ANALYSIS OF ANTIBIOTIC RESISTANCE PATTERN OF S. AUREUS STRAINS ISOLATED FROM THE ORTHOPEDICS–TRAUMATOLOGY SECTION OF “SF. SPIRIDON” CLINICAL EMERGENCY HOSPITAL, IĂȘI

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(Abstract): Aim: The retrospective analysis of antibiotic sensibility of S. aureus strains isolated from infected patients from the Orthopedics–Traumatology Clinic of “Sf. Spiridon” Clinical Emergency Hospital, Iaşi during January 2003–December 2013, in view of determining the evolution trend of the resistance phenomenon and of pinpointing the most useful treatment for these strains. Material and method: The antibiotic sensitivity test was carried out using two methods: diffusimetric–Kirby-Bauer and the MIC determination by E-test (for the strains isolated in 2013); the interpretation of the sensitivity was made in a standardized manner, in compliance with the CLSI (Clinical and Laboratory Standards Institute) standard for antibiotics testing in force. Results: The sensitivity testing for β-lactams proved that during the 11 years of the study, the average value of the frequency of resistant strains was of 41.59%±8.68. The highest frequency of MRSA (Methicillin Resistant S. aureus) strains was noticed in 2012 (58.6%), followed by 2004 (50.7%). Even if in 2013 it dropped to 38.9%, the trend calculated for 2003-2013 is slightly rising (y=0.0073x+0.372). Out of the total of 495 S. aureus strains that were isolated, 164 (33.13%) were completely sensitive to the tested antibiotics and 26 (5.25%) were resistant only to β-lactams. The other MRSA strains associated multiple resistance and MIC for vancomycin varied between 0.5-2 mg/ml. Two strains whose MIC was of 0.5 mg/ml were sensitive to most classes of tested antibiotics, including β-lactams, except for macrolides (erythromycin), and the strain whose MIC was of 2 mg/ml, was resistant to all classes of tested antibiotics, except for glycopeptides and oxazolidiones. The other tested strains had a MIC for vancomycin equal to 1mg/ml. Conclusions: Due to the fact that there are infections with SAMR strains in a rather worrying percentage (53.9%) that are resistant to the other classes of antibiotics, the only therapeutic solution being the vancomycin treatment, its use should be limited solely to those cases when it is really necessary. Fortunately, no vancomycin resistant MRSA strains have been identified in our country, but this phenomenon should be kept under close surveillance. Keywords: MRSA, VANCOMYCIN, MINIMUM INHIBITORY CONCENTRATION, E-TEST
Analysis of antibiotic resistance pattern of *S. aureus* strains isolated from the orthopedics–traumatology section of “Sf. Spiridon” Clinical Emergency Hospital, Iași

Hip or knee prostheses, fractures fixation, ligament or sinew reconstruction and other procedures requiring the use of an implant are very frequently used in view of repositioning the fractured bone segments and of recovering the articular function. Unfortunately, all these procedures are accompanied by a variable risk of infection, depending on the type of surgical intervention, ranging from 0.8% for arthroplasties and 53% for open fractures (type III Gustillo) (1). Osteoarticular infections are difficult to treat, being associated with a recurrence risk of 10–20% cases, especially in case of multi-resistant pathogens, such as methicillin resistant *Staphylococcus aureus* (MRSA). The possibility to come across a microorganism with antibiotic multi-resistance is a major concern for the clinician, in full awareness that an infection triggered by such a strain leads to the increase of morbidity and mortality (2). The retrospective analysis of the antibiotic sensitivity of *S. aureus* strains isolated from the infected patients in the Orthopedics–Traumatology Clinic of “St. Spiridon” Clinical Emergency Hospital, Iași from January 2003 to December 2013, in view of determining the evolution trend of the resistance phenomenon and of pinpointing the most useful treatment for these strains.

**MATERIAL AND METHODS**

The retrospective study was made for the period 01.01.2003–31.12.2012, and the result of the diffusimetric antibiograms was taken from the hospital’s informatics system; the prospective component of the study implied the collection of pathological products from patients admitted during 01.01.2013–31.12.2013, for whom there was a suspicion of clinical infection, in compliance with the general norms of sampling products destined for the bacteriological exam. We excluded from the database the strains from the same bacterial species and with an identical phenotype of resistance to anti-infections chemo-therapeutic products as another isolated strain from the same patient during the same infectious episode. The strains were isolated and identified in the Microbiology Labs of “Sf. Spiridon” Hospital, Iași and of the Microbiology Discipline within “Grigore T. Popa” University of Medicine and Pharmacy, Iași. Antibiotic sensitivity testing was carried out using two methods: diffusimetric–Kirby-Bauer and MIC determination by E-test (for strains isolated in 2013), and the interpretation of the sensitivity was conducted in a standardized manner, in compliance with the CLSI standard (Clinical and Laboratory Standards Institute) for antibiotic testing in force on that date (3).

**RESULTS**

From January 2003 to December 2013 a number of 555 strains of staphylococcus were identified, 495 belonging to the *S. aureus* species, the rest of 60 strains being coagulase-negative staphylococci (CoNS). The *S. aureus* strains isolated were tested for the following antibiotics: AMC (amoxicillin + clavulanic acid), OXA (oxacillin), RIF (rifampicin), CAM (chloramphenicol), TEI (teicoplanin), VAN (vancomycin), LNZ (linezolid), GEN (gentamicin), PFX (pemoxacin), LVX (levofloxacin), CIP (ciprofloxacin), CLI (clindamycin), MFX (moxifloxacin).

Sensitivity testing for β-lactams proved that during the 11 years of the study, the average value of the frequency of resistant strains was 41.59%±8.68 (tab.1).

The highest frequency of MRSA strains
was observed in 2012 (58.6%), followed by 2004 (50.7%). Even if in 2013 it dropped to 38.9%, the trend calculated for 2003-2013 is slightly rising ($y=0.7373x+37.167$) (fig. 1). Following rifampicin sensitivity testing of isolated strains of *S. aureus* the average of the frequency of resistant strains for the whole duration of the study was of 32.09±10, and the yearly distribution showed a peak in 2004 (50.7%), the trend calculated for 11 years being descending ($y=-2.1609x+44.093$) (fig. 2).

**Fig. 1.** Distribution of the frequency of MRSA strains on years of study

**Fig. 2.** Distribution of the frequency of rifampicin resistant *S. aureus* strains on years of study

Starting with 2004, the *S. aureus* strains were also tested for vancomycin and linezolid. Vancomycin sensitivity was 100% and for linezolid 100% during 2009-2013 and during 2004-2008 the percentage of resistant strains was extremely low (2004-
Analysis of antibiotic resistance pattern of *S. aureus* strains isolated from the orthopedics–traumatology section of “Sf. Spiridon” Clinical Emergency Hospital, Iași


*S.aureus* strains were tested, depending on the year, for both 2\(^{nd}\) generation quinolones, such as pefloxacin (2003-2006) and ciprofloxacin (2006-2013), and 3\(^{rd}\) generation ones, such as levofloxacin (2004-2011) and moxifloxacin (2009-2011). The average of resistant strain frequency can be seen in table I. Both for ciprofloxacin, and for levofloxacin, the tendency of identifying resistant strains, calculated for the duration of the study, was descending (y=-4.45x±51.713 for levofloxacin and y=-1.83x±34.491 for ciprofloxacin).

### TABLE I

**Distribution on years of the frequency of *S.aureus* strains resistant to various antibiotics**

<table>
<thead>
<tr>
<th>Year</th>
<th>No. of isolated strains</th>
<th>AMC</th>
<th>OXA</th>
<th>TEI</th>
<th>VAN</th>
<th>LNZ</th>
<th>GEN</th>
<th>CIP</th>
<th>PFX</th>
<th>LVX</th>
<th>MFX</th>
<th>CLI</th>
<th>RIF</th>
<th>CAM</th>
</tr>
</thead>
<tbody>
<tr>
<td>2003</td>
<td>55</td>
<td>52.7%</td>
<td>34.5%</td>
<td>3.6%</td>
<td>NT</td>
<td>NT</td>
<td>30.9%</td>
<td>NT</td>
<td>34.5%</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>32.7%</td>
<td>18.2%</td>
</tr>
<tr>
<td>2004</td>
<td>67</td>
<td>56.7%</td>
<td>50.7%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>3.0%</td>
<td>52.2%</td>
<td>NT</td>
<td>82.1%</td>
<td>55.2%</td>
<td>NT</td>
<td>NT</td>
<td>50.7%</td>
<td>50.7%</td>
</tr>
<tr>
<td>2005</td>
<td>61</td>
<td>62.3%</td>
<td>44.3%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>4.9%</td>
<td>45.9%</td>
<td>NT</td>
<td>80.3%</td>
<td>45.9%</td>
<td>NT</td>
<td>NT</td>
<td>44.3%</td>
<td>44.3%</td>
</tr>
<tr>
<td>2006</td>
<td>42</td>
<td>69.0%</td>
<td>35.7%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>7.1%</td>
<td>40.5%</td>
<td>42.9%</td>
<td>52.4%</td>
<td>35.7%</td>
<td>NT</td>
<td>NT</td>
<td>38.1%</td>
<td>NT</td>
</tr>
<tr>
<td>2007</td>
<td>59</td>
<td>35.6%</td>
<td>33.9%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>1.7%</td>
<td>27.1%</td>
<td>30.5%</td>
<td>NT</td>
<td>25.4%</td>
<td>NT</td>
<td>10.2%</td>
<td>30.5%</td>
<td>15.3%</td>
</tr>
<tr>
<td>2008</td>
<td>52</td>
<td>36.5%</td>
<td>36.5%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>1.9%</td>
<td>44.2%</td>
<td>21.2%</td>
<td>NT</td>
<td>23.1%</td>
<td>NT</td>
<td>5.8%</td>
<td>28.8%</td>
<td>NT</td>
</tr>
<tr>
<td>2009</td>
<td>40</td>
<td>30.0%</td>
<td>30.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>5.0%</td>
<td>25.0%</td>
<td>22.5%</td>
<td>NT</td>
<td>22.5%</td>
<td>22.5%</td>
<td>10.0%</td>
<td>17.5%</td>
<td>NT</td>
</tr>
<tr>
<td>2010</td>
<td>40</td>
<td>47.5%</td>
<td>47.5%</td>
<td>2.5%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>20.0%</td>
<td>20.0%</td>
<td>NT</td>
<td>17.5%</td>
<td>17.5%</td>
<td>12.5%</td>
<td>22.5%</td>
<td>NT</td>
</tr>
<tr>
<td>2011</td>
<td>32</td>
<td>50.0%</td>
<td>46.9%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>34.4%</td>
<td>28.1%</td>
<td>NT</td>
<td>28.1%</td>
<td>NT</td>
<td>18.8%</td>
<td>28.1%</td>
<td>NT</td>
</tr>
<tr>
<td>2012</td>
<td>29</td>
<td>NT</td>
<td>58.6%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>41.4%</td>
<td>17.2%</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>44.8%</td>
<td>27.6%</td>
<td>NT</td>
</tr>
<tr>
<td>2013</td>
<td>18</td>
<td>NT</td>
<td>38.9%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>0.0%</td>
<td>22.2%</td>
<td>27.8%</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>38.9%</td>
<td>22.2%</td>
<td>NT</td>
</tr>
</tbody>
</table>

The percentage of clindamycin resistant *S.aureus* strains was variable. Sensitivity testing for this antibiotic started in 2007 and continued until 2013. If, in the first years, the percentage of clindamycin resistant *S.aureus* strains was relatively low (5.8%-10.2%), one can see in figure 3 that during the last two years their frequency increased approximately four times (44.8%-2012 and 38.9%-2013), the rising trend of resistance to this antibiotic being obvious (fig. 3).

Multiple antibiotic resistance has registered an increased incidence starting with 1950. The antibiotic classes that were tested *S.aureus* strains were: β-lactams, aminoglycosides, fluoroquinolones, lincosamides, rifampicin, glycopeptides and oxazolidinones. Taking into consideration the fact that all tested strains were sensitive to glycopeptides, and oxazolidinone resistance was extremely low, cross resistance was analyzed only in the case of the first five classes of antibiotics. Out of the 495 isolated
*S. aureus* strains, 164 (33.13%) were completely sensitive to all tested antibiotics and 26 (5.25%) were only resistant to β-lactams. The other MRSA strains associated multiple resistances: 16 (3.23%) of them showed resistance to only one antibiotic class, mostly aminoglycosides (e.g. gentamicin) – 10 strains (2.02%); 103 strains (20.8%) associated resistance to two other antibiotic classes, the most frequently used being aminoglycosides and rifampicin – 92 strains (18.58%).

**Fig. 3.** Distribution of the frequency of clindamycin resistant *S. aureus* strains on years of study

**Fig. 4.** Distribution of frequency of MRSA strains according to the data from EARS-Net

Associated resistance to four antibiotic classes could be determined only starting with 2007, when the *S. aureus* strains began to be tested for clindamycin. 35 strains (7.07%) fell into this category, most of them associating resistance to lincosamides
Analysis of antibiotic resistance pattern of *S. aureus* strains isolated from the orthopedics–traumatology section of “Sf. Spiridon” Clinical Emergency Hospital, Iași

as well. We registered a number of 23 strains that were sensitive only to glycopeptides (4.64%).

For 18 strains of *S. aureus* isolated in 2013 we used the E-test to determine the MIC for vancomycin, which varied between 0.5 -2 mg/ml. Two strains whose MIC was of 0.5 mg/ml were sensitive to most tested antibiotic classes, including β-lactams, except for macrolides (erythromycin) and the strain whose MIC was of 2 mg/ml, was resistant to all tested antibiotic classes, except for glycopeptides and oxazolidinones. The other tested strains had a MIC for vancomycin equal to 1mg/ml.

**DISCUSSION**

The antibiotic sensitivity test showed high resistance levels to antimicrobial agents, with the presence of clinically and epidemiologically important phenotypes, among which the high incidence of MRSA strains (41.59%) stands out as well as the increased incidence of MDR phenotypes (31.11%). Methicillin resistant *Staphylococcus aureus* is the most important cause of infections associated to medical care round the world. In 2009, only six countries in Europe reported decreasing trends for MRSA. In 2012, although the problem seems to have been stabilized or even to have decreased in certain European countries, MRSA remains a public priority as the proportion is still over 25% in more than 1/3 of the countries (4). The latest data from EARS-Net (European Antimicrobial Resistance Surveillance Network) (2012) indicate for Romania and Portugal (the only countries in Europe with this level of resistance) a MRSA percentage of over 50% (Romania 53.9%, Portugal 53.8%).

According to our study, in 2012, the percentage of MRSA strains was of 58.6%, but in 2013 it fell under 40% (38.9%).

Upon analysis of the EARS-Net reports for 2003-2012, we assessed that: the highest frequency of MRSA was registered in 2004 (73%), followed by a marked decreased until 2007, namely 26%, in turn followed by a continuous increase until 2012, when it exceeded 50% (namely 53.9%). However, it seems that the trend calculated for 2003-2012 is slightly decreasing (y=-1,4976x+55.547) (fig 4).

By comparing the data supplied by EARS-Net and the data obtained by us, there is no statistically significant difference (t=-1,495, GL=9, p=0.169). With regard to the results concerning RIF resistance, except for 2009, when the frequencies were roughly equal (17.5% in our study and 15.6% - EARS-Net), in the other two years an increase of the incidence of RIF resistant strains was registered in our area, as compared to the data supplied by EARS-Net (2010 – 22.5% vs. 13.3%, 2011 – 28.1% vs. 7.9%).

For 2012, in Europe, the percentage of MRSA strains varied from 0.7% in Sweden to 53.9% (Romania). Except for Sweden, there are five other countries in Europe which show a percentage of SAMR strains ranging between 1.3 – 2.1%, most of them having a MRSA frequency between 10 – 25% (14 countries) (4). In addition, the same 2012 report supplies data according to which the RIF resistance for MRSA strains was of 5-7% and for MSSA (*Methicillin Sensitive S. aureus*) strains of only 0.6%. In our study, RIF resistance associated to MRSA strains was of 73.52% for the whole duration of 11 years of the study and of 47.06% for 2012, while for MSSA strains for the same period it was of 9.34%, except for 2012, when all strains were sensitive.
According to the same EARSS report (from 2013) regarding the antimicrobial resistance in Europe for 2012, the MRSA strains associated resistance to fluoroquinolones of 81% as compared to MSSA strains, with only 6%. In our study, in 2012, isolated MRSA strains associated a resistance to fluoroquinolones of 35.3% and in 2013 of 71.42%, while MSSA strains were completely sensitive to fluoroquinolones, both in 2012 and in 2013.

Although there are certain countries which militate for the use of other antibiotics than VAN, there isn’t sufficient evidence in the published randomized studies to indicate their superiority (5, 6). The data showing the efficiency of any antibiotic other than VAN in the treatment of serious infections caused by the strains whose MIC for vancomycin is situated in the “highly sensitive” area are even more limited. There is a study carried out by Soriano and col. which concluded that a MIC > 1mg/l to vancomycin through E-test is considered to be among the risk factors associated with a high mortality, but only in the case of patients who were treated empirically with vancomycin (7).

Similarly, Hidagat and col. reported that 12 out of 15 patients (80%) with MRSA determined infections, whose MIC for vancomycin was high and who failed to respond properly to the treatment with this drug, had good results when another antibiotic was used (8).

The guides for monitoring vancomycin therapy for 2009 of the Pharmacists’ Society in USA recommend alternative solutions for the treatment of MRSA infections, if MIC ≥ 2mg/l (9). On the other hand, more recent guides (2011) recommend for strains whose MIC ≥ 2mg/l, that the decisional factor in continuing vancomycin therapy should be the patient’s response, independently from MIC (10).

In conclusion, due to the fact that there are MRSA infections in a worrying percentage (53.9%) that are resistant to the other antibiotic classes, the only therapeutic solution being the vancomycin treatment, its use should be limited only to those cases when it is strictly necessary. Fortunately, in our countries vancomycin resistant MRSA strains haven’t been yet identified, but the constant surveillance of this phenomenon is a must.

REFERENCES

Analysis of antibiotic resistance pattern of *S. aureus* strains isolated from the orthopedics–traumatology section of “Sf. Spiridon” Clinical Emergency Hospital, Iași


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**NEWS**

**ANTIMICROBIAL ACTIVITY OF NOVEL 5-AMINOIMIDAZOLE-4-CARBOXAMIDRAZONES**

Ribeiro et al. developed a method to prepare a series of fifteen 5-aminoimidazole 4-carboxamidrazones, starting from 5-amino-4-cyanoformimidoyl imidazoles. When testing the antimicrobial activity of these novel amidrazones against *Candida* sp. (*C. albicans, C. krusei, C. parapsilosis*) and Gram positive (*Staphylococcus aureus*) and Gram negative (*Escherichia coli, Pseudomonas aeruginosa*) bacteria, all inhibited the growth of *Candida* sp and a subset of compounds displayed fair-moderate activity against *S. aureus* and *E. coli*. The most potent antifungal compounds were also tested against *Cryptococcus neoformans, Aspergillus fumigatus, Trichophyton rubrum, T. mentagrophytes* and *Microsporum gypseum*, showing good activity against *C. krusei* and *C. neoformans*, but less on filamentous fungi when compared to their activity on yeasts. (Ribeiro AI, Gabriel C, Cerqueira F et al. Synthesis and antimicrobial activity of novel 5-aminoimidazole-4-carboxamidrazones. *Bioorg Med Chem Lett.* 2014. pii: S0960-894X(14)00856-7. doi: 10.1016/j.bmcl.2014.08.025.)

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