



Original article

Association of microbiological factors with mortality in *Escherichia coli* bacteraemia presenting with sepsis/septic shock: a prospective cohort study

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ABSTRACT

Objectives: This study aimed to determine the association of *Escherichia coli* microbiological factors with 30-day mortality in patients with bloodstream infection (BSI) presenting with a dysregulated response to infection (i.e. sepsis or septic shock).

Methods: Whole-genome sequencing was performed on 224 *E coli* isolates of patients with sepsis/septic shock, from 22 Spanish hospitals. Phylogroup, sequence type, virulence, antibiotic resistance, and pathogenicity islands were assessed. A multivariable model for 30-day mortality including clinical and epidemiological variables was built, to which microbiological variables were hierarchically added. The predictive capacity of the models was estimated by the area under the receiver operating characteristic curve (AUROC) with 95% confidence intervals (CI).

Results: Mortality at day 30 was 31% (69 patients). The clinical model for mortality included (adjusted OR; 95% CI) age (1.04; 1.02–1.07), Charlson index ≥ 3 (1.78; 0.95–3.32), urinary BSI source (0.30; 0.16–0.57), and active empirical treatment (0.36; 0.11–1.14) with an AUROC of 0.73 (95% CI, 0.67–0.80). Addition of microbiological factors selected clone ST95 (3.64; 0.94–14.04), *eilA* gene (2.62; 1.14–6.02), and *astA* gene (2.39; 0.87–6.59) as associated with mortality, with an AUROC of 0.76 (0.69–0.82).

Discussion: Despite having a modest overall contribution, some microbiological factors were associated with increased odds of death and deserve to be studied as potential therapeutic or preventive targets.

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Introduction

Sepsis is defined as life-threatening organ dysfunction caused by a dysregulated host response to infection [1]. It is considered a major cause of mortality worldwide, being responsible for nearly 20% of global deaths [2]. Although some patients' features have been associated with mortality among patients with sepsis or septic shock, the identification of specific microbial factors influencing the outcome of patients might help in finding novel therapeutic or preventive targets.

Escherichia coli is the most common cause of bloodstream infection (BSI) in high-income countries, with an estimated incidence of 48 cases per 100 000 person-years [3] and is one of the main causes of sepsis [4]. The association of microbiological determinants with mortality has been studied in patients with *E coli* bacteraemia, with heterogeneous results [5–14]; however, most patients with *E coli* bacteraemia do not present a dysregulated response to the infection, and to our knowledge, there is a lack of information about the pathogen's role in mortality among bacteraemic patients presenting with sepsis or septic shock.

The objective of this study is to assess the association of microbiological factors of *E coli* with 30-day mortality in patients with bacteraemia presenting with sepsis or septic shock according to current definitions.

Methods

Study design and patients

PROBAC-Ec study is part of the PROBAC project, a multicentre prospective cohort of consecutive adult patients with confirmed BSI between October 4, 2016 and October 15, 2017, in 26 Spanish hospitals. The PROBAC study was approved by the ethics committees of the participating sites, which waived the need to obtain informed consent because of the observational design. Methodological details were previously reported [15]; in summary, no exclusion criteria applied; the patients' characteristics collected are shown in Table 1; patients were followed for 30 days after the BSI

and survival after hospital discharge was assessed by reviewing the charts, phone calls or consulting mortality registries.

From the PROBAC cohort, we selected the first 225 of the 1211 patients with monomicrobial *E coli* BSI presenting with sepsis or septic shock at the time of blood culture sampling according to Sepsis-3 criteria [1]. The primary endpoint was 30-day all-cause mortality.

Microbiological studies

E coli isolates were sent to the Microbiology Department of Virgen Macarena University Hospital in Seville, Spain. Antibiotic susceptibility tests were performed by broth microdilution method. EUCAST v 10.0 clinical breakpoints for susceptibility tests were used (<https://www.eucast.org/>).

Whole-genome sequencing was performed in Illumina MiSeq sequencer (Illumina Inc) and de novo assembly was done using CLC Genomics Workbench v10 software (<http://www.clcbio.com/products/>). Sequence type (ST), serotype, virulence factors (VFs), and antibiotic resistance genes were evaluated in MLST 2.0, SerotypeFinder 2.0, VirulenceFinder 2.0, and ResFinder 3.2, respectively (<https://www.genomicepidemiology.org/>). The phylogenetic group was assigned in the Clermont Typing platform (<http://clermonttyping.iame-research.center/>). Pathogenicity Islands (PAIs) were identified from the PAI-DB database (www.paidb.re.kr) (accessed on April 1, 2022). Virulence score (VS) and PAI score (PS) were calculated for each isolate as a total number of genes or islands detected, respectively. Details of the microbiological procedures and genomic analysis were reported previously, together with a full descriptive report of the microbiological characteristics of the isolates [16].

Statistical analysis

Considering a 30% 30-day mortality [4], the inclusion of 225 patients (≈ 70 deaths) would allow the inclusion of some 7–10 mortality predictors in multivariable models, of which 4–5 would

Table 1Crude association between epidemiological, clinical, and therapeutic variables and 30-day mortality among patients with bloodstream infection (BSI) due to *Escherichia coli* and presentation as sepsis/septic shock

Characteristic	Survivors (n = 155)	Nonsurvivors (n = 69)	OR (95% CI)
Demographics			
Age (y), Me (IQR)	74 (63–82)	82 (68–87)	NC
Male sex	92 (59)	43 (62)	1.13 (0.63–2.02)
Source of bacteraemia			
Urinary tract infection	93 (60)	23 (33)	0.33 (0.18–0.60)
Biliary intra-abdominal infection	34 (22)	18 (26)	1.25 (0.65–2.42)
Non-biliary intra-abdominal infection	10 (6)	11 (16)	2.75 (1.10–6.82)
Respiratory tract infections	4 (3)	11 (16)	7.15 (2.19–23.38)
Other sources	14 (9)	6 (9)	0.95 (0.35–2.61)
Urinary BSI source vs others	93 (60)	23 (33)	0.33 (0.18–0.60)
Strictly community-acquired infection	90 (58)	31 (45)	0.58 (0.33–1.04)
Underlying diseases			
Diabetes mellitus	41 (26)	17 (25)	0.90 (0.47–1.74)
Cancer	39 (25)	21 (30)	1.30 (0.69–2.43)
Chronic pulmonary disease	17 (11)	15 (22)	2.25 (1.05–4.83)
Liver disease	16 (10)	12 (17)	1.82 (0.81–4.10)
Dementia	14 (9)	13 (19)	2.33 (1.03–5.28)
Peripheral vascular disease	17 (11)	7 (10)	0.91 (0.36–2.32)
Cerebrovascular disease	15 (10)	9 (13)	1.40 (0.58–3.37)
Recurrent urinary tract infection	16 (10)	7 (10)	0.98 (0.38–2.50)
Congestive heart failure	13 (8)	10 (14)	1.82 (0.76–4.45)
Moderate to severe chronic kidney disease	12 (8)	9 (13)	1.78 (0.76–4.46)
Obstructive uropathy	16 (10)	3 (7)	0.39 (0.11–1.40)
Haematological malignancy	7 (5)	5 (7)	1.65 (0.50–5.40)
Acute myocardial infarction	6 (4)	7 (10)	2.80 (0.90–8.67)
Biliary tract obstruction	8 (5)	3 (4)	0.83 (0.21–3.24)
Immunosuppressive therapy	11 (7)	5 (7)	1.02 (0.34–3.06)
Neutropenia <100 cels/ μ L	3 (2)	4 (6)	3.11 (0.67–14.32)
Charlson index ≥ 3	59 (38)	37 (54)	1.88 (1.06–3.33)
Invasive procedures/devices			
Urinary catheter	39 (25)	19 (28)	1.13 (0.59–2.14)
Central venous catheter	31 (20)	10 (14)	0.67 (0.31–1.47)
Previous surgery	18 (12)	6 (9)	0.72 (0.27–1.91)
Mechanical ventilation	7 (5)	3 (4)	0.96 (0.24–3.83)
Biliary prosthesis	4 (3)	2 (3)	1.12 (0.20–6.30)
Nephrostomy	5 (3)	0 (0)	NC
Pitt index ≥ 3	100 (65)	55 (80)	2.16 (1.10–4.23)
Active empirical treatment	149 (96)	60 (87)	0.26 (0.09–0.78)

IQR, interquartile range; Me, median; NC, not calculated; OR, odds ratio.

be patients-related, allowing 5–6 microbiological factors in the final multivariable models.

The Charlson comorbidity index and Pitt bacteraemia score were dichotomized according to the mortality risk of different strata. All categorical variables were compared between survivors and nonsurvivors using χ^2 or Fisher's exact test as appropriate, and contingency tables were used to calculate odds ratios (ORs) with 95% CI for 30-day mortality. Age, VS, and PS were analysed as continuous variables using the Mann-Whitney U test. Clusters for virulence genes were identified by two-step cluster analysis using log-likelihood for distance measure and the Akaike information criteria for determining the number of clusters.

We first constructed an explanatory multivariable model for 30-day mortality including only patients' clinical and epidemiological characteristics using binomial logistic regression; those with bivariate $p < 0.2$ were included, and a parsimonious model was built by selecting the variables using a manual stepwise backward method. Variables with a $p < 0.1$ were kept in the model. Interactions between variables were explored and kept in the model if significant. Then, we added the microbiological variables using a hierarchical stepwise backward process, retaining the variables with a $p < 0.1$. The predictive capacity of the models on observed data was estimated by calculating the area under the receiver

operating characteristic curve (AUROC) with 95% CI, and the goodness of fit by the Hosmer-Lemeshow test.

A sensitivity analysis was performed by including the specific underlying conditions instead of the Charlson index, to check if specific conditions might modify the estimations for the microbiological characteristics. All tests were performed using SPSS (v.18; SPSS Inc). This manuscript was reported according to STROBE guidelines (Table S1).

Results

Of the 225 patients, 224 were finally analysed because the isolate from one patient could not be recovered. These patients were recruited from 22 of the 26 participating hospitals. The median age was 75 years (interquartile range [IQR] 65–84) and 60% were male. The median SOFA score at the time of blood culture sampling was six points (IQR 5–9) and the 30-day overall mortality was 31%. Urinary tract infection was the most common source of bacteraemia (116; 52%) and more than a half of the infections were community-acquired (121; 54%).

The bivariate analysis of the associations of clinical and epidemiological variables with mortality is shown in Table 1; a significant association was found for age, chronic pulmonary disease,

dementia, Charlson index ≥ 3 , Pitt score ≥ 3 , active empirical treatment, and urinary source of BSI. In addition, liver disease, obstructive uropathy, congestive heart failure, and acute myocardial infarction had $p < 0.2$ and were included in the multivariable model.

The multivariable model of clinical, epidemiological, and therapeutic characteristics for 30-day mortality included age and Charlson index ≥ 3 as associated with increased odds of death, whereas urinary source of BSI and active empirical treatment were protective factors. The AUROC of the model for observed data was 0.73 (95% CI, 0.67–0.80) (Table 2), and the p value for the Hosmer-Lemeshow test was 0.55. No significant interaction could be found between the urinary source of BSI and active empirical treatment (p 0.71).

No differences were found in phylogenetic groups, serotypes, or ST distribution between isolates from survivors and non-survivors in crude analysis; cases caused by clone ST95 were included in the multivariable analysis because of a $p < 0.2$ (Table 3). Also, no significant differences could be found between *E. coli* isolates from survivors and nonsurvivors for antibiotic susceptibility results or resistance determinants (Table S2). The median VS was 22 genes (IQR: 17–27), with no significant differences between survivors and nonsurvivors (Table S3). However, a higher proportion of some individual virulence genes was found in *E. coli* isolates from nonsurvivors, in particular, *hlyF* (hemolysin F), *tsh* (temperature-sensitive hemagglutinin), *cvaC* (microcin C), and *etsC* (putative type I secretion outer membrane protein) (Table 3). In gene-clustering analysis, five clusters were found (Table S4); none of them was individually associated with an increased risk of death (Table S5). In terms of pathogenicity islands, the median PS was 7 PAIs (IQR: 3–12), with no significant differences in median score or prevalence of PAIs between *E. coli* isolates from survivors and nonsurvivors (Table S6).

When microbiological factors were added to the first multivariable model, isolates belonging to clone ST95, *eilA* gene (encoding EilA, a HilA-like transcriptional regulator protein), *astA* gene (heat-stable enterotoxin), Charlson Index ≥ 3 and age were independently associated with increased odds for mortality, whereas urinary source of bacteraemia and active empirical treatment remain into the model as protective factors (Table 4). The AUROC of this model was 0.76 (95% CI, 0.69–0.82), and the p value for the Hosmer-Lemeshow test was 0.39. No interaction was found between clone ST95 and the presence of virulence genes *cia*, *cvaC*, *etsC*, and *hlyF* ($p > 0.99$).

A sensitivity analysis including individual comorbidities instead of the Charlson index was performed; the final model included liver disease, age, and Pitt index ≥ 3 as risk factors, and urinary source and active empirical therapy as protective factors for 30-day mortality (Table S7). The AUROC of this model was 0.73 (95% CI 0.67–0.80). When microbiological determinants were added to this model, their adjusted OR (95% CI) were as follows: for age, 1.06 per 1-year increment (1.02–1.09); liver disease, 2.42 (0.91–6.41); *astA*, 2.55 (0.93–6.96); *eilA*, 2.83 (1.22–6.53); ST95, 3.62 (0.93–14.0); urinary source of bacteraemia, 0.24 (0.12–0.48); and for active

empirical treatment, 0.31 (0.96–1.06). The AUROC of this model was 0.76 (95% CI, 0.70–0.83).

Discussion

In this prospective multicentre cohort of patients with *E. coli* bacteraemia and dysregulated response, 30-day mortality occurred in nearly one-third of patients; as expected, older age and high comorbidity index were associated with increased risk of 30-day mortality, whereas active empirical treatment and urinary BSI source were protective. The addition of microbiological determinants to the multivariable model only slightly improved its predictive ability; nevertheless, some factors, namely isolate belonging to ST95, and the virulence genes *eilA* (encoding EilA, a HilA-like transcriptional regulator protein), and *astA* (heat-stable enterotoxin) were found to be associated with increased odds for 30-day mortality even when controlling for patients' characteristics.

Because the overall contribution of microbiological factors in the predictive ability of the multivariable model was limited, we hypothesize that once the dysregulated response has occurred, patient-related factors have a stronger causal effect on mortality than microbiological factors, probably reflecting the importance of the overall patients' functional reserve.

Previous studies focused on patients with *E. coli* BSI but, to the best of our knowledge, not specifically in sepsis or septic shock, for which the outcome determinants might be different than in patients not showing a dysregulated response. However, those studies might provide some context to our data. Older age and more severe comorbidity status were associated with adverse outcomes in previous *E. coli* BSI studies [14,17], whereas urinary source of bacteraemia is typically a protective factor [5–7,10,11,13,18]. This is probably related to the high concentration of some antibiotics in urine or to an easier source control, compared to pulmonary or abdominal infections. In patients with *E. coli* BSI, an adequate empirical treatment has also been associated with lower mortality [19], in line with our results. In our cohort, extended-spectrum β -lactamase genes were not associated *per se* with 30-day mortality, as was seen in some previous studies [11,20].

In this cohort, no significant bivariate association was found between phylogenetic background and 30-day mortality, which is consistent with previous studies on BSI [6,11,13]. However, in the multivariable analysis, we found an association of ST95 with 30-day mortality, which was strong but unprecise (i.e. wide 95% CI) because of the low number of cases caused by this clonal group. To our knowledge, this is the first study to demonstrate this association. ST95 is one of the four dominant *E. coli* STs worldwide [21]. Previous studies in patients with BSI including only patients with extended-spectrum β -lactamase-producing isolates [7,9,22] excluded ST95 isolates as these are mostly associated with a low prevalence of acquired antimicrobial resistance [23]. As for other STs, ST88 was associated with mortality in a cohort of 545 patients with *E. coli* BSI [11]; there were only seven ST88 isolates in our study, one of whom died. Yoon et al. [18] found the ST131-sublineage H30Rx to be associated with early mortality, but this could not be confirmed by our study, and Brumwell et al. [14] reported an association between ST131 and increased mortality only in patients with BSI from a urinary source. Other studies found no association of specific clones with mortality [24].

A plausible biological explanation for the association between ST95 and mortality in our cohort is its particular combination of virulence traits, including adhesins, iron acquisition systems, toxins production, and survival mechanisms. Some virulence factors are more prevalent in ST95 than in the other predominant lineages [16], such as genes involved in biosynthesis and transport of the K1

Table 2

Multivariable model of clinical, epidemiological, and therapeutic variables associated with 30-day mortality in patients with bacteraemia due to *Escherichia coli* and presentation as sepsis/septic shock

Variable	p	aOR (95% CI)
Age (per year)	0.0006	1.04 (1.02–1.07)
Charlson index ≥ 3	0.069	1.78 (0.95–3.32)
Urinary tract source of bacteraemia	0.0002	0.30 (0.16–0.57)
Active empirical treatment	0.084	0.36 (0.11–1.14)

aOR, adjusted odds ratio.

Table 3Crude association between microbiological factors and 30-day mortality among patients with bloodstream infection due to *Escherichia coli* and presentation as sepsis/septic shock

Characteristics	Survivors (n = 155)	Nonsurvivors (n = 69)	OR (95% CI)
Phylogroup			
A	14 (9)	3 (4)	0.46 (0.13–1.65)
B1	14 (9)	8 (12)	1.32 (0.53–3.31)
B2	91 (59)	36 (52)	0.77 (0.43–1.36)
C	9 (6)	4 (6)	1.00 (0.30–3.36)
D	20 (13)	12 (17)	1.42 (0.65–3.10)
F	3 (2)	4 (6)	3.12 (0.68–14.33)
G	4 (3)	2 (3)	1.13 (0.20–6.30)
Phylogroup B2/non-B2	91 (59)	36 (52)	0.77 (0.43–1.36)
Serotype			
O25:H4	25 (16)	11 (16)	0.86 (0.40–1.84)
O6:H1	12 (8)	4 (6)	0.73 (0.22–2.36)
O17:H18	7 (5)	2 (4)	0.63 (0.12–3.11)
O1:H7	5 (3)	3 (3)	1.36 (0.31–5.87)
Sequence type			
ST131	27 (17)	11 (16)	0.90 (0.42–1.94)
ST131-H30Rx	8 (5)	6 (9)	1.75 (0.58–5.25)
ST95	6 (4)	6 (9)	2.37 (0.73–7.61)
ST73	19 (12)	6 (9)	0.68 (0.26–1.79)
ST69	16 (10)	7 (10)	0.98 (0.38–2.50)
ST131/ST73/ST69/ST95	68 (44)	30 (43)	0.94 (0.55–1.74)
Antibiotic resistance genes			
<i>bla</i> _{SHV-12}	1 (1)	1 (1)	2.26 (0.14–36.74)
<i>bla</i> _{OXA-48}	1 (1)	0 (0)	NC
<i>bla</i> _{CTX-M}	17 (11)	8 (12)	1.06 (0.44–2.60)
<i>bla</i> _{CTX-M15}	10 (6)	7 (10)	1.64 (0.59–4.49)
Antibiotic resistance (%)			
Ciprofloxacin	72 (46)	31 (45)	0.94 (0.53–1.66)
Amoxicillin/clavulanate	56 (36)	24 (35)	0.94 (0.52–1.71)
Piperacillin/tazoctam	13 (8)	7 (10)	1.23 (0.47–3.24)
Cefoxitin	13 (8)	7 (10)	1.23 (0.47–3.24)
Cefotaxime/Ceftriaxone	22 (14)	13 (19)	1.40 (0.66–2.98)
Cefepime	18 (12)	9 (13)	1.14 (0.49–2.69)
Amikacine	9 (6)	2 (3)	0.48 (0.10–2.30)
Gentamicine	30 (19)	11 (16)	0.79 (0.37–1.69)
Trimethoprim-sulfamethoxazole	64 (41)	30 (43)	1.09 (0.62–1.94)
Virulence genes			
<i>astA</i>	12 (8)	10 (14)	2.02 (0.83–4.93)
<i>cia</i>	26 (17)	18 (26)	1.75 (0.88–3.47)
<i>cvaC</i>	31 (20)	22 (32)	1.87 (0.99–3.56)
<i>eilA</i>	23 (15)	16 (23)	1.73 (0.85–3.54)
<i>etsC</i>	32 (21)	25 (36)	2.18 (1.17–4.09)
<i>hylF</i>	36 (23)	25 (36)	1.88 (1.01–3.48)
<i>senB</i>	38 (25)	10 (14)	0.52 (0.24–1.12)
<i>tcpC</i>	31 (20)	6 (9)	0.38 (0.15–0.96)
<i>tsh</i>	7 (5)	9 (13)	3.17 (1.13–8.90)
Virulence score (Me; IQR)	22 (17–26)	23 (18–27)	NC

NC, not calculated; OR, odds ratio.

capsule (*neuC*, *kpsMII-K1*), hemolysin F (*hylF*), putative type I secretion outer membrane protein (*etsC*) and bacteriocins production (*cia* and *cvaC*). Bacterial capsule is a major element of bacterial pathogenesis and interestingly, the genes implied in capsule synthesis were present in all ST95 isolates which could be a potential therapeutic or preventive target, although this finding deserves further investigation.

The pathogenesis of *E. coli* infection requires the display and expression of several virulence genes [25]. Our study could not confirm previously reported associations between *fyuA*, *papGIII*, *iroN*, *afa* or *iha* genes, and mortality in *E. coli* BSI [5,8–11,18]. We found *astA* gene to be an independent risk factor for 30-day mortality. This gene encodes the enteroaggregative heat-stable toxin 1 (EAST-1) toxin, a small protein broadly distributed among diarrheogenic and commensal *E. coli* strains [26]. In a collection of 174 blood isolates, *astA* gene was more frequent in isolates from patients with septic shock [27]. By contrast, Yoon et al. [18] found no association between *astA* gene and 30-day mortality in 1492 patients with *E. coli* BSI; however, only 7.6% of the patients in that

cohort were critically ill according to SOFA scoring. To date, the virulence and pathogenesis of EAST-1 toxin remain uncertain and controversial [28].

We also found the virulence gene *eilA* to be associated with increased 30-day mortality. It is a homologue of *hilA* regulator in *Salmonella enterica*, encoded by *eip* island in the *E. coli* O42 genome [25]. The putative gene product EilA was linked to modulation of expression of different genes of the *eip* island (i.e. *eipB*, *eipC*, *eipD*, *eicA* and *eaex*), to activate the genetically linked high molecular weight bacterial surface protein Air [29] and to modulating genes *eivF* and *eivA* of a pathogenicity island encoding components of type III secretion system 2 (ETT2), which are employed to deliver effector proteins into the cytoplasm of infected host cells [30]. So far, *eilA* has been linked to influencing both bacterial adhesion to epithelial cells and biofilm formation [29]; however, the contribution of *eilA* gene as virulence-related regulon to worse outcomes in patients with severe presentation remains to be elucidated and would deserve further studies.

Table 4

Multivariable model including microbiological factors associated with 30-day mortality in patients with bacteraemia due to *Escherichia coli* and presentation as sepsis/septic shock

Variable	p	aOR (95% CI)
Clone ST95	0.060	3.64 (0.94–14.04)
HilA-like transcriptional regulator gene (<i>eilA</i>)	0.023	2.62 (1.14–6.02)
Heat-stable enterotoxin gene (<i>astA</i>)	0.090	2.39 (0.87–6.59)
Charlson index ≥ 3	0.12	1.67 (0.88–3.17)
Age (per year)	0.0003	1.05 (1.02–1.08)
Urinary tract source of bacteraemia	<0.0001	0.24 (0.12–0.48)
Active empirical treatment	0.063	0.32 (0.10–1.06)

aOR, adjusted odds ratio.

This study has limitations related to the observational nature of the design, potential unmeasured confounders (e.g. patients' genetics), and lack of genome-wide association analysis. The presence of virulence genes was analysed, but not their expression. Our analyses were mostly exploratory and could not provide precise estimation of associations. The results may not be applicable to other populations. The strengths of our study include the analysis of a large prospective, multicentre and well-characterized cohort of patients with severe clinical presentation of *E coli* BSI according to current consensus definitions; and the exhaustive analysis of all *E coli* isolates by WGS.

In summary, in a cohort of 224 patients with *E coli* BSI presenting sepsis or septic shock, patient-related factors, such as older age and high comorbidity rate increase the odds of 30-day mortality, whereas the urinary source of BSI and active empirical treatment resulting in protective factors. Some bacterial factors, such as the virulence genes *astA* and *eilA*, and especially the clone ST95 showed a modest association with mortality, deserving further research.

Authors contributions

NM, IL-H, JR-B, and AP, were responsible for conceptualization, formulating the overall research questions, methodology, formal analysis, writing of original draft. NM, IL-H, and AG-M were responsible for data curation and bioinformatics analysis. LEL-C, PMM-PC, PR-G, SRR, AS-D, JG, AP-N, Md-O, CN- K, AJ-S, Ad-J, CA-C, AA, JF-S, TM-C, LB-P, AS-A, JMR-I, FG-S, AB, JMS-C, IG-L, IP-C, AR-B, and BB-C, participated by reviewing the design, recruiting patients and isolates, and thoroughly reviewed the manuscript. All authors have seen and approved the submitted version of this manuscript and accept responsibility for the decision to submit for publication. IL-H, JR-B, and AP were responsible for funding acquisition, project administration, supervision and coordinating the study. NM and IL-H contributed equally as first authors. AP and JR-B contributed equally as senior authors.

Transparency declaration

LEL-C reports consulting fees from Angelini Pharm and payments for presentations from Correvio Pharma Corp., Gilead Sciences, Inc and Viiv Healthcare. PR-G reports consulting fees and participation on a Data Safety Monitoring Board or Advisory Board from Advanz Pharma, support for attending meetings and/or travel from Gilead Sciences, Inc, and payments for presentations from Menarini Group and Shionogi & Co., Ltd. LB-P reports payments for presentations in educational events from Tillotts Pharma AG and Menarini Group and support for attending meetings and/or travel from Pfizer, Inc AG-M reports support for attending ECCMID 2022 from ESCMID. All other authors declare no competing interests. This study was funded by Instituto de Salud Carlos III through grant

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Appendix A. Supplementary data

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References

- [1] Singer M, Deutschman CS, Seymour CW, Shankar-Hari M, Annane D, Bauer M, et al. The Third International Consensus definitions for sepsis and septic shock (Sepsis-3). *JAMA* 2016;315:801–10. <https://doi.org/10.1001/jama.2016.0287>.
- [2] Rudd KE, Johnson SC, Agesa KM, Shackelford KA, Tsoi D, Kievlan DR, et al. Global, regional, and national sepsis incidence and mortality, 1990–2017: analysis for the global burden of disease study. *Lancet* 2020;395:200–11. [https://doi.org/10.1016/S0140-6736\(19\)32989-7](https://doi.org/10.1016/S0140-6736(19)32989-7).
- [3] Bonten M, Johnson JR, van den Biggelaar AHJ, Geogalis L, Geurtsen J, de Palacios PI, et al. Epidemiology of *Escherichia coli* bacteremia: a systematic literature review. *Clin Infect Dis* 2021;72:1211–9. <https://doi.org/10.1093/cid/ciaa210>.
- [4] Angus DC, van der Poll T. Severe sepsis and septic shock. *N Engl J Med* 2013;369:840–51. <https://doi.org/10.1056/NEJMra1208623>. Published correction appears in *N Engl J Med*. 2013;369:2069.
- [5] Jauréguy F, Carbonnelle E, Bonacorsi S, Clec'h C, Casassus P, Bingen E, et al. Host and bacterial determinants of initial severity and outcome of *Escherichia coli* sepsis. *Clin Microbiol Infect* 2007;13:854–62. <https://doi.org/10.1111/j.1469-0691.2007.01775.x>.
- [6] Lefort A, Panhard X, Clermont O, Woerther PL, Branger C, Mentré F, et al. Host factors and portal of entry outweigh bacterial determinants to predict the severity of *Escherichia coli* bacteremia. *J Clin Microbiol* 2011;49:777–83. <https://doi.org/10.1128/JCM.01902-10>.
- [7] Rodríguez-Baño J, Mingorance J, Fernández-Romero N, Serrano L, López-Cerero L, Pascual A, et al. Outcome of bacteraemia due to extended-spectrum β -lactamase-producing *Escherichia coli*: impact of microbiological determinants. *J Infect* 2013;67:27–34. <https://doi.org/10.1016/j.jinf.2013.04.006>.
- [8] Mora-Rillo M, Fernández-Romero N, Navarro-San Francisco C, Díez-Sebastián J, Romero-Gómez MP, Fernández FA, et al. Impact of virulence genes on sepsis severity and survival in *Escherichia coli* bacteremia. *Virulence* 2015;6:93–100. <https://doi.org/10.4161/21505594.2014.991234>. Published correction appears in *Virulence* 2016;7:491.
- [9] Hung WT, Cheng MF, Tseng FC, Chen YS, Shin-Jung Lee S, Chang TH, et al. Bloodstream infection with extended-spectrum beta-lactamase-producing *Escherichia coli*: the role of virulence genes. *J Microbiol Immunol Infect* 2019;52:947–55. <https://doi.org/10.1016/j.jmii.2019.03.005>.
- [10] Daga AP, Koga VL, Soncini JGM, de Matos CM, Perugini MRE, Pelisson M, et al. *Escherichia coli* bloodstream infections in patients at a University hospital: virulence factors and clinical characteristics. *Front Cell Infect Microbiol* 2019;9:191. <https://doi.org/10.3389/fcimb.2019.00191>.
- [11] de Lastours V, Laouénan C, Royer G, Carbonnelle E, Lepeule R, Esposito-Farèse M, et al. Mortality in *Escherichia coli* bloodstream infections: antibiotic resistance still does not make it. *J Antimicrob Chemother* 2020;75:2334–43. <https://doi.org/10.1093/jac/dkaa161>.
- [12] Tao X, Wang H, Min C, Yu T, Luo Y, Li J, et al. A retrospective study on *Escherichia coli* bacteremia in immunocompromised patients: microbiological features, clinical characteristics, and risk factors for shock and death. *J Clin Lab Anal* 2020;34:e23319. <https://doi.org/10.1002/jcla.23319>.
- [13] Denamur E, Condamine B, Esposito-Farèse M, Royer G, Clermont O, Laouénan C, et al. Genome wide association study of *Escherichia coli* bloodstream infection isolates identifies genetic determinants for the portal of entry but not fatal outcome. *PLoS Genet* 2022;18:e1010112. <https://doi.org/10.1371/journal.pgen.1010112>.
- [14] Brumwell A, Sutton G, Lantos PM, Hoffman K, Ruffin F, Brinkac L, et al. *Escherichia coli* ST131 associated with increased mortality in bloodstream infections from urinary tract source. *J Clin Microbiol* 2023;6:e0019923. <https://doi.org/10.1128/jcm.00199-23>.
- [15] Pérez-Crespo PMM, Lanz-García JF, Bravo-Ferrer J, Cantón-Bulnes ML, Sousa Domínguez A, Goikoetxea Aguirre J, et al. Revisiting the epidemiology of bloodstream infections and healthcare-associated episodes: results from a

- multicentre prospective cohort in Spain (PRO-BAC Study). *Int J Antimicrob Agents* 2021;58:106352. <https://doi.org/10.1016/j.ijantimicag.2021.106352>.
- [16] Maldonado N, López-Hernández I, García-Montaner A, López-Cortés LE, Martínez Pérez-Crespo PM, Retamar-Gentil P, et al. Whole-genome characterisation of *Escherichia coli* isolates from patients with bacteraemia presenting with sepsis or septic shock: a multicenter cross-sectional study. *Lancet Microbe* 2024. [https://doi.org/10.1016/S2666-5247\(23\)00369-5](https://doi.org/10.1016/S2666-5247(23)00369-5).
- [17] Blandy O, Honeyford K, Gharbi M, Thomas A, Ramzan F, Ellington MJ, et al. Factors that impact on the burden of *Escherichia coli* bacteraemia: multivariable regression analysis of 2011–2015 data from West London. *J Hosp Infect* 2019;101:120–8. <https://doi.org/10.1016/j.jhin.2018.10.024>.
- [18] Yoon EJ, Choi MH, Park YS, Lee HS, Kim D, Lee H, et al. Impact of host-pathogen-treatment tripartite components on early mortality of patients with *Escherichia coli* bloodstream infection: prospective observational study. *EBioMedicine* 2018;35:76–86. <https://doi.org/10.1016/j.ebiom.2018.08.029>.
- [19] Ohnuma T, Chihara S, Costin B, Treggiari MM, Bartz RR, Raghunathan K, et al. Association of appropriate empirical antimicrobial therapy with in-hospital mortality in patients with bloodstream infections in the US. *JAMA Netw Open* 2023;6:e2249353. <https://doi.org/10.1001/jamanetworkopen.2022.49353>.
- [20] Rodríguez-Baño J, Picón E, Gijón P, Hernández JR, Ruíz M, Peña C, et al. Community-onset bacteremia due to extended-spectrum beta-lactamase-producing *Escherichia coli*: risk factors and prognosis. *Clin Infect Dis* 2010;50:40–8. <https://doi.org/10.1086/649537>.
- [21] Cummins ML, Reid CJ, Djordjevic SP. F plasmid lineages in *Escherichia coli* ST95: implications for host range, antibiotic resistance, and zoonoses. *mSystems* 2022;7:e0121221. <https://doi.org/10.1128/mSystems.01212-21>. Published correction appears in *mSystems* 2022;7:e0021022.
- [22] Fröding I, Hasan B, Sylvén I, Coorens M, Naucclér P, Giske CG. Extended-spectrum-β-lactamase- and plasmid AmpC-producing *Escherichia coli* causing community-onset bloodstream infection: association of bacterial clones and virulence genes with septic shock, source of infection, and recurrence. *Antimicrob Agents Chemother* 2020;64:23511–9. <https://doi.org/10.1128/AAC.02351-19>.
- [23] Stephens CM, Adams-Sapper S, Sekhon M, Johnson JR, Riley LW. Genomic analysis of factors associated with low prevalence of antibiotic resistance in extraintestinal pathogenic *Escherichia coli* Sequence Type 95 Strains. *mSphere* 2017;2:3900–16. <https://doi.org/10.1128/mSphere.00390-16>.
- [24] Jauneikaite E, Honeyford K, Blandy O, Mosavie M, Pearson M, Ramzan FA, et al. Bacterial genotypic and patient risk factors for adverse outcomes in *Escherichia coli* bloodstream infections: a prospective molecular epidemiological study. *J Antimicrob Chemother* 2022;77:1753–61. <https://doi.org/10.1093/jac/dkac071>.
- [25] Hüttener M, Dietrich M, Paytubi S, Juárez A. HilA-like regulators in *Escherichia coli* pathotypes: the YgeH protein from the enteroaggregative strain 042. *BMC Microbiol* 2014;14:268. <https://doi.org/10.1186/s12866-014-0268-5>.
- [26] Savarino SJ, McVeigh A, Watson J, Cravioto A, Molina J, Echeverria P, et al. Enteroaggregative *Escherichia coli* heat-stable enterotoxin is not restricted to enteroaggregative *E. coli*. *J Infect Dis* 1996;173:1019–22. <https://doi.org/10.1093/infdis/173.4.1019>.
- [27] Telli M, Guiral E, Martínez JA, Almela M, Bosch J, Vila J, et al. Prevalence of enterotoxins among *Escherichia coli* isolates causing bacteraemia. *FEMS Microbiol Lett* 2010;306:117–21. <https://doi.org/10.1111/j.1574-6968.2010.01945.x>.
- [28] Dubreuil JD. EAST1 toxin: an enigmatic molecule associated with sporadic episodes of diarrhea in humans and animals. *J Microbiol* 2019;57:541–9. <https://doi.org/10.1007/s12275-019-8651-4>.
- [29] Sheikh J, Dudley EG, Sui B, Tamboura B, Suleman A, Nataro JP. EilA, a HilA-like regulator in enteroaggregative *Escherichia coli*. *Mol Microbiol* 2006;61:338–50. <https://doi.org/10.1111/j.1365-2958.2006.05234.x>.
- [30] Zhou M, Guo Z, Duan Q, Hardwidge PR, Zhu G. *Escherichia coli* type III secretion system 2: a new kind of T3SS? *Vet Res* 2014;45:32. <https://doi.org/10.1186/1297-9716-45-32>.