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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 (*mec*A in staphylococci; *van*A/*van*B/*van*C in enterococci; bla_{KPC}/bla_{VIM}/bla_{NDM} 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 *mec*A-positive, respectively. From 113 enterococci, eight were *van*A, two *van*B and two *van*C-positives. Of the total 207 carbapenem-resistant *Klebsiella pneumoniae* (73.4%), 202 carried bla_{KPC}, four bla_{KPC} and bla_{VIM} and one bla_{VIM}. A significant increase in monthly BSIs' incidence was shown (R^2: 0.449), which may be attributed to a rise of Gram-positive BSIs (R^2: 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 methicillin 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 (Koupetori *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*.,

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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 ($\geq 38.0°C$) or the clinical presentation was suggestive of bloodstream infection. The BacT/ALERT System (bioMerieux, Marcy l'Etoile, France) for blood culture incubation is used in our setting. Prolonged incubation (until 28 days) of blood culture bottles was applied in cases of suspicion for *Brucella* spp. or HACEK infection (*Haemophilus* spp., *Aggregatibacter actinomycetemcomitans*, *Cardiobacterium hominis*, *Eikenella corrodens* and *Kingella* spp.). Catheter tips from central venous lines of septic patients were processed and cultured by the rolling plate method onto blood agar plates (bioMerieux).

Isolates recovered from positive blood cultures and catheter tips were identified by Gram stain, catalase and coagulase production and by the Vitek 2 Advanced Expert System (bioMerieux). Antibiotic susceptibility testing was performed by the agar disk diffusion method against antimicrobials suggested by EUCAST according to bacterial species, whereas the E-test was applied as follows: for Enterobacteriacae and *Acinetobacter* spp. imipenem, meropenem, colistin and tigecycline were tested; for *Pseudomonas* spp. imipenem, meropenem and imipenem/EDTA; for staphylococci, enterococci and streptococci vancomycin, teicoplanin, linezolid and daptomycin; for *Streptococcus pneumoniae* penicillin, ampicillin, cefepime, cefotaxime and ceftriaxone; for anaerobic bacteria penicillin, ampicillin, amoxicillin-clavulanic acid, piperacillin, piperacillin-tazobactam, ticarcillin, imipenem, meropenem and clindamycin, whereas Gram-positive anaerobic bacteria were additionally tested for vancomycin (EUCAST, 2015). All results were interpreted according to EUCAST guidelines (EUCAST, 2015).

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, 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 *mec*A gene in phenotypically cefoxitin-resistant staphylococci, *van*A, *van*B and *van*C genes in phenotypically vancomycin-resistant enterococci and bla_{KPC}, bla_{VIM} and bla_{NDM} in phenotypically carbapenem-resistant *Klebsiella pneumo-*

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 habitants 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.

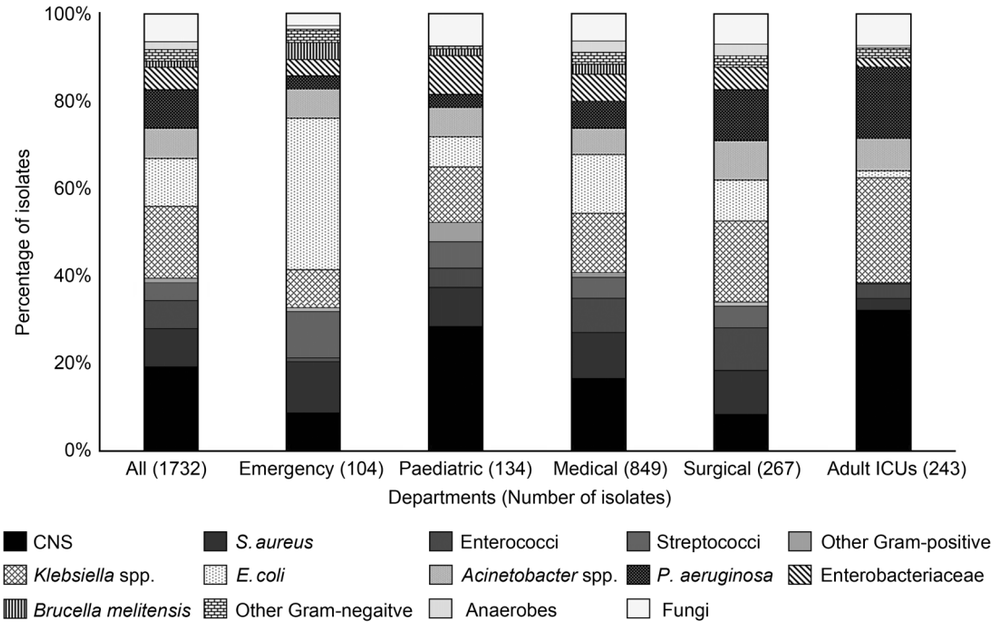

Fig. 1. Bloodstream infection rates according to pathogen isolated and the Department of patient's hospitalization. ICU, Intensive Care Unit; CNS, coagulase negative staphylococci.

Table I
Number of positive blood cultures associated with true bloodstream infections or contamination

Isolates	Positive blood cultures					
	All (4607)	Associated with contamination (1140)	Contamination rate (24.7%)	Associated with BSI (3467)	Number per BSI episode (2.0)	BSI episodes (1732)
Gram-positive	2653 (57.6%)	1121 (98.3%)	42.3%	1532 (44.2%)	2.2	685 (39.5%)
CNS	1808 (39.2%)	944 (82.8%)	52.2%	864 (2.5%)	2.6	330 (19.1%)
S. epidermidis	1115 (24.2%)	436 (38.2%)	39.1%	679 (19.6%)	2.6	265 (14.7%)
Non-*S. epidermidis*	693 (15.0%)	508 (44.6%)	73.3%	185 (5.3%)	2.8	65 (3.8%)
S. aureus	309 (6.7%)	0 (0.0%)	0.0%	309 (8.9%)	2.0	151 (8.7%)
Enterococci	167 (3.6%)	0 (0.0%)	0.0%	167 (4.8%)	1.5	113 (6.5%)
Streptococci	204 (4.4%)	52 (4.6%)	25.5%	152 (4.4%)	2.1	73 (4.2%)
Other	142[a] (3.1%)	102 (8.9%)	71.8%	40 (1.2%)	2.2	18[b] (1.0%)
Gram-negative	1693 (36.7%)	0 (0.0%)	0.0%	1693 (48.8%)	1.9	901 (52.3%)
Klebsiella spp.	632 (13.7%)	0 (0.0%)	0.0%	632 (18.2%)	2.2	282 (16.3%)
E. coli	320 (6.9%)	0 (0.0%)	0.0%	320 (9.2%)	1.7	189 (10.9%)
Acinetobacter spp.	231 (5.0%)	0 (0.0%)	0.0%	231 (6.7%)	1.5	151 (8.7%)
P. aeruginosa	232 (5.0%)	0 (0.0%)	0.0%	232 (6.7%)	1.9	120 (6.9%)
Enterobacteriaceae	151 (3.3%)	0 (0.0%)	0.0%	151 (4.4%)	1.7	90 (5.2%)
B. melitensis	59 (1.3%)	0 (0.0%)	0.0%	59 (1.7%)	2.4	25 (1.4%)
Other	68[c] (1.5%)	0 (0.0%)	0.0%	68 (2.0%)	1.5	44[d] (2.5%)
Anaerobes	64[e] (1.4%)	19 (1.7%)	29.7%	45 (1.3%)	1.5	31[f] (1.8%)
Fungi	197[g] (4.3%)	0 (0.0%)	0.0%	197 (5.7%)	1.7	115[h] (6.6%)

CNS, coagulase negative staphylococci; BSI, bloodstream infections
[a] *Corynebacterium* spp. (72), *Micrococcus* spp. (30), *Bacillus* spp. (27), *Listeria monocytogenes* (eight), *Aerococcus* spp. (three), *Leuconostoc* spp. (two)
[b] *Listeria monocytogenes* (seven), *Corynebacterium* spp. (five), *Aerococcus* spp. (two), *Bacillus* spp. (two), *Micrococcus* spp. (two)
[c] *Strenotrophomonas maltophilia* (20), *Sphingomonas* spp. (eight), *Burkholderhia cepacia* (four), *Morganella morgannii* (four), *Alcaligenes* spp. (three), *Achromobacter putrefasciens* (two), *Moraxella* spp. (two), *Neisseria meningitidis* (one), *Rhizobium radiobacter* (one), *Haemophilus influenzae* (one)
[d] *Strenotrophomonas maltophilia* (14), *Sphingomonas* spp. (13), *Morganella morgannii* (four), *Alcaligenes* spp. (three), *Burkholderhia cepacia* (three), *Achromobacter putrefasciens* (two), *Moraxella* spp (two), *Neisseria meningitidis* (one), *Rhizobium radiobacter* (one), *Haemophilus influenzae* (one)
[e] *Propionibacterium* spp. (25), *Bacteroides* spp. (22), *Peptococcus* spp. (seven), *Clostridium* spp. (three), *Peptostreptococcus* spp. (three), *Eubacterium* spp. (two), *Fusobacterium* spp. (two)
[f] *Bacteroides* spp. (14), *Peptococcus* spp. (five), *Clostridium* spp. (three), *Propionibacterium* spp. (three), *Eubacterium* spp. (two), *Fusobacterium* spp. (two), *Peptostreptococcus* spp. (two)
[g] *Candida parapsilosis* (94), *C. albicans* (70), *C. glabrata* (22), *C. tropicalis* (eight), *Cryptococcus neoformans* (two), *C. kruzei* (one)
[h] *C. parapsilosis* (50), *C. albicans* (44), *C. glabrata* (15), *C. tropicalis* (three), *Cryptococcus neoformans* (two), *C. kruzei* (one)

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 (R^2: 0.449; P 0.006) that may be attributed to the rise in Gram-positive BSIs (R^2: 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 (R^2: 0.995; P 0.033). When *B. melitensis*' 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 (R^2: 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 0.75; 95% CI 0.63–0.89) as compared to winter. BSIs due to Gram-positive bacteria was less frequent in spring ($P < 0.001$; aIRR 0.052; 95% CI 0.40–0.68), summer ($P < 0.001$; aIRR 0.58; 95% CI 10.45–0.74) and autumn ($P < 0.001$; aIRR 0.61; 95% CI 0.47–0.77), while Gram-negative bacteria ($P < 0.001$; aIRR 1.84; 95% CI 1.47–2.29) and fungi ($P < 0.001$; 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 II
Bloodstream infection isolates according to the Department of patients' hospitalization.

Isolates	Emergency Department		Paediatric Departments		Medical Departments		Surgical Departments		Adult ICUs		All Departments	
	All (104)	CR-BSI (0)	All (134)	CR-BSI (23)	All (849)	CR-BSI (102)	All (267)	CR-BSI (22)	All (378)	CR-BSI (165)	All (1732)	CR-BSI (312)
Gram-positive	34 (32.7%)	0	70 (52.2%)	22	345 (40.6%)	92	91 (34.1%)	7	145 (38.4%)	84	685 (39.5%)	205
CNS	9 (8.7%)	0	38 (28.4%)	22	140 (16.5%)	91	22 (8.2%)	3	121 (32.0%)	83	330 (19.1%)	199
S. aureus	12 (11.5%)	0	12 (9.0%)	0	89 (10.5%)	1	27 (10.1%)	3	11 (2.9%)	0	151 (8.7%)	4
Enterococci	1 (1.0%)	0	6 (4.5%)	0	68 (8.0%)	0	26 (9.7%)	0	12 (3.2%)	1	113 (6.5%)	1
Streptococci	11 (10.6%)	0	8 (6.0%)	0	40 (4.7%)	0	13 (4.9%)	0	1 (0.3%)	0	73 (4.2%)	0
Other	1 (1.0%)	0	6 (4.5%)	0	8 (0.9%)	0	3 (1.1%)	1	0 (0.0%)	0	18 (1.0%)	1
Gram-negative	66 (63.5%)	0	54 (40.3%)	0	428 (50.4%)	9	150 (56.2%)	15	203 (53.7%)	73	901 (52.3%)	97
Klebsiella spp.	9 (8.7%)	0	17 (12.7%)	0	116 (21.1%)	3	49 (18.4%)	9	91 (24.1%)	40	282 (16.3%)	52
E. coli	36 (34.6%)	0	9 (6.7%)	0	113 (13.3%)	0	25 (9.4%)	1	6 (1.6%)	0	189 (10.9%)	1
Acinetobacter spp.	3 (2.9%)	0	4 (3.0%)	0	52 (6.1%)	3	31 (11.6%)	4	61 (16.1%)	21	151 (8.7%)	28
P. aeruginosa	7 (6.7%)	0	9 (6.7%)	0	52 (6.1%)	1	24 (9.0%)	1	28 (7.4%)	11	120 (6.9%)	13
Enterobacteriaceae	4 (3.8%)	0	12 (9.0%)	0	52 (6.1%)	2	14 (5.2%)	0	8 (2.1%)	0	90 (5.2%)	2
B. melitensis	4 (3.8%)	0	2 (1.5%)	0	19 (2.2%)	0	0 (0.0%)	0	0 (0.0%)	0	25 (1.4%)	0
Other	3 (2.9%)	0	1 (0.7%)	0	24 (2.8%)	0	7 (2.6%)	0	9 (2.4%)	1	44 (2.5%)	1
Anaerobes	1 (1.0%)	0	0 (0.0%)	0	21 (2.5%)	0	7 (2.6%)	0	2 (0.5%)	0	31 (1.8%)	0
Fungi	3 (2.9%)	0	9 (6.7%)	1	55 (6.5%)	1	19 (7.1%)	0	28 (7.4%)	8	115 (6.6%)	10

ICU, Intensive Care Unit; CR-BSI, Catheter-related bloodstream infection; CNS, coagulase negative staphylococci

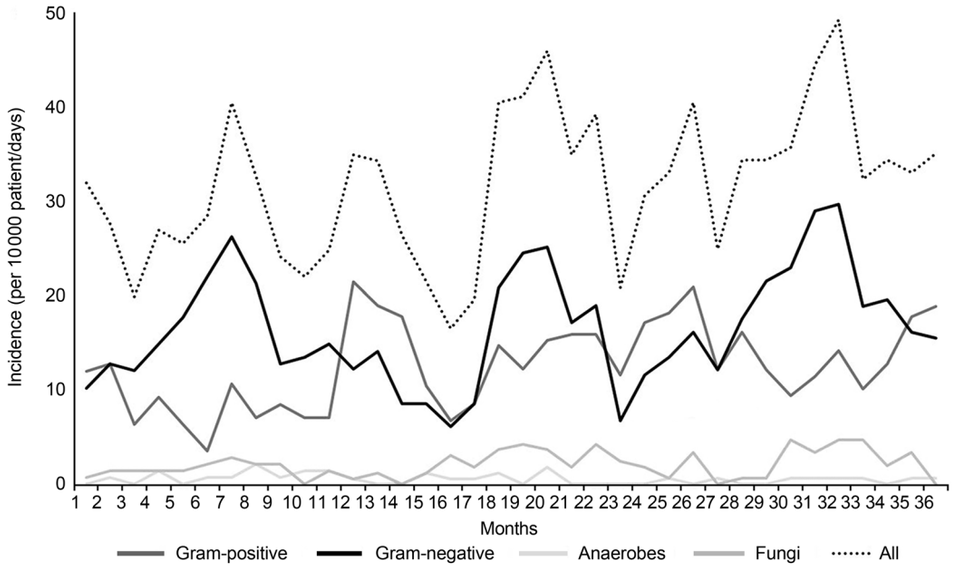

Fig. 2. Monthly variation of bloodstream infection incidence per 10000 patient-days during the study period

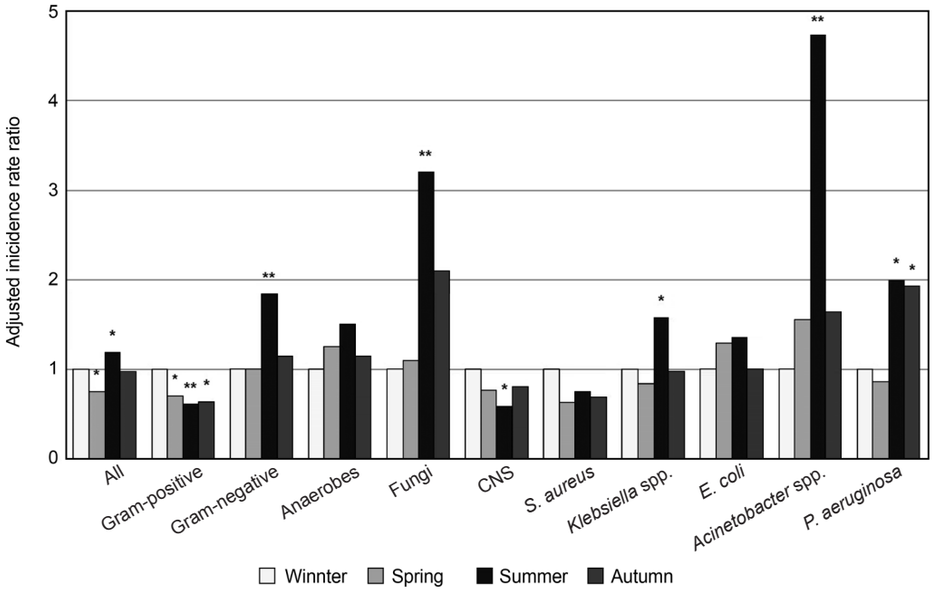

Fig. 3. Seasonal adjusted incidence of BSIs rate ratio according to isolated pathogen Winter months were defined as reference.
* $P < 0.05$; ** $P < 0.001$

(*mec*A-positive; MR-CNS and MRSA), respectively. Among the 113 enterococci, 12 (10.6%) were VRE (eight *van*A, two *van*B and two *van*C-positive). Carbapenem-resistance was observed in 207 *K. pneumoniae* (73.4%); 202 bla_{KPC}, four bla_{KPC} and bla_{VIM}, one were bla_{VIM}-positive, whereas, none carried bla_{NDM}. 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; R^2: 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; R^2: 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

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 BSI, 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, a tertiary 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 by Gram-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-Olivgeri *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-Olivgeri *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 habitants 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 shown 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; Karagiannis *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%) (Spiliopoulou *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-Olivgeris *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-Olivgeris *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-Olivgeris *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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The authors declare no conflicts of interest.

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