Resistance Profile of the Isolated Bacterial Stems in Invasive Infections in Three Hospitals in the South-East of Romania

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The purpose of this study was to determine the etiologic range of the invasive infections and the profiles of resistance to antibiotics in the isolated stems from patients hospitalized in the Sf. Apostol Andrei Emergency Hospital Galati; Sf. Cuv. Parascheva Hospital of Infectious Diseases Galati and Sf. Ioan Hospital for Children of Galati. We analysed 4029 blood cultures in the period 01.01.2014-30.12.2016. Among the bacterial stems frequently isolated were: coagulase-negative staphylococci, S. aureus, E. coli and Klebsiella pneumoniae. The level of resistance to antibiotics increased, expressing resistance phenotypes of clinic and epidemiologic importance, among which we notice the increased incidence of the stems MRSA (28.95%) and MRS (56.07%), and the production of ESBL in E. coli (30%) and Klebsiella pneumoniae (47%).

Keywords: invasive infections, resistance to antibiotics

The bacterial stems with clinical importance involved in the generalised infections belong to a wide variety of bacterial genres and species.

We noticed an increase in the incidence of systemic infections with CNS, tegument being the main source of dissemination. In the latest three decades, we noticed a constant increase in the incidence of these infections, phenomenon caused by the increase of population that presents these risk factors. More frequent are the secondary infections that derive from the urinary and digestive tracts or from infections of the soft tissues.

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Results and discussions

During the three years, we carried out 4,029 blood cultures, 15.83 % (638 samples) of which were positive. Due to the different capacity of the three hospitals, the annual tendency of the positive blood cultures was analysed for each unit separately (fig. 1-3). The tendency of the positive blood cultures represented by the regression curve has a descending trace. The regression is almost linear at the Sf. Cuv. Parascheva Hospital of Infectious Diseases because the total number of blood cultures was higher, reported to the number of beds, depending on the specificity of the hospital (fig1).

Experimental part

The study is retrospective, carried out on a number of 4,029 blood cultures sampled from the patients hospitalized in the hospitals in Galaţi, in the period 01.01.2014-30.12.2016. The blood cultures were collected according to the laboratory instructions and comprised in the Sampling Textbook, in strict aseptic conditions. We used recipients for blood cultures with special nutritive medium, whose composition favours the development of aerobic, anaerobic and microaerophilic microorganisms and were analysed in automated system (BacT/ALERT and Bactec 9050). From the positive samples, we made Gram smears and subcultures to identify and test the sensitivity to antibiotics. The biochemical identification of the microbial agents was done by using classical methods, semi-automated (RapiD™) and automated (Vitek 2 Compact) methods.

Testing the sensiteness to antibiotics was determined by using the Kirby-Bauer diffusion method, on the Mueller-Hinton standardized medium and the method of minimum inhibiting concentration obtained with the automated Vitek system, according to the Clinical Laboratory Standard Institute (CLSI). ESBL production was detected by using the test of double diffusion [1,2] and the Vitek 2 Compact software. The reference stems used for quality control of the DST were: S. aureus ATCC 25923, E.coli ATCC 25922, Ps. aeruginosa ATCC 27853 [3-5].

The statistic processing of the data was done by using our own database and Excel worksheets.

Fig. 1  Graphical representation of the annual dynamics of positive blood cultures at the Sf. Cuv. Parascheva Clinical Hospital of Infectious Diseases

Since there are studies showing that the parents’ sex influences the occurrence of sepsis [6], we carried out an analysis of the repartition on sexes of the cases analysed,
by noticing that 58% of the patients were males and only 42% were females (fig. 4).

The bacterial stems with clinical importance involved in the generalised infections belong to a wide variety of systemic infections with an incidence ranging from 15% to 30% in positive blood cultures [7, 8]. We noticed an increase in the incidence of systemic infections with CNS, tegument being the main source of dissemination.

Bacteremia with Enterococcus spp. are mainly nosocomial [9], the community ones signalling the suspicion of endocarditis. In our study, the incidence of stems was of 4.69%. Invasive enterococcal infections are caused mainly by Enterococcus faecalis (at the rate 80%) and by Enterococcus faecium (5-10%). They are normally commensal of the intestinal flora in normal conditions, but when the balance of the commensal relation is disturbed, the enterococci can cause invasion diseases. The primary systemic infections usually appear in immunocompromised patients, and they can be due to some intestinal translocations in the digestive tract [8]. In the latest three decades, we noticed a constant increase in the incidence of these infections, phenomenon caused by the increase of population that presents these risk factors. More frequent are the secondary infectious that derive from the urinary and digestive tracts or from infections of the soft tissues.

From the group of negative Gram bacilli, we isolated 36% bacterial stems, at the top being representatives of the family Enterobacteriaceae, namely: E. coli (14.56%), Klebsiella pneumoniae (10.37%), Enterobacter spp. (1.73%), Proteus spp. (3.08%), Salmonella spp. (1.23%), followed by non-fermentative negative Gram bacilli, among which Pseudomonas aeruginosa (2.71%) had the highest incidence (fig. 5). At lower frequency, Candida spp. was isolated (1.23%).

Tests for sensitivity to β-lactams of CNS stems showed high resistance rate to Penicillin of 83.16%, followed by resistance to macrolides of 65.71%, to methicillin of 56.07% and to aminoglycosides of 21.88% (fig. 6).

The isolated ones presented patterns of natural (constitutive) resistance, characteristic to those species, but also acquired resistance to antibiotics. The highest rates of sensitivity were recorded in carbapenems, glycopeptides and colimicins.

Tests for sensitivity to β-lactams of CNS stems showed high resistance rate to Penicillin of 83.16%, followed by resistance to macrolides of 65.71%, to methicillin of 56.07% and to aminoglycosides of 21.88% (fig. 6).
S. aureus showed at the rate of 28.95% resistance to methicillin (MRSA), under the maximal value 56% reported in Romania for the stems of S. aureus isolated from invasive infections in 2014 [10,11]. In 2014, the level of MRSA in Romania remained the highest in all the EU states, as it had happened in 2013 as well; in these two years, we were the only European state with MRSA level above 50%. This situation is created by the gap in efficiency of steps taken to limit inter-human transmission of microorganisms between the hospitals in Romania and hospitals of other European states and by the higher consumption of beta-lactams in Romania [11].

Tests of stems of S. aureus in macrolides and lincosamides showed that 43.42% were resistant to erythromycin, 42.11% to clindamycin, the main phenotype of resistance being Macrolide-Lincosamide-Streptogramin B (MLS B), inducible. The resistance to other classes of antibiotics was of 10.96% in fluoroquinolone and 8.06% in aminoglycosides (fig. 7).

An unwanted situation is also signalling the stems of staphylococcus aureus resistant to linezolid (1.41%), most probably selected after the extended treatment with this antibiotic.

Testing the sensitiveness to penicillin for Streptococcus pneumoniae showed in our study a resistance of 14.3% (fig. 8) comparatively with the resistance of 42.6% reported by Romania for the stems isolated from invasive infections in 2014. The resistance to beta-lactams is determined by the modification of target proteins (PBP – penicillin-binding proteins) and that is why the associations with inhibitors of beta-lactamase do not re-establish the activity of penicillin on the pneumococci resistant to them; by comparison, the cephalosporins of generation 3-4 can preserve their activity against the isolation with diminished sensitiveness to penicillin. The capacity of penicillin relation to PBP is affected cumulatively by the produced mutations and that is why the increase of CMI to penicillin is gradual. The clinic consequence is that the ENT infections or the respiratory ones, determined by stems of pneumococci with diminished sensitiveness to penicillin can be treated efficiently with higher doses of penicillin/aminopenicillins. However, the pneumococcal meningitis requires treatment with ceftriaxone or cefotaxime to which, if there is any resistance to them, vancomycin is added [11].

All the stems of pneumococci tested were sensitive to fluoroquinolones, vancomycin and linezolid (fig. 8).

Escherichia coli is the Gram-negative bacillus most frequently involved in systemic infections, being the cause of the most frequent community and nosocomial infections [11,12]. Colonizing agent of the interior digestive tract, it can determine diarrhoeal diseases, low and high urinary infections, biliary infections, spontaneous or postoperative intraabdominal infections, infections of soft parts (in the case of mixed infections), systemic infections [11].

Resistance to cephalosporins of 3rd generation is in continuous increase, reaching of percentage of 26.1% reported by Romania, comparing with 32.2% resulted from our study. For 2014, Romania had the 5th level of resistance among the EU states, and exceeds 2.2 times the estimated European average. The prevalence of stems producing ESBL was 30%, which proves the dissemination of ESBL stems in hospitals, as well as community level. In our study, we obtained an increased resistance to fluoroquinolones and aminoglycosides by 49.15% and 28.81% respectively (fig.9), comparing with 29.55% and 16.1%, data offered by Romania in 2014 [10, 11]. Identifying the stems resistant to carbapenems is still extremely rare in Europe, with an estimated average for 2014 of 0.1%. In our study, we obtained a resistance to carbapenems of 3.4%; their occurrence could be a consequence of the exchange of genetic material with other species of Enterobacteriaceae, in which such a resistance is much more frequently found.

Klebsiella pneumoniae is a major problem of public health related to the bacterial resistance to antibiotics, because it represents a laboratory to produce new carbapenemases, and then transmitting to other Enterobacteriaceae the genetic material that codifies them. A major problem was generated by treatment of infections by extending the circulation of the stems resistant to carbapenems. The therapeutic alternatives remained are extremely limited, for the invasive infections being taken into account only the colistin and tigecycline [11]. K. pneumoniae presents natural resistance to amino-penicillin due to the presence at chromosome level of the gene that codifies the TEM beta-lactamases.
to cephalosporins of 3rd generation obtained by us (fig.10) is of 67%, comparing with 69.9% as reported for Romania in 2014 [10,11]. A high percentage of stems of Klebsiella pneumoniae produced ESBL 47.5%.

Fluoroquinolones and aminoglycosides lose ever more field in front of the mechanisms of microbial resistance, for our isolations getting a resistance of 35%, respectively 60.6%, comparing with the levels of 65.7%, respectively 60.1% reported for Romania. The resistance combined to the 3rd generation of cephalosporins, fluoroquinolones, and aminoglycosides is present also in the case of this microorganism, in proportion of 37.5%. The incidence of combined resistance is in continuous increase, the result in the year 2014 of 52.5% represents the 3rd highest level in the EU states. Carbapenems were used in some places on a large scale, justified or not by the involvement of the ESBL-positive enterobacteriaceae or non-fermentative; the consequence was the emergence of the resistance to them, especially of non-fermentative and K. pneumoniae.

In our study, the level of resistance was 15%, comparing with 34.3% reported by Romania in 2014 [11].

Conclusions

The etiology of the systemic infections in this study confirmed the prevalence of positive Gram cocci as etiologic agents of (59.3%), among which the highest rate is reported for SCN (27.9%), followed by S. aureus (18.7%) and Enterococcus spp. (4.6%). The incidence of conditioned pathogenic microorganisms Gram negative of the family Enterobacteriaceae (E.coli, Klebsiella pneumoniae, Proteus, Enterobacter, Citrobacter, Salmonella), has been isolated in patients with positive blood cultures in three hospitals at the rate of 36%.

The level of resistance to antibiotics for bacterial stems isolated from blood cultures was high, with the presence of some phenotypes of clinic and epidemiologic importance, among which we notice the high incidence of stems MRSA (28.95%), MRS (56%) and the production of ESBL in E. coli (30%) and Klebsiella pneumoniae (47%). The level of multi-resistance to cephalosporins of 3rd generation, fluoroquinolones and aminoglycosides is growing, being present in 37.5% of the stems of Klebsiella pneumoniae, which is conducive to higher morbidity, mortality and costs. The excessive use of fluoroquinolones, cephalosporins and carbapenems, as well as the insufficiency of the measures to limit the inter-human transmission of the microorganisms explains the evolution of the epidemics of Clostridium difficile [13].

This study shows that the etiology of the invasive infections is due both to some species coming from the normal microbiota of the body, and some opportunistic stems, sometimes of nosocomial origin, with clinically resistant phenotypes, which can become an important taxonomic criterion in classifying the bacterial species, allowing their inclusion in groups and phenotypes, useful to the studies of epidemiology and in finding some efficient solutions to reduce the incidence of systemic infections with lethal potential.

References

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