptome of uropathogenic *Escherichia coli* during urinary tract infection. Infect Immun. 2004;72(11):6373–81.
140. Giannakopoulos X, Evangelou A, Kalfakakou V, Grammeniatis E, Papandropoulos I, Charalambopoulos K. Human bladder urine oxygen content: implications for urinary tract diseases. Int Urol Nephrol. 1997;29(4):393–401.
141. Dwyer DJ, Belenky PA, Yang JH, MacDonald IC, Martell JD, Takahashi N, et al. Antibiotics induce redox-related physiological alterations as part of their lethality. Proc Natl Acad Sci U S A. 2014;111(20):E2100-9.
142. So W, Crandon J, Nicolau D. Effects of urine matrix and pH on the potency of

delafloxacin and ciprofloxacin against urogenic *Escherichia coli and Klebsiella pneumoniae*. J Urol. 2015;194(2):563–70.
143. Martín-Gutiérrez G, Rodríguez-Beltrán J, Manuel Rodríguez-Martínez J, Costas C, Aznar J, Pascual Á, et al. Urinary tract physiological conditions promote ciprofloxacin resistance in low-level-quinolone-resistant *Escherichia coli*. Antimicrob Agents Chemother. 2016;60(7):4252–8.
144. Martin-Gutiérrez G, Docobo-Pérez F, Rodriguez-Beltŕn J, Rodriguez-Martinez JM, Aznar J, Pascual A, et al. Urinary tract conditions affect fosfomycin activity against *Escherichia coli* strains harboring chromosomal mutations involved in fosfomycin uptake. Antimicrob Agents Chemother. 2018;62(1).
145. Lesher GY, Froelich EJ, Gruett Md, Bailey JH BR. 1,8-naphthyridine derivatives. A new class of chemotherapeutic agents. J Med Pharm Chem. 1962;91:1063–5.
146. Heeb S, Fletcher MP, Chhabra SR, Diggle SP, Williams P, Cámara M. Quinolones: from antibiotics to autoinducers. FEMS Microbiol Rev. 2011;35(2):247–74.
147. King D, Malone R, Lilley S. New classification and update on the quinolone antibiotics. Am Fam Physician. 2000;61(9):2741.
148. Suaifan GARY, Mohammed AAM. Fluoroquinolones structural and medicinal developments (2013-2018): where are we now? Bioorg Med Chem. 2019;27(14):3005–60.
149. van der Putten BC, Remondini D, Pasquini G, Janes VA, Schultsz C. Quantifying the contribution of four resistance mechanisms to ciprofloxacin MIC in *Escherichia coli*: a systematic review. J Antimicrob Chemother. 2019;74:298–310.
150. Hollinger M. Chemotherapy agents. In: Introduction to Pharmacology. California: CRC Press; 1997.
151. Davis R, Markham A, Balfour JA. Ciprofloxacin. An updated review of its pharmacology, therapeutic efficacy and tolerability. Drugs. 1996;51(6):1019–74.
152. Thai T, Salisbury B, Zito P. Ciprofloxacin. In: StatPearls. Florida: Treasure Island; 2020. p. 257–64.
153. Drusano GL, Standiford HC, Plaisance K, Forrest A, Leslie J, Caldwell J. Absolute oral bioavailability of ciprofloxacin. Antimicrob Agents Chemother. 1986;30(3):444–6.
154. Gupta K, Hooton TM, Naber KG, Wullt B, Colgan R, Miller LG, et al. International clinical practice guidelines for the treatment of acute uncomplicated cystitis and pyelonephritis in women: a 2010 update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases. Clin Infect Dis. 2011;52(5):e103-20.
155. Wagenlehner FME, Kinzig-Schippers M, Sörgel F, Weidner W, Naber KG. Concentrations in plasma, urinary excretion and bactericidal activity of levofloxacin (500 mg) versus ciprofloxacin (500 mg) in healthy volunteers receiving a single oral dose. Int J Antimicrob Agents. 2006;28(6):551–9.
156. van der Starre W, van Nieuwkoop C, Paltansing S, van't Wout J, Groeneveld G, Becker M, et al. Risk factors for fluoroquinolone-resistant *Escherichia coli* in adults with community-onset febrile urinary tract infection. J Antimicrob Chemother. 2011;66(3):650–6.
157. van den Broek d'Obrenan J, Verheij TJM, Numans ME, van der Velden AW.

Antibiotic use in Dutch primary care: relation between diagnosis, consultation and treatment. J Antimicrob Chemother. 2014;69(6):1701–7.
158. Dellgren L, Claesson C, Högdahl M, Forsberg J, Hanberger H, Nilsson LE, et al. Phenotypic screening for quinolone resistance in *Escherichia coli*. Eur J Clin Microbiol Infect Dis. 2019;38(9):1765–71.
159. Cullen ME, Wyke AW, Kuroda R, Fisher LM. Cloning and characterization of a DNA gyrase A gene from *Escherichia coli* that confers clinical resistance to 4-quinolones. Antimicrob Agents Chemother. 1989;33(6):886–94.
160. Heisig P. Genetic evidence for a role of parC mutations in development of high-level fluoroquinolone resistance in *Escherichia coli*. Antimicrob Agents Chemother. 1996;40(4):879–85.
161. Takahashi A, Muratani T, Yasuda M, Takahashi S, Monden K, Ishikawa K, et al. Genetic profiles of fluoroquinolone-resistant *Escherichia coli* isolates obtained from patients with cystitis: phylogeny, virulence factors, PAIusp subtypes, and mutation patterns. J Clin Microbiol. 2009;47(3):791–5.
162. Linde HJ, Notka F, Metz M, Kochanowski B, Heisig P, Lehn N. *In vivo* increase in resistance to ciprofloxacin in *Escherichia coli* associated with deletion of the C-terminal part of MarR. Antimicrob Agents Chemother. 2000;44(7):1865–8.
163. Sulavik MC, Houseweart C, Cramer C, Jiwani N, Murgolo N, Greene J, et al. Antibiotic susceptibility profiles of *Escherichia coli* strains lacking multidrug efflux pump genes. Antimicrob Agents Chemother. 2001;45(4):1126–36.
164. Marcusson LL, Frimodt-Møller N, Hughes D. Interplay in the selection of fluoroquinolone resistance and bacterial fitness. PLoS Pathog. 2009;5(8):e1000541.
165. Oethinger M, Kern W V, Jellen-Ritter AS, McMurry LM, Levy SB. Ineffectiveness of topoisomerase mutations in mediating clinically significant fluoroquinolone resistance in *Escherichia coli* in the absence of the AcrAB efflux pump. Antimicrob Agents Chemother. 2000;44(1):10–3.
166. Mammeri H, Van De Loo M, Poirel L, Martinez-Martinez L, Nordmann P. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. Antimicrob Agents Chemother. 2005;49(1):71–6.
167. Martínez-Martínez L, Pascual A, Jacoby GA. Quinolone resistance from a transferable plasmid. Lancet. 1998;351(9105):797–9.
168. Emrich N-C, Heisig A, Stubbings W, Labischinski H, Heisig P. Antibacterial activity of finafloxacin under different pH conditions against isogenic strains of *Escherichia coli* expressing combinations of defined mechanisms of fluoroquinolone resistance. J Antimicrob Chemother. 2010;65(12):2530–3.
169. Chávez-Jacobo VM, Hernández-Ramírez KC, Romo-Rodríguez P, Pérez-Gallardo RV, Campos-García J, Gutiérrez-Corona JF, et al. CrpP is a novel ciprofloxacin-modifying enzyme encoded by the *Pseudomonas aeruginosa* pUM505 plasmid. Antimicrob Agents Chemother. 2018;62(6).
170. Pietsch F, Bergman JM, Brandis G, Marcusson LL, Zorzet A, Huseby DL, et al. Ciprofloxacin selects for RNA polymerase mutations with pleiotropic antibiotic resistance effects. J Antimicrob Chemother. 2017;72(1):75–84.
171. Hughes D, Andersson DI. Evolutionary trajectories to antibiotic resistance. Annu Rev Microbiol. 2017;71:579–96.
172. Acharya S, Foster PL, Brooks P, Fishel R. The coordinated functions of the *E. coli* MutS and MutL proteins in mismatch repair. Mol Cell. 2003;12(1):233–

46.
173. Walker GC. Mutagenesis and inducible responses to deoxyribonucleic acid damage in *Escherichia coli*. Microbiol Rev. 1984;48(1):60–93.
174. Erill I, Campoy S, Barbé J. Aeons of distress: an evolutionary perspective on the bacterial SOS response. FEMS Microbiol Rev. 2007;31(6):637–56.
175. Recacha E, Machuca J, Díaz de Alba P, Ramos-Güelfo M, Docobo-Pérez F, Rodriguez-Beltrán J, et al. Quinolone resistance reversion by targeting the SOS response. MBio. 2017;8(5).
176. Da Re S, Garnier F, Guérin E, Campoy S, Denis F, Ploy M-C. The SOS response promotes qnrB quinolone-resistance determinant expression. EMBO Rep. 2009;10(8):929–33.
177. Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of antibiotic resistance genes. Nature. 2004;427(6969):72–4.
178. Völgyi G, Vizserálek G, Takács-Novák K, Avdeef A, Tam KY. Predicting the exposure and antibacterial activity of fluoroquinolones based on physicochemical properties. Eur J Pharm Sci. 2012;47(1):21–7.
179. Nurchi VM, Crisponi G, Lachowicz JI, Zoroddu MA, Peana M, Medici S, et al. Fluoroquinolones: a micro-species equilibrium in the protonation of amphoteric compounds. Eur J Pharm Sci. 2016;93:380–91.
180. Danelon C, Nestorovich EM, Winterhalter M, Ceccarelli M, Bezrukov SM. Interaction of zwitterionic penicillins with the OmpF channel facilitates their translocation. Biophys J. 2006;90(5):1617–27.
181. Piddock LJ. Mechanism of quinolone uptake into bacterial cells. J Antimicrob Chemother. 1991;27(4):399–403.
182. O'Shea R, Moser HE. Physicochemical properties of antibacterial compounds: implications for drug discovery. J Med Chem. 2008;51(10):2871–8.
183. Delcour AH. Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta. 2009;1794(5):808–16.
184. Erdogan-Yildirim Z, Burian A, Manafi M, Zeitlinger M. Impact of pH on bacterial growth and activity of recent fluoroquinolones in pooled urine. Res Microbiol. 2011;162(3):249–52.
185. Lecomte S, Baron MH, Chenon MT, Coupry C, Moreau NJ. Effect of magnesium complexation by fluoroquinolones on their antibacterial properties. Antimicrob Agents Chemother. 1994;38(12):2810–6.
186. Lomaestro BM, Bailie GR. Quinolone-cation interactions: a review. DICP Ann Pharmacother. 1991;25(11):1249–58.
187. Kottur J, Nair DT. Reactive oxygen species play an important role in the bactericidal activity of quinolone antibiotics. Angew Chem Int Ed Engl. 2016;55(7):2397–400.
188. Martín-Gutiérrez G, Rodríguez-Martínez JM, Pascual Á, Rodríguez-Beltrán J, Blázquez J. Plasmidic qnr genes confer clinical resistance to ciprofloxacin under urinary tract physiological conditions. Antimicrob Agents Chemother. 2017;61(4):61–4.
189. EUCAST. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 10.0. [Internet]. 2020. p. 0–112. Available from: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_10.0_Breakpoint_Tables.pdf
190. EUCAST. The European Committee on Antimicrobial Susceptibility Testing

Breakpoint tables for interpretation of MICs and zone diameters European Committee on Antimicrobial Susceptibility Testing Breakpoint tables for interpretation of MICs and zone diameters. Vers [Internet]. 2022. p. 0–77. Available from: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf

191. Komp Lindgren P, Karlsson A, Hughes D. Mutation rate and evolution of fluoroquinolone resistance in *Escherichia coli* isolates from patients with urinary tract infections. Antimicrob Agents Chemother. 2003;47(10):3222–32.
192. Hombach M, Böttger EC, Roos M. The critical influence of the intermediate category on interpretation errors in revised EUCAST and CLSI antimicrobial susceptibility testing guidelines. Clin Microbiol Infect. 2013;19(2):E59-71.
193. Kalalahti I, Huotari K, Lahdensuo K, Tarkka E, Santti H, Rannikko A, et al. Rectal *E. coli* above ciprofloxacin ECOFF associate with infectious complications following prostate biopsy. Eur J Clin Microbiol Infect Dis. 2018;37(6):1055–60.
194. Longhi C, Conte MP, Marazzato M, Iebba V, Totino V, Santangelo F, et al. Plasmid-mediated fluoroquinolone resistance determinants in *Escherichia coli* from community uncomplicated urinary tract infection in an area of high prevalence of quinolone resistance. Eur J Clin Microbiol Infect Dis. 2012;31(8):1917–21.
195. Pasom W, Chanawong A, Lulitanond A, Wilailuckana C, Kenprom S, Puang-Ngern P. Plasmid-mediated quinolone resistance genes, aac(6')-Ib-cr, qnrS, qnrB, and qnrA, in urinary isolates of *Escherichia coli and Klebsiella pneumoniae* at a teaching hospital, Thailand. Jpn J Infect Dis. 2013;66(5):428–32.
196. Briales A, Rodríguez-Martínez JM, Velasco C, de Alba PD, Rodríguez-Baño J, Martínez-Martínez L, et al. Prevalence of plasmid-mediated quinolone resistance determinants qnr and aac(6')-Ib-cr in *Escherichia coli and Klebsiella pneumoniae* producing extended-spectrum β-lactamases in Spain. Int J Antimicrob Agents. 2012;39(5):431–4.
197. Raz R. Fosfomycin: an old-new antibiotic. Clin Microbiol Infect. 2012;18(1):4–7.
198. Popovic M, Steinort D, Pillai S, Joukhadar C. Fosfomycin: an old, new friend? Eur J Clin Microbiol Infect Dis. 2010;29(2):127–42.
199. Ruiz-Ramos J, Salavert Lleti M. Current key topics in fosfomycin. Fosfomycin in infections caused by multidrug-resistant Gram-negative pathogens. Rev Esp Quim. 2019;32(S1):45–54.
200. Castañeda-García A, Blázquez J, Rodríguez-Rojas A. Molecular mechanisms and clinical impact of acquired and intrinsic fosfomycin resistance. Antibiot (Basel, Switzerland). 2013;2(2):217–36.
201. Patel SS, Balfour JA, Bryson HM. Fosfomycin tromethamine. A review of its antibacterial activity, pharmacokinetic properties and therapeutic efficacy as a single-dose oral treatment for acute uncomplicated lower urinary tract infections. Drugs. 1997;53(4):637–56.
202. Wenzler E, Meyer KM, Bleasdale SC, Sikka M, Mendes RE, Bunnell KL et al. for the ARLG. *Ex vivo* urinary bactericidal activity and urinary pharmacodynamics of fosfomycin after two repeated dosing regimens of oral fosfomycin tromethamine in healthy adult subjects. Antimicrob Agents

Chemother. 2020;64(2):1–10.
203. Corvec S, Furustrand Tafin U, Betrisey B, Borens O, Trampuz A. Activities of fosfomycin, tigecycline, colistin, and gentamicin against extended-spectrum-β-lactamase-producing *Escherichia coli* in a foreign-body infection model. Antimicrob Agents Chemother. 2013;57(3):1421–7.
204. Naber KG, Thomas P, Fünfstück R. Fosfomycin trometamol in patients with renal insufficiency and in the elderly. Int Arab J Antimicrob Agents; Vol 2 No 1. 2012;
205. Oplinger M, Andrews C. Nitrofurantoin contraindication in patients with a creatinine clearance below 60 mL/min: looking for the evidence. Ann Pharmacother. 2013;47.
206. Kaye KS, Rice LB, Dane AL, Stus V, Sagan O, Fedosiuk E, et al. Fosfomycin for injection (ZTI-01) versus piperacillin-tazobactam for the treatment of complicated urinary tract infection including acute pyelonephritis: ZEUS, a phase 2/3 randomized trial. Clin Infect Dis. 2019;69(12):2045–56.
207. Dijkmans AC, Zacarías NVO, Burggraaf J, Mouton JW, Wilms EB, van Nieuwkoop C, et al. Fosfomycin: pharmacological, clinical and future perspectives. Antibiotics. 2017;6(4):1–17.
208. Wenzler E, Ellis-Grosse EJ, Rodvold KA. Pharmacokinetics, safety, and tolerability of single-dose intravenous (ZTI-01) and oral fosfomycin in healthy volunteers. Antimicrob Agents Chemother. 2017;61(9).
209. Wenzler E, Bleasdale SC, Sikka M, Bunnell KL, Finnemeyer M, Rosenkranz SL, et al. Phase I study to evaluate the pharmacokinetics, safety, and tolerability of two dosing regimens of oral fosfomycin tromethamine in healthy adult participants. Antimicrob Agents Chemother. 2018;62(8).
210. Sastry S, Doi Y. Fosfomycin: resurgence of an old companion. J Infect Chemother. 2016;22(5):273–80.
211. Silver LL. Fosfomycin: mechanism and resistance. Cold Spring Harb Perspect Med. 2017;7(2):1–12.
212. Falagas ME, Athanasaki F, Voulgaris GL, Triarides NA, Vardakas KZ. Resistance to fosfomycin: mechanisms, frequency and clinical consequences. Int J Antimicrob Agents. 2019;53(1):22–8.
213. Pullukcu H, Tasbakan M, Sipahi OR, Yamazhan T, Aydemir S, Ulusoy S. Fosfomycin in the treatment of extended spectrum beta-lactamase-producing *Escherichia coli*-related lower urinary tract infections. Int J Antimicrob Agents. 2007;29(1):62–5.
214. Falagas ME, Vouloumanou EK, Togias AG, Karadima M, Kapaskelis AM, Rafailidis PI, et al. Fosfomycin *versus* other antibiotics for the treatment of cystitis: a meta-analysis of randomized controlled trials. J Antimicrob Chemother. 2010;65(9):1862–77.
215. Wijma RA, Huttner A, Dun S Van, Kloezen W, Abbott IJ, Muller AE, et al. Urinary antibacterial activity of fosfomycin and nitrofurantoin at registered dosages in healthy volunteers. Int J Antimicrob Agents. 2019;54:435–41.
216. Stompór T. Recurrent lower urinary tract infections in adults: don't think it's *E. coli*, don't choose ciprofloxacin to treat. Polish Arch Intern Med. 2020;130(5):368–70.
217. Sojo-Dorado J, López-Hernández I, Rosso-Fernandez C, Morales IM, Palacios-Baena ZR, Hernández-Torres A, et al. Effectiveness of fosfomycin for the treatment of multidrug-resistant *Escherichia coli* bacteremic urinary tract

infections: a randomized clinical trial. JAMA Netw Open. 2022;5(1).
218. Ten Doesschate T, Kuiper S, van Nieuwkoop C, Hassing RJ, Ketels T, van Mens SP et al. Fosfomycin *vs* ciprofloxacin as oral step-down treatment for *Escherichia coli* febrile urinary tract infections in women: a randomized, placebo-controlled, double-blind, multicenter trial. Clin Infect Dis. 2022;75(2):221–9.
219. López-Montesinos, I Horcajada J. Fosfomicina oral e intravenosa en infecciones complicadas del tracto urinario. Rev Esp Quim. 2019;32(1):37–44.
220. Kadner RJ, Winkler HH. Isolation and characterization of mutations affecting the transport of hexose phosphates in *Escherichia coli*. J Bacteriol. 1973;113(2):895–900.
221. Tsuruoka T, Yamada Y. Charactertization of spontaneous fosfomycin (phosphonomycin)-resistant cells of *Escherichia coli in vitro*. J Antibiot (Tokyo). 1975;28(11):906–11.
222. Nilsson AI, Berg OG, Aspevall O, Kahlmeter G, Andersson DI. Biological costs and mechanisms of fosfomycin resistance in *Escherichia coli*. Antimicrob Agents Chemother. 2003;47(9):2850–8.
223. Grainger DC, Hurd D, Harrison M, Holdstock J, Busby SJW. Studies of the distribution of *Escherichia coli* cAMP-receptor protein and RNA polymerase along the *E. coli* chromosome. Proc Natl Acad Sci U S A. 2005;102(49):17693–8.
224. Sakamoto Y, Furukawa S, Ogihara H, Yamasaki M. Fosmidomycin resistance in adenylate cyclase deficient (cya) mutants of *Escherichia coli*. Biosci Biotechnol Biochem. 2003;67(9):2030–3.
225. Larson TJ, Cantwell JS, van Loo-Bhattacharya AT. Interaction at a distance between multiple operators controls the adjacent, divergently transcribed glpTQ-glpACB operons of *Escherichia coli* K-12. J Biol Chem. 1992;267(9):6114–21.
226. Merkel TJ, Dahl JL, Ebright RH, Kadner RJ. Transcription activation at the *Escherichia coli* uhpT promoter by the catabolite gene activator protein. J Bacteriol. 1995;177(7):1712–8.
227. Olekhnovich IN, Dahl JL, Kadner RJ. Separate contributions of UhpA and CAP to activation of transcription of the uhpT promoter of *Escherichia coli*. J Mol Biol. 1999;292(5):973–86.
228. Kim DH, Lees WJ, Kempsell KE, Lane WS, Duncan K, Walsh CT. Characterization of a Cys115 to Asp substitution in the *Escherichia coli* cell wall biosynthetic enzyme UDP-GlcNAc enolpyruvyl transferase (MurA) that confers resistance to inactivation by the antibiotic fosfomycin. Biochemistry. 1996;35(15):4923–8.
229. Horii T, Kimura T, Sato K, Shibayama K, Ohta M. Emergence of fosfomycin-resistant isolates of Shiga-like toxin-producing *Escherichia coli* O26. Antimicrob Agents Chemother. 1999;43(4):789–93.
230. Couce A, Briales A, Rodríguez-Rojas A, Costas C, Pascual A, Blázquez J. Genomewide overexpression screen for fosfomycin resistance in *Escherichia coli*: MurA confers clinical resistance at low fitness cost. Antimicrob Agents Chemother. 2012;56(5):2767–9.
231. Kobayashi S, Kuzuyama T, Seto H. Characterization of the fomA and fomB gene products from *Streptomyces wedmorensis*, which confer fosfomycin resistance on *Escherichia coli*. Antimicrob Agents Chemother.

2000;44(3):647–50.
232. Rigsby RE, Fillgrove KL, Beihoffer LA, Armstrong RN. Fosfomycin resistance proteins: a nexus of glutathione transferases and epoxide hydrolases in a metalloenzyme superfamily. Methods Enzymol. 2005;401:367–79.
233. Lupo A, Saras E, Madec J-Y, Haenni M. Emergence of blaCTX-M-55 associated with fosA, rmtB and mcr gene variants in *Escherichia coli* from various animal species in France. J Antimicrob Chemother. 2018;73(4):867–72.
234. Lee SY, Park YJ, Yu JK, Jung S, Kim Y, Jeong SH, et al. Prevalence of acquired fosfomycin resistance among extended-spectrum β-lactamase-producing *Escherichia coli and Klebsiella pneumoniae* clinical isolates in Korea and IS26-composite transposon surrounding fosA3. J Antimicrob Chemother. 2012;67(12):2843–7.
235. Wang X-M, Dong Z, Schwarz S, Zhu Y, Hua X, Zhang Y, et al. Plasmids of diverse Inc groups disseminate the fosfomycin resistance gene fosA3 among *Escherichia coli* isolates from pigs, chickens, and dairy cows in Northeast China. Antimicrob Agents Chemother. 2017;61(9).
236. Xie M, Lin D, Chen K, Chan EWC, Yao W, Chen S. Molecular characterization of *Escherichia coli* strains isolated from retail meat that harbor blaCTX-M and fosA3 genes. Antimicrob Agents Chemother. 2016;60(4):2450–5.
237. Tseng S-P, Wang S-F, Kuo C-Y, Huang J-W, Hung W-C, Ke G-M, et al. Characterization of fosfomycin resistant extended-spectrum β-lactamase-producing *Escherichia coli* isolates from human and pig in Taiwan. PLoS One. 2015;10(8):e0135864.
238. Mendoza C, Garcia JM, Llaneza J, Mendez FJ, Hardisson C, Ortiz JM. Plasmid-determined resistance to fosfomycin in *Serratia marcescens*. Antimicrob Agents Chemother. 1980;18(2):215–9.
239. Mei Q, Ye Y, Zhu Y-L, Cheng J, Chang X, Liu Y-Y, et al. Testing the mutant selection window hypothesis *in vitro* and *in vivo* with *Staphylococcus aureus* exposed to fosfomycin. Eur J Clin Microbiol Infect Dis. 2015;34(4):737–44.
240. Fu Z, Ma Y, Chen C, Guo Y, Hu F, Liu Y, et al. Prevalence of fosfomycin resistance and mutations in murA, glpT, and uhpT in methicillin-resistant *Staphylococcus aureus* strains isolated from blood and cerebrospinal fluid samples. Front Microbiol. 2015;6:1544.
241. Oteo J, Bautista V, Lara N, Cuevas O, Arroyo M, Fernández S, et al. Parallel increase in community use of fosfomycin and resistance to fosfomycin in extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli*. J Antimicrob Chemother. 2010;65(11):2459–63.
242. Greenwood D, Jones A, Eley A. Factors influencing the activity of the trometamol salt of fosfomycin. Eur J Clin Microbiol. 1986;5(1):29–34.
243. Fedrigo NH, Mazucheli J, Albiero J, Shinohara DR, Lodi FG, Machado ACDS, et al. Pharmacodynamic evaluation of fosfomycin against *Escherichia coli and Klebsiella spp.* from frinary tract tnfections and the influence of pH on fosfomycin activities. Antimicrob Agents Chemother. 2017;61(8).
244. Kurabayashi K, Tanimoto K, Fueki S, Tomita H, Hirakawa H. Elevated expression of GlpT and UhpT via FNR activation contributes to increased fosfomycin susceptibility in *Escherichia coli* under anaerobic conditions. Antimicrob Agents Chemother. 2015;59(10):6352–60.

245. Kurabayashi K, Tanimoto K, Tomita H, Hirakawa H. Cooperative actions of CRP-cAMP and FNR increase the fosfomycin susceptibility of Enterohaemorrhagic *Escherichia coli* (EHEC) by elevating the expression of glpT and uhpT under anaerobic conditions. Front Microbiol. 2017;8:426.
246. Ballestero-Téllez M, Docobo-Pérez F, Portillo-Calder On I, Rodr Iguez-Mart Inez JM, Racero L, Ramos-Guelfo MS, et al. Molecular insights into fosfomycin resistance in *Escherichia coli*. J Antimicrob Chemother. 2017;72(5):1303–9.
247. Abbott IJ, Meletiadis J, Belghanch I, Wijma RA, Kanioura L, Roberts JA, et al. Fosfomycin efficacy and emergence of resistance among *Enterobacteriaceae in an in vitro* dynamic bladder infection model. J Antimicrob Chemother. 2018;73:709–19.
248. Ohkoshi Y, Sato T, Suzuki Y, Yamamoto S, Shiraishi T, Ogasawara N, et al. Mechanism of reduced susceptibility to fosfomycin in *Escherichia coli* clinical isolates. BioMed. 2017;(5470241):1–8.
249. Carolina A, Campos C, Nath´ N, Andrade NL, Couto N, Mutters NT, et al. Characterization of fosfomycin heteroresistance among MDR *Escherichia coli* isolates from hospitalized patients in Rio de Janeiro, Brazil. J Glob Antimicrob Resist. 2020;22:584–93.
250. Pourbaix A, Guérin F, Lastours V de, Chau F, Auzou M, Boulley E, et al. Biological cost of fosfomycin resistance in *Escherichia coli* in a murine model of urinary tract infection. Int J Med Microbiol. 2017;307(8):452–9.
251. Veroux M, Giuffrida G, Corona D, Gagliano M, Scriffignano V, Vizcarra D, et al. Infective complications in renal allograft recipients: epidemiology and outcome. Transplant Proc. 2008;40(6):1873–6.
252. Alangaden G. Urinary tract infections in renal transplant recipients. Curr Infect Dis Rep. 2007;9(6):475–9.
253. Pellé G, Vimont S, Levy PP, Hertig A, Ouali N, Chassin C, et al. Acute pyelonephritis represents a risk factor impairing long-term kidney graft function. Am J Transplant. 2007;7(4):899–907.
254. Khosravi A, Montazeri E, Ghorbani A, Parhizgari N. Bacterial urinary tract infection in renal transplant recipients and their antibiotic resistance pattern: a four-year study. Iran J Microbiol. 2014;6(2):74–8.
255. Vidal E, Torre-Cisneros J, Blanes M, Montejo M, Cervera C, Aguado JM, et al. Bacterial urinary tract infection after solid organ transplantation in the RESITRA cohort. Transpl Infect Dis. 2012;14(6):595–603.
256. Alangaden GJ, Thyagarajan R, Gruber SA, Morawski K, Garnick J, El-Amm JM, et al. Infectious complications after kidney transplantation: current epidemiology and associated risk factors. Clin Transplant. 2006;20(4):401–9.
257. Anastasopoulos N-A, Duni A, Peschos D, Agnantis N, Dounousi E. The spectrum of infectious diseases in kidney transplantation: a review of the classification, pathogens and clinical manifestations. In Vivo (Brooklyn). 2015;29(4):415–22.
258. Coussement J, Abramowicz D. Should we treat asymptomatic bacteriuria after renal transplantation? Nephrol Dial Transplant. 2014;29(2):260–2.
259. Origüen J, López-Medrano F, Fernández-Ruiz M, Polanco N, Gutiérrez E, González E, et al. Should asymptomatic bacteriuria be systematically treated in kidney transplant recipients? Results from a randomized controlled trial. Am J Transplant. 2016;16(10):1–11.

260. de Souza RM, Olsburgh J. Urinary tract infection in the renal transplant patient. Nat Clin Pract Nephrol. 2008;4(5):252–64.
261. Capocasale E, Vecchi ED, Mazzoni MP, Valle RD, Pellegrino C, Ferretti S, et al. Surgical site and early urinary tract infections in 1000 kidney transplants with antimicrobial perioperative prophylaxis. Transplant Proc. 2014;46(10):3455–8.
262. Papasotiriou M, Savvidaki E, Kalliakmani P, Papachristou E, Marangos M, Fokaefs E, et al. Predisposing factors to the development of urinary tract infections in renal transplant recipients and the impact on the long-term graft function. Ren Fail. 2011;33(4):405–10.
263. Shin DH, Kim EJ, Lee S, Kim SJ, Oh J. Early-onset graft pyelonephritis is predictive of long-term outcome of renal allografts. Tohoku J Exp Med. 2015;236:175–83.
264. Naik A, Dharnidharka V, Schnitzler M, Brennan D, Segev D, Axelrod D, et al. Clinical and economic consequences of first-year UTI, sepsis and pneumonia in contemporary kidney transplantation practice. Transl Int. 2016;29(2):241–52.
265. Lentine KL, Gheorghian A, Axelrod D, Kalsekar A, L'italien G, Schnitzler MA. The implications of acute rejection for allograft survival in contemporary U.S. kidney transplantation. Transplantation. 2012;94(4):369–76.
266. Becerra BJ, Becerra MB, Safdar N. A nationwide assessment of the burden of urinary tract infection among renal transplant recipients. J Transplant. 2015;854640.
267. Fiorante S, Fernández-ruiz M, López-medrano F, Lizasoain M, Lalueza A, Morales JM, et al. Acute graft pyelonephritis in renal transplant recipients: incidence, risk factors and long-term outcome. Nephrol Dial Transplant. 2011;26(3):1065–73.
268. Parasuraman R, Julian K. Urinary tract infections in solid organ transplantation. Am J Transplant. 2013;13(S4):327–36.
269. Parapiboon W, Ingsathit A, Jirasiritham S, Sumethkul V. High incidence of bacteriuria in early post-kidney transplantation; results from a randomized controlled study. Transplant Proc. 2012;44(3):734–6.
270. Singh R, Bemelman FJ, Hodiamont CJ, Idu MM, Berge IJM, Geerlings SE. The impact of trimethoprim-sulfamethoxazole as *Pneumocystis jiroveci* pneumonia prophylaxis on the occurrence of asymptomatic bacteriuria and urinary tract infections among renal allograft recipients : a retrospective before-after study. BMC Infect Dis. 2016;16:1–10.
271. Gołębiewska J, Dębska-Ślizień A, Komarnicka J, Samet A, Rutkowski B, Gołębiewska J, et al. Urinary tract infections in renal transplant recipients. Transplant Proc. 2011;43(8):2985–90.
272. Nicolle LE. Urinary tract infections in special populations. diabetes, renal transplant, HIV infection, and spinal cord injury. Infect Dis Clin North Am. 2014;28(1):91–104.
273. Singh R, Geerlings SE, Bemelman FJ. Asymptomatic bacteriuria and urinary tract infections among renal allograft recipients. Curr Opin Infect Dis. 2015;28(1):112–6.
274. Gondos AS, Al-Moyed KA, Al-Robasi ABA, Al-Shamahy HA, Alyousefi NA. Urinary tract infection among renal transplant recipients in Yemen. PLoS One. 2015;10(12):1–10.

275. Wu X, Dong Y, Liu Y, Li Y, Sun Y, Wang J, et al. The prevalence and predictive factors of urinary tract infection in patients undergoing renal transplantation: a meta-analysis. Am J Infect Control. 2016;44(11):1261–8.
276. Gołe'biewska J, De'bska-Ślizień A, Rutkowski B. Treated asymptomatic bacteriuria during first year after renal transplantation. Transpl Infect Dis. 2014;16(4):605–15.
277. Chuang P, Parikh CR, Langone A. Urinary tract infections after renal transplantation: a retrospective review at two US transplant centers. Clin Transplant. 2005;19(2):230–5.
278. Dharnidharka VR, Agodoa LY, Abbott KC. Risk factors for hospitalization for bacterial or viral infection in renal transplant recipients-an analysis of USRDS data. Am J Transplant. 2007;7(3):653–61.
279. Giral M, Pascuariello G, Karam G, Hourmant M, Cantarovich D, Dantal J, et al. Acute graft pyelonephritis and long-term kidney allograft outcome. Kidney Int. 2002;61(5):1880–6.
280. Abbott KC, Swanson SJ, Richter ER, Bohen EM, Agodoa LY, Peters TG, et al. Late urinary tract infection after renal transplantation in the United States. Am J Kidney Dis. 2004;44(2):353–62.
281. Lorenz EC, Cosio FG. The impact of urinary tract infections in renal transplant recipients. Kidney Int. 2010;78(8):719–21.
282. Ariza-Heredia EJ, Beam EN, Lesnick TG, Cosio FG, Kremers WK, Razonable RR. Impact of urinary tract infection on allograft function after kidney transplantation. Clin Transplant. 2014;28(6):683–90.
283. Boskabadi ALI, Jalali A, Moradi M, Abbasi M, Moradi A, Boskabadi ALI, et al. Effect of antibiotic therapy on asymptomatic bacteriuria in kidney transplant recipients. Urol J. 2005;2(1):32–5.
284. Bodro M, Sanclemente G, Lipperheide I, Allali M, Marco F, Bosch J, et al. Impact of urinary tract infections on short-term kidney graft outcome. Clin Microbiol Infect. 2015;21(12):1104.e1-1104.e8.
285. Lee JJR, Bang H, Dadhania D, Hartono C, Aull MJM, Satlin M, et al. Independent risk factors for urinary tract infection and for subsequent bacteremia or acute cellular rejection: a single-center report of 1166 kidney allograft recipients. Transplantation. 2013;96(8):732–8.
286. Green H, Rahamimov R, Gafter U, Leibovitci L, Paul M. Antibiotic prophylaxis for urinary tract infections in renal transplant recipients: a systematic review and meta-analysis. Transpl Infect Dis. 2011;13(5):441–7.
287. Amari EB El, Hadaya K, Bühler L, Berney T, Rohner P, Martin PY, et al. Outcome of treated and untreated asymptomatic bacteriuria in renal transplant recipients. Nephrol Dial Transplant. 2011;26(12):4109–14.
288. Bodro M, Sanclemente G, Lipperheide I, Allali M, Marco F, Bosch J, et al. Impact of antibiotic resistance on the development of recurrent and relapsing symptomatic urinary tract infection in kidney recipients. Am J Transplant. 2015;15(4):1021–7.
289. Rice JC, Peng T, Kuo YF, Pendyala S, Simmons L, Boughton J, et al. Renal allograft injury is associated with urinary tract infection caused by *Escherichia coli* bearing adherence factors. Am J Transplant. 2006;6(10):2375–83.
290. Kamath NS, John GT, Neelakantan N, Kirubakaran MG, Jacob CK. Acute graft pyelonephritis following renal transplantation. Transpl Infect Dis. 2006;8(3):140–7.

291. Ciszek M, Paczek L, Bartłomiejczyk I, Mucha K. Urine cytokines profile in renal transplant patients with asymptomatic bacteriuria. Transplantation. 2006;81(12):1653–7.
292. Fiorante S, López-Medrano F, Lizasoain M, Lalueza A, Juan RS, Andrés A, et al. Systematic screening and treatment of asymptomatic bacteriuria in renal transplant recipients. Kidney Int. 2010;78(8):774–81.
293. Al-Hasan MN, Razonable RR, Kremers WK BL. Impact of Gram-negative bloodstream infection on long-term allograft survival after kidney transplantation. Transplantation. 2011;91(11).
294. Singh R, Bemelman FJ, Geerlings SE. Asymptomatic bacteriuria in renal allograft recipients: not so innocent after all? Futur Microbiol. 2016;11(1):1–3.
295. Nicolle LE. Asymptomatic bacteriuria: when to screen and when to treat. Infect Dis Clin North Am. 2003;17(2):367–94.
296. Nicolle L, Bradley S, Colgan R, Rice J, Schaeffer A, Hooton T. Infectious Diseases Society of America guidelines for the diagnosis and treatment of asymptomatic bacteriuria in adults. Clin Infect Dis. 2005;40(5):643–54.
297. Nicolle LE, Gupta K, Bradley SF, Colgan R, Demuri GP, Drekonja D, et al. Clinical practice guideline for the management of asymptomatic bacteriuria: 2019 update by the Infectious Diseases Society of America. 2019;1–28.
298. Fiorentino M, Pesce F, Schena A, Simone S, Castellano G, Gesualdo L. Updates on urinary tract infections in kidney transplantation. J Nephrol. 2019;0(0):1–11.
299. Gołębiewska J, Krawczyk B, Wysocka M, Dudziak A D-SA. Asymptomatic bacteriuria in kidney transplant recipients — a narrative review. Medicina (B Aires). 2023;59(108):403–15.
300. Ghosh A, Mukherjee M. Incidence of multidrug resistance, pathogenicity island markers, and pathoadaptive FimH mutations in uropathogenic *Escherichia coli* isolated from asymptomatic hospitalized patients. Folia Microbiol (Praha). 2019;64(4):587–600.
301. Muñoz P. Management of urinary tract infections and lymphocele in renal transplant recipients. Clin Infect Dis. 2001;33(1):S53-7.
302. Ramadas P, Rajendran PP, Krishnan P, Alex A, Siskind E, Kadiyala A, et al. Extended-spectrum-beta-lactamase producing bacteria related urinary tract infection in renal transplant recipients and effect on allograft function. PLoS One. 2014;9(3):1–7.
303. Origüen J, Fernández-Ruiz M, López-Medrano F, Ruiz-Merlo T, González E, Morales JM, et al. Progressive increase of resistance in *Enterobacteriaceae* urinary isolates from kidney transplant recipients over the past decade: narrowing of the therapeutic options. Transpl Infect Dis. 2016;18(4):575–84.
304. Valera B, Gentil MA, Cabello V, Fijo J, Cordero E, Cisneros JM. Epidemiology of urinary infections in renal transplant recipients. Transplant Proc. 2006;38(8):2414–5.
305. Kawecki D, Kwiatkowski A, Durlik M, Paczek L, Chmura A, Mlynarczyk G, et al. Urinary tract infections in the early posttransplant period after kidney transplantation: etiologic agents and their susceptibility. Transplant Proc. 2011;43(8):2991–3.
306. Pinheiro H, Mituiassu A, Carminatti M, Braga A, Bastos M. Urinary tract infection caused by extended-spectrum beta-lactamase-producing bacteria in kidney transplant patients. Transplant Proc. 2010;42(2):486–7.

307. Ak O, Yildirim M, Kucuk HF, Gencer S, Demir T. Infections in renal transplant patients: risk factors and infectious agents. Transplant Proc. 2013;45(3):944–8.
308. Menegueti M, Pereira MF, Bellissimo-Rodrigues F, Garcia TMP, Saber LTS, Nardim MEP, et al. Study of the risk factors related to acquisition of urinary tract infections in patients submitted to renal transplant. Rev Soc Bras Med Trop. 2015;48(3):285–90.
309. Senger S, Arslan H, Azap O, Timurkaynak F, Çağir U, Haberal M. Urinary tract infections in renal transplant recipients. Transplant Proc. 2007;39(4):1016–7.
310. Brizendine K, Richter S, Cober E, Van Duin D. Carbapenem-resistant *Klebsiella pneumoniae* urinary tract infection following solid organ transplantation. Antimicrob Agents Chemother. 2015;59(1):553–7.
311. del Rosario-Quintana C, Tosco-Núñez T, Lorenzo L, Martín-Sánchez AM, Molina-Cabrillana J. Prevalencia y factores asociados a la colonización de microorganismos multirresistentes en centros de larga estancia de Gran Canaria. Rev Esp Geriatr Gerontol. 2015;50(5):232–6.
312. Osthoff M, McGuinness SL, Wagen AZ, Eisen DP. Urinary tract infections due to extended-spectrum beta-lactamase-producing Gram-negative bacteria: identification of risk factors and outcome predictors in an Australian tertiary referral hospital. Int J Infect Dis. 2015;34:79–83.
313. Vidal E, Cervera C, Cordero E, Armiñanzas C, Carratalá J, Cisneros JM, et al. Management of urinary tract infection in solid organ transplant recipients: consensus statement of the Group for the Study of Infection in Transplant Recipients (GESITRA) of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC). Enferm Infecc Microbiol Clin. 2015;33(10):1–21.
314. Yilmaz E, Akalin H, Ozbey S, Kordan Y, Sinirtaş M, Gürcüoglu E, et al. Risk factors in community-acquired/onset urinary tract infections due to extended-spectrum beta-lactamase-producing *Escherichia coli and Klebsiella pneumoniae*. J Chemother. 2008;20(5):581–5.
315. Grover ML, Bracamonte JD, Kanodia AK, Edwards FD, Weaver AL. Urinary tract infection in women over the age of 65: is age alone a marker of complication? J Am Board Fam Med. 2009;22(3):266–71.
316. Calbo E, Romaní V, Xercavins M, Gómez L, Vidal CG, Quintana S, et al. Risk factors for community-onset urinary tract infections due to *Escherichia coli* harbouring extended-spectrum β-lactamases. J Antimicrob Chemother. 2006;57(4):780–3.
317. Barchiesi F, Montalti R, Castelli P, Nicolini D, Staffolani S, Mocchegiani F, et al. Carbapenem-resistant *Klebsiella pneumoniae* influences the outcome of early infections in liver transplant recipients. BMC Infect Dis. 2016;16(1):538.
318. Linares L, Cervera C, Cofán F, Ricart MJ, Esforzado N, Torregrosa V, et al. Epidemiology and outcomes of multiple antibiotic-resistant bacterial infection in renal transplantation. Transplant Proc. 2007;39(7):2222–4.
319. Pouch S, Kubin C, Satlin M, Tsapepas D, Lee J, Dub G, et al. Epidemiology and outcomes of carbapenem-resistant *K. pneumonia* bacteriuria in kidney trnaplsant recipients. Transpl Infect Dis. 2015;17((6)):800–9.
320. Song J, Hwang H, Yoon H, Kim J et al. Endoscopic subureteral polydimethylsiloxane injection and prevention of recurrent acute graft

pyelonephritis. Nephron Clin Pr. 2011;117(4):c385-9.
321. Memikoğlu KO, Keven K, Şengül Ş, Soypaçaci Z, Ertürk Ş, Erbay B. Urinary tract infections following renal transplantation: a single-center experience. Transplant Proc. 2007;39(10):3131–4.
322. Dupont PJ, Psimenou E, Lord R, Buscombe JR, Hilson AJ, Sweny P. Late recurrent urinary tract infections may produce renal allograft scarring even in the absence of symptoms or vesicoureteric reflux. Transplantation. 2007;84(3):351–5.
323. Albert X, Huertas I, Pereiro I, Sanfélix J, Gosalbes V, Perrotta C. Antibiotics for preventing recurrent urinary tract infection in non-pregnant women (review). Aten Primaria. 2008;(3):3–5.
324. Bodro M, Linares L, Chiang D, Moreno A, Cervera C. Managing recurrent urinary tract infections in kidney transplant patients. Expert Rev Anti Infect Ther. 2018;16(9):723–32.
325. Tavakoli A, Surange R, Pearson R, Parrott N, Augustine T, Riad H. Impact of stents on urological complications and health care expenditure in renal transplant recipients: results of a prospective, randomized clinical trial. J Urol. 2007;177(6):2260–4.
326. Britt NS, Hagopian JC, Brennan DC, Pottebaum AA, Santos CAQ, Gharabagi A, et al. Effects of recurrent urinary tract infections on graft and patient outcomes after kidney transplantation. Nephrol Dial Transplant. 2017;32(10):1758–66.
327. Tawab KA, Gheith O, Al Otaibi T, Nampoory N, Mansour H, Halim MA, et al. Recurrent urinary tract infection among renal transplant recipients: risk factors and long-term outcome. Exp Clin Transplant. 2017;15(2):157–63.
328. Sorto R, Irizar SS, Delgadillo G, Alberú J, Correa-Rotter R, Morales-Buenrostro LE. Risk factors for urinary tract infections during the first year after kidney transplantation. Transplant Proc. 2010;42(1):280–1.
329. Silva C, Afonso N, Macário F, Alves R, Mota A. Recurrent urinary tract infections in kidney transplant recipients. Transplant Proc. 2013;45(3):1092–5.
330. Pilmis B, Scemla A, Join-Lambert O, Mamzer M-F, Lortholary O, Legendre C, et al. ESBL-producing *Enterobacteriaceae*-related urinary tract infections in kidney transplant recipients: incidence and risk factors for recurrence. Infect Dis (London, England). 2015;47(10):714–8.
331. Gołębiewska JE, Dębska-Ślizień A, Rutkowski B. Urinary tract infections during the first year after renal transplantation: one center's experience and a review of the literature. Clin Transplant. 2014;28(11):1263–70.
332. Säemann M, Hörl W. Urinary tract infection in renal transplant recipients. Eur J Clin Invest. 2008;38(2):58–65.
333. Dinckan A, Aliosmanoglu I, Kocak H, Gunseren F, Mesci A, Ertug Z, et al. Surgical correction of vesico-ureteric reflux for recurrent febrile urinary tract infections after kidney transplantation. BJU Int. 2013;112(4):366–71.
334. López-Medrano F, García-Bravo M, Morales J, Andrés A, San Juan R, Lizasoain M et al. Urinary tract infection due to *Corynebacterium urealyticum* in kidney transplant recipients: an underdiagnosed etiology for obstructive uropathy and graft dysfunction — results of a prospective cohort study. Clin Infect Dis. 2008;46(6):825–30.
335. Kicia M, Wesolowska M, Kopacz Z, Jakuszko K, Sak B, Krajewska M, et al. Prevalence and molecular characteristics of urinary and intestinal

microsporidia infections in renal transplant recipients. Clin Microb Infect. 2016;22(462):5–9.
336. Terpstra M, Remmerswaal E, van Aalderen M, Wever J, Sinnige M, van der Bom-Baylon N. Circulating mucosal-associated invariant T cells in subjects with recurrent urinary tract infections are functionally impaired. Immun Inflamm Dis. Inmun Inflamm Dis. 2020;8(1):80–92.
337. Korayem GB, Zangeneh TT, Matthias KR. Recurrence of urinary tract infections and development of urinary-specific antibiogram for kidney transplant recipients. J Glob Antimicrob Resist. 2018;12:119–23.
338. Goldman JD, Julian K. Urinary tract infections in solid organ transplant recipients: Guidelines from the American Society of Transplantation Infectious Diseases Community of Practice. Clin Transplant. 2019;33:e13507.
339. Rabkin DG, Stifelman MD, Birkhoff J, Richardson KA, Cohen D, Nowygrod R, et al. Early catheter removal decreases incidence of urinary tract infections in renal transplant recipients. Transplant Proc. 1998;30(8):4314–6.
340. Wilson CH, Rix DA, Manas DM. Routine intraoperative ureteric stenting for kidney transplant recipients. Cochrane database Syst Rev. 2013;(6):CD004925.
341. Rudrabhatla P, Deepanjali S, Mandal J, Swaminathan RP, Kadhiravan T. Stopping the effective non-fluoroquinolone antibiotics at day 7 *vs* continuing until day 14 in adults with acute pyelonephritis requiring hospitalization: A randomized non-inferiority trial. PLoS One. 2018;13(5):e0197302.
342. Kim DK, Kim JH, Lee JY, Ku NS, Lee HS, Park J-Y, et al. Reappraisal of the treatment duration of antibiotic regimens for acute uncomplicated cystitis in adult women: a systematic review and network meta-analysis of 61 randomised clinical trials. Lancet Infect Dis. 2020;
343. Derington CG, Benavides N, Delate T FD. Multiple-dose oral fosfomycin for treatment of complicated urinary tract infections in the outpatient setting. Open Forum Infect Dis. 2020;7(2):ofaa034.
344. Ten Doesschate T, Van Mens SP, Van Nieuwkoop C, Geerlings SE, Hoepelman AIM, Bonten MJM, et al. Oral fosfomycin versus ciprofloxacin in women with *E.coli* febrile urinary tract infection, a double-blind placebo-controlled randomized controlled non-inferiority trial (FORECAST). BMC Infect Dis. 2018;18(1):626.
345. Sharma S, Verma P, Rawat V, Varshney U, Singh R. Fosfomycin *versus* nitrofurantoin for the treatment of lower UTI in outpatients. J Lab Physicians. 2021;13(2):118–22.
346. Mazhar A, Saliha A SH. Stewardship opportunities in the treatment of urinary tract infection using oral fosfomycin. Med J Malaysia. 2019;74(5):456–8.
347. López-Medrano FL, Tiago J, Mario S, Ruiz F, Vidal E, Origüen J, et al. Oral fosfomycin for the treatment of lower urinary tract infections among kidney transplant recipients — results of a Spanish multicenter cohort. Am J Transplant. 2020;20(2):451–62.
348. Ten Doesschate T, van Werkhoven H, Meijvis S, Stalenhoef J, Van Zuilen A, de Vries A, et al. Fosfomycin-trometamol for urinary tract infections in kidney transplant recipients. Transplantation. 2019;103(6):1272–6.
349. Kerstenetzky L, Jorgenson M, Descourouez J, Leverson G, Rose W, Redfield R, et al. Fosfomycin tromethamine for the treatment of cystitis in abdominal solid organ transplant recipients with renal dysfunction. Ann Pharmactotherapy. 2017;51(9):751–6.

350. Loethen A, Kerstenetzky L, Descourouez J, Leverson G, Smith J, Jorgenson M. Fosfomycin for the treatment of cystitis in the abdominal solid organ transplant population. Pharmacotherapy. 2017;37(5):599–606.
351. Ruiz-Ruigómez M, Fernández-Ruiz M, Silva JT, Vidal E, Origüen J, Calvo-Cano A, et al. Efficacy and safety of oral fosfomycin for asymptomatic bacteriuria in kidney transplant recipients: results from a Spanish multicenter cohort. Antimicrob Agents Chemother. 2021;65(5):1–9.
352. Cagnacci S, Gualco L, Debbia E, Schito GC, Marchese A. European emergence of ciprofloxacin-resistant *Escherichia coli* clonal groups O25:H4-ST 131 and O15:K52:H1 causing community-acquired uncomplicated cystitis. J Clin Microbiol. 2008;46(8):2605–12.
353. Aypak C, Altunsoy A, Düzgün N. Empiric antibiotic therapy in acute uncomplicated urinary tract infections and fluoroquinolone resistance: a prospective observational study. Ann Clin Microbiol Antimicrob. 2009;8:27.
354. European Medicines Agency, Pharmacovigilance Risk Assessment Committee (PRAC). Fluoroquinolone and quinolone antibiotics: PRAC recommends new restrictions on use following review of disabling and potentially long-lasting side effects. [Internet]. 2018. Available from: https://www.ema.europa.eu/en/news/disabling-potentially-permanent-side-effects-lead-suspension-restrictions-quinolone-fluoroquinolone
355. Increased risk of ruptures or tears in the aorta blood vessel with fluoroquinolone antibiotics in certain patients. FDA Drug Safety Communication. [Internet]. 2018. Available from: https://www.fda.gov/drugs/drug-safety-and-availability/fda-warns-about-increased-risk-ruptures-or-tears-aorta-blood-vessel-fluoroquinolone-antibiotics
356. European Medicines Agency. Disabling and potentially permanent side effects lead to suspension or restrictions of quinolone and fluoroquinolone antibiotics. [Internet]. 2019. p. 1–4. Available from: https://www.ema.europa.eu/en/documents/press-release/disabling-potentially-permanent-side-effects-lead-suspension-restrictions-quinolone-fluoroquinolone_en.pdf
357. Falagas ME, Kastoris AC, Kapaskelis AM, Karageorgopoulos DE. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum beta-lactamase producing, *Enterobacteriaceae* infections: a systematic review. Lancet Infect Dis. 2010;10(1):43–50.
358. García-Rodríguez J, Trujillano Martín I, Baquero F, Cisterna R, Gobernado M, Liñares F, et al. In vitro activity of fosfomycin trometamol against pathogens from urinary tract infections: a Spanish multicenter study. J Chemother. 1997;9(6):394–402.
359. Seroy JT, Grim SA, Reid GE, Wellington T, Clark NM. Treatment of MDR urinary tract infections with oral fosfomycin: a retrospective analysis. J Antimicrob Chemother. 2016;71(9):2563–8.
360. Neuner EA, Sekeres J, Hall GS, Van Duin D. Experience with fosfomycin for treatment of urinary tract infections due to multidrug-resistant organisms. Antimicrob Agents Chemother. 2012;56(11):5744–8.
361. Rosa R, Rudin SD, Rojas LJ, Hujer AM, Perez-Cardona A, Perez F, et al. “Double carbapenem” and oral fosfomycin for the treatment of complicated urinary tract infections caused by bla(NDM)-harboring *Enterobacteriaceae* in kidney transplantation. Transpl Infect Dis. 2018;20(1).

362. Cai T, Tamanini I, Tascini C, Köves B, Bonkat G, Gacci M, Novelli A, Horcajada JP, Bjerklund Johansen TE ZG. Fosfomycin trometamol *versus* comparator antibiotics for the treatment of acute uncomplicated urinary tract infections in women: a systematic review and meta-analysis. J Urol. 2020;203(3):570–8.
363. Maraki S, Samonis G, Rafailidis PI, Vouloumanou EK, Mavromanolakis E, Falagas ME. Susceptibility of urinary tract bacteria to fosfomycin. Antimicrob Agents Chemother. 2009;53(10):4508–10.
364. Senol S, Tasbakan M, Pullukcu H, Sipahi OR, Sipahi H, Yamazhan T, et al. Carbapenem *vs* fosfomicina in the treatment of *E coli* BLEE-related complicated lower urinary tract infection. J Chemother. 2010;22(5):355–7.
365. Rodríguez-Baño J, Alcalá JC, Cisneros JM, Grill F, Oliver A, Horcajada JP, et al. Community infections caused by extended-spectrum β-lactamase–producing *Escherichia coli*. Arch Intern Med. 2008;168(17):1897–902.
366. Reid GE, Grim SA, Layden JE, Akkina S, Tang I, Campara M, et al. The use of fosfomycin to treat urinary tract infections in kidney transplant recipients. Transplantation. 2013;96(3):e12-4.
367. Golebiewska JE, Debska-Ślizień A, Rutkowski B. Urinary tract infections during the first year after renal transplantation: one center's experience and a review of the literature. Clin Transplant. 2014;28(11):1263–70.
368. Moreno A, Pachon J, Codero E. Uso de fosfomicina en el tratamiento de la infección del tracto urinario bajo en el paciente trasplantado renal. Trabajo fin de grado. Universidad de Sevilla. 2018. Universidad de Sevilla;
369. Audard V, Amor M, Desvaux D, Pastural M, Baron C, Philippe R, et al. Acute graft pyelonephritis: a potential cause of acute rejection in renal transplant. Transplantation. 2005;80(8):1128–30.
370. Sabé N, Oriol I, Melilli E, Manonelles A, Bestard O, Polo C, et al. Antibiotic treatment *versus* no treatment for asymptomatic bacteriuria in kidney transplant recipients: a multicenter randomized trial. Open Forum Infect Dis. 2019;21(6):ofz24.
371. Coussement J, Scemla A, Abramowicz D, Evi N, Angela C. Antibiotics for asymptomatic bacteriuria in kidney transplant recipients (review). Cochrane Database Syst Rev. 2018;2(2):CD011357.
372. Gómez-Ochoa SA, Vega-Vera A. Systematic review and meta-analysis of asymptomatic bacteriuria after renal transplantation: incidence, risk of complications, and treatment outcomes. Transpl Infect Dis. 2020;22(1):e13221.
373. NCT02113774 The impact of antimicrobial treatment for asymptomatic bacteriuria in renal transplant patients. www.clinicaltrials.gov. www.clinicaltrials.gov.
374. Coussement J, Kamar N, Matignon M, Weekers L, Scemla A, Giral M, et al. Antibiotics *versus* no therapy in kidney transplant recipients with asymptomatic bacteriuria (BiRT): a pragmatic, multicentre, randomized, controlled trial. Clin Microbiol Infect. 2020;27:398–405.
375. Cai T, Mazzoli S, Mondaini N, Meacci F, Nesi G, D'Elia C, et al. The role of asymptomatic bacteriuria in young women with recurrent urinary tract infections: to treat or not to treat? Clin Infect Dis. 2012;55(6):771–7.
376. Kazemier BM, Koningstein FN, Schneeberger C, Ott A, Bossuyt PM, de Miranda E, et al. Maternal and neonatal consequences of treated and untreated asymptomatic bacteriuria in pregnancy: a prospective cohort study with an

embedded randomised controlled trial. Lancet Infect Dis. 2015;15(11):1324–33.
377. Sandock DS, Gothe BG, Bodner DR. Trimethoprim-sulfamethoxazole prophylaxis against urinary tract infection in the chronic spinal cord injury patient. Paraplegia. 1995;33:156–60.
378. Roghmann MC, Wallin MT, Gorman PH, Johnson JA. Prevalence and natural history of colonization with fluoroquinolone-resistant Gram-negative bacilli in community-dwelling people with spinal cord dysfunction. Arch Phys Med Rehabil. 2006;87(10):1305–9.
379. Godfrey K, Harding M, Zhanel G, Nicolle MD L, Cheang M. Antimicrobial treatmen in diabetic women with asymtpomatic bacteriuria. N Engl J Med. 2002;347(20):1576–83.
380. Origüen J, López-Medrano F, Fernández-Ruiz M, María Aguado J, Coussement J, Nagler E, et al. Reply to "Old habits die hard: screening for and treating asymptomatic bacteriuria after kidney transplantation." Am J Transplant. 2016;16(11):3301–2.
381. Gołębiewska J, Dębska-Ślizień A. Urinary tract infections in renal transplant recipients. In: Jarzembowski J, editor. Urinary tract infection - The result of the strength of the pathogen, or the weakness of the host. InTech Open; 2018.
382. Coussement J, Maggiore U, Manuel O, Scemla A, Lo F. Diagnosis and management of asymptomatic bacteriuria in kidney transplant recipients: a survey of current practice in Europe survey content. Nephrol Dial Transplant. 2018;33:1661–8.
383. Hibberd PL, Tolkoff-Rubin NE, Doran M, Delvecchio A, Cosimi AB, Delmonico FL, et al. Trimethoprim-sulfamethoxazole compared with ciprofloxacin for the prevention of urinary tract infection in renal transplant recipients. A double-blind, randomized controlled trial. Online J Curr Clin Trials. 1992;15.
384. Khorvash F, Mortazavi M, Hakamifard A, Ataei B. Comparison of the effect of co-trimoxazole and co-trimoxazole plus ciprofloxacin in urinary tract infection prophylaxis in kidney transplant patients. Adv Biomed Res. 2016;5:108.
385. Wojciechowski D, Chandran S. Effect of ciprofloxacin combined with sulfamethoxazole-trimethoprim prophylaxis on the incidence of urinary tract infections after kidney transplantation. Transplantation. 2013;96(4):400–5.
386. Patel RK, Taylor A, Jardine AG. Prophylactic ciprofloxacin for kidney transplant recipients-to add or not to add. Transplantation. 2013;96(4):370–1.
387. Arreola-Guerra JM, Rosado-Canto R, Alberú J, Maravilla E, Torres-González P, Criollo E, et al. Fosfomycin trometamol in the prophylaxis of post-kidney transplant urinary tract infection: a controlled, randomized clinical trial. Transpl Infect Dis. 2018;20(5):e12980.
388. Di Cocco P, Orlando G, Mazzotta C, Rizza V, D'Angelo M, Clemente K, et al. Incidence of urinary tract infections caused by germs resistant to antibiotics commonly used after renal transplantation. Transplant Proc. 2008;40(6):1881–4.
389. Stamm WE, Counts GW, Wagner KF, Martin D, Gregory D, McKevitt M, Turck M HK. Antimicrobial prophylaxis of recurrent urinary tract infections: a double-blind, placebo-controlled trial. Ann Intern Med. 1980;92(6):770–5.
390. Marielle A. J. Beerepoot M et al. *Lactobacilli vs* antibiotics to prevent urinary tract infections. Arch Intern Med. 2012;172(9):704–12.

391. den Heijer CDJ, Beerepoot MAJ, Prins JM, Geerlings SE, Stobberingh EE. Determinants of antimicrobial resistance in *Escherichia coli* strains isolated from faeces and urine of women with recurrent urinary tract infections. PLoS One. 2012;7(11):1–4.
392. Kahlmeter G, Menday P. Cross-resistance and associated resistance in 2478 *Escherichia coli* isolates from the Pan-European ECO.SENS Project surveying the antimicrobial susceptibility of pathogens from uncomplicated urinary tract infections. J Antimicrob Chemother. 2003;52(1):128–31.
393. Enne VI, Bennett PM, Livermore DM, Hall LMC. Enhancement of host fitness by the sul2-coding plasmid p9123 in the absence of selective pressure. J Antimicrob Chemother. 2004;53(6):958–63.
394. Bean DC, Livermore DM, Papa I HL. Resistance among *Escherichia coli* to sulphonamides and other antimicrobials now little used in man. J Antimicrob Chemother. 2005;56(5):962–4.
395. Price JR, Guran LA, Gregory WT, McDonagh MS. Nitrofurantoin *vs* other prophylactic agents in reducing recurrent urinary tract infections in adult women: a systematic review and meta-analysis. Am J Obstet Gynecol. 2016;215(5):548–60.
396. Eells SJ, Bharadwa K, McKinnell JA, Miller LG. Recurrent urinary tract infections among women: comparative effectiveness of 5 prevention and management strategies using a markov chain monte carlo model. Clin Infect Dis. 2014;58(2):147–60.
397. Ahmed H, Davies F, Francis N, Farewell D, Butler C, Paranjothy S. Long-term antibiotics for prevention of recurrent urinary tract infection in older adults: systematic review and meta-analysis of randomised trials. BMJ Open. 2017;7(5):e015233.
398. Costantini E, Zucchi A, Salvini E, Cicalese A, Li Marzi V, Filocamo MT eresa, et al. Prulifloxacin *vs* fosfomycin for prophylaxis in female patients with recurrent UTIs: a non-inferiority trial. Int Urogynecol J. 2014;25(9):1173–8.
399. Chueng T, Suarez JF, Camargo JF. Efficacy and tolerability of fosfomycin in prevention of recurrent urinary tract infections among kidney transplant recipients. Transpl Infect Dis. 2019;21(2):e13042.
400. Pagonas N, Hörstrup J, Schmidt D, Benz P, Schindler R, Reinke P, et al. Prophylaxis of recurrent urinary tract infection after renal transplantation by cranberry juice and L-methionine. Transplant Proc. 2012;44(10):3017–21.
401. Jepson RG, Craig JC. A systematic review of the evidence for cranberries and blueberries in UTI prevention. Mol Nutr Food Res. 2007;51(6):738–45.
402. Reid G, Bruce AW. Low vaginal pH and urinary-tract infection. Lancet (London, England). 1995;346(8991–8992):1704.
403. Zárate G, Nader-Macias ME. Influence of probiotic vaginal *Lactobacilli on in vitro* adhesion of urogenital pathogens to vaginal epithelial cells. Lett Appl Microbiol. 2006;43(2):174–80.
404. Uehara S, Monden K, Nomoto K, Seno Y, Kariyama R, Kumon H. A pilot study evaluating the safety and effectiveness of *Lactobacillus* vaginal suppositories in patients with recurrent urinary tract infection. Int J Antimicrob Agents. 2006;28 Suppl 1:S30-4.
405. Grodstein EI, Baggett N, Wayne S, Leverson G, D'Alessandro AM, Fernandez LA, et al. An evaluation of the safety and efficacy of simultaneous bilateral nephrectomy and renal transplantation for polycystic kidney disease: a 20-year

experience. Transplantation. 2017;101(11):2774—2779.
406. Shoma AM, Eraky I, El-Kappany HA. Pretransplant native nephrectomy in patients with end-stage renal failure: assessment of the role of laparoscopy. Urology. 2003;61(5):915–20.
407. Jean RA, Alexandre M, Yoo PS. Kidney transplantation with and without native nephrectomy for polycystic kidney disease: results of the national inpatient sample and the rational for a 2-staged procedure. J Am Coll Surg. 2018;226(6):1079–84.
408. Huttner A, Hatz C, van den Dobbelsteen G, Abbanat D, Hornacek A, Frölich R, et al. Safety, immunogenicity, and preliminary clinical efficacy of a vaccine against extraintestinal pathogenic *Escherichia coli* in women with a history of recurrent urinary tract infection: a randomised, single-blind, placebo-controlled phase 1b trial. Lancet Infect Dis. 2017;17(5):528–37.
409. Tariq R, Pardi DS, Tosh PK, Walker RC, Razonable RR, Khanna S. Fecal microbiota transplantation for recurrent *Clostridium difficile* infection reduces recurrent urinary tract infection frequency. Clin Infect Dis. 2017;65(10):1745–7.
410. Zalewska-piątek B, Piątek R. Alternative treatment approaches of urinary tract infections caused by uropathogenic *Escherichia coli* strains. Acta Biochim Pol. 2019;66(2):129–38.
411. Fonseca M, Sato J, Lima-Noronha M, Migliorini L, Fernández-Silva F, Galhardo R. Infection, genetics and evolution increased mutability to fosfomycin resistance in *Proteus mirabilis* clinical isolates. Infect Genet Evol. 2018;58:27–33.
412. Herrera-Espejo S, Del Barrio-Tofiño E, Cebrero-Cangueiro T, López-Causapé C, Álvarez-Marín R, Cisneros JM, et al. Carbapenem combinations for infections caused by carbapenemase-producing *Pseudomonas aeruginosa: experimental in vitro and in vivo* analysis. Antibiotics. 2022;11(9).
413. García J, Cantón R, García J, Gómez M, Martínez L, Rodríguez-Avial et al. Métodos básicos para el estudio de la sensibilidad a los antimicrobianos. Procedimientos en Microbiología Clínica. Vila J, editor. Recomendaciones de la Sociedad Española de Enfermedades. Infecciosas y Microbiología Clínica. 2000. 1–54 p.
414. Labrador-Herrera G, Pérez-Pulido AJ, Álvarez-Marín R, Casimiro-Soriguer CS, Cebrero-Cangueiro T, Morán-Barrio J, et al. Virulence role of the outer membrane protein CarO in carbapenem-resistant *Acinetobacter baumannii*. Virulence. 2020;11(1):1727–37.
415. Brune JE, Dickenmann M, Wehmeier C, Sidler D, Walti L, Golshayan D, et al. Impact of different urinary tract infection phenotypes within the first year post-transplant on renal allograft outcomes. Am J Transplant. 2022;22(7):1823–33.
416. Bates J, Thomas-Jones E, Moore M, Hood K, Bongard E, Llor C, et al. Variations in presentation, management, and patient outcomes of urinary tract infection: a prospective four-country primary care observational cohort study. Br J Gen Pract. 2017;67(665):e830–41.
417. Azap Ö, Togan T, Yesilkaya A, Arslan H, Haberal M. Antimicrobial susceptibilities of uropathogen *Escherichia coli* in renal transplant recipients: dramatic increase in ciprofloxacin resistance. TPS. 2013;45(3):956–7.
418. Rossignol L, Vaux S, Maugat S, Blake A, Barlier R, Heym B, et al. Incidence of urinary tract infections and antibiotic resistance in the outpatient setting: a

cross - sectional study. Infection. 2017;45(1):33–40.
419. Demilie T, Beyene G, Melaku S, Tsegaye W. Diagnostic accuracy of rapid urine dipstick test to predict urinary tract infection among pregnant women in Felege Hiwot Referral Hospital, Bahir Dar, North West Ethiopia. BMC Res Notes. 2014;7(1):1–5.
420. San-Juan R, Montejo M, Muñoz P, Torre-Cisneros J, Cervera C, Fernández-Ruiz M, et al. Impact of hepatitis C virus infection on the risk of infectious complications after kidney transplantation: data from the RESITRA/REIPI cohort. Transplantation. 2011;92(5):543–9.
421. Rosado-Canto R, Carrillo-Pérez DL, Arreola-Guerra JM, Sifuentes-Osornio J. Asymptomatic bacteriuria in kidney transplant recipients: the challenge in the first 8 weeks. Transpl Infect Dis. 2018;20(3):0–3.
422. Rosado-Canto R, Parra-Avila I, Tejeda-Maldonado J, Kauffman-Ortega C, Rodriguez-Covarrubias F, Trujeque-Matos M, et al. Perioperative fosfomycin disodium prophylaxis against urinary tract infection in renal transplant recipients: a randomized clinical trial. Nephrol Dial Transplant. 2020;35(11):1996-2003.
423. Green H, Rahamimov R, Goldberg E, Leibovici L, Gafter U, Bishara J, et al. Consequences of treated *versus* untreated asymptomatic bacteriuria in the first year following kidney transplantation: retrospective observational study. Eur J Clin Microbiol Infect Dis. 2013;32(1):127–31.
424. Lim JH, Cho JH, Lee JH, Park YJ, Jin S, Park GY, et al. Risk factors for recurrent urinary tract infection in kidney transplant recipients. Transplant Proc. 2013;45(4):1584–9.
425. Pesce F, Martino M, Fiorentino M, Rollo T, Simone S, Gallo P, et al. Recurrent urinary tract infections in kidney transplant recipients during the first-year influence long-term graft function: a single-center retrospective cohort study. J Nephrol. 2019;32(4):661–8.
426. Zhanel G, Zhanel M, Karlowsky J. Oral fosfomycin for the treatment of acute and chronic bacterial prostatitis caused by multidrug-resistant *E. coli*. Can J Infect Dis Med Microbiol. 2018;1404813.
427. Batra P, Abrol AK, Gupta S, Pushpan P KR. Susceptibility pattern of oral antimicrobials in uncomplicated UTI: does fosfomycin still stand effective? J Fam Med Prim Care. 2020;9(2):850–3.
428. Hains D, Chen X, Saxena V, Barr-Beare E, Flemming W, Easterling R, et al. Carbonic anhydrase 2 deficiency leads to increased pyelonephritis susceptibility. Am J Physiol Ren Physiol. 2014;307:869–80.
429. Halskov ACL, Dagnæs-Hansen J, Stroomberg H V., Sørensen SS, Røder A. Incidence of and risk factors for recurrent urinary tract infections in renal transplant recipients. Eur Urol Open Sci. 2023;52:115–22.
430. Abbott IJ, van Gorp E, Wyres KL, Wallis SC, Roberts JA, Meletiadis J PA. Oral fosfomycin activity against *Klebsiella pneumoniae* in a dynamic bladder infection in vitro model. J Antimicrob Chemother. 2022;77(5):1324–1333.
431. Turnidge J, Canton R, Kahlmeter G, Giske CG, Muller AE, Mouton JW. MIC-based dose adjustment: facts and fables. J Antimicrob Chemother. 2017;73(3):564–8.
432. Abbott IJ, Van Gorp E, Wijma RA, Meletiadis J, Roberts JA, Peleg AY, et al. Oral fosfomycin efficacy with variable urinary exposures following single and multiple doses against *Enterobacterales*: the importance of heteroresistance for

growth outcome. Antimicrob Agents Chemother. 2020;64(3):1–13.
433. Band VI, Hufnagel DA, Jaggavarapu S, Sherman EX, Wozniak JE, Satola SW, et al. Antibiotic combinations that exploit heteroresistance to multiple drugs effectively control infection. Nat Microbiol. 2019;4(10):1627–35.
434. Podnecky N, Fredheim E, Kloos J, Sørum V, Primicerio R, Roberts A, et al. Conserved collateral antibiotic susceptibility networks in diverse clinical strains of *Escherichia coli*. J Urol. 2019;201(5):861.
435. Fasugba O, Gardner A, Mitchell BG, Mnatzaganian G. Ciprofloxacin resistance in community-and hospital-acquired *Escherichia coli* urinary tract infections: a systematic review and meta-analysis of observational studies. BMC Infect Dis. 2015;15(1).
436. Costelloe C, Metcalfe C, Lovering A, Mant D, Hay AD. Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. BMJ. 2010;340(7756):1120.

10. ANNEXES

10.1. Publications derived from this PhD thesis:

antibiotics

MDPI

Article

Impact of Treating Asymptomatic Bacteriuria in Kidney Transplant Recipients: A Prospective Cohort Study

Sara Fontserè [1,†], Carmen Infante-Domínguez [1,†], Alejandro Suárez-Benjumea [2], Marta Suñer-Poblet [2], Carmen González-Corvillo [2], Guillermo Martín-Gutiérrez [1], Gabriel Bernal [1], Jerónimo Pachón [1,3], María Eugenia Pachón-Ibáñez [1,4,*] and Elisa Cordero [1,3,4]

1 Unit of Infectious Diseases, Microbiology, and Preventive Medicine, Virgen del Rocio University Hospital of Seville, 41013 Seville, Spain; sarafontsere@gmail.com (S.F.); carmeninfanted@gmail.com (C.I.-D.); guiller_mg86@hotmail.es (G.M.-G.); gabrielbernalblanco@gmail.com (G.B.); pachon@us.es (J.P.); elisacorderom@gmail.com (E.C.)
2 Urology and Nephrology Unit, Virgen del Rocio University Hospital, 41013 Seville, Spain; alejandro.suarez.sspa@juntadeandalucia.es (A.S.-B.); msuner@us.es (M.S.-P.); carmen.gonzalez.corvillo.sspa@juntadeandalucia.es (C.G.-C.)
3 Department of Medicine, University of Seville, 41009 Seville, Spain
4 Institute of Biomedicine of Seville (IBiS), Virgen del Rocio University Hospital/CSIC/University of Seville, 41013 Seville, Spain
* Correspondence: mpachon-ibis@us.es
† The authors contributed equally to this work.

check for updates

Citation: Fontserè, S.; Infante-Domínguez, C.; Suárez-Benjumea, A.; Suñer-Poblet, M.; González-Corvillo, C.; Martín-Gutiérrez, G.; Bernal, G.; Pachón, J.; Pachón-Ibáñez, M.E.; Cordero, E. Impact of Treating Asymptomatic Bacteriuria in Kidney Transplant Recipients: A Prospective Cohort Study. *Antibiotics* **2021**, *10*, 218. https://doi.org/10.3390/antibiotics10020218

Academic Editor: Gabriella Orlando

Received: 4 February 2021
Accepted: 19 February 2021
Published: 22 February 2021

Publisher's Note: MDPI stays neutral with regard to jurisdictional claims in published maps and institutional affiliations.

Abstract: This study aims to define the epidemiologic, clinical, and microbiological features of asymptomatic bacteriuria (AB) and cystitis in kidney transplantation recipients (KTRs), and to determine the impact of antimicrobial therapy of AB and the risk factors of cystitis. We conducted a prospective observational study of AB and cystitis in KTRs from January to June 2017. One-hundred ninety seven KTRs were included: 175 (88.8%) with AB and 22 (11.2%) with cystitis. The most frequent etiologies were *Escherichia coli*, *Klebsiella pneumoniae*, *Enterococcus faecalis*, and *Pseudomonas aeruginosa*. No differences were observed regarding the etiologies, antimicrobial susceptibility patterns, and microbiologic outcomes in AB vs. cystitis. The treatment of AB diminished the microbiological cure and increased the rates of microbiologic relapses and reinfections; in addition, treated AB patients showed a trend of developing symptomatic urinary tract infection in the following six months. The analysis of the data identified the following independent risk factors for cystitis during the six months of follow-up: AB treatment, thymoglobulin induction, previous acute pyelonephritis, and time since transplantation < 1 year. In summary, considering the lack of clinical benefits of treating AB and its impact on cystitis development in the follow-up, we support the recommendation of not screening for or treating AB.

Keywords: urinary tract infections; kidney recipients; asymptomatic bacteriuria; cystitis; prospective observational cohort

1. Introduction

Despite improved surgical techniques, antimicrobial prophylaxis, new immunosuppressive therapies, and better hygiene management of solid organ transplantation recipients (SOTRs), infectious complications remain a major cause of morbimortality in these patients. Urinary tract infections (UTIs) are among the most common infectious complications and the first cause of antibiotics treatment in kidney transplantation recipients (KTRs). The reported incidence of UTIs ranges from 4% to 75% in kidney recipients; this wide range could be explained by the heterogeneity of definitions of UTIs, follow-up times, surgical techniques, antimicrobial prophylaxis and immunosuppressive drugs, and design of the studies [1–5].

The real effect of UTIs on the outcome of KTRs is not clear. Some studies suggest the absence of association of asymptomatic bacteriuria (AB) and acute cystitis with allograft survival, rejection, renal function, and all-cause short-term mortality. The screening and treatment of AB did not improve the early outcome after transplantation and increased the risk of suffering multidrug-resistant (MDR) infections in several studies [6,7].

On the other hand, other studies have reported that the burden of UTIs in KTRs is real and high, as suffering from a UTI during the first year after transplantation increases mortality (41%) and costs per event [8]. Moreover, these patients have a higher risk of MDR infections, which also compromise the outcome [6]. The impact of acute pyelonephritis (APN) on allograft function, although uncertain, determines an adverse outcome when occurring early after transplantation [4,5,9,10].

Despite their clinical frequency, there are unanswered key points regarding the epidemiology of UTIs in KTRs, the differences in resistance patterns depending on the presence of symptoms, and the impact of treatment in the allograft survival, rejection, and mortality. In this study, we aimed to evaluate the epidemiology and clinical manifestation of UTIs and the impact of antimicrobial therapy on AB in kidney transplantation recipients.

2. Methods

2.1. Study Design

A prospective observational cohort of consecutive cases of all uncomplicated-cystitis and AB cases in KTRs that attended the outpatient clinic from January 2017 to June 2017, at the Virgen del Rocio University Hospital, Seville, Spain, was analyzed. For the study, only the first episode, in patients with reinfections or relapses, was analyzed. All interventions followed standard clinical practice. The decision to treat the AB and cystitis episodes and the antimicrobial therapy was the choice of the physicians in charge of the patients.

Urine samples were processed within 4–8 h after collection and urine pH was measured. The Microbiology Service identified the bacterial isolates and performed susceptibility testing by conventional biochemical tests (biochemical testing, pigment production, growth, and colony characteristics). The causative organism and antibiogram were identified using the MicroScan WalkAway® plus System (Beckman Coulter, Switzerland). When the identification was uncertain, it was confirmed by the Bruker Biotyper MALDI-TOF MS system (Bruker Daltonik GmbH, Leipzig, Germany). The European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria for categorizing susceptibility and resistance patterns were used [11]. Plasma creatinine, and urinary pH, leukocyturia, and nitrites determinations were also performed at inclusion. Demographics, chronic underlying diseases, time from the kidney transplantation, immunosuppressive regimens, clinical data, and antimicrobial therapy were recorded in a standardized database.

Patients were follow-up for six months after inclusion. Urine cultures were performed one and six months after inclusion. Moreover, in patients with urinary symptoms during the follow-up, urine cultures were also performed.

The study was approved by the Ethics Committee of the University Hospitals Virgen del Rocio and Virgen Macarena (2016/186), Seville, Spain.

2.2. Definitions

GESITRA/REIPI UTI guidelines [12] were used: Bacteriuria: Urine specimens isolated in quantitative counts $\geq 10^5$ colony-forming units (CFU)/mL. Asymptomatic bacteriuria: The presence of bacteriuria in the absence of any symptoms of a UTI. Cystitis: Bacteriuria and clinical manifestations such as dysuria, pollakiuria, urinary urgency, suprapubic pain, and/or hematuria, in the absence of pyelonephritis symptoms. *Acute pyelonephritis:* The simultaneous presence of bacteriuria and/or bacteremia and fever, with one or more of the following: Lumbar pain (if native kidney involved), renal allograft tenderness (if transplanted kidney involved), chills, or cystitis symptoms. *Clinical cure*: The resolution of symptoms at seven days after inclusion. *Microbiological cure (eradication)*: Negative urine culture at 7–9 days after the end of treatment. *Microbiological reinfection*: New episode of

infection by a different pathogen than the initially isolated. *Microbiological relapse*: Detection of the same initial pathogen during the month after the inclusion, with a sterile culture before. *Microbiological persistence*: No negative urine culture in the follow-up. *Mortality*: Death during the six months prior to the follow-up. *Impairment of renal function*: Elevation of creatinemia $\geq$ 0.5 mg/dl.

2.3. Statistical Analysis

A descriptive statistical analysis was performed. Continuous variables were expressed as median and interquartile range or mean and standard deviation if adjusted to a normal distribution and evaluated by Shapiro–Wilk or Kolmogorov–Smirnov tests when appropriate. For bivariate analysis, the chi-square test or the Fisher exact test was used for categorical variables; Bonferroni correction was applied when appropriate. For quantitative variables, the Mann–Whitney test or Student's t-test were used based on their distribution. If the variance was not homogeneous (Levene test), an ANOVA test was applied. The relative risks were expressed as odds ratios (ORs) and 95% confidence intervals (CI). Multivariate models were used to adjust for possible confounding variables. The clinically relevant and statistically significant variables found in the bivariate analysis were included in a matrix analysis (checked by chi-square test for categorical variables and Student's t-test for quantitative variables). Only the independent variables were finally included, which was the multivariate model that described the outcome better. Significance was established at $p < 0.05$. All reported p-values are based on two-tailed tests. Statistical analyses were performed using SPSS version 18.0 software (SPSS, Chicago, IL, USA).

3. Results

3.1. Characteristics and Outcomes of the Entire Cohort

Our study included a total of 197 patients, 175 (88.8%) with AB and 22 (11.2%) with cystitis. Out of the total, 104 (52.8%) were women, and the median age was 59 years (IQR: 48–69). The median time after transplantation was 3.8 years (IQR: 0.8–10). Our study included 58 (29.4%) and 10 (6.9%) patients who received the transplantation in the previous year and month, respectively. The most common immunosuppressive drug combination was mycophenolate (MMF), prednisone, and tacrolimus (n = 124, 62.9%). Induction therapy was performed for 91 (46.1%) patients: 67 (34.0%) with basiliximab or daclizumab and 24 (12.1%) with thymoglobulin. Instrumentation of the urinary tract took place in 40 (20.3%) patients, 24 (12.2%) within the previous six months of the UTI diagnosis.

In the previous six months, 80 patients were diagnosed with at least one episode of bacteriuria (40.6%), 15 with APN (7.6%), and 10 with cystitis (5.1%). Patients with cystitis had more frequently detectable urinary nitrites than patients with AB (35.7% vs. 9.1%, p = 0.01, OR 3.4, 95% CI 1.3–9.1). At inclusion, 70 (35.5%) patients had viral co-infections: Cytomegalovirus infection in 45 (22.8%) cases and BK virus infection in 19 (9.6%). Patients with cystitis were more frequently co-infected with the hepatitis C virus (HCV) (33.3% vs. 3.3%, p = 0.01, OR 2.3, 95% CI 2.3–18.5). No differences were found in other demographics and transplant-related variables (Table 1).

Most frequent etiologies in all patients were *Escherichia coli* (n = 89, 45.2%), *Klebsiella pneumoniae* (n = 30, 15.2%), *Enterococcus faecalis* (n = 23, 11.6%), and *Pseudomonas aeruginosa* (n = 13, 6.6%) (Table 1). Etiologies were similar in patients with cystitis and AB. There were 60 (30.4%) isolates resistant to cotrimoxazole, 55 (27.9%) to ciprofloxacin, 38 (19.2%) to amoxicillin-clavulanate, 21 (10.6%) to third- and/or fourth generation cephalosporins, and 19 (9.6%) to fosfomycin. No differences were observed in antimicrobial susceptibility between isolates from patients with cystitis and AB (Table 1).

Table 1. Baseline, clinical and microbiological features of kidney recipients with bacteriuria.

Variable	All Cases *n* = 197	Asymptomatic Bacteriuria *n* = 175	Cystitis *n* = 22	*p* Value
Time from transplant to inclusion (years; median, IQR)	3.76 (0.78–10.3)	3.85 (0.77–9.92)	2.35 (0.63–11.4)	0.48
Diabetes mellitus- *n* (%)	46 (23.4)	42 (24.0)	4 (18.2)	0.79
Transplant indication- *n* (%)				0.97
Tubulointerstitial	40 (20.3)	35 (20.0)	5 (22.7)	-
Glomerulonephritis	40 (20.3)	36 (20.6)	4 (18.2)	-
Polycystic kidney disease	36 (18.3)	32 (18.3)	4 (18.2)	-
Diabetic nephropathy	11 (5.6)	9 (5.1)	2 (9.1)	-
Hypertension/renovascular	16 (8.1)	15 (8.6)	1 (4.5)	-
Tumoral	4 (2.0)	4 (2.3)	0 (0)	-
Etiology uncertain/unknown	49 (24.9)	43 (24.6)	6 (27.3)	-
Charlson index (median, IQR)	3 (2-5)	3(2-5)	4(2-5)	-
Induction drug- *n* (%)				
None	99 (50.3)	87 (49.7)	12 (54.5)	-
Basiliximab	56 (28.4)	49 (28.0)	7 (31.8)	-
Daclizumab	11 (5.6)	10 (5.7)	1 (4.5)	-
Thymoglobulin	24 (12.2)	23 (13.1)	1 (4.5)	-
Current immunosuppression- *n* (%)				
MMF	142 (72.1)	2.0)	16 (72.7)	-
Prednisone	180 (91.4)	161 (92.0)	19 (86.4)	-
Tacrolimus	174 (88.3)	155 (88.6)	19 (86.4)	-
mTOR inhibitors	10 (5.1)	9 (5.1)	1 (4.5)	-
Cyclosporine	12 (6.1)	10 (5.7)	2 (9.1)	-
Urinary instrumentation- *n* (%)	40 (20.3)	35 (20.0)	5 (22.7)	0.84
Double J stent	34 (17.3)	29 (16.6)	5 (22.7)	-
Urethral catheter	3 (1.5)	3 (1.7)	0 (0)	-
Nephrostomy	3 (1.5)	3 (1.7)	0 (0)	-
Length of instrumentation (days, median, IQR)	0 (0-26)	0 (0–26)	0 (0–43.5)	0.52
Cotrimoxazole prophylaxis- *n* (%)	32 (16.2)	28 (16%)	4 (18.1)	-
Etiology- *n* (%)				
Escherichia coli	89 (45.2)	79 (45.1)	10 (45.5)	0.93
E. coli ESBL-producers	5 (2.5)	5 (2.9)	0 (0)	0.55
Klebsiella pneumoniae	30 (15.2)	28 (16.0)	1 (4.5)	0.15
K. pneumoniae ESBL-producers	5 (2.5)	4 (2.3)	1 (4.5)	0.54
Enterococcus faecalis	23 (11.6)	20 (11.4)	3 (13.6)	0.76
Pseudomonas aeruginosa	13 (6.6)	11 (6.3)	2 (9.1)	0.62
Klebsiella oxytoca	8 (4.0)	6 (3.4)	2 (9.1)	0.27
Proteus mirabilis	7 (3.6)	6 (3.4)	1(4.5)	0.75
Morganella morganii	4 (2.0)	4 (2.3)	0 (0)	0.62

Table 1. *Cont.*

Variable	All Cases n = 197	Asymptomatic Bacteriuria n = 175	Cystitis n = 22	p Value
Enterobacter aerogenes	4 (2.0)	3 (1.7)	1(4.5)	0.44
Enterobacter cloacae	3 (1.5)	2 (1.4)	1 (<1)	0.33
Treatment- *n* (%)	75 (38.1)	54 (30.9)	21 (95.5)	
Ciprofloxacin	22 (11.2)	16 (9.1)	6 (27.3)	0.01
Fosfomycin	29 (14.7)	19 (10.9)	10 (45.5)	<0.01
Amoxicillin-clavulanate	16 (8.1)	12 (6.9)	4 (18.2)	0.07
Cephalosporins	5 (2.5)	4 (2.3)	1 (4.5)	0.53
Cotrimoxazole	3 (1.5)	3 (1.7)	0 (0)	0.54
Antibiotic resistance- *n* (%)				
Ciprofloxacin	55 (27.9)	45 (25.7)	10 (45.5)	0.16
Fosfomycin	19 (9.6)	17 (9.7)	2 (9)	0.94
Amoxicillin-clavulanate	38 (19.2)	31 (17.7)	7 (31.8)	0.12
Cephalosporins	21 (10.6)	16 (9.1)	5 (22.7)	0.08
Cotrimoxazole	60 (30.4)	53 (30.5)	7 (31.8)	0.73

MMF: Mycophenolate, mTOR: Mammalian target of rapamycin, ESBL-producers: extended spectrum beta-lactamases-producers. IQR: Interquartile range.

Seventy-five (38.1%) patients received antimicrobial treatment, with differences in AB (n = 54, 30.8%) and cystitis (n = 21, 95.4%) cases. The most common antibiotics prescribed were fosfomycin, ciprofloxacin, and amoxicillin-clavulanate, without differences between cystitis and AB (Table 1).

At the one-month follow-up, 191 (96.9%) out of the 197 patients were cured, and 4 (2.0%) and 2 (1.0%) had cystitis and APN, respectively, without differences between patients with AB or cystitis at inclusion. At the six-month follow-up, 181 (91.8%) patients were cured, and 8 (4.0%) and 8 (4.0%) had cystitis and APN, respectively, without differences between patients with AB or cystitis at inclusion (Table 2). The most frequent etiologies of symptomatic UTIs during the follow-up were *E. coli* (64.7%) and *E. faecalis* (29.2%).

Table 2. Microbiological and clinical outcomes of the total events, asymptomatic bacteriuria and cystitis.

Variables	Bacteriuria n = 197	AB n = 175	Cystitis n = 22	p Value
One month follow up outcome Microbiological- *n* (%)				
Cure	111 (56.3)	99 (56.7)	12 (54.5)	0.51
Persistence	40 (20.3)	35 (20.0)	5 (22.7)	0.75
Relapse	11 (5.5)	11 (6.3)	0 (0)	0.26
Re-infection	14 (7.1)	10 (5.7)	4 (18.2)	0.07
Without follow up data	21 (10.6)	20 (11.4)	1 (4.5)	0.35
Clinical- *n* (%)				
Asymptomatic	191 (96.9%)	170 (97.1%)	21 (95.4%)	0.5
Cystitis	4 (2.0)	3 (2)	1 (4.5)	0.6
APN	2 (1.0)	2 (1)	0 (0)	0.6

Table 2. *Cont.*

Variables	Bacteriuria *n* = 197	AB *n* = 175	Cystitis *n* = 22	*p* Value
Six months follow up outcome Microbiological- *n* (%)				
Cure	53 (26.9)	48 (27.4)	5 (22.7)	0.45
Persistence	34 (17.2)	31 (17.7)	3 (13.6)	0.68
Relapse	27 (13.7)	24 (13.7)	3 (13.6)	0.96
Re-infection	37 (18.7)	31 (17.7)	6 (27.2)	0.29
Without follow up data	58 (29.4)	49 (28.0)	9 (40.9)	0.3
Clinical- *n* (%)				
Asymptomatic	181 (91.8)	163 (93.1%)	19 (86.3)	0.22
Cystitis	8 (4.0)	6 (3.4)	1 (4.5)	0.26
APN	8 (4.0)	6 (3.4)	2 (9)	0.26
Graft outcome- *n* (%)				
Graft dysfunction	10 (5.1)	8 (4.6)	2 (9.1)	0.29
Graft rejection	4 (2.0)	4 (2.3)	0 (0)	0.17
Graft loss	1 (0.5)	1 (0.6)	0 (0)	0.17

AB: Asymptomatic bacteriuria, APN: Acute pyelonephritis.

Regarding the microbiological outcome, at the one-month follow-up, 111 (56.3%) patients were microbiologically cured. In 40 (20.3%) patients, the bacteriuria persisted, 11 (5.5%) patients relapsed, and 14 (7.1%) patients were re-infected; 21 (10.6%) patients had no urine cultures at this time. At the six-month follow-up, 53 (26.9%) were cured, and 34 (17.2%), 27 (13.7%), and 37 (18.7%) had persistence, relapse, and re-infection, respectively. No differences were found in microbiological cure at any follow-up time-points regarding AB or cystitis diagnosis at inclusion (Table 2).

At the six-month follow-up, renal function worsened in 10 (5.1%) patients, four (2.0%) had a graft rejection, and one (0.5%) lost the graft. Six (3.0%) patients died during the six months of follow-up (five AB and one cystitis), none because of the UTI. The graft and survival outcomes of patients with AB and cystitis were similar (Table 2).

3.2. Impact of Antibiotic Treatment in AB Outcome

Among patients with AB, 54 (30.8%) received antimicrobial therapy; most common treatments were fosfomycin ($n = 19$, 10.8%), ciprofloxacin ($n = 16$, 9.1%), and amoxicillin-clavulanate ($n = 12$, 6.8%). A higher proportion of treated AB patients, when compared to those untreated, received the transplant in the six months before inclusion (30.2% vs. 16.7%, $p = 0.04$, OR 2.16, 95% CI 1–4.6) and had isolates resistant to cotrimoxazole (54.1% vs. 23.2%, $p < 0.01$, OR 2.24, 95% CI 1.4–3.4). Moreover, there were trends to higher creatinine levels before and during the actual episode in vs. untreated AB patients: 1.78 vs. 1.54 mg/dL ($p = 0.07$) and 1.77 vs. 1.57 mg/dL ($p = 0.08$), respectively. No differences were found among the rest of the analyzed variables (Table 3).

The episodes of symptomatic UTIs in AB patients during the follow-up are summarized in Table 3. After one month, 4 (7.4%) out of 54 treated AB patients presented with a symptomatic UTI (two cystitis and two APN), whereas 1 (0.8%) out of 121 untreated AB patients had a cystitis episode ($p = 0.06$). After six months, 6 (11.1%) treated vs. 4 (3.3%) untreated AB patients had UTI episodes ($p = 0.07$, OR 3.65, 95% CI 0.98–13.53).

Table 3. Characteristics of treated and untreated patients with asymptomatic bacteriuria.

Variables	Treated AB n = 54	Untreated AB n = 121	OR (95%CI)	p Value
Previous creatininemia (mg/dL, median, IQR)	1.78 (0.82–2.75)	1.54 (0.86–2.22)	0.08 (0.048–0.212)	0.07
Creatininemia at the time of inclusion (mg/dL)	1.77 (0.98–2.57)	1.57 (0.88–2.25)	0.02 (0.126–0.157)	0.08
Time since transplant < 6 months	16 (30.2)	20 (16.7)	2.16 (1.013–4.614)	0.04
One month follow up outcome Microbiological- n (%)				
Cure	24 (44.4)	75 (61.9)	0.49 (0.25–0.94)	<0.01
Persistence	10 (18.5)	25 (20.6)	0.9 (0.51–1.6)	0.7
Relapse	7 (12.9)	4 (3.3)	2.2 (1.3–3.69)	0.04
Re-infection	7 (12.9)	3 (2.4)	2.4 (1.5–3.9)	<0.01
Without follow up data	6 (11.1)	14 (11.6)	0.97 (0.48–1.9)	0.95
Clinical- n (%)				
Symptomatic UTI	4 (7.4)	1 (0.8)		
Cystitis	2 (3.7)	1 (0.8)	2.2 (0.96–5.1)	0.25
APN	2 (3.7)	2 (3.7)	3.3 (2.65–4.2)	0.09
Six months follow up outcome Microbiological- n (%)				
Cure	13 (24.1)	37 (30.6)	0.8 (0.4–1.3)	0.3
Persistence	12 (22.2)	25 (20.6)	1.06 (0.6–1.8)	0.8
Relapse	12 (22.2)	14 (11.6)	1.6 (1.01–2.7)	0.06
Re-infection	14 (25.9)	23 (19)	1.3 (0.80–2.12)	0.11
Without follow up data	13 (24.1)	36 (29.8)	0.8 (0.48–1.38)	0.45
Clinical- n (%)				
Cystitis	2 (3.7)	3 (2.5)	1.3 (0.44–3.92)	0.66
APN	4 (7.4)	1 (0.8)	2.8 (1.8–4.3)	0.03
Graft outcome- n (%)				
Graft rejection	1 (1.8)	3 (2.5)	0.8 (0.14–4.5)	0.8
Graft dysfunction	2 (3.7)	6 (4.9)	0.7 (0.14–3.8)	0.7
Graft loss	0 (0.0)	1 (0.8)	-	0.7

AB: Asymptomatic bacteriuria, APN: Acute pyelonephritis. IQR: Interquartile range.

A multivariate analysis was performed to evaluate possible confounding variables of the effect of treating AB at the risk of developing symptomatic UTIs in the six months after inclusion. The following variables were identified as independent risk factors: Use of thymoglobulin as the induction drug ($p < 0.01$, OR 8, 95% CI 1.9–34.2), APN after the transplant ($p < 0.01$, OR 12, 95% CI 2.7–53.5), antimicrobial treatment of AB ($p = 0.02$, OR 5, 95% CI 1.2–20.6), and time since transplantation less than one year ($p = 0.01$, OR 5.7, 95% CI 1.5–22.2) (Table 4).

Table 4. Risk factors of symptomatic UTI during the 6 months of follow-up in patients with asymptomatic bacteriuria.

Variables	Symptomatic UTI (n = 15)	No Symptomatic UTI (n = 182)	Crude OR (95% CI)	p Value	Adjusted OR (95% CI)
Urinary pH (median, IQR)	6.7 (6.2–7.2)	6.4 (5.9–6.9)	0.11 (0.01–0.2)	0.06	-
Previous creatininemia (mg/dl, median, IQR)	2.07 (0.87–3.27)	1.55 (0.84–2.27)	0.06 (0.01–0.12)	0.02	-
Time after transplant < 1 year (median, IQR)	9 (60)	49 (27.22)	4.01 (1.4–11.9)	0.01	5.7 (1.4–22.2)
Recurrent UTI previous transplant- n (%)	4(26.7)	28 (15.3)	-	0.3	-
Urinary reflux- n (%)	0 (0.0)	13 (7.1)	-	0.3	-
MMF doses (median, IQR)	700 (435–1045)	750 (329–1268)	-	0.6	-
Induction treatment- n (%)	11 (73.3)	80 (43.9)	3.2 (1.1–9.7)	0.01	-
No drug	4 (26.7)	95 (52.2)	0.36 (0.12-1)	0.05	-
Basiliximab	4 (26.7)	52 (28.7)	-	1.0	-
Daclizumab	1 (6.6)	10 (5.5)	-	1.0	-
Thymoglobulin	6 (40)	18 (9.9)	4.6 (1.8–11.8)	<0.01	8 (1.9–34.2)
Previous APN post-transplant- n (%)	11 (73.3)	60 (32.9)	4.8 (1.6–14.7)	<0.01	12 (2.7–53.5)
Developing UTI 2 months after transplant- n (%)	13 (86.7)	93 (51.1)	4.6 (1.1–19.8)	0.03	-
Previous rejection- n (%)	2 (13.3)	12 (6.6)	-	0.49	-
Urinary instrumentation- n (%)	5 (33.3)	35 (19.2)	-	0.36	-
Obstructive uropathy post-transplant- n (%)	0 (0.0)	10 (5.5)	-	0.59	-
Nosocomial acquisition of the AB- n (%)	11 (73.3)	167 (91.7)	0.24 (0.06–0.99)	0.04	-
Antibiotic therapy of the AB- n (%)	11 (73.3)	64 (35.2)	4.7 (1.5–13.5)	0.02	5 (1.2–20.6)
Microbiological cure at 1 month- n (%)	4 (26.6)	107 (58.8)	0.2 (0.09–0.854)	0.01	-

UTI: Urinary tract infection, MMF: Mycophenolate, APN: Acute pyelonephritis, AB: Asymptomatic bacteriuria.

Regarding the microbiological outcome, at one-month follow-up, patients with treated AB experienced a microbiological cure less frequently than those untreated (44.4% vs. 61.9%, $p < 0.01$, OR 0.49, 95% CI 0.25–0.94). These patients also had a higher number of relapses (12.9% vs. 3.3%, $p < 0.05$, OR 2.2, 95% CI 1.3–3.7), and re-infections (12.9% vs. 2.4%, $p < 0.01$, OR 2.4, 95% CI 1.5–3.9). At the six-month follow-up, microbiological outcomes were similar in treated and untreated AB (Table 3).

4. Discussion

This study shows that antimicrobial resistance is a major issue in kidney recipients with a UTI and that treating AB in kidney recipients diminish the microbiological cure and increases the rates of microbiologic relapses and reinfections; in addition, treated AB patients showed a trend of developing symptomatic UTIs in the following six months. To our knowledge, this is the largest study to examine the epidemiology and clinical manifestation and impact of antimicrobial therapy on non-complicated UTI in kidney recipients, prospectively.

The most common etiology of non-complicated UTI was *E. coli*, as described for the general population [13,14]; however, the spectrum of etiologies was more diverse than in non-immunocompromised hosts, with a higher frequency of *Enterococcus* spp. and *Pseudomonas aeruginosa* infections [1,15]. The high proportion of antimicrobial resistance found must also be highlighted; it is in the range of the proportion described in other studies of kidney recipients (44–77%) [15–17] and clearly higher than the incidence described in the general population (18–25%) [14,18].

One-third of the episodes included occurred during the first year after the transplant. It has been described that most episodes of bacteriuria occur early after transplantation [13]. Several reasons have been hypothesized to explain these findings, including immunological net status or urinary instrumentation. The close follow-up of early kidney recipients could also have contributed to this finding.

No differences were found regarding the etiology and antimicrobial susceptibility of bacteriuria according to the presence of symptoms; however, in patients with bacteriuria, the presence of nitrites was associated with urinary symptoms. This finding has not been previously described in kidney recipients; however, a higher sensitivity of urinary nitrites at diagnosing cystitis when screening for bacteriuria in pregnant women, rather than AB, has been reported [19].

Hepatitis C virus co-infection also occurred more frequently in patients with cystitis. In the RESITRA cohort, an association is reported between hepatitis C virus serostatus and receiving thymoglobulin or experiencing an upper UTI, which are, at the same time, risk factors for developing a symptomatic UTI in our cohort [20].

Treating AB did not improve 1-month and 6-month microbiological outcomes. It did not have any impact on the survival of either patients or grafts. This is in accordance with what has been previously described: The persistence of bacteriuria, relapse, or reinfection did not affect the survival, renal function, or allograft function [2,4,10,21–23]. Preventive measures to reduce UTIs in transplant recipients, as antimicrobial prophylaxis, have not been reported to affect either the graft's or patient's survival; however, antimicrobial prophylaxis reduced the incidence of bacteriuria and sepsis in a meta-analysis study [24].

In the present study, treating AB did not prevent the development of symptomatic UTI in the follow-up, as other studies had already reported [4,25,26]. On the contrary, independently treating AB increased the risk of symptomatic UTIs in the following six months. Some factors were also identified to independently increase the risk of developing a symptomatic UTI during the six months of follow-up: Induction therapy with thymoglobulin, "early" post-transplant period bacteriuria, and previous APN. These factors might be, as previously stated, surrogate markers of the global net-state of immunosuppression and urinary predisposing factors, which might have contributed to a higher risk of symptomatic infection [2,3,8].

The open design of the study is a limitation to be considered. It might explain the increased risk of symptomatic UTI in the follow-up in treated patients with AB. The physician in charge of the patient decided when to treat and, therefore, they more frequently treated patients who had AB, were recently transplanted, had previous episodes of APN, or received thymoglobulin. All these are factors associated with a higher risk of symptomatic UTI in the follow-up, as previously stated. Although treatment of AB was an independent risk factor of developing a symptomatic UTI within six months, the presence of other confounding factors not considered in the present analysis could not be ruled out.

Some randomized trials assessing the impact of treatment AB on kidney recipients have already been reported, with results against treating, within small samples [4,27]. Some others are in the process of being concluded or published and might clarify this issue (NCT01871753 and NCT02113774) [27].

5. Conclusions

In summary, the high rate of resistant UTIs in kidney recipients and the lack of clinical benefits of treating AB in the present study support the recommendation of stopping screening and treating AB, while waiting for robust incoming assay results. The risk factors for developing a symptomatic UTI observed in this study might help define a subpopulation that could benefit from specific strategies, such as close follow-up, antimicrobial prophylaxis, or self-antibiotic initiation once symptoms are present.

Author Contributions: S.F. and C.I.-D. complete the data base, analyzed the results and wrote the manuscript. A.S.-B., M.S.-P. and C.G.-C., included the patients in the study and reviewed the manuscript. G.M.-G. worked in the data base. M.E.P.-I. and E.C. conceived the study, designed the experiments, analyzed the results and wrote and revised the manuscript. G.B. investigated. J.P. conceptualized, supervised, wrote and reviewed the manuscript. S.F. and C.I.-D. contributed equally to this work (joint first authors). All authors have read and agreed to the published version of the manuscript.

Funding: This work was supported by the Instituto de Salud Carlos III, Subdirección General de Evaluación y Fomento de la Investigación, Ministerio de Economía, Industria y Competitividad (PI17-01405) and by Plan Nacional de I + D + i 2013–2016 and Instituto de Salud Carlos III, Subdirección General de Redes y Centros de Investigación Cooperativa, Ministerio de Ciencia, Innovación y Universidades, Spanish Network for Research in Infectious Diseases (REIPI RD16/0016/0009)-co-financed by European Development Regional Fund "A way to achieve Europe", Operative program Intelligent Growth 2014–2020. G.M.-G. was supported by a Río Hortega research contract from the Instituto de Salud Carlos III.). M.E.P.I. is supported by the "Contract to Access to the Spanish System of Research and Innovation, V Research Program of the University of Seville" (USE13901-D) grant.

Conflicts of Interest: None of the authors has a conflict of interest to declare.

References

1. Vidal, E.; Torre-Cisneros, J.; Blanes, M.; Montejo, M.; Cervera, C.; Aguado, J.; Len, O.; Carratalá, J.; Cordero, E.; Bou, G.; et al. Bacterial urinary tract infection after solid organ transplantation in the RESITRA cohort. *Transpl. Infect. Dis.* **2012**, *14*, 595–603. [CrossRef] [PubMed]
2. Pellé, G.; Vimont, S.; Levy, P.P.; Hertig, A.; Ouali, N.; Chassin, C.; Arlet, G.; Rondeau, E.; Vandewalle, A. Acute Pyelonephritis Represents a Risk Factor Impairing Long-Term Kidney Graft Function. *Arab. Archaeol. Epigr.* **2007**, *7*, 899–907. [CrossRef] [PubMed]
3. Alangaden, G.J.; Thyagarajan, R.; Gruber, S.A.; Morawski, K.; Garnick, J.; El-Amm, J.M.; West, M.S.; Sillix, D.H.; Chandrasekar, P.H.; Haririan, A. Infectious complications after kidney transplantation: Current epidemiology and associated risk factors. *Clin. Transplant.* **2006**, *20*, 401–409. [CrossRef]
4. Origüen, J.; López-Medrano, F.; Fernández-Ruiz, M.; Polanco, N.; Gutiérrez, E.; González, E.; Mérida, E.; Ruiz-Merlo, T.; Morales-Cartagena, A.; Asín, M.A.P.; et al. Should Asymptomatic Bacteriuria Be Systematically Treated in Kidney Transplant Recipients? Results from a Randomized Controlled Trial. *Arab. Archaeol. Epigr.* **2016**, *16*, 2943–2953. [CrossRef]
5. Singh, R.; Bemelman, F.J.; Geerlings, S.E. Asymptomatic bacteriuria in renal allograft recipients: Not so innocent after all? *Futur. Microbiol.* **2016**, *11*, 1–3. [CrossRef]
6. Linares, L.; Cervera, C.; Cofán, F.; Ricart, M.; Esforzado, N.; Torregrosa, V.; Oppenheimer, F.; Campistol, J.; Marco, F.; Moreno, A. Epidemiology and Outcomes of Multiple Antibiotic–Resistant Bacterial Infection in Renal Transplantation. *Transplant. Proc.* **2007**, *39*, 2222–2224. [CrossRef]
7. Fontserè, S.; Chacón-Mora, N.; Cordero, E. Review of Bacterial Urinary Tract Infection in Kidney Transplant Recipients: Incidence, Risk Factors and Impact on the Graft Survival. *Int. J. Transplant. Res. Med.* **2017**, *3*, 1–4. [CrossRef]
8. Naik, A.S.; Dharnidharka, V.R.; Schnitzler, M.A.; Brennan, D.C.; Segev, D.L.; Axelrod, D.; Xiao, H.; Kucirka, L.; Chen, J.; Lentine, K.L. Clinical and economic consequences of first-year urinary tract infections, sepsis, and pneumonia in contemporary kidney transplantation practice. *Transpl. Int.* **2016**, *29*, 241–252. [CrossRef] [PubMed]
9. Singh, R.; Geerlings, S.E.; Bemelman, F.J. Asymptomatic bacteriuria and urinary tract infections among renal allograft recipients. *Curr. Opin. Infect. Dis.* **2015**, *28*, 112–116. [CrossRef]
10. Fiorante, S.; Fernández-Ruiz, M.; López-Medrano, F.; Lizasoain, M.; Lalueza, A.; Morales, J.M.; San-Juan, R.; Andrés, A.; Otero, J.R.; Aguado, J.M. Acute graft pyelonephritis in renal transplant recipients: Incidence, risk factors and long-term outcome. *Nephrol. Dial. Transplant.* **2010**, *26*, 1065–1073. [CrossRef]
11. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 11.0. 2021. Available online: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_11.0_Breakpoint_Tables.pdf (accessed on 1 January 2021).
12. Vidal, E.; Cervera, C.; Cordero, E.; Armiñanzas, C.; Carratalá, J.; Cisneros, J.M.; Fariñas, M.C.; López-Medrano, F.; Moreno, A.; Muñoz, P.; et al. Management of urinary tract infection in solid organ transplant recipients: Consensus statement of the Group for the Study of Infection in Transplant Recipients (GESITRA) of the Spanish Society of Infectious Diseases and Clinical Microbiology (SEIMC) and the Spanish Network for Research in Infectious Diseases (REIPI). *Enferm. Infecc. y Microbiol. Clín.* **2015**, *33*, 679.e1–679.e21.
13. Fiorentino, M.; Pesce, F.; Schena, A.; Simone, S.; Castellano, G.; Gesualdo, L. Updates on urinary tract infections in kidney transplantation. *J. Nephrol.* **2019**, *32*, 751–761. [CrossRef] [PubMed]
14. Butler, C.C.; Francis, N.; Thomas-Jones, E.; Llor, C.; Bongard, E.; Moore, M.; Little, P.; Bates, J.; Lau, M.; Pickles, T.; et al. Variations in presentation, management, and patient outcomes of urinary tract infection: A prospective four-country primary care observational cohort study. *Br. J. Gen. Pract.* **2017**, *67*, e830–e841. [CrossRef]
15. Khosravi, A.D.; Montazeri, E.A.; Ghorbani, A.; Parhizgari, N. Bacterial urinary tract infection in renal transplant recipients and their antibiotic resistance pattern: A four-year study. *Iran J. Microbiol.* **2014**, *6*, 74–78. [PubMed]
16. Origüen, J.; Fernández-Ruiz, M.; López-Medrano, F.; Ruiz-Merlo, T.; González, E.; Morales, J.; Fiorante, S.; San-Juan, R.; Villa, J.; Orellana, M.; et al. Progressive increase of resistance in Enterobacteriaceae urinary isolates from kidney transplant recipients over the past decade: Narrowing of the therapeutic options. *Transpl. Infect. Dis.* **2016**, *18*, 575–584. [CrossRef]

17. Azap, Ö.; Togan, T.; Yeşilkaya, A.; Arslan, H.; Haberal, M. Antimicrobial Susceptibilities of Uropathogen Escherichia coli in Renal Transplant Recipients: Dramatic Increase in Ciprofloxacin Resistance. *Transplant. Proc.* **2013**, *45*, 956–957. [CrossRef]
18. Rossignol, L.; Vaux, S.; Maugat, S.; Blake, A.; Barlier, R.; Heym, B.; Le Strat, Y.; Blanchon, T.; Hanslik, T.; Coignard, B. Incidence of urinary tract infections and antibiotic resistance in the outpatient setting: A cross-sectional study. *Infection* **2016**, *45*, 33–40. [CrossRef]
19. Demilie, T.; Beyene, G.; Melaku, S.; Tsegaye, W. Diagnostic accuracy of rapid urine dipstick test to predict urinary tract infection among pregnant women in Felege Hiwot Referral Hospital, Bahir Dar, North West Ethiopia. *BMC Res. Notes* **2014**, *7*, 481. [CrossRef] [PubMed]
20. López-Medrano, F.; Fernández-Ruiz, M.; Morales, J.M.; San-Juan, R.; Cervera, C.; Carratalá, J.; Torre-Cisneros, J.; Gavaldá, J.; Muñoz, P.; Len, Ó.; et al. Impact of Hepatitis C Virus Infection on the Risk of Infectious Complications After Kidney Transplantation: Data From the RESITRA/REIPI Cohort. *Transplantation* **2011**, *92*, 543–549. [CrossRef]
21. Moradi, M.; Abbasi, M.; Moradi, A.; Boskabadi, A.; Jalali, A. Effect of antibiotic therapy on asymptomatic bacteriuria in kidney transplant recipients. *Urol. J.* **2005**, *2*, 32–35. [PubMed]
22. Ariza-Heredia, E.J.; Beam, E.N.; Lesnick, T.G.; Cosio, F.G.; Kremers, W.K.; Razonable, R.R. Impact of urinary tract infection on allograft function after kidney transplantation. *Clin. Transplant.* **2014**, *28*, 683–690. [CrossRef]
23. Capocasale, E.; De Vecchi, E.; Mazzoni, M.; Valle, R.D.; Pellegrino, C.; Ferretti, S.; Sianesi, M.; Iaria, M. Surgical Site and Early Urinary Tract Infections in 1000 Kidney Transplants With Antimicrobial Perioperative Prophylaxis. *Transplant. Proc.* **2014**, *46*, 3455–3458. [CrossRef]
24. Green, H.; Rahamimov, R.; Gafter, U.; Leibovitci, L.; Paul, M. Antibiotic prophylaxis for urinary tract infections in renal transplant recipients: A systematic review and meta-analysis. *Transpl. Infect. Dis.* **2011**, *13*, 441–447. [CrossRef] [PubMed]
25. Green, H.; Rahamimov, R.; Goldberg, E.; Leibovici, L.; Gafter, U.; Bishara, J.; Mor, E.; Paul, M. Consequences of treated versus untreated asymptomatic bacteriuria in the first year following kidney transplantation: Retrospective observational study. *Eur. J. Clin. Microbiol. Infect. Dis.* **2012**, *32*, 127–131. [CrossRef]
26. Sabé, N.; Oriol, I.; Melilli, E.; Manonelles, A.; Bestard, O.; Polo, C.; Arcos, I.L.; Perelló, M.; Garcia, D.; Riera, L.; et al. Antibiotic Treatment Versus No Treatment for Asymptomatic Bacteriuria in Kidney Transplant Recipients: A Multicenter Randomized Trial. *Open Forum Infect. Dis.* **2019**, *6*, ofz243. [CrossRef] [PubMed]
27. Rosado-Canto, R.; Carrillo-Pérez, D.L.; Arreola-Guerra, J.M.; Sifuentes-Osornio, J. Asymptomatic bacteriuria in kidney transplant recipients: The challenge in the first 8 weeks. *Transpl. Infect. Dis.* **2018**, *20*, e12895. [CrossRef] [PubMed]

10.2. Database form:

Nº HISTORIA:

Nombre:

DATOS BASALES:

- Fecha nacimiento:
- Fecha petición del urocultivo:
- Fecha inclusión al estudio:
- Indicación de trasplante:
    0. Nefropatía diabética
    1. Nefroangioesclerosis
    2. Nefropatía tubulointersticial
    3. Glomerulonefritis
    4. Poliquistosis
    5. Tumoral/obstructiva
    6. Desconocida
- Índice de Charlson
- Profilaxis con cotrimoxazol: 0=no, 1=sí.

FACTORES DE RIESGO:

- Fecha de trasplante: 0. Trasplante menor a 1 mes; 1. Trasplante menor a 6 meses; 2. Trasplante menor a 1 año.
- Tipo de donante: 0. Cadáver; 1. Vivo no emparentado; 2. Vivo emparentado.
- Tratamiento Inmunosupresor:

0. En el primer mes post-trasplante y en la inclusión.

1. Tipo de tratamiento: 0. prednisona; 1. tacrolimus; 2. MMF (o equivalente MPA);

3. ciclosporina; 4. azatioprina; 5. Inhibidores tipo m-TOR; y sus combinaciones.

2. Dosis de MMF o equivalente (mg/día).

- Tratamiento de inducción tres meses antes a la inclusión: 0: basiliximab, 1: daclizumab o similar, 2: timoglobulina, 3: ninguno.
- Episodios de rechazo previos a la inclusión:

1. Fecha.

2. Tratamiento: a. bolos corticoides, b. timoglobulina. c. otros.

- Re-intervención

VARIABLES DEPENDIENTES PARA ITU:

- Sexo mujer: 0=no; 1=sí.
- pH urinario: 0: ácido, 1: neutro, 2: básico.
- Diabetes Mellitus: 0=no; 1=sí.
- Enfermedad renal poliquística: 0=no; 1=sí.
- Enfermedad sistémica tratada con corticoides o inmunosupresores: 0=no; 1=sí.
- Anomalías del tracto urinario: 0=no; 1=sí (reflujo urinario, uropatía obstructiva, volumen resiudal elevado…)
- Diálisis extensa (más de un año): 0=no; 1=sí.
- Factores intraquirúrgicos: 0=donante cadáver; 1=órgano donante infectado: 2=duplicidad ureteral del órgano trasplantado; 3=sondaje urinario prolongado; 4= retrasplante

- Factores postquirúrgicos: 0= inmunosupresión; 1=rechazo o disfunción del injerto; 2=infección del injerto; 3=instrumentalización de la vía urinaria prolongada (mayor a 10 días).

- Tipo de complicación post-quirúrgica en el lecho: linfocele, fuga, estenosis ureteral, sangrado/hematoma, dehiscencia, linfocele, infección de la herida quirúrgica.

- Trasplante renal previo: 0=no; 1=sí.

- Antecedente de ITU los seis meses previos a la inclusión: 0=no; 1=AB, 2= cistitis, 3=PNA

- Microorganismo responsable de la ITU previa.

- Tratamiento usado para ITUs previas en los tres meses previos a la inclusión

- Días de tratamiento usado para la ITU

- Antecedente de ITU recurrente pre-trasplante: 0=no; 1=sí.

- Co-infección concomitante: 0=CMV, 2= BK virus, 3= tuberculosis, 4=ninguna.

- ITU nosocomial: 0=no; 1=sí.

- Resistencias iniciales (0=no; 1=sí.): ciprofloxacino, fosfomicina, amoxilina-clavulánico, cefalosporinas, cotrimoxazol, productoras de BLEE o carbapenemasas.

- CMI a fosfomicina y ciprofloxacino: LLFR, LLQR, resistencia y plena sensibilidad a ciprofloxacino y/o fosfomicina.

- Filtrado glomerular a la inclusión y previa a la misma.

VARIABLES RESULTADO:

- Nº de episodio

- Germen aislado en urocultivo.

- pH urinario de inclusión, leucocitosis y nitritos.

- ITU precoz (<2 mes post-trasplante): 0=no; 1=sí.

- ITU tardía (<1 año post-trasplante): 0=no; 1=sí.
- Clínica: 0=Bacteriuria asintomática; 1=Síntomas bajos
- Adquisisión nosocomial del evento: 0=no; 1=sí.
- Tratamiento del episodio (en objetivo 1). Antibiótico empleado: 0: ciprofloxacino; 1: fosfomicina/trometamol (en el resto de objetivos).
- Duración del tratamiento del episodio (en objetivo 1).
- Adherencia correcta al tratamiento prescrito: 0=no; 1=sí.
- Curación clínica y microbiológica a los 14 días del tratamiento: 0=no; 1=sí.
- Urocultivo de control: 0=no (negativo); 1=sí (positivo, reinfección/recidiva/contaminado/desconocido)
- pH urinario de control.
- Nuevo episodio de ITU en los 30 días posteriores: 0=no; 1=sí. Reinfección, persistencia, recidiva, curación microbiológica. Síntomas al mes: 0=no; 1=cistitis, 2= PNA.
- Nuevo episodio de ITU durante los 6 meses posteriores: 0=no; 1=sí. Reinfección, persistencia, recidiva, curación microbiológica. Síntomas durante los seis meses: 0=no; 1=cistitis, 2= PNA.
- Germen de la re-infección.
- Complicación: 0=no; 1=sí (sepsis, obstrucción urinaria, etc.).
- Deterioro de la función renal en >0,5mg/dL de Cr al final del seguimiento: 0=no; 1=sí.
- Efectos adversos del antibiótico: 0=no; 1=sí.

FIN DE SEGUIMIENTO DEL PACIENTE:

- Fecha fin seguimiento

- Desenlace: 0. estable; 1. pielonefritis; 2. pérdida del injerto; 3. muerte.

- Motivo fin de seguimiento:

    0. Fin de estudio

    1. Abandono paciente y fecha

    2. Pérdida injerto y fecha

    3. Exitus relacionado y fecha

    4. Exitus no relacionado y fecha

10.3. Informed consent:

CONSENTIMIENTO INFORMADO - INFORMACIÓN AL PACIENTE

Antes de proceder a la firma de este consentimiento informado, lea atentamente la información que a continuación se le facilita y realice las preguntas que considere oportunas.

Naturaleza:

Este estudio se está realizando en el Hospital Universitario Virgen del Rocío. Con las muestras recogidas, se obtendrá una población lo suficientemente amplia para poder realizar estudios que nos proporcionen una información fiable sobre diferentes aspectos relacionados con la respuesta a diferentes tratamiento antibióticos en función de las características de la orina de receptores de trasplante renal con cultivos de orina positivos.

Este estudio pretende determinar si existe o no mejor respuesta a determinados antibióticos (ciprofloxacino y fosfomicina/trometamol) si la orina es menos ácida.

Con ello intentaremos comprobar la hipótesis de trabajo de que una orina ácida reduce la eficacia del tratamiento con ciprofloxacino pero no con fosfomicina/trometamol de las infecciones urinarias en el receptor de trasplante renal.

El estudio empezará una vez usted haya sido sometido a un trasplante y exista un cultivo positivo de orina. Durante este periodo se le programarán visitas para

comprobar la respuesta al tratamiento antibiótico mediante una entrevista y una toma de muestra de orina para cultivo.

Importancia:

El estudio pretende confirmar datos no validados hasta ahora. Por lo tanto, los resultados del estudio supondrán, a corto plazo, una ventaja para la colectividad de pacientes sometidos a un trasplante.

Implicaciones para el donante/paciente:

- La participación es totalmente voluntaria.
- El paciente puede retirarse del estudio cuando así lo manifieste, sin dar explicaciones y sin que esto repercuta en sus cuidados médicos.
- Todos los datos carácter personal, obtenidos en este estudio son confidenciales y se tratarán conforme a la Ley Orgánica de Protección de Datos de Carácter Personal 15/99.
- La donación/información obtenida se utilizará exclusivamente para los fines específicos de este estudio.

Riesgos de la investigación para el donante/paciente:

No es previsible un riesgo adicional para Ud. respecto del propio de la intervención de trasplante al que será sometido. Puesto que el estudio no plantea intervenciones en el

tratamiento diferentes a las que se realizan habitualmente en estas circunstancias, no habrá efectos indeseables derivados.

Su participación en el estudio es enteramente voluntaria y usted puede, en cualquier momento, retirarse del estudio sin justificar su decisión. Esto no afectará a la calidad de su posterior atención médica.

Si requiere información adicional se puede poner en contacto con nuestro personal del Servicio de Enfermedades Infecciosas en el teléfono 955012185 o en el correo electrónico: mariae.cordero.sspa@juntadeandalucia.es

CONSENTIMIENTO INFORMADO - CONSENTIMIENTO POR ESCRITO DEL PACIENTE

TÍTULO: Efecto del pH urinario en la efectividad clínica y / o microbiológica del tratamiento antibiótico en infecciones urinarias en receptores de trasplante renal.

.

Yo (Nombre y Apellidos):.....

• He leído el documento informativo que acompaña a este consentimiento (Información al Paciente)

• He podido hacer preguntas sobre el estudio “Efecto del pH de la orina en la eficacia clínica y/o microbiológica del tratamiento de infecciones urinarias en receptores de transplante de riñón.”

• He recibido suficiente información sobre el estudio: “Efecto del pH de la orina en la eficacia clínica y/o microbiológica del tratamiento de infecciones urinarias en receptores de transplante de riñón.”

• He hablado con el profesional sanitario informador: médico facultativo.

• Comprendo que mi participación es voluntaria y soy libre de participar o no en el estudio.

• Se me ha informado que todos los datos obtenidos en este estudio serán confidenciales y se tratarán conforme establece la Ley Orgánica de Protección de Datos de Carácter Personal 15/99.

• Se me ha informado de que la donación/información obtenida sólo se utilizará para los fines específicos del estudio.

• **Deseo** ser informado/a de mis datos genéticos y otros de carácter personal que se obtengan en el curso de la investigación, incluidos los descubrimientos inesperados

que se puedan producir, siempre que esta información sea necesaria para evitar un grave perjuicio para mi salud o la de mis familiares biológicos.

Si No

Comprendo que puedo retirarme del estudio:

• Cuando quiera

• Sin tener que dar explicaciones

• Sin que esto repercuta en mis cuidados médicos

Presto libremente mi conformidad para participar en el *proyecto titulado*

"Efecto del pH de la orina en la eficacia clínica y/o microbiológica del tratamiento antibiótico de infecciones urinarias en receptores de transplante de riñón."

Firma del paciente (o representante legal en su caso)

Firma del profesional sanitario informador

Nombre y apellidos:……. Nombre y apellidos: ……..

Fecha: …… Fecha: …….

10.4. Approval of the local ethics committee:

JUNTA DE ANDALUCIA

CONSEJERÍA DE SALUD
Dirección General de Investigación y Gestión del Conocimiento
Comité Coordinador de Ética de la Investigación Biomédica de Andalucía

RESOLUCIÓN

Visto el procedimiento de autorización administrativa para la realización del estudio posautorización de seguimiento prospectivo con medicamentos código de protocolo FIS-CIP-2016-01 titulado: *"Efecto del ph de la orina en la eficacia clínica y/o microbiológica del tratamiento de infecciones urinarias en receptores de trasplante de riñón"*, Protocolo versión 1.0 de 7 de julio de 2016, HIP/CI versión 1.0 de 7 de julio de 2016, se constata lo siguiente:

HECHOS

PRIMERO.- Con fecha 13 de octubre de 2016, D. Carlos García Pérez, en nombre y representación del promotor solicita la autorización administrativa para la realización del estudio anteriormente mencionado, cuyo Promotor es FUNDACIÓN PÚBLICA ANDALUZA PARA LA GESTIÓN DE LA INVESTIGACIÓN EN SALUD DE SEVILLA (FISEVI).

SEGUNDO.- Que el Comité Coordinador de Ética de la Investigación Biomédica de Andalucía en su reunión del 25 de octubre de 2016 (Acta 11/16), ha evaluado el mencionado estudio considerándolo adecuado y emitiendo el correspondiente informe favorable,

FUNDAMENTOS JURÍDICOS

PRIMERO.- Este Comité Coordinador de Ética de la Investigación Biomédica de Andalucía es competente para la emisión de la presente Resolución en virtud de las competencias atribuidas en el artículo 7.3d) del Decreto 439/2012, de 14 de diciembre, por el que se regulan los órganos de ética asistencial y de la investigación biomédica de Andalucía, BOJA núm. 251 de 27 de diciembre de 2010.

SEGUNDO.- De conformidad con lo establecido en el artículo 24 del Real Decreto 577/2013, de 26 de julio, por el que se regula la farmacovigilancia de medicamentos de uso humano, BOE núm. 179, de 27 de julio de 2013, el estudio *"Efecto del ph de la orina en la eficacia clínica y/o microbiológica del tratamiento de infecciones urinarias en receptores de trasplante de riñón"* cumple con la finalidad de completar la información obtenida durante el desarrollo clínico de los medicamentos previo a su autorización.

TERCERO.- Consta en el procedimiento tramitado al efecto que el estudio " *Efecto del ph de la orina en la eficacia clínica y/o microbiológica del tratamiento de infecciones urinarias en receptores de trasplante de riñón"* respeta las directrices publicadas en la Orden SAS 3470/2009, de 16 de diciembre, por la que se publican las directrices sobre estudios posautorización de tipo observacional para medicamentos de uso humano, BOE núm. 310, de 25 de diciembre de 2009.

Avda. de la Innovación, s/n Edificio Arena 1. Apdo. Correos 17.111, 41080 Sevilla
Telef. 95 500 63 00 Fax 95 500 63 31