

Implementation of a Bundle for the Management of Patients With Gram-Negative Bloodstream Infection: A Pre-Post Quasi-experimental Study

Cecilia Bonazzetti,^{1,2,a} Andrea Grechi,^{1,a} Dino Gibertoni,³ Ludovica Mangione,¹ Fulvia Todisco,¹ Matteo Tonnini,^{1,4} Fabio Piscaglia,^{1,4} Paolo Caraceni,^{1,5} Pietro Calogero,⁶ Claudio Borghi,^{1,7} Giovanni Barbara,^{1,8} Natascia Carocchia,² Matteo Rinaldi,^{1,2} Renato Pascale,^{1,2} Beatrice Tazza,² Alice Toschi,² Armando Amicucci,¹ Caterina Campoli,² Zeno Pasquini,² Federica Calò,² Simone Ambretti,^{1,9} Pierluigi Viale,^{1,2} and Maddalena Giannella^{1,2,e}, for the FICO-BUG Study Group^b

¹Department of Medical and Surgical Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy, ²Infectious Diseases Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, ³Epidemiology and Statistics, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, ⁴Division of Internal Medicine, Hepatobiliary and Immunoallergic Diseases, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, ⁵Unit of Semeiotics, Liver and Alcohol-Related Disease, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, ⁶Department of Medical and Surgical Sciences, IRCCS-Sant'Orsola-Malpighi Hospital, Bologna, Italy, ⁷Cardiovascular Medicine Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, ⁸Gastroenterology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy, and ⁹Microbiology Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy

Background. Lack of international guidelines for gram-negative bloodstream infection (GN-BSI) management poses clinical challenges. We aimed to assess whether implementing a management bundle for patients with GN-BSI affected mortality and/or emergence of multidrug resistance (MDR).

Methods. This was a single-center, quasi-experimental study including adult patients hospitalized with monomicrobial GN-BSI. In the preimplementation phase (“pre-phase,” 2018–2019), no standardized protocol was used. In the postimplementation phase (“post-phase,” 2022–2023), a bundle was applied including predefined rules for follow-up blood cultures (FU-BCs), imaging, β -lactam administration schedule, and treatment duration. Primary endpoint was 30-day all-cause mortality; secondary endpoint was 30-day MDR-GN carriage or infection. Treatment-effect lasso models were used to estimate bundle impact.

Results. A total of 2683 patients were included: 1430 in the pre-phase and 1253 in the post-phase. FU-BCs were performed in 22.5% (n = 322) versus 47% (n = 589), $P < .001$, and imaging in 65.5% (n = 936) versus 71.6% (n = 897), $P < .001$; median treatment duration was 10 days in both phases ($P = .625$). Thirty-day mortality was 10.7% (n = 153) versus 11.6% (n = 143), $P = .467$. New MDR carriage/infection was 140 (11.0%) versus 78 (6.5%), $P < .001$. The bundle had no overall effect on 30-day mortality (average treatment effect [ATE] = 0.008, $P = .568$), despite reduced mortality in BSIs due to carbapenem-resistant organisms (ATE = -0.074 , $P = .197$); the effect was not significant. MDR emergence was significantly reduced (ATE = -0.038 , $P = .031$) in both overall and high-risk populations.

Conclusions. The GN-BSI management bundle reduced MDR.

Keywords. antibiotic resistance; bundle; gram negative bloodstream infection; MDR carriage; MDR infection.

The management of bloodstream infections (BSIs) is complex, requiring timely antimicrobial therapy, a targeted diagnostic approach, effective source control, and ongoing follow-up [1]. While established guidelines exist for infections caused by

pathogens such as *Staphylococcus aureus* and *Candida* spp [2, 3], the management of gram-negative (GN) BSIs remains highly differentiated due to the absence of standardized protocols [4].

The use of follow-up blood cultures (FU-BCs) is debated in GN-BSIs due to concerns over cost and extended hospital stays [5]. Nevertheless, observational studies and meta-analyses suggest that FU-BCs may reduce mortality, particularly in cases involving severe infection sources, antibiotic resistance, or suboptimal initial therapy [6]. Beyond FU-BCs, radiological assessment is critical in identifying BSI sources and complications [1, 7]. Indeed, several imaging modalities such as computed tomography (CT) and contrast-enhanced ultrasound have proven useful for diagnosing and managing complications of GN-BSIs, such as pyelonephritis and intra-abdominal abscesses [8, 9]. Additionally, BSI source identification may enhance source control, which remains a cornerstone of successful treatment and is useful in reducing antibiotic use [10]. Intravenous β -lactam antibiotics are usually

Received 23 October 2025; accepted 27 October 2025; published online 15 November 2025
 aC. B. and A. G. contributed equally to this work.

^bMembers of the FICO-BUG Study Group are listed in the Acknowledgments.

Correspondence: Maddalena Giannella, MD, PhD, Department of Medical and Surgical Sciences, University of Bologna, Infectious Diseases Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Via Massarenti 11, Bologna 40137, Italy (maddalena.giannella@unibo.it).

Open Forum Infectious Diseases®

© The Author(s) 2025. Published by Oxford University Press on behalf of Infectious Diseases Society of America. This is an Open Access article distributed under the terms of the Creative Commons Attribution-NonCommercial-NoDerivs licence (<https://creativecommons.org/licenses/by-nc-nd/4.0/>), which permits non-commercial reproduction and distribution of the work, in any medium, provided the original work is not altered or transformed in any way, and that the work is properly cited. For commercial re-use, please contact reprints@oup.com for reprints and translation rights for reprints. All other permissions can be obtained through our RightsLink service via the Permissions link on the article page on our site—for further information please contact journals.permissions@oup.com.
<https://doi.org/10.1093/ofid/ofaf680>

administered for initial treatment of GN-BSIs, with transition to targeted therapy once susceptibility results are available [1]. Pharmacokinetic/pharmacodynamic (PK/PD) approaches, including extended or continuous β -lactam infusion, are promising in achieving higher rates of cure and minimizing resistance [11]. Finally, for uncomplicated GN-BSIs, evidence supports noninferiority of shorter antibiotic courses, theoretically reducing the risks associated with prolonged therapy [7, 12, 13].

Structured, evidence-based care bundles have emerged as an effective approach to standardizing management and improving outcomes [14]. While such bundles have been successfully implemented for infections like *Staphylococcus aureus* BSI [15], limited data are available on their use in GN-BSIs [16, 17]. Our hypothesis was that introducing a bundle for the management of patients with GN-BSI could improve patient outcome and optimize antibiotic use, reducing the emergence of further antibiotic resistance.

With this premise, we performed a pre-post quasi-experimental study to assess the impact of a tailored bundle on clinical outcome and emergence of multidrug resistance (MDR) in patients with GN-BSI, focusing on key interventions including FU-BCs, imaging investigations, optimized antibiotic administration, and streamlined treatment duration.

METHODS

Setting and Study Population

The study was conducted at IRCCS Azienda Ospedaliero-Universitaria of Bologna, a 1400-bed tertiary teaching hospital. Inclusion criteria consisted of hospitalized patients with GN-BSI, aged 18 years or older, who provided informed consent to participate in the study. Exclusion criteria were palliative care or high likelihood of death within 72 hours from index blood culture (BC), or hospital discharge within 72 hours after index BC. Polymicrobial BSIs involving gram-positive bacteria or fungi were not eligible, although patients with BSI caused by multiple GN bacteria were included.

Study Design

We conducted a quasi-experimental study with pre-phase and post-phase periods. In the pre-phase (January 2018 to December 2019), the management of patients with GN-BSI was not dictated by clinical protocol. This cohort includes patients who had been prospectively enrolled in an observational study already ongoing at our center before the implementation of the bundle [18]. During the postintervention phase (January 2022 to December 2023), patients with GN-BSI were prospectively identified through an alert system, which consisted of an electronic report generated by the microbiology laboratory for positive BCs. This report was accessed daily, from Monday to Friday, by the infectious disease (ID) consultants, who

subsequently proposed the management of BSI according to the predefined bundle to the attending physicians. The bundle consisted of the following components:

1. Follow-up blood cultures: FU-BCs were recommended within 7 days for high-risk patients, such as immunocompromised individuals, those with device-related BSIs, patients who received inappropriate empirical therapy, those with incomplete or absent source control, and those with infections caused by pathogens with difficult-to-treat resistance (DTR) profiles.
2. Imaging and source control: Imaging was performed to assess the BSI source and complications. For urinary-origin BSI, contrast-enhanced imaging was recommended to rule out pyelonephritic foci. Chest X-ray and/or CT were recommended for suspected pneumonia. In the presence of central venous catheter, color-Doppler ultrasonography was advised to exclude thrombophlebitis. Echocardiography was suggested for patients with suspected endocarditis, and positron emission tomography-CT scan was used in patients suspected of having vascular or valvular prosthesis infections or when no clear alternative focus for the GN-BSI was evident. Source control is intended as interventional measures aimed at eliminating the infectious focus (such as removal of infected hardware or drainage of infected fluid via surgery or other procedural interventions).
3. Optimized antibiotic administration: Loading dose followed by extended or continuous infusion of β -lactams was recommended where appropriate (eg, this rule was not applied to ceftriaxone and ertapenem).
4. Shortened treatment duration for uncomplicated BSIs: For uncomplicated GN-BSIs (see definition in “Study Variables and Definitions” below), a treatment duration of 7 days with at least 1 in vitro active agent was recommended. For other cases, treatment duration was individualized based on clinical features.

Before the bundle implementation, education and training sessions with all physicians in the hospital involved in the management of patients with GN-BSI were performed to present the scope and the components of the bundle, and to set up its implementation (eg, microbiology laboratory workflow for providing results of index BC and agreement on FU-BC criteria, radiology unit agreement on imaging investigations).

The ID consultation service, available upon request by the attending physician, was accessible during both phases. Follow-up procedures were identical in both study phases. Patients in both the pre-phase and post-phase were followed from the day of index BCs up to 1 year through inpatient or outpatient visits, medical record reviews, or telephone calls. Patients were included in the study only once at the time of index BCs.

Microbiology

During the study period, BCs were incubated using the automated BACTEC FX blood culture system (Becton Dickinson, Franklin Lakes, New Jersey, USA). Positive blood cultures were processed using matrix-assisted laser desorption/ionization–time of flight for rapid pathogen identification. Susceptibility testing of strains was performed using the automated Vitek 2 system (bioMérieux, Marcy l'Étoile, France) during the pre-phase period, while the MicroScan WalkAway system (Beckman Coulter, Brea, California, USA) was used in the post-phase period. Minimum inhibitory concentration values were interpreted according to European Committee on Antimicrobial Susceptibility Testing clinical breakpoints.

Rectal swabs for carbapenemase-producing Enterobacterales (CPE) were performed weekly to all patients as screening program.

Study Variables and Definitions

The primary endpoint was 30-day all-cause mortality. Secondary endpoints included (1) colonization and/or infection with MDR-GN (defined as resistance to at least 1 agent in ≥ 3 antimicrobial categories) [19] and DTR-GN (defined by resistance to all first-line antibiotics) [20], assessed at 30 and 90 days by standardized surveillance cultures and/or clinical cultures if needed; (2) all-cause mortality at 90, 180, and 360 days from the date of index BCs; (3) persistent BSI (ie, a BSI that remained positive over time, despite appropriate antimicrobial therapy and initial management efforts); (4) breakthrough BSI (defined as a new episode of BSI that occurred while the patient was receiving appropriate antimicrobial therapy, specifically active in vitro against the identified pathogen); (5) recurrent BSI (new episode of BSI caused by the same pathogen that occurred after resolution of a previous BSI episode, typically following a documented period of negative blood cultures and clinical improvement); and (6) documented *Clostridioides difficile* infection at 30 and 90 days from the index BCs.

Other study variables included demographic data (age and sex), baseline clinical parameters such as McCabe-Jackson criteria [21] and Charlson Comorbidity Index (CCI) [22], immunosuppression status (including neutropenia, defined as neutrophils < 500 cells/ μL ; solid organ transplantation; corticosteroid therapy at a dosage of ≥ 16 mg/day for ≥ 15 days; and human immunodeficiency virus-1 infection with CD4 count < 200 cells/ μL). Date of hospital admission and discharge, the ward of stay at the time of the first positive BCs, and rectal swab positivity for CPE at the time of the index BCs were also obtained. Degree of clinical severity at the time of index BCs based on the Sequential Organ Failure Assessment (SOFA) score [23], quick SOFA score, Pitt bacteremia score, and septic shock criteria was also assessed [24]. As for BSI characteristics, uncomplicated BSI was defined as BSI that met all 4 criteria: (1) secondary to urinary tract, intrabdominal, respiratory, catheter-related, and skin and soft tissue; (2) complete source control; (3) absence of

immunocompromising condition; and (4) clinical improvement within 72 hours of effective antibiotic treatment—at a minimum including defervescence and hemodynamic stability [7]. Site of BSI acquisition (community-acquired, healthcare-associated, and nosocomial) [25] and pathogen characteristics including species and resistance mechanisms were recorded. Data on BSI management included execution of FU-BCs, type and date of imaging, identification of the infection source, identification of local or embolic complications, and source control (classified as partial, complete, or not performed). Appropriateness of empirical therapy was defined as the administration of at least 1 antibiotic active in vitro; failure to administer antimicrobial therapy or administration beyond 24 hours from infection onset was considered inappropriate. Last, bundle compliance was categorized as follows: no adherence, partial adherence (1 or more recommendations followed), or total adherence (all recommendations followed).

Statistical Analysis

All enrolled patients were described by expressing categorical variables as absolute frequencies and percentages, and continuous variables as mean (\pm standard deviation) or median (interquartile range). Categorical variables were compared using χ^2 or Fisher test, and continuous variables were compared using Student *t*-test or Mann-Whitney test, based on the variable distribution. To reduce survival bias, we excluded from the analysis patients who died within 5 days from the index blood culture. Moreover, follow-up blood cultures and imaging that were carried > 5 days after outbreak of BSI were considered as not performed. To evaluate the impact of the bundle on clinical outcomes, a treatment-effect lasso model was used, a method designed to estimate the causal effects of an intervention on an outcome by leveraging the lasso model to manage high-dimensional data and identify relevant variables. In this model, the average treatment effect (ATE; the average difference in outcomes between treated and untreated groups) and the potential-outcome means (POM; the expected outcome if all patients were not treated) are estimated for the treatment variable and for each level of the outcome by combining lasso methods for inference with augmented inverse-probability weighting, and lasso methods are used to select variables to be included in the model. This method allows us to model treatment (ie, having been managed with the bundle) causally and at the same time using a large number of covariates potentially affecting the outcome to efficiently estimate the effect of treatment on the outcome. We estimated the treatment effect of bundle on the primary outcome (30-day mortality) and on colonization and/or infection by MDR/DTR microorganisms at 30 days in the overall population. We also performed the analysis on subgroups identified by the BSI's causative microorganisms (*Escherichia coli*, *Klebsiella* spp, *Enterobacter* spp, and *Pseudomonas* spp) and resistance profiles (carbapenem resistant and non-carbapenem resistant). In all models, the controls

Table 1. Characteristics of the Population

Characteristic	Pre-phase (n = 1430)	Post-phase (n = 1253)	Test (P Value)
Age, y, mean \pm SD	70.4 \pm 16.4	71.1 \pm 16.2	.222
Male sex	789 (55.2)	733 (58.5)	.083
Myocardial infarction	225 (15.7)	174 (13.9)	.180
COPD	212 (14.8)	176 (14.0)	.567
Moderate to severe CKD	305 (21.3)	287 (22.9)	.326
Uncomplicated DM	251 (17.6)	249 (19.9)	.124
End-organ damage DM	75 (5.2)	54 (4.3)	.259
Moderate to severe liver disease	146 (10.2)	90 (7.2)	.006
Localized solid tumor	216 (15.1)	191 (15.2)	.921
Metastatic solid tumor	110 (7.7)	106 (8.5)	.466
Leukemia	64 (4.5)	60 (4.8)	.700
CCI score, mean \pm SD	5.6 \pm 3.0	5.6 \pm 2.7	.479
McCabe-Jackson classification			.869
Rapidly fatal	148 (10.8)	120 (10.2)	
Moderately fatal	644 (46.8)	551 (46.7)	
Not fatal	584 (42.5)	509 (43.1)	
Immunosuppression	235 (16.4)	226 (18.0)	.272
Neutropenia	69 (4.8)	58 (4.6)	.811
SOT	104 (7.3)	116 (9.3)	.062
Corticosteroids	59 (4.1)	45 (3.6)	.474
Inpatient ward			<.001
Internal medicine	1052 (73.6)	770 (61.5)	
Surgery	159 (11.1)	180 (14.4)	
ICU	117 (8.2)	136 (10.9)	
Emergency	102 (7.1)	166 (13.3)	
Length of hospital stay, d, median (IQR)	18.0 (10–34)	21.0 (11–39)	<.001
Time from admission to BSI, d, median (IQR)	1.0 (0–12)	1.0 (0–14)	.519
Pitt bacteremia score, mean \pm SD	0.9 \pm 1.6	1.0 \pm 1.5	.027
SOFA score, mean \pm SD	3.4 \pm 2.9	3.5 \pm 2.7	.404
Septic shock	119 (8.3)	123 (9.8)	.178
Uncomplicated BSI	134 (9.4)	112 (8.9)	.646
Rectal swab positivity	170 (11)	90 (7.1)	<.001
Site of BSI acquisition			.346
Community-acquired	469 (33.0)	438 (35.1)	
Healthcare-associated	373 (26.2)	334 (26.8)	
Nosocomial	580 (40.8)	476 (38.1)	

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: BSI, bloodstream infection; CCI, Charlson Comorbidity Index; CKD, chronic kidney disease; COPD, chronic obstructive pulmonary disease; DM, diabetes mellitus; ICU, intensive care unit; IQR, interquartile range; SD, standard deviation; SOFA, Sequential Organ Failure Assessment; SOT, solid organ transplantation.

included were demographic, clinical, comorbidities, bundle components, pathogens, resistance variables, and the square of continuous variables to account for potential nonlinearity (list provided in [Supplementary Table 1](#)). Lasso estimation was carried out using the double robust estimator [26], which calculates a unique value for λ , the smallest one that dominates the noise in the estimating equations.

Stata version 18.5 software was used for all analyses, with statistical significance set at $P = .05$.

RESULTS

A total of 1430 patients were included in the preimplementation phase (pre-phase) and 1253 in the postimplementation

phase (post-phase), as shown in the study flowchart ([Supplementary Figure 1](#)). The characteristics of these subpopulations are compared in [Table 1](#). Mean age was similar in the 2 groups (70.4 \pm 16.4 vs 71.1 \pm 16.2 years; $P = .222$), as was the proportion of male patients (55.2% vs 58.5%; $P = .083$). Mean CCI score was lower in the post-phase (5.6 \pm 3.0 vs 5.6 \pm 2.7; $P = .479$) and the McCabe-Jackson classification showed a higher proportion of “not fatal” cases (42.5% vs 43.1%; $P = .869$), whereas no significant differences were observed in Pitt and SOFA scores. Median hospital length of stay was significantly longer in the post-phase (18.0 vs 21.0 days; $P < .001$), while the time from admission to BSI onset was shorter (1.0 vs 1.0 days; $P = .519$). However, there were fewer nosocomial cases (40.8% vs 38.1%) in the post-phase.

Table 2. Source of Bloodstream Infection, Microorganism and Mechanism of Resistance

Characteristic	Pre-phase (n = 1430)	Post-phase (n = 1253)	Test (P Value)
Primary	280 (19.6)	292 (23.6)	.013
Secondary			
Lung infection	96 (6.7)	81 (6.5)	.844
IAI	313 (21.9)	232 (18.7)	.040
UTI	585 (41.0)	482 (38.9)	.271
SSTI	13 (0.9)	10 (0.8)	.772
Other source	51 (3.6)	50 (4.0)	.524
Intravascular device-related	89 (6.2)	92 (7.4)	.224
Microorganism			
<i>Klebsiella</i> spp	371 (26)	355 (28.4)	.152
<i>Escherichia coli</i>	797 (55.8)	596 (47.8)	<.001
<i>Enterobacter</i> spp	93 (6.5)	67 (5.4)	.213
<i>Citrobacter</i> spp	2 (0.1)	7 (0.6)	.061
<i>Serratia</i> spp	0	25 (2.0)	<.001
<i>Morganella</i> spp	2 (0.1)	10 (0.8)	.011
<i>Proteus</i> spp	55 (3.9)	51 (4.1)	.756
<i>Salmonella</i> spp	0 (0.0)	2 (0.2)	.130
<i>Pseudomonas</i> spp	67 (4.7)	96 (7.7)	.001
<i>Acinetobacter</i> spp	26 (1.8)	15 (1.2)	.194
<i>Stenotrophomonas</i> spp	15 (1.1)	4 (0.3)	.025
Other GN bacteria	0	20 (1.6)	<.001
Mechanism of resistance			
None	397 (27.8)	431 (34.4)	<.001
3GC resistance	533 (37.3)	446 (35.6)	.368
FQ resistance	600 (42)	332 (26.5)	<.001
Carbapenem resistance	140 (9.8)	62 (4.9)	<.001
BL/BLI resistance	739 (51.7)	614 (49)	.167
Amoxicillin/clavulanic acid	696 (48.7)	580 (46.3)	.218
Piperacillin/tazobactam	345 (24.1)	260 (20.8)	.037
DTR	168 (12.1)	91 (7.5)	<.001
Carbapenem resistance			
KPC	80 (5.6)	23 (1.8)	<.001
VIM	0	4 (0.3)	.033
NDM	10 (0.7)	7 (0.6)	.647
OXA-48-like	2 (0.1)	14 (1.1)	.001
Other	7 (0.5)	7 (0.6)	.804

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: 3GC, third-generation cephalosporin; BL/BLI, β -lactam/ β -lactamase inhibitor; DTR, difficult-to-treat resistance; FQ, fluoroquinolones; GN, gram-negative; IAI, intrabdominal infection; KPC, *Klebsiella pneumoniae* carbapenemase; NDM, New Delhi metallo- β -lactamase; OXA-48-like, oxacillinase-48-like; SSTI, skin and soft tissue infection; UTI, urinary tract infection; VIM, Verona integron-encoded metallo- β -lactamase.

Regarding BSI characteristics (Table 2), primary BSIs were more frequent in the post-phase (19.6% vs 23.6%; $P = .013$), whereas intra-abdominal infections decreased (21.9% vs 18.7%; $P = .040$). *Escherichia coli* remained the most common pathogen but was less frequent in the post-phase (55.8% vs 47.8%; $P < .001$), while *Klebsiella* spp were stable (26% vs 28.4%; $P = .152$). Resistance profiles differed between the 2 study periods. Carbapenemase-producing strains were significantly less common in the post-phase, especially *Klebsiella pneumoniae* carbapenemase (5.6% vs 1.8%; $P < .001$). The prevalence of DTR also decreased significantly (12.1% vs 7.5%; $P < .001$).

Regarding BSI management (Table 3), the rate of appropriate empirical therapy increased in the post-phase (68.9% vs 77.0%; $P < .001$), as did the execution of FU-BCs within 5

days (22.5% vs 47%; $P < .001$) and the use of imaging (65.5% vs 71.6%; $P < .001$). Source control was completely performed in a slightly higher proportion of patients in the post-phase (40.3% vs 41.9%; $P = .223$). Median treatment duration was similar between the 2 groups. Empirical and definitive treatment regimens by antibiotic class for the pre- and post-phases and the ID consultation are reported in Supplementary Table 2.

As illustrated in Figure 1, in the post-phase the adherence to individual components of the management bundle varied substantially among patients.

Clinical outcomes are summarized in Table 4. There were no statistically significant differences between the pre- and post-phase cohorts in terms of mortality at day 30 (10.7% vs 11.4%; $P = .556$), 90 days (19.1% vs 20.9%; $P = .239$),

Table 3. Management of Bloodstream Infection

Characteristic	Pre-phase (n = 1430)	Post-phase (n = 1253)	Test (P Value)
Treatment duration, d, median (IQR)	10.0 (8–14)	10.0 (8–13)	.625
Appropriate empirical therapy	944 (68.9)	923 (77.0)	<.001
Appropriate targeted therapy	1308 (96.2)	1102 (98.9)	<.001
Execution of FU-BCs within 5 d	322 (22.5)	589 (47.0)	<.001
Imaging performance within 5 d	936 (65.5)	897 (71.6)	<.001
Source control			.223
Completely performed	249 (40.3)	228 (41.9)	
Partially performed	98 (15.9)	102 (18.8)	
Not performed	271 (43.9)	214 (39.3)	

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: FU-BC, follow-up blood culture; IQR, interquartile range.

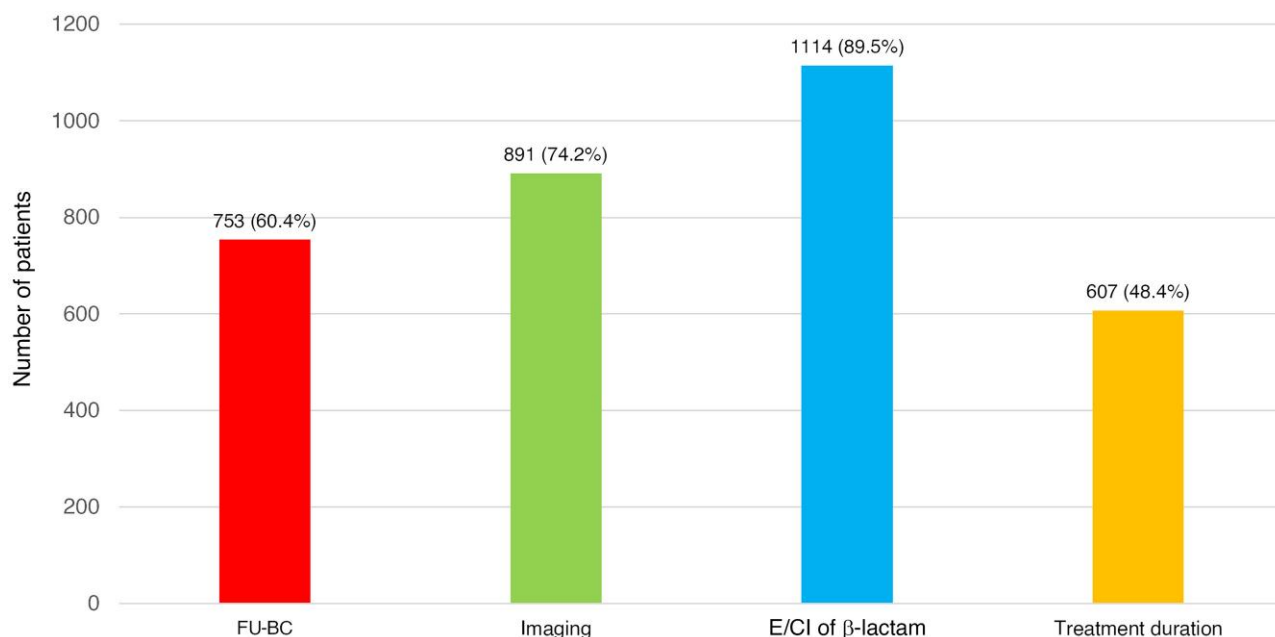


Figure 1. Bundle adherence. Abbreviations: E/CI: extended/continuous infusion; FU-BC, follow-up blood culture.

180 days (23.2% vs 26.8%; $P = .031$), and 360 days (27.1% vs 30.9%; $P = .032$). New carriage or infection with MDR or DTR organisms at 30 and 90 days was significantly reduced in the post-phase (11% vs 6.5%, $P < .001$; and 5.7% vs 3.2%, $P < .003$, respectively). *Clostridioides difficile* infections remained infrequent, with no significant difference at 30 days (1.8% vs 2.1%; $P = .497$), but were slightly more common in the post-phase at 90 days (0.6% vs 1.2%; $P = .131$).

The treatment-effect lasso model confirmed that no significant difference in 30-day mortality was observed between the 2 periods in the overall population (ATE = 0.006; $P = .675$, with POM = 0.112). Subgroup analyses revealed a marked reduction in 30-day mortality among patients infected with

carbapenem-resistant organisms (ATE = -0.077 ; $P = .178$), albeit not statistically significant likely because of the limited number of patients in this group ($n = 167$). Regarding secondary outcomes, the rate of new MDR/DTR carriage or infection at 30 days was significantly lower in the post-phase (ATE = -0.038 ; $P = .034$, with POM = 0.111). Subgroup analyses of this outcome were consistent with the overall findings, showing reductions across most pathogen groups: *E coli* (ATE = 0.002; $P = .889$), *Klebsiella* spp (ATE = -0.056 ; $P = .114$), *Pseudomonas* spp (ATE = -0.163 ; $P = .457$), carbapenem-sensitive strains (ATE = -0.038 ; $P = .037$) and carbapenem-resistant strains (ATE = -0.208 ; $P = .038$). These results are illustrated in Figure 2.

Table 4. Comparison of Outcomes Between Cohorts

Outcome	Pre-phase (n = 2092)	Post-phase (n = 1263)	Test (P Value)
30-d mortality	153 (10.7)	143 (11.4)	.467
90-d mortality	273 (19.1)	262 (20.9)	.239
180-d mortality	332 (23.2)	336 (26.8)	.031
360-d mortality	388 (27.1)	387 (30.9)	.032
Persistent BSI	82 (6.2)	59 (4.8)	.150
Breakthrough BSI	25 (1.9)	52 (4.3)	<.001
Recurrent BSI within 90 d	135 (9.4)	120 (9.6)	.904
30-d MDR/DTR carriage/infection	140 (11.0)	78 (6.5)	<.001
90-d MDR/DTR carriage/infection	68 (5.7)	38 (3.2)	.003
30-d <i>C difficile</i> infection	22 (1.8)	26 (2.1)	.497
90-d <i>C difficile</i> infection	7 (0.6)	14 (1.2)	.131

Data are presented as No. (%) unless otherwise indicated.

Abbreviations: BSI, bloodstream infection; *C difficile*, *Clostridioides difficile*; DTR, difficult-to-treat resistance; MDR, multidrug resistance.

DISCUSSION

In this quasi-experimental study, a structured GN-BSI care bundle did not lower overall 30-day mortality but improved outcomes in high-risk subgroups such as carbapenem-resistant BSIs and significantly reduced new MDR/DTR colonization and infection in both overall and subgroup populations.

Our study differs from previous studies focused on bundle management for GN-BSI for a larger sample size and multiple key components, including FU-BCs. Thus, it offers a comprehensive and structured approach to GN-BSI management [16, 17].

One of the most significant improvements following bundle adoption was the increased execution of FU-BCs within 5 days (22.5% vs 47%). Although their routine use in GN-BSI remains debated [5], recent evidence suggests that FU-BCs may offer a survival benefit in specific populations, such as those with high bacterial load, resistant organisms, or suboptimal source control [6, 27]. In our study, enhanced FU-BC use may have enabled earlier recognition of persistent infection or treatment failure, contributing to more informed clinical decisions.

Imaging was significantly increased in the post-phase, with >70% of patients undergoing diagnostics within 5 days from index BCs. Enhanced imaging supports infection source identification and detection of complications like abscesses or embolic events [28]. In this regard, despite the performance of source control was similar in the 2 study phases, we did not collect the date of source control to assess an impact on timing.

Extended or continuous infusion of β -lactams was implemented in nearly 90% of patients, aligning with growing evidence supporting PK/PD-optimized therapy [29]. In addition, we implemented the therapeutic drug monitoring in specific settings, pathogens, and/or in patients receiving new drugs showing a significant benefit in terms of microbiological clearance and prevention of resistance [30]. Moreover, TDM was applied more frequently in the postintervention phase. This reflects the substantial growth of supporting

evidence that emerged in the years following the pre-phase, showing that TDM-guided optimization of β -lactam therapy can significantly improve patient outcomes, as demonstrated in recent meta-analyses [31].

Regarding the mode of β -lactam administration, we have also added data on the proportion of patients treated with extended or continuous infusion in each phase. In both study periods, virtually all eligible patients received β -lactams via extended infusion or continuous infusion whenever this was feasible (depending on drug stability, venous access, and infusion compatibility).

Adherence to shortened treatment was suboptimal (48.4%), likely due to concerns about recurrence. However, trials have confirmed that shorter courses are effective for uncomplicated GN-BSIs, highlighting the need for ongoing education and stewardship support [12, 13].

Importantly, suboptimal adherence to some bundle components—particularly shortened treatment duration and systematic FU-BC execution—may have limited the overall prognostic impact of the intervention, potentially attenuating the expected benefits despite improvements in other areas.

Our main findings demonstrate that while the bundle did not significantly reduce overall 30-day mortality in the general population, it seemed to produce improvements in patients with carbapenem-resistant BSIs; however, this should be confirmed in a larger study. These findings reflect the heterogeneity of the GN-BSI population including patients with *E coli* BSI from urinary source carrying generally low level of resistance and associated with low rates of mortality, to immunocompromised patients with drug-resistant GN bacteria associated with higher rates of mortality [32]. Computational models able to stratify GN-BSI patients according to their risk of death, or complications such as relapse/recurrence, could be useful to prioritize patients to be managed according to a structured bundle.

Indeed, since novel antibiotics for the treatment of carbapenem-resistant GN infections were not yet available

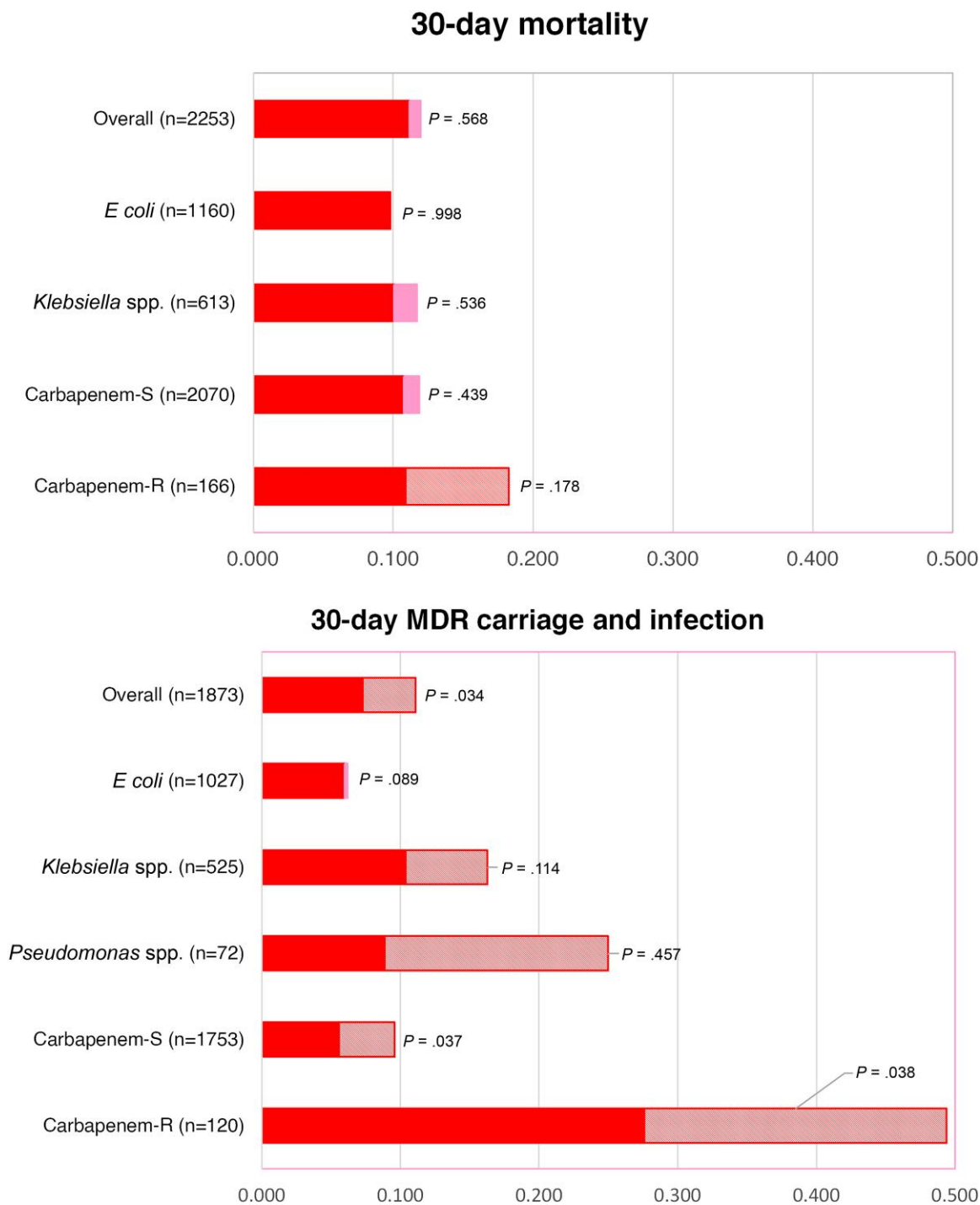


Figure 2. Treatment-effect lasso on 30-day mortality and 30-day multidrug resistance (MDR) carriage infection. Solid (filled) bar represents the potential-outcome means (POM); positive average treatment effect (ATE) is shown by the open bar (no fill) extending beyond the POM, while negative ATE is represented by the dashed bar inside the POM; P value is of the ATE. The analysis on mortality for patients with *Pseudomonas* spp infection is not reported because it produced inconsistent results (POM >1).

before 2018, we decided to exclude patients with BSI occurring prior to 1 January 2018. Although this reduced the sample size, it minimized the risk of selection bias related to unequal access to these new therapeutic options. We expected a reduction in the emergence of further resistance as a consequence of better

antibiotic use. Despite achieving this goal, we must recognize that some key elements of the bundle for this purpose, such as reduction of treatment duration and increased source control, were not achieved. On the contrary, there was an increase in appropriate empirical therapy favoring de-escalation [33],

probably as a consequence of educational and training activities preceding the bundle implementation. Moreover, several bundle components likely shaped MDR colonization outcomes—promoting appropriate empiric therapy, using PK/PD-optimized β -lactam dosing, and applying stewardship measures such as shorter courses for uncomplicated infections.

Finally, the lower prevalence of antibiotic resistance during the post-phase may have influenced this endpoint, even if the model was adjusted for baseline resistance. Moreover, the finding that the bundle was protective against MDR infection and colonization also in the subgroup with carbapenem-susceptible organisms highlights its potential role in preventing the development of resistance even among pathogens for which multiple therapeutic options are still available. This is particularly relevant as inappropriate or excessive use of carbapenems in such settings could otherwise promote unnecessary selective pressure [34, 35].

Our findings indicate that the bundle's benefits—most evident in carbapenem-resistant BSIs—are amplified when paired with structured, intensified ID consultation, consistent with prior evidence that systematic ID consultation input improves early complication recognition, optimizes antibiotic choice and dosing, reinforces evidence-based practice, and ultimately enhances patient outcomes [36–38].

Last, the analytical strength of this study is enhanced by the application of the treatment-effects lasso model, an estimation method that is particularly well suited for real-world, high-dimensional data. By addressing collinearity and limiting overfitting, it allowed for a more robust and interpretable analysis of the impact of treatment, especially in subgroup assessments that might otherwise be underpowered or confounded.

However, several limitations must be acknowledged. The quasi-experimental design, while pragmatic, cannot fully eliminate the risk of unmeasured confounding. The intervention was implemented in a single tertiary center with established ID support, potentially limiting external validity.

Furthermore, not all bundle components were consistently adopted—particularly FU-BCs and treatment duration—possibly attenuating the observed benefits. Moreover, leaving decisions on FU-BCs or specific imaging to the ID physician may limit reproducibility, but this pragmatic, individualized approach reflects real-world practice in complex, comorbid patients. Last, long-term resistance trends and cost-effectiveness were not evaluated, which are important metrics for future studies.

In conclusion, this study shows that bundle-based GN-BSI management, while not universally reducing mortality, curbs antimicrobial resistance and improves outcomes in patients with carbapenem-resistant BSI. It is a valuable stewardship tool, warranting focus on patient selection, adherence, and long-term economic impact. Limited adherence may have blunted overall prognosis. Future work should define an optimal, compliance-friendly bundle for GN-BSIs.

Supplementary Data

Supplementary materials are available at [Open Forum Infectious Diseases](https://doi.org/10.1093/ofid/ofad001) online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

Acknowledgments. Members of the FICO-BUG Study Group are as follows: Giuseppe Ferraro (Infectious Diseases Unit, Azienda Ospedaliero-Universitaria di Ferrara, Ferrara, Italy); Federica Cosentino (Unit of Infectious Diseases, ARNAS Garibaldi Hospital, University of Catania, Catania, Italy); Teresa Miani, Giacomo Beci, Michele Cantini, Maria Clara Chionsini, Irene Grassi, Marta Malosso, Giulia Menotti, Beatrice Miani, Filippo Miselli, Riccardo Pasquali, Anna Pozzi, Riccardo Riccardi, Daniele Riccucci, Valeria Viscusi, Manuel Zagarrigo, Alberto Zuppiroli, Roberta Capozzi, Assunta Cocchiarella, Pietro Fait, Caterina Gatto, Maria Eugenia Giacomini, Alessia Krawczyk, Francesco Marrella, Matteo Montironi, Carolina Patrucco, Alberto Pecorelli, Beatrice Profitti, Dario Santoro, Ilaria Sartini, Oana Vatamanu, Giacomo Ambrosi, Samantha Babini, Michele Bacchiega, Simone Boccia, Chiara Calgarini, Agnese Poma, Maria Teresa Presutti, Carlotta Sgarella, and Lorenzo Santangelo (Department of Medical and Surgical Sciences, Alma Mater Studiorum, University of Bologna, Bologna, Italy); Michela di Chiara and Francesca Simone (Infectious Diseases Unit, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy); and Chiara Faggiano and Alessandro Venturi (Division of Internal Medicine, Hepatobiliary and Immunoallergic Diseases, IRCCS Azienda Ospedaliero-Universitaria di Bologna, Bologna, Italy).

Patient consent. The study was conducted according to the Declaration of Helsinki and the Good Clinical Practice guidelines and was approved by the local ethics committee (number 894/2021/Oss/AOUBo).

Potential conflicts of interest. All authors: No reported conflicts.

References

- Rodríguez-Baño J, De Cueto M, Retamar P, Gálvez-Acebal J. Current management of bloodstream infections. *Expert Rev Anti Infect Ther* 2010; 8:815–29.
- Liu C, Bayer A, Cosgrove SE, et al. Clinical practice guidelines by the Infectious Diseases Society of America for the treatment of methicillin-resistant *Staphylococcus aureus* infections in adults and children: executive summary. *Clin Infect Dis* 2011; 52:285–92.
- Cornely OA, Sprute R, Bassetti M, et al. Global guideline for the diagnosis and management of candidiasis: an initiative of the ECMM in cooperation with ISHAM and ASM. *Lancet Infect Dis* 2025; 25:e280–93.
- Diallo K, Thilly N, Luc A, et al. Management of bloodstream infections by infection specialists: an international ESCMID cross-sectional survey. *Int J Antimicrob Agents* 2018; 51:794–8.
- Canzoneri CN, Akhavan BJ, Tosur Z, Andrade PEA, Aisenberg GM. Follow-up blood cultures in gram-negative bacteremia: are they needed? *Clin Infect Dis* 2017; 65:1776–9.
- Gatti M, Bonazzetti C, Tazza B, et al. Impact on clinical outcome of follow-up blood cultures and risk factors for persistent bacteraemia in patients with gram-negative bloodstream infections: a systematic review with meta-analysis. *Clin Microbiol Infect* 2023; 29:1150–8.
- Heil EL, Bork JT, Abbo LM, et al. Optimizing the management of uncomplicated gram-negative bloodstream infections: consensus guidance using a modified Delphi process. *Open Forum Infect Dis* 2021; 8:ofab434.
- Boccatonda A, Stupia R, Serra C. Ultrasound, contrast-enhanced ultrasound and pyelonephritis: a narrative review. *World J Nephrol* 2024; 13:98300.
- El-Ghar MA, Farg H, Sharaf DE, El-Diasty T. CT and MRI in urinary tract infections: a spectrum of different imaging findings. *Medicina (Kaunas)* 2021; 57:32.
- De Waele JJ. Importance of timely and adequate source control in sepsis and septic shock. *J Intensive Med* 2024; 4:281–6.
- Giannella M, Malosso P, Scudeller L, et al. Quality of care indicators in the MAnAgeMent of BLOODstream infections caused by Enterobacteriaceae (MAMBOO-E study): state of the art and research agenda. *Int J Antimicrob Agents* 2021; 57:106320.
- The BALANCE Investigators, for the Canadian Critical Care Trials Group, the Association of Medical Microbiology and Infectious Disease Canada Clinical

- Research Network, the Australian and New Zealand Intensive Care Society Clinical Trials Group, and the Australasian Society for Infectious Diseases Clinical Research Network. Antibiotic treatment for 7 versus 14 days in patients with bloodstream infections. *N Engl J Med* **2025**; 392:1065–78.
13. Molina J, Montero-Mateos E, Praena-Segovia J, et al. Seven-versus 14-day course of antibiotics for the treatment of bloodstream infections by Enterobacterales: a randomized, controlled trial. *Clin Microbiol Infect* **2022**; 28:550–7.
 14. Institute for Healthcare Improvement. Innovation series: using care bundles to improve health care quality. **2012**. Available at: <https://www.ihl.org/library/white-papers/using-care-bundles-improve-health-care-quality>.
 15. Lopez-Cortes LE, Del Toro MD, Galvez-Acebal J, et al. Impact of an evidence-based bundle intervention in the quality-of-care management and outcome of *Staphylococcus aureus* bacteremia. *Clin Infect Dis* **2013**; 57:1225–33.
 16. Bavaro DF, Diella L, Belati A, et al. Impact of a multistep bundles intervention in the management and outcome of gram-negative bloodstream infections: a single-center “proof-of-concept” study. *Open Forum Infect Dis* **2022**; 9:ofac488.
 17. DiPietro J, Dubrovskaya Y, Marsh K, et al. Antibiotic stewardship bundle for uncomplicated gram-negative bacteremia at an academic health system: a quasi-experimental study. *Antimicrob Steward Healthc Epidemiol* **2024**; 4:e171.
 18. Toschi A, Pascale R, Gibertoni D, et al. Epidemiological characteristics and management of gram-negative bacteraemia in different immunocompromised hosts: observational single-center study. *PLoS One* **2025**; 20:e0327535.
 19. Magiorakos AP, Srinivasan A, Carey RB, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* **2012**; 18:268–81.
 20. Kadri SS, Adjemian J, Lai YL, et al. Difficult-to-treat resistance in gram-negative bacteremia at 173 US hospitals: retrospective cohort analysis of prevalence, predictors, and outcome of resistance to all first-line agents. *Clin Infect Dis* **2018**; 67:1803–14.
 21. Reilly J, Coignard B, Price L, et al. The reliability of the McCabe score as a marker of co-morbidity in healthcare-associated infection point prevalence studies. *J Infect Prev* **2016**; 17:127–9.
 22. Charlson ME, Pompei P, Ales KL, MacKenzie CR. A new method of classifying prognostic comorbidity in longitudinal studies: development and validation. *J Chronic Dis* **1987**; 40:373–83.
 23. Vincent JL, Moreno R, Takala J, et al. The SOFA (Sepsis-related Organ Failure Assessment) score to describe organ dysfunction/failure: on behalf of the Working Group on Sepsis-Related Problems of the European Society of Intensive Care Medicine. *Intensive Care Med* **1996**; 22:707–10.
 24. Singer M, Deutschman CS, Seymour CW, et al. The Third International Consensus Definitions for Sepsis and Septic Shock (Sepsis-3). *JAMA* **2016**; 315:801.
 25. Friedman ND. Health care-associated bloodstream infections in adults: a reason to change the accepted definition of community-acquired infections. *Ann Intern Med* **2002**; 137:791.
 26. Chernozhukov V, Chetverikov D, Demirer M, et al. Double/debiased machine learning for treatment and structural parameters. *Econom J* **2018**; 21:C1–C68.
 27. Thaden JT, Cantrell S, Dagher M, et al. Association of follow-up blood cultures with mortality in patients with gram-negative bloodstream infections: a systematic review and meta-analysis. *JAMA Netw Open* **2022**; 5:e2232576.
 28. Mondal U, Warren E, Bookstaver PB, Kohn J, Al-Hasan MN. Incidence and predictors of complications in gram-negative bloodstream infection. *Infection* **2024**; 52:1725–31.
 29. Gatti M, Cojutti PG, Pea F. Impact of attaining aggressive vs. conservative PK/PD target on the clinical efficacy of beta-lactams for the treatment of gram-negative infections in the critically ill patients: a systematic review and meta-analysis. *Crit Care* **2024**; 28:123.
 30. Gatti M, Rinaldi M, Cojutti PG, et al. A pre-post quasi-experimental study of antimicrobial stewardship exploring the impact of a multidisciplinary approach aimed at attaining an aggressive joint pharmacokinetic/pharmacodynamic target with ceftazidime/avibactam on treatment outcome of KPC-producing *Klebsiella pneumoniae* infections and on ceftazidime/avibactam resistance development. *Antimicrob Agents Chemother* **2025**; 69:e0048825.
 31. Pai Mangalore R, Ashok A, Lee SJ, et al. Beta-lactam antibiotic therapeutic drug monitoring in critically ill patients: a systematic review and meta-analysis. *Clin Infect Dis* **2022**; 75:1848–60.
 32. Holmes CL, Anderson MT, Mobley HLT, Bachman MA. Pathogenesis of gram-negative bacteremia. *Clin Microbiol Rev* **2021**; 34:e00234–20.
 33. López-Cortés LE, Delgado-Valverde M, Moreno-Mellado E, et al. Efficacy and safety of a structured de-escalation from antipseudomonal β -lactams in bloodstream infections due to Enterobacterales (SIMPLIFY): an open-label, multi-centre, randomised trial. *Lancet Infect Dis* **2024**; 24:375–85.
 34. Segagni Lusignani L, Presterl E, Zatorska B, Van Den Nest M, Diab-Elschahawi M. Infection control and risk factors for acquisition of carbapenemase-producing Enterobacteriaceae. A 5 year (2011–2016) case-control study. *Antimicrob Resist Infect Control* **2020**; 9:18.
 35. Budia-Silva M, Kostyanev T, Ayala-Montano S, et al. International and regional spread of carbapenem-resistant *Klebsiella pneumoniae* in Europe. *Nat Commun* **2024**; 15:5092.
 36. Tang G, Huang L, Zong Z. Impact of infectious disease consultation on clinical management and outcome of patients with bloodstream infection: a retrospective cohort study. *Sci Rep* **2017**; 7:12898.
 37. Ong SWX, Luo J, Fridman DJ, et al. Association between infectious diseases consultation and mortality in hospitalized patients with gram-negative bloodstream infection: a retrospective population-wide cohort study. *Clin Infect Dis* **2024**; 79:855–63.
 38. Papadimitriou-Olivgeris M, Senn L, Jacot D, Guery B. Predictors of mortality of *Pseudomonas aeruginosa* bacteraemia and the role of infectious diseases consultation and source control; a retrospective cohort study. *Infection* **2025**; 53:117–24.