Trends of Bloodstream Infections in a University Greek Hospital during a Three-Year Period: Incidence of Multidrug-Resistant Bacteria and Seasonality in Gram-negative Predominance

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Abstract

The aim of the study was to assess the epidemiology, the incidence of multidrug-resistant bacteria and bloodstream infections’ (BSIs) seasonality in a university hospital. This retrospective study was carried out in the University General Hospital of Patras, Greece, during 2011–13 y. Blood cultures from patients with clinical presentation suggestive of bloodstream infection were performed by the Bact/ALERT System. Isolates were identified by Vitek 2 Advanced Expert System. Antibiotic susceptibility testing was performed by the disk diffusion method and E-test. Resistance genes (meca in staphylococci; vanA/vanB/vanC in enterococci; blavac,blaesp,blaox in Klebsiella spp.) were detected by PCR. In total, 4607 (9.7%) blood cultures were positive from 47451 sets sent to Department of Microbiology, representing 1732 BSIs. Gram-negative bacteria (52.3%) were the most commonly isolated, followed by Gram-positive (39.5%), fungi (6.6%) and anaerobes bacteria (1.8%). The highest contamination rate was observed among Gram-positive bacteria (42.3%). Among 330 CNS and 150 Staphylococcus aureus, 281 (85.2%) and 60 (40.0%) were mecA-positive, respectively. From 113 enterococci, eight were vanA, two vanB and two vanC-positive. Of the total 207 carbapenem-resistant Klebsiella pneumoniae (73.4%), 202 carbamycin-resistant Klebsiella pneumoniae; vanA/vanB/vanC, respectively. Two blaox, four blavac, and blaox in one blafr. A significant increase in monthly BSIs’ incidence was shown (R<0.449), which may be attributed to a rise of Gram-positive BSIs (R<0.337). Gram-positive BSIs were less frequent in spring (P<0.001), summer (P<0.001), and autumn (P<0.001), as compared to winter months, while Gram-negative bacteria (P<0.001) and fungi (P<0.001) were more frequent in summer months. BSIs due to carbapenem-resistant S. aureus and carbapenem-resistant Gram-negative bacteria increased during the study period. The increasing incidence of BSIs can be attributed to an increase of Gram-positive BSI incidence, even though Gram-negative bacteria remained the predominant ones. Seasonality may play a role in the predominance of Gram-negative’s BSI.

Key words: bacteremia, Candida non-albicans, Staphylococcus aureus, carbapenem-resistance, methicillin resistant vancomycin resistant enterococci (VRE)

Introduction

Bloodstream infections (BSIs) remain a crucial public health problem of increasing incidence and importance in the modern world, whereas, they are characterized by high morbidity and mortality (Wisplinghoff et al., 2004; Bouza et al., 2014; Koupetori et al., 2014). The reported raising incidence can be explained by the ageing of hospitalized patients, the increasing number of immunosuppressed patients and the acquisition of virulence factors by common pathogens (Goto and Al-Hasan, 2013; Papadimitriou-Olivgeri et al., 2015). The wide use of invasive devices, such as central venous catheters, is another important factor leading to the rise of the number of catheter-related bloodstream infections (CR-BSIs) (Rodriguez-Creixems et al., 2013).

Resistance to antimicrobial agents played a determining role in the epidemiology of bloodstream infections during the last decades (Kouchetor et al., 2014). While Gram-negatives were the most common cause of BSIs before 90s (Karchmer, 2000), this changed after the dissemination of resistant Gram-positive bacteria, such as methicillin-resistant Staphylococcus aureus and vancomycin resistant enterococci (VRE) (de Kraker et al., * Corresponding author: I. Spiliopoulou, Department of Microbiology, School of Medicine University of Patras, University Campus, Rion, Patras, Greece; e-mail: spiliop1@upatras.gr
2013). Thus, the last two decades Gram-positive bacteria were the most prominent worldwide (Wisplinghoff et al., 2004; de Kraker et al., 2013; Gubbels et al., 2015). The dissemination of carbapenem-resistant bacteria has changed the situation and currently Gram-negative ones have become the most common cause of BSIs in countries where these bacteria have invaded (Koupetori et al., 2014). Besides the role of resistance, studies have shown that environmental factors, such as, higher temperatures, can influence the epidemiology (Eber et al., 2011; Paul, 2012). It has been reported that Escherichia coli is more prominent in higher temperatures (Eber et al., 2011; Paul, 2012). In a multicenter study, it was shown that countries with low distance from the equator have higher proportion of Gram-negative bacteremias as compared to Gram-positive ones (Fisman et al., 2014).

The aim of the present study was to assess the evolution of bloodstream infections, especially, those related with the use of intravenous catheters, to determine the causative agents and spread of multidrug resistant (MDR) isolates, as well as, to evaluate the role of seasonality in trends of microorganisms isolation during a three-year period in a tertiary university Greek hospital.

**Experimental**

**Materials and Methods**

This retrospective observational study has taken place in the University General Hospital of Patras (UGHP), Greece, a 770 bed tertiary hospital. The UGHP is the only tertiary hospital in Southwestern Greece, receiving patients from eight prefectures with 921 852 total population served. The study was carried out under the Hospital Surveillance Programme for multi-drug resistant infections of hospitalized patients, and was approved by the University Hospital Ethics Committee (HEC No: 571).

During a three-year period (from January 2011 till December 2013), two blood sets (consisted of an aerobic and an anaerobic blood culture bottle) from peripheral sites and one from the central venous line (if present) were obtained and sent to the Department of Microbiology, whenever a patient developed fever (≥38.0°C) or the clinical presentation was suggestive of bloodstream infection. The BacT/ALERT System (≥ 38.0°C) or the clinical presentation was suggestive of Microbiology, whenever a patient developed fever (if present) were obtained and sent to the Department Committee (HEC No: 571).

BSI was defined according to CDC definition (Horan et al., 2008). Isolation of a common commensal organism from blood cultures, such as Aerococcus spp., Bacillus spp., coagulase-negative staphylococci (CNS), Corynebacterium spp., Micrococcus spp., Propionibacterium spp., and viridans group streptococci (viridans group streptococci), was characterized as true BSI if the pathogen was isolated from at least two blood culture sets, as described by CDC guidelines (Horan et al., 2008). For all other pathogens, only one positive blood culture associated with clinical signs of infection were needed in order to be defined as BSI. CR-BSI was defined when the central venous catheter tip grew over 15 colony-forming units of the phenotypically same strain as the blood culture isolate. Positive blood cultures that were not associated with BSI were characterized as contamination.

Phenotypic identification of carbapenemase production among imipenem-resistant Enterobacteriaceae was performed by the EDTA synergy test (MER/MER-EDTA) and the boronic acid synergy test (MER/MER-boronic acid) by the disk diffusion method, which distinguishes the production of MBL and serine carbapenemase (Tsakris et al., 2010). All imipenem-non-susceptible (IMP-NS) Pseudomonas aeruginosa isolates (MICs ≤ 1 mg/l) were examined for metallo-beta-lactamase (MBL) production using the E-test MBL assay (bioMerieux). PCR was used to identify mecA gene in phenotypically cefoxitin-resistant staphylococci, vanA, vanB and vanC genes in phenotypically vancomycin-resistant enterococci and bla_KPC, bla_VIM and bla_GIM in phenotypically carbapenem-resistant Klebsiella pneumo...
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niae isolates (Queenan and Bush, 2007; Papadimitriou-Olivgeris et al., 2015).

SPSS version 19.0 (SPSS, Chicago, IL) software was used for all analyses. Bacterial BSI trends that were assessed by Spearman’s correlation analysis. Incidence was defined as the number of BSI of a pathogen per 10 000 patient-days for all pathogens, while for Brucella spp. BSI yearly incidence was defined per 100 000 inhabitants of western Greece. Poisson regression distribution with general log-linear analysis was used to estimate the adjusted incidence rate ratios (aIRR) with 95% confidence intervals (CI) by comparing the BSI incidence according to the season of occurrence. P < 0.05 was considered statistically significant.

Results

During the study period, in total 47457 blood cultures sets were processed in the Microbiology Department, corresponding to 15292, 16335 and 15824 the years 2011, 2012 and 2013, respectively. Among them, 1464 (9.6%), 1619 (9.9%) and 1524 (9.6%) were positive collected from 903, 1010 and 1024 patients, accordingly. Gram-positive bacteria represented the majority of isolates (2653, 57.6%), followed by Gram-negative ones (1693, 36.7%), fungi (197, 4.3%) and anaerobes (64, 1.4%) (Table I). More specifically, CNS (n = 1808), especially Staphylococcus epidermidis (n = 1115) was the most common species among Gram-positive bacteria followed by S. aureus (n = 309), enterococci (n = 167), streptococci (n = 204), and others Gram-positive (n = 142). Klebsiella spp. was isolated from 632 blood cultures, while E. coli, P. aeruginosa, Acinetobacter spp., other Enterobacteriaceae, Brucella melitensis and other Gram-negative bacteria were isolated from 632, 320, 232, 231, 151, 59 and 68, respectively.

During the study period, 2115 catheter tips were evaluated (761 in 2011, 686 in 2012 and 668 in 2013). Among them, 267 (35.1%), 272 (39.7%) and 240 (35.9%) were culture-positive, recovered from 230, 253 and 224 patients, respectively. The isolated pathogens in declining order were: CNS (358), Klebsiella spp. (145), Acinetobacter spp. (110), fungi (43), P. aeruginosa (37), S. aureus (23), enterococci (17), Enterobacteriaceae other than Klebsiella spp. and E. coli (16), streptococci (eight), E. coli (eight), other Gram-positive (seven), other Gram-negative (seven).

Aforementioned positive blood cultures represented 1732 BSIs (486, 606 and 640 in 2011, 2012 and 2013, respectively), of which 312 (18.0%) where catheter-related. Gram-negative bacteria were the main cause of BSI (901, 52.3%), followed by Gram-positive (685, 39.5%), fungi (115, 6.6%) and anaerobes (31, 1.8%).

The number of positive blood cultures associated with true BSI or contamination is shown in Table I. The highest contamination rate was observed for Gram-positive bacteria (42.3%). No blood culture positive for Gram-negative bacteria or fungi was considered as contamination. Table II and Fig. 1 depict the rates of isolates from BSIs according to pathogen isolated and the Department of patients’ hospitalization.

Fig. 1. Bloodstream infection rates according to pathogen isolated and the Department of patient's hospitalization. ICU, Intensive Care Unit; CNS, coagulase negative staphylococci.
In the Paediatric Departments, including the neonatal ICU, Gram-positive bacteria predominate, whereas, in all adult Departments Gram-negatives prevailed (Table II, Fig. 1).

Figure 2 shows the monthly variation of BSIs’ incidence per 10000 patient-days during the study period. A significant rise was observed (R²: 0.449; P = 0.006) that may be attributed to the rise in Gram-positive BSIs (R²: 0.337; P = 0.044). No significant variation was observed for Gram-negatives, anaerobes or fungi. No pathogen had a significant variation in BSI’s incidence during the study period, with the exception of BSIs caused by S. aureus which increased from 2.0 per 10 000 patient-days in 2011 to 3.4 in 2013 (R²: 0.995; P = 0.033).

When B. melitensii’s BSI yearly incidence was calculated according to the population of Southwestern Greece a significant rise was found, from 0.4 per 100 000 habitants in 2011 to 1.5 in 2013 (R²: 0.974; P < 0.001).

Figure 3 depicts the seasonal adjusted incidence rate ratio of BSIs according to isolated pathogen category (Gram-positive, Gram-negative, anaerobes, fungi), as compared to winter. The incidence of BSIs was 19% higher in summer months (P = 0.024; aIRR 1.19; 95% CI 1.02–1.39) and 25% less frequent in autumn (P = 0.001; aIRR 0.61; 95% CI 0.47–0.77), while Gram-negative bacteria (P < 0.001; aIRR 3.20; 95% CI 1.57–6.51) were more frequent in summer months. No significant difference was observed in BSIs’ incidence due to anaerobes.

Among the 330 CNS and the 150 S. aureus BSIs, 281 (85.2%) and 60 (40.0%) were methicillin-resistant

Table I

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Positive blood cultures</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (4607)</td>
</tr>
<tr>
<td>----------</td>
<td>------------</td>
</tr>
<tr>
<td>Gram-positive</td>
<td>2653 (57.6%)</td>
</tr>
<tr>
<td>CNS</td>
<td>1808 (39.2%)</td>
</tr>
<tr>
<td>S. epidermidis</td>
<td>1115 (24.2%)</td>
</tr>
<tr>
<td>Non-S. epidermidis</td>
<td>693 (15.0%)</td>
</tr>
<tr>
<td>S. aureus</td>
<td>309 (6.7%)</td>
</tr>
<tr>
<td>Enterococci</td>
<td>167 (3.6%)</td>
</tr>
<tr>
<td>Streptococci</td>
<td>204 (4.4%)</td>
</tr>
<tr>
<td>Other</td>
<td>142 (3.1%)</td>
</tr>
<tr>
<td>Gram-negative</td>
<td>1693 (36.7%)</td>
</tr>
<tr>
<td>Klebsiella spp.</td>
<td>632 (13.7%)</td>
</tr>
<tr>
<td>E. coli</td>
<td>320 (6.9%)</td>
</tr>
<tr>
<td>Acinetobacter spp.</td>
<td>231 (5.0%)</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>232 (5.0%)</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>151 (3.3%)</td>
</tr>
<tr>
<td>B. melitensii</td>
<td>59 (1.3%)</td>
</tr>
<tr>
<td>Other</td>
<td>68 (1.5%)</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>64 (1.4%)</td>
</tr>
<tr>
<td>Fungi</td>
<td>197 (4.3%)</td>
</tr>
</tbody>
</table>

CNS, coagulase negative staphylococci; BSI, bloodstream infections

* Corynebacterium spp. (72), Micrococcus spp. (30), Bacillus spp. (27), Listeria monocytogenes (eight), Aerococcus spp. (three), Leuconostoc spp. (two)
* Listeria monocytogenes (seven), Corynebacterium spp. (five), Aerococcus spp. (two), Bacillus spp. (two), Micrococcus spp. (two)
* Streptococcus mutans (20), Staphylococcus spp. (eight), Burkholderia cepacia (four), Morganella morgannii (four), Alcaligenes spp. (three), Acinetobacter pittsburghensis (two), Morganella spp. (two), Neisseria meningitidis (one), Rhizobium radiobacter (one), Haemophilus influenzae (one)
* Streptococcus mutans (14), Staphylococcus spp. (13), Morganella morgannii (four), Alcaligenes spp. (three), Burkholderia cepacia (three), Acinetobacter pittsburghensis (two), Morganella spp. (two), Neisseria meningitidis (one), Rhizobium radiobacter (one), Haemophilus influenzae (one)
* Propionibacterium spp. (25), Bacteroides spp. (22), Peptococcus spp. (seven), Clostridium spp. (three), Peptostreptococcus spp. (three), Fusobacterium spp. (two)
* Bacteroides spp. (14), Peptococcus spp. (five), Clostridium spp. (three), Propionibacterium spp. (three), Eubacterium spp. (two), Fusobacterium spp. (two), Peptostreptococcus spp. (two)
* Candida parapsilosis (94), C. albicans (70), C. glabrata (22), C. tropicalis (eight), Cryptococcus neoformans (two), C. krusei (one)
* C. parapsilosis (50), C. albicans (44), C. glabrata (15), C. tropicalis (three), Cryptococcus neoformans (two), C. krusei (one)
Increase in incidence of BSI and MDR bacteria

Table II
Bloodstream infection isolates according to the Department of patients’ hospitalization.

<table>
<thead>
<tr>
<th>Isolates</th>
<th>Emergency Department</th>
<th>Paediatric Departments</th>
<th>Medical Departments</th>
<th>Surgical Departments</th>
<th>Adult ICUs</th>
<th>All Departments</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>All (104)</td>
<td>CR-BSI (0)</td>
<td>All (134)</td>
<td>CR-BSI (23)</td>
<td>All (849)</td>
<td>CR-BSI (102)</td>
</tr>
<tr>
<td>Gram-positive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CNS</td>
<td>34 (32.7%)</td>
<td>0</td>
<td>345 (40.6%)</td>
<td>92</td>
<td>91 (34.1%)</td>
<td>7</td>
</tr>
<tr>
<td>S. aureus</td>
<td>9 (8.7%)</td>
<td>0</td>
<td>89 (10.5%)</td>
<td>1</td>
<td>27 (10.1%)</td>
<td>3</td>
</tr>
<tr>
<td>Enterococci</td>
<td>12 (11.5%)</td>
<td>0</td>
<td>68 (8.0%)</td>
<td>0</td>
<td>82 (29.5%)</td>
<td>0</td>
</tr>
<tr>
<td>Streptococci</td>
<td>11 (10.6%)</td>
<td>0</td>
<td>40 (4.7%)</td>
<td>0</td>
<td>13 (4.9%)</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>1 (1.0%)</td>
<td>0</td>
<td>8 (0.9%)</td>
<td>0</td>
<td>5 (1.1%)</td>
<td>1</td>
</tr>
<tr>
<td>Gram-negative</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Klebsiella spp</td>
<td>66 (63.5%)</td>
<td>0</td>
<td>428 (50.4%)</td>
<td>9</td>
<td>150 (56.2%)</td>
<td>15</td>
</tr>
<tr>
<td>E. coli</td>
<td>9 (8.7%)</td>
<td>0</td>
<td>116 (21.1%)</td>
<td>3</td>
<td>49 (18.4%)</td>
<td>9</td>
</tr>
<tr>
<td>Acinetobacter spp</td>
<td>36 (34.6%)</td>
<td>0</td>
<td>113 (13.3%)</td>
<td>0</td>
<td>25 (9.4%)</td>
<td>1</td>
</tr>
<tr>
<td>P. aeruginosa</td>
<td>3 (2.9%)</td>
<td>0</td>
<td>52 (6.1%)</td>
<td>3</td>
<td>31 (11.6%)</td>
<td>4</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>4 (3.8%)</td>
<td>0</td>
<td>52 (6.1%)</td>
<td>1</td>
<td>24 (9.0%)</td>
<td>1</td>
</tr>
<tr>
<td>B. melitensis</td>
<td>4 (3.8%)</td>
<td>0</td>
<td>19 (2.2%)</td>
<td>0</td>
<td>0 (0.0%)</td>
<td>0</td>
</tr>
<tr>
<td>Other</td>
<td>3 (2.9%)</td>
<td>0</td>
<td>24 (2.8%)</td>
<td>0</td>
<td>7 (2.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Anaerobes</td>
<td>1 (1.0%)</td>
<td>0</td>
<td>21 (2.5%)</td>
<td>0</td>
<td>7 (2.6%)</td>
<td>0</td>
</tr>
<tr>
<td>Fungi</td>
<td>3 (2.9%)</td>
<td>0</td>
<td>55 (6.5%)</td>
<td>1</td>
<td>19 (7.1%)</td>
<td>0</td>
</tr>
</tbody>
</table>

ICU: Intensive Care Unit; CR-BSI: Catheter-related bloodstream infection; CNS: Coagulase-negative staphylococci
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Among the 113 enterococci, 12 (10.6%) were VRE (eight vanA, two vanB and two vanC-positive). Carbapenem-resistance was observed in 207 K. pneumoniae (73.4%); 202 blaKPC, four blaKPC and blaVIM, one were blaVIM-positive, whereas, none carried blaNDM. Moreover, 96 (63.6%) Acinetobacter spp., 43 (35.8%) P. aeruginosa and 12 (13.3%) Enterobacteriaceae were MBL-positive. No E. coli was carbapenem-resistant. An increase in MRSA BSIs incidence was observed during the study period (0.9 per 10000 patient-days in 2011 to 1.3 in 2013; R²: 0.987; P < 0.014), while no significant variation was observed in MR-CNS and VRE incidence. In total, BSIs due to carbapenem-resistant Gram-negative bacteria increased from 5.3 to 7.4 per 10000 patient-days; R²: 0.818; P < 0.042).

Among the 115 patients with fungemia, 113 episodes (98.2%) were caused by Candida spp. (50 Candida parapsilosis, 44 Candida albicans, 15 Candida glabrata, three Candida tropicalis, one Candida kruzei). Among patients with candidemia, 38 (33.6%) had a previous episode of bacteremia (21 due to KPC-producing

Fig. 2. Monthly variation of bloodstream infection incidence per 10000 patient-days during the study period

Fig. 3. Seasonal adjusted incidence of BSI rate ratio according to isolated pathogen. Winter months were defined as reference.

* P < 0.05; ** P < 0.001

(meC-positive; MR-CNS and MRSA), respectively. Among the 113 enterococci, 12 (10.6%) were VRE (eight vanA, two vanB and two vanC-positive). Carbapenem-resistance was observed in 207 K. pneumoniae (73.4%); 202 blaKPC, four blaKPC and blaVIM, one were blaVIM-positive, whereas, none carried blaNDM. Moreover, 96 (63.6%) Acinetobacter spp., 43 (35.8%) P. aeruginosa and 12 (13.3%) Enterobacteriaceae were MBL-positive. No E. coli was carbapenem-resistant. An increase in MRSA BSIs incidence was observed during the study period (0.9 per 10000 patient-days in 2011 to 1.3 in 2013; R²: 0.987; P < 0.014), while no significant variation was observed in MR-CNS and VRE incidence. In total, BSIs due to carbapenem-resistant Gram-negative bacteria increased from 5.3 to 7.4 per 10000 patient-days; R²: 0.818; P < 0.042).

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Fig. 3. Seasonal adjusted incidence of BSI rate ratio according to isolated pathogen. Winter months were defined as reference.

* P < 0.05; ** P < 0.001
K. pneumoniae, six Staphylococcus spp. four Acinetobacter spp., three enterococci, two P. aeruginosa and two E. coli). Previous KPC-producing K. pneumoniae BSI was significantly higher among Candida non-albicans BSIs as compared to those caused by C. albicans (19 of 69, 27.5% vs two of 44, 4.5%; P 0.002).

Discussion

BSIs comprise one of the commonest types of infections in hospitalized patients, associated with high mortality (12.0–43.0%) (Goto and Al-Hasan, 2013; Bouza et al., 2014). The incidence of BSIs (community and healthcare-associated) in our study was 13.6 per 1000 admissions. A point prevalence study of healthcare-associated infections in European hospitals in 2011–12 found that 1.7% of patients from Greek hospitals developed a BS, percentage comparable to ours (1.4%) (ECDC, 2013). BSIs rate in Greece was the highest (18.9%) among the European countries (10.7%) (ECDC, 2013). The incidence of BSIs was higher in our study as compared to those reported in a review from healthcare-associated BSIs from European countries and USA (2.7–8.4 per 1000 admissions), but was significantly lower to that reported from ‘Laikon’ General Hospital in Athens, Greece, from 1995 to 2002 (26.9 per 1000 admissions) (Hadziyannis et al., 2004; Goto and Al-Hasan, 2013). Overall, Southern European countries, including Greece, show higher prevalence of healthcare-associated infections as compared to those of Central or North Europe (ECDC, 2013). A significant rise in BSIs incidence was observed during the study period, a finding similar to that in other studies, probably due to ageing of hospitalized population, growing number of immunocompromised patients and increased use of invasive devices (Hadziyannis et al., 2004; de Kraker et al., 2013; Bouza et al., 2014).

The pathogens responsible for BSIs vary widely between continents and countries. (Wisplinghoff et al., 2004; ECDC, 2013; Anderson et al., 2014). In our study, Gram-negative bacteria were the predominant pathogens (52.3%), followed byGram-positive (39.5%), fungi (6.6%) and anaerobes (1.8%). Even though, our results are close to those reported in related studies from Greece and Spain (Bouza et al., 2014; Koupetori et al., 2014), these findings differ from those of other European countries and North America reporting that during the last two decades Gram-positive bacteria have become the most common cause of sepsis (Hadziyannis et al., 2004; de Kraker et al., 2013; ECDC, 2013; Anderson et al., 2014; Gubbels et al., 2015). On the other hand, countries with closer proximity to equator exhibit higher percentages of Gram-negative BSIs (Fisman et al., 2014). Despite the predominance of Gram negatives in our and other Greek studies, a significant increase of Gram-positive incidence was detected in our setting, mainly attributed mainly to an increase of S. aureus BSIs. The same rising trend in BSIs due to Gram-positive bacteria was reported in a study from 31 Greek hospitals due to an increase in CNS and S. aureus incidence (Koupetori et al., 2014). Taking into account results from aforementioned study and the present one, we may hypothesize that in the future we will witness in Greece a shift in BSIs etiology from Gram-negative towards Gram-positive bacteria.

In most studies, E. coli, S. aureus, enterococci and streptococci constitute the most commonly isolated pathogens from BSIs, findings that contradict our results, where CNS and Klebsiella spp. were the main pathogens, followed by E. coli, Acinetobacter spp. and S. aureus (de Kraker et al., 2013; ECDC, 2013; Anderson et al., 2014; Gubbels et al., 2015; Khatib et al., 2015). A great disparity of isolated pathogens rates among the different Departments of our Hospital was found. In the Emergency Department, E. coli, S. aureus and streptococci were the most common cause of BSIs, reflecting the epidemiology of community-associated bacteremic infections (E. coli from urinary tract and abdominal infections, streptococci from respiratory tract infections, and S. aureus from skin and soft tissue infections) (ECDC, 2013). Similarly to previous reports, he highest rate of CNS was reported in the adult ICUs (32.0%) and Paediatric Departments (28.4%) due to the high usage of central venous catheters in adult critically ill patients and premature neonates (Wisplinghoff et al., 2004; Pereira et al., 2013).

Contamination rate among positive blood cultures (24.7%) was comparable to that reported in previous studies (16.5–19.5%) (Kitaura et al., 2014; Chang et al., 2015; Khatib et al., 2015). This finding may be explained by the low adherence to antiseptic procedures due to low nurse-to-patient ratio in Greek hospitals (Papadimitriou-Oliveri et al., 2015). The probability of contamination and true bloodstream infection depends on the identity of the isolated pathogen, therefore, no blood culture positive for Gram-negative bacteria or fungi was judged to be contaminated in our Setting. (Kitaura et al., 2014; Chang et al., 2015; Khatib et al., 2015). On the contrary, among 72 Corynebacterium spp., 30 Micrococcus spp., 27 Bacillus spp. and 25 Propionibacterium spp. isolated from positive blood cultures, only five (6.9%), two (6.7%), two (7.4%) and three (12.0%) true BSIs were verified, respectively, results comparable to those previously reported (Urban, 2012; Kitaura et al., 2014; Chang et al., 2015). Despite the fact that CNS were the most commonly isolated pathogen from positive blood cultures (39.2%), they accounted for only 19.1% of BSIs. Since CNS are normal skin commensals, the
contamination rate of blood cultures was high (52.2%). Moreover, among CNS, *S. epidermidis* isolates were associated with a significantly lower contamination rate as compared to non-*S. epidermidis* (39.1% vs 73.3%; P < 0.001), in accordance with other investigators. (Uyanik et al., 2014; Papadimitriou-Olivgeri et al., 2015) Thus, it is imperative to distinguish true BSIs, since contamination may lead to unnecessary antibiotic consumption. (Papadimitriou-Olivgeris et al., 2015)

A low percentage of BSIs were catheter-related (18.0%), comparable to that reported from other investigators (8.2–24.0%), but lower to that from the point prevalence study in Europe (39.5%) (Wisplinghoff et al., 2004; Rodriguez-Creixems et al., 2013; ECDC, 2013). Gram-positive bacteria were the most commonly isolated pathogens (65.7%) from CR-BSIs, as previously shown, whereas, CNS predominated (Rodriguez-Creixems et al., 2013; Papadimitriou-Olivgeri et al., 2015). In a study from Israel, Gram-negative bacteria accounted for 76.4% of CR-BSIs, whereas, the low percentage of Gram-positive bacteria was due to the low percentage of CNS CR-BSIs (0.8%), which in our setting accounted for 63.8% of cases (Braun et al., 2014).

Another important finding of the present study was the high incidence of bacteremic brucellosis in Southwestern Greece. While brucellosis remains a rare infection in Europe, high notification rates were reported from Mediterranean countries that were not officially brucellosis-free in animal populations (cattle, sheep or goats) (EFSA and ECDC, 2015). The highest rate of human brucellosis was reported from Greece which increased from 0.9 per 100,000 inhabitants in 2011 to 1.4 in 2013, comparable to that found in the present study (EFSA and ECDC, 2015). Even though, no significant variation in brucellosis trends was found in Europe during 2011–3, we showed a significant increase in *B. melitensis* bacteremia, which may be attributed to lack of animal vaccination and consumption of raw non-heat treated cheese that remains the main risk factor for brucellosis (EFSA and ECDC, 2015; Kariagiannis et al., 2012). As previously shown, in addition to population health education, the complete and proper animal vaccination can lead to reduction of human brucellosis (Jelastopulu et al., 2008).

Resistance among tested pathogens was high in the present study and coincided to the rates reported for Greece from the ECDC (ECDC, 2015). MRSA rates among *S. aureus* were 40.0%, a fact that may be attributed to the dissemination of the highly successful ST80 clone not only in the community, but also in the hospital (Drougka et al., 2014; Papadimitriou-Olivgeris et al., 2015). Methicillin-resistance among CNS was even higher, as previously shown, while VRE rates were lower (Papadimitriou-Olivgeri et al., 2015; Papadimitriou-Olivgeris et al., 2015; ECDC, 2015).

The most important issue in our study was the presence of carbapenem-resistance among Gram-negative bacteria. The rates were higher among *K. pneumoniae*, which in their majority produced the carbapenemase KPC. This is in accordance with a previous Greek study from 31 hospitals (Koupetori et al., 2014). As compared to other Gram-negative nosocomial bacteria, *Klebsiella* spp. were associated with persistent BSI (2.2 positive blood culture bottles per BSI as compared to 1.9).

In a previous study in our adult general ICU during 2010–12, persistence of *K. pneumoniae* BSIs was due to inappropriate empiric therapy, which may also explain the findings of the present study, since only 67.9% of patients with KPC-producing *K. pneumoniae* BSIs received appropriate antibiotic therapy (Kang et al., 2013; Papadimitriou-Olivgeris et al., 2014a). Carbapenem-resistance rates among *P. aeruginosa* and *Acinetobacter* spp. were lower than that reported from ECDC (ECDC, 2015). Even though Gram-negative BSI incidence remained stable over time, a significant increase was observed in carbapenem-resistant BSI incidence. This was propagated by the low nurse-to-patient ratio, the absence of isolation rooms (<5%) and the high antibiotic consumption in Greek hospitals (rate 54.7%) (ECDC, 2013).

Seasonality is an important issue for healthcare-associated infections (Eber et al., 2011; Paul, 2012). In our study, total BSI incidence was higher during summer months due to higher incidence of *Candida* spp. and Gram-negative bacteria during these months, especially *Klebsiella* spp., *Acinetobacter* spp., and *P. aeruginosa*. It was previously proven that Gram-negative BSI incidence increases proportionately to temperature’s increase (Paul, 2012; Schwab et al., 2014). Even though, this was shown for *E. coli*, we found no difference in seasonal distribution of *E. coli* (de Kraker et al., 2013; Schwab et al., 2014). This is the first study to demonstrate higher CNS BSIs incidence in winter months, which cannot be explained by temperature-dependence of CNS. Seasonality was previously found among *S. aureus* infections, with higher incidence during summer months and autumn (Paul, 2012; Schwab et al., 2014). No such association was found in the present study.

Candidemia comprises an important cause of BSIs worldwide, associated with high mortality rates (Wisplinghoff et al., 2004; Falagas et al., 2010). In our study, BSIs due to *Candida* spp. accounted for 6.5% of all BSI, with a predominance of *C. non-albicans* species (61.1%). A great variability in distribution of different *Candida* spp., with *C. albicans* being the dominant species in North and Central Europe and *C. non-albicans* species in South America, South Europe, and Asia has been reported in a systematic review (Falagas et al., 2010). It is also shown that the percentage of *C. non-
albicans falls steadily during the last decade worldwide (Falagas et al., 2010). During an eleven-year period (1998–2008), candidemias accounted for 3.3% of BSIs in UGHP with C. albicans being the predominant species (64.0%) (Spiiliopoulou et al., 2010). This abrupt change in Candida spp. epidemiology in our Institution can be explained by the dissemination of KPC-producing K. pneumoniae BSIs. In a previous study among ICU patients we showed that KPC-producing K. pneumoniae BSIs predispose to candidemia and selection of C. non-albicans species (Papadimitriou-Olivergeris et al., 2014b). Similarly, same results are shown in the present study, since previous episodes of KPC-producing K. pneumoniae was significantly higher among candidemia due to C. non-albicans species as compared to C. albicans. Candidemia showed seasonality in the present study, being significantly higher in summer months as compared to winter ones, a finding that reinforces previously published results of a previous study, where hospitalization during summer months was an independent risk factor for Candida spp. isolation. (Papadimitriou-Olivergeris et al., 2014b)

The present study has several limitations. No clinical data were included rendering the calculation of BSIs mortality or the role of appropriate treatment in survival impossible. Second, this is a single center study; however, it reflects not only the local epidemiology but also the national one (ECDC, 2013; Koupetori et al., 2014; Papadimitriou-Olivergeris et al., 2015). Despite the evaluation of seasonality, no temperature data were included.

In conclusion, BSIs remain an important cause of healthcare-associated infections. The increasing incidence of BSIs was due to similar increase of Gram-positive BSI incidence, even though Gram-negative bacteria were the predominant ones. CNS was the most commonly isolated pathogen from bacteremic patients and was more common during winter months, in contrast to summer occurrence of Gram-negative bacteria especially Klebsiella spp. Antibiotic resistance of both Gram-positive cocci and Gram-negative bacilli was high, contributing in their dissemination in the hospital environment. Candida epidemiology changed abruptly the last years, with C. non-albicans species being the predominant ones, probably due to the high incidence of KPC-producing K. pneumoniae since it is shown to be a prelude of C. non-albicans candidemia. In the context of budgetary crisis, absence of isolation rooms, low nurse-to-patient ratio and high antibiotic consumption, even though intensive effort in infection control measures is made, they remain inefficacious.

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