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ORIGINAL ARTICLE

Molecular characteristics and predictors of mortality among Gram-positive bacteria isolated from bloodstream infections in critically ill patients during a 5-year period (2012–2016)

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Abstract

To identify the molecular characteristics of Gram-positive cocci isolated from blood cultures and clinical outcome among critically ill patients. This retrospective study was conducted in the general intensive care unit of the University General Hospital of Patras, Greece, during a 5-year period (2012–2016). All adult patients with a Gram-positive BSI were included. PCR was applied to identify *mecA* gene (staphylococci); *vanA*, *vanB*, and *vanC* genes (enterococci). Linezolid-resistant *S. epidermidis*, MRSA, and VRE were further typed by multilocus sequence typing. Mutations in region V of 23S rDNA and ribosomal protein L4were investigated by PCR and sequencing analysis. The presence of the *cfr* gene was tested by PCR. In total, 141 Gram-positive BSIs were included. Coagulase-negative staphylococci predominated ($n = 69$; 65 methicillin-resistant, 23 linezolid-resistant carrying both C2534T and T2504A mutations and belonging to the ST22 clone), followed by enterococci ($n = 46$; 11 vancomycin-resistant carrying *vanA* gene, classified into four clones), *S. aureus* ($n = 22$; 10 methicillin-resistant, classified into three clones) and streptococci ($n = 4$). The most common type of infection was catheter-related (66; 46.8%), followed by primary BSI (28; 19.9%). Overall 14-day fatality was 24.8%. Multivariate analysis revealed septic shock as independent predictor of fatality, while appropriate empiric antimicrobial treatment and catheter-related BSI were identified as a predictor of good prognosis. Even though most of Gram-positive cocci were multidrug-resistant, fatality rate was low, associated with catheter-related BSIs. Among CNS, LR isolates represented one-third of BSIs due to the dissemination of ST22 *S. epidermidis* propagated by utilization of linezolid.

Keywords Methicillin-resistant *S. aureus* (MRSA) · Coagulase-negative staphylococci · Vancomycin-resistant enterococci (VRE) · Linezolid resistance · Septic shock

Introduction

Bloodstream infections (BSIs) are a common occurrence among critically ill patients, being associated with increased morbidity and mortality [1–3]. Even though Gram-negative bacteria predominate among aforementioned population, Gram-positive cocci provoke a considerable proportion of BSIs especially catheter-related (CR-BSIs) [4]. According to EPIC II, among Gram-positive cocci, staphylococci predominated, followed by enterococci and streptococci [3].

Due to widespread use of antibiotics, in addition to failing infection control practices, multidrug-resistant Gram-positive cocci are endemic in many countries, including Greece [2, 5, 6]. *Staphylococcus aureus* represents the most virulent among Gram-positive cocci and accounts for 9% of BSI in Greece, of which more than 40% were caused by strains resistant to

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methicillin (MRSA) [5]. MRSA incidence progressively declined from 2000 to 2015 [7]. Occurrence of vancomycin resistance among enterococci declines in Greece, representing 7.5–8.5% of isolates in the UGHP during the study period (personal data). According to the European Center for Disease Prevention and Control, vancomycin resistance among *Enterococcus faecalis* decreased from 5.2% in 2006 to 0% in 2017; vancomycin resistance among *E. faecium* decreased during the same period from 42.5 to 30.8% [8]. The high prevalence of multidrug-resistant Gram-positive cocci has led to increased use of antibiotics with enhanced anti-Gram-positive activity, such as glycopeptides, and newer ones like linezolid and daptomycin [1, 9]. The use of linezolid contributed to the rise and dissemination of strains resistant to that antibiotic, especially in CNS [10–13].

We performed a retrospective study in order to elucidate the epidemiology and mortality of BSIs due to Gram-positive cocci among critically ill patients.

Methods This retrospective study was conducted in the general intensive care unit (ICU) of the University General Hospital of Patras, Greece, during a 5-year period (2012 to 2016). The study was approved by the Bioethics' Committee of the University General Hospital of Patras (No 434).

All adult patients (≥ 18 years old) with positive blood cultures by Gram-positive bacteria were eligible. Isolation of a common commensal organism from blood cultures, such as *Bacillus* spp., coagulase-negative staphylococci (CNS), *Corynebacterium* spp., *Micrococcus* spp., and *Cutibacterium* spp., was characterized as true BSI if the pathogen was isolated from at least two blood culture sets, as described by US Centers for Disease Control and Prevention (CDC) guidelines; for all other pathogens, only one positive blood culture associated with clinical signs of infection were sufficient for the identification of BSI. The CDC definition was used to characterize BSI as primary or secondary (urinary, respiratory, catheter-related, abdominal, skin and soft tissue infections, endocarditis, meningitis) [14]. Infection was categorized as sepsis or septic shock according to new sepsis definition. The date of collection of the first positive blood culture was defined as infection onset [15]. Appropriate antibiotic treatment was defined as one that included an antimicrobial agent with in vitro activity against the infecting isolates, initiated within 72 h from the onset of infection, at an adequate dosage.

Antibiotic susceptibility testing was performed by the agar disk diffusion method and the Etest according to EUCAST guidelines. PCR was applied to detect *mecA* gene in phenotypically cefoxitin-resistant staphylococci; *vanA*, *vanB*, and *vanC* genes in phenotypically vancomycin-resistant enterococci (VRE), and *cfr* in linezolid non-susceptible CNS [16–18]. Mutations in region V of 23S rDNA were investigated by PCR and sequencing analysis [19]. Sequence data were analyzed using Chromas (www.technelysium.com.au/chromas.html). The possible presence of mutations in ribosomal protein L4 was investigated by PCR followed by sequence analysis [20]. Linezolid-resistant *S. epidermidis*, MRSA and vancomycin-resistant *E. faecium* and *E. faecalis* (VRE) were further typed by multilocus sequence typing (http://www.mlst.net).

ICU's computerized database (Criticus TM, University of Patras, Greece) and patients' chart reviews were used in order to collect epidemiologic data. Parameters assessed included demographic characteristics (age, sex), co-morbidities, severity scores of illness on admission and upon onset of infection (SAPS II (Simplified Acute Physiology Score II) and SOFA (Sequential Organ Failure Assessment) scores), prior surgery, length of hospitalization, type of antibiotic administration, corticosteroid administration, and enteral or parenteral nutrition.

SPSS version 23.0 (SPSS, Chicago, IL) was used for data analyses. Categorical variables were analyzed by using the Fisher exact test and continuous variables with Mann-Whitney *U* test. Multiple logistic regression analysis was performed. Factors contributing to multicollinearity were excluded from the multivariate analysis. Odds ratios (ORs) and 95% confidence intervals (CIs) were calculated to evaluate the strength of any association. All statistic tests were 2-tailed and $P < 0.05$ was considered statistically significant.

Results

Among the 1665 patients admitted to ICU during the study period, 575 episodes of BSI (from 403 patients) were recorded from which 141 were due to Gram-positive bacteraemia (129 patients), accounting for an incidence of 8.3 Gram-positive BSIs per 1000 patient-days. The most common type of infection was catheter-related (66; 46.8%), followed by primary BSI (28; 19.9%), urinary tract infections (16; 11.3%), abdominal infection (12; 8.5%), and ventilator-associated pneumonia (10; 7.1%) (Table 1). Coagulase negative staphylococci (CNS) predominated ($n = 69$; 63 *S. epidermidis*, two *S. haemolyticus*, two *S. lugdunensis*, one *S. capitis*, and one *S. hominis*; 65 methicillin-resistant, 23 linezolid-resistant), followed by enterococci ($n = 46$; 11 VRE), *S. aureus* ($n = 22$; 10 methicillin-resistant carrying *mecA* gene, MRSA), and streptococci ($n = 4$; two *S. agalactiae*, one *S. gallolyticus*, one *S. mitis*). All 23 coagulase-negative staphylococci that showed linezolid MIC > 256 mg/l, were *S. epidermidis*, carried both C2534T and T2504A mutations and belonged to the ST22 clone. None of them carried the *cfr* gene or any mutation in the L4 ribosomal protein gene. Among the 11 VRE, eight were *E. faecium* carrying the *vanA* gene and belonging to ST117 ($n = 4$), ST17 ($n = 3$), and ST203 ($n = 1$), two were *E. gallinarum*, *vanC*-positive, and one *E. faecalis vanA*-positive belonging to ST28. MRSA belonged to ST80 ($n = 4$), ST30 ($n = 4$), and ST239 ($n = 2$). Overall 14-day fatality was

Table 1 Univariate analyses for predictors of fatality in patients with bacteraemia due to Gram-positive cocci

Characteristics	Univariate analysis			Multivariate analysis	
	Survivors ($n = 106$)	Non-survivors ($n = 35$)	P	P	OR (95% CI)
Demographics					
Age (years)	55.3 ± 17.9	59.8 ± 17.6	0.240		
Male gender	81 (76.4%)	25 (71.4%)	0.652		
Chronic diseases					
Diabetes mellitus	9 (8.5%)	5 (14.3%)	0.336		
Chronic obstructive pulmonary disease	4 (3.8%)	5 (14.3%)	0.042	–	–
Chronic heart failure	7 (6.6%)	3 (8.6%)	0.709		
Chronic kidney disease	4 (3.8%)	0 (0.0%)	0.572		
Malignancy (solid organ or hematologic)	8 (7.5%)	2 (5.7%)	1.000		
Immunosuppression	6 (5.7%)	1 (2.9%)	0.681		
Obesity	30 (28.3%)	8 (22.9%)	0.662		
Charlson comorbidity index	3.1 ± 2.9	3.4 ± 2.9	0.476		
Admission data					
SAPS II upon admission	36.1 ± 10.7	41.9 ± 14.4	0.099		
SOFA score upon admission	8.2 ± 3.1	9.7 ± 3.7	0.035		
Prior surgery	52 (49.1%)	11 (31.4%)	0.080		
Infection data					
Days at risk	11.5 ± 10.2	11.2 ± 9.9	0.879		
Septic shock	29 (27.4%)	28 (80.0%)	< 0.001	0.039	4.0 (1.1–10.9)
Noradrenaline dose (μg/kg/min)	5.6 ± 12.5	30.5 ± 22.3	< 0.001		
Source of infection					
Catheter-related bateraemia	58 (54.7%)	8 (22.9%)	0.002	0.024	0.28 (0.09–0.85)
Abdominal infection	9 (8.5%)	3 (8.6%)	1.000		
Urinary tract infection	13 (12.3%)	3 (8.6%)	0.761		
Ventilator-associated pneumonia	5 (4.7%)	5 (14.3%)	0.120		
Primary bacteraemia	15 (14.2%)	13 (37.1%)	0.006		
Other[a]	6 (5.7%)	3 (8.6%)	0.690		
SAPS II upon onset of infection	36.1 ± 10.1	48.4 ± 11.8	< 0.001		
SOFA score upon onset of infection	6.5 ± 3.0	9.6 ± 3.6	< 0.001		
Appropriate empiric treatment	100 (94.3%)	22 (62.9%)	< 0.001	0.011	0.20 (0.06–0.69)
Beta-lactam-containing regimen	91 (85.8%)	30 (85.7%)	1.000		
Glycopeptide-containing regimen	70 (66.0%)	21 (60.0%)	0.545		
Linezolide-containing regimen	32 (30.2%)	11 (31.4%)	1.000		
Daptomycin-containing regimen	6 (5.7%)	1 (2.9%)	0.681		
Corticosteroid administration during infection	55 (51.9%)	18 (51.4%)	1.000		
Parenteral nutrition	38 (35.8%)	13 (37.1%)	1.000		
Enteral nutrition	74 (69.8%)	17 (48.6%)	0.027		
Acute kidney injury	17 (16.0%)	21 (60.0%)	< 0.001	–	–
Hemodialysis	6 (5.7%)	7 (20.0%)	0.018		
Thrombopenia ($< 100 \times 10^9$/l)	15 (14.2%)	16 (48.5%)	< 0.001	–	–
Microbiologic data					
Species					
Coagulase negative staphylococci	53 (50.0%)	16 (45.7%)	0.700		
S. aureus	15 (14.2%)	7 (20.0%)	0.426		
Enterococci	34 (32.1%)	12 (34.3%)	0.837		
Streptococci	4 (3.8%)	0 (0.0%)	0.572		
Resistance					

Table 1 (continued)

Characteristics	Univariate analysis			Multivariate analysis	
	Survivors ($n = 106$)	Non-survivors ($n = 35$)	P	P	OR (95% CI)
Methicillin resistance[b]	58 (85.3%)	17 (73.9%)	1.000		
Vancomycin resistance[c]	7 (20.0%)	4 (33.3%)	0.435		
Linezolid resistance[d]	19 (35.8%)	4 (25.0%)	0.550		

Data are number (%) of patients or mean ± standard deviation

APACHE II: Acute Physiology and Chronic Health Evaluation II, SAPS II: Simplified Acute Physiology Score II, SOFA: Sequential Organ Failure Assessment

[a] Three nosocomial meningitis, three surgical site infections, two endocarditis, one septic arthritis

[b] Among all staphylococci ($n = 91$)

[c] Among all enterococci ($n = 46$)

[d] Among coagulase negative staphylococci ($n = 69$)

24.8% (35 patients). Table 1 shows univariate and multivariate analyses of predictors of BSI fatality. Multivariate analysis revealed septic shock (P 0.039; OR 4.0, CI 1.1–10.9) as independent predictor of fatality, while appropriate empiric antimicrobial treatment (P 0.011; OR 0.20, CI 0.06–0.69) and catheter-related BSI (P 0.024; OR 0.28, CI 0.09–0.85) were identified as a predictor of good prognosis.

Twenty-three among the 69 coagulase-negative staphylococci were methicillin and linezolid-resistant, therefore, we have performed a secondary analysis for risk factors for development of bacteraemia by linezolid-resistant strains. Multivariate analysis revealed administration of linezolid (P0.015; OR 4.9, CI 1.4–18.1) as the only independent risk factor for development of bacteraemia by linezolid-resistant coagulase-negative staphylococci Table 2.

Discussion

During the study period, infections due to Gram-positive cocci accounted for 24.5% of all BSIs, with carbapenemase-producing Gram-negative bacteria being the most prominent (55.0%). In general, the incidence of BSIs independently of the pathogen isolated is high in our study (24.2%) in comparison to the literature (7.8% in EPIC II) [3]. A shift in BSI's epidemiology in Greek hospitals was observed in the last two decades towards predominance of Gram-negative bacteria, due to emergence and dissemination of very successful clones of carbapenemase-producing Gram-negative bacilli [5.6]. Even though in the EPIC II study infections due to Gram-negative bacteria predominated, the epidemiology of BSI varies widely between countries, with many showing a predominance of Gram-positive cocci [1, 6, 21].

As compared to carbapenemase-producing Gram-negative bacilli, fatality of BSIs due to Gram-positive cocci was lower (24.8%), as previously shown [2]. The most consistent predictor of survival in the literature was administration of appropriate empiric antibiotic therapy [1, 22]. In the present study, despite the high rate of multi-drug resistant pathogens (86; 61.0%; 10 MRSA, 65 MR-CNS, 11 VRE), 86.5% of patients received appropriate empiric treatment. This high percentage can be explained by the fact that these pathogens are prevalent in the Greek healthcare system, leading to an empiric initiation of anti-Gram-positive antibiotics, such as glycopeptides (vancomycin or teicoplanin), linezolid, or daptomycin in all patients with a severe infection [5]. Glycopeptides were the preferred agent as empiric coverage in our cohort, with only 11 isolates (8.7%) being resistant. In the EUROBACT study, vancomycin was also the most commonly used antibiotic with anti-Gram positive action, followed by linezolid and daptomycin [1]. Even though many studies have shown during the last decades an increase of vancomycin MIC among MRSA [23], a phenomenon known as MIC creep, our isolates had an MIC ≤ 1 mg/l, for which vancomycin remains the preferred agent. A previous Greek multicenter study showed a decrease of MIC among MRSA from 2008 to 2012, which was probably due to the reduction of vancomycin utilization in favor to newer therapeutic options, such as linezolid and daptomycin [8].

Another reason for the low fatality was the fact that Gram-positive bacteria, as compared to carbapenemase-producing Gram-negative bacteria, were more commonly associated with CR-BSIs [24], and better clinical outcome due to easy and rapid source control by removing the infected catheter [1, 4, 21, 25]. In the present study, CR-BSIs accounted for more than half of BSIs, and can explain the fact that CNS predominated among Gram-positive cocci, since they have a propensity to colonize indwelling devices and subsequently provoke infection. Reinforcing strategies for prevention of CR-BSI are the following: training of medical personnel on antisepsis, introduction of checklist for CVC insertion, and education of nursing personnel for disinfecting techniques for inserted catheters [25].

Table 2 Univariate and multivariate analyses of risk factors for infection due to linezolid-resistant coagulase negative staphylococci

Characteristics	Univariate analysis			Multivariate analysis	
	LS-CNS ($n = 46$)	LR-CNS ($n = 23$)	*P*	*P*	OR (95% CI)
Demographics					
Age (years)	58.0 ± 16.6	54.4 ± 22.5	0.660		
Male gender	34 (73.9%)	21 (91.3%)	0.119		
Chronic diseases					
Diabetes mellitus	6 (13.0%)	3 (13.0%)	1.000		
Chronic obstructive pulmonary disease	3 (6.5%)	1 (4.3%)	1.000		
Chronic heart failure	5 (10.9%)	0 (0.0%)	0.161		
Chronic kidney disease	1 (2.2%)	0 (0.0%)	1.000		
Malignancy (solid organ or haematologic)	4 (8.7%)	2 (8.7%)	1.000		
Immunosuppression	1 (2.2%)	1 (4.3%)	1.000		
Obesity	14 (30.4%)	6 (26.1%)	0.784		
Charlson comorbidity index	3.4 ± 2.7	3.1 ± 3.2	0.575		
Admission data					
SAPS II upon admission	38.8 ± 12.8	32.4 ± 8.2	0.058		
SOFA score upon admission	9.3 ± 3.5	7.4 ± 2.4	0.064		
Prior surgery	18 (39.1%)	10 (43.5%)	0.798		
Prior antibiotic administration					
Penicillins	24 (52.2%)	11 (47.8%)	0.802		
Cephalosporins	6 (13.0%)	0 (0.0%)	0.168		
Carbapenems	34 (73.9%)	22 (95.7%)	0.047		
Glycoptetides	38 (82.6%)	19 (82.6%)	1.000		
Linezolid	8 (17.4%)	14 (60.9%)	0.001	0.015	4.9 (1.4–18.1)
Daptomycin	0 (0.0%)	1 (4.3%)	0.333		
Tigecycline	1 (2.2%)	7 (30.4%)	0.001		
Colistin	10 (21.7%)	17 (73.9%)	<0.001		
Aminoglycosides	15 (32.6%)	12 (52.2%)	0.128		
Quinolones	3 (6.5%)	1 (4.3%)	1.000		
Number of antibiotics administered	3.1 ± 1.6	4.8 ± 2.0	0.001	–	–
Infection data					
Days at risk	11.2 ± 8.3	16.5 ± 10.1	0.022	–	–
Septic shock	14 (30.4%)	6 (26.1%)	0.784		
Noradrenaline dose (μg/kg/min)	9.3 ± 15.7	6.3 ± 13.8	0.261		
Source of infection					
Catheter-related bateraemia	39 (84.8%)	17 (77.3%)	0.505		
Primary bacteraemia	7 (15.2%)	5 (22.7%)			
SAPS II upon onset of infection	39.7 ± 12.7	35.5 ± 11.0	0.239		
SOFA score upon onset of infection	6.5 ± 3.5	6.1 ± 2.5	0.890		
Prior corticosteroid administration	18 (39.1%)	12 (52.2%)	0.318		
Prior parenteral nutrition	14 (30.2%)	2 (52.2%)	0.114		
Prior enteral nutrition	28 (60.9%)	15 (65.2%)	0.796		
Methicillin resistance	43 (93.5%)	22 (95.7%)	1.000		

Data are number (%) of patients or mean ± standard deviation

APACHE II, Acute Physiology and Chronic Health Evaluation II; *CNS*, coagulase negative staphylococci; *LS*, linezolid-susceptible; LR, linezolid-resistant; SAPS II, Simplified Acute Physiology Score II; SOFA: Sequential Organ Failure Assessment

One-third of infections due to CNS were linezolid resistant (LR). Consistently with the literature, prior administration of linezolid was the only risk factor for development of infection by LR isolates [11, 12, 26]. Among patients with LR-CNS

BSI, 39.1% did not receive linezolid in the last 2 months prior to BSI development, indicating that such isolates disseminated to patients in nearby beds, as previously shown [11, 26]. All LR *S. epidermidis* belonged to ST22 and carried both C2534T, T2504A; aforementioned ST and mutations were found in previous studies from our setting and other Greek and European hospitals underlying the success of that clone [11, 13, 26]. The efficacy of LR-CNS in disseminating and becoming endemic in different hospital wards, including those with low linezolid consumption, was shown in a Spanish university hospital. In that study, LR *S. epidermidis* belonged to ST2 and apart from G2576 T 23S rDNA mutation, they also carried a variety of mutations in the genes encoding L3 and L4 ribosomal proteins [27]. No isolate in the present study carried the transferable *cfr* gene which encodes an rRNA methyltransferase; this gene has been detected in CNS, *S. aureus* and even enterococci, especially in central and north America [28].

Clones of vancomycin-resistant *E. faecium* found in the present study (ST117, ST17, ST203) represent previously found ones in Greece, belonging to the highly worldwide successful clonal complex 17 [29]. Epidemiology of ST types of MRSA strains represents the current Greek epidemiology with the invasion of the community-acquired ST80 clone in the healthcare system supplanting the previous nosocomial clone of ST239 [7, 30]. The four infections due to ST30 depict this clone's dissemination among patients and personnel of the ICU [31].

The present study has several limitations. First, this is a retrospective study in one ICU with high incidence of infections. Second, since a high rate of BSIs was related to CVC, and due to high rate of multidrug-resistant pathogens, our results may not be directly extrapolated to regions with lower incidence of CR-BSI or multidrug-resistant pathogens. No data on vancomycin's trough levels were included in our analysis since they were not routinely measured.

Gram-positive BSIs represented approximately one-fourth of all BSIs in a setting with endemic carbapenemase-producing Gram-negative bacteria, justifying an empiric utilization of an anti-Gram-positive antibiotic. Even though most of them were multidrug-resistant, fatality was low, associated with CR-BSIs. Among CNS, LR isolates represented one-third of BSIs due to dissemination of ST22 *S. epidermidis* propagated by utilization of linezolid.

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Compliance with ethical standards

Conflict of interest The authors declare that there are no conflicts of interest.

Ethical approval The study was approved by the Bioethics' Committee of the University General Hospital of Patras (No 434).

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