Antibioresistance of Microbial Strains Implicated in the Etiology of Sepsis with Oro-Maxillofacial Portal of Entry

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Methicillin-resistance phenomenon regarding Staphylococcus aureus which is often met as etiologic agent of severe systemic infections with oral-maxillofacial portal of entry imposes the first-line therapeutic schemes readjustment in patients with significant risk factors. Minimum inhibitory concentration (MIC) determination for every isolated S.aureus strain is useful for the antibiotherapy guiding, in order to choose the appropriate antimicrobial substances and to avoid the selection of resistant mutants. There have been studied and tested 9036 bacterial strains isolated from patients hospitalized in the Sf.Spiridon Emergency County Hospital between 2013-2016. Minimum inhibitory concentrations (MIC), MIC 50 and MIC 90 values were determined for the following antibiotics: Penycilline, Erithromycin, Oxacylline, Tetracycline, Gentamycin, Tobramycine, Kanamycin, Ryfampicyn, Trimethoprim-Sulfamethoxazole, Ofloxacine, Ciprofloxacine and Vancomycin.The classification of each identified bacterial strain into sensitive or resistant was accomplished according to the breakpoints recommended by CLSI 2016 (Clinical and Laboratory Standard Institute). We considered intermediately susceptible isolates as being resistant. S.aureus antibioresistance was high to tetracycline, erythromycin and kanamycin, with elevated MIC 90 values (64µg). The rate of resistance to penicillin in the case of S.aureus was 94.7%. The lowest MIC values regarding Pseudomonas aeruginosa were for imipenem, meropenem and colistin and the highest ones for piperacillin-tazobactam, ceftazidime and amikacin. Third generation cephalosporins demonstrated their inefficiency in the staphylococcal infections' treatment as a consequence of an increasing resistance to this category of betalactams. Vancomycin remains a saving in-hospital therapeutic option in the case of MRSA implication, next to teicoplanin and linezolid.

Keywords: antibioresistance, oro-dental sepsis, minimum inhibitory concentration

Focal disease of oro-dental origin home signifies that a focus of infection in the oral cavity may be at the origin of some distant lesions. This concept is less controversial because it is difficult to prove with certainty the oro-maxillofacial portal of entry of the microorganisms involved in extraoral infections. Animal experiments and clinical studies on human subjects have described several pathophysiological mechanisms: bacteremia, toxic and immunological mechanisms and suction phenomenon. Various dental procedures, such as oral-dental prophylaxis, tooth brushing, chewing, favor the passage of bacterial flora and its toxins into the bloodstream. All these conditions are aggravated by poor oral hygiene or presence of a possible dental infection. Typically, the involved bacterial germs are destroyed by the reticuloendothelial system of the host, but an impaired immune system, valvular heart disease, and the presence of associated diseases, such as respiratory infections, diabetes, periprosthetic infections and brain abscesses favor bacterial colonization. Current treatment trends require antibiotic prophylactic measures only in patients at high risk of infection (Persac S., et al., 2011) [1].

The aim of this study was to determine the minimum inhibitory concentrations and interpret the results in view of facilitating the early etiologic diagnosis and early initiation of appropriate therapy in patients with sepsis with oro-maxillofacial portal of entry.

Experimental part

Material and methods

Oral cavity involvement was recorded in 16% of patients, most often in the form of lesions extending away from the floor of the mouth (7.5%) and maxillary sinusitis (5.5%). According to epidemiological characteristics, it was more common in women younger than 50 years, and most frequently of streptococcal etiology. A total of 9036 bacterial strains isolated from patients admitted to the Iasi Sf. Spiridon Emergency County Hospital during 2013-2016 were tested.

MIC values were determined by using E-test strips or automated microbiological analyzers: Vitek 2 C or Phoenix. E-TEST: it is a method for determining the susceptibility of microorganisms to antibiotics and antifungals (by measuring the MIC value). An exponential antibiotic or antifungal gradient is immobilized on the surface of 5/50 mm inert plastic strips. Strips are applied in a radial arrangement to the surface of an agar plate pre-seeded with the test strain. After incubation at 35°C for 18-24 h, the MIC value is read. MIC value is indicated by the point of intersection between the inhibition ellipse of bacterial culture and the graded scale on the strip.

Results were interpreted according to CLSI guidelines [2].

Vitek 2C is a high-performance automated microbiology system that has been developed for the identification and testing of antibiotic susceptibility of bacteria isolated from clinical samples or environment. The system includes: VITEK 2 Compact (Cabinet), computer (workstation), monitor, printer, barcode reader, UPS. *Advanced Expert System* (AES) for antibiograms/antifungigrams is a new concept that offers a permanent *expert*, ready at any moment to interpret at the most advanced level of knowledge the complexity of antibiotic susceptibility test results. Tests are performed by using the cards available for automatic Vitek 2C system, namely: those for the identification of bacteria and fungi; for antibiotic/antifungal

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susceptibility testing of bacteria/fungi significantly clinically involved.

The analyzer has the ability to simultaneously perform 30 assays (identification and susceptibility to antibiotics/ antifungal agents).

The cards are read turbidimetrically for antibiotic susceptibility and colorimetrically for identifying bacterial species.

When testing is complete, the analyzer automatically reports the results that can be printed manually/ automatically on paper.

The results for Gram-negative bacilli identification as well as for antibiogram/antifungigram are available in the 2-10 hours, for Gram-positive cocci in 2-8 hours, for nutritionally fastidious species 18-24 h anaerobes (included), and for fungi 18 hours.

Card quality control is carried out with reference microbial strains for each type of card, strains recommended by the manufacturer.

Reference strains: *Escherichia coli* ATCC 25922, *Escherichia coli* ATCC 35218, *Acinetobacter baumannii* ATCC BAA-747, *Staphylococcus aureus* ATCC 29213, *Candida parapsilosis* ATCC 22019.

The analyzer automatically provides antibiogram results, according to MIC values, interpreting the results as: SUSCEPTIBLE/INTERMEDIATE/ RESISTANCE.

Antifungigram was performed by MIC determination on VITEK 2C automatic analyzer. Identification cards used: YST (*Candida)* and AST -YST 06 YST 07 (antifungigram). The cards included the following antifungal agents: Voriconazole, Fluconazole, Amphotericin B, Flucytosine, Caspofungin, Micafungin.

BD PHOENIX analyzer has the ability to simultaneously perform identification and antibiotic susceptibility of 100 bacterial isolates. The results of identification are available in 3-4 h, and of susceptibility testing in 6-10 h. Antibiotic susceptibility was determined by dilution method (MIC). When the test is complete, the analyzer automatically reports the results by displaying them on the screen and printing them on paper. The system includes: BD PHOENIX 100, with a rotor in which 100 single-use panels can be inserted simultaneously, *BD EpiCenter* system for data processing, BD Xpert system which verifies the discordant results, interprets and shows special messages (if further tests are needed) and prints the results. Susceptibility of Gram negative bacteria can be tested against 21 antibiotics and of Gram-positive bacteria against 24 antibiotics. Each new batch of panels is checked with reference ATCC strains.

Interpretation of antibiotic susceptibility:

S = Susceptible

I = Intermediate

R = Resistant

N = Nonsusceptible, indicates the absence of criteria for categorizing the strain as intermediate or resistant. It often occurs when there are not known resistant strains of a microorganism.

X = Invalid (MIC cannot be interpreted).

Results and discussions

In this prospective study, distribution by the etiological agent that caused sepsis was: *S. aureus* (19%), *Streptococcus salivarius* (15%), *Streptococcus mitis* (13%), *Streptococcus anginosus* (12%), *β-hemolytic streptococcus* (approximately 16%), *Candida* and *P. aeruginosa* species (9%), anaerobic flora - cocci or bacilli (7%), *S. epidermidis* (5.5%), *Acinetobacter* (6.5%), *E. coli* and *Klebsiella* (5%).

The obtained data are in agreement with data in the literature. A study by Poeschl PW et al., 2011 [3]on a total of 142 strains isolated from 76 patients reported the following results: streptococci (36%), staphylococci (13%), *Prevotella* species (8%) and *Peptostreptococcus* species (6%). Most patients presented anaerobic microbial flora (63%).

Antimicrobial agents. MIC 50 and MIC 90 values of the tested antimicrobial agents for the studies *S. aureus* strains are presented in tables1, 2 and 3 and figures 1, 2 and 3.

The *S. aureus* strains tested in this study showed elevated MIC 90 values (64µg / mL) for kanamycin and tetracycline and high percentages of resistance to kanamycin, erythromycin, trimethoprimsulfamethoxazole. For penicillin the resistance rate was 94.7% (fig. 1 and table 1).

Resistance to third generation cephalosporins and aztreonam has progressively increased in Romania after their introduction in therapy. Within a 10 year-interval, the percentage of isolates resistant to ceftazidime has doubled [2].

According to data in the literature, *S. aureus* is the most common etiologic agent in mono- and polymicrobial infections, approximately 1/2 of the isolates being methicillin-resistant. In polymicrobial infections, Enterococcus and Gram negative bacillus species have been identified in percentages of 37% and 67%, respectively [4].

MIC interpretation. In the studied cases, all *S. aureus* strains were susceptible to: vancomycin, linezolid, teicoplanin, and trimethoprim-sulfamethoxazole (fig. 4).

The literature indicates that *S. aureus* is the etiologic agent most commonly detected in mono- and polymicrobial infections, nearly 1/2 being methicillin-resistant forms. Enterococcus and Gram-negative bacillus

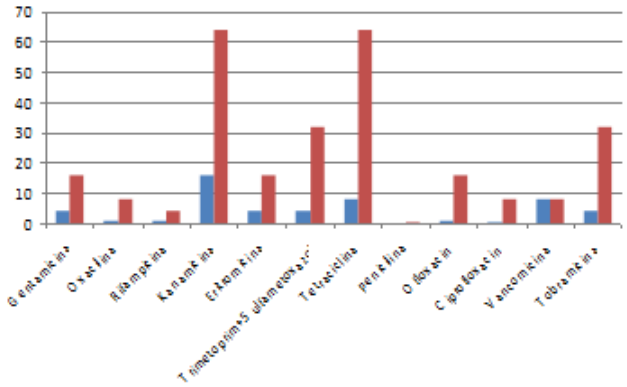

Fig. 1. *In vitro* activity of the tested antimicrobial agents against the *A. aureus* strains included in the study

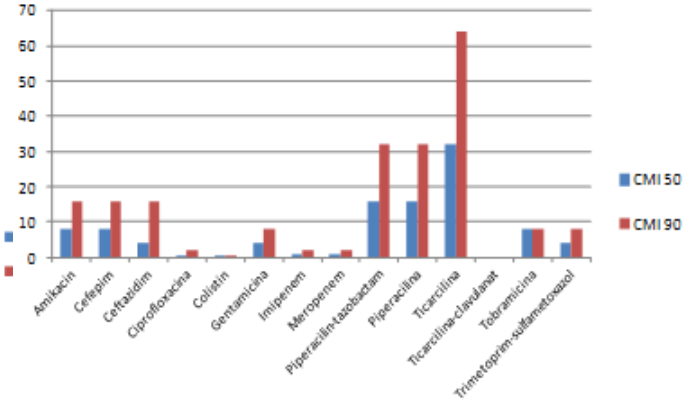

Fig. 2. *In vitro* activity of the tested antimicrobial agents against *the Pseudomonas aeruginosa* strains included in the study (n=18)

Antibiotics used in susceptibility testing	CMI 50	CMI 90
Gentamicin	4	16
Oxacillin	1	8
Rifampicin	1	4
Kanamycin	16	64
Eythromycin	4	16
Trimethoprim-Sulfamethoxazole	4	32
Tetracycline	8	64
Penicillin	0.06	0.25
Ofloxacin	1	16
Ciprofloxacin	0.5	8
Vancomycin	8	8
Tobramycin	4	32

Table 1
In vitro aCTIVITY OF THE TESTED ANTIMICROBIAL AGENTS AGAINST THE *S. AUREUS* STRAINS INCLUDED IN THE STUDY

Antibiotic used in susceptibility testing	CMI 50	CMI 90
Amikacin	8	16
Cefepime	8	16
Ceftazidime	4	16
Ciprofloxacin	0.5	2
Colistin	0.5	0.5
Gentamicin	4	8
Imipenem	1	2
Meropenem	1	2
Piperacillin/Tazobactam	16	32
Piperacillin	16	32
Ticarcillin	32	64
Ticarcillin-clavulanate	64	64
Tobramycin	8	8
Trimethoprim-sulfamethoxazole	4	8

Table 2
In vitro ACTIVITY OF THE TESTED ANTIMICROBIAL AGENTS AGAINST THE *PSEUDOMONAS AERUGINOSA* STRAINS INCLUDED IN THE STUDY (n=18)

Used antibiotic	CMI 50	CMI 90
Amikacin	4	8
Aztreonam	4	8
Ceftazidime	4	8
Ciprofloxacin	1	2
Gentamicin	4	8
Imipenem	1	2
Meropenem	0.5	1
Tobramicyn	4	8
Trimethoprim-sulfamethoxazole	1	2

Table 3
In vitro ACTIVITY OF THE TESTED ANTIMICROBIAL AGENTS AGAINST *THE KLEBSIELLA* SPP. STRAINS INCLUDED IN THE STUDY (n=13)

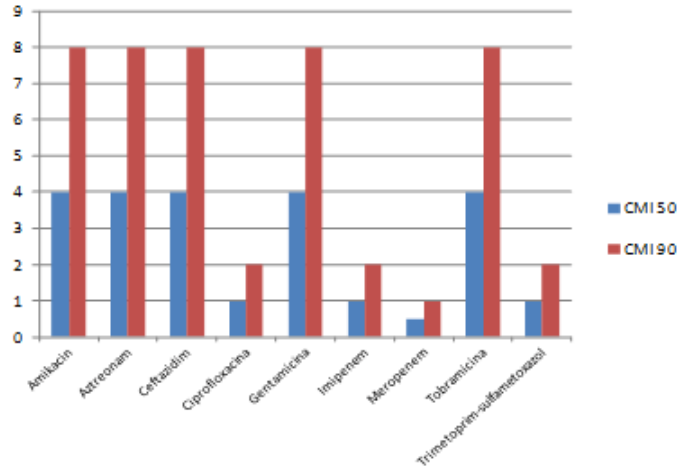

Fig. 3. *In vitro* activity of the tested antimicrobial agents against *the Klebsiella* spp. strains included in the study (n=13)

species were most frequently isolated in polymicrobial infection (37 and 67%, respectively) [4].

An Australian study showed that MRSA was isolated in large proportions (36%) in polymicrobial infections and demonstrated resistance to a wide range of antibiotics.

Most patients enrolled in this study received antibiotic therapy prior to various surgeries. It was found that antibiotic prophylaxis was not effective against the isolated pathogenic strains, particularly MRSA, enterococcus and Gram-negative bacillus species, additional prophylactic measures being necessary [5].

In this study MRSA was isolated in 45% of all periarticular infections.

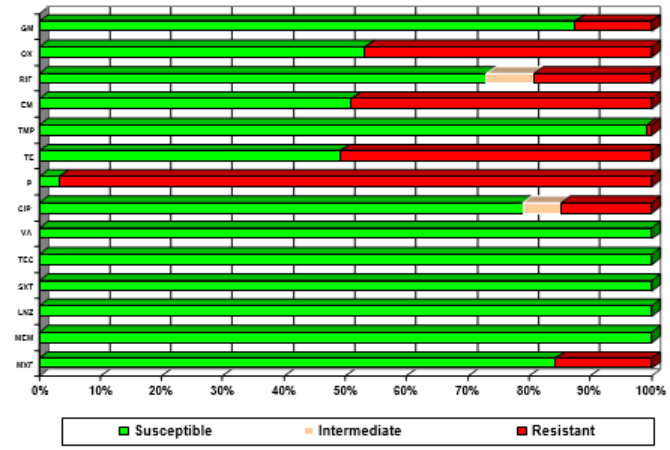

Fig. 4. Antibiotic susceptibility and resistance of *S. aureus* strains

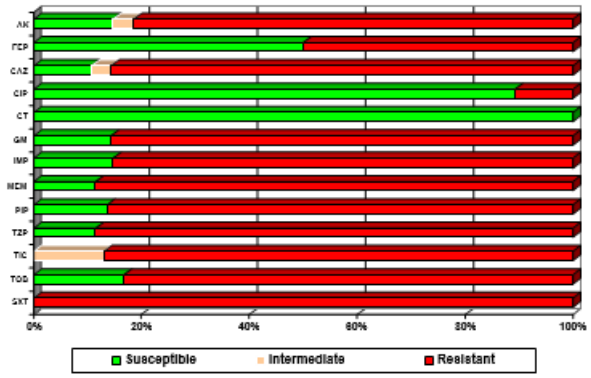

Fig. 5. Antibiotic susceptibility and resistance of *Pseudomonas aeruginosa* strains

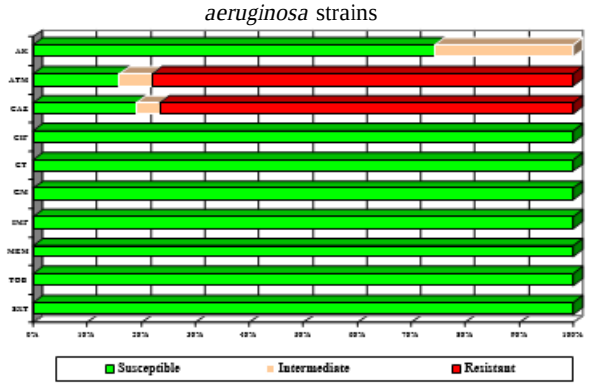

Fig. 6. Antibiotic susceptibility and resistance of *Klebsiella* spp. strains

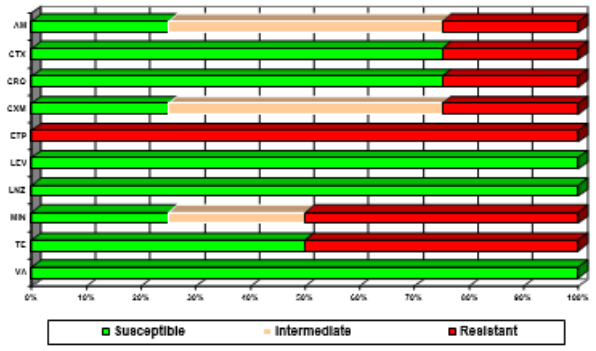

Fig. 7. Antibiotic susceptibility and resistance of *Streptococcus salivarius* strains

Currently, international treatment guidelines recommend cefazolin or flucloxacillin as prophylactic antibiotics, vancomycin being indicated only in patients at high risk for MRSA colonization (patients admitted for more than 5 days) and in patients with beta-lactam hypersensitivity.

The study demonstrated that 45% of all infections could be prevented with vancomycin.

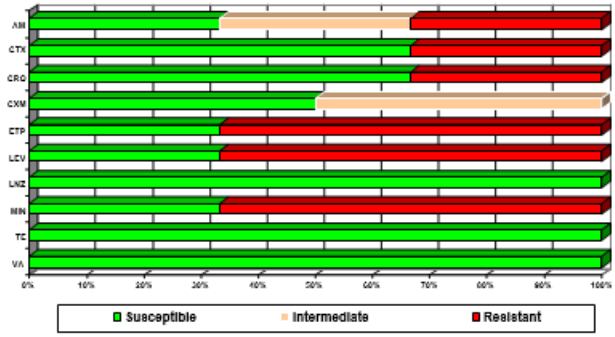

Fig. 8. Antibiotic susceptibility and resistance of *Streptococcus mitis* strains

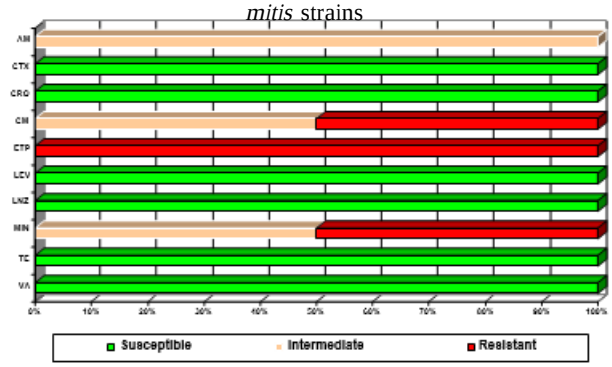

Fig. 9. Antibiotic susceptibility and resistance of *Streptococcus anginosus* strains

Another study by Finkelstein et al. compared vancomycin and cefazolin in the treatment of MRSA polymicrobial infections. The conclusion of the study was: surgical prophylaxis and vancomycin administration are problematic, forcing the association between these and cefazolin [6-9].

Consequently, in MRSA infections vancomycin used in combination with cefazolin could be effective.

The literature mentions concerns regarding the adverse effects of and bacterial resistance to vancomycin [10].

Other studies have demonstrated the efficacy of teicoplanin and daptomycin in the prophylaxis of MRSA infections[6,10-12].

Pseudomonas aeruginosa was sensitive to colistin (100%) and ciprofloxacin (90%) (fig. 5).

In the studied cases, all *Klebsiella* spp strains were susceptible to ciprofloxacin, colistin, gentamicin, meropenem, tobramycin and trimethoprim + sulfamethoxazole, and resistant to ATM (Aztreonam) and CAZ (ceftazidime). Almost 30% of strains showed intermediate resistance to amikacin (fig. 6).

Antibiotic susceptibility of the tested *Streptococcus salivarius* strains revealed: high resistance to ertapenem (100%), minocycline (50%) and tetracycline (50%); increased increased susceptibility to cefotaxime (75%) and ceftriaxone (75%); 100% susceptibility to levofloxacin, linezolid and vancomycin (fig. 7).

For the tested *Streptococcus mitis* strains the following were found: high resistance to ertapenem (66.7%), levofloxacin (66.7%) and minocycline (66.7%); increased susceptibility to cefotaxime (66.7%) and ceftriaxone (66.7%); 100% susceptibility to linezolid, vancomycin, and tetracycline (fig. 8).

For the tested *Streptococcus anginosus* strains the following were recorded: high resistance to ertapenem (100%), clindamycin (50%), and minocycline (50%); 100% susceptibility to cefotaxime, ceftazidime, levofloxacin, linezolid, vancomycin, and tetracycline (fig. 9).

In France, amoxicillin is the antibiotic of choice in preventing oro-maxillofacial infections, reducing the risk of bacteremia due to oral streptococci (*S. mitis*, *S. salivarius*,

S. mutans, *S. anginosus*), anaerobic microbial flora (*Prevotella sp*, *Fusobacterium sp.*, *Peptostreptococcus sp.*) or staphylococci (MRSA, MSSA, SCN)[13].

Conclusions

The conducted prospective study revealed that *S. aureus* was 100% sensitive to vancomycin, 90% to imipenem and 80% to tobramycin.

The tested *S. aureus* strains showed high MIC 90 values (64μg/mL) for tetracycline and kanamycin and high-level resistance to kanamycin, erythromycin, trimethoprim-sulfamethoxazole, for penicillin the resistance rate being 94.7%.

Resistance to third generation cephalosporins and aztreonam has progressively increased in Romania after their introduction in therapy. Over a 10-year interval, the percentage of isolates resistant to ceftazidime has doubled.

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