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Proof–of–concept study to quantify changes in intestinal loads of KPC-producing *Klebsiella pneumoniae* in colonised patients following selective digestive decontamination with oral gentamicin

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ABSTRACT

Objectives: To monitor quantitatively the extent of intestinal colonisation by KPC-producing *Klebsiella pneumoniae* (KPC-Kp) in colonised patients who receive selective digestive decontamination (SDD) with oral gentamicin.

Methods: We developed a real-time quantitative PCR (qPCR) method for determination of the relative load of bla_{KPC} (RL_{KPC}) within the gut microbiota. Clinical validation was performed using a culture method as the gold standard and receiver operating curve (ROC) analysis. Fifteen patients were observationally and prospectively followed for one year. Clinical, microbiological variables and rectal swab samples were collected at 0 (baseline), 14 and 30 days and monthly thereafter.

Results: Clinical validation performed on 111 rectal swab samples demonstrated that the PCR method detected 17% more positives than the culture method. ROC curve analysis documented excellent agreement between both methods (area under the curve, 0.96; 95% confidence interval 0.93–0.99). The RL_{KPC} decreased in 6/15 (40%) and 7/12 (58.3%) patients on days 14 and 30, respectively. Persistent eradication was observed in 2/12 (16.7%), 3/9 (33.3%), 4/8 (50%) and 7/8 (87.5%) patients at 1, 3, 6 and 12 months, respectively, with a median time of 150 days (range 30–270) to persistent eradication. Gentamicin-resistant KPC-Kp isolates were identified in 4/15 (26.7%) patients. The rates of infections (57.1% vs. 12.5%, $P = 0.119$) and deaths (71.4% vs. 0%, $P = 0.007$) were higher among patients with high baseline RL_{KPC}.

Conclusion: Following SDD, a rapid reduction on intestinal load is observed when the colonising KPC-Kp isolate is susceptible to gentamicin; however, persistent eradication at the end of SDD is low. Intestinal carriage of KPC-Kp persists after three months in about one third of patients.

Introduction

Colonisation by carbapenem-resistant *Enterobacterales* (CRE) is associated with increased risk of infection and death, especially in vulnerable patients, i.e., critical, surgical, haematological or immunosuppressed patients [1–5]. A recent study provided evidence that the relative intestinal load of CRE may be an important fac-

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tor affecting the subsequent risk of infection [2]. Recent studies have employed either culture on CRE selective medium or real-time quantitative polymerase chain reaction (qPCR) for quantification of rectal colonisation by CRE; however, there are no standardised methods so far [2,6–9].

The use of oral antibiotics to decolonise patients has been widely studied, although its clinical (avoiding infection), microbiological (eradicating resistant bacteria) and epidemiological (avoiding transmission) efficacy are debatable [5,10,11]. The most used antibiotics in this situation are aminoglycosides and colistin. Our group has shown that selective digestive decontamination (SDD) with oral gentamicin reduces infection and crude mortality in patients colonised by *Klebsiella peumoniae* carbapenemase (KPC)-producing *Klebsiella pneumoniae* [3]. It is logical to hypothesise that SDD has an impact on the kinetics of intestinal colonisation by reducing the relative bacterial load. This would explain that even if complete microbiological eradication is not achieved, clinical benefits can be obtained on events related to colonisation, especially in patients at high risk of infection. In this study, we aimed to develop and validate a qPCR method for determining the relative intestinal load of bla_{KPC} gene (RL_{KPC}) in rectal swabs, study the kinetics of intestinal loads of KPC-Kp following SDD with oral gentamicin, and describe relevant epidemiological outcomes, i.e., rates of decolonisation, persistent eradication, detection of gentamicin-resistant isolates, infection and mortality.

2. Materials and Methods

2.1. Ethics

All patients or their legal representatives provided written informed consent before this study was reported. The study protocol was approved by Reina Sofía University Hospital Ethical Committee (KLEBCOM study, Reference 3197, Act 284).

2.2. Study design and patients

Prospective, observational study of 15 patients with KPC-Kp colonisation who received SDD with oral gentamicin at Reina Sofía University Hospital (Cordoba, Spain) between January 2018 and March 2019, with a 1-year follow-up. Patients fulfilled previously reported criteria for clinical indication of selective digestive decontamination [3]. Patients with signs or symptoms of concomitant infection at baseline were excluded. SDD consisted of a gentamicin solution (8 mg/mL) administered in a dose of 80 mg gentamicin every 6 hours PO for 14 days. An orabase paste of gentamicin (1.6 mg/g) was also applied to oral mucosa, gums, palate and tracheal stoma (0.5 g every 6 h for 2 weeks). Clinical infections were defined according to Center for Disease Control (CDC) criteria [12]. Rectal swabs were obtained on days 0, 14, 30 and monthly thereafter, until 1 year later, death or lost to follow-up. When clinically feasible, additional rectal swab samples were collected during SDD or infection episodes and were included for clinical validation of the qPCR method.

2.3. Primary, secondary outcomes and definitions

The primary outcome was the intestinal relative load of KPC-Kp (RL_{KPC}) (see below for definition) at day 0 (baseline, before SDD), and at 14 and 30 days (post-SDD). Secondary outcomes were persistent eradication at 1, 3, 6 and 12 months of follow-up, detection of gentamicin-resistant KPC-Kp isolates, infection and mortality rates during follow-up. Intestinal colonisation was defined as a qPCR positive for bla_{KPC} together with the isolation of a KPC-Kp isolate from a rectal swab. Gastrointestinal decolonisation at a specific time-point was defined as the absence of KPC-Kp in a patient's rectal swab sample both by culture and PCR. Persistent eradication was defined as the absence of KPC-Kp both by culture and qPCR in two consecutive rectal swabs, with at least 2-week separation, and no subsequent recolonisation during the remaining follow-up period.

2.4. Rectal swab sample collection and DNA extraction

We used rectal swabs containing one millilitre of liquid Amies transport media (ESwabs, Copan Group, Brescia, Italy) for sampling of the patient's intestinal microbiota. Rectal swabs were vortexed for one minute, and 50 μL of the Amies transport medium were submitted to culture-based quantification [9] and standard microbiological procedures (see Section 2.5 and Supplementary Materials for detailed protocols). The remaining volume (approximately 900 µL) was transferred to a microcentrifuge tube, centrifuged for 1 min at 500 $\times$ *g* to eliminate the pelleted cell debris, and the supernatant was further centrifuged for 10 min at 10,000 $\times$ *g* to collect the bacteria. Total genomic DNA (gDNA) was extracted from bacterial pellets using UltraClean Microbial DNA Isolation Kit (Qiagen, Germany), following the manufacturer's instructions, and submitted to qPCR quantification (Section 2.6).

2.5. Standard microbiological procedures

Identification of the KPC-Kp isolates was confirmed using MALDI Biotyper (Bruker Daltonics). KPC carbapenemase production was confirmed with the NG-Test CARBA 5 (NGBiotech, France), and the first KPC-Kp isolate from each patient was submitted to bla_{KPC} gene Sanger sequencing for identification of the bla_{KPC-3} allele. Antimicrobial susceptibility testing was performed using commercial microdilution methods, i.e., MicroScan WalkAway system with panels NC53 (Beckman Coulter, USA) or Sensititre DKMGN panels (Thermo Fisher, USA), for colistin, meropenem, fosfomycin, ceftolozane-tazobactam and ceftazidime-avibactam. For determination of gentamicin MICs, we used broth microdilution [13] and gradient strips (Liofilchem, Italy), according to the manufacturer's instructions. *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as control strains. Clinical categories were determined according to EUCAST version 9.0 (2019). Multilocus sequence typing was performed according to the protocol of the Pasteur Institute [14].

2.6. Relative quantification of the intestinal load of bla_{KPC} gene by qPCR in rectal swabs

Optimisation of cycling conditions, specificity, linearity ranges, efficiency, limits of detection of the qPCR reactions and bacterial suspensions are described in Supplementary Materials. qPCR assays were performed in 96-well plates with the CFX-connect TM Real-Time System (Bio-Rad Laboratories, USA). We performed two parallel qPCR reactions for each patient's gDNA sample in a single 96-well plate, i.e., for amplification of bla_{KPC} target gene (upper half of the plate) and 16S rRNA normalizer gene (lower half of the plate), with primer pairs KPC-15F/KPC-177R and 515F/685R, respectively, described elsewhere [9,15,16]. Each plate assay included the following template samples: (i) gDNA (diluted at a concentration of 0.01 ng/µL), extracted from a pure 100% KPC-Kp bacterial suspension, set as reference (six replicates); (ii) double-distilled water, used as non-template control (NTC, six replicates); and the undiluted gDNA extracted from rectal swabs (a maximum of 18 samples, each in duplicate). One hundred-fold dilutions of gDNA from rectal swabs were used when the Ct values were out of the qPCR linearity ranges. Each qPCR reaction contained 2 microliters of template DNA, 10 microliters of SYBR-green mix (iTaq Universal SYBR® Green Supermix 2X, Biorad, USA), 0.5 µM primers (KPC-

15F/KPC-177R for bla_{KPC} and 515F/685R for 16S rRNA, described elsewhere [15,16]), and molecular biology grade water up to 20 µL. Cycling conditions consisted of one holding step at 95°C for 5 seconds, followed by 40 cycles of 5 seconds at 95°C and 30 seconds at 60°C, and a final Melt Curve Analysis (5 seconds at 65°C up to 95°C, with 0.5°C increments at each step). The threshold cycles (Ct) for bla_{KPC} and 16SrRNA genes were automatically calculated by the PCR system. We used the delta-delta threshold cycle (ΔΔCt) algorithm to estimate the relative load of bla_{KPC} (RL_{KPC}) in rectal swabs, compared to the 100% KPC-Kp reference bacterial culture, i.e., $RL_{KPC} = 2^{-\Delta\Delta Ct}$, where ΔΔCt = (ΔCt [gDNA from a patient's rectal swab]–ΔCt [gDNA from the 100% KPC-Kp bacterial suspension]) and ΔCt = (ΔCt [16S rRNA]–ΔCt [bla_{KPC}]).

2.7. Statistical analysis

Epidemiological values were expressed as the median and interquartile range for continuous variables and as percentages for categorical variables. Linearity of the qPCR was validated by linear regression. Sensitivity, specificity, positive predictive value, negative predictive value and receiver operating characteristic (ROC) curve analyses were used to assess the accuracy of the qPCR relative to the culture-based quantification method. The optimal diagnostic cut-off value was determined by calculating the Youden Index of the ROC curve. Correlation among bacterial load values obtained by both methods was assessed by means of Kendall's tau and Spearman's rho correlation coefficients and clinical agreement by means of Kendall's concordance (W) coefficient and the intraclass correlation coefficient (ICC). We considered a P value of <0.05 to be significant. Statistical analysis was performed using the SPSS version 25.0 software (IBM, USA).

3. Results

3.1. Diagnostic accuracy of the qPCR assay for determination of RL_{KPC} in rectal swabs

We performed clinical validation of the qPCR test on 111 rectal swab samples obtained from 15 patients during 1 year of follow-up [6]. The median (range, minimum, maximum) intestinal load of KPC-Kp relative to total aerobic bacteria, according to the quantitative standard culture method (set as the gold standard for this study) was 0.0002% (0.00%–0.64%, 0.00%, 100.00%). The median intestinal load of *bla*KPC relative to 16SrRNA genes (RL_{KPC}) according to qPCR analysis was 0.0004% (0.00%–0.10%, 0.00%, 96.27%). The area under the ROC (AUROC) curve documented excellent agreement between both methods (AUROC 0.96; 95% confidence interval 0.93–0.99, $P < 0.001$), with sensitivity and specificity of 100% and 76.4%, respectively. The positive predictive value and negative predictive value were 81.3% and 100%, respectively. The accuracy of KPC-Kp detection by qPCR relative to culture was 85.43%. The Youden Index for the ROC curve reached a maximum (0.768) with a qPCR RL_{KPC} value of 0.001%, which was established as the optimal diagnostic cut-off value relative to the culture method. We observed strong positive correlation (Kendall's tau = 0.794, $P < 0.001$; Spearman's rho = 0.900, $P < 0.001$) between the two assays, while statistical estimates showed low concordance (Kendall's W = 0.137, $P < 0.001$; ICC 0.288, 95% confidence interval 0.100–0.455, $P = 0.002$). All discordant samples (N = 12) were qPCR-positive and culture-negative (Supplementary Table S1) and showed qPCR-based RL_{KPC} below or equal to the calculated diagnostic cut-off value of 0.001%. These findings suggest that the qPCR is consistent with conventional culture for determination of KPC-Kp RL_{KPC} above the diagnostic threshold of 0.001%.

Table 1
Characteristics of 15 patients who received selective digestive decolonisation (SDD) therapy with oral gentamicin

	No. (%)
Age (years), median (range)	70 (55–73)
Male	10 (66.7)
Intensive care unit admission	5 (33.3)
Length of first hospital stay (days), median (IQR)	54 (38–86)
Prior hospitalisation (3 months)	7 (46.7)
Prior immunosuppressive therapy (3 months)	4 (26.7)
Prior antibiotic exposure (1 month), n (%)	12 (80.0)
β-lactam ± β-lactamase inhibitors	7 (46.7)
cephalosporins	3 (20.0)
carbapenems	2 (13.3)
aminoglycosides	1 (6.7)
fluoroquinolones	5 (33.3)
Invasive procedures prior to recruitment	
surgical (3 months)	7 (46.7)
mechanical ventilation (1 month)	7 (46.7)
central venous catheter (1 month)	10 (66.7)
urinary catheterization (1 month)	11 (73.3)
Comorbidities	
diabetes mellitus	6 (40.0)
tumour	5 (33.3)
chronic kidney disease	3 (20.0)
solid organ transplant recipient	2 (13.3)
recurrent urinary tract infection	1 (6.7)
Charlson's score, median (range)	2 (1-2)
McCabe's score, median (range)	
non-fatal	3 (20.0)
rapidly fatal	7 (46.7)
ultimately fatal	5 (33.3)
Risk factor that indicated decolonisation	
Major surgery in the previous month	4 (26.0)
Multi-comorbid patients [a]	8 (53.3)
Previous recurrent, severe KPC-Kp infections	3 (20.0)
Systemic antibiotics in the first 30 days of follow-up	10 (66.7)
β-lactam ± β-lactamase inhibitors	8 (53.3)
aminoglycosides	1 (6.7)
Colistin	1 (6.7)
Primary outcome: RL_{KPC} (%, median, range)	
Baseline (day 0)	0.68 (0.001–9.83)
Day 14	<0.001 (0.00–0.06)
Day 30	0.01 (0.00–2.71)
Secondary outcomes	
Decolonisation on day 14 (among survivors)	
Day 14	5/15 (33.3)
Day 30	3/12 (25.0)
Persistent eradication (among survivors)	
Day 30	2/12 (16.7)
Day 90	3/9 (33.3)
6 months	4/8 (50.0)
12 months	7/8 (87.5)
Detection of gentamicin-resistant KPC-Kp isolates	4 (26.7)
KPC-Kp-related infections	5 (33.3)
Crude mortality	
Day 30	2 (13.1%)
1 year	5 (33.3)

RL_{KPC}, relative intestinal load of bla_{KPC}.
[a] Multi-comorbid patients: patients with more than two chronic debilitating diseases including diabetes mellitus, chronic pulmonary disease, chronic liver disease, renal insufficiency, chronic cardiac insufficiency and immunodepression.

3.2. Kinetics of RL_{KPC} following SDD with oral gentamicin

The clinical characteristics of the 15 patients included in this study are summarised in Table 1. The kinetics of RL_{KPC} during 1-year follow-up, based on qPCR determination, are graphically represented in Fig. 1. Study outcomes have been recorded in Table 2.

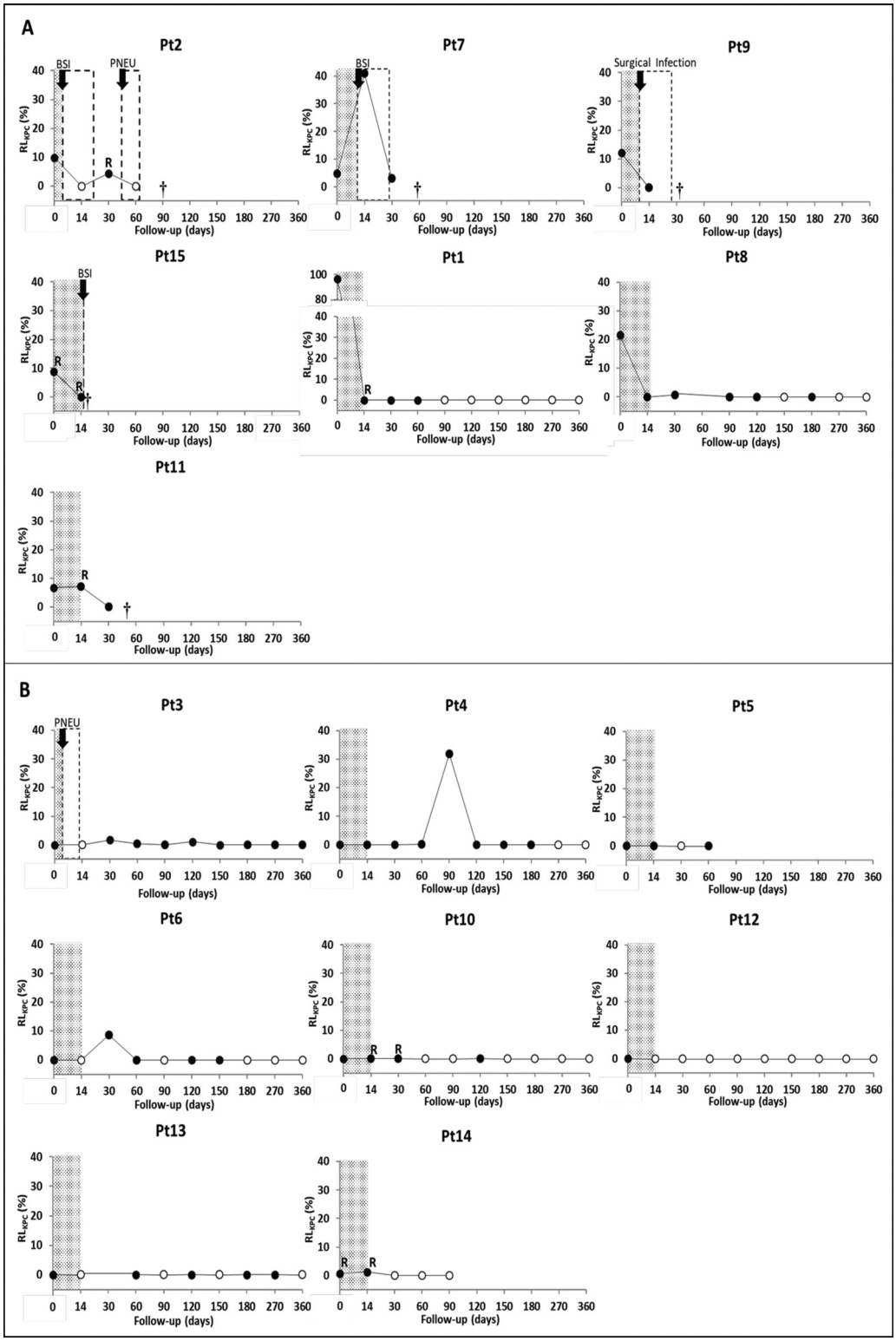

Fig. 1. Evolution of the relative intestinal load of *bla*$_{KPC}$ (RL_{KPC}) in rectal swabs, estimated by qPCR. Patients were classified as having (A) high or (B) low baseline-RL_{KPC}. The duration of selective digestive decolonisation (SDD) is represented with a dotted area. Onset of an infection episode is indicated by an arrow, and the duration of targeted antibiotic therapy is indicated by a discontinuous rectangle. White circles represent rectal swabs negative for KPC-Kp (0% RL_{KPC}). BSI, bloodstream infection; PNEU, pneumonia; R, gentamicin-resistant KPC-Kp isolate.

Regarding primary endpoints, the median baseline-RL_{KPC} (day 0) was 0.68% (range 0.001%–9.83%, minimum <0.001%, maximum 96.27%). This value decreased to <0.001% (range 0.00%–0.06%, minimum 0%, maximum 40.90%, $P = 0.173$) on day 14, and to 0.01% (range <0.001%–2.71%, minimum 0%, maximum 8.67%; $P = 0.071$) on day 30 of follow-up. Based on potential clinical significance, we classified patients into two groups, according to their baseline-RL_{KPC} being above (7/15, high baseline-RL_{KPC}) or below (8/15, low baseline-RL_{KPC}) the global median baseline-RL_{KPC} for this cohort (Fig. 1). The observed reduction in this median value with regard

Table 2
Study outcomes of 15 patients who received SDD with oral gentamicin (reported relative intestinal bla_{KPC} loads (RL_{KPC}) are based on qPCR assay)

Patient	Age (sex)	Underlaying disease	Risk factor for SDD [a]	SDD (duration, days)	RL_{KPC} (%)			Microbiological eradication (first negative swab, days)	Type of Infection (onset, days)	Death (time, days)
					0 d (baseline)	14 d	30 d			
Pt1	55 (M)	Fournier gangrene	MS	14	96.27	<0.001	0.002	Yes (90)	-	alive
Pt8	61 (F)	Diabetes mellitus	MC	14	21.61	0.001	0.67	Yes (270)	-	alive
Pt9	71 (F)	Diabetes mellitus, Rheumatoid arthritis	MS	8	12.10	<0.001	NA	No	SI (8)	29
Pt2	70 (M)	Pneumonia in heart transplant patient	MS	3	9.83	0	4.38(R)	No	BSI & PNEU (3) PNEU (49)	83
Pt15	65 (M)	Hypertension, chronic kidney disease, metabolic syndrome	MC	7	8.80(R)	<0.001 (R)	NA	No	BSI (7)	16
Pt11	81 (M)	Diabetes mellitus, lymphoma	MC	14	6.76	7.20 (R)	0.01	No	-	49
Pt7	71 (M)	Septic shock (biliary source)	MC	13	4.95	40.90	3.05	No	BSI (13)	55
Pt14	60 (M)	Severe head trauma	MS	14	0.68(R)	1.26 (R)	0	Yes (30)	-	alive
Pt10	46(M)	Pancreatic adenocarcinoma	RSI	14	0.06	<0.001 (R)	<0.001 (R)	Yes (150)	-	alive
Pt4	73 (M)	Kidney transplant	MC	14	0.05	0.06	<0.001	Yes (270)	-	alive
Pt3	51 (F)	Severe head trauma	RSI	2	0.02	0	1.69	No	PNEU (2)	alive
Pt6	84 (F)	Lung cancer	MC	14	0.001	0	8.67	Yes (180)	-	alive
Pt13	90 (M)	Colon cancer	RSI	14	<0.001	0	NA	No	-	alive
Pt5	51 (F)	Diabetic nephropathy (dialysis)	MC	14	<0.001	< 0.001	0	No	-	alive
Pt12	72 (M)	Lymphoma	MC	14	<0.001	0	0	Yes (14)	-	alive

BSI, bloodstream infection; F, female; M, male; MC, multiple comorbidities; MS, major surgery; PNEU, pneumonia; R, gentamicin-resistant KPC-Kp isolate; RSI, recurrent, severe KPC-Kp infections SDD, selective digestive decolonisation; SI, surgical infection.

[a] Risk factor for SDD [3]: MS (including transplantation) performed within the following 2 weeks; MC, i.e. more than two chronic debilitating diseases, including diabetes mellitus, chronic pulmonary disease, chronic liver disease, renal insufficiency, chronic cardiac insufficiency and immunodepression).

to day 0 was statistically significant only on day 30 in the group of patients with high baseline-RL_{KPC} ($P = 0.043$, Supplementary Table S2).

On day 14, we observed a reduction in RL_{KPC} in 11/15 (73.3%) patients (Table 2); however, gastrointestinal decolonisation was observed in only 5/15 (33.3%) patients, 1/7 (14.3%) in the high baseline-RL_{KPC} and 4/8 (50%) in the low baseline-RL_{KPC} groups ($P = 0.282$). In addition, 1/15 (6.7%) patients in the low baseline group (Pt4) showed no substantial RL_{KPC} change, and 3/15 (20%) showed increased RL_{KPC}, possibly related to the presence of gentamicin-resistant KPC-Kp isolates (Pt11 and Pt14) or development of a KPC-Kp-related bloodstream infection episode on day 13 (Pt7) (Fig. 1 and Table 2). Among the five patients who were decolonised on day 14, only one (Pt12) remained decolonised for the rest of the follow-up; another one (Pt2) showed a RL_{KPC} rebound on day 30, likely related to the presence of a gentamicin-resistant KPC-Kp isolate, and the last two (Pt3 and Pt6) showed recolonisation with no detection of gentamicin-resistant isolates on day 30 (Table 2 and Fig. 1). On day 30, 7/12 (58.3%) patients showed reduced RL_{KPC} compared to baseline values; however, only 3/12 (25.0%, Pt5, Pt12 and Pt14) showed decolonisation, and they all were included in the low baseline-RL_{KPC} group (Fig. 1). Among them, two remained decolonised during follow-up (Pt12 and Pt14), and one (Pt5) showed recolonisation with a low RL_{KPC} value.

Regarding secondary outcomes, the rate of persistent eradication at 1, 3, 6 and 12 months was 2/12 (16.7%), 3/9 (33.3%), 4/8 (50%) and 7/8 (87.5%), respectively, with a median time of 150 days (range, 30–270) to persistent eradication. De novo detection of gentamicin-resistant KPC-Kp isolates was observed in 4/15 (26.7%) patients within the first 30 days of follow-up; however, we did not identify gentamicin-resistant colonisation in subsequent points of follow-up in these patients (Fig. 1, Pt2, Pt1, Pt11 and Pt14). The antibiotic sensitivity results obtained for the complete KPC-Kp isolate collection (N = 60) are shown in Supplementary Table S3. Of note, two of the baseline KPC-Kp isolates were resistant to gentamicin (MIC >16 mg/L), but patients were treated because of the relevance of decolonisation in these cases. Multilocus-sequence typing of baseline, gentamicin-resistant and infecting KPC-Kp isolates in all patients who developed gentamicin-resistant colonisation or KPC-Kp-related infection confirmed that they all belonged to the KPC-3-producing *Klebsiella pneumoniae* sequence type (ST)-512 clone, endemic in our hospital.

The rate of patients who developed KPC-Kp-related infections was 5/15 (33.3%), i.e., 4/7 (57.1%) vs. 1/8 (12.5%) in the high vs. low baseline-RL_{KPC}, respectively ($P = 0.119$). One patient (Pt2) developed two infection episodes during follow-up (Table 2 and Fig. 1). Most (4/6) infection episodes occurred within 14 days of recruitment, resulting in interruption of SDD and administration of ceftazidime-avibactam-based systemic therapy. Interestingly, the RL_{KPC} was reduced during or at the end of all infection episodes for which follow-up rectal swabs were available (Fig. 1). Crude mortality was 5/15 (33.3%). All patients who died were within the high baseline-RL_{KPC} group, i.e., 5/7 (71.4%) vs. 0% in the low baseline-RL_{KPC} ($P = 0.007$).

4. Discussion

In this study, we aimed to: (i) characterise quantitatively the kinetics of KPC-Kp intestinal colonisation by using real-time quantitative PCR; (ii) clinically validate our qPCR, using a culture-based method as gold standard; and (iii) describe relevant epidemiological outcomes.

Regarding validation of the qPCR assay, we found good clinical correlation with conventional culture; however, concordance was

low, as a result of discrepancies in 12 samples that were culture-negative and qPCR-positive. On the one hand, the qPCR assay may be more sensitive than the culture-based assay, as supported by previous studies [17,18]. However, in our experimental conditions, it is not possible to rule out that the positive qPCR test may be highlighting the presence of residual KPC-Kp gDNA derived from dead cells and not necessarily correlate with a low concentration of viable bacteria that may go undetected by the culture-based method. Most importantly, it remains to be established the potential clinical significance of detecting such low levels of KPC-Kp colonisation, below the detection limit of conventional culture—for instance, with regard to surveillance and infection control purposes. Finally, the turnaround time was significantly shorter for the qPCR-based method than for culture, with a mean of 6 h vs. 30 h for culture, under optimal conditions. The utility of qPCR for estimating the relative intestinal load of *K. pneumoniae* producing KPC or OXA-48 has been previously demonstrated [2,6–9].

Regarding the potential impact of SDD on KPC-Kp intestinal loads, we report that the RL_{KPC} was reduced in about 60% of patients in our cohort on day 30 in the absence of gentamicin-resistant KPC-Kp isolates. However, as previously observed [5,19–21], the effect of SDD in reduction of CRE load is not long-lasting and colonisation reappears after a window period. We observed a high rate of recolonisation in our study, although at low intestinal load levels possibly due to the greater sensitivity of the qPCR-based method.

Our group has shown previously that SDD reduces the risk of infection and death in patients with rectal colonisation by KPC-Kp [3]. We observed a high number of infections and higher mortality in patients with high baseline-RL_{KPC}. We hypothesise that the protective effect of SDD may be achieved by reducing the intestinal RL_{KPC} during the risk period for infection. A recent prospective, longitudinal, observational study has shown that increased RL_{KPC} is associated with KPC-Kp bacteraemia [2]. Another report indicates that hospitalised patients who developed a hospital infection by OXA-producing *Klebsiella pneumoniae* have, on average, a higher relative load of bla_{OXA-48} than patients without infections [8]. Further studies are needed to clarify whether SDD is equally beneficial to patients with high or low $RL_{KPC,}$ as well as to establish the clinical risk factors associated with having different RL_{KPC}.

Our findings also support previous data from our group indicating that SDD with gentamicin has an impact on development of gentamicin resistance, i.e., 4/15 (26.7%) of patients in our present cohort versus 6/28 (21.4%) in our previous study [3], although here we observed that the increase in gentamicin MIC was transient. The risk of resistance development is one of the reasons why the 2019 European Society of Clinical Microbiology and Infectious Diseases (ESCMID)-European Committee of Infection Control (EUCIC) clinical guidelines on decolonisation of multidrug-resistant Gram-negative bacteria carriers do not recommend routine decolonisation in clinical practice [11].

Our study has limitations. The sample size is small. In our hospital, SDD is currently prescribed by the infectious disease specialist only to patients fulfilling risk-based criteria for developing a KPC-Kp infection [3,10], and all consecutive patients fulfilling these criteria between January 2018 and January 2019 were invited to participate. The study was designed as a proof-of-concept study of the applicability of our qPCR for monitorization of intestinal loads of KPC-Kp in this clinically relevant context and thus reflects real clinical practice in a large tertiary teaching hospital with current endemicity by KPC-Kp during this period. Importantly, we also made all possible efforts to ensure completion of follow-up by incorporating a dedicated nurse to the research team, who visited frail, elderly or bedridden patients at their locations if they were unable to return to hospital for rectal swab sample collection. A second limitation is that the study was not designed to account for the contribution of spontaneous reduction or decolonisation of intestinal KPC-Kp, which may mask the direct impact of SDD. Finally, another limitation of this study is that we performed molecular typing of KPC-Kp isolates only in a subset of patients with gentamicin-resistant KPC-Kp or infecting KPC-Kp isolates; thus it is not possible to rule out the acquisition of new strains other than the endemic KPC-3-producing *Klebsiella pneumoniae* ST-512 clone during follow-up in the remaining patients.

Our observations may have important implications for clinical management. First, new studies are necessary to confirm the potential association of baseline CRE load with the risk of infection or death, which may serve to discriminate in the clinic those patients susceptible to receiving SDD. Second, we observed that the rate of reduction in RL_{KPC} is not homogeneous after the first dose of SDD. It may take a few days to have a maximum effect. Thus, SDD may be started a few days before the start of a risk period (i.e., chemotherapy course involving a period of neutropenia, HSCT, scheduled intervention, etc.) and may be maintained for the full duration of the risk period (e.g., period of neutropenia, postoperative), with close monitoring of resistance development. Finally, SDD may be repeated every time the patient is at high risk of infection.

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Competing interests

None of the authors reports any conflicts of interest.

Ethical approval

Not required.

Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.jgar.2022.04.010.

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