



Original Contribution

SPECIES AFFILIATION AND ANTIBIOTIC RESISTANCE OF THE CLINICAL ISOLATES OF HAEMOCULTURES

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ABSTRACT

PURPOSE: The data on the etiological structure of isolates from haemocultures for 5 years have been summarized. **METHODS:** Here were investigated 2557 haemocultures, from which 193 were positive (7.5 %). **RESULTS:** Among the isolated strains prevailed as follows: *CNS* – 49.4%, *S. aureus* – 14.9%, *E. coli* – 12.1%, other enterobacteria – 6.9%; followed by *P. aeruginosa* – 5.7%, *E. faecalis* – 4.0%. The antibacterial resistance of the leading isolates was analyzed: more than the half of *CNS* /71.4%/ were MR, 22.5% were *Amikacin* - R and 26% were *Levofloxacin* – R. From the isolates *S. aureus* 15.4% were *MRS*, there were no resistant isolates to *Amikacin* and *Levofloxacin*. *E. coli* *ESBL_s* were 43%. The data from previous and current our studies were compared and analyzed. **CONCLUSIONS:** The data for species affiliation of the isolates from haemocultures in the current survey confirmed the established in the last decades tendency for prevalence of Gram- positive bacteria as *CNS*, *S.aureus*, *Enterococcus* spp.

Key words: blood stream infections, etiology, drug susceptibility.

INTRODUCTION

Clinically significant bacteremia is always related to acute clinical manifestation and high lethality. Discovering microorganisms in patient's blood is of extreme importance for the diagnose, prognosis and therapy. Urgent empiric therapy is required in most of the cases before the laboratory identification of the isolated microorganisms and the ascertaining of their antibiotic sensitivity. The antibiotic resistance is one of the most frequent reasons for unsuccessful treatment of the bacteremia (1). A major therapeutical problem are Oxacillin-resistant staphylococci (ORSA, ORCNS), the enterococci with high level of resistance to Aminoglycosides (HLAR), Vancomycin-resistant enterococci (VRE), Extended Spectrum β -Lactamase (ESBL)-producing species of the family of Enterobacteriaceae and polyresistant strains *P.aeruginosa* (2, 3). In this respect the knowledge of the most frequent etiological agents and their expected types of resistance is

the basis for the proper and successful treatment of blood infections.

MATERIALS AND METHODS

For the period 2008-2012, 2557 haemocultures from hospitalised patients, treated in Multi-profile Hospital for Active Treatment - Stara Zagora were studied. The Bactec and the BBL systems as well as the conventional methods were used. The identification of the isolated microorganisms was carried out by an automated system Crystal, BBL and routine methods. The study included only the estimated as clinically important first isolates of a hospitalised patient. The antibiotic sensibility was defined by Kirby- Bauer disc diffusion method. Oxacillin-screen agar was used to identify ORSA. The double-disc synergy method with discs of Amoxicillin-Clavulanic acid/Ceftazidime and Erythromycin/Clindamycin, respectively, was applied to detect ESBL-producing isolates from Enterobacteriaceae family and MLS-phenotypes of Gram-positive cocci. Gentamicin discs of 120 mcg were used to ascertain HLAR, respectively. The aim of the study: Survey of the etiological structure and the antibiotic resistance of isolates

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from haemocultures for 5-year period. Comparative analyze of the etiological structure and the antibiotic resistance of the isolates compared with data from a previous similar study (2002-2007).

RESULTS AND DISCUSSION

In 193 cases from the studied 2557 (7.54%) haemocultures, presence of microorganisms was ascertained. They were estimated as cause of bacteremia/fungemia. Cases of polymicrobial bacteremia were not registered. The received

values were lower than our previous 5-year study - 10.9% (4), and also as compared to the values in other University hospitals in the country - 11.2% (5) and European data - 13.4%÷19.1% (2, 6, 7). The lower number of isolated strains is explained with the obligatory examination required for the clinical paths and the reorganized structure of the hospital. The etiological structure and the relative share of the isolated microorganisms are presented in **Table 1**.

Table 1. Etiological structure of haemocultures' isolates

Microorganisms	Number	Percent
Coagulase-negative staphylococci	86	48
<i>S. aureus</i>	26	14.5
<i>E. faecalis</i>	7	3.9
<i>E. faecium</i>	2	1.1
Viridans streptococci	5	2.8
<i>S. pneumoniae</i>	3	1.7
<i>E. coli</i>	21	11.7
<i>K. pneumoniae</i>	7	3.9
Other from Enterobacteriaceae	5	2.8
<i>P. aeruginosa</i>	10	5.6
<i>A. baumannii</i>	5	2.8
<i>Candida</i> spp.	2	1.1
Total	179	100

Gram-positive bacteria prevail and they are 72 % of all isolates, and Gram-negative ones are 27 %, which corresponds to the current tendencies for isolates distribution in haemocultures (8): the average values for Gram-positive isolates are between 50-62%, and for Gram-negative - 24-41% for Europe and the USA, up to 47% in Bulgaria (2, 5, 9). In view of the increasing frequency of the fungemia, on average for European Union - 4.6% (2), in our study *Candida* were isolated in 1.1 % - values close to a similar studies in the country (0.04% - 1.8%) (9, 10).

Among Gram-positive bacteria, coagulate-negative staphylococci (*CNS*) - 48 % predominate, followed by *S. aureus* - 14.5 % and *E. faecalis* - 3.9. The leader is *CNS*. Our values are higher than the data, published in BulSTAR (11) as compared to other sources in Bulgaria (34%÷40%) (5, 9) are higher than our previous study (4) and higher than the average values for the European Union - 17.8% (2). According to European studies 67% of the isolated *CNS* haemocultures are contaminates (12). In our data we have included only strains, estimated as clinically important - isolated from

2 or 3 haemocultures for an episode, presence of favorable factors and clinical data for bacteremia. The isolates *S. aureus* have lower values as compared to BulSTAR and the average values for the European Union and are a bit above the values from the previously studied period.

Gram+/- bacteria predomination can be explained with the occurrence of new mechanisms of resistance among them, the use of broad-spectrum antibiotics, influencing mainly gram +/- bacteria, enhancement of the risk groups of patients - with valve and vascular prosthesis, iv-catheter and others . (6, 12, 13, 14)

E. coli is the third most frequent isolate from haemocultures - 11.7 % and the most frequent one among Gram negative microorganisms. It is followed by *P. aeruginosa* - 5.6% and *K. pneumoniae* - 3.9. The data are in accordance with the literature. (1, 2, 5, 9)

The antibiotic resistance of isolates is interesting for the purposes of adequate antibiotic treatment.

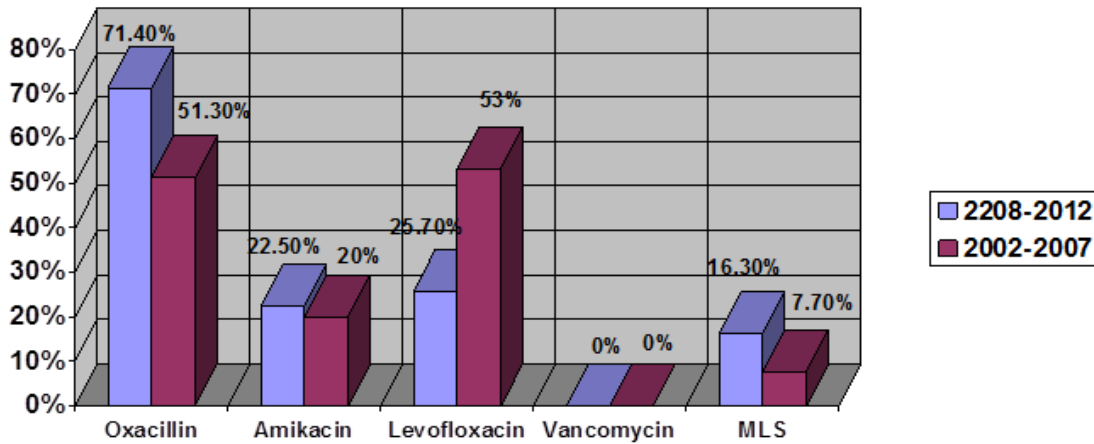


Figure 1. Antibiotic resistance of CNS

From the data presented in **Figure 1**, it is evident that 71.40% of CNS are resistant to oxacillin, 22.50% - to amikacin and 25.70% - to

levofloxacin. MLS-resistance is ascertained in 16.30%.

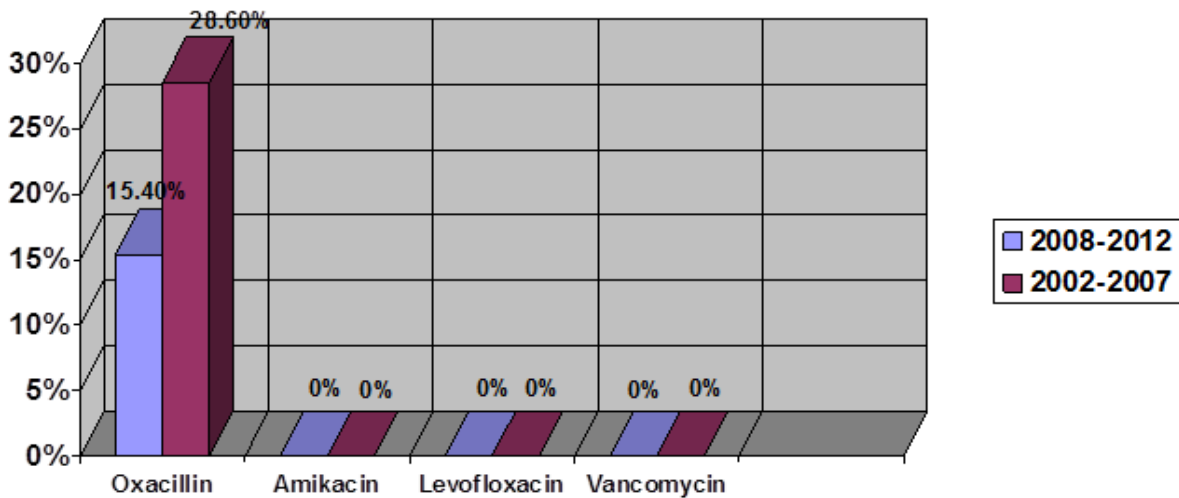


Figure 2. Antibiotic resistance of S. aureus

Oxacillin-resistant *S. aureus* (ORSA) are 15.40%. All are sensible to amikacin, levofloxacin and vancomycin. Half of the ORSA and 7.70% of all *S. aureus* demonstrate MLS – constitutive resistance.

The isolated now CNS are by 20% less resistant to oxacillin and for *S. aureus* there is reduction in the resistance from 28.60% to 15.40%. Thus, the frequency of ORSA and ORCNS is in

accordance with the data from other university hospitals - 7.10%÷29.60% and 31.20%÷52.20% respectively (5, 9), but is less when compared to the USA and the European researches – 23-57% for *S. aureus* and up to 76% for CNS (2, 15). *S. aureus* shows maintained resistance to aminoglycosides and levofloxacin, lower MLS-resistance as compared to the average isolates in Europe. (2)

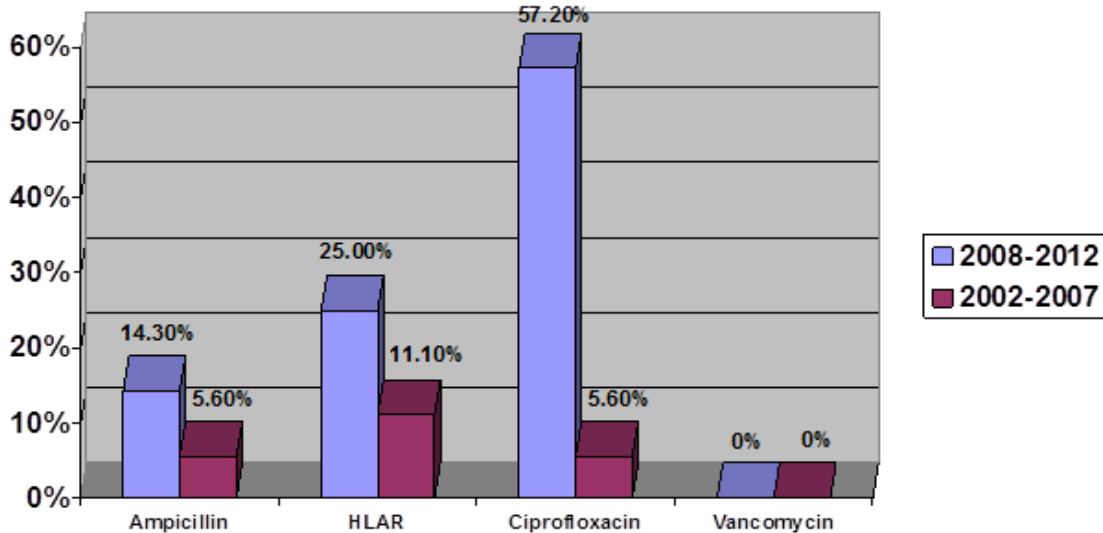


Figure 3. Antibiotic resistance to *Enterococcus faecalis*

As show on **Figure 3**, 14.30% of the isolates *E. faecalis* are resistant to ampicillin, 57.20 % - to ciprofloxacin, and 25% show HLAR. Vancomycin-resistant enterococci were not ascertained. The resistance to ampicillin and HLAR increases as compared to our previous survey (11.1%) and the other available benchmarking data (below 4%). Despite the tendency for increase of HLAR in the current period as compared to the previous one /by

14%/, it still remains lower as compared to the average values for Europe that reach up to 68 % (2.16)

All isolates of viridans *Streptococci*, *S. pneumoniae* and *S. pyogenes* are sensible to penicillin, erythromycin and levofloxacin. Among the Gram-positive isolates strains resistant to glycolipid antibiotics were not determined.

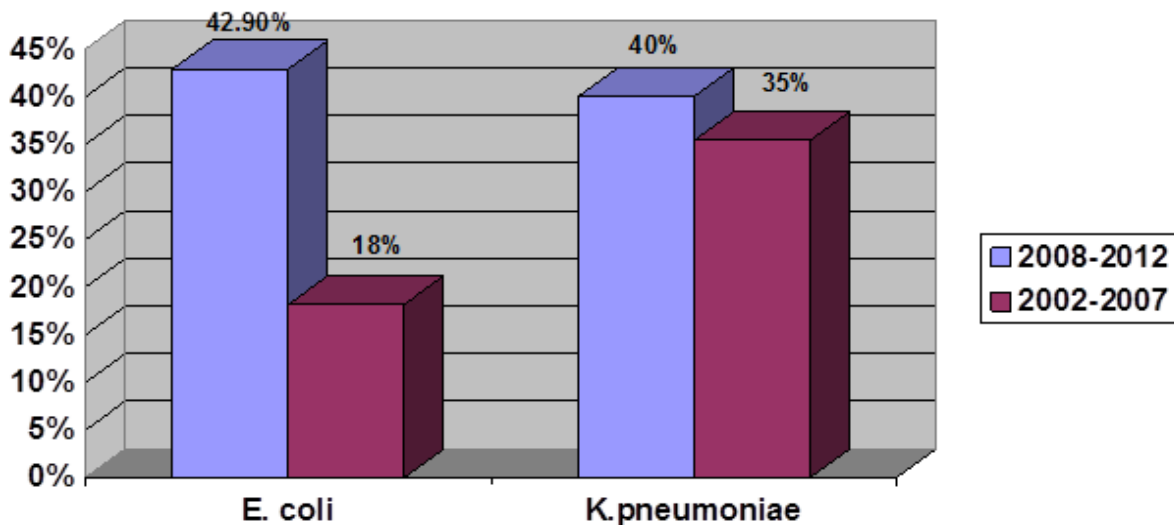


Figure 4. ESBLs-producing isolates from *Enterobacteriaceae* family

For 36.40 % of the representatives of the family of *Enterobacteriaceae* ESBL_s were ascertained. The most frequent ESBLs-producing are: *E. coli* – 42.90 % and *K. pneumoniae* - 40%. As compared to the data from the previous study

there is increase by two times of the ESBL_s producing *E. coli* having less increase for *K. pneumoniae* – ESBL-positive ones (5%). Isolates resistant to carbapenems were not verified.

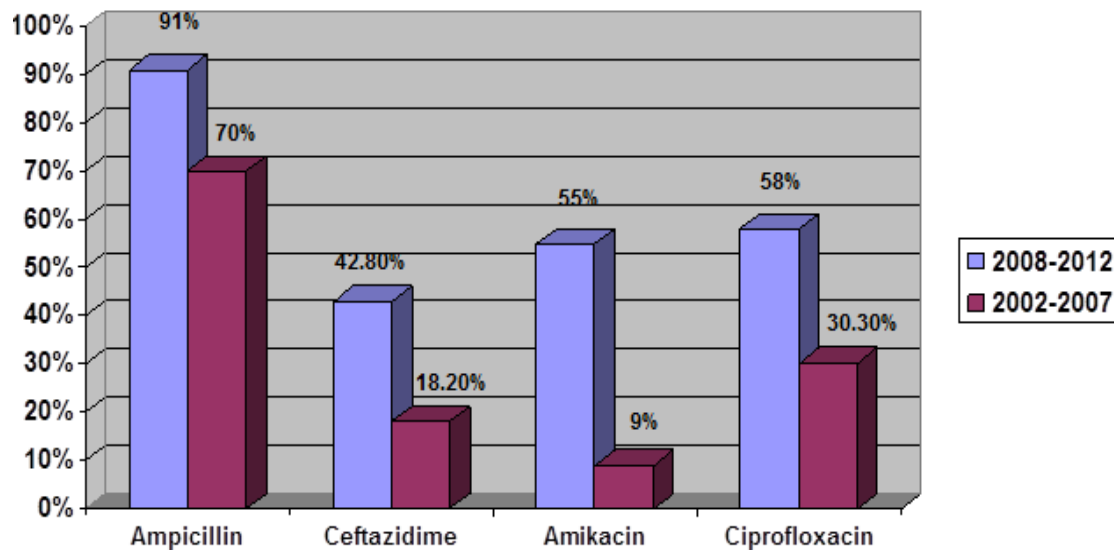


Figure 5. Antibiotic resistance of *E. coli*

Ampicillin resistant *E. coli* isolates are 91%, 55% of them – resistant to amikacin and 58% - to ciprofloxacin. All ESBLs positive *E. coli* are resistant to both to amikacin and ciprofloxacin.

There is an obvious tendency for increase of the resistance of isolates to all antibiotics during the period 2008-2012.

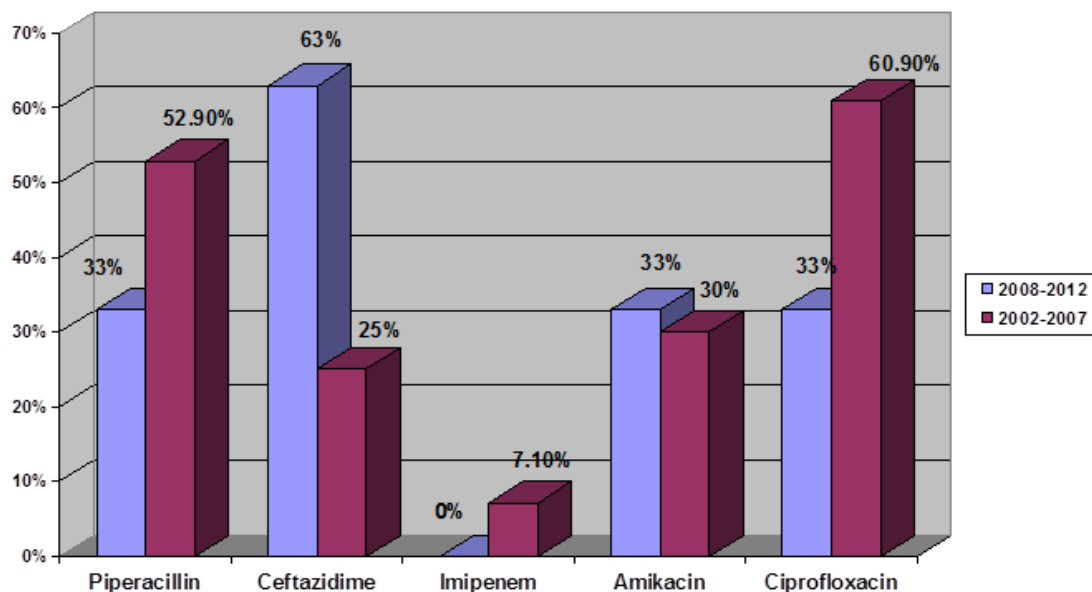


Figure 6. Antibiotic resistance of *P. aeruginosa*

The resistance of the isolated *P. aeruginosa* showed the following values: 33% - to piperacillin, 63% - to ceftazidime, 33% - to amikacin and 33% - to ciprofloxacin. Isolates, resistant to Imipenem were not ascertained. The increased resistance to ceftazidime should be noted /by two times/ the resistance to amikacin had kept and the resistance to piperacillin and ciprofloxacin reduced.

When comparing the resistance of Gram-negative isolates with the ones from other sources, they do not show one and the same tendencies in the levels of resistance to the different groups of antibiotics (2, 3, 5, 7, 9, 15, 16). Most probably it is due to the connection with the frequency of application of antimicrobials in the different hospitals.

CONCLUSIONS

1. The data for species affiliation of the isolates from haemocultures in the current survey confirmed the established in the last decades the tendency for prevalence of Gram- positive bacteria as *CNS*, *S.aureus*, *Enterococcus spp.*
2. The level of resistance of Gram/+/ bacteria is close to or lower than the average values, registered in Bulgaria and the European countries. ORSA and ORCNS continue to be a problem for the treatment of bacteremias.
3. Despite the significant resistance of Gram -/ isolates to third-generation cephalosporins, resistance to carbapenems was not ascertained and they remain a drug of choice.

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