The Lancet Infectious Diseases

Effectiveness and safety of a structured de-escalation from antipseudomonal β-lactams in bloodstream infections due to Enterobacterales: a multicentre randomised clinical trial (SIMPLIFY).

--Manuscript Draft--

Manuscript Number:	THELANCETID-D-23-01225R3
Article Type:	Article (Clinical Trials)
Keywords:	de-escalation; Antimicrobial Stewardship; antimicrobial use; bloodstream infection; ecological impact; Enterobacterales
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Abstract:	Background: De-escalation from broad-spectrum to narrow-spectrum antibiotics is considered an important measure to reduce the selective pressure of antibiotics, but is performed much less frequently than desired. Lack of adequate evidence is a barrier to its implementation. The objective was to determine whether de-escalation from an anti-pseudomonal β-lactam to a narrower-spectrum drug is non-inferior to continuing the antipseudomonal drug in patients with Enterobacterales bacteraemia. Methods: An open-label, pragmatic, randomised trial was performed between October 2016 and January 2020 in 21 Spanish hospitals. Patients with bacteraemia caused by Enterobacterales susceptible to one of the de-escalation options and treated empirically with an antipseudomonal β-lactam were eligible. Patients were assigned (1:1) to de-escalate to ampicillin, trimethoprim-sulfamethoxazole (only urinary tract infections), cefuroxime, cefotaxime or ceftriaxone, amoxicillin-clavulanic acid, ciprofloxacin or ertapenem in that order according to susceptibility, or to continue with the empiric antipseudomonal β-lactam. Oral switching was allowed. The primary outcome was clinical cure 3–5 days after end of treatment. The non-inferiority margin was 10%. Findings: Of 344 randomised patients, 331 (164 and 167 in the experimental and control arms, respectively) formed the modified intention-to-treat population and were included in the primary analysis. The primary outcome was achieved by 148 patients (90·2%) in the de-escalation arm, and 148 (88·6%) in the non-de-escalation arm (risk difference, 1·60%; 95% CI, -5·03 to 8·23; p=0·39 for non-inferiority). The effect was consistent in adjusted and subgroup analyses. Severe adverse events were reported in 53 (24·2%) and 56 (32%) patients in the de-escalation and non-de-escalation arms, respectively. Interpretation: De-escalation from an antipseudomonal β-lactam in Enterobacterales bacteraemia following a predefined rule was non-inferior to continuing the empiric antipseudomonal drug. These results support de-escalation in this setting. Trial registration: EudraCT number 2015-004219-19. Clinicaltrials. gov identifier NCT02795949).

Seville, 25th October 2023

Dear Dr Hall,

Editor, The Lancet Infectious Diseases

Thank you for the opportunity to submit a new version of our manuscript entitled *"Effectiveness and safety of a structured de-escalation from antipseudomonal β-lactams in bloodstream infections due to Enterobacterales: a multicentre randomised clinical trial (SIMPLIFY)"* (manuscript ID: THELANCETID-D-23-01225). Please see our responses to your comments below.

Luis Eduardo López-Cortés

On behalf of all authors

Comments' to the Author:

1. Do you wish the names of the collaborators in the SIMPLIFY study group to be listed on PubMed? If your answer is yes, please upload with your revision a list of names of all study group members presented as a two-column table in Word. First and middle names or initials should be placed in the first column, and surnames in the second column. Names should be ordered as you wish them to appear on PubMed. The table will not be included in the paper itself - it's simply used to make sure that PubMed adds the names correctly.

   Response: we add a table. Thank you for the opportunity to include all the authors; It is very important for us.

1. Please can you provide completed ICMJE (coi) forms for Patricia Capon Gonzalez and Diego Vicente Anza.

   Response: Sure. Attached are both signed documents.

2. Please can you provide signed author statement forms for Maria Teresa Perez-Rodriguez and Mariona Xercavins-Valls.

   Response: Attached are both signed documents.

3. Please modify the Contributors statement to describe who has access to the data in the study, who verified the data in the study, and who was responsible for the decision to submit for publication.

   Response: we change it following your recommendation (line 548).

4. For the newly described literature search in the Evidence before this study paragraph of the Research in context panel, please list the databases searched and the date range of the search

   Response: we change the sentence as follows: “A systematic review including studies published in MEDLINE via PubMed from inception until January 2020 found low level evidence for recommending de-escalation in short-term treatments”.

5. Please check the number randomised to each arm in the Summary (there are two sentences, which contradict each other)

   Response: we deleted the first sentence and corrected the one in results as follows: “171 patients were randomised to the experimental arm and

173 to the control arm; 164 and 167, respectively, formed the mITTP and were included in the primary analysis".

6. Please include Supplement A (the protocol) and Supplement B (the CONSORT statement extension) in the main supplementary file and resupply

   Response: we include both documents.

7. How were 'acquisition type' and 'source of bacteraemia' determined? I apologise if I missed a description of this in the protocol

   Response: You are right, these variables were not defined in the study protocol but only in the CRF. Acquisition type was defined as by Friedman's criteria (Friedman ND, Kaye KS, Stout JE, et al. Health care—associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. Ann Intern Med. 2002 Nov 19;137(10):791-7). Source of bacteraemia was defined as the site of infection, according to organ-related signs and symptoms, and when appropriate, isolation of the bacteria from an appropriate sample from the infection site. We included both in Methods (line 245).

8. Could the trial profile show flow through all of the different analysis populations - eg, the CEP and MEP?

   Response: We included the data on the flow chart (Figure 1)

9. In the authors' list, please check the name of Jose Manuel Guerra Glaso (or Laso?)

   Response: You are right, thanks for review it. We corrected it.

10. Is it possible to include ethnicity data in table 1?

    Response: We are sorry because we did not include this data in the protocol.

11. Can you make it clearer in the paper (Summary and methods) how the 95% CI for the treatment difference should be interpreted in relation to the non-inferiority margin - ie, non-inferiority is declared when the upper bound/lower bound is below/above the NI margin

    Response: We included the interpretation as suggested. In the abstract, we changed "The non-inferiority margin was -10%" for "Non-inferiority was declared when the lower bound of the 95% CI of the absolute

difference in cure rate is below the -10% non-inferiority margin". We also included the same sentence in Methods.

12. The figure files you have provided are not editable, containing images whose content cannot be selected. Please can you provide editable files? More information can be found here: https://www.thelancet.com/for-authors/forms?section=artwork.

    Response: we provide figures as editable files.

TITLE: Effectiveness and safety of a structured de-escalation from antipseudomonal β-lactams in bloodstream infections due to Enterobacterales: a multicentre randomised clinical trial (SIMPLIFY).

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KEY WORDS: de-escalation, antimicrobial stewardship, antimicrobial use, bloodstream infection, ecological impact, Enterobacterales.

RUNNING TITLE: De-escalation in bacteraemia

WORD COUNT: 3771 words

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Research in context

Evidence before this study: De-escalation is an antimicrobial stewardship strategy in which empiric antimicrobials are either replaced by narrower spectrum agents or discontinued if used in unneeded combination, with the aim to reduce selection pressure on microorganisms to contribute to the control of antimicrobial resistance. Despite considered as standard of practice by many infectious diseases specialists, there is a lack of high-level evidence for the non-inferiority effectiveness of de-escalation. A systematic review including studies published in MEDLINE via PubMed from inception until January 2020 found low level evidence for recommending de-escalation in short-term treatments; we performed a literature search including the terms “de-escalation” or “streamlining”, and “antimicrobial therapy” or “antibiotics” but could not find any randomised trial published thereafter. As de-escalation is a broad concept, randomised trials in specific clinical situations using structured de-escalation protocols are needed.

Added value of this study: De-escalation was investigated in a specific clinical situation (bacteraemia due to Enterobacterales receiving empirical therapy with an antipseudomonal β-lactams) and using a pragmatic, predefined rule to select the narrower-spectrum drug. De-escalation was proved to be non-inferior in clinical effectiveness and safety to continuing with the initial antipseudomonal drug.

Implications of all the available evidence: Beyond other considerations, an important barrier to de-escalate is the lack of adequate evidence for its efficacy and safety in specific clinical situations. To our knowledge, this is the first randomised trial investigating the effectiveness of antibiotic de-escalation in a specific clinical situation.

These results should facilitate the implementation of de-escalation in clinical practice and facilitating sequential oral treatment, which may help reducing the ecological impact of antipseudomonal β-lactams.

Summary

Background: De-escalation from broad-spectrum to narrow-spectrum antibiotics is considered an important measure to reduce the selective pressure of antibiotics, but is performed much less frequently than desired. Lack of adequate evidence is a barrier to its implementation. The objective was to determine whether de-escalation from an anti-pseudomonal β-lactam to a narrower-spectrum drug is non-inferior to continuing the antipseudomonal drug in patients with Enterobacterales bacteraemia.

Methods: An open-label, pragmatic, randomised trial was performed between in 21 Spanish hospitals. Patients with bacteraemia caused by Enterobacterales susceptible to one of the de-escalation options and treated empirically with an antipseudomonal β-lactam were eligible. Patients were randomised (1:1) stratified by urinary source to de-escalate to ampicillin, trimethoprim-sulfamethoxazole (only urinary tract infections), cefuroxime, cefotaxime or ceftriaxone, amoxicillin-clavulanic acid, ciprofloxacin or ertapenem in that order according to susceptibility, or to continue with the empiric antipseudomonal β-lactam. Oral switching was allowed. The primary outcome was clinical cure 3–5 days after end of treatment in the modified intention-to-treat population (mITTP), formed by patients who received at least one dose of study drugs. Safety was assessed in all participants. Non-inferiority was declared when the lower bound of the 95% CI of the absolute difference in cure rate is below the -10% non-inferiority margin~~The non-inferiority margin was -10%~~. This trial is registered with EudraCT number 2015 004219 19 and Clinicaltrials.gov identifier NCT02795949. It was completed on July 2020. ~~One hundred seventy-three patients were randomised to the control arm and 171 to the experimental one.~~

Findings: Between October 2016 and January 2020, 1713 patients were randomised to the experimental arm and 1731 to the control arm; 164 and 167, respectively, formed the mITTP and were included in the primary analysis. The primary outcome was achieved by 148 patients (90·2%) in the de-escalation arm, and 148 (88·6%) in the non-de-escalation arm (risk difference, 1·60%; 95% CI, -5·03 to 8·23).. Severe adverse events were reported in 53 (24·2%) and 56 (32%) patients in the de-escalation and non-de-escalation arms, respectively. Six (4·6%) and 9 (5·6%) patients in the experimental and control arm died during the 60-day follow-up, respectively.

Interpretation: De-escalation from an antipseudomonal β-lactam in Enterobacterales bacteraemia following a predefined rule was non-inferior to continuing the empiric antipseudomonal drug. These results support de-escalation in this setting.

Funding: The study was funded by the 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 RD16/0016/0001; 0002; 0003; 0005; 0007; 0008; 0009; 0011; 0012; 0015); Spanish Clinical Research and Clinical Trials Platform, (SCReN, PT13/0002/0010 and PT17/0017/0012, and PI15/00439), co-financed by European Union; European Development Regional Fund “A way to achieve Europe”, Operative Program Intelligence Growth 2014-2020.

INTRODUCTION

Inappropriate antimicrobial treatment of patients with invasive infections is associated with higher mortality;[1] therefore, empiric treatment with broad-spectrum antibiotics (BSA) is recommended in severe infections to ensure appropriate coverage of the causative microorganism(s).[2] However, these drugs also exert a significant selection pressure that contributes to the spread of multidrug-resistant (MDR) bacteria.[3] This is particularly important in the case of antipseudomonal beta-lactams which in addition to selection pressure, may also induce the expression of resistance mechanisms in *Pseudomonas aeruginosa*[4,5], and are associated with an increased risk of infections caused by *Clostridiodes difficile*[6] and carbapenem-resistant Enterobacterales.[7] Unfortunately, exposure to antipseudomonal drugs is very common in hospitalised patients.

De-escalation to a narrow-spectrum drugs in patients initially treated with BSA once the aetiology of an infection is known is advocated as a key measure to reduce exposure to the latter. Although considered standard of care by most infectious diseases specialists, de-escalation is performed much less frequently than desired.[6, 8-10] An important barrier to de-escalate for many physicians is the lack of adequate evidence on its efficacy and safety, particularly when early improvement with empirical therapy is not evident. In fact, a Cochrane review found insufficient evidence to recommend for or against de-escalation in adults with sepsis,[11] and a recent systematic review found low level evidence for recommending de-escalation in short-term treatments.[12] Therefore, randomised trials are needed. Because de-escalation is a broad concept, trials should include specific clinical situations and

structured de-escalation protocols to be applicable in clinical practice.

Antipseudomonal agents are frequently recommended as empirical drugs in patients with severe healthcare-associated or nosocomial infections; if the infection is shown to be caused by an Enterobacterales, the question whether de-escalation to an *in vitro* active non-antipseudomonal agents should be performed is then typically raised. We hypothesised that de-escalation to an active non-antipseudomonal drug in these patients, using a predefined rule, would be non-inferior to continuing with the empirical drug.

METHODS

Study design

The SIMPLIFY trial (EudraCT 2015-004219-19; ClinicalTrials.gov NCT02795949) is an investigator-driven, pragmatic, multicentre, open-label, controlled clinical trial in patients with bacteraemia caused by Enterobacterales who received empirical treatment with an antipseudomonal β-lactam. The effectiveness of two strategies as targeted treatment was compared: the experimental strategy, in which patients are de-escalated to a non-antipseudomonal drug according to isolate susceptibility, following a prespecified list of drugs; and the control strategy, in which patients are continued with the empirical antipseudomonal drug. The Hospital Universitario Virgen Macarena Ethics Committee approved the study; written informed consent was obtained from all participants. The study protocol was published[13] and is available in Supplement A. The results are reported in accordance

with the CONSORT statement extension (supplementary material B) for non-inferiority and equivalence trials.[14]

The trial was conducted in 21 Spanish public tertiary hospitals between October 2016 and January 2020, with the support of the Spanish Network for Research in Infectious Diseases (REIPI) and the Spanish Clinical Research Network (SCReN).

Participants

Hospitalised patients aged ≥ 18 years with monomicrobial Enterobacterales bacteraemia were eligible for enrolment if they fulfilled all the following inclusion criteria: (1) receipt of empiric monotherapy (started <24 hours after blood cultures were taken) with an antipseudomonal β-lactam with *in vitro* activity against the causative bacteria, including meropenem, imipenem, piperacillin-tazobactam, cefepime, ceftazidime or aztreonam; (2) the causative organism was susceptible *in vitro* to at least one of the antibiotics included in the experimental arm (see below); and (3) intravenous antimicrobial treatment was planned for at least 5 days from the first active drug received. A negative pregnancy test was required for women of childbearing age. Exclusion criteria were: (1) life expectancy <30 days; (2) pregnancy or breastfeeding; (3) isolation of carbapenemase-producing Enterobacterales; (4) recruitment >48 hours after the antimicrobial susceptibility report was available; (5) neutropenia <500 cells/μL at randomization; (6) and planned duration of treatment >28 days (e.g., osteomyelitis or infectious endocarditis). Baseline patient characteristics were collected on the day of randomisation, except for clinical severity

variables, which were collected retrospectively for the day the first blood cultures were draw. Acquisition type was defined as by Friedman's criteria.[15] Source of bacteraemia was defined as the site of infection, according to organ-related signs and symptoms, and when appropriate, isolation of the bacteria from an appropriate sample from the infection site. Written informed consent for participation was obtained from patients. The recruitment period took place between October 5th 2016 and January 23rd 2020.

Randomization and masking

In the first 48 hours after the antimicrobial susceptibility report was available, patients were randomly assigned (1:1) to continue with the same empiric intravenous antipseudomonal drug (control group) or to switch to the first *in vitro* active drug from a predefined de-escalation list (de-escalation group), both in monotherapy.

Assignment to treatment group was performed centrally by a web-based automated system integrated with the electronic case report form (eCRF). Simple randomisation was performed, stratified only by source of bacteraemia (UTI or other). Blinding was not possible due to multiple options in both arms.

Procedures

The order of the de-escalation list was: ampicillin; trimethoprim-sulfamethoxazole (TMP-SMX; only urinary tract infections [UTI]); cefuroxime; cefotaxime or ceftriaxone; amoxicillin-clavulanic acid; ciprofloxacin; and ertapenem

(de-escalation group). Amoxicillin-clavulanic acid was placed after the cephalosporins because of its anti-anaerobic activity. Ciprofloxacin, despite being potentially active against *P. aeruginosa*, was included as per standard de-escalation practice. As an exception to the rule, the options for AmpC or ESBL producers were restricted to meropenem or imipenem in the control arm, and TMP-SMX, ciprofloxacin or ertapenem in the de-escalation arm, regardless of susceptibility to other drugs. Standard doses of all drugs for invasive infections were used in both arms (available in the study protocol).

The recommended duration of treatment was 7 to 14 days. Switching to oral therapy was allowed after 5 days of active intravenous therapy in stable patients showing clinical improvement with adequate source control if it was necessary, and if the patient was tolerant of oral intake. Permitted oral drugs were chosen following standard practice and are listed in the study protocol.

Outcomes

The primary end point was clinical cure assessed 3-5 days after completion of antibiotic treatment (test-of-cure, TOC) in the modified intention-to-treat population (mITTP), which included patients who received at least one dose of study drugs. Secondary end points included a per-protocol comparison of clinical cure rates at TOC, performed in the clinically evaluable population (CEP), microbiological cure at TOC in the microbiologically evaluable population (MEP), recurrence, *Clostridioides difficile* infection, adverse events (AE), mortality at day 60. AEs were defined as any incident detrimental to health in a randomised patient, regardless the potential causal relationship with the treatment assigned. AEs were considered severe if life-

threatening or causing death, relevant disability, lengthening of hospital stay or new hospitalisation.

Clinical cure was defined as resolution of all symptoms and signs of infection and no need for treatment modification due to unfavourable clinical response or AE. Patients who died were considered not to have reached clinical cure. Microbiological cure was defined as negative follow-up blood cultures and, when applicable, from the site of infection. Recurrence was defined as a new bacteraemia episode caused by the same bacterial species as the initial episode within 60 days of randomisation.

The following populations were considered: the mITTP included all correctly randomised patients who had received at least one dose of intravenous antibiotics after randomization; the CEP included all patients evaluated at TOC who had completed at least 5 days of intravenous therapy (or who died earlier having received at least one dose of intravenous antibiotic) and a total treatment duration of at least 7 days. The microbiologically evaluable population (MEP) included those in the CEP with at least one follow-up blood culture taken ≥48 hours after randomization. In addition, all patients recruited from 8 sites were proposed as participants in an exploratory sub-study of rectal colonization with MDR gram-negative bacteria (see below).

Patients were followed for 60 days; 30- and 60-day visits could be performed by telephone. SCReN monitors verified eCRF data against original data sources.

Microbiological Studies

Blood isolates were frozen at -80ºC and sent to the reference laboratory (Hospital Universitario Virgen Macarena, Sevilla), where identification was confirmed

using MALDI-TOF (Bruker®), and antimicrobial susceptibility testing was performed by manual broth microdilution, interpreted according to EUCAST guidelines.[165,176]

For patients participating in the colonization study, rectal swabs were taken at randomization, end of treatment, and TOC. The microorganisms investigated were Enterobacterales producing ESBLs, carbapenemases and/or plasmid-mediated AmpC or hyperproducing chromosomal AmpC (cAmpC), carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *P. aeruginosa,* and *Stenotrophomonas maltophilia*. Rectal swabs were cultured in MacConkey agar with 2 mg/L cefotaxime. ESBL, cAmpC, pAmpC and carbapenemase production were determined by standard phenotypic methods.[187] PCR assays were used to characterize β-lactamase genes. In *P. aeruginosa*, disk diffusion was used to detect carbapenem resistance.

Statistical analyses

Assuming an 85% cure rate in both arms (based on previous observational data,[10] 344 patients (172 per arm) were needed to reject the inferiority of de-escalation considering a 10% margin, 80% power, 2-sided alpha of 5%, and 5% dropout rate. The -10% non-inferiority margin followed recent trials of complicated UTI and intra-abdominal infections.[198,2019]

Differences in proportions were calculated with 2-sided 95% confidence intervals (CI) for the endpoints, using the control group as reference. For the primary analysis in the mITTP, all patients not reaching clinical cure or missing evaluation at TOC were considered as not cured. Non-inferiority was declared when the lower bound of the 95% CI of the absolute difference in cure rate is below the -10% non-

inferiority margin. Multivariable analysis using logistic regression was performed to control for the effect of potential residual confounders on the primary outcome. A sensitivity analysis according to source of infection was performed, as planned in the study protocol. In addition, post hoc were also performed in subgroups according to age, acquisition type, severe sepsis/shock, and microorganism. Likewise, a desirability of outcome ranking (DOOR) analysis was also performed.[20] Patient outcomes evaluated at 60 days were classified according to an ordinal scale with five mutually exclusive hierarchical levels in descending order of desirability (the lower the DOOR rank, the more desirable the outcome): (1) cured without events; (2) cured with non-severe event(s); (3) cured with severe event(s); (4) not cured; and (5) death. The DOOR analysis was performed on the CEP population. The probability of patients in the experimental arm having better DOOR scores compared to those in the control group was calculated with 95% CI. In addition, a pre-planned superiority analysis was performed by adding a response adjusted for duration of antibiotic risk (RADAR) in which the duration of exposure to antipseudomonal β-lactams was added as a tie-breaking variable for the DOOR rank.[210] Data were analysed using SPSS Statistics 26 (IBM Corp).

Role of the funding source

The funder had no influence in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

RESULTS

In total, 2,030 patients were evaluated for inclusion during the study period; of the 765 who had no exclusion criteria, 344 (44·9%) were randomised (Figure 1). Thirteen of the randomised patients were not included in the mITT populaton, including 5 patients who withdrew their consent, 5 randomized in error due to polymicrobial bacteraemia, and 3 who were withdrawn by the physician in charge. Therefore, the mITT population included 331 patients: 164 were randomised to the experimental arm (de-escalation) and 167 to the control arm (non-de-escalation).

The median age of patients was 72 (interquartile range [IQR], 64-80); 196 (59·2%) were male, and the median Charlson index was 3 (IQR 1-5); the most frequent comorbidities were diabetes mellitus (120 patients [36·3%]) and solid organ cancer (109 [32·9%]); 168 patients (50·7%) had a community-acquired infection. The biliary and urinary tracts (129 [38·9%] and 126 [38%], respectively) were the most frequent sources of bacteraemia. *Escherichia coli* (215 [65%]) and *Klebsiella pneumoniae* (54 [16·3%]) were the most frequent pathogens.

Patient characteristics in the two study arms are summarised in Table 1. More patients in the control arm had chronic pulmonary disease (29 [17·4%] vs 21 [12·8%]) and diabetes mellitus (64 [38·3%] vs 56 [34·1%]), while the opposite was true for use of immunosuppressive drugs (17 [10·2%] vs 30 [18·3%]). More patients in the experimental arm had nosocomial infection (44 [26·3%] vs 27 [16·5%]), but fewer presented with severe sepsis or septic shock (35 [20·9%] vs 48 [29·2%]). The median (IQR) number of days of antipseudomonal drugs was 2 (2-3) in the

388 experimental arm and 7 (6-9) in the control arm (p<0.001).

389 The most frequent empirical antipseudomonal drugs used in both groups was
390 piperacillin/tazobactam (104 [63·4] and 107 [64·1] in the experimental and in the
391 control arms, respectively (Table 1). The drugs to which patients were de-escalated in
392 the experimental arm are shown in Table 1. Ninety-six (58·5%) patients in the
393 experimental arm and 118 (70·7%) in the control arm were later switched to oral
394 drugs (Table 1).

395 Clinical cure rates at TOC in de-escalated versus non-de-escalated patients,
396 were, respectively, 90·2% (148 patients) and 88·6% (148 patients) (risk difference,
397 1·6%; 95% CI, -5·0 to 8·2; Table 2); thus, de-escalation met the prespecified non-
398 inferiority criteria. The reasons for not reaching clinical cure were similar in both
399 groups and are also specified in Table 2. AEs leading to drug discontinuation are
400 reported below.

401 In multivariable analysis, the adjusted OR of de-escalation for reaching clinical
402 cure at TOC (controlling for chronic pulmonary disease, diabetes,
403 immunosuppressants, acquisition type, and severe sepsis or shock) was 0·56 (95%
404 CI 0·16-2·02; p=0·38).

405 No significant differences were found for secondary endpoints. Clinical cure at
406 TOC in the CEP (per-protocol analysis) was reached by 143 of 148 (96·6%) patients
407 in the experimental arm and 144 of 156 (92·3%) in the control arm (risk difference,
408 4·3, 95% CI -0·9 to 9·5). Microbiological cure in the MEP at TOC occurred in 124 of
409 128 (96·9%) and 125 of 132 (94·7%) patients in the de-escalation and non-de-
410 escalation arms, respectively (risk difference, 2·2, 95% CI -2·7 to 7·1) (Table 2).
411 Sixty-day mortality was 4·6% (7 of 153) among de-escalated patients versus 5·6% (9

412 of 160) among non-de-escalated patients. At day 60, relapse occurred in 9 of 153
413 (5·9%) de-escalated and 18 of 160 (11·3%) non-de-escalated patients; only 1 case of
414 *C. difficile* infection was diagnosed in each arm.

415 Subgroup analyses were performed on the mITT (Table 3). Overall, the results
416 were consistent with the primary analysis. Of note, clinical cure rates at TOC
417 between patients in the de-escalation and non-de-escalation groups presenting with
418 severe sepsis or septic shock at randomization were similar [44/47 (93·6%) vs· 31/34
419 (91·2%), risk difference, 3·3 (-3·0 to 9·5)]. Among patients with UTI, clinical cure was
420 reached in 59/60 patients (98·3%) in the experimental arm and 55/63 (87·3%) in the
421 control arm (risk difference, 11·0 [1·8 to 20·2]). Interactions between the subgroups
422 and treatment arm were analysed; none were statistically significant (data not shown).

423 A DOOR analysis was performed on 146 patients in the experimental arm and
424 155 patients in the control arm for whom an assessment of all variables at 60 days
425 was available. Among de-escalated patients, 55 (37·7%) were cured without events,
426 53 (36·3%) were cured with only non-severe events, 27 (18·5%) with one or more
427 severe events, 4 (2·7%) were not cured and 7 died (4·8%). Among non-de-escalated
428 patients, the corresponding figures were 59 (38·1%), 57 (36·8%), 26 (16·8%), 6
429 (3·9%) and 7 (4·5%), respectively (Figure 2 and appendix p 3). The proportion of
430 cases with better DOOR scores among patients in the experimental arm was 0·50
431 (95% CI, 0·44 to 0·56). When duration of exposure to antipseudomonal β-lactams
432 was used to break ties (RADAR analysis), the probability of a better DOOR in de-
433 escalated patients was 0·68 (95% CI, 0·63 to 0·73).

434 Ninety-nine patients (60·3%) in the experimental arm and 94 (56·3%) in the
435 control arm were reported to have at least one AE (risk difference, 4·0; 95% CI: -6·6

to 14·6; p=0·23). The total number of reported AEs was 219 in de-escalated and 175 in non-de-escalated patients, and 53 (24·2%) and 56 (32%) respectively, were considered severe. Five patients were withdrawn due to AEs: 3 in the experimental arm (persistent fever; development of septic shock; hepatic abscess) and 2 in the control arm (renal and hepatic toxicity; and pancreatitis with abdominal abscess). Table 4 and appendix p 4 lists the AEs and severe AEs. Two hundred and four minor and 10 major protocol deviations were documented without associated safety risks after being assessed by the study team and the monitoring group.

Rectal samples were taken at recruitment and TOC from 46 and 64 patients who gave their consent in the experimental and control groups, respectively. Of these, 7 in the experimental group (15·2%) and 15 in the control group (23·4%) acquired any MDR gram negative bacteria (p=0·28). MDR organisms acquired in the experimental group were 6 Enterobacterales (2 AmpC- and 4 ESBL-producing) and 1 *S. maltophilia*; and in the control group, 13 Enterobacterales (6 AmpC-, 6 ESBL-, 1 carbapenemase-producing) and 2 *S. maltophilia* (appendix p 5).

DISCUSSION

In this pragmatic randomised trial in patients with bacteraemia caused by Enterobacterales, de-escalation following a prespecified rule from an antipseudomonal β-lactam to a narrower-spectrum antibiotic active *in vitro* was non-inferior in efficacy to continuing with the empiric drug. The results of the different outcomes, subgroups and DOOR analyses were consistent and supported the non-inferiority hypothesis; when time of exposure to antipseudomonal β-lactams was

considered, de-escalation was superior. Notably, the 95%CI of the risk difference was >1 for the experimental arm among patients with UTI, which is relevant because randomisation was stratified for urinary source.

De-escalation is a broad concept that includes discontinuation of unnecessary drugs and/or switching to narrower spectrum antibiotics as targeted therapy, based on microbiological data or clinical re-evaluation.[221] Therefore, it includes heterogeneous drugs (those used initially and for de-escalation) and very diverse clinical situations (different infection sources, infection severity, causative pathogens and patient populations). This heterogeneity poses a challenge for the design of randomised trials with results that can be applied to decisions in individual patients.

We found only three previous randomised trials comparing de-escalation with continuation of initial therapy, all with limited statistical power, and none in patients with bacteraemia. Falguera *et al* evaluated de-escalation based on urine antigens in patients admitted with community-acquired pneumonia in one Spanish hospital;[232] relapse occurred in 3 of 25 de-escalated patients (12%) after a positive urine antigen result, 1 of 63 (1·6%) among those not de-escalated because of a negative urine test, and 2 of 89 (2·2%) not de-escalated because assigned to no urine test. Leone *et al* compared de-escalation and continuation of empiric therapy in 59 and 57 ICU patients, respectively, with sepsis of various origins and microorganisms;[243] in that study, de-escalation could not demonstrate to be non-inferior for the duration if ICU stay of the patients, without differences in mortality. The limitations of this study were discussed in detail elsewhere.[254] Rattanaumpawan *et al* compared de-escalation to ertapenem vs continuing with type-2 carbapenems in 32 and 34 patients, respectively, with diverse infections caused by ESBL producers, around 50% of which were

bacteremic.[265] The trial was stopped because of low recruitment, and no significant differences in outcome were observed in the patients tested. In a meta-analysis of mainly observational studies investigating de-escalation in patients with sepsis or bacteraemia, a possible protective effect of de-escalation for mortality was found, although this effect was no longer significant when studies providing adjusted estimates were considered.[276] We recently performed an observational study in patients with Enterobacterales bacteraemia initially treated with antipseudomonal agents, in which de-escalation was not associated with worse outcomes in propensity-score adjusted analysis.[10]

It is recognised that the suggested rank order of drugs used for de-escalation is open to debate because high-level evidence on the differential ecological impact of the drugs is lacking; previously published rankings have in fact been based on expert opinion.[287] Also, while different antipseudomonals will also have heterogeneous impacts, these drugs have consistently been associated with an increased risk of colonization and/or infection caused by MDR *P. aeruginosa* and Enterobacterales.[4,5,7] Despite being biologically sound, the possible reduction of risk of acquisition of MDR organisms by using de-escalation has not been investigated in depth, and may differ depending on the initial and final drug, the patient's baseline microbiota and the epidemiological situation. In this study, we performed an exploratory study on the acquisition of MDR Gram-negative bacteria in a small subset of patients. The results, although limited, are encouraging, and suggest that once the effectiveness of de-escalation has been demonstrated, it could be a primary endpoint in future trials of de-escalation from antipseudomonal drugs. Regarding *C. difficile* infection, we could not show any impact because the number of reported cases was very low. An

American cohort published in 2019 showed that the use of empirical anti-pseudomonal β-lactam for more than 48 hours was an independent risk factor for *C. difficile* infection.[6]

The pragmatic design of the study raises issues about the potential different efficacy of each drug used by intravenous and oral routes. However, to our knowledge, significant differences in efficacy have not been shown in direct comparisons of the drugs used in randomised trials for susceptible Enterobacterales. Although more patients in the control group were switched to oral drugs, almost 30% of patients in this group were only treated by the intravenous route, and most of those who were stepped down to an oral drug received ciprofloxacin. Switch to oral fluoroquinolones was much less frequent in the experimental group as this option was only second last in the list of options for that group with the intention to avoid it whenever possible because of its antipseudomonal activity. While the efficacy of all oral drugs used seems similar at least in bacteraemic urinary tract infection,[2,9,30,8] even if fluoroquinolones are considered better, the design would have in any case favoured the control group. Overall, we think that the fact that different drugs were used in both groups did not have a relevant impact on the results of the strategies compared.

This study has several limitations. There were various antibiotic options in both arms, reflecting actual practice; the study was not blinded for the same reason. Duration of treatment in most patients was longer than currently recommended for Enterobacterales bacteraemia, based on recent trial results supporting 7 days as an appropriate duration in most patients.[3,10] The rectal colonization study was performed on a limited number of patients. Some strengths of the study include its randomised

pragmatic design, and exclusion of stable, low-risk patients who would benefit from very early oral switching. The characteristics of the patients enrolled, including the elderly with comorbidities, are probably representative of the general population in whom de-escalation would be considered.

In conclusion, de-escalation from antipseudomonal β-lactams in patients with bacteraemia caused by Enterobacterales was non-inferior in clinical effectiveness and safety to continuing with the initial drug. Because the most frequent sources of bacteraemia were the urinary and biliary tracts, these results apply mostly to these infections. Given their potential ecological benefit, these results provide evidence to implement active actions to promote de-escalation in this clinical setting.

Contributors: Study design and funds proposal: JR-B, LEL-C, PR-G. Critical review of study design: all other authors. Study coordination: LEL-C, JR-B, CR-F, JB-F. Monitoring coordination: CR-F, EM-M. Microbiological studies: MD-V; all other authors: recruitment of patients, follow-up and collection of patients' data. Analyses of data: JRB, LEL-C. Drafting of the manuscript: LEL-C, JR-B. Critical review of manuscript: all other authors.

Declaration of interest: LELC has served as scientific advisor for Angelini, speaker for Angelini, ViiV, Gilead and Correvio, and has served as trainer for ViiV, outside the submitted work; LBP reports other from Pfizer, and Tillotts Pharma Spain, outside

the submitted work; PRG has served as advisor for Advanz, and speaker for Menarini, Shionogui and Angelini. All other authors had no conflicts to disclose.

Access to data and data sharing: Luis Eduardo López-Cortés and Jesús Rodríguez-Baño had ~~full access to all the data in the study and takes responsibility for the integrity of the data and the accuracy of the data analysis~~ full access to the data in the study, verified the data in the study, and were responsible for the decision to submit for publication. Individual, anonymised data would be shared after a signed agreement with Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla if requested with the objective of performing a meta-analysis with individual patients' data. Requests should be submitted to the corresponding author. Interested researchers should obtain the approval of the Ethic Committee CEIM Provincial de Sevilla. A database in SPSS file with the requested data and a dictionary of terms would be provided.

ACKNOWLEDGMENTS: Scren (Spanish Clinical research network, funded by Instituto de Salud Carlos III (PT17/0017/0012 and PT20/00123).

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Data sharing statement

Individual, anonymised data would be shared after a signed agreement with Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla if requested with the objective of performing a meta-analysis with individual patients' data. Requests should be submitted to the corresponding author. Interested researchers should obtain the approval of the Ethic Committee CEIM Provincial de Sevilla. A database in SPSS file with the requested data and a dictionary of terms would be provided.

REFERENCES:

1. Ohnuma T, Chihara S, Costin B, et al. Association of appropriate empirical antimicrobial therapy with in-hospital mortality in patients with bloodstream infections in the US. *JAMA Netw Open*. 2023;6(1):e2249353. doi: 10.1001/jamanetworkopen.2022.49353.
2. Evans L, Rhodes A, Alhazzani W, et al. Surviving Sepsis Campaign: International guidelines for management of sepsis and septic shock 2021. *Crit Care Med*. 2021;49(11):e1063-e1143. doi: 10.1097/CCM.0000000000005337.
3. Karam G, Chastre J, Wilcox MH, Vincent JL. Antibiotic strategies in the era of multidrug resistance. *Crit Care*. 2016;20(1):136. doi: 10.1186/s13054-016-1320-7.
4. Solé M, Fábrega A, Cobos-Trigueros N, et al. In vivo evolution of resistance of *Pseudomonas aeruginosa* strains isolated from patients admitted to an intensive care unit: mechanisms of resistance and antimicrobial exposure. *J Antimicrob Chemother.* 2015;70(11):3004-13. doi: 10.1093/jac/dkv228.
5. Boyer A, Doussau A, Thiébault R, et al. *Pseudomonas aeruginosa* acquisition on an intensive care unit: Relationship between antibiotic selective pressure and patients' environment. *Crit Care*. 2011;15(1):R55. doi: 10.1186/cc10026.
6. Seddon MM, Bookstaver PB, Justo JA, et al. Role of early de-escalation of antimicrobial therapy on risk of *Clostridioides difficile* infection following Enterobacteriaceae bloodstream infections. *Clin Infect Dis*. 2019;69(3):414-420. doi: 10.1093/cid/ciy863.
7. Pérez-Galera S, Bravo-Ferrer JM, Paniagua M, et al; COMBACTE-CARE-EURECA Team. Risk factors for infections caused by carbapenem-resistant Enterobacterales: an international matched case-control-control study (EURECA). *EClinicalMedicine*. 2023;57:101871. doi: 10.1016/j.eclinm.2023.101871.
8. De Waele JJ, Schouten J, Beovic B, Tabah A, Leone M. Antimicrobial de-escalation as part of antimicrobial stewardship in intensive care: No simple answers to simple questions-a viewpoint of experts. *Intensive Care Med*. 2020;46(2):236-244. doi: 10.1007/s00134-019-05871-z.
9. Moehring RW, Yarrington ME, Warren BG, et al; Centers for Disease Control and Prevention's Prevention Epicenters Program. Evaluation of an opt-out protocol for antibiotic de-escalation in patients with suspected sepsis: a multicenter,

randomized, controlled trial. *Clin Infect Dis*. 2023;76(3):433-442. doi: 10.1093/cid/ciac787.

10. Palacios-Baena ZR, Delgado-Valverde M, Valiente Méndez A, et al; REIPI/GEIRAS-SEIMC Bacteraemia-MIC Group. Impact of de-escalation on prognosis of patients with bacteremia due to Enterobacteriaceae: A post hoc analysis from a multicenter prospective cohort. *Clin Infect Dis*. 2019;69(6):956-962. doi: 10.1093/cid/ciy1032.
11. Silva BN. Cochrane Database Syst Rev. 2013 Mar 28;2013(3):CD007934. doi: 10.1002/14651858.CD007934.pub3.
12. Lakbar I, De Waele JJ, Tabah A, Einav S, Martin-Loeches I, Leone M. Antimicrobial de-escalation in the ICU: From recommendations to level of evidence. *Adv Ther*. 2020;37(7):3083-3096. doi: 10.1007/s12325-020-01390-2.
13. López-Cortés LE, Rosso-Fernández C, Núñez-Núñez M, et al; SIMPLIFY Study Group. Targeted simplification versus antipseudomonal broad-spectrum beta-lactams in patients with bloodstream infections due to Enterobacteriaceae (SIMPLIFY): a study protocol for a multicentre, open-label, phase III randomised, controlled, non-inferiority clinical trial. *BMJ Open*. 2017;7(6):e015439. doi: 10.1136/bmjopen-2016-015439.
14. Piaggio G, Elbourne DR, Pocock SJ, Evans SJ, Altman DG; CONSORT Group. Reporting of noninferiority and equivalence randomized trials: extension of the CONSORT 2010 statement. *JAMA*. 2012;308(24):2594-604. doi: 10.1001/jama.2012.87802.
15. Friedman ND, Kaye KS, Stout JE, et al. Health care—associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. *Ann Intern Med*. 2002 Nov 19;137(10):791-7.
~~15.~~16. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.1, 2018. http://www.eucast.org.
~~16.~~17. EUCAST Guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, Version 2.0, 2017.
~~17.~~18. ISO 20776-1:2006. Clinical laboratory testing and in vitro diagnostic test systems — Susceptibility testing of infectious agents and evaluation of

performance of antimicrobial susceptibility test devices — Part 1: Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.

~~18.~~19. Solomkin J, Hershberger E, Miller B, et al. Ceftolozane/tazobactam plus metronidazole for complicated intra-abdominal infections in an era of multidrug resistance: Results from a randomized, double-blind, phase 3 trial (ASPECT-cIAI). *Clin Infect Dis*. 2015;60(10):1462-71. doi: 10.1093/cid/civ097.

~~19.~~20. Wagenlehner FM, Sobel JD, Newell P, et al. Ceftazidime-avibactam versus doripenem for the treatment of complicated urinary tract infections, including acute pyelonephritis: RECAPTURE, a phase 3 randomized trial program. *Clin Infect Dis*. 2016;63(6):754-762. doi: 10.1093/cid/ciw378.

~~20.~~21. Evans SR, Rubin D, Follmann D, et al. Desirability of Outcome Ranking (DOOR) and Response Adjusted for Duration of Antibiotic Risk (RADAR). *Clin Infect Dis*. 2015;61(5):800-6. doi: 10.1093/cid/civ495.

~~21.~~22. Tabah A, Cotta MO, Garnacho-Montero J, et al. A systematic review of the definitions, determinants, and clinical outcomes of antimicrobial de-escalation in the Intensive Care Unit. *Clin Infect Dis*. 2016;62(8):1009-1017. doi: 10.1093/cid/civ1199.

~~22.~~23. Falguera M, Ruiz-González A, Schoenenberger JA, et al. Prospective, randomised study to compare empirical treatment versus targeted treatment on the basis of the urine antigen results in hospitalised patients with community-acquired pneumonia. *Thorax*. 2010;65(2):101-6. doi: 10.1136/thx.2009.118588.

~~23.~~24. Leone M, Bechis C, Baumstarck K, et al; AZUREA Network Investigators. De-escalation versus continuation of empirical antimicrobial treatment in severe sepsis: a multicenter non-blinded randomized noninferiority trial. *Intensive Care Med*. 2014;40(10):1399-408. doi: 10.1007/s00134-014-3411-8.

~~24.~~25. Timsit JF, Harbarth S, Carlet J. De-escalation as a potential way of reducing antibiotic use and antimicrobial resistance in ICU. *Intensive Care Med*. 2014;40(10):1580-2. doi: 10.1007/s00134-014-3485-3.

~~25.~~26. Rattanaumpawan P, Werarak P, Jitmuang A, Kiratisin P, Thamlikitkul V. Efficacy and safety of de-escalation therapy to ertapenem for treatment of infections caused by extended-spectrum-β-lactamase-producing

Enterobacteriaceae: an open-label randomized controlled trial. *BMC Infect Dis*. 2017;17(1):183. doi: 10.1186/s12879-017-2284-1.

~~26.~~27. Paul M, Dickstein Y, Raz-Pasteur A. Antibiotic de-escalation for bloodstream infections and pneumonia: systematic review and meta-analysis. *Clin Microbiol Infect*. 2016;22(12):960-967. doi: 10.1016/j.cmi.2016.05.023.

~~27.~~28. Weiss E, Zahar JR, Lesprit P, et al; De-escalation Study Group. Elaboration of a consensual definition of de-escalation allowing a ranking of β-lactams. *Clin Microbiol Infect*. 2015;21(7):649.e1-10. doi: 10.1016/j.cmi.2015.03.013.

~~28.~~29. Sutton JD, Stevens VW, Chang NN, Khader K, Timbrook TT, Spivak ES. Oral β-Lactam Antibiotics vs Fluoroquinolones or Trimethoprim-Sulfamethoxazole for Definitive Treatment of Enterobacterales Bacteremia From a Urine Source. *JAMA Netw Open.* 2020;3(10):e2020166. doi: 10.1001/jamanetworkopen.2020.20166.

~~29.~~30. Tamma PD, Conley AT, Cosgrove SE, et al. Association of 30-day mortality with oral step-down vs continued intravenous therapy in patients hospitalized with Enterobacteriaceae bacteremia. *JAMA Intern Med.* 2019;179(3):316-323. doi: 10.1001/jamainternmed.2018.6226.

~~30.~~31. Turjeman A, von Dach E, Molina J, et al. Duration of antibiotic treatment for Gram-negative bacteremia - Systematic review and individual participant data (IPD) meta-analysis. *EClinicalMedicine*. 2022;55:101750. doi: 10.1016/j.eclinm.2022.101750.

715 Figure 1. Patient recruitment and randomization

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Figure 2. Breakdown of desirability of outcome rank (DOOR) scores by treatment group. Categories are: (1) Cured, no events; (2) cured, at least one non-severe event; (3) cured, at least one severe event; (4) Not cured; (5) Death.

725 Table 1. Baseline characteristics of patients in the modified intention-to-treat
726 population.

Characteristic	**De-escalation arm (n=164)**	**Non-de-escalation arm (n=167)**
Age in years, mean (SD)	69·5 (12·8)	71·9 (12·2)
Female gender	64 (39·0)	71 (42·5)
Charlson index, median (IQR)	2·5 (1-4)	3 (1-5)
Congestive heart failure	19 (11·6)	21 (12·6)
Chronic pulmonary disease	21 (12·8)	29 (17·4)
Solid organ cancer	55 (33·5)	54 (32·3)
Haematologic cancer	3 (1·8)	2 (1·2)
Diabetes mellitus	56 (34·1)	64 (38·3)
Chronic kidney disease	37 (22·6)	39 (23·4)
Obstructive uropathy	18 (11·0)	15 (9·0)
Chronic liver disease	18 (11·0)	19 (11·4)
Obstructive biliary tract disease	29 (17·7)	38 (22·8)
Inflammatory intestinal disease	4 (2·4)	6 (3·6)
Immunosuppressive drug use	30 (18·3)	17 (10·2)
Fully dependent for basic activities	13 (7·9)	19 (11·4)
Acquisition type		
Community-acquired	89 (54·3)	79 (47·3)
Community-onset, healthcare-associated	48 (29·3)	44 (26·3)
Nosocomial	27 (16·5)	44 (26·3)
Severity of infection at presentation		
Severe sepsis	31 (18·9)	28 (16·8)
Septic sock	17 (10·4)	7 (4·2)
Source of bacteraemia		
Biliary tract	62 (37·8)	67 (40·1)
Urinary tract	61 (37·2)	65 (38·9)
Abdominal other than biliary tract	16 (9·8)	14 (8·4)
Vascular catheter	7 (4·3)	12 (7·2)
Skin and skin structure	4 (2·4)	0 (0·0)
Respiratory tract	2 (1·2)	2 (1·2)
Other	2 (1·2)	1 (0·6)
Unknown	10 (6·1)	6 (3·6)
Aetiology		
Escherichia coli	103 (62·8)	112 (67·1)
Klebsiella pneumoniae	30 (18·3)	24 (14·4)
Klebsiella oxytoca	9 (5·5)	7 (4·2)
Enterobacter cloacae	3 (1·8)	11 (6·6)
Proteus mirabilis	6 (3·7)	7 (4·2)
Other	13 (7·9)	6 (3·6)
Pitt score, median (IQR)	0 (0-2)	0 (0-1)
Days from blood cultures to administration of empirical therapy, median (IQR)	0 (0-0)	0 (0-0)
Days from blood cultures to randomisation, median (IQR) [1]	2 (2-3)	2 (2-3)
Days of intravenous therapy, median (IQR)	7 (6-10)	7 (6-9)
Days of oral therapy, median (IQR)	3 (0-6)	3 (0-6)

Total days of therapy, median (IQR)	11 (9-14)	11 (9-14)
Source control within 72 hours		
Not required	98 (59·8)	106 (63·5)
Required and performed	54 (32·9)	57 (34·1)
Required and not performed	12 (7·3)	4 (2·4)
Empirical drugs used		
Imipenem or meropemem	45 (27·4)	47 (28·1)
Piperacillin/tazobactam	104 (63·4)	107 (64·1)
Cefepime or ceftazidime	14 (8·5)	11 (6·6)
Aztreonam	1 (0·6)	2 (1·2)
De-escalation intravenous drug		
Ampicillin	Not applicable	23 (14)
Trimetoprim-sulfamethoxazole		6 (3·7)
Cefuroxime		23 (14)
Cefotaxime or ceftriaxone		52 (31·7)
Amoxicillin-clavulanate		24 (14·6)
Ciprofloxacin		18 (10·9)
Ertapenem		18 (10·9)
Switched to oral drugs, n (%)	96 (58·3)	118 (70·7)
Ciprofloxacin	21 (20·4)	97 (78·2)
Cefuroxime	26 (25·2)	17 (13·7)
Amoxicillin	19 (18·4)	1 (0·8)
Amoxicillin-clavulanate	17 (16·5)	4 (3·2)
Cefixime	14 (13·6)	1 (0·8)
Trimethoprim-sulfamethoxazole	6 (5·8)	2 (1·6)
Ertapenem	0 (0·0)	2 (1·6)

Data are number of patients (%) except where specified.

[1] Equals the time to de-escalation in the de-escalation arm.

Table 2. Primary and secondary endpoint analyses.

Outcome	De-escalation arm	Non de-escalation arm	Difference in percentage points (2-sided 95% CI)
Primary analysis (modified intention-to-treat population)			
Clinical cure at TOC, n (%)	148/164 (90·2)	148/167 (88·6)	1·6 (-5·0 to 8·2)
Reasons for not reaching clinical cure at TOC			
Clinical failure	7/164 (4·3)	12/167 (7·2)	-2·9 (-7·9 to 2·1)
Missed assessment	6/164 (3·7)	5/167 (3)	0·7 (-3·2 to 4·6)
Withdrawn due to adverse events	3/164 (1·8)	2/167 (1·2)	0·6 (-2·0 to 3·2)
Secondary analyses			
Clinical cure at TOC (clinically evaluable population)	143/148[1] (96·6)	144/156[2] (92·3)	4·3 (-0·9 to 9·5)
Microbiological cure at TOC (microbiologically evaluable population)	124/128 (96·9)	125/132 (94·7)	2·2 (-2·7 to 7·1)
Clinical cure at day 60	142/153[3] (92·8)	144/160[4] (90)	2·8 (-3·4 to 9·0)
Relapses until day 60	9/153[3] (5·9)	18/160[4] (11·3)	-5·5 (-11·6 to 0·8)
Death at day 60	7/153[3] (4·6)	9/160[4] (5·6)	-1·0 (-5·9 to 3·9)
Clostridioides difficile infection until day 60	1/153[3] (0·7)	1/160[4] (0·6)	0·10 (-1·7 to 1·9)

TOC: test of cure.

[1]16 patients were excluded from the modified intention-to-treat population for failing to complete 5 days of treatment (13) and for missing assessment at TOC (3)

[2]9 patients were excluded from the modified intention-to-treat population: failure to complete 5 days of treatment (7), withdrawn due to adverse events (2), and missed assessment at TOC (3)

[3]11 patients excluded from the modified intention-to-treat population due to missed assessment

[4]7 patients excluded from the modified intention-to-treat population due to missed assessment

Table 3. Subgroup analyses for clinical cure at test of cure in the modified intention-to-treat population.

CHARACTERISTIC	**De-escalation arm**	**Non-de-escalation arm**	**Difference in percentage points (2-sided 95% CI)**
Age < 80 years	117/124 (94·4)	112/121 (92·6)	1·8 (4·4 to 8·0)
Age ≥ 80 years	31/32 (96·9)	36/40 (90·0)	6·9 (-4·9 to 18·7)
Community acquisition	82/85 (96·5)	70/75 (93·3)	3·1 (-3·6 to 9·9)
Non-community acquisition	66/71 (93)	78/86 (90·7)	2·3 (-6·4 to 10·9)
Severe sepsis or septic shock	44/47 (93·6)	31/34 (91·2)	2·4 (-9·1 to 14)
Non-severe sepsis or septic shock	104/109 (95·4)	117/127 (92·1)	3·3 (-3·0 to 9·5)
Urinary source	59/60 (98·3)	55/63 (87·3)	11·0 (1·8 to 20·2)
Non-urinary source	89/96 (92·7)	93/98 (94·9)	2·2 (-9·0 to 4·6)
Biliary source	57/58 (98·3)	61/64 (95·3)	3·0 (-3·4 to 9·3)
Non-biliary source	89/96 (92·7)	86/96 (89·6)	3·1 (-4·9 to 11·2)
Urinary or biliary source	113/118 (95·8)	122/126 (96·8)	1·1 (-3·7 to 5·8)
Non-urinary or biliary source	36/39 (92·3)	33/33 (100)	7·7 (7·7 to 7·7)
Bacteraemia caused by *E. coli*	95/100 (95)	98/106 (92·5)	2·5 (-4·1 to 9·2)
Bacteraemia caused by non-*E. coli* Enterobacterales	53/56 (94·6)	50/55 (90·9)	3·7 (5·9 to 13·4)

Table 4. Adverse events reported.

	Experimental arm (n=164)	**Control arm (n=167)**
Gastrointestinal disorders		
Nausea/vomiting	4 (2·4)	5 (2·9)
Diarrhoea	6 (3·6)	11 (6·5)
Pancreatitis	0	5 (2·9)
Abdominal pain	5 (3)	5 (2·9)
Oral mucosis	3 (1·8)	1 (0·6)
Constipation	3 (1·8)	1 (0·6)
Cholangitis	3 (1·8)	2 (1·2)
Cholecystitis	2 (1·2)	2 (1·2)
Gastrointestinal bleeding	0	2 (1·2)
Liver abscess	1 (0·6)	1 (0·6)
Cirrhotic decompensation	2 (1·2)	3 (1·8)
Liver toxicity	0	2 (1·2)
Intestinal occlusion	0	1 (0·6)
Jaundice	1 (0·6)	0
Dehiscence of ileal anastomosis	1 (0·6)	0
Infections and infestations		
Respiratory tract	6 (3·6)	4 (2·4)
Primary bacteremia/sepsis with unknown source	8 (4·8)	2 (1·2)
C. difficile infection	1 (0·6)	1 (0·6)
Sepsis	3 (1·8)	1 (0·6)
UTI	11 (6·7)	6 (3·6)
Bacteraemia due to *E. coli*	0	6 (3·6)
Otitis	0	1 (0·6)
Skin soft tissue	1 (0·6)	0
Septic shock	1 (0·6)	0
Conjunctivitis	1 (0·6)	0
Candidemia	3 (1·8)	0
Surgical wound infection	1 (0·6)	0
Biliary prosthesis infection	1 (0·6)	0
Varicella Zoster virus reactivation	1 (0·6)	0
Influenza A infection	1 (0·6)	0

General disorders and administration site conditions		
Edema	1 (0·6)	3 (1·8)
Phlebitis or extravasation	9 (5·4)	1 (0·6)
Fever	12 (7·3)	8 (4·8)
Multi-organ failure	0	1 (0·6)
Fatigue	1 (0·6)	0
Exanthema	1 (0·6)	0
Abdominal haematoma	1 (0·6)	0
Blood and lymphatic system disorders		
Anaemia	2 (1·2)	2 (1·2)
Thrombocytopenia	2 (1·2)	1 (0·6)
Leucopoenia	4 (2·4)	1 (0·6)
Neutropenia	6 (3·6)	1 (0·6)
Thrombocytosis	1 (0·6)	0
Bicytopenia	1 (0·6)	0
Investigations		
Hypopotassaemia	0	2 (1·2)
Hypophosphatemia	0	1 (0·6)
AST, ALT elevation	1 (0·6)	2 (1·2)
Hyperuricemia	0	1 (0·6)
Hypernatremia	0	1 (0·6)
Hyperbilirubinemia	0	1 (0·6)
Creatinine elevation	1 (0·6)	0
Hypoalbuminemia	1 (0·6)	0
Nervous system disorders		
Acute vestibular neuritis	0	1 (0·6)
Epileptic seizures	0	1 (0·6)
Syncope	1 (0·6)	0
Headache	2 (1·2)	0
Asthenia	3 (1·8)	2 (1·2)
Collection in the anterior epidural space	1 (0·6)	0
Respiratory, thoracic and mediastinal disorders		
Hydropneumothorax	0	1 (0·6)
Pleural effusion	0	1 (0·6)
Dyspnea	5 (3)	3 (1·8)
Bronchospasm	0	1 (0·6)
Respiratory distress	1 (0·6)	1 (0·6)

Massive haemoptysis	0	1 (0·6)
Cough	2 (1·2)	0
Epistaxis	2 (1·2)	0
Renal and urinary disorders		
Renal insufficiency	6 (3·6)	2 (1·2)
Hepatorenal syndrome	1 (0·6)	0
Haematuria	3 (1·8)	2 (1·2)
Dysuria	1 (0·6)	1 (0·6)
Urinary retention	2 (1·2)	1 (0·6)
Leukocyturia	0	1 (0·6)
Complicated renal cyst	1 (0·6)	0
Musculoeskeletal and connective tissue disorders		
Chest muscle pain	1 (0·6)	1 (0·6)
Cardiac disorders		
Heart failure	3 (1·8)	2 (1·2)
Atrial flutter	0	1 (0·6)
Angor	0	1 (0·6)
Heart failure	3 (1·8)	0
Pericardial effusion	1 (0·6)	0
Acute coronary syndrome	1 (0·6)	0
Skin and subcutaneous tissue complications		
Rash/urticarial	1 (0·6)	2 (1·2)
Pruritus	1 (0·6)	1 (0·6)
Dermatitis	1 (0·6)	0
Endocrine disorders		
Gout attack	0	1 (0·6)
Hyperglycaemia	1 (0·6)	0
Vascular disorders		
Hypotension	3 (1·8)	2 (1·2)
Ictus	1 (0·6)	0
Neoplasms benign, malignant and unspecified		
Cancer progression	2 (1·2)	1 (0·6)
Chemotherapy toxicity	1 (0·6)	1 (0·6)
Myelodysplastic syndrome	1 (0·6)	0

TITLE: Effectiveness and safety of a structured de-escalation from antipseudomonal β-lactams in bloodstream infections due to Enterobacterales: a multicentre randomised clinical trial (SIMPLIFY).

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KEY WORDS: de-escalation, antimicrobial stewardship, antimicrobial use, bloodstream infection, ecological impact, Enterobacterales.

RUNNING TITLE: De-escalation in bacteraemia

WORD COUNT: 3771 words

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Research in context

Evidence before this study: De-escalation is an antimicrobial stewardship strategy in which empiric antimicrobials are either replaced by narrower spectrum agents or discontinued if used in unneeded combination, with the aim to reduce selection pressure on microorganisms to contribute to the control of antimicrobial resistance. Despite considered as standard of practice by many infectious diseases specialists, there is a lack of high-level evidence for the non-inferiority effectiveness of de-escalation. A systematic review including studies published in MEDLINE via PubMed from inception until January 2020 found low level evidence for recommending de-escalation in short-term treatments; we performed a literature search including the terms “de-escalation” or “streamlining”, and “antimicrobial therapy” or “antibiotics” but could not find any randomised trial published thereafter. As de-escalation is a broad concept, randomised trials in specific clinical situations using structured de-escalation protocols are needed.

Added value of this study: De-escalation was investigated in a specific clinical situation (bacteraemia due to Enterobacterales receiving empirical therapy with an antipseudomonal β-lactams) and using a pragmatic, predefined rule to select the narrower-spectrum drug. De-escalation was proved to be non-inferior in clinical effectiveness and safety to continuing with the initial antipseudomonal drug.

Implications of all the available evidence: Beyond other considerations, an important barrier to de-escalate is the lack of adequate evidence for its efficacy and safety in specific clinical situations. To our knowledge, this is the first randomised trial investigating the effectiveness of antibiotic de-escalation in a specific clinical situation.

These results should facilitate the implementation of de-escalation in clinical practice and facilitating sequential oral treatment, which may help reducing the ecological impact of antipseudomonal β-lactams.

Summary

Background: De-escalation from broad-spectrum to narrow-spectrum antibiotics is considered an important measure to reduce the selective pressure of antibiotics, but is performed much less frequently than desired. Lack of adequate evidence is a barrier to its implementation. The objective was to determine whether de-escalation from an anti-pseudomonal β-lactam to a narrower-spectrum drug is non-inferior to continuing the antipseudomonal drug in patients with Enterobacterales bacteraemia.

Methods: An open-label, pragmatic, randomised trial was performed between in 21 Spanish hospitals. Patients with bacteraemia caused by Enterobacterales susceptible to one of the de-escalation options and treated empirically with an antipseudomonal β-lactam were eligible. Patients were randomised (1:1) stratified by urinary source to de-escalate to ampicillin, trimethoprim-sulfamethoxazole (only urinary tract infections), cefuroxime, cefotaxime or ceftriaxone, amoxicillin-clavulanic acid, ciprofloxacin or ertapenem in that order according to susceptibility, or to continue with the empiric antipseudomonal β-lactam. Oral switching was allowed. The primary outcome was clinical cure 3–5 days after end of treatment in the modified intention-to-treat population (mITTP), formed by patients who received at least one dose of study drugs. Safety was assessed in all participants. Non-inferiority was declared when the lower bound of the 95% CI of the absolute difference in cure rate is below the -10% non-inferiority margin. This trial is registered with EudraCT number 2015 004219 19 and Clinicaltrials.gov identifier NCT02795949. It was completed on July 2020.

Findings: Between October 2016 and January 2020, 171 patients were randomised to the experimental arm and 173 to the control arm; 164 and 167, respectively,

formed the mITTP and were included in the primary analysis. The primary outcome was achieved by 148 patients (90·2%) in the de-escalation arm, and 148 (88·6%) in the non-de-escalation arm (risk difference, 1·60%; 95% CI, -5·03 to 8·23).. Severe adverse events were reported in 53 (24·2%) and 56 (32%) patients in the de-escalation and non-de-escalation arms, respectively. Six (4·6%) and 9 (5·6%) patients in the experimental and control arm died during the 60-day follow-up, respectively.

Interpretation: De-escalation from an antipseudomonal β-lactam in Enterobacterales bacteraemia following a predefined rule was non-inferior to continuing the empiric antipseudomonal drug. These results support de-escalation in this setting.

Funding: The study was funded by the 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 RD16/0016/0001; 0002; 0003; 0005; 0007; 0008; 0009; 0011; 0012; 0015); Spanish Clinical Research and Clinical Trials Platform, (SCReN, PT13/0002/0010 and PT17/0017/0012, and PI15/00439), co-financed by European Union; European Development Regional Fund “A way to achieve Europe”, Operative Program Intelligence Growth 2014-2020.

INTRODUCTION

Inappropriate antimicrobial treatment of patients with invasive infections is associated with higher mortality;[1] therefore, empiric treatment with broad-spectrum antibiotics (BSA) is recommended in severe infections to ensure appropriate coverage of the causative microorganism(s).[2] However, these drugs also exert a significant selection pressure that contributes to the spread of multidrug-resistant (MDR) bacteria.[3] This is particularly important in the case of antipseudomonal beta-lactams which in addition to selection pressure, may also induce the expression of resistance mechanisms in *Pseudomonas aeruginosa*[4,5], and are associated with an increased risk of infections caused by *Clostridiodes difficile*[6] and carbapenem-resistant Enterobacterales.[7] Unfortunately, exposure to antipseudomonal drugs is very common in hospitalised patients.

De-escalation to a narrow-spectrum drugs in patients initially treated with BSA once the aetiology of an infection is known is advocated as a key measure to reduce exposure to the latter. Although considered standard of care by most infectious diseases specialists, de-escalation is performed much less frequently than desired.[6, 8-10] An important barrier to de-escalate for many physicians is the lack of adequate evidence on its efficacy and safety, particularly when early improvement with empirical therapy is not evident. In fact, a Cochrane review found insufficient evidence to recommend for or against de-escalation in adults with sepsis,[11] and a recent systematic review found low level evidence for recommending de-escalation in short-term treatments.[12] Therefore, randomised trials are needed. Because de-escalation is a broad concept, trials should include specific clinical situations and

structured de-escalation protocols to be applicable in clinical practice.

Antipseudomonal agents are frequently recommended as empirical drugs in patients with severe healthcare-associated or nosocomial infections; if the infection is shown to be caused by an Enterobacterales, the question whether de-escalation to an *in vitro* active non-antipseudomonal agents should be performed is then typically raised. We hypothesised that de-escalation to an active non-antipseudomonal drug in these patients, using a predefined rule, would be non-inferior to continuing with the empirical drug.

METHODS

Study design

The SIMPLIFY trial (EudraCT 2015-004219-19; ClinicalTrials.gov NCT02795949) is an investigator-driven, pragmatic, multicentre, open-label, controlled clinical trial in patients with bacteraemia caused by Enterobacterales who received empirical treatment with an antipseudomonal β-lactam. The effectiveness of two strategies as targeted treatment was compared: the experimental strategy, in which patients are de-escalated to a non-antipseudomonal drug according to isolate susceptibility, following a prespecified list of drugs; and the control strategy, in which patients are continued with the empirical antipseudomonal drug. The Hospital Universitario Virgen Macarena Ethics Committee approved the study; written informed consent was obtained from all participants. The study protocol was published[13] and is available in Supplement A. The results are reported in accordance

with the CONSORT statement extension (supplementary material B) for non-inferiority and equivalence trials.[14]

The trial was conducted in 21 Spanish public tertiary hospitals between October 2016 and January 2020, with the support of the Spanish Network for Research in Infectious Diseases (REIPI) and the Spanish Clinical Research Network (SCReN).

Participants

Hospitalised patients aged ≥ 18 years with monomicrobial Enterobacterales bacteraemia were eligible for enrolment if they fulfilled all the following inclusion criteria: (1) receipt of empiric monotherapy (started <24 hours after blood cultures were taken) with an antipseudomonal β-lactam with *in vitro* activity against the causative bacteria, including meropenem, imipenem, piperacillin-tazobactam, cefepime, ceftazidime or aztreonam; (2) the causative organism was susceptible *in vitro* to at least one of the antibiotics included in the experimental arm (see below); and (3) intravenous antimicrobial treatment was planned for at least 5 days from the first active drug received. A negative pregnancy test was required for women of childbearing age. Exclusion criteria were: (1) life expectancy <30 days; (2) pregnancy or breastfeeding; (3) isolation of carbapenemase-producing Enterobacterales; (4) recruitment >48 hours after the antimicrobial susceptibility report was available; (5) neutropenia <500 cells/μL at randomization; (6) and planned duration of treatment >28 days (e.g., osteomyelitis or infectious endocarditis). Baseline patient characteristics were collected on the day of randomisation, except for clinical severity

variables, which were collected retrospectively for the day the first blood cultures were draw. Acquisition type was defined as by Friedman's criteria.[15] Source of bacteraemia was defined as the site of infection, according to organ-related signs and symptoms, and when appropriate, isolation of the bacteria from an appropriate sample from the infection site. Written informed consent for participation was obtained from patients. The recruitment period took place between October 5th 2016 and January 23rd 2020.

Randomization and masking

In the first 48 hours after the antimicrobial susceptibility report was available, patients were randomly assigned (1:1) to continue with the same empiric intravenous antipseudomonal drug (control group) or to switch to the first *in vitro* active drug from a predefined de-escalation list (de-escalation group), both in monotherapy.

Assignment to treatment group was performed centrally by a web-based automated system integrated with the electronic case report form (eCRF). Simple randomisation was performed, stratified only by source of bacteraemia (UTI or other). Blinding was not possible due to multiple options in both arms.

Procedures

The order of the de-escalation list was: ampicillin; trimethoprim-sulfamethoxazole (TMP-SMX; only urinary tract infections [UTI]); cefuroxime; cefotaxime or ceftriaxone; amoxicillin-clavulanic acid; ciprofloxacin; and ertapenem

(de-escalation group). Amoxicillin-clavulanic acid was placed after the cephalosporins because of its anti-anaerobic activity. Ciprofloxacin, despite being potentially active against *P. aeruginosa*, was included as per standard de-escalation practice. As an exception to the rule, the options for AmpC or ESBL producers were restricted to meropenem or imipenem in the control arm, and TMP-SMX, ciprofloxacin or ertapenem in the de-escalation arm, regardless of susceptibility to other drugs. Standard doses of all drugs for invasive infections were used in both arms (available in the study protocol).

The recommended duration of treatment was 7 to 14 days. Switching to oral therapy was allowed after 5 days of active intravenous therapy in stable patients showing clinical improvement with adequate source control if it was necessary, and if the patient was tolerant of oral intake. Permitted oral drugs were chosen following standard practice and are listed in the study protocol.

Outcomes

The primary end point was clinical cure assessed 3-5 days after completion of antibiotic treatment (test-of-cure, TOC) in the modified intention-to-treat population (mITTP), which included patients who received at least one dose of study drugs. Secondary end points included a per-protocol comparison of clinical cure rates at TOC, performed in the clinically evaluable population (CEP), microbiological cure at TOC in the microbiologically evaluable population (MEP), recurrence, *Clostridioides difficile* infection, adverse events (AE), mortality at day 60. AEs were defined as any incident detrimental to health in a randomised patient, regardless the potential causal relationship with the treatment assigned. AEs were considered severe if life-

threatening or causing death, relevant disability, lengthening of hospital stay or new hospitalisation.

Clinical cure was defined as resolution of all symptoms and signs of infection and no need for treatment modification due to unfavourable clinical response or AE. Patients who died were considered not to have reached clinical cure. Microbiological cure was defined as negative follow-up blood cultures and, when applicable, from the site of infection. Recurrence was defined as a new bacteraemia episode caused by the same bacterial species as the initial episode within 60 days of randomisation.

The following populations were considered: the mITTP included all correctly randomised patients who had received at least one dose of intravenous antibiotics after randomization; the CEP included all patients evaluated at TOC who had completed at least 5 days of intravenous therapy (or who died earlier having received at least one dose of intravenous antibiotic) and a total treatment duration of at least 7 days. The microbiologically evaluable population (MEP) included those in the CEP with at least one follow-up blood culture taken ≥48 hours after randomization. In addition, all patients recruited from 8 sites were proposed as participants in an exploratory sub-study of rectal colonization with MDR gram-negative bacteria (see below).

Patients were followed for 60 days; 30- and 60-day visits could be performed by telephone. SCReN monitors verified eCRF data against original data sources.

Microbiological Studies

Blood isolates were frozen at -80ºC and sent to the reference laboratory (Hospital Universitario Virgen Macarena, Sevilla), where identification was confirmed

using MALDI-TOF (Bruker®), and antimicrobial susceptibility testing was performed by manual broth microdilution, interpreted according to EUCAST guidelines.[16,17]

For patients participating in the colonization study, rectal swabs were taken at randomization, end of treatment, and TOC. The microorganisms investigated were Enterobacterales producing ESBLs, carbapenemases and/or plasmid-mediated AmpC or hyperproducing chromosomal AmpC (cAmpC), carbapenem-resistant *Acinetobacter baumannii*, carbapenem-resistant *P. aeruginosa,* and *Stenotrophomonas maltophilia*. Rectal swabs were cultured in MacConkey agar with 2 mg/L cefotaxime. ESBL, cAmpC, pAmpC and carbapenemase production were determined by standard phenotypic methods.[18] PCR assays were used to characterize β-lactamase genes. In *P. aeruginosa*, disk diffusion was used to detect carbapenem resistance.

Statistical analyses

Assuming an 85% cure rate in both arms (based on previous observational data,[10] 344 patients (172 per arm) were needed to reject the inferiority of de-escalation considering a 10% margin, 80% power, 2-sided alpha of 5%, and 5% dropout rate. The -10% non-inferiority margin followed recent trials of complicated UTI and intra-abdominal infections.[19,20]

Differences in proportions were calculated with 2-sided 95% confidence intervals (CI) for the endpoints, using the control group as reference. For the primary analysis in the mITTP, all patients not reaching clinical cure or missing evaluation at TOC were considered as not cured. Non-inferiority was declared when the lower bound of the 95% CI of the absolute difference in cure rate is below the -10% non-

inferiority margin. Multivariable analysis using logistic regression was performed to control for the effect of potential residual confounders on the primary outcome. A sensitivity analysis according to source of infection was performed, as planned in the study protocol. In addition, post hoc were also performed in subgroups according to age, acquisition type, severe sepsis/shock, and microorganism. Likewise, a desirability of outcome ranking (DOOR) analysis was also performed.[20] Patient outcomes evaluated at 60 days were classified according to an ordinal scale with five mutually exclusive hierarchical levels in descending order of desirability (the lower the DOOR rank, the more desirable the outcome): (1) cured without events; (2) cured with non-severe event(s); (3) cured with severe event(s); (4) not cured; and (5) death. The DOOR analysis was performed on the CEP population. The probability of patients in the experimental arm having better DOOR scores compared to those in the control group was calculated with 95% CI. In addition, a pre-planned superiority analysis was performed by adding a response adjusted for duration of antibiotic risk (RADAR) in which the duration of exposure to antipseudomonal β-lactams was added as a tie-breaking variable for the DOOR rank.[21] Data were analysed using SPSS Statistics 26 (IBM Corp).

Role of the funding source

The funder had no influence in the design and conduct of the study; collection, management, analysis, and interpretation of the data; preparation, review, or approval of the manuscript; and decision to submit the manuscript for publication.

RESULTS

In total, 2,030 patients were evaluated for inclusion during the study period; of the 765 who had no exclusion criteria, 344 (44·9%) were randomised (Figure 1). Thirteen of the randomised patients were not included in the mITT populaton, including 5 patients who withdrew their consent, 5 randomized in error due to polymicrobial bacteraemia, and 3 who were withdrawn by the physician in charge. Therefore, the mITT population included 331 patients: 164 were randomised to the experimental arm (de-escalation) and 167 to the control arm (non-de-escalation).

The median age of patients was 72 (interquartile range [IQR], 64-80); 196 (59·2%) were male, and the median Charlson index was 3 (IQR 1-5); the most frequent comorbidities were diabetes mellitus (120 patients [36·3%]) and solid organ cancer (109 [32·9%]); 168 patients (50·7%) had a community-acquired infection. The biliary and urinary tracts (129 [38·9%] and 126 [38%], respectively) were the most frequent sources of bacteraemia. *Escherichia coli* (215 [65%]) and *Klebsiella pneumoniae* (54 [16·3%]) were the most frequent pathogens.

Patient characteristics in the two study arms are summarised in Table 1. More patients in the control arm had chronic pulmonary disease (29 [17·4%] vs 21 [12·8%]) and diabetes mellitus (64 [38·3%] vs 56 [34·1%]), while the opposite was true for use of immunosuppressive drugs (17 [10·2%] vs 30 [18·3%]). More patients in the experimental arm had nosocomial infection (44 [26·3%] vs 27 [16·5%]), but fewer presented with severe sepsis or septic shock (35 [20·9%] vs 48 [29·2%]). The median (IQR) number of days of antipseudomonal drugs was 2 (2-3) in the

386 experimental arm and 7 (6-9) in the control arm (p<0.001).

387 The most frequent empirical antipseudomonal drugs used in both groups was
388 piperacillin/tazobactam (104 [63·4] and 107 [64·1] in the experimental and in the
389 control arms, respectively (Table 1). The drugs to which patients were de-escalated in
390 the experimental arm are shown in Table 1. Ninety-six (58·5%) patients in the
391 experimental arm and 118 (70·7%) in the control arm were later switched to oral
392 drugs (Table 1).

393 Clinical cure rates at TOC in de-escalated versus non-de-escalated patients,
394 were, respectively, 90·2% (148 patients) and 88·6% (148 patients) (risk difference,
395 1·6%; 95% CI, -5·0 to 8·2; Table 2); thus, de-escalation met the prespecified non-
396 inferiority criteria. The reasons for not reaching clinical cure were similar in both
397 groups and are also specified in Table 2. AEs leading to drug discontinuation are
398 reported below.

399 In multivariable analysis, the adjusted OR of de-escalation for reaching clinical
400 cure at TOC (controlling for chronic pulmonary disease, diabetes,
401 immunosuppressants, acquisition type, and severe sepsis or shock) was 0·56 (95%
402 CI 0·16-2·02; p=0·38).

403 No significant differences were found for secondary endpoints. Clinical cure at
404 TOC in the CEP (per-protocol analysis) was reached by 143 of 148 (96·6%) patients
405 in the experimental arm and 144 of 156 (92·3%) in the control arm (risk difference,
406 4·3, 95% CI -0·9 to 9·5). Microbiological cure in the MEP at TOC occurred in 124 of
407 128 (96·9%) and 125 of 132 (94·7%) patients in the de-escalation and non-de-
408 escalation arms, respectively (risk difference, 2·2, 95% CI -2·7 to 7·1) (Table 2).
409 Sixty-day mortality was 4·6% (7 of 153) among de-escalated patients versus 5·6% (9

of 160) among non-de-escalated patients. At day 60, relapse occurred in 9 of 153 (5·9%) de-escalated and 18 of 160 (11·3%) non-de-escalated patients; only 1 case of *C. difficile* infection was diagnosed in each arm.

Subgroup analyses were performed on the mITT (Table 3). Overall, the results were consistent with the primary analysis. Of note, clinical cure rates at TOC between patients in the de-escalation and non-de-escalation groups presenting with severe sepsis or septic shock at randomization were similar [44/47 (93·6%) vs· 31/34 (91·2%), risk difference, 3·3 (-3·0 to 9·5)]. Among patients with UTI, clinical cure was reached in 59/60 patients (98·3%) in the experimental arm and 55/63 (87·3%) in the control arm (risk difference, 11·0 [1·8 to 20·2]). Interactions between the subgroups and treatment arm were analysed; none were statistically significant (data not shown).

A DOOR analysis was performed on 146 patients in the experimental arm and 155 patients in the control arm for whom an assessment of all variables at 60 days was available. Among de-escalated patients, 55 (37·7%) were cured without events, 53 (36·3%) were cured with only non-severe events, 27 (18·5%) with one or more severe events, 4 (2·7%) were not cured and 7 died (4·8%). Among non-de-escalated patients, the corresponding figures were 59 (38·1%), 57 (36·8%), 26 (16·8%), 6 (3·9%) and 7 (4·5%), respectively (Figure 2 and appendix p 3). The proportion of cases with better DOOR scores among patients in the experimental arm was 0·50 (95% CI, 0·44 to 0·56). When duration of exposure to antipseudomonal β-lactams was used to break ties (RADAR analysis), the probability of a better DOOR in de-escalated patients was 0·68 (95% CI, 0·63 to 0·73).

Ninety-nine patients (60·3%) in the experimental arm and 94 (56·3%) in the control arm were reported to have at least one AE (risk difference, 4·0; 95% CI: -6·6

to 14·6; p=0·23). The total number of reported AEs was 219 in de-escalated and 175 in non-de-escalated patients, and 53 (24·2%) and 56 (32%) respectively, were considered severe. Five patients were withdrawn due to AEs: 3 in the experimental arm (persistent fever; development of septic shock; hepatic abscess) and 2 in the control arm (renal and hepatic toxicity; and pancreatitis with abdominal abscess). Table 4 and appendix p 4 lists the AEs and severe AEs. Two hundred and four minor and 10 major protocol deviations were documented without associated safety risks after being assessed by the study team and the monitoring group.

Rectal samples were taken at recruitment and TOC from 46 and 64 patients who gave their consent in the experimental and control groups, respectively. Of these, 7 in the experimental group (15·2%) and 15 in the control group (23·4%) acquired any MDR gram negative bacteria (p=0·28). MDR organisms acquired in the experimental group were 6 Enterobacterales (2 AmpC- and 4 ESBL-producing) and 1 *S. maltophilia*; and in the control group, 13 Enterobacterales (6 AmpC-, 6 ESBL-, 1 carbapenemase-producing) and 2 *S. maltophilia* (appendix p 5).

DISCUSSION

In this pragmatic randomised trial in patients with bacteraemia caused by Enterobacterales, de-escalation following a prespecified rule from an antipseudomonal β-lactam to a narrower-spectrum antibiotic active *in vitro* was non-inferior in efficacy to continuing with the empiric drug. The results of the different outcomes, subgroups and DOOR analyses were consistent and supported the non-inferiority hypothesis; when time of exposure to antipseudomonal β-lactams was

458 considered, de-escalation was superior. Notably, the 95%CI of the risk difference
459 was >1 for the experimental arm among patients with UTI, which is relevant because
460 randomisation was stratified for urinary source.

461 De-escalation is a broad concept that includes discontinuation of unnecessary
462 drugs and/or switching to narrower spectrum antibiotics as targeted therapy, based
463 on microbiological data or clinical re-evaluation.[22] Therefore, it includes
464 heterogeneous drugs (those used initially and for de-escalation) and very diverse
465 clinical situations (different infection sources, infection severity, causative pathogens
466 and patient populations). This heterogeneity poses a challenge for the design of
467 randomised trials with results that can be applied to decisions in individual patients.

468 We found only three previous randomised trials comparing de-escalation with
469 continuation of initial therapy, all with limited statistical power, and none in patients
470 with bacteraemia. Falguera *et al* evaluated de-escalation based on urine antigens in
471 patients admitted with community-acquired pneumonia in one Spanish hospital;[23]
472 relapse occurred in 3 of 25 de-escalated patients (12%) after a positive urine antigen
473 result, 1 of 63 (1·6%) among those not de-escalated because of a negative urine test,
474 and 2 of 89 (2·2%) not de-escalated because assigned to no urine test. Leone *et al*
475 compared de-escalation and continuation of empiric therapy in 59 and 57 ICU
476 patients, respectively, with sepsis of various origins and microorganisms;[24] in that
477 study, de-escalation could not demonstrate to be non-inferior for the duration if ICU
478 stay of the patients, without differences in mortality. The limitations of this study were
479 discussed in detail elsewhere.[25] Rattanaumpawan *et al* compared de-escalation to
480 ertapenem vs continuing with type-2 carbapenems in 32 and 34 patients, respectively,
481 with diverse infections caused by ESBL producers, around 50% of which were

bacteremic.[26] The trial was stopped because of low recruitment, and no significant differences in outcome were observed in the patients tested. In a meta-analysis of mainly observational studies investigating de-escalation in patients with sepsis or bacteraemia, a possible protective effect of de-escalation for mortality was found, although this effect was no longer significant when studies providing adjusted estimates were considered.[27] We recently performed an observational study in patients with Enterobacterales bacteraemia initially treated with antipseudomonal agents, in which de-escalation was not associated with worse outcomes in propensity-score adjusted analysis.[10]

It is recognised that the suggested rank order of drugs used for de-escalation is open to debate because high-level evidence on the differential ecological impact of the drugs is lacking; previously published rankings have in fact been based on expert opinion.[28] Also, while different antipseudomonals will also have heterogeneous impacts, these drugs have consistently been associated with an increased risk of colonization and/or infection caused by MDR *P. aeruginosa* and Enterobacterales.[4,5,7] Despite being biologically sound, the possible reduction of risk of acquisition of MDR organisms by using de-escalation has not been investigated in depth, and may differ depending on the initial and final drug, the patient's baseline microbiota and the epidemiological situation. In this study, we performed an exploratory study on the acquisition of MDR Gram-negative bacteria in a small subset of patients. The results, although limited, are encouraging, and suggest that once the effectiveness of de-escalation has been demonstrated, it could be a primary endpoint in future trials of de-escalation from antipseudomonal drugs. Regarding *C. difficile* infection, we could not show any impact because the number of reported cases was very low. An

American cohort published in 2019 showed that the use of empirical anti-pseudomonal β-lactam for more than 48 hours was an independent risk factor for *C. difficile* infection.[6]

The pragmatic design of the study raises issues about the potential different efficacy of each drug used by intravenous and oral routes. However, to our knowledge, significant differences in efficacy have not been shown in direct comparisons of the drugs used in randomised trials for susceptible Enterobacterales. Although more patients in the control group were switched to oral drugs, almost 30% of patients in this group were only treated by the intravenous route, and most of those who were stepped down to an oral drug received ciprofloxacin. Switch to oral fluoroquinolones was much less frequent in the experimental group as this option was only second last in the list of options for that group with the intention to avoid it whenever possible because of its antipseudomonal activity. While the efficacy of all oral drugs used seems similar at least in bacteraemic urinary tract infection,[29,30] even if fluoroquinolones are considered better, the design would have in any case favoured the control group. Overall, we think that the fact that different drugs were used in both groups did not have a relevant impact on the results of the strategies compared.

This study has several limitations. There were various antibiotic options in both arms, reflecting actual practice; the study was not blinded for the same reason. Duration of treatment in most patients was longer than currently recommended for Enterobacterales bacteraemia, based on recent trial results supporting 7 days as an appropriate duration in most patients.[31] The rectal colonization study was performed on a limited number of patients. Some strengths of the study include its randomised

pragmatic design, and exclusion of stable, low-risk patients who would benefit from very early oral switching. The characteristics of the patients enrolled, including the elderly with comorbidities, are probably representative of the general population in whom de-escalation would be considered.

In conclusion, de-escalation from antipseudomonal β-lactams in patients with bacteraemia caused by Enterobacterales was non-inferior in clinical effectiveness and safety to continuing with the initial drug. Because the most frequent sources of bacteraemia were the urinary and biliary tracts, these results apply mostly to these infections. Given their potential ecological benefit, these results provide evidence to implement active actions to promote de-escalation in this clinical setting.

Contributors: Study design and funds proposal: JR-B, LEL-C, PR-G. Critical review of study design: all other authors. Study coordination: LEL-C, JR-B, CR-F, JB-F. Monitoring coordination: CR-F, EM-M. Microbiological studies: MD-V; all other authors: recruitment of patients, follow-up and collection of patients' data. Analyses of data: JRB, LEL-C. Drafting of the manuscript: LEL-C, JR-B. Critical review of manuscript: all other authors.

Declaration of interest: LELC has served as scientific advisor for Angelini, speaker for Angelini, ViiV, Gilead and Correvio, and has served as trainer for ViiV, outside the submitted work; LBP reports other from Pfizer, and Tillotts Pharma Spain, outside

the submitted work; PRG has served as advisor for Advanz, and speaker for Menarini, Shionogui and Angelini. All other authors had no conflicts to disclose.

Access to data and data sharing: Luis Eduardo López-Cortés and Jesús Rodríguez-Baño had full access to the data in the study, verified the data in the study, and were responsible for the decision to submit for publication. Individual, anonymised data would be shared after a signed agreement with Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla if requested with the objective of performing a meta-analysis with individual patients' data. Requests should be submitted to the corresponding author. Interested researchers should obtain the approval of the Ethic Committee CEIM Provincial de Sevilla. A database in SPSS file with the requested data and a dictionary of terms would be provided.

ACKNOWLEDGMENTS: Scren (Spanish Clinical research network, funded by Instituto de Salud Carlos III (PT17/0017/0012 and PT20/00123).

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Data sharing statement

Individual, anonymised data would be shared after a signed agreement with Fundación Pública Andaluza para la Gestión de la Investigación en Salud de Sevilla if requested with the objective of performing a meta-analysis with individual patients' data. Requests should be submitted to the corresponding author. Interested researchers should obtain the approval of the Ethic Committee CEIM Provincial de Sevilla. A database in SPSS file with the requested data and a dictionary of terms would be provided.

REFERENCES:

1. Ohnuma T, Chihara S, Costin B, et al. Association of appropriate empirical antimicrobial therapy with in-hospital mortality in patients with bloodstream infections in the US. *JAMA Netw Open*. 2023;6(1):e2249353. doi: 10.1001/jamanetworkopen.2022.49353.
2. Evans L, Rhodes A, Alhazzani W, et al. Surviving Sepsis Campaign: International guidelines for management of sepsis and septic shock 2021. *Crit Care Med*. 2021;49(11):e1063-e1143. doi: 10.1097/CCM.0000000000005337.
3. Karam G, Chastre J, Wilcox MH, Vincent JL. Antibiotic strategies in the era of multidrug resistance. *Crit Care*. 2016;20(1):136. doi: 10.1186/s13054-016-1320-7.
4. Solé M, Fábrega A, Cobos-Trigueros N, et al. In vivo evolution of resistance of *Pseudomonas aeruginosa* strains isolated from patients admitted to an intensive care unit: mechanisms of resistance and antimicrobial exposure. *J Antimicrob Chemother.* 2015;70(11):3004-13. doi: 10.1093/jac/dkv228.
5. Boyer A, Doussau A, Thiébault R, et al. *Pseudomonas aeruginosa* acquisition on an intensive care unit: Relationship between antibiotic selective pressure and patients' environment. *Crit Care*. 2011;15(1):R55. doi: 10.1186/cc10026.
6. Seddon MM, Bookstaver PB, Justo JA, et al. Role of early de-escalation of antimicrobial therapy on risk of *Clostridioides difficile* infection following Enterobacteriaceae bloodstream infections. *Clin Infect Dis*. 2019;69(3):414-420. doi: 10.1093/cid/ciy863.
7. Pérez-Galera S, Bravo-Ferrer JM, Paniagua M, et al; COMBACTE-CARE-EURECA Team. Risk factors for infections caused by carbapenem-resistant Enterobacterales: an international matched case-control-control study (EURECA). *EClinicalMedicine*. 2023;57:101871. doi: 10.1016/j.eclinm.2023.101871.
8. De Waele JJ, Schouten J, Beovic B, Tabah A, Leone M. Antimicrobial de-escalation as part of antimicrobial stewardship in intensive care: No simple answers to simple questions-a viewpoint of experts. *Intensive Care Med*. 2020;46(2):236-244. doi: 10.1007/s00134-019-05871-z.
9. Moehring RW, Yarrington ME, Warren BG, et al; Centers for Disease Control and Prevention's Prevention Epicenters Program. Evaluation of an opt-out protocol for antibiotic de-escalation in patients with suspected sepsis: a multicenter,

randomized, controlled trial. *Clin Infect Dis*. 2023;76(3):433-442. doi: 10.1093/cid/ciac787.

10. Palacios-Baena ZR, Delgado-Valverde M, Valiente Méndez A, et al; REIPI/GEIRAS-SEIMC Bacteraemia-MIC Group. Impact of de-escalation on prognosis of patients with bacteremia due to Enterobacteriaceae: A post hoc analysis from a multicenter prospective cohort. *Clin Infect Dis*. 2019;69(6):956-962. doi: 10.1093/cid/ciy1032.
11. Silva BN. Cochrane Database Syst Rev. 2013 Mar 28;2013(3):CD007934. doi: 10.1002/14651858.CD007934.pub3.
12. Lakbar I, De Waele JJ, Tabah A, Einav S, Martin-Loeches I, Leone M. Antimicrobial de-escalation in the ICU: From recommendations to level of evidence. *Adv Ther*. 2020;37(7):3083-3096. doi: 10.1007/s12325-020-01390-2.
13. López-Cortés LE, Rosso-Fernández C, Núñez-Núñez M, et al; SIMPLIFY Study Group. Targeted simplification versus antipseudomonal broad-spectrum beta-lactams in patients with bloodstream infections due to Enterobacteriaceae (SIMPLIFY): a study protocol for a multicentre, open-label, phase III randomised, controlled, non-inferiority clinical trial. *BMJ Open*. 2017;7(6):e015439. doi: 10.1136/bmjopen-2016-015439.
14. Piaggio G, Elbourne DR, Pocock SJ, Evans SJ, Altman DG; CONSORT Group. Reporting of noninferiority and equivalence randomized trials: extension of the CONSORT 2010 statement. *JAMA*. 2012;308(24):2594-604. doi: 10.1001/jama.2012.87802.
15. Friedman ND, Kaye KS, Stout JE, et al. Health care—associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. *Ann Intern Med*. 2002 Nov 19;137(10):791-7.
16. The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 8.1, 2018. http://www.eucast.org.
17. EUCAST Guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance, Version 2.0, 2017.
18. ISO 20776-1:2006. Clinical laboratory testing and in vitro diagnostic test systems — Susceptibility testing of infectious agents and evaluation of performance of

antimicrobial susceptibility test devices — Part 1: Reference method for testing the in vitro activity of antimicrobial agents against rapidly growing aerobic bacteria involved in infectious diseases.

19. Solomkin J, Hershberger E, Miller B, et al. Ceftolozane/tazobactam plus metronidazole for complicated intra-abdominal infections in an era of multidrug resistance: Results from a randomized, double-blind, phase 3 trial (ASPECT-cIAI). *Clin Infect Dis*. 2015;60(10):1462-71. doi: 10.1093/cid/civ097.
20. Wagenlehner FM, Sobel JD, Newell P, et al. Ceftazidime-avibactam versus doripenem for the treatment of complicated urinary tract infections, including acute pyelonephritis: RECAPTURE, a phase 3 randomized trial program. *Clin Infect Dis*. 2016;63(6):754-762. doi: 10.1093/cid/ciw378.
21. Evans SR, Rubin D, Follmann D, et al. Desirability of Outcome Ranking (DOOR) and Response Adjusted for Duration of Antibiotic Risk (RADAR). *Clin Infect Dis*. 2015;61(5):800-6. doi: 10.1093/cid/civ495.
22. Tabah A, Cotta MO, Garnacho-Montero J, et al. A systematic review of the definitions, determinants, and clinical outcomes of antimicrobial de-escalation in the Intensive Care Unit. *Clin Infect Dis*. 2016;62(8):1009-1017. doi: 10.1093/cid/civ1199.
23. Falguera M, Ruiz-González A, Schoenenberger JA, et al. Prospective, randomised study to compare empirical treatment versus targeted treatment on the basis of the urine antigen results in hospitalised patients with community-acquired pneumonia. *Thorax*. 2010;65(2):101-6. doi: 10.1136/thx.2009.118588.
24. Leone M, Bechis C, Baumstarck K, et al; AZUREA Network Investigators. De-escalation versus continuation of empirical antimicrobial treatment in severe sepsis: a multicenter non-blinded randomized noninferiority trial. *Intensive Care Med*. 2014;40(10):1399-408. doi: 10.1007/s00134-014-3411-8.
25. Timsit JF, Harbarth S, Carlet J. De-escalation as a potential way of reducing antibiotic use and antimicrobial resistance in ICU. *Intensive Care Med*. 2014;40(10):1580-2. doi: 10.1007/s00134-014-3485-3.
26. Rattanaumpawan P, Werarak P, Jitmuang A, Kiratisin P, Thamlikitkul V. Efficacy and safety of de-escalation therapy to ertapenem for treatment of infections caused by extended-spectrum-β-lactamase-producing Enterobacteriaceae: an

open-label randomized controlled trial. *BMC Infect Dis*. 2017;17(1):183. doi: 10.1186/s12879-017-2284-1.

27. Paul M, Dickstein Y, Raz-Pasteur A. Antibiotic de-escalation for bloodstream infections and pneumonia: systematic review and meta-analysis. *Clin Microbiol Infect*. 2016;22(12):960-967. doi: 10.1016/j.cmi.2016.05.023.
28. Weiss E, Zahar JR, Lesprit P, et al; De-escalation Study Group. Elaboration of a consensual definition of de-escalation allowing a ranking of β-lactams. *Clin Microbiol Infect*. 2015;21(7):649.e1-10. doi: 10.1016/j.cmi.2015.03.013.
29. Sutton JD, Stevens VW, Chang NN, Khader K, Timbrook TT, Spivak ES. Oral β-Lactam Antibiotics vs Fluoroquinolones or Trimethoprim-Sulfamethoxazole for Definitive Treatment of Enterobacterales Bacteremia From a Urine Source. *JAMA Netw Open.* 2020;3(10):e2020166. doi: 10.1001/jamanetworkopen.2020.20166.
30. Tamma PD, Conley AT, Cosgrove SE, et al. Association of 30-day mortality with oral step-down vs continued intravenous therapy in patients hospitalized with Enterobacteriaceae bacteremia. *JAMA Intern Med.* 2019;179(3):316-323. doi: 10.1001/jamainternmed.2018.6226.
31. Turjeman A, von Dach E, Molina J, et al. Duration of antibiotic treatment for Gram-negative bacteremia - Systematic review and individual participant data (IPD) meta-analysis. *EClinicalMedicine*. 2022;55:101750. doi: 10.1016/j.eclinm.2022.101750.

711 Figure 1. Patient recruitment and randomisation

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Figure 2. Breakdown of desirability of outcome rank (DOOR) scores by treatment group. Categories are: (1) Cured, no events; (2) cured, at least one non-severe event; (3) cured, at least one severe event; (4) Not cured; (5) Death.

721 Table 1. Baseline characteristics of patients in the modified intention-to-treat
722 population.

Characteristic	**De-escalation arm (n=164)**	**Non-de-escalation arm (n=167)**
Age in years, mean (SD)	69·5 (12·8)	71·9 (12·2)
Female gender	64 (39·0)	71 (42·5)
Charlson index, median (IQR)	2·5 (1-4)	3 (1-5)
Congestive heart failure	19 (11·6)	21 (12·6)
Chronic pulmonary disease	21 (12·8)	29 (17·4)
Solid organ cancer	55 (33·5)	54 (32·3)
Haematologic cancer	3 (1·8)	2 (1·2)
Diabetes mellitus	56 (34·1)	64 (38·3)
Chronic kidney disease	37 (22·6)	39 (23·4)
Obstructive uropathy	18 (11·0)	15 (9·0)
Chronic liver disease	18 (11·0)	19 (11·4)
Obstructive biliary tract disease	29 (17·7)	38 (22·8)
Inflammatory intestinal disease	4 (2·4)	6 (3·6)
Immunosuppressive drug use	30 (18·3)	17 (10·2)
Fully dependent for basic activities	13 (7·9)	19 (11·4)
Acquisition type		
Community-acquired	89 (54·3)	79 (47·3)
Community-onset, healthcare-associated	48 (29·3)	44 (26·3)
Nosocomial	27 (16·5)	44 (26·3)
Severity of infection at presentation		
Severe sepsis	31 (18·9)	28 (16·8)
Septic sock	17 (10·4)	7 (4·2)
Source of bacteraemia		
Biliary tract	62 (37·8)	67 (40·1)
Urinary tract	61 (37·2)	65 (38·9)
Abdominal other than biliary tract	16 (9·8)	14 (8·4)
Vascular catheter	7 (4·3)	12 (7·2)
Skin and skin structure	4 (2·4)	0 (0·0)
Respiratory tract	2 (1·2)	2 (1·2)
Other	2 (1·2)	1 (0·6)
Unknown	10 (6·1)	6 (3·6)
Aetiology		
Escherichia coli	103 (62·8)	112 (67·1)
Klebsiella pneumoniae	30 (18·3)	24 (14·4)
Klebsiella oxytoca	9 (5·5)	7 (4·2)
Enterobacter cloacae	3 (1·8)	11 (6·6)
Proteus mirabilis	6 (3·7)	7 (4·2)
Other	13 (7·9)	6 (3·6)
Pitt score, median (IQR)	0 (0-2)	0 (0-1)
Days from blood cultures to administration of empirical therapy, median (IQR)	0 (0-0)	0 (0-0)
Days from blood cultures to randomisation, median (IQR) [1]	2 (2-3)	2 (2-3)
Days of intravenous therapy, median (IQR)	7 (6-10)	7 (6-9)
Days of oral therapy, median (IQR)	3 (0-6)	3 (0-6)

Total days of therapy, median (IQR)	11 (9-14)	11 (9-14)
Source control within 72 hours		
Not required	98 (59·8)	106 (63·5)
Required and performed	54 (32·9)	57 (34·1)
Required and not performed	12 (7·3)	4 (2·4)
Empirical drugs used		
Imipenem or meropemem	45 (27·4)	47 (28·1)
Piperacillin/tazobactam	104 (63·4)	107 (64·1)
Cefepime or ceftazidime	14 (8·5)	11 (6·6)
Aztreonam	1 (0·6)	2 (1·2)
De-escalation intravenous drug		
Ampicillin	Not applicable	23 (14)
Trimetoprim-sulfamethoxazole		6 (3·7)
Cefuroxime		23 (14)
Cefotaxime or ceftriaxone		52 (31·7)
Amoxicillin-clavulanate		24 (14·6)
Ciprofloxacin		18 (10·9)
Ertapenem		18 (10·9)
Switched to oral drugs, n (%)	96 (58·3)	118 (70·7)
Ciprofloxacin	21 (20·4)	97 (78·2)
Cefuroxime	26 (25·2)	17 (13·7)
Amoxicillin	19 (18·4)	1 (0·8)
Amoxicillin-clavulanate	17 (16·5)	4 (3·2)
Cefixime	14 (13·6)	1 (0·8)
Trimethoprim-sulfamethoxazole	6 (5·8)	2 (1·6)
Ertapenem	0 (0·0)	2 (1·6)

Data are number of patients (%) except where specified.

[1] Equals the time to de-escalation in the de-escalation arm.

Table 2. Primary and secondary endpoint analyses.

Outcome	De-escalation arm	Non de-escalation arm	Difference in percentage points (2-sided 95% CI)
Primary analysis (modified intention-to-treat population)			
Clinical cure at TOC, n (%)	148/164 (90·2)	148/167 (88·6)	1·6 (-5·0 to 8·2)
Reasons for not reaching clinical cure at TOC			
Clinical failure	7/164 (4·3)	12/167 (7·2)	-2·9 (-7·9 to 2·1)
Missed assessment	6/164 (3·7)	5/167 (3)	0·7 (-3·2 to 4·6)
Withdrawn due to adverse events	3/164 (1·8)	2/167 (1·2)	0·6 (-2·0 to 3·2)
Secondary analyses			
Clinical cure at TOC (clinically evaluable population)	143/148[1] (96·6)	144/156[2] (92·3)	4·3 (-0·9 to 9·5)
Microbiological cure at TOC (microbiologically evaluable population)	124/128 (96·9)	125/132 (94·7)	2·2 (-2·7 to 7·1)
Clinical cure at day 60	142/153[3] (92·8)	144/160[4] (90)	2·8 (-3·4 to 9·0)
Relapses until day 60	9/153[3] (5·9)	18/160[4] (11·3)	-5·5 (-11·6 to 0·8)
Death at day 60	7/153[3] (4·6)	9/160[4] (5·6)	-1·0 (-5·9 to 3·9)
Clostridioides difficile infection until day 60	1/153[3] (0·7)	1/160[4] (0·6)	0·10 (-1·7 to 1·9)

TOC: test of cure.

[1]16 patients were excluded from the modified intention-to-treat population for failing to complete 5 days of treatment (13) and for missing assessment at TOC (3)

[2]9 patients were excluded from the modified intention-to-treat population: failure to complete 5 days of treatment (7), withdrawn due to adverse events (2), and missed assessment at TOC (3)

[3]11 patients excluded from the modified intention-to-treat population due to missed assessment

[4]7 patients excluded from the modified intention-to-treat population due to missed assessment

Table 3. Subgroup analyses for clinical cure at test of cure in the modified intention-to-treat population.

CHARACTERISTIC	**De-escalation arm**	**Non-de-escalation arm**	**Difference in percentage points (2-sided 95% CI)**
Age < 80 years	117/124 (94·4)	112/121 (92·6)	1·8 (4·4 to 8·0)
Age ≥ 80 years	31/32 (96·9)	36/40 (90·0)	6·9 (-4·9 to 18·7)
Community acquisition	82/85 (96·5)	70/75 (93·3)	3·1 (-3·6 to 9·9)
Non-community acquisition	66/71 (93)	78/86 (90·7)	2·3 (-6·4 to 10·9)
Severe sepsis or septic shock	44/47 (93·6)	31/34 (91·2)	2·4 (-9·1 to 14)
Non-severe sepsis or septic shock	104/109 (95·4)	117/127 (92·1)	3·3 (-3·0 to 9·5)
Urinary source	59/60 (98·3)	55/63 (87·3)	11·0 (1·8 to 20·2)
Non-urinary source	89/96 (92·7)	93/98 (94·9)	2·2 (-9·0 to 4·6)
Biliary source	57/58 (98·3)	61/64 (95·3)	3·0 (-3·4 to 9·3)
Non-biliary source	89/96 (92·7)	86/96 (89·6)	3·1 (-4·9 to 11·2)
Urinary or biliary source	113/118 (95·8)	122/126 (96·8)	1·1 (-3·7 to 5·8)
Non-urinary or biliary source	36/39 (92·3)	33/33 (100)	7·7 (7·7 to 7·7)
Bacteraemia caused by *E. coli*	95/100 (95)	98/106 (92·5)	2·5 (-4·1 to 9·2)
Bacteraemia caused by non-*E. coli* Enterobacterales	53/56 (94·6)	50/55 (90·9)	3·7 (5·9 to 13·4)

Table 4. Adverse events reported.

	Experimental arm (n=164)	**Control arm (n=167)**
Gastrointestinal disorders		
Nausea/vomiting	4 (2·4)	5 (2·9)
Diarrhoea	6 (3·6)	11 (6·5)
Pancreatitis	0	5 (2·9)
Abdominal pain	5 (3)	5 (2·9)
Oral mucosis	3 (1·8)	1 (0·6)
Constipation	3 (1·8)	1 (0·6)
Cholangitis	3 (1·8)	2 (1·2)
Cholecystitis	2 (1·2)	2 (1·2)
Gastrointestinal bleeding	0	2 (1·2)
Liver abscess	1 (0·6)	1 (0·6)
Cirrhotic decompensation	2 (1·2)	3 (1·8)
Liver toxicity	0	2 (1·2)
Intestinal occlusion	0	1 (0·6)
Jaundice	1 (0·6)	0
Dehiscence of ileal anastomosis	1 (0·6)	0
Infections and infestations		
Respiratory tract	6 (3·6)	4 (2·4)
Primary bacteremia/sepsis with unknown source	8 (4·8)	2 (1·2)
C. difficile infection	1 (0·6)	1 (0·6)
Sepsis	3 (1·8)	1 (0·6)
UTI	11 (6·7)	6 (3·6)
Bacteraemia due to *E. coli*	0	6 (3·6)
Otitis	0	1 (0·6)
Skin soft tissue	1 (0·6)	0
Septic shock	1 (0·6)	0
Conjunctivitis	1 (0·6)	0
Candidemia	3 (1·8)	0
Surgical wound infection	1 (0·6)	0
Biliary prosthesis infection	1 (0·6)	0
Varicella Zoster virus reactivation	1 (0·6)	0
Influenza A infection	1 (0·6)	0

General disorders and administration site conditions		
Edema	1 (0·6)	3 (1·8)
Phlebitis or extravasation	9 (5·4)	1 (0·6)
Fever	12 (7·3)	8 (4·8)
Multi-organ failure	0	1 (0·6)
Fatigue	1 (0·6)	0
Exanthema	1 (0·6)	0
Abdominal haematoma	1 (0·6)	0
Blood and lymphatic system disorders		
Anaemia	2 (1·2)	2 (1·2)
Thrombocytopenia	2 (1·2)	1 (0·6)
Leucopoenia	4 (2·4)	1 (0·6)
Neutropenia	6 (3·6)	1 (0·6)
Thrombocytosis	1 (0·6)	0
Bicytopenia	1 (0·6)	0
Investigations		
Hypopotassaemia	0	2 (1·2)
Hypophosphatemia	0	1 (0·6)
AST, ALT elevation	1 (0·6)	2 (1·2)
Hyperuricemia	0	1 (0·6)
Hypernatremia	0	1 (0·6)
Hyperbilirubinemia	0	1 (0·6)
Creatinine elevation	1 (0·6)	0
Hypoalbuminemia	1 (0·6)	0
Nervous system disorders		
Acute vestibular neuritis	0	1 (0·6)
Epileptic seizures	0	1 (0·6)
Syncope	1 (0·6)	0
Headache	2 (1·2)	0
Asthenia	3 (1·8)	2 (1·2)
Collection in the anterior epidural space	1 (0·6)	0
Respiratory, thoracic and mediastinal disorders		
Hydropneumothorax	0	1 (0·6)
Pleural effusion	0	1 (0·6)
Dyspnea	5 (3)	3 (1·8)
Bronchospasm	0	1 (0·6)
Respiratory distress	1 (0·6)	1 (0·6)

Massive haemoptysis	0	1 (0·6)
Cough	2 (1·2)	0
Epistaxis	2 (1·2)	0
Renal and urinary disorders		
Renal insufficiency	6 (3·6)	2 (1·2)
Hepatorenal syndrome	1 (0·6)	0
Haematuria	3 (1·8)	2 (1·2)
Dysuria	1 (0·6)	1 (0·6)
Urinary retention	2 (1·2)	1 (0·6)
Leukocyturia	0	1 (0·6)
Complicated renal cyst	1 (0·6)	0
Musculoeskeletal and connective tissue disorders		
Chest muscle pain	1 (0·6)	1 (0·6)
Cardiac disorders		
Heart failure	3 (1·8)	2 (1·2)
Atrial flutter	0	1 (0·6)
Angor	0	1 (0·6)
Heart failure	3 (1·8)	0
Pericardial effusion	1 (0·6)	0
Acute coronary syndrome	1 (0·6)	0
Skin and subcutaneous tissue complications		
Rash/urticarial	1 (0·6)	2 (1·2)
Pruritus	1 (0·6)	1 (0·6)
Dermatitis	1 (0·6)	0
Endocrine disorders		
Gout attack	0	1 (0·6)
Hyperglycaemia	1 (0·6)	0
Vascular disorders		
Hypotension	3 (1·8)	2 (1·2)
Ictus	1 (0·6)	0
Neoplasms benign, malignant and unspecified		
Cancer progression	2 (1·2)	1 (0·6)
Chemotherapy toxicity	1 (0·6)	1 (0·6)
Myelodysplastic syndrome	1 (0·6)	0

SUPPLEMENTARY MATERIAL A

CLINICAL TRIAL PROTOCOL

RANDOMISED CONTROLLED, OPEN-LABEL, MULTI-CENTRE PHASE 3 CLINICAL TRIAL TO DEMONSTRATE NON-INFERIORITY OF TARGETED NARROW-SPECTRUM ANTIBIOTIC THERAPY VERSUS BROAD-SPECTRUM THERAPY WITH AN ANTIPSEUDOMONAL BETA-LACTAM IN THE TREATMENT OF PATIENTS WITH ENTEROBACTERALES BACTERAEMIA

CODENAME: SIMPLIFY

Nº EUDRACT:2015-004219-19

VERSION: 5.0 dated 30 October 2019 (Relevant amendment No.5).

1.- SUMMARY

1.1 Clinical trial type

Clinical trial on medicinal products with active substances under authorised conditions of use in different treatment strategies.

1.2 Sponsor identification

Fundación Investigación Sevilla (FISEVI).
Hospital Universitario Virgen del Rocío.
Edificio de laboratorios, 6th floor.
Avda. Manuel Siurot S/N
41013 Sevilla
Tel. 955013134/ 955013284 (Macarena site)
Fax: 955008016 (Macarena site)

1.3 Title of clinical trial

A randomised controlled, open-label, multi-centre phase 3 clinical trial to demonstrate the non-inferiority of targeted narrow-spectrum antibiotic therapy versus broad-spectrum therapy with an antipseudomonal beta-lactam in the treatment of patients with Enterobacterales bacteraemia.

1.4 Protocol code

SIMPLIFY

1.5 Coordinating investigator

Dr. Luis Eduardo López Cortés
Hospital Universitario Virgen Macarena
UGC de Enfermedades Infecciosas y Microbiología
Avda. Dr. Fedriani, 3,
41071 Seville.

1.6 Centres in which the trial is planned to be conducted

This is a multi-centre study with 21 participating hospitals, 5 of which belong to the Spanish Network for Research in Infectious Diseases.

1.7 Clinical Research Ethics Committee(s)

Reference committee: Ethics Committee for Research with Medicinal Products in the province of Seville

1.8 Responsible for monitoring

The monitoring unit will be the Clinical Research and Trials Unit of the Virgen del Rocio and Virgen Macarena University Hospitals.

1.9 Experimental and control treatments

Experimental treatment: De-escalation to a narrower-spectrum antibiotic (non-antipseudomonal beta-lactam, trimethoprim-sulfamethoxazole, or ciprofloxacin) (see below), based on the susceptibility data of the isolated microorganism.

Control treatment: Continuation with the same antipseudomonal beta-lactam that was started empirically until at least day 3 of treatment after randomisation.

1.10 Phase of the clinical trial

Phase 3

1.11 Objectives

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Primary objective: To demonstrate non-inferiority of targeted narrow-spectrum antibiotic therapy versus broader-spectrum therapy with an antipseudomonal beta-lactam with respect to clinical cure 3-5 days after completion of antibiotic therapy in Enterobacterales bacteraemias.

Secondary objectives:

- To evaluate short-term (day 5) and long-term (day 60) clinical response, mortality until day 60, hospital stay and recurrences of both strategies: targeted treatment (narrow-spectrum, based on antibiogram) versus maintenance of broad-spectrum empirical treatment.

- To assess the number of days of antibiotic treatment with an antipseudomonal beta-lactam avoided.

- To assess the total duration of treatment.
- To assess the development of secondary infections other than the initial bacteraemia.
- To assess the safety of the antibiotics used in the protocol for the treatment of bacteraemia.
- More specifically, in a subgroup of patients, to study the effect of both strategies on intestinal colonisation with multidrug-resistant Gram-negative bacilli, assessing the effect of duration of treatment on colonisation with these bacteria on day 30 after diagnosis of bacteraemia.

1.12 Study design

Randomised controlled, open-label, multi-centre phase 3 clinical trial to demonstrate non-inferiority of an experimental strategy with antibiotics in an approved indication.

1.13 Study disease or condition

Monomicrobial Enterobacterales bacteraemia of any origin.

1.14 Primary outcome variable

Primary outcome variable: Clinical cure 3-5 days after completion of treatment in the modified intention-to-treat population (mITTP). This will also apply to patients with undrained abscesses, who will be assessed no later than day 31-33 (28 +/- 3-5 days) after initiation of appropriate antibiotic treatment; patients with drained abscess after day 7 will be assessed 7 days after drainage.

1.15 Study population and total number of patients

The study population will consist of hospitalised adult patients with monomicrobial Enterobacterales bacteraemia of any origin in blood cultures. The sample size required to achieve the objectives using a non-inferiority design is 344 patients (172 per arm).

1.16 Duration of treatment

The standard duration of treatment will be between 7 and 14 days, but in no case less than 7 days. In cases with an undrained abscess focus of infection or a complex focus (high inoculum, non-removable device, etc.), duration may be longer than 14 days, based on the justified criteria of the clinician in charge.

1.17 Timing and anticipated completion date

The total trial duration is anticipated to be four years from the start of the recruitment period. Submission of documentation to the Spanish Agency of Medicines and Medical Products and the Ethics Committees, as well as initial training at the participating centres will take 6 months. Patient recruitment will be 41 months, with an estimated further 6 months for analysis and subsequent dissemination of results.

3.- GENERAL INFORMATION

3.1 Trial identification

Study codename: SIMPLIFY
EudraCT: 2015-004219-19

3.2 Clinical trial type

A randomised controlled, open-label, multi-centre phase 3 clinical trial to demonstrate the non-inferiority of targeted narrow-spectrum antibiotic therapy versus broad-spectrum therapy with an antipseudomonal beta-lactam in the treatment of patients with Enterobacterales bacteraemia.

3.3 Sponsor details

Fundación Investigación Sevilla (FISEVI).
Hospital Universitario Virgen del Rocío.
Edificio de laboratorios, 6ª planta.
Avda. Manuel Siurot S/N
41013/Seville
Tel. 955013645 / 955013284(Macarena site)
Fax: 955008016 (Macarena site)

3.3.1 Sponsor representative

Dr. Clara M. Rosso Fernández
Clinical Research and Clinical Trials Unit
Hospital Universitario Virgen del Rocío,
Avda. Manuel Siurot s/n
41013. Seville
Tel.:955313414
Fax: 954232992

3.3.2 Responsible monitoring unit

Unidad de Investigación Clínica y Ensayos Clínicos
University Hospitals Virgen del Rocío and Virgen Macarena
Avda. Manuel Siurot S/N
41013. Seville
Tel.:955313414
Fax: 955095338

3.4 Description of study products

Included patients will have been treated empirically for infection with an antipseudomonal beta-lactam antibiotic.

Experimental group

Rule-based de-escalation to a non-antipseudomonal beta-lactam antibiotic, trimethoprim-sulfamethoxazole or ciprofloxacin once the sensitivity of the Enterobacterales causing the bacteraemia is known. Study drugs are ampicillin, trimethoprim-sulfamethoxazole, cefuroxime, cefotaxime, amoxicillin/clavulanic acid, ciprofloxacin and ertapenem. De-escalation will be based on a pre-established rule (see below) that considers the sensitivity of the isolated microorganism.

Control group

Continuation with the same antipseudomonal beta-lactam antibiotic that was started empirically, at least until completion of 3 days of treatment after randomisation. These drugs are: ceftazidime, cefepime, aztreonam, piperacillin/tazobactam, imipenem, meropenem.

3.5 Details of study investigators

See annex

3.6 Laboratories, Medical Department or Related Institutions

Inter-centre Clinical Unit of Infectious Diseases, Microbiology and Preventive Medicine
University Hospitals Virgen Macarena and Virgen del Rocío.
Avenida del Doctor Fedriani s/n
41009.Sevilla
Contact telephone: 600162310
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Pharmacy Clinical Management Unit
University Hospital Virgen Macarena
Avenida Doctor Fedriani s/n
41009. Sevilla

4.- RATIONALE

Bacteraemia is a pathological entity associated with a high mortality rate, which depends directly on factors such as patient comorbidity, severity of presentation, the causative pathogen and clinical management [1,2]. Its incidence is high overall, although it occurs more frequently in the nosocomial and healthcare settings. Enterobacterales as a group cause the majority of community bacteraemias and a high percentage of nosocomial infections, with an associated mortality of around 15% [3].

The use of empirical and targeted antibiotic therapy appropriate to the antibiotic sensitivity of the isolated pathogen is associated with a better prognosis [4]. Empirical treatment of sepsis potentially caused by Enterobacterales usually includes a beta-lactam (less frequently a quinolone because of the high resistance rate). For nosocomial infections and some healthcare-associated infections, the selected regimen should include an antibiotic with adequate activity against *Pseudomonas aeruginosa* [5,6].

The increase in multidrug-resistant pathogens in turn involves the use of combination and/or broad-spectrum antibiotic therapy, especially in nosocomial infections and severe sepsis or septic shock [7,8]. As a result, antipseudomonal beta-lactams and combinations are frequently used in clinical practice for the empirical treatment of sepsis potentially caused by Enterobacterales. The frequency with which treatment is subsequently simplified to a narrower-spectrum antibiotic based on antibiogram data is low [9-12]. The effect of sustained antibiotic pressure (where the antibiotic spectrum is greater than necessary) is the selection or induction of resistance, which depletes therapeutic resources [13-6]. In response to this phenomenon, a worldwide scientific movement has emerged in recent years with the aim of halting the advance of this “antibiotic crisis”. One strategy is to implement programmes that optimise the use of antibiotics (PROA) [17]. Among the activities included as part of PROA programmes are those aimed at reducing the use of certain antibiotic families, such as antipseudomonals. One of the potentially most useful strategies for doing this is to modify empirical therapy to a targeted, narrower-spectrum antibiotic based on clinical parameters and/or antibiotic sensitivity results (“de-escalation”) [13]. This can be achieved by switching from combination therapy to monotherapy, or by replacing the initial drug with a different one. Many experts in the pathology of infectious diseases assume that this is both the normal as well as the most rational course of action, given that the drug to which treatment will be de-escalated has already been shown to be effective against the infection for which it is to be indicated. In reality, however, the strategy is applied far less frequently than it should be [13-6]. This is because the practice is supported only by expert recommendations [18-20], a few observational studies suggesting its safety [21-5] and use on a rational basis. Thus, many prescribers have serious doubts about its efficacy and safety.

Some of the arguments raised by those who are doubtful about de-escalation can be summarised as follows: 1) antimicrobial susceptibility tests have a margin of error of at least one dilution. This may mean that simplification is less safe than broad-spectrum treatment because the minimum inhibitory concentrations (MIC) of the most commonly used de-escalated antibiotics are closer to the cut-off point and are therefore reported as sensitive when in fact they may not be. 2) Strains or subpopulations resistant to narrow-spectrum drugs may be selected during the time when the empirical treatment is being taken, which could lead to treatment failure if de-escalation is chosen. 3) In the case of polymicrobial infections, it is not uncommon for only one to be isolated in a blood culture, so that simplification of treatment may be less safe and effective than a broad-spectrum regimen. 4) There are doubts about the real efficacy of certain narrower-spectrum drugs against beta-lactamase-producing strains (e.g. beta-lactamase inhibitors against extended-spectrum beta-lactamase (ESBL)-producing strains). Furthermore, while it is accepted that broad-spectrum treatment has a greater impact on the selection of multidrug-resistant strains, some studies suggest that this may depend more on the duration of treatment than on the antibiotic spectrum [26, 27].

Based on these unresolved questions, a recent systematic review in the *Cochrane Library* concluded that there were not enough studies to support de-escalation as a therapeutic strategy and that randomised studies were essential [28]. Following a search of clinical trial registers, we detected two studies on this topic [29-30]. One was a clinical trial focusing on patients with severe sepsis admitted to intensive care units, which ended in August 2014; the other was a non-randomised interventional study in patients with severe infections treated with meropenem or piperacillin/tazobactam. The latter was initiated in 2010 and its status is currently unknown. There are therefore no trials underway using the same methodology and objectives as ours.

Given the frequency and clinical importance of bacteraemia caused by Enterobacterales and the large number of patients being treated with a broader spectrum than necessary, we believe that the study we are proposing is necessary to assess the impact of de-escalation. Our study is intended to be a proof-of-concept (POC) trial in “real clinical practice” and will provide a solid foundation on which to continue developing strategies to optimise antimicrobial use. Other designs would clearly not provide the evidence needed to change current clinical practice.

This project would enable us to demonstrate whether antibiotic de-escalation based on microbiological data in patients with Enterobacterales bacteraemia is as safe and effective as maintenance of adequate broader-spectrum empirical therapy. If that is the case, it would reduce the consumption of broad-spectrum antibiotics very significantly and have a direct protective effect against resistance development.

5.- HYPOTHESIS AND OBJECTIVES OF THE TRIAL

5.1 Hypothesis

The efficacy and safety of treatment with targeted narrow-spectrum antibiotics against susceptible Enterobacterales is non-inferior to broad-spectrum treatment with an antipseudomonal beta-lactam in patients with bacteraemia caused by these microorganisms.

5.2 Objectives

Primary objective: To demonstrate the non-inferiority of targeted narrow-spectrum antibiotic treatment versus broad-spectrum treatment with an antipseudomonal beta-lactam with respect to clinical cure 3 to 5 days after the end of antibiotic treatment for Enterobacterales bacteraemia.

Secondary objectives:

- To assess short- (day 5) and long-term (day 60) clinical response, mortality until day 60, hospital stay and recurrence rates for both targeted treatment strategies (reduced-spectrum based on antibiogram versus maintenance of broad-spectrum empirical therapy.
- To assess the number of days of antibiotic treatment with an antipseudomonal antibiotic avoided.
- To assess total duration of treatment.
- To assess the development of secondary infections other than the initial bacteraemia.
- To assess the safety of antibiotics included in the protocol for the treatment of bacteraemia.
- More specifically, in a subgroup of patients, to study the effect of both strategies on colonisation of the intestinal tract with multidrug-resistant (MDR) Gram-negative bacilli, and to assess the effect of duration of treatment on colonisation with these bacteria on day 30 after diagnosis of bacteraemia.

6.- STUDY DESIGN

6.1 Study variables

6.1.1 Primary endpoint

Clinical cure 3 to 5 days after the end of antibiotic treatment in the modified intention-to-treat population (mITTP). This will also apply to patients with undrained abscesses, who will be assessed no later than day 31-33 after initiation of appropriate

antibiotic treatment; patients with drained abscess beyond day 7 will be assessed 7 days after abscess drainage.

6.1.2 Secondary variables

- Clinical response and early microbiological cure (day 5) in the mITTP, and in the clinically and microbiologically evaluable populations (CMEP), respectively.
- Late clinical and microbiological cure (day 60) in the mITTP, respectively.
- Recurrences in the first 60 days in the mITTP. Multidrug-resistant (MDR) infection within 60 days in the CEP. Frequency of *Clostridium difficile* infection.
- Frequency and severity of adverse reactions in the mITT population. Proportion of rectal colonisation with MDR Gram-negative bacilli (see Microbiological Studies section).

6.2 Design

A randomised controlled, open-label multi-centre phase 3 clinical trial to demonstrate non-inferiority of targeted narrow-spectrum antibiotic treatment versus broad-spectrum treatment with an antipseudomonal beta-lactam with respect to clinical cure 3 to 5 days after the end of antibiotic treatment in Enterobacterales bacteraemia.

- **Experimental group:** de-escalation to a non-antipseudomonal antibiotic based on a pre-established rule that considers the sensitivity data of the isolated microorganism.
- **Control group:** continuation for at least 3 days after randomisation with the same antipseudomonal beta-lactam that was started empirically. In specific cases, switching to another antipseudomonal beta-lactam on the day of randomisation would be allowed (see below).

6.3 Randomisation procedure

Patients will be detected through daily review of blood culture results. An antibiogram of the isolate, based on one of the methods recommended by EUCAST or CLSI, will be essential, even in centres where rapid genotypic or proteomic tests are available.

If all the inclusion criteria are met and none of the exclusion criteria apply, informed consent will be sought. When informed consent is obtained, randomisation will be performed. Randomisation will be centralised and made available on a web site designed for the purpose. Stratified randomisation will be performed by focus of bacteraemia (urinary versus non-urinary), with a 1:1 allocation ratio to ensure the inclusion of a similar number of cases with non-urinary source in both treatment arms.

The number of cases of urinary bacteraemia will be in a 1:1 allocation ratio; there will be no maximum number of urinary focus patients included in the trial.

6.4 Blinding

There will be no blinding, as this is an open-label study. Therefore, no blinding procedure is applicable either.

6.5 Trial treatments

Experimental group

For those included in the experimental group, the responsible clinician will de-escalate antibiotic therapy in accordance with the de-escalation protocol on the same day that the microbiological sensitivity data become available. The de-escalation protocol will include drugs without antipseudomonal activity, with the exception of ciprofloxacin due to its special suitability for oral treatment.

The options for de-escalation will be as follows: the first drug to which the microorganism is sensitive (for this purpose, those with MICs in the intermediate susceptibility range will be interpreted as resistant) will be indicated, preferably according to EUCAST or CLSI criteria, otherwise, in the following order:

1. Ampicillin 2 g IV/6h 2.
2. TMP/SMX 160/800 mg IV/8 -12h
3. Cefuroxime 750-1000 mg/8h
4. Cefotaxime 1-2g IV/8h or ceftriaxone 1 g/12-24h
5. Amoxicillin/clavulanate 1000/125 mg IV/8h
6. Ciprofloxacin 400 mg IV/12h
7. Ertapenem 1-2g/24h.

* In the case of urinary focus, trimethoprim-sulfamethoxazole should only be used as long as the focus of infection is not an undrained abscess, due to its lower efficacy in these conditions.

Control Group

In the control group, the following treatment options will be allowed (the patient should continue with the empirical treatment that they were already taking):

- Piperacillin/tazobactam 4/0.5 g IV/6-8h
- Meropenem 1-2 g IV/8h

- Imipenem 0.5 g IV/6h - 1g IV/6h
- Aztreonam 1-2 g IV/8h
- Ceftazidime 1-2 g IV/8h
- Cefepime 2 g IV/8-12h.

In both arms of the study and for the antibiotics specified, dosing is allowed to be set at the discretion of the clinician responsible for the patient at each centre. All dosages are included in the technical fact sheet, and even the lowest doses are appropriate based on PK/PD and clinical studies for the treatment of Enterobacterales bacteraemia. The possibility of variable dosage is necessary, firstly, because the trial design is based on real clinical practice, and secondly, because it includes any source of bacteraemia, and high doses will be necessary for high-inoculum foci. All patients will be evaluated by physicians who are specialists in infectious diseases to ensure that the optimal individualised dose is selected for each patient.

Provided that all inclusion criteria are met and none of the exclusion criteria apply, patients with bacteraemia caused by extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales may be included at the discretion of the responsible clinician, in which case the use of maximum doses of the antibiotics under study is recommended.

The inclusion of Enterobacterales with inducible AmpC beta-lactamases (*Enterobacter* spp., *Providencia* spp., *Morganella morganii*, *Serratia marcescens*, *Citrobacter freundii*) is also allowed. In this situation, the use of third-generation cephalosporins will be avoided in both treatment arms, even if the isolated strain is susceptible to these antibiotics. Consequently, on the day of randomisation, patients on empirical treatment with ceftazidime will be allowed to switch to another antipseudomonal beta-lactam in the control arm. For Enterobacterales bacteraemia with derepressed ampC, trimethoprim-sulfamethoxazole (only for urinary focus), ciprofloxacin or ertapenem will be used for patients in the experimental arm, at the discretion of the responsible clinician. The use of piperacillin/tazobactam in Enterobacterales with derepressed ampC is at the discretion of the responsible clinician.

When the focus of bacteraemia is potentially polymicrobial (regardless of whether the blood culture isolate was monomicrobial), combined treatment with metronidazole 500 mg IV/8h or clindamycin 600 mg IV/8h is permitted if the clinician responsible judges that additional anti-anaerobic coverage would be necessary.

If coverage against Gram-positive pathogens resistant to the drugs listed above is required, the use of any of the following is permitted: vancomycin, teicoplanin, daptomycin, or linezolid (dosage adjusted to the focus of the bacteraemia and the characteristics of the patient).

In both arms, dose adjustments will be based on the patient’s renal clearance (and/or liver function, if applicable) at that time.

6.6. Patient follow-up

Patient follow-up will be performed at the visits defined in this protocol and will continue until day 60 ± 5 from the start of antibiotic treatment. The schedule of visits is specified in section 8.4.

6.7. Criteria for termination or discontinuation of the trial

Premature discontinuation of the clinical trial may be triggered by a decision by the regulatory authorities, a change in the opinion of the Clinical Research Ethics Committees, by safety and/or drug safety concerns, or indications of inefficacy.

Both the investigator and the sponsor reserve the right to discontinue the trial at any time for reasonable medical and/or administrative reasons.

6.8. Acquisition, packaging and labelling of medicines

Medicines used in the treatment of patients in both arms of the study are approved for the treatment of the indication for which they are used in this study.

This study has an open-label design, and the medicines will be supplied from the pharmacy at each centre as standard treatment. Masking and specific labelling of the drugs will not be necessary.

6.9 Storage and dispensing of medicines

The drugs will be stored in the Pharmacy Service at each centre in accordance with the storage conditions specified by the product manufacturers.

Dispensing will be carried out in accordance with standard procedures at each participating site, and with administrative control at all times. A record of dispensing will be kept on the dispensing sheets, noting the batch, expiry date, and number of units dispensed.

6.10 End of trial

The day of the final visit of the last patient included in the study will be considered the end of the trial.

7.- SELECTION CRITERIA

Adult patients hospitalised with bacteraemia of any source with monomicrobial blood culture isolation of an Enterobacterales (including *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Morganella* spp., *Salmonella* spp., *Enterobacter* spp., *Serratia* spp., *Providencia* spp., *Serratia* spp. and *Citrobacter* spp.).

7.1 Inclusion criteria

1. Hospitalised adult patients (18 years of age or older) having bacteraemia of any source and monomicrobial blood culture isolation of an Enterobacterales.
2. Empirical treatment, in monotherapy or in combination, with an antipseudomonal beta-lactam antibiotic (piperacillin/tazobactam, meropenem, imipenem, aztreonam, ceftazidime or cefepime) active against the isolated microorganism, initiated within 24 hours after the appearance of signs and symptoms compatible with sepsis and collection of the blood culture in which the Enterobacterales was isolated. Antibiotic treatment could have been initiated prior to blood culture collection, as long as the inclusion criteria below are met. The patient could have received any other type of antibiotic therapy up to 24 hours after blood culture collection.
3. The microorganism is sensitive to at least one of the drugs in the experimental arm.
4. Patients diagnosed with potentially includable Enterobacterales bacteraemia in whom it is deemed necessary to maintain intravenous therapy for at least 3 days after randomisation, or 5 days from the initial blood culture collection.
5. Patients who have signed informed consent forms prior to the start of the trial.

7.2 Exclusion criteria

1. Terminal condition, or estimated expected life expectancy less than 30 days, or in purely palliative treatment for their underlying disease.
2. Pregnant or breastfeeding women.
3. Isolation of carbapenemase-producing Enterobacterales (CRE).
4. Delay in inclusion of more than 48 hours after the availability of antibiotic sensitivity data for the Enterobacterales isolated in blood culture.
5. Severe neutropenia (<500 cells/mm^3) at the time of randomisation.
6. Infections with treatment duration potentially exceeding 28 days (endocarditis and osteomyelitis), or meningitis.

7.3 Criteria for withdrawal

In accordance with the Helsinki Declaration patients have the right to withdraw from the study at any time and for any reason, either personally or through their representative.

7.3.1 Efficacy criteria

Clinical failure. Clinical failure will be considered when, after 72 hours of treatment in the experimental or control arm, any of the following circumstances occur:

- Worsening of the symptoms directly related to the bacteraemia that led to inclusion in the trial.

- Non-disappearance of sepsis (if present at baseline) in the absence of any second infection that would justify its persistence or recurrence.

- Need to discontinue the antibiotic or add another antibiotic due to lack of efficacy based on the above criteria, provided that this does not occur as a result of a second infection unrelated to the one for which inclusion in the trial was requested.

In these circumstances, it will be considered therapeutic failure for the purposes of the primary outcome variable.

7.3.2 Safety criteria

Any adverse event that, in the judgement of the clinician, warrants withdrawal of the study antibiotic.

When, for any reason, the treatment is no longer safe for the patient, or may endanger the patient's life, or have serious consequences for the patient.

7.3.3 For non-compliance or violation of the rules contained in the protocol

When the patient no longer complies with the trial guidelines, the patient may be withdrawn at the discretion of the investigator-in-charge, or the patient is lost to follow-up.

The need, for any reason, to add an antibiotic with activity against Gram-negative bacilli different from those used in the study before the end of trial treatment, provided that this is not due to the appearance of a second infection unrelated to the one that led to inclusion in the trial.

7.3.4 Follow-up of prematurely withdrawn patients

If a patient is prematurely withdrawn from the trial, the investigator should give the primary reason for the withdrawal and, as specified in the GCP guidelines, procedures should be followed in accordance with the standard protocols for treatment of the patient's condition at the discretion of the responsible clinician.

7.4 Definitions

Bacteraemia: blood culture isolation of one or more microorganism(s) by standard processing techniques.

Bacteraemia focus: The primary focus of bacteraemia will be determined according to CDC definitions (Garner JS, Jarvis WR, Emori TG, et al. CDC definitions for

nosocomial infections. *Am J Infect Contro*l 1998; 16:128-40). In case of doubt, it will be agreed in consensus by two clinical investigators from the same centre.

Sepsis:

- Temperature > 38º C or < 36º C.
- HR > 90 bpm.
- RR > 20 rpm or PaCO2 < 32 mmHg or need for mechanical ventilation.
- Leukocytosis > 12,000 cells/ mm^3, or leukopenia < 4,000 cells/ mm^3, or immature forms>10%.

Severe Sepsis:

- Sepsis associated with organ dysfunction, hypotension* or hypoperfusion. The sequential organ failure assessment (SOFA) score includes the respiratory, renal, hepatic, cardiovascular, haematological and neurological systems.
- *BP> 90 mmHg or any drop in BP greater than 40mmHG from baseline, in the absence of other causes of hypotension.

Septic shock:

- Hypotension due to sepsis, persisting despite fluid administration, accompanied by perfusion alterations (metabolic acidosis or hyperlactatemia), or organ dysfunction, or need for vasoactive drugs to maintain blood pressure.

Clinical cure: the situation in which all of the following conditions are met: survival at the time of assessment; disappearance of all signs and symptoms of infection (or return to the situation prior to the current infection); no need for modification of treatment due to unfavourable clinical course directly related to the bacteraemia that led to inclusion in the trial.

Microbiological cure: blood culture taken at second visit is negative, with absence of microbiological isolates in direct microbiological samples from the focus, if applicable. Presumptive microbiological cure is accepted in cases where negative culture results from the initial focus of infection cannot be demonstrated.

Presumptive microbiological cure: in cases where it is not possible to obtain a control sample from the initial focus of infection (e.g. drained collection) and the clinical and/or radiological course has been satisfactory.

Clinical recurrence of infection: recurrence within the first 60 days of at least one clinical criterion of sepsis plus one analytical criterion related to the bacteraemia that justified inclusion in the trial, with or without bacteraemia.

Microbiological recurrence of infection: new isolation of the original Enterobacterales in blood cultures or at the focus of infection following negative blood cultures, or after being classified as presumptive microbiological cure.

Multidrug-resistant infection: caused by organisms that are non-susceptible to at least one agent in 3 or more antimicrobial categories (Magiorakos et al. *ClinMicrobiolInfect*2012; 18:268-81); in our study, based on parameters established by EUCAST.

8.- TREATMENT OF SUBJECTS

Experimental treatment:

For those included in the experimental group, antibiotic treatment will be simplified in accordance with the antibiogram and the de-escalation protocol, which includes drugs without antipseudomonal activity, except for ciprofloxacin, due to its special suitability for oral treatments.

Following the criteria of the clinician responsible for the patient, the de-escalation options will be as follows: the first drug to which the microorganism is sensitive will be indicated (for this purpose, those with MICs in the intermediate susceptibility range will be interpreted as resistant), preferably in accordance with the criteria of EUCAST or CLSI, otherwise, in this order:

1. Ampicillin 2 g IV/6h
2. TMP/SMX* 160/800 mg IV/8 -12h
3. Cefuroxime 750-1000 mg/8h
4. Cefotaxime 1-2g IV/8h or ceftriaxone 1 g/12-24h
5. Amoxicillin/clavulanic acid 1000/125 mg IV/8h
6. Ciprofloxacin 400 mg IV/12h
7. Ertapenem 1-2g/24h.

*Trimethoprim-sulfamethoxazole should only be used in urinary tract infections if the focus is not an undrained abscess, due to its lower efficacy in these conditions.

Control treatment:

In the control group, the following treatment options will be allowed (the patient should continue with the empirical treatment that he/she was already taking):

- Piperacillin/tazobactam 4/0.5 g IV/6-8h
- Meropenem 1-2 g IV/8h
- Imipenem 0.5 g IV/6h - 1g IV/6h
- Aztreonam 1-2 g IV/8h
- Ceftazidime 1-2 g IV/8h
- Cefepime 2 g IV/8-12h.

In both arms of the study and for the antibiotics specified, dosing is allowed to be established at the discretion of the clinician responsible for the patient at each

centre. All dosages are included in the technical fact sheet, and even the lowest doses are appropriate based on PK/PD and clinical studies for the treatment of Enterobacterales bacteraemia. The possibility of variable dosage is necessary, firstly, because the trial design is based on real clinical practice, and secondly, because it includes any source of bacteraemia, and high doses will be necessary for a high-inoculum focus. All patients will be evaluated by physicians who are specialists in infectious diseases to ensure that the optimal individualised dose is selected for each patient.

Provided that all inclusion criteria are met and none of the exclusion criteria apply, the inclusion of bacteraemia caused by extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales (ESBL) is allowed at the discretion of the responsible clinician, in which case, the use of maximum doses of the antibiotics under study is recommended.

The inclusion of Enterobacterales with inducible AmpC beta-lactamases (*Enterobacter* spp., *Providencia* spp., *Morganella morganii*, *Serratia marcescens*, *Citrobacter freundii*) is also allowed, in which case the use of third-generation cephalosporins in either treatment arm will be avoided, even if the isolated strain is susceptible to these antibiotics. Consequently, on the day of randomisation, patients on empirical treatment with ceftazidime will be allowed to switch to another antipseudomonal beta-lactam in the control arm. For Enterobacterales bacteraemia with derepressed ampC, trimethoprim-sulfamethoxazole (only if the focus is urinary), ciprofloxacin or ertapenem will be used for patients in the experimental arm, at the discretion of the responsible clinician. The use of piperacillin/tazobactam in Enterobacterales with derepressed ampC is at the discretion of the responsible clinician.

If coverage against Gram-positive pathogens resistant to the drugs listed above is required, the use of any of the following is allowed (standard doses adjusted to the focus of the bacteraemia and patient characteristics):

- Vancomycin
- Teicoplanin
- Daptomycin
- Linezolid.

In both arms, dose adjustment will be based on the patient's renal (and/or hepatic if necessary) clearance at that time.

8.1 Duration of treatment and treatment adjustments

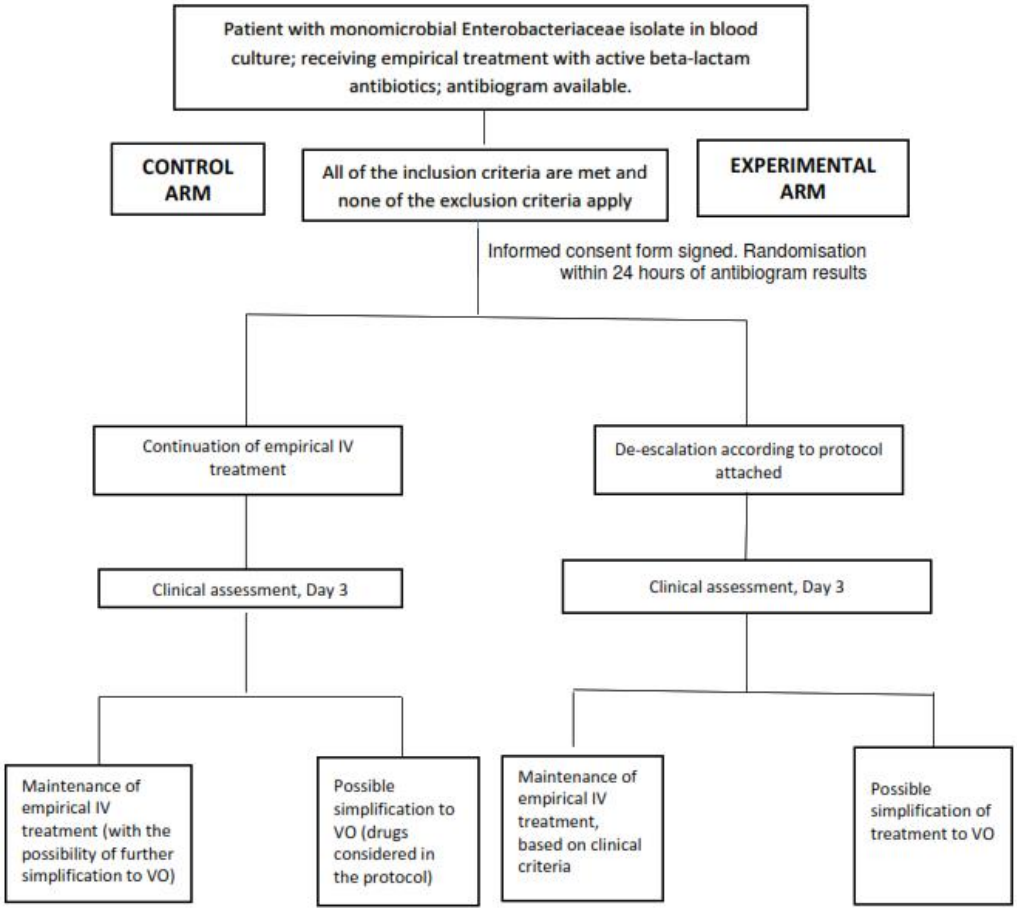
Duration of treatment should be between 7 and 14 days, but in no case should it be less than this interval. In the case of an undrained abscess or complex focus (high inoculum, non-removable prosthetic material), duration may be longer than 14 days but no longer than 28 days, based on the justified clinical reasoning of the responsible

clinician.

After completion of at least 5 days of intravenous treatment (in other words, at least 3 days of treatment after de-escalation), sequential oral therapy will be allowed if the following conditions are met:

- Clinical improvement, including absence of fever above 38ºC
- Control of primary focus
- Absence of secondary foci
- Haemodynamic stability

Tolerance to the oral route and absence of any gastrointestinal pathology that could lead to poor absorption of the drug



Simplification of treatment to the oral route in the experimental arm will always be performed using the same antibiotic as by the IV route, and at the following doses: amoxicillin 1 g/8h, TMP/SMX 160/800 mg/8-12h, cefuroxime axetil 500 mg/8-12h, amoxicillin/clavulanic acid 875/125 mg/8h or ciprofloxacin 500 mg/12h. In the case of intravenous treatment with cefotaxime or ceftriaxone, the options are ceftibuten 400 mg/12-24h or cefixime 400 mg/12-24h. The reasons for offering different dosages have been given above. If IV ertapenem has been used previously in the absence of other treatment options, IM or IV treatment will be allowed to continue, even after discharge from hospital.

Since there are no equivalent oral drugs in the control arm, the possibility of simplifying treatment to ciprofloxacin 500-750 mg VO/12h on day 3 after randomisation (i.e., 5th day of IV treatment) will be allowed at the discretion of the clinician responsible for the patient; if the microorganism is resistant to ciprofloxacin, cefuroxime axetil 500 mg/8-12h, ceftibuten 400 mg/12-24h, or cefixime 400 mg/12-24h could be used; if resistant to all of these, ertapenem 1g IM or IV on an outpatient basis.

In both arms, and if antibiotic therapy against anaerobic or resistant Gram-positive pathogens has been used, oral treatment against these pathogens could be continued, at the decision of the responsible physician, with metronidazole, clindamycin or linezolid. Traceability will follow the methodology of clinical trials.

8.2 Concomitant medication

Concomitant use of systemic antibiotics with activity against the Enterobacterales isolated in blood cultures is not permitted. Administration of such antibiotics during the antibiotic treatment phase is a criterion for withdrawal from the study. Their administration in the post-treatment follow-up phase will be taken into account in the statistical analysis.

In general, there are no contraindications to the use of other drugs; the investigators should however take into account the contraindications, warnings, and precautions for use, as well as possible interactions with other drugs, as specified on the data sheets of the investigational drugs.

8.3 Rescue medication

The use of rescue medication is not envisaged. If a patient is withdrawn due to lack of efficacy or similar, he/she will be treated according to the clinical guidelines and standard clinical practice for those cases.

8.4 Scheduled visits and assessments

Procedures	Screening visit (Day 0)	Visit 1 (Day1)	Visit 2 (Days 3-5)	End of treatment[1,2,4]	Test of cure (10-19)[1,3]	Day 30(±5)[4,6]	Day 60(±5)[4,6]
Randomisation	X						
Informed Consent	X						
Inclusion/exclusion criteria	X						
Pregnancy test[7]	X						
Clinical history / anamnesis	X	X	X	X	X	X	X
Physical examination	X	X	X	X[4]	X	X[4]	X[4]
Haematology / biochemistry		X[9]	X	X[4]	X		
Blood culture	X		X	X[5]			
Rectal swab[8]	X			X	X	X	
Concomitant medication	X	X	X	X		X	X
Dispensing control	X	X	X	X			
Adverse events		X	X	X	X	X	X

1 If the focus is an undrained abscess or complex (high inoculum, non-removable prosthetic material, etc.), duration may be longer then 14 days, based on the reasoned clinical judgment of the responsible clinician. Patients with undrained abscesses will be evaluated between 3 and 5 days after the end of antibiotic treatment (i.e., maximum day 31-33); patients with a drained abscess beyond day 7 will be evaluated 7 days after drainage.
2 To be performed on days 7 to 14 after the start of appropriate antibiotic treatment.
3 3-5 days after the end of treatment.
4 These check-ups may be performed by telephone if the patient is not admitted. In this case, it is not necessary to perform a physical examination, haematology or biochemistry.
5 Only if the previous blood culture was positive, or if symptoms persist.
6 To be performed on days 30 (±5) and 60(±5) after randomisation day.
7 If applicable.
8 In-person visits only (and at selected centres) in the case of rectal swabs.
9 If not available, data corresponding to day 0 would be collected.

8.5 Procedures per visit

The follow-up of patients in the present study consists of 6 scheduled visits, although not all of them are in-person. The visits in this protocol are listed in chronological order; the day, or range of days, of the study on which they should be carried out is indicated in brackets.

For the purposes of this study, Day 0 is considered to be the day of inclusion and the initiation of any of the study treatments. The following sections detail the procedures to be followed at each visit

8.5.1 Screening visit (Day 0)

At the screening visit, or Day 0, certain actions should be carried out as a pre-inclusion step, as well as pre-inclusion data collection. Actions to be carried out at each visit are listed below:

- Identification of cases through the microbiology laboratory at each centre.
- Assessment of inclusion/exclusion criteria.
- Signing of informed consent sheet and randomisation.
- In the case of women of childbearing age, a pregnancy test will be requested.
- Demographic data.
- Clinical history /anamnesis, with collection of personal history: hospital admissions in the previous month, bacteraemia in the previous month, invasive procedures in the previous week, antibiotherapy received in the 48 hours prior to inclusion, Charlson comorbidity index, type of acquisition, suspected source of bacteraemia, clinical symptoms, Pitt score, treatment and doses used prior to randomisation.
- Physical examination with equipment, including weight, blood pressure, heart rate, respiratory rate, body temperature.
- Collect most recent haematology and blood biochemistry data.
- Blood culture: this should be done prior to the administration of study medication.
- Rectal swab: this test will be performed only at selected centres.
- Medication administration / dispensing control.
- Review of concomitant medication.

8.5.2 Visit 1 (Day1 after randomisation)

- Clinical symptoms in relation to the clinical course of the patient.
- Physical examination: at least blood pressure, heart rate, respiratory rate, temperature.
- Haemogram: at least leukocyte, neutrophil and platelet counts. If not available, data corresponding to day 0 should be collected.

- Blood biochemistry: at least with creatinine and urea. If not available, day 0 data should be collected.
- Medication administration / dispensing control
- Review of concomitant medication.
- Assessment of adverse events.

8.5.3 Visit 2 (Day 3-5 after randomisation)

- Clinical symptoms in relation to the clinical course of the patient.
- Physical examination: at least blood pressure, heart rate, respiratory rate, temperature.
- Control blood culture, even if the patient remains febrile.
- Medication administration / dispensing control.
- Review of concomitant medication.
- Assessment of adverse events.

8.5.4 End-of-treatment visit (Day 7-14 after start of antibiotic treatment; may be by phone call or in-person)

To estimate duration of treatment, all days of active treatment received by the patient will be taken into account, including the 24 hours prior to randomisation.

- Clinical symptoms in relation to the clinical course of the patient.
- Physical examination: collecting blood pressure, heart rate, respiratory rate, temperature.
- Haemogram: leukocytes, neutrophils and platelets if the patient is admitted.
- Blood biochemistry: at least creatinine, urea, C-reactive protein if the patient is admitted.
- Blood culture: only if the previous blood culture was positive or symptoms persist.
- Rectal swab: only patients at selected centres.
- Medication administration / dispensing control.
- Review of concomitant medication.
- Assessment of adverse events.

8.5.5 Test-of-cure (ROC) visit (must be in-person)

To be performed between 3-5 days after completion of antibiotic treatment. This will also apply to patients with undrained abscesses, whose assessment will be performed no later than day 31-33 (28 +/- 3-5 days) after the start of appropriate antibiotic treatment.

- Clinical symptoms in relation to the clinical course of the patient.
- Physical examination: collecting blood pressure, heart rate, respiratory rate, temperature.

- Haemogram: leukocytes, neutrophils and platelet counts.
- Blood biochemistry: at least creatinine, urea, C-reactive protein.
- Rectal swab: only in-person visits and at selected centres.
- Medication administration / dispensing control.
- Review of concomitant medication.
- Assessment of adverse events.

8.5.6 Visit Day 30 ± 5 (after randomisation; may be by phone call or in-person)

- Clinical symptoms in relation to the clinical course of the patient.
- Physical examination: collecting blood pressure, heart rate, respiratory rate, temperature if the visit is in-person.
- Rectal smear: only in-person visits at selected centres.
- Review of concomitant medication.
- Assessment of adverse events.

8.5.7 Visit Day 60 ± 5 (after randomisation; can be by telephone or in-person)

- Clinical symptoms in relation to the clinical course of the patient.
- Physical examination, recording blood pressure, heart rate, respiratory rate, temperature if the visit is in-person.
- Review of concomitant medication.
- Assessment of adverse events.

9.- ASSESSMENT OF EFFECTIVENESS

9.1 Primary efficacy endpoint

The primary variable is clinical cure 3 to 5 days after the end of antibiotic treatment in the mITTP. For the estimation of treatment duration, all days of active treatment received by the patient will be taken into account, including the 24 hours prior to randomisation. This will also apply to patients with undrained abscesses, who will be assessed no later than day 31-33 from the start of appropriate antibiotic treatment; patients with drained abscesses beyond day 7 will be evaluated 7 days after drainage. Given that the primary outcome of the trial is not a hard variable and has a certain element of subjectivity, as does the methodology employed (non-blinded trial), a complementary assessment based on the variables collected at test-of-cure will be carried out by a blinded external investigator. This assessment will be performed at least twice: i) coinciding with the interim safety analysis and ii) prior to closure of the study database.

Clinical cure is defined as follows: survival at the time of assessment; disappearance

of all signs and symptoms of infection (or return to the situation prior to the current infection); and no need for modification of treatment due to an unfavourable clinical evolution directly related to the bacteraemia that led to inclusion in the trial.

9.2 Laboratory tests

Blood tests are envisaged at the initial visit and subsequently, as specified in the visit schedule. These tests will be performed locally at each centre in accordance with standard clinical practice. The following tests will be performed:

- Haemogram including leukocyte, neutrophil and platelet counts.
- Blood biochemistry including at least the determination of creatinine, urea, and C-reactive protein.
- Blood culture.

9.3 Microbiological studies

All clinical isolates will be kept frozen and sent to the laboratory of the Virgen Macarena University Hospital for antibiotic sensitivity testing. If transport to the laboratory will take more than 2 hours, the sample will be kept at 4ºC (in a refrigerator) until its arrival in the laboratory. Once the sample is received in the laboratory, it should be kept at -80ºC until it is sent to the reference laboratory (HU Virgen Macarena).

At certain centres (University Hospital (HU) Virgen Macarena, HU Puerta del Mar, HU Marqués de Valdecilla, Hospital Clínico Universitario Lozano Blesa, HU de La Princesa, HU La Paz, Complejo Asistencial Universitario de León, HU de Cruces and HU Donosita), specific samples will be taken to carry out the secondary objective associated with selection for MDR GNB. Rectal swab samples will be taken from patients in both treatment arms on the day of randomisation, at the test-of-cure visit, at the end of treatment, and at the visit on day 30, whenever feasible, to detect the presence of *P. aeruginosa* resistant to carbapenems or piperacillin/tazobactam, *Stenotrophomonas* spp., multidrug-resistant *A. baumannii* and ESBL-producing Enterobacterales, carbapenemases and chromosomal (hyperproducing) and plasmid-mediated AmpC. Rectal swabs will be stored at -80ºC.

Rectal swab samples will be sent (at room temperature) to the coordinating centre in accordance with the established timeline. For processing, they will be inoculated into Mac Conkey agar supplemented with 4 mg/L cefotaxime, chromogenic medium for detection of carbapenemase-producing Enterobacterales, and blood agar as a control sample.

After incubation for 48 hours in aerobiosis at 35°C ± 2°C, all morphotypes will be selected for subsequent identification by MALDI-TOF or standard biochemical tests, followed by characterisation of resistance mechanisms (presence of ESBLs, hyperproducing chromosomal or plasmid-mediated AmpC and carbapenemases). Phenotypic (double disk,

algorithm for detection of carbapenemases based on inhibition disks, synergy studies) and genotypic methods (PCR and sequencing) will be used on approximately 150 strains.

An analysis of clonal relationships among the corresponding clinical isolates will be carried out using pulsed field gel electrophoresis (PFGE).

Guidelines for the collection and processing of samples can be found in Annex II.

10.- SAFETY ASSESSMENT

10.1 Safety assessments

The following clinical assessments should be performed to evaluate the safety profile of the trial treatment.

10.1.1 Physical examination, vital signs

At each visit, a physical examination will be performed, including taking of vital signs.

10.1.2 Laboratory testing

Blood samples will be drawn for the haemogram (haemoglobin, leukocyte count, neutrophils and platelets) and biochemistry (including at least creatinine, urea and C-reactive protein). Microbiological tests will be performed to identify pathogenic strains.

10.2 Definitions

Adverse event (AE) means any untoward or unfavourable medical occurrence in a patient or clinical trial subject treated with a medicinal product, even if it does not have a causal relationship with that treatment.

An adverse event, therefore, can be any undesirable or unintended sign (including an abnormal laboratory finding), symptom, or disease that is associated temporally with the use of an investigational medicinal product, whether or not it is related to the investigational medicinal product.

Adverse reaction (AR): Any unintended harmful reaction to an investigational medicinal product, regardless of the dose administered.

In the case of an adverse reaction, unlike an adverse event, there is a suspected causal relationship between the investigational medicinal product and the adverse event.

Imputability criteria: The promoter will classify AEs based on their causal relationship with the medicinal product, as:

- **Definite**: there is a reasonable time relationship between administration of the drug and the occurrence of the AE. This event coincides with the ARs described for the drug, improves with drug withdrawal, reappears after re-administration and cannot be explained by alternative causes.
- **Probable**: there is a reasonable time relationship between the administration of the drug and the occurrence of the AE. The event coincides with the ARs described for the drug, improves after discontinuation of treatment and cannot be explained by other alternatives.
- **Possible**: there is a reasonable time relationship between the administration of the drug and the occurrence of the AE. The event coincides with the ARs described for the drug but can be explained by alternative causes.
- **Unlikely, conditional**: there is a reasonable time relationship between the administration of the drug and the occurrence of the AE. The event however does not coincide with the ARs described for the drug and can be explained by alternative causes.
- **Not related**: there is no reasonable time sequence between administration of the drug and the occurrence of the AE. The event does not coincide with the ARs described for the drug and can be explained by alternative causes.

To expedite reporting, adverse reactions classed as definite, probable and possible will be considered to be causally related; and the unlikely / conditional category will be considered to be not causally related.

Determining the possible relationship with the study treatment is the responsibility of the site Principal Investigator or the person designated by the PI.

Severity: Is defined as any **serious** adverse event or adverse reaction at any dose that:

- Causes the death of the patient
- Is life-threatening[1]
- Requires hospitalisation or prolongation of the patient's hospitalisation.
- Causes permanent or significant disability or incapacity
- Results in a congenital anomaly or malformation.

[1] The concept of "life-threatening" means that, in the opinion of the investigator, the patient at the time of the AE or AR is at real risk of death; it does not refer to the fact that the AE/AR could hypothetically have resulted in death if it been more severe.

10.3 Serious adverse events: reporting and collection

In the case of a serious adverse event (SAE), it must be reported to the Sponsor using the form designed for this purpose. A member of the investigation team will complete and sign the SAE notification form, which will be sent, by fax or e-mail (by the monitoring staff), immediately, and always within 24 hours of becoming aware of the event, to the:

Unidad de Investigación Clínica y Ensayos Clínicos
Hospital Universitario Virgen del Rocío
Avda. Manuel Siurot S/N
41013. Sevilla
Tel.: 955 01 34 14
Fax: 955095338

Staff at the unit will review the form received and, if necessary, request further information from the investigator. When further information on the SAE is obtained, or the SAE is resolved or unlikely to change, a follow-up report will be completed.

If there is suspicion that the SAE may be a Suspected Unexpected Serious Adverse Reaction (SUSAR), the investigator should provide follow-up information as requested by the sponsor.

The initial notification will be followed by detailed written reports. The sponsor will notify the *Agencia Española de Medicamentos y Productos Sanitarios* (AEMPS) of all suspected adverse reactions associated with investigational medicinal products that are both serious and unexpected. The maximum notification period will be 15 calendar days from the time the sponsor becomes aware of the suspected adverse reaction.

When the suspected and unexpected serious adverse reaction has caused the death of the subject or endangered his/her life, the sponsor will inform the AEMPS within a maximum of seven calendar days from the moment the sponsor becomes aware of the case. This information shall be completed, as far as possible, within the following eight days.

10.4 Notification to investigators

The sponsor must communicate to the investigators any information that may affect the safety of trial subjects as soon as possible.

11.- STATISTICS

11.1 Sample size determination

Epidat 4.0 software was used to calculate the sample size. The following parameters were used for the estimation: power: 80%; alpha error: 5%; estimated clinical cure rate in both groups: 85%; allocation ratio 1:1; accepted non-inferiority margin: 10%. The estimated cure rate was obtained from a recently published article by our group (Retamar P, SAEI/SAMPAC Bacteremia Group, et al; *AntimicrobAgentsChemother*2012; 56:472-8). Since this is a non-inferiority study, both groups were assigned the same value. Based on these assumptions, the sample size would be 326 cases (163 per group). It was decided to add an additional 5% to allow for possible losses due to lack of complete follow-up. Accordingly, the final sample size is 344 cases (172 per arm).

11.2 Statistical analysis

In a feasibility study carried out at participating centres during 2014, all had more than 10 episodes of Enterobacterales bacteraemia per month. Given the number of participating hospitals, this number ensures that the sample size will be achieved. The absolute difference in cure rates, with 95% confidence intervals, between patients in the experimental and control groups will be calculated. Multivariate logistic regression analyses will be performed for the primary outcome variable to ensure the independence of treatment effect, including centre. A superiority analysis will also be performed, based on a composite categorical variable made up of the following: survival at day 14, cure 3-5 days after completion of antibiotic treatment, days of treatment with an antipseudomonal beta-lactam avoided, and presence or absence of adverse effects. This variable will include the presence of any degree of *C. difficil*e infection, the presence of secondary infections with multidrug-resistant pathogens and the presence of adverse effects of any degree associated with antibiotic treatment. Following the methodology outlined in a recent publication in *Clinical Infectious Diseases* (Evans SR et al. *ClinInfectDis* 2015; 61:800-6), a superiority analysis of experimental treatment versus control will be conducted, using ordinal logistic regression. Since no specific dosing of the antibiotics under study will be used, a sensitivity analysis will be performed to adequately control for this aspect of the study.
Since the final number of patients with urinary focus bacteraemia will exceed the limit previously defined in an earlier version of the protocol (30% of the sample size), “focus of bacteraemia” will be included as a variable in multivariate analysis, and a sensitivity analysis will be performed on low- and high-risk foci.

11.3 Definitions of study populations for analysis

Intention-to-Treat (ITT) population: all randomised patients.

Modified Intention-to-Treat population (mITTP): patients who have received at least one intravenous dose of antibiotics. This population will include those patients whose treatment was modified due to the development of a second infection unrelated to the one that led to inclusion in the study, or whose treatment was prolonged beyond day 14 (or beyond day 28 for undrained abscesses) without sufficient clinical justification.

Clinically evaluable population (CEP): patients who have completed 5 days of intravenous treatment after randomisation (or who die earlier having received at least one dose of intravenous antibiotic) and a total treatment duration of at least 7 days.

Clinically and microbiologically evaluable population (CMEP): as above, plus at least one blood culture was performed after 48 hours after randomisation.

11.4 Independent Review Board

To mitigate possible bias deriving from the open-label design of the trial, the evaluation of results will be performed by an independent committee blinded to treatment allocation. This committee will be formed of 3 expert investigators belonging to the *Red Española de Investigación en Patología Infecciosa* (REIPI) [Spanish Network for Research in Infectious Diseases] and will reach its conclusions by consensus.

A preliminary analysis (interim analysis) will be performed when 50% of the planned patients have been included and monitored to ensure that there are no safety or efficacy reasons for discontinuing the trial.

12.- ETHICAL ASPECTS

The trial will be conducted in accordance with the principles of the Declaration of Helsinki, and according to current legal regulations (*Real Decreto* 1090/2015) and will not be initiated until the approval of the reference Clinical Research Ethics Committee, the agreement of the Directors of the Institutions, and the authorisation of the Spanish Agency of Medicines and Health Products] has been obtained.

The investigator must comply with all requirements of the protocol. If a situation arises in which a temporary deviation from the protocol is required, the investigator or other physician responsible for the patient should contact the monitor as soon as possible to discuss the situation and agree on an appropriate course of action. The investigator will document the deviation from the protocol and the circumstances that prompted the deviation.

12.1 Informed consent

The patient should give consent before being admitted to the clinical trial. The physician should clearly explain the nature, purpose and possible consequences of the clinical trial in a way that is understandable to the patient. The information provided by the physician should also be recorded. In obtaining and documenting consent, investigators will comply with the relevant legislation (Royal Decree 1090/2015), the standards of Good Clinical Practice and the ethical principles derived from the Declaration of Helsinki.

The study subject will give consent by signing the form provided for this purpose. The investigator will receive an appropriate number of informed consent forms from the sponsor; each form should be signed by investigator and patient.

The investigator will not initiate any research pertaining to the trial until the investigator has obtained the patient's consent.

12.2 Data protection

The processing, communication and transfer of the personal data of all participating subjects shall comply with the provisions of Organic Law 15/1999 of 13 December 1999 on the Protection of Personal Data. In accordance with the provisions of the aforementioned legislation, patients may exercise their rights of access, modification, opposition to and deletion of data by contacting their study doctor.

The anonymity of the subjects participating in the study will be maintained at all times. The data collected for the study will be identified by a code and only the investigator and collaborators will be able to associate these data with the patient and their medical history.

The patient's identity will not be revealed to any person, with the following exceptions: i) personnel authorised by the sponsor, when necessary, to check study data and procedures, but always maintaining patient confidentiality in accordance with current legislation; ii) in the event of a medical emergency or legal requirement (Health Authorities: Spanish Agency of Medicines and Health Products, and Local Clinical Trials Committee).

The data from this study will only be used for the specific purposes of the study.

12.3 Monitoring and auditing

The study will be monitored through local visits, telephone calls and periodic inspection of case report forms (CRFs) with sufficient frequency to check the following:

- Rate of patient inclusion, compliance with protocol procedure standards, completeness and accuracy of data entered into data collection notebooks, verification against original documents, occurrence of adverse events.

- Monitoring visits will be conducted by study monitors. It is intended that the monitors will have access to patient records at the investigator's request. The investigator will devote sufficient time to these visits and will grant authorised persons access to all documentation.

- The trial may be independently audited. Members of the Clinical Research Ethics Committee may also supervise the trial.

12.4 Premature termination or discontinuation of the trial

If the trial is prematurely terminated or suspended, the sponsor should promptly inform the investigator and the regulatory authority(ies) of the termination or suspension, and the reason for its termination or suspension. The sponsor or investigator should promptly inform the IRB/IEC, providing it with the reason for termination or suspension as specified in the relevant regulatory requirement(s).

13.- FUNDING AND INSURANCE

13.1 Funding

The project has received funding through the public call for Strategic Action in Health of the Instituto de Salud Carlos III 2015.

13.2 Insurance

The promoter has taken out a civil liability insurance policy in accordance with the requirements specified in the RD 1090/2015.

14.- PUBLICATION POLICY

Publications will be in accordance with the provisions of Royal Decree 1090/2015 of December 4, which regulates clinical trials with drugs; the Ethics Committees for Research with drugs and the Spanish Registry of Clinical Studies, article 42, which contains the following text:

1. The sponsor is obliged to publish the results, both positive and negative, of the authorized clinical trials, preferably in scientific journals before being disclosed to the non-healthcare public, regardless of the obligations to publish the results report in the Spanish Registry of clinical studies (REec) and the provisions in this regard in Regulation (EU) No. 536/2014 of the European Parliament and of the Council of April 16, 2014.

2. *When studies and research papers on medicines aimed at the scientific community are made public, the funds obtained by the author, by or for their realization, and the source of funding will be stated.*

3. The anonymity of the subjects participating in the trial will be maintained at all times.

4. *Treatments whose efficacy has not yet been determined will not be made known in a premature or sensational way, nor will it [efficacy] be exaggerated. Interim results that may compromise the reliability of the final results of the trial will not be publicised.*

5. The advertising of medicines for human use in research is strictly prohibited, as established in the consolidated text of the Law on guarantees and rational use of medicines and health products, in Royal Decree 1416/1994, of June 25 , which regulates the advertising of medicines for human use, in Royal Decree 1907/1996, of August 2, on advertising and commercial promotion of products, activities or services with an alleged health purpose, and in Law 34/ 1988, of November

11, General Publicity.

6. In all cases, to make public the general results of the investigations, once completed, the guidelines of the European Commission and, where appropriate, the instructions of the Spanish Agency of Medicines and Health Products will be followed.

7. When a substudy of a clinical trial ends later than the rest of the trial, it will be necessary for the summary of its results to be published in the year following its completion, without delaying the reporting of the results of the rest of the trial."

ANNEX I. BIBLIOGRAPHY

1. Garnacho-Montero J, et al. Impact of adequate empirical antibiotic therapy on the outcome of patients admitted to the intensive care unit with sepsis. CriticalCare Medicine **2003**; 31:2742-51.
2. Vincent JL, et al. Sepsis in European intensive care units: results of the SOAP study. Crit Care Med **2006**; 34:344 -353.
3. Rodríguez-Baño J, et al. Epidemiology and clinical features of community-acquired, healthcare-associated and nosocomial bloodstream infections in tertiary-care and community hospitals. ClinMicrobiolInfect**2010**; 16:1408-13.
4. Retamar P, et al; SAEI/SAMPAC. Impact of inadequate empirical therapy on the mortality of patients with bloodstream infections: a propensity score-based analysis. AntimicrobAgentsChemother**2012**; 56:472.
5. Al-Hasan MN, et al. Impact of healthcare-associated acquisition on community-onset Gram-negative bloodstream infection: a population-based study: healthcare-associated Gram-negative BSI. Eur J ClinMicrobiol Infect Dis **2012**; 31:1163-71.
6. Schechner V, et al. Gram-negative bacteremia upon hospital admission: when should *Pseudomonas aeruginosa* be suspected?. Clin Infect Dis **2009**; 48:580.
7. Rodríguez-Baño J, et al. β-Lactam/β-lactam inhibitor combinations for the treatment of bacteremia due to extended-spectrum β- lactamase-producing Escherichia coli: a post hoc analysis of prospective cohorts. ClinInfectDis**2012**; 54:167-74.
8. Tumbarello M, et al. Predictive models for identification of hospitalized patients harboring KPC-producing *Klebsiella pneumoniae*. Antimicrob Agents Chemother**2014**; 58:3514-3520.
9. Khasawneh FA, et al. Antibiotic de-escalation in bacteremic urinary tract infections: potential opportunities and effect on outcome. Infection. **2014**; 42:829-34.
10. Heenen S, et al. Antibiotic strategies in severe nosocomial sepsis: why do we not de-escalate more often? Crit Care Med **2012**; 40:1404-9.
11. Bal AM, et al. Antibiotic stewardship: overcoming implementation barriers. CurrOpin Infect Dis **2011**; 24:357-62.
12. De Waele JJ, et al. De-escalation after empirical meropenem treatment in the intensive care unit: fiction or reality? J CritCare**2010**; 25:641-6.
13. Paterson DL, et al. Empirical antibiotic choice for the seriously ill patient: are minimization of selection of resistant organisms and maximization of individual outcome mutually exclusive? Clin Infect Dis **2003**; 36:1006-12.
14. Martínez-Martínez L, et al. Development of resistances to antibiotic drugs: causes,

consequences and importance to the public health system. EnfermInfeccMicrobiolClin**2010**; 28 Suppl 4:4-9.

15. Disponible en: http://www.ecdc.europa.eu/en/publications/Publications/0909_TER_The_Bacterial_Challenge_Time_to_React.pdf.
16. Livermore DL. Has the era of untreatable infections arrived?. J AntimicrobChemother**2009**; 64 Suppl 1:i29 -36.
17. Rodríguez-Baño J, et al. Programs for optimizing the use of antibiotics (PROA) in Spanish hospitals: GEIH-SEIMC, SEFH and SEMPSPH consensus document. EnfermInfeccMicrobiolClin**2012**; 30:22.e1-22.e23.
18. Mosier MJ, et al. Making the case for de-escalation therapy in ventilator-associated pneumonia once again. Crit Care Med **2013**; 41:1810-1.
19. Montravers P, et al. De-escalation: in drug we trust. Crit Care Med **2012**; 40:1645-6.
20. KayeKS. Antimicrobial de-escalation strategies in hospitalized patients with pneumonia, intra-abdominal infections, and bacteremia. J HospMed**2012**; 7 Suppl1:S13-21.
21. Garnacho-Montero J, et al. De-escalation of empirical therapy is associated with lower mortality in patients with severe sepsisand septic shock. IntensiveCareMed**2014**; 40:32-40.
22. Mokart D, et al. De-escalation of antimicrobial treatment in neutropenic patients with severe sepsis: results from an observational study. Intensive Care Med **2014**; 40:41-9.
23. Shime N, et al. De-escalation of antimicrobial therapy for bacteraemia due to difficult-to-treat Gram-negative bacilli. Infection**2013**; 41:203-10.
24. Shime N, et al. De-escalation of antimicrobials in the treatment of bacteraemia due to antibiotic-sensitive pathogens inimmuno competent patients. Infection **2011**; 39:319-25.
25. Morel J, et al. De-escalation as part of a global strategy of empiric antibiotherapy management. A retrospective study in a medico-surgical intensive care unit. Crit Care **2010**;14:R225.
26. DiNubile MJ, et al. Acquisition of resistant bowel flora during a double-blind randomized clinical trial of ertapenem versus piperacillin-tazobactam therapy for intraabdominal infections. Antimicrob Agents Chemother**2005**; 49:3217-21.1.
27. Rice LB. The Maxwell Finland Lecture: for the duration-rational antibiotic administration in an era of antimicrobial resistance and clostridium difficile. Clin Infect Dis **2008**; 46:491-6.
28. Silva BN, et al. De-escalation of antimicrobial treatment for adults with sepsis, severe sepsis or septic shock. Cochrane DatabaseSystRev**2013**; 3:CD007934.
29. Disponible en: https://clinicaltrials.gov/ct2/show/NCT01626612. (Consultado el 7 de Marzo de 2015).
30. Disponible en: https://clinicaltrials.gov/ct2/show/NCT01066013. (Consultado el 7 de Marzo de 2015).

CONSORT

Figure 2

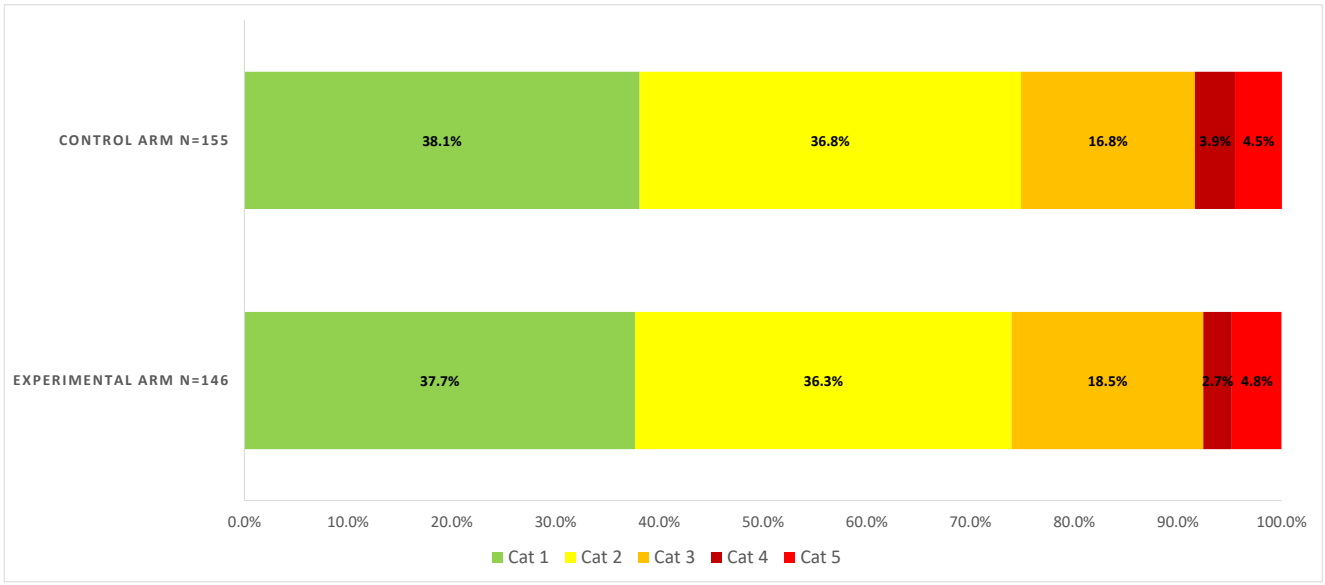

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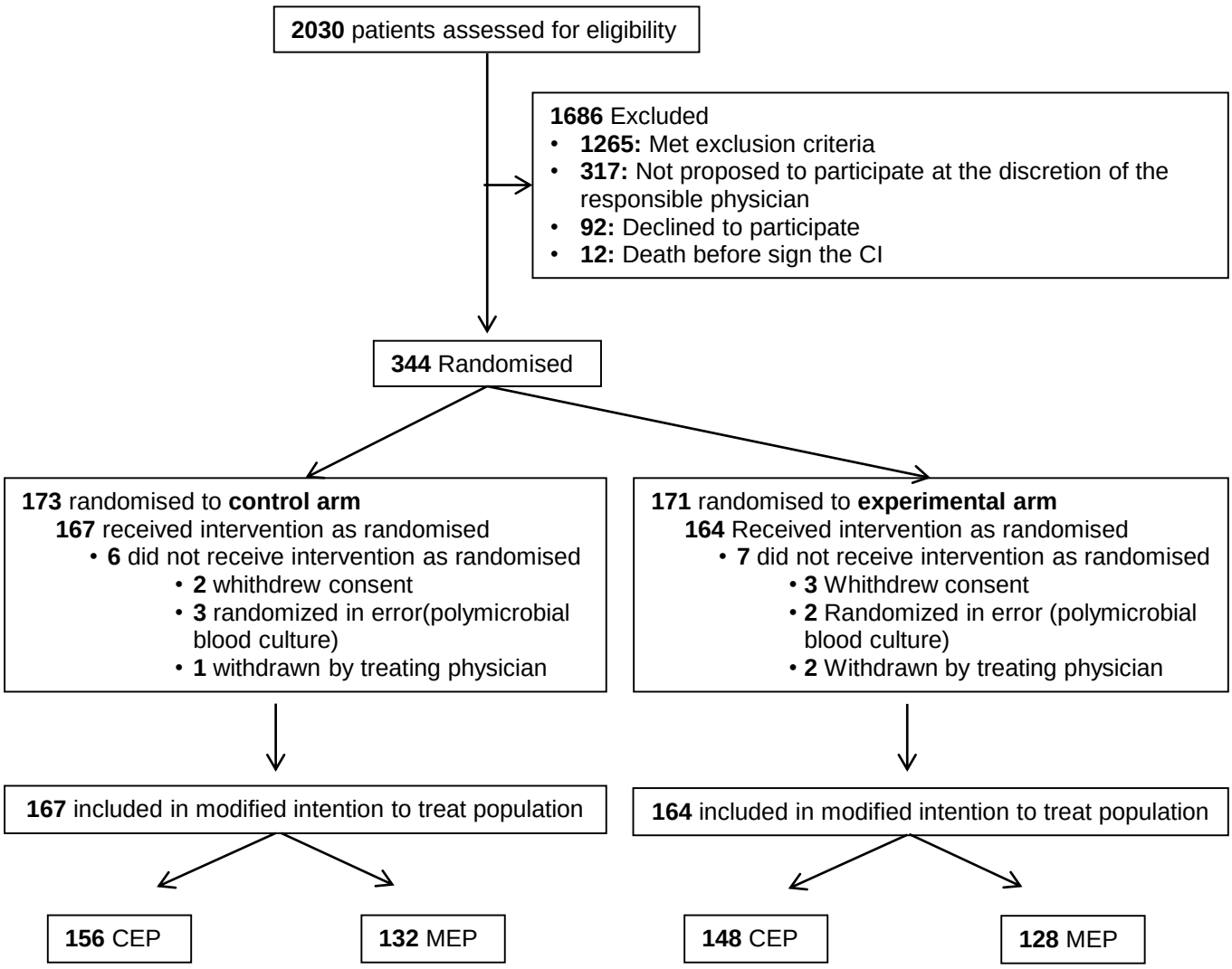
Figure 1

Figure 1.



CEP: all patients evaluated at TOC who had completed at least 5 days of intravenous therapy. MEP: those patients in the CEP who had at least one follow-up blood culture taken ≥48 hours after randomization

Appendix

Authors table

First and middle names	Surnames
Luis Eduardo	López Cortés
Mercedes	Delgado-Valverde
Elisa	Moreno-Mellado
Josune	Goikoetxea Aguirre
Laura	Guio Carrión
María José	Blanco Vidal
Leyre Mónica	López Soria
María Teresa	Pérez- Rodríguez
Lucía	Martínez Lamas
Francisco	Arnaiz de las Revillas
Carlos	Armiñanzas
Carlos	Ruiz de Alegría-Puig
Patricia	Jiménez Aguilar
María del Carmen	Martínez-Rubio
Carmen	Sáez-Bejar
Carmen	de las Cuevas
Andrés	Martín-Aspas
Fátima	Galán
José	Ramón Yuste
José	José Leiva-León
Germán	Germán Bou
Patricia	Capón González
Lucía	Boix-Palop
Mariona	Xercavins-Valls
Miguel Ángel	Goenaga-Sánchez
Diego	Vicente Anza
Juan José	Castón
Manuel	Recio Rufián
Esperanza	Merino
Juan Carlos	Rodríguez
Belén	Loeches
Guillermo	Cuervo
José Manuel	Guerra Laso
Antonio	Plata
Salvador	Pérez Cortés
Pablo	López Mato
José Luis	Sierra Monzón
Clara	Rosso-Fernández
José María	Bravo-Ferrer
Pilar	Retamar-Gentil
Jesús	Rodríguez Baño