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Etiological investigation and antibiotic resistance profiles encountered in systemic bacterial infections

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ABSTRACT

Systemic bacterial infections are representing a great medical problem, both because of the severity and high mortality, and also of the controversies related to diagnosis and treatment. The purpose of this study was to evaluate the incidence of positive blood cultures and to determine the etiologic spectrum of systemic bacterial infections and antibiotic susceptibility profiles of the isolated strains in patients admitted to three hospitals located in Galati city, i.e. Emergency Hospital "Apostle Andrew", Hospital of Infectious Diseases "St. Parascheva" and Children Hospital "St. John". Of the 3,337 blood cultures analyzed during 2009-2012, 292 were true positive (8.75%) with the etiology dominated by Gram-positive cocci (60%) followed by Gram-negative bacilli (35.92%). The rates of resistance to antimicrobial agents for blood cultures isolates were increased, with resistance phenotypes of clinical and epidemiological importance, most notably, a high incidence of MRSA strains (37.6%) and MRS (46.8%) and production of ESBL in *Escherichia coli* (19%) and *Klebsiella pneumoniae* (63%) strains.

Keywords: *bacteremia, etiology, antibiotic resistance, MRSA, ESBL.*

1. INTRODUCTION

Sepsis has a growing incidence and represents the leading cause of mortality in Intensive Care Units and the third cause of death in developed countries, equaling the number of deaths due to acute myocardial infarction [1, 2, 3]. The etiologic spectrum of bacteremia and septicemia is very diverse, being represented less by classical pathogens, but dominated by opportunistic microorganisms, residents of normal skin and digestive tract microbiota or commensals, exhibiting a high degree of virulence and antibiotic resistance [4, 5, 6]. Uncontrolled and widespread use of antibiotics has favored the increased incidence of antibiotic-resistant bacteria, which have a major and decisive role in the management of systemic bacterial infections.

2. EXPERIMENTAL SECTION

We have performed a retrospective study on a total number of 3,337 blood cultures from patients with febrile syndrome, admitted between January 2009 - June 2012, in three hospitals of Galati city. Blood samples were collected under strict aseptic conditions, seeded in the standardized blood culture bottles containing special a culture medium, whose composition is favoring the development

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of micro-aerobic, anaerobic and microaerophilic micro-organisms and analyzed in semiautomatic (*Oxoid Signal System*) and automated (*BacT/ALERT* and Bactec 9050) systems. From the positive samples there were performed Gram stained smears, subcultures identification and the antibiotic susceptibility assay [4].

The clinical significance of isolates was determined by the following criteria: isolate identity at the primary infection, the number of consecutive, positive blood cultures sets, timing of blood culture positivity and clinical characteristics (predisposing factors, therapeutic efficiency) [4, 7].

Biochemical identification of the isolated microbial agents was performed using conventional (phenylalanine desaminase, urease, citrate degradation in aerobiosis, indole, lysine decarboxylase) [8], multitest: TSI (triple sugar iron), MIU (mobility, indole, urease) and MILF (mobility, indole, lysine decarboxylase, phenylalanine desaminase) [5, 9], semiautomatic (microtest API) and automated (Vitek 2 Compact) systems.

Antibiotic susceptibility testing was determined using the disk diffusion method, according to CLSI (2009, 2010, 2011, 2012) and the minimum inhibitory concentration value was obtained with the automatic Vitek system. The reference strains used for quality control of the antibiotic susceptibility assay were: *Staphylococcus aureus* ATCC 25923, *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 [10, 11, 12, 13].

Statistical analysis was performed using its own database and Excel software.

3. RESULTS SECTION

Of the total of 3,337 analyzed blood cultures, 16.81% (561 samples) were positive (Table 1).

Table 1: Distribution of blood cultures analyzed in three hospitals of the Galati city

Blood cultures	Number	The positivity rate (%)
Positive	561	16,81
True positive	292	8,75
False positive	269	8,06
Negative	2797	83,19
Total	3337	

The etiology and incidence of different microbial species in the cases of true positive systemic infections is shown in Table 2.

Table 2: Distribution of infectious agents isolated from blood cultures

Infectious agents	Number	(%)	Infectious agents	Number	(%)
Coagulase-negative *Staphylococcus*	60	20.56	*Proteus spp.*	9	3,08
S. aureus	54	18,5	*Enterobacter spp.*	7	2,39
Enterococcus spp.	34	11,6	*Citrobacter spp.*	4	1,36
Streptococcus viridians	3	1.02	*Salmonella spp.*	1	0,34
Streptococcus pyogenes	1	0,34	*Acinetobacter spp.*	10	3,42
Streptococcus viridians	23	7,88	*Pseudomonas spp.*	1	0,34
Bacillus spp.	2	0,68	*Stenotrophomonas maltophilia*	1	0,34
E.coli	38	13,01	*Neisseria meningitidis*	2	0,68
K. pneumoniae	34	11,64	*Candida spp.*	8	2,74
			TOTAL	**292**	

The isolated strains showed both patterns of natural (constitutive) resistance, as well as markers of acquired resistance to antibiotics.

Coagulase-negative *Staphylococcus* strains were more frequently isolated (20.56%) than *S. aureus* (18.5%) (Table 2).

S. aureus strains showed a rate of 37.6% resistance to methicillin, 40% to erythromycin, 35.1% to clindamycin, the most encountered resistance phenotype to macrolides and lincosamides being the MLSBi. Concerning other classes of antibiotics, the resistance rates were of 12% to fluoroquinolones and 17% to aminoglycosides (Figure 1).

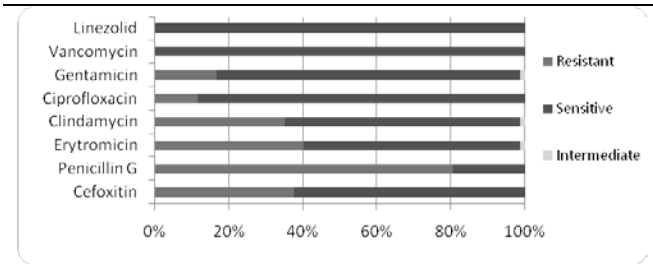

Figure 1: The distribution of resistance markers in the analyzed *S. aureus* strains

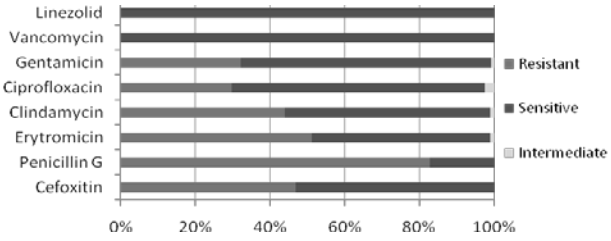

Figure 2: The distribution of resistance markers in CNS strains

The ß-lactams susceptibility testing of coagulase-negative *Staphylococcus* (CNS) strains showed a high rate of resistance to penicillin of 82.7%, followed by macrolides (51%), methicillin (46.8%) and aminoglycosides (31%) (Fig. 2). All strains of *Staphylococcus* sp. proved to be susceptible to Vancomycin and Linezolid.

The *Enterococcus spp.* strains accounted for 11.6% of all positive samples (Table 2). *Enterococcus* spp. strains showed a high-level resistance (HLAR) to gentamicin, of 18.5% and of 20.3% to ciprofloxacin. *Enterococcus* strains showed 100% sensitivity to vancomycin, being also susceptible to ampicillin and penicillin in a high proportion (79.6% and 74%, respectively) (Fig. 3).

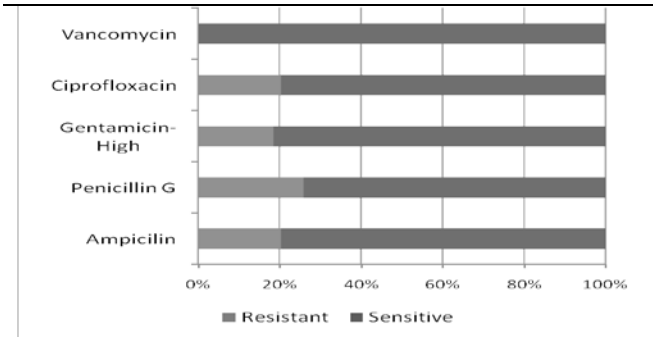

Figure 3: The distribution of resistance markers in the *Enterococcus* spp. strains

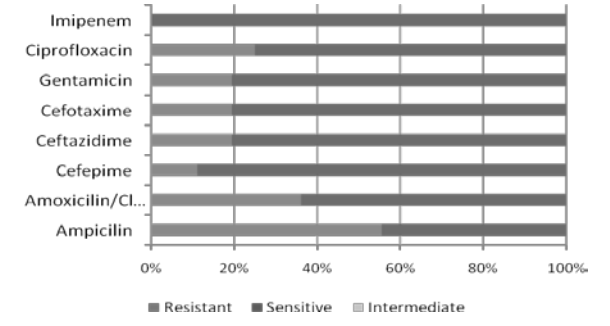

Figure 4: The distribution of resistance markers in the analyzed *E.coli* strains

The *Enterobacteriaceae* strains represented 31.8% of the positive samples, out of which 13.01% were *E. coli*, 11.64% *K. pneumoniae* and 3.08% *Proteus* spp. (Table 2).

The *E. coli* strains were resistant to ciprofloxacin by 25% and to beta-lactamine inhibitors by 36%, due to the production of chromosomally encoded beta-lactamases of AmpC type. By contrast, 19% of the ESBL-producing strains were resistant to penicillins and third generation cephalosporins (Fig. 4).

A high percentage of *Klebsiella pneumoniae* strains were ESBL (63%) and AmpC (53%) producers and 40% were resistant to ciprofloxacin and gentamicin (Fig. 5).

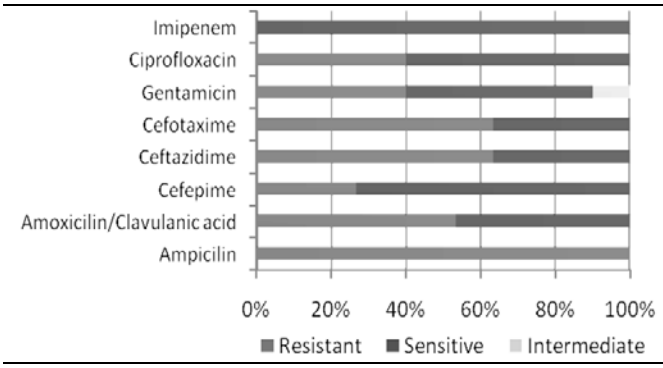

Figure 5: The distribution of resistance markers in the analyzed *K. pneumonia* strains

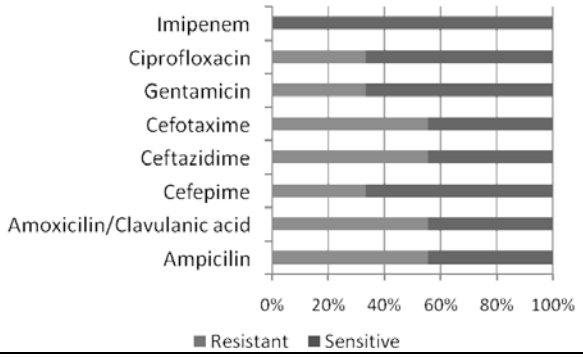

Figure 6: The distribution of resistance markers in the analyzed *Proteus* spp. strains

The *Proteus* spp. isolates were ESBL producers in a proportion of 56%, and resistance rates to ciprofloxacin and gentamicin were of 33% (Fig. 5). All tested *Enterobacteriaceae* strains showed 100% susceptibility to imipenem.

Among the non-fermentative Gram-negative bacilli, 3.42% were represented by *Acinetobacter* spp. strains, 63% of them being resistant to ciprofloxacin and 50% to amikacin and piperacillin with tazobactam, 38% to gentamicin and 25% to imipenem (Fig. 7).

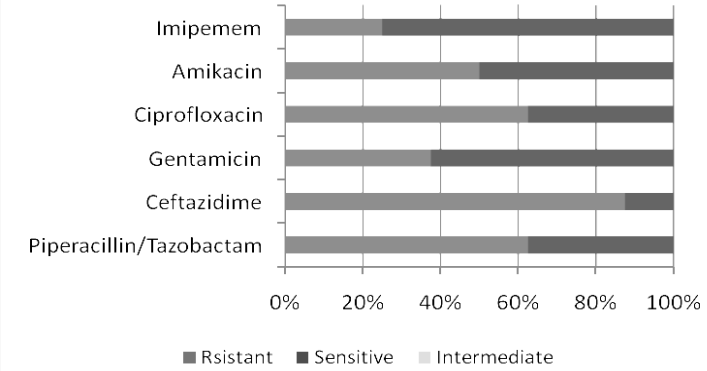

Figure 7: The distribution of resistance markers in the analyzed *Acinetobacter* spp. strains

Sepsis is characterized by clinical signs caused by the systemic inflammatory response triggered by an infection. The infection occurs when microorganisms exceed the body's defense mechanisms and are multiply within host tissues [14], with the increasing progression in the adjacent tissues, often by using blood dissemination to produce secondary infections in remote anatomical sites.

The *S. aureus* strains were isolated in our study in 18.5% of cases, the level methicillin resistance being 37.6%, which is below the maximum level of 50.5% reported by Romania for *S. aureus* strains isolated from invasive infections in 2011 [15]. In the last three decades the incidence of MRSA caused an increased concern in hospitals institutions worldwide, with geographical variations. The highest prevalence was observed in Portugal hospitals (54%), while the lowest prevalence was detected in the Netherlands (1.4%) and Scandinavia (<1%). These geographical variations of MRSA isolation rates are on one hand due to the clonal variation of MRSA strains circulating in a particular geographic area, with different virulence and pathogenicity potentials, and on the other hand, they reflect differences in the use of antibiotics in hospitals, and the effectiveness of the policies used to monitor nosocomial infections.

Although systemic infections with coagulase-negative *Staphylococcus* spp. strains (CNS) are usually nosocomial, in 8% of cases CNS were also implicated in community systemic infections [4, 16]. Published data highlight the highest prevalence of SCN in systemic infections with an incidence

which varies from 15% to 30% [4, 16]. In this study, SCN were isolated in 20.9% of the true positive samples, and 46.8% were resistant to methicillin.

Bactereemia cases with *Enterococcus* spp. are mainly nosocomial [4, 17], the community ones being those of suspected endocarditis. In the U.S., three out of four by 10,000 hospitalizations for nosocomial bacteremia caused by *Enterococcus* sp. lead to death. Most of the enterococcal infections are caused by *Enterococcus faecalis* (up to 80%) and *Enterococcus faecium* (5-10%). Under normal conditions these strains are commensal, but when the balance of the commensal relationship is disrupted, *Enterococcus* sp. strains could cause an invasive disease. Primary systemic infections usually occur in immunocompromised patients and may be due to the bacterial translocation from the digestive tract [4].

The most common secondary infections are derived from the urinary tract, digestive or soft tissue infections. *Enterococcus* spp. exhibits resistance to multiple classes of natural antibiotics, such as cephalosporins, penicillins, sulfonamides and aminoglycosides (low level resistance). The successful treatment of enterococcal infections critically depends on the clinical resistance markers of the respective strains, acquired by horizontally mediated transfer by mobile genetic elements (plasmids, transposons) and by genetic recombination or mutations.

In the present study, the *Enterococcus* sp. strains presented 20% resistance to ampicillin and ciprofloxacin, associated with 18.5% high-level resistance to gentamicin; all *Enterococcus* spp. strains showed sensitivity to vancomycin. According to ECDC EARS.net, the high-level resistance to aminoglycosides (HLAR) rates in *Enterococcus faecalis* strains are increasing in all European countries, excepting Iceland, which did not report in 2011 any resistant strain. The highest rate of vancomycin resistance in *Enterococcus faecium* strains has been reported in Ireland (34.9%), while in Romania, Sweden, Iceland and Bulgaria there has been no reported case of resistance.

The *Enterobacteriaceae* family represents an important etiologic cause of systemic infections (17%) [4,7] occupying the second place after CNS. In the present study the proportion of *Enterobacteriaceae* strains was 31.8%.

E. coli is the most frequently isolated Gram-negative pathogen from blood cultures, and also the most common cause of community and nosocomial urinary tract infections (7-11%) [4, 18] associated with spontaneous and surgical peritonitis and wound infections. The *E. coli* strains implicated in systemic infections proved to have the same antibiotic resistance phenotype as the ones isolated from urinary tract infections. Source of infection to patients with nosocomial infections could be: infected catheters, urinary, gastrointestinal and respiratory tract. In our evaluations, *E. coli* was isolated in 13% of positive cases. Data provided by ECDC EARS.net (2011) for Romania present a higher resistance rate to aminopenicillins (68%), compared to that of 56%, obtained in our study and to fluoroquinolones, 30% as compared with 25%, in our study. Resistance to third generation cephalosporins is increasing, reaching 22% in strains reported by Romania, comparable with the 19% value, resulted from our study, which shows extensive dissemination of ESBL producing strains, both at communiy level and in the hospital.

Klebsiella spp. is a common cause of community (10%) and systemic nosocomial (6%) infections [4, 19]. Systemic infections with *Klebsiella* spp. originate from remote sites, and usually derives from urinary, respiratory tract, bile ducts or soft tissue infections. *Klebsiella pneumoniae* is the second cause, among Gram-negative bacilli, of systemic infections, after *E. coli.* The incidence of *Klebsiella pneumoniae* isolates in our study was 11.64%. *K. pneumoniae* presents natural resistance to aminopenicillins, through the presence of the chromosomally encoded TEM beta-lactamases. The

level of resistance to third generation cephalosporins was higer (63%) than that of 44%, reported by Romania to ECDC EARS.net in 2011.

Fluoroquinolones and aminoglycosides are increasingly losing their efficiency due to the emergence of variate microbial resistance mechanisms. Our isolates yielded a resistance of 40% for both classes, towards the levels of 30% and 50%, respectively, reported by Romania to ECDC EARS.net in 2011. Combined resistance to third generation cephalosporins, fluoroquinolones and aminoglycosides were present 26% strains. Carbapenems retained their clinical effectiveness in *Enterobacteriaceae* strains, but a close monitoring of their evolution must be considered with the maximum rigor, to preserve their activity.

Proteus spp. is part of the normal microbiota of the human gastrointestinal tract and the third leading cause of urinary tract infections, particularly the nosocomial ones. *Proteus vulgaris* cause lung infections, urinary tract infections or bacteremia only when it gets an extra-intestinal localization [20]. In our study, *Proteus* spp. showed an incidence of 3.08%. Our blood cultures isolates showed 55.5% resistance to aminopenicillins, to beta lactamase inhibitors and third generation cephalosporins and 33% to aminoglycosides and fluoroquinolones.

Acinetobacter spp. was isolated from several opportunistic infections (septicemia, 3-5% cases of nosocomial pneumonia, endocarditis, meningitis, skin infections, eye surgical wounds and urinary tract infections), particularly in patients with deficient host defense mechanisms [20]. Although most strains of *Acinetobacter* spp. are contaminants, when isolated from systemic infections, they should be considered pathogenic. Wisplinghoff et al. reported an incidence of 1-2% for *Acinetobacter* spp., but however these organisms are in the top 10 of the major etiological causes of systemic bacterial infections [21]. Of particular concern is the growing resistance to most classes of antibiotics used to treat nosocomial infections, including carbapenems, the only antibiotics remaining effective being colimicines. In our study, the isolates of *Acinetobacter* spp. accounted for 3.42% of all isolates and showed a high level of resistance to ceftazidime (87%), ciprofloxacin (62.5%) and imipenem (25%).

The European experience shows that *Acinetobacter baumannii* spreads under the pressure of high concentrations of antibiotics and poor hygiene in hospitals [4, 22]. Since resistance of this microorganism occurs very rapidly during treatment with antibiotics, it can be isolated from blood cultures harvested after prolonged exposure to antibiotic therapy, being predictable for a multidrug-resistance phenotype.

4. CONCLUSIONS

The present study confirmed the prevalence of Gram-positive cocci in the etiology of systemic infections (60%), of which a large proportion was the SCN (20.56%), followed by *S. aureus* (18.5%) and *Enterococcus* spp. (11.6%). The opportunistic Gram-negative strains belonging to *Enterobacteriaceae* family (*E. coli, Klebsiella* sp., *Proteus* spp., *Enterobacter* sp., *Citrobacter* sp., *Salmonella* sp.) were isolated from patients with positive blood cultures in a proportion of 31.8% in the city of Galati.

The level of resistance to antimicrobial agents was increased among the analyzed isolates with the presence of phenotypes with clinical and epidemiological importance, most notably is the high incidence of MRSA strains (37.6%), MRS (46.8%) and ESBL production to *E. coli* (19%) and to *Klebsiella pneumoniae* (63%). The level of multiple resistance to the third generation cephalosporins, fluoroquinolones and aminoglycosides is increasing, the highest rate of 26% being registered in *Klebsiella pneumoniae* strains. Many of these multiple resistant strains were of nosocomial origin, causing severe difficulties in choosing the optimal treatment of patients and

reflecting the increased selective pressure, due to excessive and injudicious antibiotics consumption. *Acinetobacter* spp. strains were highly resistant to imipenem, ceftazidime and fluoroquinolones, the only antibiotics remaining effective being colimicines.

The present study demonstrated that systemic infections etiology is represented both by species from the normal microbiota of the body, and also by opportunistic strains, sometimes of nosocomial origin, which exhibit clinical resistance phenotypes, that could even become an important taxonomic criterion in the bacterial species identification, allowing their inclusion in groups and phenotypes useful in epidemiological studies and in identifying effective solutions for the early detection and monitoring the incidence of systemic, potentially lethal infections.

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