



Depósito de investigación de la Universidad de Sevilla

<https://idus.us.es/>

Esta es la versión aceptada del artículo publicado en Oxford Academic

This is a accepted manuscript of a paper published in Oxford Academic

Clinical Infectious Diseases (2019): 15 September of 2019

**DOI:** [https://doi.org/ 10.1093/cid/ciy1032](https://doi.org/10.1093/cid/ciy1032)

**Copyright:** The Author(s) 2018. Published by Oxford University Press for the Infectious Diseases Society of America. All rights reserved.

El acceso a la versión publicada del artículo puede requerir la suscripción de la revista.

Access to the published version may require subscription.

“This is a pre-copyedited, author-produced version of an article accepted for publication in [Clinical Infectious Diseases] following peer review. The version of record [Clinical Infectious Diseases, *Volume 69, Issue 6*, 15 September 2019, Pages 956–962, <https://doi.org/10.1093/cid/ciy1032> ] is available online at: Oxford Academic [<https://academic.oup.com/ajcp/article/152/4/446/5532317> and DOI <https://doi.org/10.1093/cid/ciy1032> ]”

# Clinical Infectious Diseases

## Impact of de-escalation on prognosis of patients with bacteraemia due to Enterobacteriaceae: a post-hoc analysis from a multicenter prospective cohort --Manuscript Draft--

<b>Manuscript Number:</b>	CID-91871R1
<b>Full Title:</b>	Impact of de-escalation on prognosis of patients with bacteraemia due to Enterobacteriaceae: a post-hoc analysis from a multicenter prospective cohort
<b>Short Title:</b>	De-escalation in Enterobacteriaceae
<b>Article Type:</b>	Major Article
<b>Corresponding Author:</b>	Jesus Rodriguez-Bano, PhD, MD Hospital Universitario Virgen Macarena Sevilla, SPAIN
<b>Corresponding Author Secondary Information:</b>	
<b>Corresponding Author's Institution:</b>	Hospital Universitario Virgen Macarena
<b>Corresponding Author's Secondary Institution:</b>	
<b>First Author:</b>	Zaira Raquel Palacios Baena
<b>First Author Secondary Information:</b>	
<b>Order of Authors:</b>	Zaira Raquel Palacios Baena Mercedes Delgado Valverde Adoración Valiente Méndez Benito Almirante Silvia Gómez Zorrilla Nuria borrell Juan E Corzo Mercedes Gurguí Cristina de la Calle Lara García Álvarez Lucía Ramos Mónica Gozalo M. Isabel Morosini José Molina Manuel Causse Álvaro Pascual Jesus Rodriguez-Bano, PhD, MD
<b>Order of Authors Secondary Information:</b>	
<b>Manuscript Region of Origin:</b>	SPAIN
<b>Abstract:</b>	Background: More data are needed about the safety of antibiotic de-escalation in specific clinical situations as a strategy to reduce exposure to broad-spectrum antibiotics. The aims of this study were to investigate predictors of de-escalation and its impact on the outcome of patients with bloodstream infection due to Enterobacteriaceae (BSI-E).

	<p>Methods: A post-hoc analysis was performed of a prospective, multicenter cohort of patients with BSI-E initially treated with ertapenem or antipseudomonal <math>\beta</math>-lactams. Logistic regression was used to analyze factors associated with early de-escalation (EDE) and Cox regression for the impact of EDE and late de-escalation (LDE) on 30-day all-cause mortality. A propensity score (PS) for EDE vs. no de-escalation (NDE) was calculated. Failure at end of treatment and length of hospital stay were also analyzed.</p> <p>Results: Overall, 516 patients were included; EDE was performed in 241 patients (46%), LDE in 95 (18%) and NDE in 180 (35%). Variables independently associated with a lower probability of EDE were multidrug-resistant isolates (OR 0.50, 95% CI 0.30-0.83) and nosocomial infection empirically treated with imipenem or meropenem (OR 0.35, 95% CI 0.14-0.87). After controlling for confounders, EDE was not associated with increased risk of mortality; Hazard ratios (HR) and (95% CI) were: general model, 0.58 (0.25-1.31), model with PS, 0.69 (0.29-1.65), and PS-matched pairs, 0.98 (0.76-1.26). LDE was not associated with mortality. De-escalation was not associated with clinical failure or length of hospital stay.</p> <p>Conclusions: De-escalation in patients with monomicrobial bacteraemia due to Enterobacteriaceae was not associated with a detrimental impact on clinical outcome.</p>
<p><b>Response to Reviewers:</b></p>	<p>Sevilla, 18 November 2018</p> <p>Dear Dr. Paterson,</p> <p>We are submitting the revised version of the manuscript entitled "Impact of de-escalation on prognosis of patients with bacteraemia due to Enterobacteriaceae: a post-hoc analysis from a multicenter prospective cohort" (manuscript ID: CID-91871) according to your and the reviewers' comments and suggestions. We thank you and the reviewers for their kind and useful comments which have been of help to improve the paper.</p> <p>Please find below our responses to all the comments.</p> <p>Jesús Rodríguez-Baño On behalf of all authors</p> <p>REVIEWER COMMENTS</p> <p>Reviewer #1: This manuscript provides a post-hoc analysis of the effect of de-escalation on the outcome of bacteremic patients who were infected with a single species of Enterobacteriaceae. The conclusion that de-escalation, either early or late, does not affect mortality is important, in that this is the largest study documenting these kinds of data. Although this is a retrospective study and not a randomized, control trial, the data appear to be valid with appropriate statistical analyses. A few minor suggestions have been provided for the consideration of the authors:</p> <p>1.Line 140. Define BSD: Response: please note that BSD is already defined in line 100 (BSD=broad-spectrum drugs).</p> <p>2.Lines 140-142. The phrase "or ertapenem" is confusing as it stands. It appears that this is in the list of antipseudomonal drugs. Perhaps "ertapenem" could be the first drug mentioned followed by the "antipseudomonal beta-lactams such as.." Response: thank you for your comment. We changed this as suggested in line 140, and also in line 285 and in Summary of the main points.</p> <p>3.Line 180. Please provide further details or a reference for your "phenotypic methods". Response to the reviewer: We used the EUCAST recommendations; we added this information and the appropriate reference.</p> <p>4.Line 263. The statement that "a urinary or biliary source were protective factors" needs further explanation - they were associated with 40% of the failures. Response to the reviewer: The reviewer is right: 40% of failures occurred in patients with a urinary or biliary tract source (therefore 60% of failures occurred in patients with</p>

other sources). The protective association of urinary and biliary tract with failure was significant and was confirmed in the multivariate analysis. Please note that the proportion of failures in the whole series was low (6.7%), and in patients with urinary or biliary tract source was 3.7% while for other sources it was 14.3%. If the low rate of failures is not considered, a false impression that failures in such sources was frequent. We added the data for failure rates to avoid such impression.

Reviewer #2:

In this study, the authors evaluated variables associated with de-escalation, and the impact of de-escalation on prognosis among patients with monomicrobial bacteraemia due to Enterobacterales who received early active empirical monotherapy with antipseudomonal beta-lactams or ertapenem at 13 University hospitals in Spain. They concluded that overall, de-escalation in patients with monomicrobial bacteraemia due to Enterobacteriaceae was not associated with detrimental impact on clinical outcome. Such studies are of clear interest, as they support stewardship activities, which are very important to restrict resistance selection and ultimately the ever increasing incidence of MDR/XDR/PDR infections. There are some limitations, clearly admitted by the authors, but the very large number of cases studied is a strong advantage and the analysis is appropriate, making the manuscript of interest for the literature. I have some suggestions:

1. As the authors performed detailed (phenotypic and molecular) bacteriological analysis of the isolates in a single central laboratory, they could present some data on the resistance patterns (with MICs) and mechanisms of the monomicrobial pathogens of the BSIs, per bacterial species.

Response: We added this information in a new supplementary Table (Table S2).

2. The resistance patterns of the community vs hospital Enterobacterales in the study hospitals or, if not available for all hospitals, for Spain overall, would be of interest for the readers.

Response: We provide the susceptibility data as requested in a supplementary Table (Table S7).

3. It would be very informative if the authors could also present, further to the existing analysis as a total (to retain the very large number of cases), their results separately for nosocomial/healthcare-associated vs. community BSIs, as nosocomial/healthcare-associated infections are more complicated, as stated also in the text.

Response: We performed a stratified analysis for nosocomial and non-nosocomial cases as suggested and provided the results of the estimations of the effect of de-escalation on mortality in the text.

The Editor, *Clinical Infectious Diseases*

Sevilla, Spain, September 18<sup>th</sup>, 2018

Dear Dr. Schooley,

We are submitting our manuscript entitled: **“Impact of de-escalation on prognosis of patients with bacteraemia due to Enterobacteriaceae: a post-hoc analysis from a multicenter prospective cohort”** to be considered for publication in *Clinical Infectious Diseases*.

This paper is the result of a big multicenter, prospective cohort study investigating the the safety of de-escalation strategy in patients with bacteremia due to Enterobacteriaceae. De-escalation is consider by most infectious diseases specialist as a safe and appropriate strategy for better use of antibiotics; however, it is performed much less that desirable due to different reasons, including scarcity of well-performed studies on its safety. One of the problems of previous studies is the inclusion of different aspects of de-escalation, such as stopping redundant drugs or combination regimens. Our objective was to analyze de-escalation in a specific situation, bacteremia due to Enterobacteriaceae treated empirically with an anti-pseudomonal beta-lactam or ertapenem in monotherapy. We hope our results will reinforce the practice of de-escalation in this situation.

The paper only includes original results, have not been submitted elsewhere and have been seen and approved by all authors.

Sincerely,

Jesús Rodríguez-Baño, on behalf of all authors

1 **Impact of de-escalation on prognosis of patients with bacteraemia due to**

2 **Enterobacteriaceae: a post-hoc analysis from a multicenter prospective cohort**

3

4 **Authors:** Zaira R. (ZRPB) Palacios-Baena<sup>1</sup>, Mercedes (MDV) Delgado-Valverde<sup>1</sup>,  
5 Adoración (AVM) Valiente Méndez<sup>1</sup>, Benito (BA) Almirante<sup>2</sup>, Silvia (SGZ) Gómez-  
6 Zorrilla<sup>3</sup>, Núria (NB) Borrell<sup>4</sup>, Juan E. (JEC) Corzo<sup>5</sup>, Mercedes (MG) Gurguí<sup>6</sup>, Cristina  
7 (CC) de la Calle<sup>7</sup>, Lara (LGA) García-Álvarez<sup>8</sup>, Lucía (LR) Ramos<sup>9</sup>, Mónica (MG)  
8 Gozalo<sup>10</sup>, María Isabel (MIM) Morosini<sup>11</sup>, José (JM) Molina<sup>12</sup>, Manuel (MC) Causse<sup>13</sup>,  
9 Álvaro (AP) Pascual<sup>1</sup>, Jesús (JRB) Rodríguez-Baño<sup>1,\*</sup> on behalf of the REIPI/GEIRAS-  
10 SEIMC BACTERIEMIA-MIC group.

11

12 <sup>1</sup>Unidad Clínica de Enfermedades Infecciosas, Microbiología y Medicina Preventiva,  
13 Hospital Universitario Virgen Macarena/Departamento de Medicina y Microbiología,  
14 Universidad de Sevilla/Instituto de Biomedicina de Sevilla (IBiS). Sevilla, Spain.

15 <sup>2</sup>Hospital Universitari Vall d'Hebron. Barcelona, España.

16 <sup>3</sup>Hospital de Bellvitge. Barcelona, Spain.

17 <sup>4</sup>Hospital Universitario Son Espases. Palma de Mallorca, Islas Baleares, España.

18 <sup>5</sup>Unidad Clínica Enfermedades Infecciosas y Microbiología. Hospital Universitario  
19 Virgen de Valme. Sevilla, Spain.

20 <sup>6</sup>Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

21 <sup>7</sup>Hospital Clinic i Provincial, Barcelona, Spain.

22 <sup>8</sup>Departamento de Enfermedades Infecciosas. Hospital San Pedro-CIBIR. Logroño,  
23 Spain.

24 <sup>9</sup>Hospital Universitario A Coruña. A Coruña, Spain.

25 <sup>10</sup>Hospital Marqués de Valdecilla-IDIVAL, Santander. Spain.

26 <sup>11</sup>Hospital Ramón y Cajal. Madrid, Spain.

27 <sup>12</sup>Unidad Clínica de Enfermedades Infecciosas, Microbiología y Medicina Preventiva,  
28 Hospital Universitario Virgen del Rocío/Universidad de Sevilla/Instituto de  
29 Biomedicina de Sevilla (IBIS). Seville, Spain.

30 <sup>13</sup>Unidad de Gestión Clínica de Microbiología. Hospital Universitario Reina Sofía.  
31 Instituto Maimónides de Investigación Clínica (IMIBIC), Universidad de Córdoba.  
32 Córdoba, Spain.

33

34

35 **\*Corresponding author:** Jesús Rodríguez-Baño. Unidad Clínica de Enfermedades  
36 Infecciosas, Microbiología y Medicina Preventiva, Hospital Universitario Virgen  
37 Macarena, Avda Dr. Fedriani, 3, 41009 Sevilla, Spain. Phone: +34 677906512. E-mail  
38 address: [jesusrb@us.es](mailto:jesusrb@us.es)

39

40 **Running title:** De-escalation in Enterobacteriaceae bacteraemia.

41

## 42 **SUMMARY OF THE MAIN POINTS**

43 De-escalation from empirical antipseudomonal  $\beta$ -lactams or ertapenem to lower  
44 spectrum antibiotics in patients with bacteremia due to Enterobacteriaceae was not  
45 associated with any detrimental impact in terms of mortality, clinical failure or length of  
46 hospital stay

47

48

49

50

51 **ABSTRACT**

52 **Background:** More data are needed about the safety of antibiotic de-escalation in  
53 specific clinical situations as a strategy to reduce exposure to broad-spectrum  
54 antibiotics. The aims of this study were to investigate predictors of de-escalation and its  
55 impact on the outcome of patients with bloodstream infection due to Enterobacteriaceae  
56 (BSI-E).

57 **Methods:** A post-hoc analysis was performed of a prospective, multicenter cohort of  
58 patients with BSI-E initially treated with ertapenem or antipseudomonal  $\beta$ -lactams.  
59 Logistic regression was used to analyze factors associated with early de-escalation  
60 (EDE) and Cox regression for the impact of EDE and late de-escalation (LDE) on 30-  
61 day all-cause mortality. A propensity score (PS) for EDE vs. no de-escalation (NDE)  
62 was calculated. Failure at end of treatment and length of hospital stay were also  
63 analyzed.

64 **Results:** Overall, 516 patients were included; EDE was performed in 241 patients  
65 (46%), LDE in 98 (18%) and NDE in 180 (35%). Variables independently associated  
66 with a lower probability of EDE were multidrug-resistant isolates (OR 0.50, 95% CI  
67 0.30-0.83) and nosocomial infection empirically treated with imipenem or meropenem  
68 (OR 0.35, 95% CI 0.14-0.87). After controlling for confounders, EDE was not  
69 associated with increased risk of mortality; Hazard ratios (HR) and (95% CI) were:  
70 general model, 0.58 (0.25-1.31), model with PS, 0.69 (0.29-1.65), and PS-matched  
71 pairs, 0.98 (0.76-1.26). LDE was not associated with mortality. De-escalation was not  
72 associated with clinical failure or length of hospital stay.

73 **Conclusions:** De-escalation in patients with monomicrobial bacteraemia due to  
74 Enterobacteriaceae was not associated with a detrimental impact on clinical outcome.



75 **Keywords:** De-escalation, streamlining, Enterobacteriaceae, bloodstream infections,

76 mortality.

77

78 **INTRODUCTION**

79

80 Patients with sepsis are frequently treated empirically with broad-spectrum  
81 drugs (BSD) because the early administration of active drugs has been associated with  
82 improved outcome, particularly in the presence of septic shock [1]. This can lead to  
83 overuse of these drugs, which is usually considered to be one of the contributing factors  
84 for the spread of multidrug-resistant (MDR) bacteria [2]. To minimize this problem,  
85 streamlining or de-escalation from broad- to narrower-spectrum drugs is usually  
86 advocated once the susceptibility of the causative agent of the infection is known, and  
87 antimicrobial stewardship programs frequently include interventions facilitating or  
88 recommending this practice [3]. However, de-escalation is performed less frequently  
89 than is desirable. Barriers include uncertainty among many prescribers; indeed,  
90 although de-escalation is considered standard of care for most infectious diseases  
91 specialists, a recent systematic review concluded that there is no adequate evidence as  
92 to whether de-escalation of antimicrobial agents is effective and safe for adults with  
93 sepsis [4]. Hence, providing more information about the safety of de-escalation would  
94 help increase implementation, and knowledge of the variables influencing the  
95 performance of de-escalation would lead to better targeting of interventions promoting  
96 this practice.

97 Bloodstream infections (BSI) are an ideal model for de-escalation, since etiology  
98 and susceptibility are known, and a more specialized evaluation of patients is possible  
99 [5]. A meta-analysis including studies of sepsis, bacteraemia and pneumonia found a  
100 trend towards higher mortality with de-escalation in 3 randomized trials, but lower  
101 mortality in observational studies [6]. However, the studies were heterogeneous with  
102 respect to type of patient and infection, etiology, definitions used and interventions,

103 which precludes high confidence in the meta-analytic estimates. Studies of specific  
104 populations and etiologies are needed therefore. A randomized trial of patients with  
105 bacteraemia due to Enterobacteriaceae is now recruiting [7], although the results will  
106 not be available for 2 years. The objectives of this study were to evaluate the frequency  
107 of variables associated with de-escalation, and the impact of de-escalation on prognosis  
108 only among patients with bacteraemia due to Enterobacteriaceae.

109

## 110 **METHODS**

111

### 112 **Study design, sites and study population**

113 This is a post-hoc analysis of the prospective Bacteraemia-MIC cohort, which  
114 included BSI episodes due to Enterobacteriaceae at 13 University hospitals in Spain.  
115 The methods are detailed in previous reports [8, 9]. Briefly, consecutive adult patients  
116 with monomicrobial bacteraemia due to Enterobacteriaceae who received empirical  
117 treatment in the first 12 hours after the blood cultures were drawn were included. The  
118 original study was conducted between January 2011 and December 2013. Exclusion  
119 criteria were polymicrobial bacteraemia, non-hospitalized patients, do-not resuscitate  
120 orders, neutropenia ( $<500/\mu\text{L}$ ) and survival  $<24$  hours after blood cultures were drawn.  
121 For this analysis, patients from the Bacteraemia-MIC cohort were selected if: (1) initial  
122 treatment was monotherapy with an in vitro active BSD, including antipseudomonal  $\beta$ -  
123 lactams such as meropenem, imipenem, doripenem, ceftazidime, cefepime or  
124 piperacillin/tazobactam, or ertapenem; and (2) the causative microorganism was  
125 susceptible to any of the following narrower-spectrum drugs (NSD): ampicillin,  
126 amoxicillin/clavulanic acid, non-antipseudomonal cephalosporins such as cefazolin,  
127 cefuroxime, cefotaxime or ceftriaxone, trimethoprim/sulfamethoxazole,

128 aminoglycosides, fosfomycin and fluoroquinolones. The classification of antibiotics as  
129 BSD or NSD was based on a previously published consensus ranking of  $\beta$ -lactams  
130 according to spectrum and resistance-promoting potential [10]. Exclusion criteria were  
131 treatment change to another broader-spectrum drug between days 2 and 5 (as we were  
132 unable to rule out patients having secondary infections that would overestimate the  
133 comparative efficacy of NSD), and death before the susceptibility tests were available  
134 (since these patients did not have the opportunity to de-escalate). All patients were  
135 followed for 30 days.

136 The Institutional Review Board of the University Hospital Virgen Macarena,  
137 Seville, Spain, approved the study and waived the need to obtain informed consent due  
138 to the observational nature of the study. This analysis was reported according to  
139 STROBE recommendations (Supplementary Table S1) [11].

140

#### 141 **Variables and definitions**

142 The main outcome variable was 30-day all-cause mortality. Secondary outcomes  
143 were: clinical response at day 21, and length of hospital stay among survivors. Clinical  
144 response was classified as clinical cure if all signs and symptoms of infection had been  
145 completely resolved, and failure if there were any persistent, recurrent or new signs and  
146 symptoms related to infection, or if death occurred.

147 The main exposure of interest was de-escalation, defined as switching from the  
148 empirical BSD to any of the NSDs, or from piperacillin-tazobactam, imipenem or  
149 meropenem to ertapenem. De-escalation was classified as early de-escalation (EDE) if  
150 performed in  $\leq 4$  days (the day when blood cultures were drawn was considered day 0),  
151 late de-escalation (LDE) it was from day 5 to day 7, or non-de-escalation (NDE) if the  
152 empirical drug was continued for at least  $\geq 7$  days.

153 Other exposure variables included demographic data, type of onset of infection  
154 (nosocomial, healthcare-associated or community), chronic underlying conditions and  
155 severity according to the Charlson index [12], acute severity of underlying condition  
156 according to Pitt score [13] measured on day -1, SOFA score measured on day 0 [14],  
157 severe sepsis or septic shock at day 0 [15], source of infection using CDC criteria, [16]  
158 and microorganism.

159 All isolates were sent to the Hospital Universitario Virgen Macarena, where  
160 identification was confirmed and susceptibility to antimicrobials was studied using  
161 microdilution, and interpreted according to EUCAST breakpoints [17]. Extended-  
162 spectrum beta-lactamase (ESBL), AmpC and carbapenemase production were studied  
163 by phenotypic methods, followed by PCR amplification and molecular sequencing. For  
164 the sake of simplicity, isolates producing ESBLs, AmpC or carbapenem resistance were  
165 considered as MDR.

166

### 167 **Statistical analysis**

168 The chi-square test or Fisher's exact test was used to compare categorical  
169 variables. The Mann-Whitney U test was used to compare continuous variables. When  
170 appropriate, continuous variables were dichotomized according to their association with  
171 death, using Classification and Regression Tree (CART) analysis. Multivariate Cox  
172 regression analysis was used to analyze the impact of EDE and LDE on 30-day  
173 mortality. Logistic regression and linear regression were used to identify the impact of  
174 EDE and LDE on failure and length of hospital stay among survivors, respectively.  
175 Variables with a P value of <0.2 in univariate comparisons and those considered of  
176 clinical importance were manually entered into the multivariate model. The variables in  
177 the models were selected manually using a backward stepwise process. Interactions and

178 collinearity were evaluated. Sensitivity analyses were performed by reclassifying the  
179 main exposure as EDE vs LDE+NDE, and as EDE+LDE vs NDE.

180 In addition, a propensity score (PS) was calculated for receiving EDE instead of  
181 NDE. Its predictive ability was calculated using the area under the receiver operating  
182 curve (AUROC) with 95% confidence intervals (CI), and the Hosmer-Lemeshow test  
183 was used for goodness of fit. The PS was used in two ways: as a covariate to control for  
184 residual confounding in multivariate models after checking for collinearity, and to  
185 perform a matched cohort analysis in which patients undergoing EDE and NDE were  
186 matched (1:1) according to their propensity scores using calipers of width 0.007.  
187 Statistical analysis was carried out using the SPSS program (SPSS 25.0, IBM Corp,  
188 Armonk, NY, USA).

189

## 190 **RESULTS**

191

192 The Bacteraemia-MIC cohort included 1058 patients with BSI due to  
193 Enterobacteriaceae; of these, 516 (48.7%) patients fulfilled the criteria for the de-  
194 escalation analysis (Figure 1). The number of patients per hospital ranged from 8 (1.6%)  
195 to 69 (13.4%). Overall, 241 (46.7%) patients received EDE, 95 (18.4%) LDE, and 180  
196 (34.8%) were not de-escalated. The proportion of EDE among hospitals with >20 cases  
197 ranged from 13% to 75.4%. The patients' characteristics are shown in Table 1.  
198 Compared to patients who underwent EDE, those in the NDE group more frequently  
199 had nosocomial infections, had been admitted to the ICU, had respiratory tract  
200 infections and received empiric therapy with meropenem. Overall, 70 (13.6%) isolates  
201 were ESBL producers, 26 (5.1%) AmpC producers, and 3 (0.6%) were carbapenem-  
202 resistant (none were carbapenemase producers). Among patients undergoing de-

203 escalation, the most frequent empirical drugs were piperacillin-tazobactam, and  
204 imipenem or meropenem and the most frequent drugs used for de-escalation were  
205 fluoroquinolones (68 patients in EDE and 38 in LDE), cefotaxime or ceftriaxone (92  
206 and 20) and amoxicillin-clavulanic acid (43 and 26) (supplementary Table S2).

207

### 208 **Variables associated with EDE**

209 The association of different variables with EDE is shown in Table 2. The  
210 variable “center” was dichotomized into low and high proportions of patients with EDE.  
211 In multivariate analysis, bacteraemia caused by MDR isolates and nosocomial episodes  
212 empirically treated with imipenem or meropenem were associated with a lower  
213 probability of receiving EDE. Even after controlling for these variables, patients  
214 hospitalized in centers with a high proportion of EDE still had a higher probability of  
215 receiving EDE. The AUROC for the model was 0.72 (95% CI 0.66-0.75).

216

### 217 **Mortality analysis**

218 Mortality rates were 4.1% (10/241), 6.3% (6/95) and 9.4% (17/180) in patients  
219 with EDE, LDE and NDE, respectively (Table 1). The univariate and multivariate  
220 analysis of variables associated with 30-day mortality are shown in Table 3. Source of  
221 bacteraemia was dichotomized into urinary or biliary tract vs others, according to their  
222 association with mortality. Hospitals were also classified into those with lower and  
223 higher mortality, and this variable was retained in the models. Multivariate analysis  
224 (Table 3) selected Charlson >3, source other than urinary or biliary tract, presentation  
225 with severe sepsis or shock, and SOFA >4 as associated with mortality. Among de-  
226 escalated patients, no trend toward higher mortality was found, although the model  
227 showed poor discrimination (AUROC=0.64; 95% CI: 0.52-0.75). In sensitivity analysis,

228 the adjusted HR for mortality were: 0.67 (95% CI 0.33-1.36, p=0.27) for EDE or LDE  
229 vs. NDE, and 0.60 (95% CI 0.27-1.30, p=0.19) for EDE vs LDE-NDE. No significant  
230 interactions were found in either model.

231 We then investigated the impact of EDE versus NDE including the PS for EDE  
232 (LDE patients were excluded from this analysis) (Table 3). No significant collinearity  
233 was found between the PS and other variables. Again, EDE did not show an association  
234 with higher 30-day mortality (adjusted HR=0.69; 95% CI: 0.29-1.65; p=0.41); the  
235 AUROC of this model was higher (0.72; 95% CI: 0.61-0.82). Finally, we matched 137  
236 pairs of patients receiving EDE or NDE according to PS. Matched sub-cohorts had  
237 exposure to all other variables (supplementary Table S3). Mortality was 5.4% (n=7) in  
238 EDE and 7.7% in NDE (n=10) (HR=0.98; 95% CI: 0.76-1.26; p=0.84).

239

#### 240 **Clinical cure and length of stay**

241 Overall, 35 patients showed failure at the end of antibiotic treatment (6.7%):  
242 11/2421 (4.6%) with EDE, 6/95 (6.3%) with LDE, and 18/180 (10%) with NDE. The  
243 univariate and multivariate analyses of variables associated with failure are shown in  
244 Table 4. The multivariate model showed that Charlson index >3, severe sepsis/septic  
245 shock at presentation and SOFA score at day 0 were associated with higher treatment  
246 failure, while a urinary or biliary source were protective factors. De-escalation was not  
247 found to be associated with failure (Table 4).

248 The median hospital stay after BSI was 14 days (IQR 9-24) and according to  
249 group, it was 14 days (9-28) for EDE, 13 (7-20) for LDE and 15 days (IQR 10-25) for  
250 NDE. The univariate analysis of variables associated with length of hospital stay is  
251 shown at Supplementary Table S4. Linear regression model of variables associated with  
252 length of hospital stay showed that nosocomial acquisition, Charlson index >3 and the



253 presence of severe sepsis/septic shock at presentation were associated with more days of  
254 hospitalization (p values 0.006, <0.001 and 0.01 respectively). EDE and NDE were not  
255 found to be associated with longer hospital stay (p=0.56 and 0.67, respectively)  
256 (Supplementary Table S5).

257

## 258 **DISCUSSION**

259

260 In this cohort, less than half the candidate patients received early de-escalation,  
261 and one third of patients were never de-escalated. Patients with MDR isolates or  
262 nosocomial infections empirically treated with imipenem or meropenem had a lower  
263 probability of de-escalation. Finally, neither EDE nor LDE were shown to be associated  
264 with worse outcomes.

265 To our knowledge, this is by far the biggest study of de-escalation among  
266 patients with bacteraemia [6]. It is important to note that we only included non-  
267 neutropenic, adult patients with monomicrobial bacteraemia due to Enterobacteriaceae  
268 who received early active empirical monotherapy with antipseudomonal beta-lactams or  
269 ertapenem. We are not therefore addressing the impact of changing from combination  
270 therapy to monotherapy, but only of changes in the empirical drug used. This  
271 population is somewhat more homogeneous than those considered in most previous  
272 studies.

273 The definition of de-escalation used is open to debate [18]. Unfortunately, many  
274 previous studies did not provide a specific definition of de-escalation and/or the drugs  
275 considered. The objective of de-escalation is to reduce exposure at the individual and  
276 group levels to drugs with a high negative ecological impact. However, the ecological  
277 impact of the drugs may depend on different variables, including local epidemiology,

278 the previous colonization status of patients, microbiota composition, and the dosing or  
279 duration of antibiotic therapy. In this study we used a classification of beta-lactams  
280 developed by consensus [10]; in this consensus, imipenem and meropenem ranked  
281 highest in terms of spectrum width and highest resistance selecting potential, followed  
282 by ertapenem and piperacillin-tazobactam or antipseudomonal cephalosporins. As drugs  
283 for de-escalation, we included the lower-ranked beta-lactams in the consensus, in  
284 addition to other drugs suitable for oral use [19] that would allow the earlier discharge  
285 of patients, such as fluoroquinolones and trimethoprim-sulfamethoxazole. We also  
286 analyzed late de-escalation.

287 De-escalation is performed less frequently than is desirable [20]. The main  
288 barriers identified for de-escalations are uncertainties about etiology, inadequate  
289 empirical therapy and isolation of MDR bacteria [19, 20]. In patients with  
290 monomicrobial bacteraemia due to Enterobacteriaceae, the only uncertainty about  
291 etiology is the possibility of polymicrobial infection in certain types of infection,  
292 typically intraabdominal and some skin/skin structure-associated infections. In our  
293 study, source of BSI was not associated with higher rates of de-escalation when  
294 controlling for confounders, and inadequate empirical therapy was an exclusion  
295 criterion. However, MDR bacteria were associated with a lower probability of de-  
296 escalation. We also identified nosocomial infection as a predictor for no de-escalation  
297 when these patients had been empirically treated with imipenem or meropenem, which  
298 may be a marker for more complex clinical situations. We suspect that other factors,  
299 such as stewardship interventions, less awareness of susceptibility results at weekends,  
300 and the training and opinions of individual prescribers could also play a role in de-  
301 escalation practice and merit specific studies.

302           In crude analysis, de-escalation was associated with lower mortality and failure.  
303   This was probably due to confounding by indication as the associations were no longer  
304   significant when other mortality predictors were considered in multivariate analysis,  
305   which is similar to the results found in the meta-analysis by Paul et al for observational  
306   studies of patients with severe sepsis or bacteraemia [6]. The results are reinforced by  
307   the fact that all our estimates in different analyses were consistent, and that we included  
308   mortality, failure of treatment and length of stay as outcome variables. Interestingly, the  
309   estimates provided by multivariate analysis were much less accurate than those  
310   provided by the PS-based matched pairs analysis. Our results strongly suggest therefore  
311   that de-escalation is safe. In fact, theoretically, it may have some individual beneficial  
312   effects if secondary infections caused by MDR bacteria are reduced, although  
313   demonstrating such an effect would require specific studies with a very large number of  
314   patients. Any analysis of population-level benefits would also require specific studies.

315           Our study has several limitations. Because it is not a randomized controlled trial,  
316   unmeasured confounding variables or residual confounding cannot be ruled out. The  
317   data were collected several years ago and changes in antimicrobial resistance may  
318   influence the results. Moreover, despite being controlled in the analysis, differences in  
319   clinical practice at each center might have influenced the outcomes. Some strengths of  
320   the study are its multicenter character, the use of clearly specified definitions, and the  
321   use of advance statistical methodologies to control for confounders.

322           In conclusion, the results of this study reinforce the fact that antibiotic de-  
323   escalation in patients with monomicrobial bacteraemia due to Enterobacteriaceae does  
324   not have a detrimental impact on outcome, 30-day all-cause mortality, failure, or length  
325   of hospital stay when compared with continuation with broad-spectrum antibiotics.  
326   These results may be useful for antibiotic stewardship activities.

327 **ACKNOWLEDGEMENTS**

328 **Other investigators from the REIPI/GEIH-SEIMC BACTERAEEMIA-MIC group:**

329 M. de Cueto (Unidad Clínica Intercentros de Enfermedades Infecciosas, Microbiología  
330 y Medicina Preventiva, Hospital Universitario Virgen Macarena, Seville, Spain), AM  
331 Planes Reig (Departamento de Microbiología, Hospital Universitari Valld'Hebron,  
332 Barcelona, Spain), F. Tubau Quintano (Servicio de Microbiología, Hospital  
333 Universitario de Bellvitge-IDIBELL, Barcelona, Spain), C. Peña (Servicio de  
334 Enfermedades Infecciosas, Hospital Universitario de Bellvitge-IDIBELL, Barcelona,  
335 Spain), ME Galán Otalora (Hospital de la Santa Creu i Sant Pau, Barcelona, Spain), C.  
336 Ruíz de Alegría (Servicio de Microbiología, Hospital Universitario Marqués de  
337 Valdecilla, Santander, Spain), R. Cantón (Servicio de Microbiología, Hospital  
338 Universitario Ramón y Cajal and Instituto Ramón y Cajal de Investigación Sanitaria  
339 (IRYCIS), Madrid, Spain), JA Lepe and JM Cisneros (Unidad Clínica de Enfermedades  
340 Infecciosas, Microbiología y Medicina Preventiva, Hospital Virgen del Rocío, Seville,  
341 Spain), J. Torre-Cisneros, R. Lara (Unidad Clínica de Enfermedades infecciosas  
342 Hospital Universitario Reina Sofía. Instituto Maimónides de Investigación Clínica  
343 (IMIBIC), Universidad de Córdoba, Córdoba, Spain).

344 **FINANCIAL SUPPORT**

345 This work was supported by the Instituto de Salud Carlos III, Ministry of  
346 Economy and Competitiveness, Spain (FIS; PI10/02021) co-financed by European  
347 Development Regional Fund 'A way to achieve Europe' ERDF, Spanish Network for  
348 Research in Infectious Diseases (REIPI RD12/0015).

349

350 **Potential Conflicts of Interest**

351 Dr. Zaira Palacios Baena reports personal fees from Gilead, outside the submitted work.  
352 Dr. Rodríguez-Baño reports personal fees from Merck, personal fees from AstraZeneca  
353 for a nondrug related research projects, and grants from Innovative Medicines Initiative  
354 (IMI), outside the submitted work. All other authors have no potential conflict of  
355 interest to declare.  
356  
357

358 **REFERENCES:**

- 359 1. Kollef MH. Broad-spectrum antimicrobials and the treatment of serious bacterial  
360 infections: getting it right up front. *Clin Infect Dis* 2008; 47: S3–13.
- 361 2. Karam G, Chastre J, Wilcox MH, Vincent JL. Antibiotic strategies in the era of  
362 multidrug resistance. *Crit Care*. 2016 Jun 22;20(1):136.
- 363 3. Barlam TF, Cosgrove SE, Abbo LM, MacDougall C, Schuetz AN, Septimus EJ,  
364 et al. Implementing an Antibiotic Stewardship Program: Guidelines by the  
365 Infectious Diseases Society of America and the Society for Healthcare  
366 Epidemiology of America. *Clin Infect Dis* 2016; 62: e51-77.
- 367 4. Silva BN, Andriolo RB, Atallah AN, Salomão R. De-escalation of antimicrobial  
368 treatment for adults with sepsis, severe sepsis or septic shock. *Cochrane*  
369 *Database Syst Rev*. 2013; 3: CD007934.
- 370 5. López-Cortés LE, Cueto M, Rodríguez-Baño J. How should we best treat  
371 patients  
372 with bloodstream infections? *Future Microbiol* 2017; 12: 927-930.
- 373 6. Paul M, Dickstein Y, Raz-Pasteur A. Antibiotic de-escalation for bloodstream  
374 infections and pneumonia: systematic review and meta-analysis. *Clin Microbiol*  
375 *Infect* 2016; 22: 960-967.
- 376 7. López-Cortés LE, Rosso-Fernández C, Núñez-Núñez M, et al. Targeted  
377 simplification versus antipseudomonal broad-spectrum beta-lactams in patients  
378 with bloodstream infections due to Enterobacteriaceae (SIMPLIFY): a study  
379 protocol for a multicentre, open-label, phase III randomised, controlled, non-  
380 inferiority clinical trial. *BMJ Open* 2017; 7: e015439.
- 381 8. Delgado-Valverde M, Torres E, Valiente-Mendez A, et al. Impact of the MIC of  
382 piperacillin/tazobactam on the outcome for patients with bacteraemia due to

- 383 Enterobacteriaceae: the Bacteraemia-MIC project. *J Antimicrob Chemother.*  
384 2016 Feb;71(2):521-30.
- 385 9. Delgado-Valverde M, Valiente-Mendez A, Torres E, et al. MIC of  
386 amoxicillin/clavulanate according to CLSI and EUCAST: discrepancies and  
387 clinical impact in patients with bloodstream infections due to  
388 Enterobacteriaceae. *J Antimicrob Chemother.* 2017 May 1;72(5):1478-1487.
- 389 10. Weiss E, Zahar JR, Lesprit P, Ruppe E, Leone M, Chastre J, Lucet JC, Paugam-  
390 Burtz C, Brun-Buisson C, Timsit JF; De-escalation Study Group. Elaboration of  
391 a consensual definition of de-escalation allowing a ranking of  $\beta$ -lactams. *Clin*  
392 *Microbiol Infect* 2015; 21: 649.e1-10.
- 393 11. Von Elm E, et al. The strengthening the reporting of observational studies in  
394 epidemiology (STROBE) statement: guidelines for reporting observational  
395 studies. *J. Clin Epidemiol.* 2008;61:344-349.
- 396 12. Charlson ME, Pompei P, Ales KL et al. A new method of classifying prognostic  
397 co-morbidity in longitudinal studies: development and validation. *J Chron Dis.*  
398 1987; 40: 373-83.
- 399 13. Hilf M, Yu Vh, Sharp J, et al. Antibiotic therapy for *Pseudomonas aeruginosa*  
400 bacteremia: outcome correlations in a prospective study of 200 patients. *Am J*  
401 *Med.* 1989; 87: 540-546.
- 402 14. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure  
403 Assessment) score to describe organ dysfunction/failure. On behalf of the  
404 Working Group on Sepsis-Related Problems of the European Society of  
405 Intensive Care Medicine. *Intensive Care Med.* 1996 Jul;22(7):707-10.

- 406 15. American College of Chest Physicians/Society of Critical Care Medicine  
407 Consensus Conference: definitions for sepsis and organ failure and guidelines  
408 for the use of innovative therapies in sepsis. *Crit Care Med.* 1992; 20: 864–74.
- 409 16. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of  
410 health care-associated infection and criteria for specific types of infections in the  
411 acute care setting. *Am J Infect Control* 2008; 36: 309-32.
- 412 17. EUCAST. Breakpoint tables for interpretation of MICs and zone diameters,  
413 version 5.0. 2015. Available at:  
414 [http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Breakpoint\\_ta](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf)  
415 [bles/v\\_5.0\\_Breakpoint\\_Table\\_01.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf).
- 416 18. Huttner B, Pulcini C, Schouten J. De-constructing de-escalation. *Clin Microbiol*  
417 *Infect* 2016; 22: 958-959.
- 418 19. Garnacho-Montero J, Escoresca-Ortega A, Fernández-Delgado E. Antibiotic de-  
419 escalation in the ICU: how is it best done? *Curr Opin Infect Dis* 2015; 28:193-8.
- 420 20. Masterton RG. Antibiotic de-escalation. *Crit Care Clin* 2011; 27: 149-62.

421

422

423

424 Figure 1: Flowchart



## 1 TABLES

## 2 Table 1. Features of patients with bacteraemia due to Enterobacteriaceae according to de-escalation group.

3

Variable	Early de-escalation (n=241)	Late de-escalation (n=95)	No de-escalation (n=180)	<i>P</i> value early vs no de-escalation	<i>P</i> value late vs no de-escalation
High-mortality hospital	81 (33.6)	31(32.6)	69 (38.3)	0.31	0.35
Etiology: <i>Escherichia coli</i>	164 (68)	60(63.2)	111 (61.7)	0.17	0.80
ESBL-producing Enterobacteriaceae	26 (10.6)	11 (11.6)	31 (17.2)	0.05	0.21
AmpC-producing Enterobacteriaceae	7 (2.9)	3 (3.2)	16 (8.9)	0.01	0.12
Carbapenem-resistant Enterobacteriaceae	0	2 (2.1)	1 (0.6)	0.88	0.57
Multidrug-resistant Enterobacteriaceae <sup>1</sup>	33 (13.7)	16 (16.8)	48 (26.7)	<0.001	0.06
Male gender	158 (65.6)	64 (67.4)	117 (65)	0.90	0.69
Age >60 years	187 (77.6)	79(83.2)	126 (70)	0.07	0.01
Nosocomial acquisition	57 (23.7)	23 (24.2)	76 (42.2)	<0.001	0.003
Intensive care unit admission	7 (2.9)	1 (1.1)	14 (7.8)	0.02	0.02
Previous surgery	32 (13.3)	14 (14.7)	41 (22.8)	0.01	0.11
Previous antimicrobial therapy	104 (43.2)	33 (34.7)	75 (41.7)	0.76	0.26
Charlson index >3	60 (24.9)	17 (17.9)	42 (23.3)	0.71	0.29
Source					
Urinary tract	104 (43.2)	30 (31.6)	59 (32.8)	0.03	0.94
Biliary tract	86 (35.7)	39 (41.1)	52 (28.9)	0.17	0.39
Other intrabdominal source	18 (7.5)	15 (15.8)	26 (14.4)	0.03	0.90
Skin and skin structures	4 (1.7)	2 (2.1)	8 (4.4)	0.16	0.51
Catheter-related	10 (4.1)	3 (3.2)	9 (5)	0.85	0.68
Respiratory tract	5 (2.1)	2 (2.1)	11 (6.1)	0.05	0.54
Others	2 (0.8)	1 (1.1)	3 (1.7)	0.74	0.68
Unknown source	12 (5)	1 (1.1)	11 (6.1)	0.77	0.10
Pitt score >3	25 (10.4)	3 (3.2)	22 (12.2)	0.55	0.01
Severe sepsis/septic shock	83 (34.4)	24 (25.3)	67 (37.2)	0.55	0.04
SOFA score >4 (day 0)	59 (24.5)	14 (14.7)	45 (25)	0.90	0.04

Empirical therapy					
Piperacillin/tazobactam	124 (51.5)	54 (56.8)	82 (45.6)	0.27	0.09
Ceftazidime	10 (4.1)	1 (1.1)	6 (3.3)	0.86	0.45
Cefepime	5 (2.1)	0	2 (1.1)	0.70	0.77
Ertapenem	37 (15.4)	14 (14.7)	22 (12.2)	0.43	0.68
Imipenem	29 (12)	2 (2.1)	15 (8.3)	0.28	0.07
Meropenem	36 (14.9)	24 (25.3)	53 (29.4)	<0.001	0.55
Mortality at day 30	10 (4.1)	6 (6.3)	17 (9.4)	0.02	0.37
Failure at the end of treatment	11 (4.6)	6 (6.3)	162 (10)	0.02	0.30
Median days of hospital stay (IQR)	14 (9-28)	13 (7-20)	15 (10-25)	0.21	0.003

4  
5  
6  
7

IQR: interquartile range.

<sup>1</sup>Multidrug-resistant isolates were those producing ESBL or AmpC or carbapenem-resistant.

8 **Table 2. Analysis of the association of different variables with early de-escalation.**

9

Variable	Early de-escalation (n=241)	Late or not de-escalation (n=275)	Crude OR (95% CI)	P value	Adjusted OR (95% CI) <sup>1</sup>	P value
Hospital with high rate of de-escalation	131 (54.4)	59 (21.5)	4.36 (2.97-6.39)	<0.001	4.34 (2.93-6.45)	<0.001
Etiology: <i>Escherichia coli</i>	164 (68)	171 (62.2)	1.29 (0.90-1.86)	0.16		
Multidrug-resistant isolate <sup>2</sup>	33 (13.7)	64 (23.3)	0.52 (0.33-0.83)	0.006	0.50 (0.30-0.83)	0.007
Empirical treatment with piperacillin-tazobactam	124 (51.5)	136 (49.5)	1.08 (0.76-1.53)	0.65		
Empirical treatment with ertapenem	37 (15.4)	36 (13.1)	1.20 (0.73-1.97)	0.46		
Empirical treatment with imipenem or meropenem	65 (27)	94 (34.2)	1.40 (0.96-2.05)	0.07	1.20 (0.73-1.99)	0.46
Male gender	158 (65.7)	181 (65.8)	0.98 (0.68-1.42)	0.95		
Age >60 years	187 (77.6)	205 (74.5)	1.18 (0.78-1.77)	0.41		
Nosocomial infection	57 (23.7)	99 (36)	0.55 (0.37-0.81)	0.002	0.83 (0.50-1.39)	0.49
Intensive care unit admission	7 (2.9)	15 (5.5)	0.51 (0.20-1.29)	0.15		
Previous surgery	32 (13.3)	55 (20)	0.61 (0.38-0.98)	0.04		
Charlson index >3	60 (24.9)	59 (21.5)	1.21 (0.80-1.82)	0.35		
Urinary and biliary tract source	190 (78.8)	180 (65.5)	1.96 (1.32-2.92)	<0.001		
Pitt score >3	25 (10.4)	25 (9.1)	1.15 (0.64-2.07)	0.62		
Severe sepsis/septic shock	83 (34.4)	91 (33.1)	1.06 (0.73-1.53)	0.74		
SOFA score >4 (day 0)	59 (24.5)	59 (21.5)	1.18 (0.78-1.79)	0.41		
Interaction: nosocomial infection and empirical treatment with imipenem or meropenem					0.35 (0.14-0.87)	0.02

10

11

12

13

<sup>1</sup>Hosmer-Lemeshow test, P value = 0.99; AUROC = 0.71 (0.66-0.75), p<0.001.

<sup>2</sup>Multidrug-resistant isolates were those producing ESBLs or AmpC, or carbapenem-resistant.

14 **Table 3. Univariate and multivariate analyses of risk factors associated with all-cause 30-day mortality using Cox regression.**

Variable			Crude analysis		Adjusted analysis <sup>2</sup>		EDE vs NDE, adjusted by PS <sup>3</sup>	
	No. deceased (%) N= 33	No. alive (%) N= 483	HR (95 CI%)	P value	HR (95% CI)	P value	HR (95% CI)	P value
Hospital with high mortality	19 (57.6)	162 (33.5)	2.56 (1.28-5.12)	0.007	1.68 (0.80-3.53)	0.16	1.91 (0.83-4.36)	0.12
Etiology: <i>Escherichia coli</i>	17 (51.5)	318 (65.8)	0.56 (0.281-1.12)	0.10				
Male gender	22 (66.7)	317 (65.6)	1.05 (0.51-2.17)	0.88				
Age >60 years	27 (81.8)	365 (75.6)	1.45 (0.61-3.52)	0.40				
Nosocomial acquisition	18 (54.5)	138 (28.6)	2.84 (1.43-5.64)	0.003				
Intensive care unit admission	5 (15.2)	17 (3.5)	4.15 (1.60-10.76)	0.003				
Charlson index >3	14 (42.4)	105 (21.7)	0.54 (1.27-5.07)	0.008	3.02 (1.50-6.09)	0.002	3.69 (1.65-8.24)	0.001
Urinary or biliary source	14 (42.4)	356 (73.7)	0.28 (1.14-0.56)	<0.001	0.35 (0.17-0.74)	0.006	0.23 (0.08-0.61)	0.004
Pitt score >3	10 (30.3)	40 (8.3)	4.33 (2.06-9.10)	<0.001				
Severe sepsis/septic shock	23 (69.7)	151 (31.3)	4.76 (2.26-10.01)	<0.001	3.06 (1.32-7.09)	0.009	3.29 (1.25-8.63)	0.01
SOFA score >4 (day 0)	17 (51.5)	101 (21)	3.81 (1.92-7.54)	<0.001	2.18 (1.03-4.57)	0.03	2.73 (1.20-6.23)	0.01
Empirical meropenem	9 (27.3)	104 (21.5)	1.35 (1.62-2.91)	0.44				
De-escalation								
No de-escalation	17 (51.5)	163 (33.7)	Reference	0.10	Reference	0.41	Reference	
Early de-escalation	10 (30.3)	231 (47.8)	0.42 (0.19-0.93)	0.03	0.58 (0.25-1.31)	0.19	0.69 (0.29-1.65)	0.41
Late de-escalation	6 (18.2)	89 (18.4)	0.65 (0.25-1.66)	0.37	0.89 (0.35-2.26)	0.80	Excluded	
Propensity score <sup>1</sup>							0.81 (0.06-10.42)	0.87

15  
16  
17  
18  
19  
20  
21  
22

Abbreviations: PS, propensity score; EDE, early de-escalation; NDE, no de-escalation.

<sup>1</sup>Calculated only for patients in the early de-escalation and no de-escalation groups. The variables included in the propensity score were: high-risk hospital, microorganism, gender, age, acquisition, department, Charlson index, previous antibiotic therapy, urinary and biliary source, Pitt score, SOFA score at day 0, severe sepsis and septic shock, and empirical therapy. The AUROC of the PS model was 0.68 (95%CI 0.63-0.73), *p value*=0.001, Hosmer-Lemeshow test=0.84.

<sup>2</sup>The AUROC of the model was 0.64 (0.52-0.75), *p value*=0.007

<sup>3</sup>Patients in the late de-escalation group were excluded from this analysis.

23

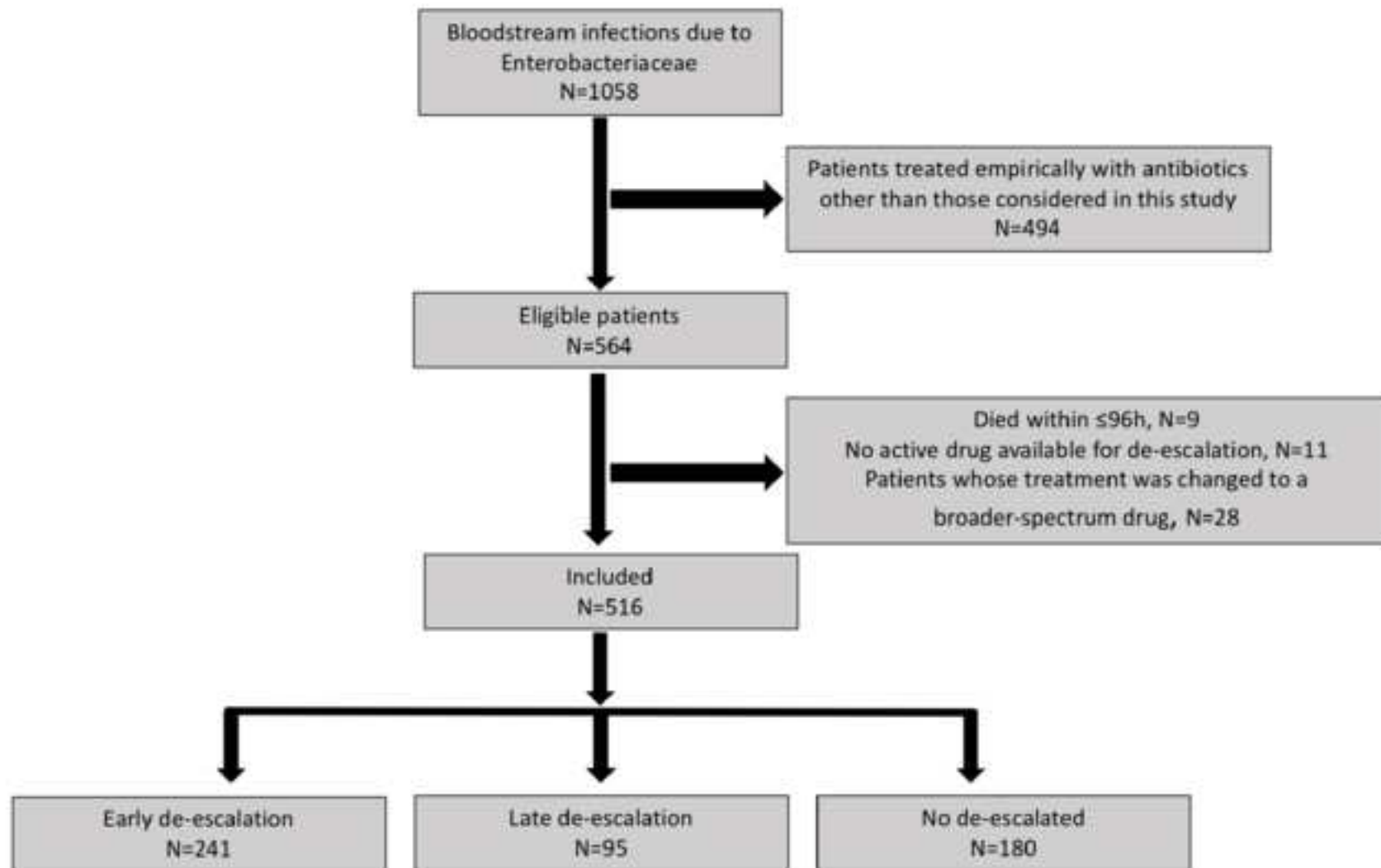
24 **Table 4. Univariate and multivariate model of variables associated with failure at the end of antibiotic treatment.**

Variable	Crude analysis				Adjusted analysis <sup>1</sup>	
	Failure (%) (N= 35)	Cure (%) (N= 481)	OR (95% CI)	P value	OR (95% CI)	P value
Hospital with high proportion of failure	17 (48.6)	131 (27.2)	2.52 (1.26-5.04)	0.009	1.70 (0.78-3.70)	0.17
Etiology: <i>Escherichia coli</i>	20 (57.1)	315 (65.5)	0.70 (0.35-1.40)	0.32		
Male gender	22 (62.9)	317 (65.9)	0.87 (0.43-1.78)	0.71		
Age >60 years	29 (82.9)	363 (75.5)	1.57 (0.63-3.87)	0.32		
Nosocomial acquisition	17 (48.6)	139 (28.9)	2.32 (1.16-4.64)	0.01		
Intensive care unit admission	4 (11.4)	18/ (3.7)	3.31 (1.05-10.40)	0.04		
Charlson index >3	14 (40)	105 (21.8)	2.38 (1.17-4.85)	0.01	2.87 (1.31-6.29)	0.008
Urinary or biliary tract source	14 (40)	356 (74)	0.23 (0.11-0.47)	<0.001	0.24 (0.11-0.52)	<0.001
Pitt score >3	10 (28.6)	40 (4.3)	4.41 (1.97-9.83)	<0.001		
Severe sepsis/septic shock	24 (68.6)	150 (31.2)	4.81 (2.29-10.08)	<0.001	3.09 (1.27-7.50)	0.01
SOFA score >4 (Day 0)	19 (54.3)	99 (20.6)	4.58 (2.27-9.23)	<0.001	2.76 (1.21-6.25)	0.01
De-escalation						
No de-escalation	18 (51.4)	162 (33.7)	Reference		Reference	
Early de-escalation	11 (31.4)	230 (47.8)	0.43 (0.19-0.93)	0.03	0.56 (0.24-1.32)	0.18
Late de-escalation	6 (17.1)	89 (18.5)	0.60 (0.23-1.58)	0.30	0.98 (0.34-2.83)	0.98

25 Abbreviations: ICU, intensive care unit.

26 <sup>1</sup>AUROC of this model 0.81 (0.74-0.89), p<0.001, Hosmer-Lemeshow test 0.61.

27



**Supplementary material:**

- **Supplementary Table S1.** Checklist of items according to STROBE recommendations.
- **Supplementary Table S2.** Susceptibility data and production of key beta-lactamase among Enterobacteriaceae causing bloodstream infection in the patients included in the study.
- **Supplementary Table S3.** Different de-escalation schemes.
- **Supplementary Table S4.** Comparison of patients matched according to propensity score.
- **Supplementary Table S5.** Univariate analysis of variables associated with length of hospital stay.
- **Supplementary Table S6.** Linear regression model of variables associated with length of hospital stay.
- **Supplementary Table S7.** Susceptibility data and production of key beta-lactamase among Enterobacteriaceae causing bloodstream infection in the whole Bacteremia-MIC cohort and stratified according to the type of acquisition.

**Supplementary Table S1.** Checklist of items according to STROBE document.

	<b>RECOMMENDATION</b>	<b>ASSESSMENT IN ARTICLE</b>
<b>TITLE AND ABSTRACT</b>	<p><i>a)</i> Indicate the study design with a commonly used term in the title or abstract</p> <p><i>b)</i> Provide an informative and balanced summary in the abstract of what was done and what was found</p>	<p>a) Study design specified in title and abstract</p> <p>b) Balanced summary included in the abstract</p>
<b>BACKGROUND/ RATIONALE</b>	Explain the scientific background and rationale for the investigation being reported	The scientific background and rationale are included in the introduction
<b>OBJECTIVES</b>	State specific objectives, including any pre-specified hypotheses	Pre-specified hypothesis and objectives are stated in Methods
<b>STUDY DESIGN</b>	Present key elements of study design early in the paper	Study design described in the first part of Methods
<b>SETTING</b>	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	Described in Methods
<b>PARTICIPANTS</b>	<p><i>(a)</i> Give the eligibility criteria and the sources and methods of selection of participants. Describe methods of follow-up</p> <p><i>(b)</i> For matched studies, give matching criteria and number of exposed and unexposed</p>	<p>a) Described in Methods</p> <p>b) This is not a matched study</p>
<b>VARIABLES</b>	Clearly define all outcomes, exposures, predictors, potential confounders and effect modifiers. Give diagnostic criteria, if applicable	Defined in Methods
<b>DATA SOURCES/ MEASUREMENT</b>	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	Specified in Methods. The same methods for data collection were used in groups.
<b>BIAS</b>	Describe any efforts to address potential sources of bias	<p>Selection bias: inclusion of consecutive cases.</p> <p>Information bias: use of standard, well-defined, easy-to-collect variables (piloted).</p> <p>Use of soft and hard outcome variables.</p>



<b>STUDY SIZE</b>	Explain how the study size was arrived at	Explained in Methods
<b>QUANTITATIVE VARIABLES</b>	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why.	Quantitative variables were handled as such. No groupings were made
<b>STATISTICAL METHODS</b>	<p>(a) Describe all statistical methods, including those used to control for confounding</p> <p>(b) Describe any methods used to examine subgroups and interactions</p> <p>(c) Explain how missing data were addressed</p> <p>(d) If applicable, explain how loss to follow-up was addressed</p> <p>(e) Describe any sensitivity analyses</p>	<p>a) Quantitative variables were handled as such. No groupings were made</p> <p>b) Included in Methods</p> <p>c) Included in Methods</p> <p>d) No patient was lost to follow-up</p> <p>e) Included in methods</p>
<b>PARTICIPANTS</b>	<p>(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible,</p> <p>(b) Give reasons for non-participation at each stage.</p> <p>(c) Consider use of a flow diagram</p>	a), b) and c) Included in results.
<b>DESCRIPTIVE DATA</b>	<p>(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders</p> <p>b) Indicate number of participants with missing data for each variable of interest</p> <p>(c) Summarise follow-up time (eg, average and total amount)</p>	a), b), c) Table 1
<b>OUTCOME DATA</b>	Report numbers of outcome events or summary measures over time	Table 3
<b>MAIN RESULTS</b>	<p>(a) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included</p> <p>(b) Report category boundaries when continuous variables were categorized</p> <p>c) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time</p>	a), b), c) Table 3

	period	
<b>OTHER ANALYSES</b>	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	Included in Results
<b>KEY RESULTS</b>	Summarize key results with reference to study objectives	Specified in Abstract and Discussion
<b>LIMITATIONS</b>	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	Included in Discussion
<b>INTERPRETATION</b>	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	Included in Discussion
<b>GENERALIZABILITY</b>	Discuss the generalizability (external validity) of the study results	Included in Discussion
<b>FUNDING</b>	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	Included

**Supplementary Table S2. Susceptibility data and production of key beta-lactamases among the Enterobacteriaceae causing bloodstream infections in the patients included in the study. Data are number of susceptible isolates (or beta-lactamase producers) per isolates tested (percentage per group).**

	<i>Escherichia coli</i> (N=335)	<i>Klebsiella spp.</i> (n=101)	<i>Proteus spp.</i> (n=12)	<i>Enterobacter spp.</i> (n=40)	<i>Serratia spp.</i> (n=14)	Others (n=14)
<b>Amoxicillin-clavulanic acid</b>	163/329 (49.5)	77/101 (76.2)	10/11 (90.9)	3/39 (7.7)	1/14 (7.1)	1/14 (7.1)
<b>Piperacillin-tazobactam</b>	307/329 (93.3)	91/101 (90.1)	11/11 (100)	33/40 (82.5)	12/14 (85.7)	12/14 (85.7)
<b>Cefotaxime</b>	282/334 (84.4)	91/101 (90.1)	12/12 (100)	28/40 (70)	12/14 (85.7)	11/14 (78.6)
<b>Cefepime</b>	286/334 (85.6)	95/101 (94.1)	12/12 (100)	33/40 (82.5)	14/14 (100)	14/14 (100)
<b>Ertapenem</b>	334/334 (100)	100/101 (99)	12/12 (100)	38/40 (95)	14/14 (100)	14/14 (100)
<b>Meropenem</b>	334/334 (100)	101/101 (100)	12/12 (100)	40/40 (100)	14/14 (100)	14/14 (100)
<b>Ciprofloxacin</b>	194/334 (58.1)	89/101 (88.1)	11/12 (91.7)	35/40 (87.5)	14/14 (100)	13/14 (92.9)
<b>ESBL-producers</b>	55/335 (16.4)	8/101 (7.9)	0/12 (0)	5/40 (12.5)	0/14 (0)	0/14 (0)
<b>AmpC-producer</b>	12/335 (3.6)	2/101 (2)	0/12 (0)	6/40 (15)	2/14 (14.3)	4/14 (28.6)
<b>Carbapenemase-producer</b>	0/335 (0)	0 (101 (0)	0/12 (0)	0/40 (0)	0/14 (0)	0/14 (0)

**Supplementary Table S3. Distribution of de-escalation schemes.**

		De-escalation drug					
		Ampicillin (n=5)	Amoxicillin- clavulanic acid (n=69)	Cefotaxime, ceftriaxone (n=112)	Ertapenem (n=33)	Ciprofloxacin, levofloxacin (n=106)	Trimethoprim- sulfamethoxazole (n=10)
Empirical drug							
<b>Early De- escalation</b>	<b>Piperacillin-tazobactam (n=124)</b>	3	21	44	18	34	4
	<b>Ceftazidime (n=10)</b>		2	7	-	1	-
	<b>Cefepime (n=5)</b>	1	-	2	-	2	-
	<b>Ertapenem (n=37)</b>	1	5	19	-	9	3
	<b>Imipenem, meropenem (n=65)</b>	-	15	20	8	22	-
	Total	5	43	92	26	68	7
<b>Late De- escalation</b>	<b>Piperacillin-tazobactam (n=54)</b>	-	18	7	2	27	-
	<b>Ceftazidime (n=1)</b>	-	-	1	-	-	-
	<b>Ertapenem (n= 14)</b>	-	4	3	-	4	3
	<b>Imipenem, meropenem (n=25)</b>	-	4	9	5	7	-
	Total	0	26	20	7	38	3

**Supplementary Table S4. Comparison of matched patients according to propensity score.**

Variable	Early de-escalation (n=130)	No de-escalation (n=130)	<i>P value</i>
<b>High mortality hospital</b>	46/130 (35.4)	45/130 (34.6)	0.89
<b>Etiology: <i>Escherichia coli</i></b>	89/130 (68.5)	88/130 (67.7)	0.89
<b>Male gender</b>	82/130 (63.1)	83/130 (63.8)	0.89
<b>Age &gt;60 years</b>	101/130 (77.7)	96/130 (73.8)	0.46
<b>Nosocomial acquisition</b>	40/130 (30.8)	43/130 (33.1)	0.69
<b>ICU admission</b>	7/130 (5.4)	6/130 (4.6)	0.77
<b>Previous surgery</b>	20/130 (15.4)	24/130 (18.5)	0.50
<b>Previous antimicrobial therapy</b>	54/130 (41.5)	52/130 (40)	0.80
<b>Charlson index &gt;3</b>	28/130 (21.5)	31/130 (23.8)	0.65
<b>Source</b>			0.52
<b>Urinary tract</b>	58/130 (44.6)	48/130 (36.9)	
<b>Biliary tract</b>	38/130 (29.2)	45/130 (34.6)	
<b>Other intraabdominal Infection</b>	13/130 (10)	13/130 (10)	
<b>Skin and skin structures</b>	2/130 (1.5)	5/130 (3.8)	
<b>Catheter-related</b>	7/130 (5.4)	6/130 (4.6)	
<b>Respiratory tract</b>	2/130 (1.5)	4/130 (3.1)	
<b>Others</b>	0	2/130 (1.5)	
<b>Unknown source</b>	10/130 (7.7)	7/130 (5.4)	
<b>Pitt score &gt;3</b>	16/130 (12.3)	14/130 (10.8)	0.69
<b>Severe sepsis/septic shock</b>	48/130 (36.9)	47/130 (36.2)	0.89
<b>SOFA score &gt;4 (day 0)</b>	35/130 (26.9)	35/130 (26.9)	1
<b>Empirical therapy</b>			0.83
<b>Piperacillin/tazobactam</b>	66/130 (50.8)	68/130 (52.3)	
<b>Ceftazidime</b>	2/130 (1.5)	5/130 (3.8)	
<b>Cefepime</b>	2/130 (1.5)	2/130 (1.5)	
<b>Ertapenem</b>	19/130 (14.6)	19/130 (14.6)	
<b>Imipenem</b>	14/130 (10.8)	15/130 (11.5)	
<b>Meropenem</b>	27/130 (20.8)	21/130 (16.2)	
<b>Mortality at day 30</b>	7/130 (5.4)	10/130 (7.7)	0.45
<b>Failure at end of treatment</b>	6/130 (4.6)	10/130 (7.7)	0.30
<b>Median days of hospital stay (IQR)</b>	13 (8-26)	15 (10-24)	0.38

IQR: interquartile range

**Supplementary Table S5. Univariate analysis of variables associated with length of hospital stay.**

<b>Variable</b>	<b>P value</b>
<b>Etiology: <i>Escherichia coli</i></b>	0.06 <sup>1</sup>
<b>Male gender</b>	0.27 <sup>1</sup>
<b>Age &gt;60 years</b>	0.34 <sup>1</sup>
<b>Nosocomial acquisition</b>	0.002 <sup>1</sup>
<b>Intensive care unit admission</b>	0.01 <sup>1</sup>
<b>Charlson index &gt;3</b>	0.001 <sup>1</sup>
<b>Urinary or biliary tract source</b>	0.002 <sup>1</sup>
<b>Pitt score &gt;3</b>	0.16 <sup>1</sup>
<b>Severe sepsis/septic shock</b>	0.001 <sup>1</sup>
<b>SOFA score &gt;4 (day 0)</b>	0.06 <sup>1</sup>
<b>Empirical treatment with meropenem</b>	0.04 <sup>1</sup>
<b>De-escalation</b>	0.02 <sup>2</sup>
<b>No de-escalation</b>	
<b>Early de-escalation</b>	
<b>Late de-escalation</b>	
<b>Failure at end of treatment</b>	0.91 <sup>1</sup>
<b>Mortality at day 30</b>	0.27 <sup>1</sup>

<sup>1</sup>U Mann-Whitney test

<sup>2</sup>Kruskal-Wallis test

**Supplementary Table S6. Linear regression model of variables associated with length of hospital stay.**

<b>Variable</b>	<b>Coefficient</b>	<b><i>P</i> value</b>	<b>95% CI</b>
<b>Nosocomial acquisition</b>	2.78	0.006	1.17-6.79
<b>Charlson index &gt;3</b>	3.78	<0.001	2.79-8.85
<b>Severe sepsis/septic shock</b>	2.45	0.01	0.67-6.04
<b>Early de-escalation vs. no de-escalation<sup>1</sup></b>	-1.03	0.56	(-4.51)-2.45
<b>No de-escalation vs. global de-escalation<sup>2</sup></b>	0.79	0.67	(-2.89)-4.47

<sup>1</sup>This group includes the no de-escalation group and the late de-escalation group

<sup>2</sup>This group includes the early and late de-escalation groups

**Supplementary Table S7. Susceptibility data and production of key carbapenemases among Enterobacteriaceae causing bloodstream infection in the whole Bacteremia-MIC cohort and stratified according to the type of acquisition. Data are number of susceptible isolates (or beta-lactamase producers) per isolates tested (percentage).**

	<b>All episodes (n=516)</b>	<b>Community-onset episodes (n=190)</b>	<b>Nosocomial episodes (n=157)</b>	<b>Health-care associated episodes (n=169)</b>
<b>Amoxicillin-clavulanic acid</b>	255/505 (50.5)	110/186 (59.1)	64/153 (41.8)	81/166 (48.8)
<b>Piperacillin-tazobactam</b>	466/506 (92.1)	182/186 (97.8)	135/154 (87.7)	149/166 (89.8)
<b>Cefotaxime</b>	438/515 (85)	171/190 (90)	125/156 (80.1)	142/169 (84)
<b>Cefepime</b>	454/515 (88.2)	173/190 (91.1)	133/156 (85.3)	148/169 (87.6)
<b>Ertapenem</b>	509/515 (98.8)	190/190 (100)	153/156 (98.1)	166/169 (98.2)
<b>Meropenem</b>	514/514 (100)	189/189 (100)	156/156 (100)	169/169 (100)
<b>Ciprofloxacin</b>	356/515 (69.1)	146/190 (76.8)	107/156 (68.6)	103/169 (60.9)
<b>ESBL-producer</b>	68/516 (13.2)	24/190 (12.6)	23/157 (14.6)	21/169 (14.6)
<b>AmpC-producer</b>	26/516 (5)	2/190 (1.1)	15/157 (9.6)	9/169 (5.3)
<b>Carbapenemase producers</b>	0/516 (0)	0/190 (0)	0/157 (0)	0/169 (0)

MIC breakpoint for considering susceptible to amoxicillin-clavulanic acid and piperacillin-tazobactam was  $\leq 8$  mg/L, for cefotaxime, ceftazidime, cefepime and levofloxacin  $\leq 1$  mg/L, for ertapenem and ciprofloxacin  $\leq 0.5$  mg/L and for meropenem  $\leq 2$  mg/L. Intermediate isolates were considered as resistant for this analysis.

This analysis was performed using EUCAST 2015 breakpoints.



1           **Impact of de-escalation on prognosis of patients with bacteraemia due to**  
2           **Enterobacteriaceae: a post-hoc analysis from a multicenter prospective cohort**

3

4           **Running title:** De-escalation in Enterobacteriaceae bacteraemia.

5

6           **Authors:** Zaira R. (ZRPB) Palacios-Baena<sup>1</sup>, Mercedes (MDV) Delgado-Valverde<sup>1</sup>,  
7           Adoración (AVM) Valiente Méndez<sup>1</sup>, Benito (BA) Almirante<sup>2</sup>, Silvia (SGZ) Gómez-  
8           Zorrilla<sup>3</sup>, Núria (NB) Borrell<sup>4</sup>, Juan E. (JEC) Corzo<sup>5</sup>, Mercedes (MG) Gurguí<sup>6</sup>, Cristina  
9           (CC) de la Calle<sup>7</sup>, Lara (LGA) García-Álvarez<sup>8</sup>, Lucía (LR) Ramos<sup>9</sup>, Mónica (MG)  
10          Gozalo<sup>10</sup>, María Isabel (MIM) Morosini<sup>11</sup>, José (JM) Molina<sup>12</sup>, Manuel (MC) Causse<sup>13</sup>,  
11          Álvaro (AP) Pascual<sup>1</sup>, Jesús (JRB) Rodríguez-Baño<sup>1,\*</sup> on behalf of the REIPI/GEIRAS-  
12          SEIMC BACTERIEMIA-MIC group.

13

14          <sup>1</sup>Unidad Clínica de Enfermedades Infecciosas, Microbiología y Medicina Preventiva,  
15          Hospital Universitario Virgen Macarena/Departamento de Medicina y Microbiología,  
16          Universidad de Sevilla/Instituto de Biomedicina de Sevilla (IBiS). Sevilla, Spain.

17          <sup>2</sup>Hospital Universitari Vall d'Hebron. Barcelona, España.

18          <sup>3</sup>Hospital de Bellvitge. Barcelona, Spain.

19          <sup>4</sup>Hospital Universitario Son Espases. Palma de Mallorca, Islas Baleares, España.

20          <sup>5</sup>Unidad Clínica Enfermedades Infecciosas y Microbiología. Hospital Universitario  
21          Virgen de Valme. Sevilla, Spain.

22          <sup>6</sup>Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

23          <sup>7</sup>Hospital Clinic i Provincial, Barcelona, Spain.

24          <sup>8</sup>Departamento de Enfermedades Infecciosas. Hospital San Pedro-CIBIR. Logroño,  
25          Spain.

26 <sup>9</sup>Hospital Universitario A Coruña. A Coruña, Spain.

27 <sup>10</sup>Hospital Marqués de Valdecilla-IDIVAL, Santander. Spain.

28 <sup>11</sup>Hospital Ramón y Cajal. Madrid, Spain.

29 <sup>12</sup>Unidad Clínica de Enfermedades Infecciosas, Microbiología y Medicina Preventiva,  
30 Hospital Universitario Virgen del Rocío/Universidad de Sevilla/Instituto de Biomedicina  
31 de Sevilla (IBIS). Seville, Spain.

32 <sup>13</sup>Unidad de Gestión Clínica de Microbiología. Hospital Universitario Reina Sofía.  
33 Instituto Maimónides de Investigación Clínica (IMIBIC), Universidad de Córdoba.  
34 Córdoba, Spain.

35

36 **Keywords:** De-escalation, streamlining, Enterobacteriaceae, bloodstream infections,  
37 mortality.

38

39 **\*Corresponding author:** Jesús Rodríguez-Baño. Unidad Clínica de Enfermedades  
40 Infecciosas, Microbiología y Medicina Preventiva, Hospital Universitario Virgen  
41 Macarena, Avda Dr. Fedriani, 3, 41009 Sevilla, Spain. Phone: +34 677906512. E-mail  
42 address: [jesusrb@us.es](mailto:jesusrb@us.es)

43 **Alternative corresponding author:** Zaira R. Palacios-Baena, Unidad Clínica de  
44 Enfermedades Infecciosas, Microbiología y Medicina Preventiva, Hospital Universitario  
45 Virgen Macarena, Avda Dr. Fedriani, 3, 41009 Sevilla, Spain. Phone: +34 653276353.  
46 E-mail address: [Zaira.palacios.baena@hotmail.com](mailto:Zaira.palacios.baena@hotmail.com)

47

48

49 **SUMMARY OF THE MAIN POINTS**

50 De-escalation from empirical **ertapenem or antipseudomonal  $\beta$ -lactams** to lower spectrum

51 antibiotics in patients with bacteremia due to Enterobacteriaceae was not associated with

52 any detrimental impact in terms of mortality, clinical failure or length of hospital stay

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74 **ABSTRACT**

75

76 **Background:** More data are needed about the safety of antibiotic de-escalation in specific  
77 clinical situations as a strategy to reduce exposure to broad-spectrum antibiotics. The  
78 aims of this study were to investigate predictors of de-escalation and its impact on the  
79 outcome of patients with bloodstream infection due to Enterobacteriaceae (BSI-E).

80 **Methods:** A post-hoc analysis was performed of a prospective, multicenter cohort of  
81 patients with BSI-E initially treated with ertapenem or antipseudomonal  $\beta$ -lactams.  
82 Logistic regression was used to analyze factors associated with early de-escalation (EDE)  
83 and Cox regression for the impact of EDE and late de-escalation (LDE) on 30-day all-  
84 cause mortality. A propensity score (PS) for EDE vs. no de-escalation (NDE) was  
85 calculated. Failure at end of treatment and length of hospital stay were also analyzed.

86 **Results:** Overall, 516 patients were included; EDE was performed in 241 patients (46%),  
87 LDE in 95 (18%) and NDE in 180 (35%). Variables independently associated with a  
88 lower probability of EDE were multidrug-resistant isolates (OR 0.50, 95% CI 0.30-0.83)  
89 and nosocomial infection empirically treated with imipenem or meropenem (OR 0.35,  
90 95% CI 0.14-0.87). After controlling for confounders, EDE was not associated with  
91 increased risk of mortality; Hazard ratios (HR) and (95% CI) were: general model, 0.58  
92 (0.25-1.31), model with PS, 0.69 (0.29-1.65), and PS-matched pairs, 0.98 (0.76-1.26).  
93 LDE was not associated with mortality. De-escalation was not associated with clinical  
94 failure or length of hospital stay.

95 **Conclusions:** De-escalation in patients with monomicrobial bacteraemia due to  
96 Enterobacteriaceae was not associated with a detrimental impact on clinical outcome.

97 **INTRODUCTION**

98

99 Patients with sepsis are frequently treated empirically with broad-spectrum drugs  
100 (BSD) because the early administration of active drugs has been associated with improved  
101 outcome, particularly in the presence of septic shock [1]. This can lead to overuse of these  
102 drugs, which is usually considered to be one of the contributing factors for the spread of  
103 multidrug-resistant (MDR) bacteria [2]. To minimize this problem, streamlining or de-  
104 escalation from broad- to narrower-spectrum drugs is usually advocated once the  
105 susceptibility of the causative agent of the infection is known, and antimicrobial  
106 stewardship programs frequently include interventions facilitating or recommending this  
107 practice [3]. However, de-escalation is performed less frequently than is desirable.  
108 Barriers include uncertainty among many prescribers; indeed, although de-escalation is  
109 considered standard of care for most infectious diseases specialists, a recent systematic  
110 review concluded that there is no adequate evidence as to whether de-escalation of  
111 antimicrobial agents is effective and safe for adults with sepsis [4]. Hence, providing  
112 more information about the safety of de-escalation would help increase implementation,  
113 and knowledge of the variables influencing the performance of de-escalation would lead  
114 to better targeting of interventions promoting this practice.

115 Bloodstream infections (BSI) are an ideal model for de-escalation, since etiology  
116 and susceptibility are known, and a more specialized evaluation of patients is possible  
117 [5]. A meta-analysis including studies of sepsis, bacteraemia and pneumonia found a  
118 trend towards higher mortality with de-escalation in 3 randomized trials, but lower  
119 mortality in observational studies [6]. However, the studies were heterogeneous with  
120 respect to type of patient and infection, etiology, definitions used and interventions, which  
121 precludes high confidence in the meta-analytic estimates. Studies of specific populations

122 and etiologies are needed therefore. A randomized trial of patients with bacteraemia due  
123 to Enterobacteriaceae is now recruiting [7], although the results will not be available for  
124 2 years. The objectives of this study were to evaluate the frequency of variables associated  
125 with de-escalation, and the impact of de-escalation on prognosis only among patients with  
126 bacteraemia due to Enterobacteriaceae.

127

## 128 **METHODS**

129

### 130 **Study design, sites and study population**

131 This is a post-hoc analysis of the prospective Bacteraemia-MIC cohort, which  
132 included BSI episodes due to Enterobacteriaceae at 13 University hospitals in Spain. The  
133 methods are detailed in previous reports [8, 9]. Briefly, consecutive adult patients with  
134 monomicrobial bacteraemia due to Enterobacteriaceae who received empirical treatment  
135 in the first 12 hours after the blood cultures were drawn were included. The original study  
136 was conducted between January 2011 and December 2013. Exclusion criteria were  
137 polymicrobial bacteraemia, non-hospitalized patients, do-not resuscitate orders,  
138 neutropenia (<500/ $\mu$ L) and survival <24 hours after blood cultures were drawn. For this  
139 analysis, patients from the Bacteraemia-MIC cohort were selected if: (1) initial treatment  
140 was monotherapy with an in vitro active BSD, including ertapenem or antipseudomonal  
141  $\beta$ -lactams such as meropenem, imipenem, doripenem, ceftazidime, cefepime or  
142 piperacillin/tazobactam; and (2) the causative microorganism was susceptible to any of  
143 the following narrower-spectrum drugs (NSD): ampicillin, amoxicillin/clavulanic acid,  
144 non-antipseudomonal cephalosporins such as cefazolin, cefuroxime, cefotaxime or  
145 ceftriaxone, trimethoprim/sulfamethoxazole, aminoglycosides, fosfomicin and  
146 fluoroquinolones. The classification of antibiotics as BSD or NSD was based on a

147 previously published consensus ranking of  $\beta$ -lactams according to spectrum and  
148 resistance-promoting potential [10]. Exclusion criteria were treatment change to another  
149 broader-spectrum drug between days 2 and 5 (as we were unable to rule out patients  
150 having secondary infections that would overestimate the comparative efficacy of NSD),  
151 and death before the susceptibility tests were available (since these patients did not have  
152 the opportunity to de-escalate). All patients were followed for 30 days.

153 The Institutional Review Board of the University Hospital Virgen Macarena,  
154 Seville, Spain, approved the study and waived the need to obtain informed consent due  
155 to the observational nature of the study. This analysis was reported according to STROBE  
156 recommendations (Supplementary Table S1) [11].

157

## 158 **Variables and definitions**

159 The main outcome variable was 30-day all-cause mortality. Secondary outcomes  
160 were: clinical response at day 21, and length of hospital stay among survivors. Clinical  
161 response was classified as clinical cure if all signs and symptoms of infection had been  
162 completely resolved, and failure if there were any persistent, recurrent or new signs and  
163 symptoms related to infection, or if death occurred.

164 The main exposure of interest was de-escalation, defined as switching from the  
165 empirical BSD to any of the NSDs, or from piperacillin-tazobactam, imipenem or  
166 meropenem to ertapenem. De-escalation was classified as early de-escalation (EDE) if  
167 performed in  $\leq 4$  days (the day when blood cultures were drawn was considered day 0),  
168 late de-escalation (LDE) it was from day 5 to day 7, or non-de-escalation (NDE) if the  
169 empirical drug was continued for at least  $\geq 7$  days.

170 Other exposure variables included demographic data, type of onset of infection  
171 (nosocomial or community-onset, the latter including non-nosocomial but healthare-

172 associated), chronic underlying conditions and severity according to the Charlson index  
173 [12], acute severity of underlying condition according to Pitt score [13] measured on day  
174 -1, SOFA score measured on day 0 [14], severe sepsis or septic shock at day 0 [15], source  
175 of infection using CDC criteria, [16] and microorganism.

176 All isolates were sent to the Hospital Universitario Virgen Macarena, where  
177 identification was confirmed and susceptibility to antimicrobials was studied using  
178 microdilution and interpreted according to EUCAST breakpoints [17]. Extended-  
179 spectrum beta-lactamase (ESBL), AmpC and carbapenemase production were studied by  
180 phenotypic methods according to EUCAST guideline on detection of resistance  
181 mechanisms [18], followed by PCR amplification and molecular sequencing. For the sake  
182 of simplicity, isolates producing ESBLs, AmpC or carbapenem resistance were  
183 considered as MDR.

184

### 185 **Statistical analysis**

186 The chi-square test or Fisher's exact test was used to compare categorical  
187 variables. The Mann-Whitney U test was used to compare continuous variables. When  
188 appropriate, continuous variables were dichotomized according to their association with  
189 death, using Classification and Regression Tree (CART) analysis. Multivariate Cox  
190 regression analysis was used to analyze the impact of EDE and LDE on 30-day mortality.  
191 Logistic regression and linear regression were used to identify the impact of EDE and  
192 LDE on failure and length of hospital stay among survivors, respectively. Variables with  
193 a P value of <0.2 in univariate comparisons and those considered of clinical importance  
194 were manually entered into the multivariate model. The variables in the models were  
195 selected manually using a backward stepwise process. Interactions and collinearity were



196 evaluated. Sensitivity analyses were performed by reclassifying the main exposure as  
197 EDE vs LDE+NDE, and as EDE+LDE vs NDE.

198 In addition, a propensity score (PS) was calculated for receiving EDE instead of  
199 NDE. Its predictive ability was calculated using the area under the receiver operating  
200 curve (AUROC) with 95% confidence intervals (CI), and the Hosmer-Lemeshow test was  
201 used for goodness of fit. The PS was used in two ways: as a covariate to control for  
202 residual confounding in multivariate models after checking for collinearity, and to  
203 perform a matched cohort analysis in which patients undergoing EDE and NDE were  
204 matched (1:1) according to their propensity scores using calipers of width 0.007.  
205 Statistical analysis was carried out using the SPSS program (SPSS 25.0, IBM Corp,  
206 Armonk, NY, USA).

207

## 208 **RESULTS**

209

210 The Bacteraemia-MIC cohort included 1058 patients with BSI due to  
211 Enterobacteriaceae; of these, 516 (48.7%) patients fulfilled the criteria for the de-  
212 escalation analysis (Figure 1). The number of patients per hospital ranged from 8 (1.6%)  
213 to 69 (13.4%). Overall, 241 (46.7%) patients received EDE, 95 (18.4%) LDE, and 180  
214 (34.8%) were not de-escalated. The proportion of EDE among hospitals with >20 cases  
215 ranged from 13% to 75.4%. The patients' characteristics are shown in Table 1, and the  
216 susceptibility data per microorganism is shown in Supplementary Table S2. Compared to  
217 patients who underwent EDE, those in the NDE group more frequently had nosocomial  
218 infections, had been admitted to the ICU, had respiratory tract infections and received  
219 empiric therapy with meropenem. Overall, 68 (13.2%) isolates were ESBL producers, 26  
220 (5%) AmpC producers, and 3 (0.6%) were carbapenem-resistant (none were

221 carbapenemase producers). Among patients undergoing de-escalation, the most frequent  
222 empirical drugs were piperacillin-tazobactam, and imipenem or meropenem and the most  
223 frequent drugs used for de-escalation were fluoroquinolones (68 patients in EDE and 38  
224 in LDE), cefotaxime or ceftriaxone (92 and 20) and amoxicillin-clavulanic acid (43 and  
225 26) (supplementary **Table S3**).

226

### 227 **Variables associated with EDE**

228 The association of different variables with EDE is shown in Table 2. The variable  
229 “center” was dichotomized into low and high proportions of patients with EDE. In  
230 multivariate analysis, bacteraemia caused by MDR isolates and nosocomial episodes  
231 empirically treated with imipenem or meropenem were associated with a lower  
232 probability of receiving EDE. Even after controlling for these variables, patients  
233 hospitalized in centers with a high proportion of EDE still had a higher probability of  
234 receiving EDE. The AUROC for the model was 0.72 (95% CI 0.66-0.75).

235

### 236 **Mortality analysis**

237 Mortality rates were 4.1% (10/241), 6.3% (6/95) and 9.4% (17/180) in patients  
238 with EDE, LDE and NDE, respectively (Table 1). The univariate and multivariate  
239 analysis of variables associated with 30-day mortality are shown in Table 3. Source of  
240 bacteraemia was dichotomized into urinary or biliary tract vs others, according to their  
241 association with mortality. Hospitals were also classified into those with lower and higher  
242 mortality, and this variable was retained in the models. Multivariate analysis (Table 3)  
243 selected Charlson >3, source other than urinary or biliary tract, presentation with severe  
244 sepsis or shock, and SOFA >4 as associated with mortality. Among de-escalated patients,  
245 no trend toward higher mortality was found, although the model showed poor

246 discrimination (AUROC=0.64; 95% CI: 0.52-0.75). In sensitivity analysis, the adjusted  
247 HR (95% CI) for mortality were: 0.67 (0.33-1.36, p=0.27) for EDE or LDE vs. NDE, and  
248 0.60 (0.27-1.30, p=0.19) for EDE vs LDE-NDE. In nosocomial episodes, the adjusted HR  
249 (95% CI) for mortality in EDE and LDE were 0.46 (0.13-1.60) and 1.78 (0.53-5.92),  
250 respectively, and in community-onset, 0.70 (0.22-2.14) and 0.52 (0.10-2.56),  
251 respectively. No significant interactions were found in either model.

252 We then investigated the impact of EDE versus NDE including the PS for EDE  
253 (LDE patients were excluded from this analysis) (Table 3). No significant collinearity  
254 was found between the PS and other variables. Again, EDE did not show an association  
255 with higher 30-day mortality (adjusted HR=0.69; 95% CI: 0.29-1.65; p=0.41); the  
256 AUROC of this model was higher (0.72; 95% CI: 0.61-0.82). Finally, we matched 137  
257 pairs of patients receiving EDE or NDE according to PS. Matched sub-cohorts had  
258 exposure to all other variables (supplementary Table S4). Mortality was 5.4% (n=7) in  
259 EDE and 7.7% in NDE (n=10) (HR=0.98; 95% CI: 0.76-1.26; p=0.84).

260

### 261 **Clinical cure and length of stay**

262 Overall, 35 patients showed failure at the end of antibiotic treatment (6.7%):  
263 11/2421 (4.6%) with EDE, 6/95 (6.3%) with LDE, and 18/180 (10%) with NDE. The  
264 univariate and multivariate analyses of variables associated with failure are shown in  
265 Table 4. The multivariate model showed that Charlson index >3, severe sepsis/septic  
266 shock at presentation and SOFA score at day 0 were associated with higher treatment  
267 failure, while a urinary or biliary source was protective. The failure rate for these sources  
268 was 3.7%, while it was 14.3% for other sources. De-escalation was not found to be  
269 associated with failure (Table 4).

270 The median hospital stay after BSI was 14 days (IQR 9-24) and according to  
271 group, it was 14 days (9-28) for EDE, 13 (7-20) for LDE and 15 days (IQR 10-25) for  
272 NDE. The univariate analysis of variables associated with length of hospital stay is shown  
273 at Supplementary **Table S5**. Linear regression model of variables associated with length  
274 of hospital stay showed that nosocomial acquisition, Charlson index >3 and the presence  
275 of severe sepsis/septic shock at presentation were associated with more days of  
276 hospitalization (p values 0.006, <0.001 and 0.01 respectively). EDE and NDE were not  
277 found to be associated with longer hospital stay (p=0.56 and 0.67, respectively)  
278 (Supplementary **Table S6**).

279

## 280 **DISCUSSION**

281

282 In this cohort, less than half the candidate patients received early de-escalation,  
283 and one third of patients were never de-escalated. Patients with MDR isolates or  
284 nosocomial infections empirically treated with imipenem or meropenem had a lower  
285 probability of de-escalation. Finally, neither EDE nor LDE were shown to be associated  
286 with worse outcomes.

287 To our knowledge, this is by far the biggest study of de-escalation among patients  
288 with bacteraemia [6]. It is important to note that we only included non-neutropenic, adult  
289 patients with monomicrobial bacteraemia due to Enterobacteriaceae who received early  
290 active empirical monotherapy with **ertapenem or antipseudomonal beta-lactams**. We are  
291 not therefore addressing the impact of changing from combination therapy to  
292 monotherapy, but only of changes in the empirical drug used. This population is  
293 somewhat more homogeneous than those considered in most previous studies. **In any**  
294 **setting, the proportion of patients with BSI-E who are candidates to de-escalation in**

295 according to the criteria used would depend on the antibiotics used empirically and the  
296 susceptibility of the isolates; to provide more information in this regard, the susceptibility  
297 data of the Enterobacteriaceae isolated in the whole Bacteremia-MIC cohort is shown in  
298 Supplementary Table S7.

299 The definition of de-escalation used is open to debate [19]. Unfortunately, many  
300 previous studies did not provide a specific definition of de-escalation and/or the drugs  
301 considered. The objective of de-escalation is to reduce exposure at the individual and  
302 group levels to drugs with a high negative ecological impact. However, the ecological  
303 impact of the drugs may depend on different variables, including local epidemiology, the  
304 previous colonization status of patients, microbiota composition, and the dosing or  
305 duration of antibiotic therapy. In this study we used a classification of beta-lactams  
306 developed by consensus [10]; in this consensus, imipenem and meropenem ranked  
307 highest in terms of spectrum width and highest resistance selecting potential, followed by  
308 ertapenem and piperacillin-tazobactam or antipseudomonal cephalosporins. As drugs for  
309 de-escalation, we included the lower-ranked beta-lactams in the consensus, in addition to  
310 other drugs suitable for oral use [20] that would allow the earlier discharge of patients,  
311 such as fluoroquinolones and trimethoprim-sulfamethoxazole. We also analyzed late de-  
312 escalation.

313 De-escalation is performed less frequently than is desirable [21]. The main  
314 barriers identified for de-escalations are uncertainties about etiology, inadequate  
315 empirical therapy and isolation of MDR bacteria [20, 21]. In patients with monomicrobial  
316 bacteraemia due to Enterobacteriaceae, the only uncertainty about etiology is the  
317 possibility of polymicrobial infection in certain types of infection, typically  
318 intraabdominal and some skin/skin structure-associated infections. In our study, source  
319 of BSI was not associated with higher rates of de-escalation when controlling for

320 confounders, and inadequate empirical therapy was an exclusion criterion. However,  
321 MDR bacteria were associated with a lower probability of de-escalation. We also  
322 identified nosocomial infection as a predictor for no de-escalation when these patients  
323 had been empirically treated with imipenem or meropenem, which may be a marker for  
324 more complex clinical situations. We suspect that other factors, such as stewardship  
325 interventions, less awareness of susceptibility results at weekends, and the training and  
326 opinions of individual prescribers could also play a role in de-escalation practice and  
327 merit specific studies.

328         In crude analysis, de-escalation was associated with lower mortality and failure.  
329 This was probably due to confounding by indication as the associations were no longer  
330 significant when other mortality predictors were considered in multivariate analysis,  
331 which is similar to the results found in the meta-analysis by Paul et al for observational  
332 studies of patients with severe sepsis or bacteraemia [6]. The results are reinforced by the  
333 fact that all our estimates in different analyses were consistent, and that we included  
334 mortality, failure of treatment and length of stay as outcome variables. Interestingly, the  
335 estimates provided by multivariate analysis were much less accurate than those provided  
336 by the PS-based matched pairs analysis. Our results strongly suggest therefore that de-  
337 escalation is safe. In fact, theoretically, it may have some individual beneficial effects if  
338 secondary infections caused by MDR bacteria are reduced, although demonstrating such  
339 an effect would require specific studies with a very large number of patients. Any analysis  
340 of population-level benefits would also require specific studies.

341         Our study has several limitations. Because it is not a randomized controlled trial,  
342 unmeasured confounding variables or residual confounding cannot be ruled out. The data  
343 were collected several years ago and changes in antimicrobial resistance may influence  
344 the results. Moreover, despite being controlled in the analysis, differences in clinical

345 practice at each center might have influenced the outcomes. Some strengths of the study  
346 are its multicenter character, the use of clearly specified definitions, and the use of  
347 advance statistical methodologies to control for confounders.

348 In conclusion, the results of this study reinforce the fact that antibiotic de-  
349 escalation in patients with monomicrobial bacteraemia due to Enterobacteriaceae does  
350 not have a detrimental impact on outcome, 30-day all-cause mortality, failure, or length  
351 of hospital stay when compared with continuation with broad-spectrum antibiotics. These  
352 results may be useful for antibiotic stewardship activities.

353

#### 354 **CONFLICT OF INTEREST**

355

356 ZRPB has received honoraria for educational talks funded by Gilead. JRB has been an  
357 advisor for AstraZeneca, Merck, InfectoPharm, Achaogen and Basilea, and a speaker at  
358 educational courses for AstraZeneca and Merck. All other authors declare not to have  
359 conflict of interest.

360

#### 361 **ACKNOWLEDGEMENTS**

362 **Other investigators from the REIPI/GEIH-SEIMC BACTERAEEMIA-MIC group:**

363 M. de Cueto (Unidad Clínica Intercentros de Enfermedades Infecciosas, Microbiología y  
364 Medicina Preventiva, Hospital Universitario Virgen Macarena, Seville, Spain), AM  
365 Planes Reig (Departamento de Microbiología, Hospital Universitari Vall d'Hebron,  
366 Barcelona, Spain), F. Tubau Quintano (Servicio de Microbiología, Hospital Universitario  
367 de Bellvitge-IDIBELL, Barcelona, Spain), C. Peña (Servicio de Enfermedades  
368 Infecciosas, Hospital Universitario de Bellvitge-IDIBELL, Barcelona, Spain), ME Galán  
369 Otalora (Hospital de la Santa Creu i Sant Pau, Barcelona, Spain), C. Ruíz de Alegría

370 (Servicio de Microbiología, Hospital Universitario Marqués de Valdecilla, Santander,  
371 Spain), R. Cantón (Servicio de Microbiología, Hospital Universitario Ramón y Cajal and  
372 Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain), JA Lepe  
373 and JM Cisneros (Unidad Clínica de Enfermedades Infecciosas, Microbiología y  
374 Medicina Preventiva, Hospital Virgen del Rocío, Seville, Spain), J. Torre-Cisneros, R.  
375 Lara (Unidad Clínica de Enfermedades infecciosas Hospital Universitario Reina Sofía.  
376 Instituto Maimónides de Investigación Clínica (IMIBIC), Universidad de Córdoba,  
377 Córdoba, Spain).

### 378 **FINANCIAL SUPPORT**

379 This work was supported by the Instituto de Salud Carlos III, Ministry of  
380 Economy and Competitiveness, Spain (FIS; PI10/02021) co-financed by European  
381 Development Regional Fund ‘A way to achieve Europe’ ERDF, Spanish Network for  
382 Research in Infectious Diseases (REIPI RD12/0015).

383

### 384 **REFERENCES:**

- 385 1. Kollef MH. Broad-spectrum antimicrobials and the treatment of serious bacterial  
386 infections: getting it right up front. *Clin Infect Dis* 2008; 47: S3–13.
- 387 2. Karam G, Chastre J, Wilcox MH, Vincent JL. Antibiotic strategies in the era of  
388 multidrug resistance. *Crit Care*. 2016 Jun 22;20(1):136.
- 389 3. Barlam TF, Cosgrove SE, Abbo LM, MacDougall C, Schuetz AN, Septimus EJ,  
390 et al. Implementing an Antibiotic Stewardship Program: Guidelines by the  
391 Infectious Diseases Society of America and the Society for Healthcare  
392 Epidemiology of America. *Clin Infect Dis* 2016; 62: e51-77.



- 393 4. Silva BN, Andriolo RB, Atallah AN, Salomão R. De-escalation of antimicrobial  
394 treatment for adults with sepsis, severe sepsis or septic shock. *Cochrane Database*  
395 *Syst Rev.* 2013; 3: CD007934.
- 396 5. López-Cortés LE, Cueto M, Rodríguez-Baño J. How should we best treat patients  
397 with bloodstream infections? *Future Microbiol* 2017; 12: 927-930.
- 398 6. Paul M, Dickstein Y, Raz-Pasteur A. Antibiotic de-escalation for bloodstream  
399 infections and pneumonia: systematic review and meta-analysis. *Clin Microbiol*  
400 *Infect* 2016; 22: 960-967.
- 401 7. López-Cortés LE, Rosso-Fernández C, Núñez-Núñez M, et al. Targeted  
402 simplification versus antipseudomonal broad-spectrum beta-lactams in patients  
403 with bloodstream infections due to Enterobacteriaceae (SIMPLIFY): a study  
404 protocol for a multicentre, open-label, phase III randomised, controlled, non-  
405 inferiority clinical trial. *BMJ Open* 2017; 7: e015439.
- 406 8. Delgado-Valverde M, Torres E, Valiente-Mendez A, et al. Impact of the MIC of  
407 piperacillin/tazobactam on the outcome for patients with bacteraemia due to  
408 Enterobacteriaceae: the Bacteraemia-MIC project. *J Antimicrob Chemother.* 2016  
409 Feb;71(2):521-30.
- 410 9. Delgado-Valverde M, Valiente-Mendez A, Torres E, et al. MIC of  
411 amoxicillin/clavulanate according to CLSI and EUCAST: discrepancies and  
412 clinical impact in patients with bloodstream infections due to Enterobacteriaceae.  
413 *J Antimicrob Chemother.* 2017 May 1;72(5):1478-1487.
- 414 10. Weiss E, Zahar JR, Lesprit P, Ruppe E, Leone M, Chastre J, Lucet JC, Paugam-  
415 Burtz C, Brun-Buisson C, Timsit JF; De-escalation Study Group. Elaboration of  
416 a consensual definition of de-escalation allowing a ranking of  $\beta$ -lactams. *Clin*  
417 *Microbiol Infect* 2015; 21: 649.e1-10.

- 418 11. Von Elm E, et al. The strengthening the reporting of observational studies in  
419 epidemiology (STROBE) statement: guidelines for reporting observational  
420 studies. *J. Clin Epidemiol.* 2008;61:344-349.
- 421 12. Charlson ME, Pompei P, Ales KL et al. A new method of classifying prognostic  
422 co-morbidity in longitudinal studies: development and validation. *J Chron Dis.*  
423 1987; 40: 373–83.
- 424 13. Hilf M, Yu Vh, Sharp J, et al. Antibiotic therapy for *Pseudomonas aeruginosa*  
425 bacteremia: outcome correlations in a prospective study of 200 patients. *Am J*  
426 *Med.* 1989; 87: 540-546.
- 427 14. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure  
428 Assessment) score to describe organ dysfunction/failure. On behalf of the  
429 Working Group on Sepsis-Related Problems of the European Society of Intensive  
430 Care Medicine. *Intensive Care Med.* 1996 Jul;22(7):707-10.
- 431 15. American College of Chest Physicians/Society of Critical Care Medicine  
432 Consensus Conference: definitions for sepsis and organ failure and guidelines for  
433 the use of innovative therapies in sepsis. *Crit Care Med.* 1992; 20: 864–74.
- 434 16. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health  
435 care-associated infection and criteria for specific types of infections in the acute  
436 care setting. *Am J Infect Control* 2008; 36: 309-32.
- 437 17. EUCAST. Breakpoint tables for interpretation of MICs and zone diameters,  
438 version 5.0. 2015. Available at:  
439 [http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Breakpoint ta](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf)  
440 [bles/v\\_5.0\\_Breakpoint\\_Table\\_01.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf).
- 441 18. The EUCAST guideline on detection of resistance mechanisms, version 1.0. 2013.  
442 Available at:

- 443 [http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Resistance\\_mechanisms/EUCAST\\_detection\\_of\\_resistance\\_mechanisms\\_v1.0\\_20131211.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Resistance_mechanisms/EUCAST_detection_of_resistance_mechanisms_v1.0_20131211.pdf).
- 444
- 445 19. Huttner B, Pulcini C, Schouten J. De-constructing de-escalation. *Clin Microbiol Infect* 2016; 22: 958-959.
- 446
- 447 20. Garnacho-Montero J, Escorezca-Ortega A, Fernández-Delgado E. Antibiotic de-escalation in the ICU: how is it best done? *Curr Opin Infect Dis* 2015; 28:193-8.
- 448
- 449 21. Masterton RG. Antibiotic de-escalation. *Crit Care Clin* 2011; 27: 149-62.
- 450

1           **Impact of de-escalation on prognosis of patients with bacteraemia due to**  
2           **Enterobacteriaceae: a post-hoc analysis from a multicenter prospective cohort**

3  
4           **Running title:** De-escalation in Enterobacteriaceae bacteraemia.

5  
6           **Authors:** Zaira R. (ZRPB) Palacios-Baena<sup>1</sup>, Mercedes (MDV) Delgado-Valverde<sup>1</sup>,  
7           Adoración (AVM) Valiente Méndez<sup>1</sup>, Benito (BA) Almirante<sup>2</sup>, Silvia (SGZ) Gómez-  
8           Zorrilla<sup>3</sup>, Núria (NB) Borrell<sup>4</sup>, Juan E. (JEC) Corzo<sup>5</sup>, Mercedes (MG) Gurguí<sup>6</sup>, Cristina  
9           (CC) de la Calle<sup>7</sup>, Lara (LGA) García-Álvarez<sup>8</sup>, Lucía (LR) Ramos<sup>9</sup>, Mónica (MG)  
10           Gozalo<sup>10</sup>, María Isabel (MIM) Morosini<sup>11</sup>, José (JM) Molina<sup>12</sup>, Manuel (MC) Causse<sup>13</sup>,  
11           Álvaro (AP) Pascual<sup>1</sup>, Jesús (JRB) Rodríguez-Baño<sup>1,\*</sup> on behalf of the REIPI/GEIRAS-  
12           SEIMC BACTERIEMIA-MIC group.

13  
14           <sup>1</sup>Unidad Clínica de Enfermedades Infecciosas, Microbiología y Medicina Preventiva,  
15           Hospital Universitario Virgen Macarena/Departamento de Medicina y Microbiología,  
16           Universidad de Sevilla/Instituto de Biomedicina de Sevilla (IBiS). Sevilla, Spain.

17           <sup>2</sup>Hospital Universitari Vall d'Hebron. Barcelona, España.

18           <sup>3</sup>Hospital de Bellvitge. Barcelona, Spain.

19           <sup>4</sup>Hospital Universitario Son Espases. Palma de Mallorca, Islas Baleares, España.

20           <sup>5</sup>Unidad Clínica Enfermedades Infecciosas y Microbiología. Hospital Universitario  
21           Virgen de Valme. Sevilla, Spain.

22           <sup>6</sup>Hospital de la Santa Creu i Sant Pau, Barcelona, Spain.

23           <sup>7</sup>Hospital Clínic i Provincial, Barcelona, Spain.

24           <sup>8</sup>Departamento de Enfermedades Infecciosas. Hospital San Pedro-CIBIR. Logroño,  
25           Spain.

26 <sup>9</sup>Hospital Universitario A Coruña. A Coruña, Spain.

27 <sup>10</sup>Hospital Marqués de Valdecilla-IDIVAL, Santander. Spain.

28 <sup>11</sup>Hospital Ramón y Cajal. Madrid, Spain.

29 <sup>12</sup>Unidad Clínica de Enfermedades Infecciosas, Microbiología y Medicina Preventiva,  
30 Hospital Universitario Virgen del Rocío/Universidad de Sevilla/Instituto de Biomedicina  
31 de Sevilla (IBIS). Seville, Spain.

32 <sup>13</sup>Unidad de Gestión Clínica de Microbiología. Hospital Universitario Reina Sofía.  
33 Instituto Maimónides de Investigación Clínica (IMIBIC), Universidad de Córdoba.  
34 Córdoba, Spain.

35

36 **Keywords:** De-escalation, streamlining, Enterobacteriaceae, bloodstream infections,  
37 mortality.

38

39 **\*Corresponding author:** Jesús Rodríguez-Baño. Unidad Clínica de Enfermedades  
40 Infecciosas, Microbiología y Medicina Preventiva, Hospital Universitario Virgen  
41 Macarena, Avda Dr. Fedriani, 3, 41009 Sevilla, Spain. Phone: +34 677906512. E-mail  
42 address: [jesusrb@us.es](mailto:jesusrb@us.es)

43 **Alternative corresponding author:** Zaira R. Palacios-Baena, Unidad Clínica de  
44 Enfermedades Infecciosas, Microbiología y Medicina Preventiva, Hospital Universitario  
45 Virgen Macarena, Avda Dr. Fedriani, 3, 41009 Sevilla, Spain. Phone: +34 653276353.  
46 E-mail address: [Zaira.palacios.baena@hotmail.com](mailto:Zaira.palacios.baena@hotmail.com)

47

48

49 **SUMMARY OF THE MAIN POINTS**

50 De-escalation from empirical antipseudomonal  $\beta$ -lactams or ertapenem to lower spectrum  
51 antibiotics in patients with bacteremia due to Enterobacteriaceae was not associated with  
52 any detrimental impact in terms of mortality, clinical failure or length of hospital stay

53

54

55

56

57

58

59

60

61

62

63

64

65

66

67

68

69

70

71

72

73

74 **ABSTRACT**

75

76 **Background:** More data are needed about the safety of antibiotic de-escalation in specific  
77 clinical situations as a strategy to reduce exposure to broad-spectrum antibiotics. The  
78 aims of this study were to investigate predictors of de-escalation and its impact on the  
79 outcome of patients with bloodstream infection due to Enterobacteriaceae (BSI-E).

80 **Methods:** A post-hoc analysis was performed of a prospective, multicenter cohort of  
81 patients with BSI-E initially treated with ertapenem or antipseudomonal  $\beta$ -lactams.  
82 Logistic regression was used to analyze factors associated with early de-escalation (EDE)  
83 and Cox regression for the impact of EDE and late de-escalation (LDE) on 30-day all-  
84 cause mortality. A propensity score (PS) for EDE vs. no de-escalation (NDE) was  
85 calculated. Failure at end of treatment and length of hospital stay were also analyzed.

86 **Results:** Overall, 516 patients were included; EDE was performed in 241 patients (46%),  
87 LDE in 98 (18%) and NDE in 180 (35%). Variables independently associated with a  
88 lower probability of EDE were multidrug-resistant isolates (OR 0.50, 95% CI 0.30-0.83)  
89 and nosocomial infection empirically treated with imipenem or meropenem (OR 0.35,  
90 95% CI 0.14-0.87). After controlling for confounders, EDE was not associated with  
91 increased risk of mortality; Hazard ratios (HR) and (95% CI) were: general model, 0.58  
92 (0.25-1.31), model with PS, 0.69 (0.29-1.65), and PS-matched pairs, 0.98 (0.76-1.26).  
93 LDE was not associated with mortality. De-escalation was not associated with clinical  
94 failure or length of hospital stay.

95 **Conclusions:** De-escalation in patients with monomicrobial bacteraemia due to  
96 Enterobacteriaceae was not associated with a detrimental impact on clinical outcome.

97 **INTRODUCTION**

98

99 Patients with sepsis are frequently treated empirically with broad-spectrum drugs  
100 (BSD) because the early administration of active drugs has been associated with improved  
101 outcome, particularly in the presence of septic shock [1]. This can lead to overuse of these  
102 drugs, which is usually considered to be one of the contributing factors for the spread of  
103 multidrug-resistant (MDR) bacteria [2]. To minimize this problem, streamlining or de-  
104 escalation from broad- to narrower-spectrum drugs is usually advocated once the  
105 susceptibility of the causative agent of the infection is known, and antimicrobial  
106 stewardship programs frequently include interventions facilitating or recommending this  
107 practice [3]. However, de-escalation is performed less frequently than is desirable.  
108 Barriers include uncertainty among many prescribers; indeed, although de-escalation is  
109 considered standard of care for most infectious diseases specialists, a recent systematic  
110 review concluded that there is no adequate evidence as to whether de-escalation of  
111 antimicrobial agents is effective and safe for adults with sepsis [4]. Hence, providing  
112 more information about the safety of de-escalation would help increase implementation,  
113 and knowledge of the variables influencing the performance of de-escalation would lead  
114 to better targeting of interventions promoting this practice.

115 Bloodstream infections (BSI) are an ideal model for de-escalation, since etiology  
116 and susceptibility are known, and a more specialized evaluation of patients is possible  
117 [5]. A meta-analysis including studies of sepsis, bacteraemia and pneumonia found a  
118 trend towards higher mortality with de-escalation in 3 randomized trials, but lower  
119 mortality in observational studies [6]. However, the studies were heterogeneous with  
120 respect to type of patient and infection, etiology, definitions used and interventions, which  
121 precludes high confidence in the meta-analytic estimates. Studies of specific populations



122 and etiologies are needed therefore. A randomized trial of patients with bacteraemia due  
123 to Enterobacteriaceae is now recruiting [7], although the results will not be available for  
124 2 years. The objectives of this study were to evaluate the frequency of variables associated  
125 with de-escalation, and the impact of de-escalation on prognosis only among patients with  
126 bacteraemia due to Enterobacteriaceae.

127

## 128 **METHODS**

129

### 130 **Study design, sites and study population**

131 This is a post-hoc analysis of the prospective Bacteraemia-MIC cohort, which  
132 included BSI episodes due to Enterobacteriaceae at 13 University hospitals in Spain. The  
133 methods are detailed in previous reports [8, 9]. Briefly, consecutive adult patients with  
134 monomicrobial bacteraemia due to Enterobacteriaceae who received empirical treatment  
135 in the first 12 hours after the blood cultures were drawn were included. The original study  
136 was conducted between January 2011 and December 2013. Exclusion criteria were  
137 polymicrobial bacteraemia, non-hospitalized patients, do-not resuscitate orders,  
138 neutropenia ( $<500/\mu\text{L}$ ) and survival  $<24$  hours after blood cultures were drawn. For this  
139 analysis, patients from the Bacteraemia-MIC cohort were selected if: (1) initial treatment  
140 was monotherapy with an in vitro active BSD, including antipseudomonal  $\beta$ -lactams such  
141 as meropenem, imipenem, doripenem, ceftazidime, cefepime or piperacillin/tazobactam,  
142 or ertapenem; and (2) the causative microorganism was susceptible to any of the  
143 following narrower-spectrum drugs (NSD): ampicillin, amoxicillin/clavulanic acid, non-  
144 antipseudomonal cephalosporins such as cefazolin, cefuroxime, cefotaxime or  
145 ceftriaxone, trimethoprim/sulfamethoxazole, aminoglycosides, fosfomycin and  
146 fluoroquinolones. The classification of antibiotics as BSD or NSD was based on a

147 previously published consensus ranking of  $\beta$ -lactams according to spectrum and  
148 resistance-promoting potential [10]. Exclusion criteria were treatment change to another  
149 broader-spectrum drug between days 2 and 5 (as we were unable to rule out patients  
150 having secondary infections that would overestimate the comparative efficacy of NSD),  
151 and death before the susceptibility tests were available (since these patients did not have  
152 the opportunity to de-escalate). All patients were followed for 30 days.

153 The Institutional Review Board of the University Hospital Virgen Macarena,  
154 Seville, Spain, approved the study and waived the need to obtain informed consent due  
155 to the observational nature of the study. This analysis was reported according to STROBE  
156 recommendations (Supplementary Table S1) [11].

157

#### 158 **Variables and definitions**

159 The main outcome variable was 30-day all-cause mortality. Secondary outcomes  
160 were: clinical response at day 21, and length of hospital stay among survivors. Clinical  
161 response was classified as clinical cure if all signs and symptoms of infection had been  
162 completely resolved, and failure if there were any persistent, recurrent or new signs and  
163 symptoms related to infection, or if death occurred.

164 The main exposure of interest was de-escalation, defined as switching from the  
165 empirical BSD to any of the NSDs, or from piperacillin-tazobactam, imipenem or  
166 meropenem to ertapenem. De-escalation was classified as early de-escalation (EDE) if  
167 performed in  $\leq 4$  days (the day when blood cultures were drawn was considered day 0),  
168 late de-escalation (LDE) it was from day 5 to day 7, or non-de-escalation (NDE) if the  
169 empirical drug was continued for at least  $\geq 7$  days.

170 Other exposure variables included demographic data, type of onset of infection  
171 (nosocomial, healthcare-associated or community), chronic underlying conditions and

172 severity according to the Charlson index [12], acute severity of underlying condition  
173 according to Pitt score [13] measured on day -1, SOFA score measured on day 0 [14],  
174 severe sepsis or septic shock at day 0 [15], source of infection using CDC criteria, [16]  
175 and microorganism.

176 All isolates were sent to the Hospital Universitario Virgen Macarena, where  
177 identification was confirmed and susceptibility to antimicrobials was studied using  
178 microdilution, and interpreted according to EUCAST breakpoints [17]. Extended-  
179 spectrum beta-lactamase (ESBL), AmpC and carbapenemase production were studied by  
180 phenotypic methods, followed by PCR amplification and molecular sequencing. For the  
181 sake of simplicity, isolates producing ESBLs, AmpC or carbapenem resistance were  
182 considered as MDR.

183

#### 184 **Statistical analysis**

185 The chi-square test or Fisher's exact test was used to compare categorical  
186 variables. The Mann-Whitney U test was used to compare continuous variables. When  
187 appropriate, continuous variables were dichotomized according to their association with  
188 death, using Classification and Regression Tree (CART) analysis. Multivariate Cox  
189 regression analysis was used to analyze the impact of EDE and LDE on 30-day mortality.  
190 Logistic regression and linear regression were used to identify the impact of EDE and  
191 LDE on failure and length of hospital stay among survivors, respectively. Variables with  
192 a P value of <0.2 in univariate comparisons and those considered of clinical importance  
193 were manually entered into the multivariate model. The variables in the models were  
194 selected manually using a backward stepwise process. Interactions and collinearity were  
195 evaluated. Sensitivity analyses were performed by reclassifying the main exposure as  
196 EDE vs LDE+NDE, and as EDE+LDE vs NDE.

197 In addition, a propensity score (PS) was calculated for receiving EDE instead of  
198 NDE. Its predictive ability was calculated using the area under the receiver operating  
199 curve (AUROC) with 95% confidence intervals (CI), and the Hosmer-Lemeshow test was  
200 used for goodness of fit. The PS was used in two ways: as a covariate to control for  
201 residual confounding in multivariate models after checking for collinearity, and to  
202 perform a matched cohort analysis in which patients undergoing EDE and NDE were  
203 matched (1:1) according to their propensity scores using calipers of width 0.007.  
204 Statistical analysis was carried out using the SPSS program (SPSS 25.0, IBM Corp,  
205 Armonk, NY, USA).

206

## 207 **RESULTS**

208

209 The Bacteraemia-MIC cohort included 1058 patients with BSI due to  
210 Enterobacteriaceae; of these, 516 (48.7%) patients fulfilled the criteria for the de-  
211 escalation analysis (Figure 1). The number of patients per hospital ranged from 8 (1.6%)  
212 to 69 (13.4%). Overall, 241 (46.7%) patients received EDE, 95 (18.4%) LDE, and 180  
213 (34.8%) were not de-escalated. The proportion of EDE among hospitals with >20 cases  
214 ranged from 13% to 75.4%. The patients' characteristics are shown in Table 1. Compared  
215 to patients who underwent EDE, those in the NDE group more frequently had nosocomial  
216 infections, had been admitted to the ICU, had respiratory tract infections and received  
217 empiric therapy with meropenem. Overall, 70 (13.6%) isolates were ESBL producers, 26  
218 (5.1%) AmpC producers, and 3 (0.6%) were carbapenem-resistant (none were  
219 carbapenemase producers). Among patients undergoing de-escalation, the most frequent  
220 empirical drugs were piperacillin-tazobactam, and imipenem or meropenem and the most  
221 frequent drugs used for de-escalation were fluoroquinolones (68 patients in EDE and 38

222 in LDE), cefotaxime or ceftriaxone (92 and 20) and amoxicillin-clavulanic acid (43 and  
223 26) (supplementary Table S2).

224

#### 225 **Variables associated with EDE**

226 The association of different variables with EDE is shown in Table 2. The variable  
227 “center” was dichotomized into low and high proportions of patients with EDE. In  
228 multivariate analysis, bacteraemia caused by MDR isolates and nosocomial episodes  
229 empirically treated with imipenem or meropenem were associated with a lower  
230 probability of receiving EDE. Even after controlling for these variables, patients  
231 hospitalized in centers with a high proportion of EDE still had a higher probability of  
232 receiving EDE. The AUROC for the model was 0.72 (95% CI 0.66-0.75).

233

#### 234 **Mortality analysis**

235 Mortality rates were 4.1% (10/241), 6.3% (6/95) and 9.4% (17/180) in patients  
236 with EDE, LDE and NDE, respectively (Table 1). The univariate and multivariate  
237 analysis of variables associated with 30-day mortality are shown in Table 3. Source of  
238 bacteraemia was dichotomized into urinary or biliary tract vs others, according to their  
239 association with mortality. Hospitals were also classified into those with lower and higher  
240 mortality, and this variable was retained in the models. Multivariate analysis (Table 3)  
241 selected Charlson >3, source other than urinary or biliary tract, presentation with severe  
242 sepsis or shock, and SOFA >4 as associated with mortality. Among de-escalated patients,  
243 no trend toward higher mortality was found, although the model showed poor  
244 discrimination (AUROC=0.64; 95% CI: 0.52-0.75). In sensitivity analysis, the adjusted  
245 HR for mortality were: 0.67 (95% CI 0.33-1.36, p=0.27) for EDE or LDE vs. NDE, and

246 0.60 (95% CI 0.27-1.30, p=0.19) for EDE vs LDE-NDE. No significant interactions were  
247 found in either model.

248 We then investigated the impact of EDE versus NDE including the PS for EDE  
249 (LDE patients were excluded from this analysis) (Table 3). No significant collinearity  
250 was found between the PS and other variables. Again, EDE did not show an association  
251 with higher 30-day mortality (adjusted HR=0.69; 95% CI: 0.29-1.65; p=0.41); the  
252 AUROC of this model was higher (0.72; 95% CI: 0.61-0.82). Finally, we matched 137  
253 pairs of patients receiving EDE or NDE according to PS. Matched sub-cohorts had  
254 exposure to all other variables (supplementary Table S3). Mortality was 5.4% (n=7) in  
255 EDE and 7.7% in NDE (n=10) (HR=0.98; 95% CI: 0.76-1.26; p=0.84).

256

#### 257 **Clinical cure and length of stay**

258 Overall, 35 patients showed failure at the end of antibiotic treatment (6.7%):  
259 11/2421 (4.6%) with EDE, 6/95 (6.3%) with LDE, and 18/180 (10%) with NDE. The  
260 univariate and multivariate analyses of variables associated with failure are shown in  
261 Table 4. The multivariate model showed that Charlson index >3, severe sepsis/septic  
262 shock at presentation and SOFA score at day 0 were associated with higher treatment  
263 failure, while a urinary or biliary source were protective factors. De-escalation was not  
264 found to be associated with failure (Table 4).

265 The median hospital stay after BSI was 14 days (IQR 9-24) and according to  
266 group, it was 14 days (9-28) for EDE, 13 (7-20) for LDE and 15 days (IQR 10-25) for  
267 NDE. The univariate analysis of variables associated with length of hospital stay is shown  
268 at Supplementary Table S4. Linear regression model of variables associated with length  
269 of hospital stay showed that nosocomial acquisition, Charlson index >3 and the presence  
270 of severe sepsis/septic shock at presentation were associated with more days of

271 hospitalization (p values 0.006, <0.001 and 0.01 respectively). EDE and NDE were not  
272 found to be associated with longer hospital stay (p=0.56 and 0.67, respectively)  
273 (Supplementary Table S5).

274

## 275 **DISCUSSION**

276

277 In this cohort, less than half the candidate patients received early de-escalation,  
278 and one third of patients were never de-escalated. Patients with MDR isolates or  
279 nosocomial infections empirically treated with imipenem or meropenem had a lower  
280 probability of de-escalation. Finally, neither EDE nor LDE were shown to be associated  
281 with worse outcomes.

282 To our knowledge, this is by far the biggest study of de-escalation among patients  
283 with bacteraemia [6]. It is important to note that we only included non-neutropenic, adult  
284 patients with monomicrobial bacteraemia due to Enterobacteriaceae who received early  
285 active empirical monotherapy with antipseudomonal beta-lactams or ertapenem. We are  
286 not therefore addressing the impact of changing from combination therapy to  
287 monotherapy, but only of changes in the empirical drug used. This population is  
288 somewhat more homogeneous than those considered in most previous studies.

289 The definition of de-escalation used is open to debate [18]. Unfortunately, many  
290 previous studies did not provide a specific definition of de-escalation and/or the drugs  
291 considered. The objective of de-escalation is to reduce exposure at the individual and  
292 group levels to drugs with a high negative ecological impact. However, the ecological  
293 impact of the drugs may depend on different variables, including local epidemiology, the  
294 previous colonization status of patients, microbiota composition, and the dosing or  
295 duration of antibiotic therapy. In this study we used a classification of beta-lactams

296 developed by consensus [10]; in this consensus, imipenem and meropenem ranked  
297 highest in terms of spectrum width and highest resistance selecting potential, followed by  
298 ertapenem and piperacillin-tazobactam or antipseudomonal cephalosporins. As drugs for  
299 de-escalation, we included the lower-ranked beta-lactams in the consensus, in addition to  
300 other drugs suitable for oral use [19] that would allow the earlier discharge of patients,  
301 such as fluoroquinolones and trimethoprim-sulfamethoxazole. We also analyzed late de-  
302 escalation.

303 De-escalation is performed less frequently than is desirable [20]. The main  
304 barriers identified for de-escalations are uncertainties about etiology, inadequate  
305 empirical therapy and isolation of MDR bacteria [19, 20]. In patients with monomicrobial  
306 bacteraemia due to Enterobacteriaceae, the only uncertainty about etiology is the  
307 possibility of polymicrobial infection in certain types of infection, typically  
308 intraabdominal and some skin/skin structure-associated infections. In our study, source  
309 of BSI was not associated with higher rates of de-escalation when controlling for  
310 confounders, and inadequate empirical therapy was an exclusion criterion. However,  
311 MDR bacteria were associated with a lower probability of de-escalation. We also  
312 identified nosocomial infection as a predictor for no de-escalation when these patients  
313 had been empirically treated with imipenem or meropenem, which may be a marker for  
314 more complex clinical situations. We suspect that other factors, such as stewardship  
315 interventions, less awareness of susceptibility results at weekends, and the training and  
316 opinions of individual prescribers could also play a role in de-escalation practice and  
317 merit specific studies.

318 In crude analysis, de-escalation was associated with lower mortality and failure.  
319 This was probably due to confounding by indication as the associations were no longer  
320 significant when other mortality predictors were considered in multivariate analysis,



321 which is similar to the results found in the meta-analysis by Paul et al for observational  
322 studies of patients with severe sepsis or bacteraemia [6]. The results are reinforced by the  
323 fact that all our estimates in different analyses were consistent, and that we included  
324 mortality, failure of treatment and length of stay as outcome variables. Interestingly, the  
325 estimates provided by multivariate analysis were much less accurate than those provided  
326 by the PS-based matched pairs analysis. Our results strongly suggest therefore that de-  
327 escalation is safe. In fact, theoretically, it may have some individual beneficial effects if  
328 secondary infections caused by MDR bacteria are reduced, although demonstrating such  
329 an effect would require specific studies with a very large number of patients. Any analysis  
330 of population-level benefits would also require specific studies.

331 Our study has several limitations. Because it is not a randomized controlled trial,  
332 unmeasured confounding variables or residual confounding cannot be ruled out. The data  
333 were collected several years ago and changes in antimicrobial resistance may influence  
334 the results. Moreover, despite being controlled in the analysis, differences in clinical  
335 practice at each center might have influenced the outcomes. Some strengths of the study  
336 are its multicenter character, the use of clearly specified definitions, and the use of  
337 advance statistical methodologies to control for confounders.

338 In conclusion, the results of this study reinforce the fact that antibiotic de-  
339 escalation in patients with monomicrobial bacteraemia due to Enterobacteriaceae does  
340 not have a detrimental impact on outcome, 30-day all-cause mortality, failure, or length  
341 of hospital stay when compared with continuation with broad-spectrum antibiotics. These  
342 results may be useful for antibiotic stewardship activities.

343

344

345 **ACKNOWLEDGEMENTS**

346 **Other investigators from the REIPI/GEIH-SEIMC BACTERAEEMIA-MIC group:**  
347 M. de Cueto (Unidad Clínica Intercentros de Enfermedades Infecciosas, Microbiología y  
348 Medicina Preventiva, Hospital Universitario Virgen Macarena, Seville, Spain), AM  
349 Planes Reig (Departamento de Microbiología, Hospital Universitari Vall d'Hebron,  
350 Barcelona, Spain), F. Tubau Quintano (Servicio de Microbiología, Hospital Universitario  
351 de Bellvitge-IDIBELL, Barcelona, Spain), C. Peña (Servicio de Enfermedades  
352 Infecciosas, Hospital Universitario de Bellvitge-IDIBELL, Barcelona, Spain), ME Galán  
353 Otalora (Hospital de la Santa Creu i Sant Pau, Barcelona, Spain), C. Ruíz de Alegría  
354 (Servicio de Microbiología, Hospital Universitario Marqués de Valdecilla, Santander,  
355 Spain), R. Cantón (Servicio de Microbiología, Hospital Universitario Ramón y Cajal and  
356 Instituto Ramón y Cajal de Investigación Sanitaria (IRYCIS), Madrid, Spain), JA Lepe  
357 and JM Cisneros (Unidad Clínica de Enfermedades Infecciosas, Microbiología y  
358 Medicina Preventiva, Hospital Virgen del Rocío, Seville, Spain), J. Torre-Cisneros, R.  
359 Lara (Unidad Clínica de Enfermedades infecciosas Hospital Universitario Reina Sofía.  
360 Instituto Maimónides de Investigación Clínica (IMIBIC), Universidad de Córdoba,  
361 Córdoba, Spain).

## 362 **FINANCIAL SUPPORT**

363 This work was supported by the Instituto de Salud Carlos III, Ministry of  
364 Economy and Competitiveness, Spain (FIS; PI10/02021) co-financed by European  
365 Development Regional Fund 'A way to achieve Europe' ERDF, Spanish Network for  
366 Research in Infectious Diseases (REIPI RD12/0015).

367

### 368 **Potential Conflicts of Interest**

369 Dr. Zaira Palacios Baena reports personal fees from Gilead, outside the submitted work.

370 Dr. Rodríguez-Baño reports personal fees from Merck, personal fees from AstraZeneca

Formatted: Font: Bold

Formatted: Font: Not Bold

Formatted: Font: Not Bold

Formatted: Indent: First line: 0"

Formatted: Font: Not Bold

371 for a nondrug related research projects, and grants from Innovative Medicines Initiative  
372 (IMI), outside the submitted work. All other authors have no potential conflict of interest  
373 to declare.

Formatted: Font: Not Bold

Formatted: Font: Not Bold

Formatted: Font: Bold

374

375 **REFERENCES:**

- 376 1. Kollef MH. Broad-spectrum antimicrobials and the treatment of serious bacterial  
377 infections: getting it right up front. Clin Infect Dis 2008; 47: S3–13.
- 378 2. Karam G, Chastre J, Wilcox MH, Vincent JL. Antibiotic strategies in the era of  
379 multidrug resistance. Crit Care. 2016 Jun 22;20(1):136.
- 380 3. Barlam TF, Cosgrove SE, Abbo LM, MacDougall C, Schuetz AN, Septimus EJ,  
381 et al. Implementing an Antibiotic Stewardship Program: Guidelines by the  
382 Infectious Diseases Society of America and the Society for Healthcare  
383 Epidemiology of America. Clin Infect Dis 2016; 62: e51-77.
- 384 4. Silva BN, Andriolo RB, Atallah AN, Salomão R. De-escalation of antimicrobial  
385 treatment for adults with sepsis, severe sepsis or septic shock. Cochrane Database  
386 Syst Rev. 2013; 3: CD007934.
- 387 5. López-Cortés LE, Cueto M, Rodríguez-Baño J. How should we best treat patients  
388 with bloodstream infections? Future Microbiol 2017; 12: 927-930.
- 389 6. Paul M, Dickstein Y, Raz-Pasteur A. Antibiotic de-escalation for bloodstream  
390 infections and pneumonia: systematic review and meta-analysis. Clin Microbiol  
391 Infect 2016; 22: 960-967.
- 392 7. López-Cortés LE, Rosso-Fernández C, Núñez-Núñez M, et al. Targeted  
393 simplification versus antipseudomonal broad-spectrum beta-lactams in patients  
394 with bloodstream infections due to Enterobacteriaceae (SIMPLIFY): a study

- 395 protocol for a multicentre, open-label, phase III randomised, controlled, non-  
396 inferiority clinical trial. *BMJ Open* 2017; 7: e015439.
- 397 8. Delgado-Valverde M, Torres E, Valiente-Mendez A, et al. Impact of the MIC of  
398 piperacillin/tazobactam on the outcome for patients with bacteraemia due to  
399 Enterobacteriaceae: the Bacteraemia-MIC project. *J Antimicrob Chemother.* 2016  
400 Feb;71(2):521-30.
- 401 9. Delgado-Valverde M, Valiente-Mendez A, Torres E, et al. MIC of  
402 amoxicillin/clavulanate according to CLSI and EUCAST: discrepancies and  
403 clinical impact in patients with bloodstream infections due to Enterobacteriaceae.  
404 *J Antimicrob Chemother.* 2017 May 1;72(5):1478-1487.
- 405 10. Weiss E, Zahar JR, Lesprit P, Ruppe E, Leone M, Chastre J, Lucet JC, Paugam-  
406 Burtz C, Brun-Buisson C, Timsit JF; De-escalation Study Group. Elaboration of  
407 a consensual definition of de-escalation allowing a ranking of  $\beta$ -lactams. *Clin*  
408 *Microbiol Infect* 2015; 21: 649.e1-10.
- 409 11. Von Elm E, et al. The strengthening the reporting of observational studies in  
410 epidemiology (STROBE) statement: guidelines for reporting observational  
411 studies. *J. Clin Epidemiol.* 2008;61:344-349.
- 412 12. Charlson ME, Pompei P, Ales KL et al. A new method of classifying prognostic  
413 co-morbidity in longitudinal studies: development and validation. *J Chron Dis.*  
414 1987; 40: 373–83.
- 415 13. Hilf M, Yu Vh, Sharp J, et al. Antibiotic therapy for *Pseudomonas aeruginosa*  
416 bacteremia: outcome correlations in a prospective study of 200 patients. *Am J*  
417 *Med.* 1989; 87: 540-546.
- 418 14. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure  
419 Assessment) score to describe organ dysfunction/failure. On behalf of the

- 420 Working Group on Sepsis-Related Problems of the European Society of Intensive  
421 Care Medicine. *Intensive Care Med.* 1996 Jul;22(7):707-10.
- 422 15. American College of Chest Physicians/Society of Critical Care Medicine  
423 Consensus Conference: definitions for sepsis and organ failure and guidelines for  
424 the use of innovative therapies in sepsis. *Crit Care Med.* 1992; 20: 864–74.
- 425 16. Horan TC, Andrus M, Dudeck MA. CDC/NHSN surveillance definition of health  
426 care-associated infection and criteria for specific types of infections in the acute  
427 care setting. *Am J Infect Control* 2008; 36: 309-32.
- 428 17. EUCAST. Breakpoint tables for interpretation of MICs and zone diameters,  
429 version 5.0. 2015. Available at:  
430 [http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST\\_files/Breakpoint\\_ta](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf)  
431 [bles/v\\_5.0\\_Breakpoint\\_Table\\_01.pdf](http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_5.0_Breakpoint_Table_01.pdf).
- 432 18. Huttner B, Pulcini C, Schouten J. De-constructing de-escalation. *Clin Microbiol*  
433 *Infect* 2016; 22: 958-959.
- 434 19. Garnacho-Montero J, Escoreca-Ortega A, Fernández-Delgado E. Antibiotic de-  
435 escalation in the ICU: how is it best done? *Curr Opin Infect Dis* 2015; 28:193-8.
- 436 20. Masterton RG. Antibiotic de-escalation. *Crit Care Clin* 2011; 27: 149-62.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.



# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.



# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.



## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Instructions

The purpose of this form is to provide readers of your manuscript with information about your other interests that could influence how they receive and understand your work. The form is designed to be completed electronically and stored electronically. It contains programming that allows appropriate data display. Each author should submit a separate form and is responsible for the accuracy and completeness of the submitted information. The form is in six parts.

#### 1. Identifying information.

#### 2. The work under consideration for publication.

This section asks for information about the work that you have submitted for publication. The time frame for this reporting is that of the work itself, from the initial conception and planning to the present. The requested information is about resources that you received, either directly or indirectly (via your institution), to enable you to complete the work. Checking "No" means that you did the work without receiving any financial support from any third party -- that is, the work was supported by funds from the same institution that pays your salary and that institution did not receive third-party funds with which to pay you. If you or your institution received funds from a third party to support the work, such as a government granting agency, charitable foundation or commercial sponsor, check "Yes".

#### 3. Relevant financial activities outside the submitted work.

This section asks about your financial relationships with entities in the bio-medical arena that could be perceived to influence, or that give the appearance of potentially influencing, what you wrote in the submitted work. You should disclose interactions with ANY entity that could be considered broadly relevant to the work. For example, if your article is about testing an epidermal growth factor receptor (EGFR) antagonist in lung cancer, you should report all associations with entities pursuing diagnostic or therapeutic strategies in cancer in general, not just in the area of EGFR or lung cancer.

Report all sources of revenue paid (or promised to be paid) directly to you or your institution on your behalf over the 36 months prior to submission of the work. This should include all monies from sources with relevance to the submitted work, not just monies from the entity that sponsored the research. Please note that your interactions with the work's sponsor that are outside the submitted work should also be listed here. If there is any question, it is usually better to disclose a relationship than not to do so.

For grants you have received for work outside the submitted work, you should disclose support ONLY from entities that could be perceived to be affected financially by the published work, such as drug companies, or foundations supported by entities that could be perceived to have a financial stake in the outcome. Public funding sources, such as government agencies, charitable foundations or academic institutions, need not be disclosed. For example, if a government agency sponsored a study in which you have been involved and drugs were provided by a pharmaceutical company, you need only list the pharmaceutical company.

#### 4. Intellectual Property.

This section asks about patents and copyrights, whether pending, issued, licensed and/or receiving royalties.

#### 5. Relationships not covered above.

Use this section to report other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work.

#### Definitions.

**Entity:** government agency, foundation, commercial sponsor, academic institution, etc.

**Grant:** A grant from an entity, generally [but not always] paid to your organization

**Personal Fees:** Monies paid to you for services rendered, generally honoraria, royalties, or fees for consulting, lectures, speakers bureaus, expert testimony, employment, or other affiliations

**Non-Financial Support:** Examples include drugs/equipment supplied by the entity, travel paid by the entity, writing assistance, administrative support, etc.

**Other:** Anything not covered under the previous three boxes

**Pending:** The patent has been filed but not issued

**Issued:** The patent has been issued by the agency

**Licensed:** The patent has been licensed to an entity, whether earning royalties or not

**Royalties:** Funds are coming in to you or your institution due to your patent



## ICMJE Form for Disclosure of Potential Conflicts of Interest

### Section 1. Identifying Information

1. Given Name (First Name) CRISTINA      2. Surname (Last Name) DE LA CALLE      3. Date 21/09/2018

4. Are you the corresponding author?     Yes     No

5. Manuscript Title

"Predictors and prognosis impact of de-escalation in patients with bacteraemia

6. Manuscript Identifying Number (if you know it)

due to Enterobacteriaceae: a post-hoc analysis from a multicenter prospective cohort".

### Section 2. The Work Under Consideration for Publication

Did you or your institution **at any time** receive payment or services from a third party (government, commercial, private foundation, etc.) for any aspect of the submitted work (including but not limited to grants, data monitoring board, study design, manuscript preparation, statistical analysis, etc.)?

Are there any relevant conflicts of interest?     Yes     No

### Section 3. Relevant financial activities outside the submitted work.

Place a check in the appropriate boxes in the table to indicate whether you have financial relationships (regardless of amount of compensation) with entities as described in the instructions. Use one line for each entity; add as many lines as you need by clicking the "Add +" box. You should report relationships that were **present during the 36 months prior to publication.**

Are there any relevant conflicts of interest?     Yes     No

### Section 4. Intellectual Property -- Patents & Copyrights

Do you have any patents, whether planned, pending or issued, broadly relevant to the work?     Yes     No

## ICMJE Form for Disclosure of Potential Conflicts of Interest

---

### Section 5. Relationships not covered above

Are there other relationships or activities that readers could perceive to have influenced, or that give the appearance of potentially influencing, what you wrote in the submitted work?

- Yes, the following relationships/conditions/circumstances are present (explain below):
- No other relationships/conditions/circumstances that present a potential conflict of interest

At the time of manuscript acceptance, journals will ask authors to confirm and, if necessary, update their disclosure statements. On occasion, journals may ask authors to disclose further information about reported relationships.

### Section 6. Disclosure Statement

Based on the above disclosures, this form will automatically generate a disclosure statement, which will appear in the box below.

### Evaluation and Feedback

Please visit <http://www.icmje.org/cgi-bin/feedback> to provide feedback on your experience with completing this form.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.

# Please wait...

If this message is not eventually replaced by the proper contents of the document, your PDF viewer may not be able to display this type of document.

You can upgrade to the latest version of Adobe Reader for Windows®, Mac, or Linux® by visiting [http://www.adobe.com/go/reader\\_download](http://www.adobe.com/go/reader_download).

For more assistance with Adobe Reader visit <http://www.adobe.com/go/acrreader>.

Windows is either a registered trademark or a trademark of Microsoft Corporation in the United States and/or other countries. Mac is a trademark of Apple Inc., registered in the United States and other countries. Linux is the registered trademark of Linus Torvalds in the U.S. and other countries.