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Etiological Structure and *In Vitro* Susceptibility to Antimicrobial Agents from Blood Cultures in Bulgarian Multiprofile Hospital, 2015-2016

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Abstract

During the period 2015-2016, a total of 1490 haemocultures were tested, 24.4% of which were positive. Among the 363 isolated microbial agents, 224 (62% or 15% of all) were considered clinically significant with equal distribution of *Gram-positive* (48.2%) and *Gram-negative bacteria* (47.8%). This finding differs from European and US practice where *Gram positive bacteria* are dominant. Fungi were isolated in 4%. The etiological structure was as follows: Coagulase Negative *Staphylococcus* – 20.1%, *E. coli* - 14.3%, *Enterococcus* spp. - 14.3%, Klebsiella spp. - 11.2%, S. aureus - 10.3%, *Acinetobacter baumannii* - 9.4%, *Enterobacter* spp. - 5.8%, P. *aeruginosa* – 4.9%. A trend toward increase of *Klebsiella* spp, *E. coli*, *S. aureus* и Candida spp. and decrease of coagulase-negative *Staphylococci*, *Enterococcus* spp. and *Acinetobacter baumannii* was observed. Regarding "ESKAPE" pathogens – we had a similar rate of *Enterococci,* and lower rates for the rest compared to USA practice, but in contrast to European data we had lower rate of *S. aureus*, similar rates of Enterobacter and P. aeruginosa, and higher rates of *Enterococci, Klebsiella* and *Acinetobacter*. Multidrug resistance was found in 11% of *Gram positive* and 47% of *Gram negative flora*. Resistance rates were similar to the European, but higher in *Gram negative* and lower in *Gram positive* when compared to USA. Owing to failure of the other approaches we introduced a stronger stewardship and restrictive policy regarding prescription of antibiotics.

Keywords

Blood stream infections; Haemocultures; ESKAPE pathogens; Multi drug resistance

Introduction

Despite the recent advance in medicine bloodstream infections (BSI) leading to severe sepsis and septic shock represent a major source of morbidity and are still associated with a high mortality exceeding 50% [1]. According to population-based studies, which are claimed to be the best of defining epidemiology in non-selected population, the rate of BSI is 140-160 per 100 000 in high-income countries [2]. The estimated rates for North America and Europe are up to 67,7 000 and 1.4 million episodes per year with case-related mortality 12-18% (94,000 deaths) and 13-20% (27,6 000 deaths) respectively [3]. The annual cost in USA is estimated at 24 $ billion [1].

Moreover, we are witnesses of a dramatic increase of multidrug resistant organisms (MDR), especially in a hospital setting [4]. Particularly, it is valid for the so-called „ESKAPE" pathogens (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacteriaceae),* which increasingly become a major health care problem worldwide. The infections caused by MDR are associated with up to 30,000$ increase of the additional hospital costs per case [5]. Commonly, MDR strains differ between the different health care providers and geographical areas, so evaluation of their etiologic structure and type of resistance for any particular instituttion and geographical area is of a paramount importance for the successful management. BSI often require starting of empiric antimicrobial therapy before the final microbiological analysis along with removal of the primary source and supportive care. According to the US Center for Disease Control (CDC), the rate of inadequately prescribed antimicrobial agenst is approximatelly 50% [1]. Delayed and/or inadequate antimicrobial therapy is associated with poorer outcome and an increased rate of MDR. In this light, the analysis of the etiological structure and type of resistance are of a paramount importance to guide the initial empiric therapy. The aim of the present study was to determine the etiological structure and type of resistance to antimicrobial agents of the pathogens isolated form blood cultures in order to actualize the guideline for empiric therapy and to compare our data with the current trends worldwide.

Material and Methods

The study was conducted in a Multiprofile hospital for active treatment with 840 beds, average 36,000 admissions annually and serving as a tertiary center for the whole country. Since 2015 a strict antimicrobial stewardship and restrictive antibiotic policy were implemented into our practice. A total 1490 blood cultures during 2015-2016 were tested. All repeated isolates from any given patient were excluded from the analysis.

All specimens were taken only in the presence of clinical indications through aseptic technique according to the good clinical practice rules. The volume of blood taken (18-20 ml) was distributed equally in two sets for aerobic and anaerobic cultivation. The cultivation was performed through BACTEC 9050 (Becton Dickinson) and BacT/ALERT 3D® (Select Link) according to the instructions of manifacturer. The positive cultures were stained by Gram and were subcultivated by conventional methods [6]. The identification of the isolated pathogens was performed conventionaly [6] and/or by VITEK® 2 (bioMerieux) [7,8]. The antimcrobial susceptibility was examined through the method of Bauer-Kirby or by VITEK® 2 (bioMerieux). Examining of phenotypes of resistance was performed according to recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST). In *Enterobacteriaceae* spp. extended spectrum *β-lactamase* (ESBL)

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screening was performed by double-disk test [9]. Criteria for MDR were resistance to more than three groups of antibiotics for both *Gram positive and negative flora* or methicyllin resistance of *Staphylococci* (MRSA). All results were analyzed according to the criteria of EUCAST [7,8].

Results and Discussion

Etiological structure

The results are given on Table 1. Overall 24.4% (363/1490) of a total 1490 haemocultures were positive. However, only 62% of them (n=224) or 15% of all were assessed as clinically significant. Thirty eight percent (139/363), mainly coagulase-*negative Staphyloccoci* (CNS), were considered a contamination of skin flora which is similar to others reported CNS as the main contaminant (75%) [5,10]. The positive culture rate is similar to the ICU survey conducted in inner-city hospital in New York (12.6%) [5] European rate (15.5%) [11] and others (17-20%) [12,13], but significantly higher than reported for the rest country (7.5-11%) [14-16]. There was almost equal rate of *Gram positive and Gram negative flora* – 48.2% *vs*. 47.8%. This finding differs sifnificantly from other large bulgarian hospitals data indicating preponderance of Gram positive flora (62-72%) [14-16], from a recent US survey and a large study, encompassing 112 European hospitals, reporting rates 59% vs. 31% [5] and 53% *vs*. 41% [11], respectively. However, a large US survey of National Healthcare Safety Network (NHSN), evaluating only central line-associated BSI (CLABSI), reported significantly higher rate of Gram positive versus Gram negative bacteria (51% *vs.* 26%) [17].

In the present series, CNS was the leading *Gram positive* pathogen (20%), followed by *Enterococcus* spp. (14%) and *S. aureus* (10%). The proportion of CNS leading to clinically manifested infection was significantly lower when compared to the average for our country 34-50% [14-16], but is comparable with the reported rates for the European Union and US [10,17]. The rate of S. aureus was lower than the other Bulgarian studies (15%) [14,15], Europe [11] and USA [5]. Despite the drop of *Enterococcus spp*. (from 17% to 12%) it still represents a major challenge due to frequent MDR. Our rate is similar to USA (17-18%) [5,17], but higher than Europe (4.6%) [11].

Most of the *Gram negative* isolates in our series were *Enterobacteriaceae spp*. (31% of all isolated strains), which is confirmed by some institutions [14], although other reported preponderance of non-fermentive *Gram negative flora* at national level [15].

A major concern for our Institution are *Acinetobacter baumannii*, *Klebsiella spp*. and P. *Aeruginosa*, although the rate is similar to other reports [14,18,19]. There was significant increase of *Klebsiella spp*., while *A. baumannii* and *P. aeruginosa* dropped significantly. European study reported lower rates of these pathogens [11], whereas the single center US report showed significantly higher rate of *Klebsiella* and *A. baumannii* [5]. NHSN reported lower rates of *Klebsiella* (8%), *A. baumannii* (2%) and *P. aeruginosa* (4%), but significantly higher *Candida spp*. (14.6%) in CLABSI [17]. The rate of fungi was 4% in the present series, with increase from 2.8% to 5.1%, versus 5% and 10% in European and US series [5,11].

Resistance to antimicrobial agents

MDR appears to be the major problem of the contemporary health care system leading to increased morbidity, mortality and hospital costs. This was the reason Infectious Diseases Society of America (IDSAS) to initiate the 10×′20 initiative for development of ten new antibiotics by 2020 [20]. The resistance rate for *β-lactams* of S. aureus (MRSA) was 11% and was significantly lower in comparison to the average for all hospital isolates (40%), and similar to other Bulgarian authors – 7-31% [14,15]. Our rate is similar to other European reports, but is significantly lower than reported rates in Canada (22%) and USA (53-55%) [5,17]. *Enterococcus spp*. was characterized with a high level of gentamycin resistance (HLR-gentamicin) (89% of all), which is significantly higher than the average for our country [14,15], but is comparable with other centers (74%) [21]. There were no *S. aureus* и *Enterococcus spp*. resistant to glycopeptides (VRE) and Linezolid in contrast to Orsini et al. who reported VRE in 67% of the *Enterococci* [5] and 83% rate reported by Sievert et al. [17]. *Enterococcus spp* became particularly problematic, especially as a cause of nosocomial infections. *VRE* appeared in USA and Europe during the late 1980[-ies]. It was the first bacteria developed acquired Vancomycin resistance and could be a great threat due to its ability to transfer resistance

Table 1: Etiological Structure of Bloodstream Infections in MMA, 2015-2016.

Isolates	2015[1] (n=106)	2016[1] (n=118)	Total[1] (n=224)
Bacteria			
Gram negative			
Escherichia coli	12.3 (13)	16.1 (19)	14.3 (32)
Klebsiella spp.	5.7 (6)	16.1 (19)	11.2 (25)
Enterobacter spp.	6.6 (7)	5.1 (6)	5.8 (13)
other *Enterobacteriaceae*	0	0.8 (1)	0.4 (1)
Acinetobacter baumannii	12.2 (13)	6.8 (8)	9.4 (21)
Pseudomonas aeruginosa	6.6 (7)	3.4 (4)	4.9 (11)
other non-fermentative	0.9 (1)	2.5 (3)	1.8 (4)
Total	**44.3 (47)**	**50.8 (60)**	**47.8 (107)**
Gram positive			
Coagulase negative Staphylococcus (CNS)	26.4 (28)	14.4 (17)	20.1 (45)
Staphylococcus aureus	8.5 (9)	11.9 (14)	10.3 (23)
Enterococcus spp.	17.0 (18)	11.9 (14)	14.3 (32)
Other *Gram positive*	0.9 (1)	5.9 (7)	3.6 (8)
Total	**52.8 (56)**	**44.1 (52)**	**48.2 (108)**
Fungi			
Candida spp.	2.8 (3)	5.1 (6)	4.0 (9)

genes to other Gram positive and negative species [22,23]. Actually, the rate of MDR among *Gram positive flora* was 11% compared to 19% in others [5].

Among *Enterobacteriaceae*, ESBL-producers were widespread, frequently with resistance to other antimicrobial groups. ESBL and MDR were 31% of E. coli and 36% of *K. pneumoniae*, which is similar to the literature data [5,14-16,18]. In fact, 46.7% of Gram negative flora in our series were MDR. Orsini et al. showed MDR in 34% of *Gram negative* organisms with ESBL in 92% [5]. All *A. Baumannii* and *P. aeruginosa* strains in our series were MDR, including carbapenems, aminoglycosides, quinolones and Piperacilin/Tasobactam, but susceptible to colistin. There were no carbapenem-resistant *Klebsiella* strains. The multicenter US survey reported Carbapenem resistance in 63%, 26% and 13% for these pathogens without significant difference between ICU and non-ICU [17]. However, the single center study including all hemocultures, showed Carbapenem resistance in 75% of the Gram negative organisms, all of them *A. baumannii* and *K. pneumoniae* [5]. In an US survey spanning 2006-2008, Kallen et al. reported MDR rates for *A. Baumannii P. aeruginosa* and *K. pneumoniae* – 60%, 10% and 15%, respectively [5].

Conclusion

The empiric therapy of severe infections should be based on up-to-date reports of the etiological structure at institutional and national level. A trend toward increase of *Klebsiella* spp., *E. coli*, S. aureus и Candida spp. and decrease of coagulase-*negative Staphylococci*, *Enterococcus spp*. and *Acinetobacter baumanni* was observed for our Institution. Regarding "ESKAPE" pathogens – we had similar rate of *Enterococci*, and lower rates for the rest compared to USA practice, but in contrast to the European data we had lower rate of *S. aureus*, similar rates of *Enterobacter*, *P. aeruginosa* and higher rates of *Enterococci*, *Klebsiella* and *Acinetobacter*.

MDR was observed in 11% of *Gram positive* and 47% of *Gram negative* organisms. MDR rates were similar to the European, but higher in Gram negative and lower in Gram positive when compared to USA. We consider that is the incorrect use of the antimicrobial drugs. Owing to failure of the other approaches we introduced a stronger stewardship and restrictive policy regarding antimicrobiological prescription.

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