

## TESTING THE SENSITIVITY OF *Staphylococcus aureus* ANTIBIOTICS

Marioara Nicoleta FILIMON<sup>\*</sup>, Aurica Breica BOROZAN<sup>\*\*</sup>, Smaranda Laura GOȚIA<sup>\*\*\*</sup>,  
Roxana POPESCU<sup>\*\*\*\*</sup>, Vasile Daniel GHERMAN<sup>\*\*\*\*\*</sup>

<sup>\*</sup> Western University of Timișoara, Faculty of Chemistry-Biology-Geography, Department of Biology, Timișoara, Romania

<sup>\*\*</sup> Banat University of Agricultural Sciences and Veterinary Medicine from Timișoara, Department of Horticulture, Timișoara, Romania

<sup>\*\*\*</sup> University of Medicine and Pharmacy "Victor Babeș" Timișoara, Department of Physiology, Timișoara, Romania

<sup>\*\*\*\*</sup> University of Medicine and Pharmacy "Victor Babeș" Timișoara, Cellular and Molecular Biology, Timișoara, Romania

<sup>\*\*\*\*\*</sup> University of Polytechnic, Timișoara, Faculty of Hydrotechnic, Department of Microbiology, Timișoara, Romania

Corresponding author: Marioara Nicoleta Filimon, Western University of Timișoara, Faculty of Chemistry-Biology-Geography, Department of Biology, 16 Pestalozzi, 300115 Timișoara, Romania, Tel.: 0040256592646, fax:0040256592622, e-mail: nicoleta\_filimon@yahoo.com

**Abstract.** This study has in view to establish and test the sensitivity of *Staphylococcus aureus* antibiotics. There are different injuries caused by superficial skin infections: from simple pimples to infections that endanger our lives, like an abscess, furuncle septicemia, meningitis, toxic food, urinary tract infection at sexually active young women. Samples have been taken from 30 people with staphylococcus infections. They were nineteen women and eleven men, between the age of 2 and 79. During this study some antibiograms have been made, based on pharyngeal exudates, acne secretion and urine culture. It has been established that the most efficient recommended antibiotics are: oxacilin, erythromycin, rifampicin and ciprofloxacin. The penicillin turned out to be less efficient to remove and destroy the *Staphylococcus aureus* species.

**Keywords:** antibiogram, antibiotics, *Staphylococcus aureus*

### INTRODUCTION

This study had in view to establish and test the sensitivity of *Staphylococcus aureus* bacteria, because of its multiple effect on skin, nasal lining, pharynx and the urine culture.

*Staphylococcus* can cause a wide range of infections. Their severity and location vary from superficial skin infections, to infections which endanger our lives like septicemia and meningitis. Mostly, the *Staphylococcus* produces penicillinase. Some people can even resist without any kind of problems to analogues of penicillin.

The three most common types of *Staphylococcus* are: *Staphylococcus aureus*, *Staphylococcus epidermidis* (resistance of the nasal vestibule and teguments, accidental pathogen, found in subacute bacterial endocarditis, followed by surgery and cardiovascular explorations at the level of the infected acne) and *Staphylococcus saprophyticus* (external environment saprophyte may contaminate the lining and teguments).

*Staphylococcus aureus* is a bacteria widely spread in the environment, like in air, dust and on the household items. Healthy people have it, in a percentage of 30% on their nasal lining. The percentage is even higher at people, who work in the hospital, at patients who need dialysis, those who have diabetes and those who use intravenous drugs.

*Staphylococcus aureus* is also a pathogen species and presents superficial skin infections from simple pimples to infections that endanger our lives, like an abscess, furuncle septicemia, meningitis, toxic food, urinary tract infection at sexually active young women. It can cause different angina, a bronchitis and lung infection which, in case of children, forms bronchopneumonia and is often fatale [1].

*Staphylococcus aureus* can be treated with a wide range of antibiotics. Efficient and inefficient antibiotics were enlisted. In specialized literature, we identified 10 groups of antibiotics: penicillin, oxacilin, gentamicin, tobromicin, tetracycline, erythromycin, clindamycin,

vancomycin, rifampicin, linezolid and ciprofloxacin. Taking into consideration the last years, branches of *Staphylococcus aureus* and *Staphylococcus epidermidis* have been developed. These are resistant to methicillin, to all kind of penicillin, to almost every cephalosporin, to streptomycin and tetracycline. Penicillin, represented by ampicillin and amoxicillin, is an efficient antibiotic from this species.

Oxacilin can be used as a mono therapy in the treatment of staphylococcus infections. Oxacilin might be useful in case of dangerous staphylococcus infections, endocarditis and septicemia in association with active aminoglycoside. Gentamicin and tobromicin have a large antimicrobial spectrum. The tetracycline is formed of Gram positive and Gram negative, aerobe and anaerobe bacteria. Brand new and resistant branches were selected. This is the reason why they are rarely used as antibiotics. Gram positive coccus are inhibited by small concentrations. The MIC for *Staphylococcus*, *Streptococcus* and *Pneumococcus* is 0.2 and 3.1 mcg/ml [18].

Patients who are allergic to penicillin can use eritromycin. These patients have slight infections of staphylococcus, streptococcus, pneumococcus, anthrax and syphilis. Recently, it has been discovered that the eritromycin can be used with success in vulgar acne therapy [18].

Clindamycin is useful in sever staphylococcus infections, like in case of staphylococcus infections resistant to G penicillin, but sensitive to clindamycin. Several branches of *Staphylococcus aureus* are inhibited at a concentration of 0.4 mcg/ml [7].

Vancomycin and teicoplanina have a major effect on staphylococcus infections resistant to methicillin. Linezolid is used in *Staphylococcus* infections (resistant to methicillin), pneumonia (caused by *Staphylococcus aureus*), communitary pneumonia, skin infections, soft tissue infections and bacterial endocarditis with sensitive germs [7].

*Staphylococcus* has a great sensitivity. For *Staphylococcus aureus* the MIC is 0.005-0.001 mcg/ml. The antibiotic is active also in the case of poli

resistant branches - *Staphylococcus* resistant to G penicillin and methicillin and/or vancomycin. It may have a synergistic reaction, when the penicillin is resistant to penicillinase and vancomycin [18].

Ciprofloxacin presents a high efficiency against bacteria resistant to aminoglycoside, penicillin, cephalosporine, tetracycline and other antibiotics. Infections of the middle ear (middle otitis) and paranasal sinuses (sinusitis) may appear, especially if these are generated by *Pseudomonas* and *Staphylococcus* [18].

The efficacy of chlorhexidine digluconate was determined against some strains of collected and clinically isolated bacteria and fungi. The efficacy was evaluated either by calculating a minimum inhibitory concentration (MIC). The MIC values of chlorhexidine for *Staphylococcus aureus*, *Microsporum gypseum*, *Microsporum canis* and *Trichophyton mentagrophytes* were 0.625 µg/ml, 12.5 µg/ml, 50 µg/ml and 6.25 µg/ml, respectively. The *in vitro* efficacy of chlorhexidine was higher against the strains of *Staphylococcus aureus* and *Pseudomonas aeruginosa* (0.5 mg/ml for 5 min and 0.5 mg/ml for 10 min) than against clinical isolates [15].

It is generally accepted that methicillin-resistant *Staphylococcus aureus* is also resistant to aminoglycoside antibiotics. We investigated trends of gentamicin and arbekacin susceptibilities and the prevalence of the genes encoding aminoglycoside-modifying enzymes (AMEs) for a total of 218 strains of MRSA isolated from blood specimens obtained from 1978 through 2002 in one hospital. The minimum inhibitory concentrations of gentamicin at which 50% of the strains that were inhibited (MIC<sub>50</sub>) are 0.5 µg/ml for the isolates obtained from 1990 to 2002. The MIC<sub>90</sub> of gentamicin was consistently ≥128 µg/ml. Our results imply that gentamicin-resistant and arbekacin-resistant MRSA's have consistently decreased for the past 25 years [3].

## MATERIALS AND METHODS

Thirty persons with staphylococcus infections have been studied, 19 women and 11 men, between the age of 2 and 79. Patients have been examined. Nasal and pharynx secretion, acne secretion and urine culture has been collected. It's compulsory to establish the sensitivity of antibiotics and chemotherapy of every bacterial branch in order to determine the right treatment.

Usually, a branch can be considered sensitive if the germs are efficiently affected by the antibiotic. The therapeutic effect may be obtained with the help of "usual" doses and administration. The branch may be considered slightly sensitive if the germs are little affected. The therapeutic effect cannot be obtained but in special conditions (like, to recommend a higher doses than as usual, the use of some special administration: intravenous injection and intraradicular injection) [16].

Bacteria sensitivity towards antibiotics is tested "in vitro", assuring them standard culture condition (culture environment, inoculum and incubation time) in

the presence of a smaller quantity of antibiotic. Basically, a branch is resistant if the result of the sensitivity tested "in vitro" is negative.

The antibiogram can be made by a test "in vitro." It represents the lab method, showing the antibiotic sensitivity of germs, collected from patients with bacterial infections. These are cultivated in special environments (for instance on the Mueller-Hinton agar). We have to use pure cultures for antibiograms (a single bacterial branch), even in case of multibacterial infections.

The isolation of *Staphylococcus aureus* is based on its growth in a simple environment and in a hypercholesterol environment. On the agarose, the blood is developed in colonies of 2-3 mm. The colonies have a creamy consistence. Usually, it contains β-hemolysis [19].

We submitted a disk with antibiotics, on the surface of a solid environment, planted with a bacterial culture. The active antimicrobial substance will run into the environment, having a small concentration, started from the edge of the microtablet.

After a certain time of incubation, there are two different ways to follow: the first one, because here the microbial growth is inhibited by a concentration of antimicrobial substance. Second, a growing area, where the antibiotic concentration is too small for a growing inhibition.

If the diameter of the inhibitive area is bigger, the germ is more sensitive. The quantity of the antibiotic necessary to the inhibition of the tested bacteria (minimum inhibited concentration=MIC), is smaller. There is an inversely proportional relation between the diameter of the inhibited area and MIC [10, 11].

## RESULTS

*Staphylococcus* frequently produces infections which can be treated only in hospital. This is the reason why it is recommended to test their sensitivity to antibiotics for a right treatment.

In this order, there are: pharynx and nasal perspire, acne secretion and urine culture. Then, the so-called antibiograms. As concerning the pharynx perspire, a group of 10 people were tested: 4 men and 6 women with ages between 20 and 49. This test was made on 7 children (4 girls and 3 boys) between the ages of 2 and 14.

The nasal perspires has been made on a group of 10 people (4 men and 6 women), between the age of 18 and 52. Only one nasal perspire has been made on children. In order to test the resistance of *Staphylococcus aureus* on antibiotics, the acne secretion has been made on a single woman (aged 25). The urine culture, has been made on a single woman, too (aged 79).

In the case of antibiograms, the following antibiotics have been used: penicillin, oxacilin, gentamicin, tobramycin, tetracycline, erythromycin, clindamycin, vancomycin, rifampicin, linezolid and ciprofloxacin.

The concentration / microtablets of the antibiotics were already known. After the antibiograms were made

(based on the above mentioned method), and after the inhibition was established, meaning the minimum inhibitive concentration (MIC), the obtained results were statistically processed, permitting to achieve the following figure.

Using the statistical tests, we established 4 most efficient antibiotics: oxacilin, erythromycin, rifampicin and ciprofloxacin. These antibiotics may be recommended and used in different kind of disease caused by *Staphylococcus aureus* (Fig. 1). Different kind of disease caused by *Staphylococcus aureus* may be treated and used with other antibiotics, like the linezolid (Fig. 2).

As concerning the tested antibiotics, we established that penicillin is less efficient (63.33% cases) in order to treat *Staphylococcus aureus* (Fig. 3.).

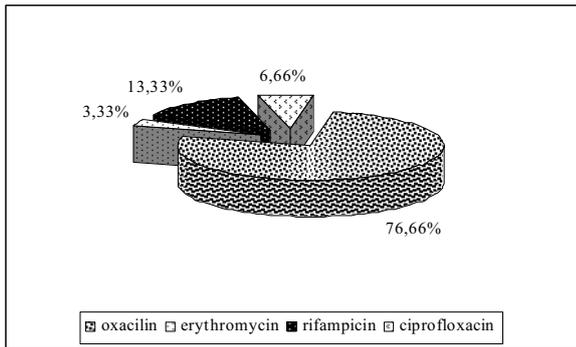


Figure 1. The most efficient antibiotic used in the case of *Staphylococcus aureus*.

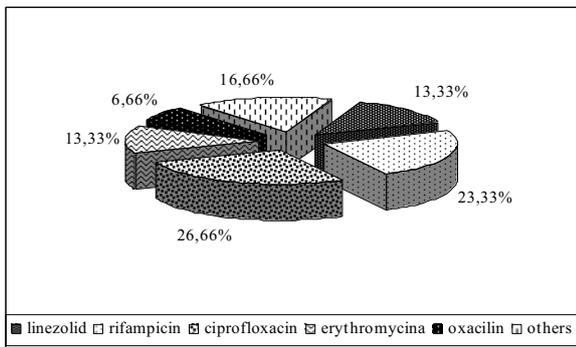


Figure 2. Other antibiotics which can be used in the case of *Staphylococcus aureus*.

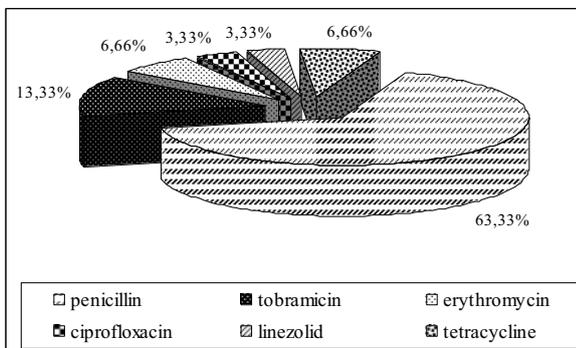


Figure 3. Inefficient antibiotics in the case of *Staphylococcus aureus*.

Depending on every person, on penicillin and on every person's clinical picture, there are other inefficient antibiotics, but on a lower rate. People had a certain clinical picture, a kind of specificity in disease

initiated by *Staphylococcus aureus*. This is the reason why some antibiotics appear in both groups, as efficient and inefficient, but of course, on a lower rate.

## DISCUSSIONS

Infections caused by methicillin-resistant strains may be more difficult to manage or more expensive to treat, because vancomycin is inherently less efficacious. The increasing prevalence of MRSA will inevitably increase vancomycin use, adding further to the problem of antibiotic-resistant gram-positive bacteria [8].

Antimicrobial resistance to penicillin, methicillin, or vancomycin is an unavoidable consequence of the selective pressure of antibiotic exposure. Although the details of the epidemiology of staphylococcal drug resistance may change, the fundamental forces driving it are similar. The question is not whether resistance will occur, but how prevalent resistance will become. Minimizing the antibiotic pressure that favors the selection of resistant strains is essential to controlling the emergence of these strains in the hospital and the community, regardless of their origins [17].

Resistance to these antibiotics has also led to the use of new, broad-spectrum anti-Gram positive antibiotics such as linezolid because of its availability as an oral drug [4].

Vancomycin-resistant *S. aureus* (VRSA) is a strain of *S. aureus* that has become resistant to the glycopeptides. The first case of vancomycin-intermediate *S. aureus* (VISA) was reported in Japan in 1996 [13]; but the first case of *S. aureus* truly resistant to glycopeptide antibiotics was only reported in 2002 [6]. Three cases of VRSA infection have been reported in the United States as of 2005[14].

The treatment of choice for *S. aureus* infection is penicillin; but in most countries, penicillin-resistance is extremely common and first-line therapy is most commonly a penicillinase-resistant penicillin (for example, oxacilin or flucloxacillin). Combination therapy with gentamicin may be used to treat serious infections like endocarditis, but its use is controversial because of the high risk of damage to the kidneys. The duration of treatment depends on the site of infection and on severity [2].

Antibiotic resistance in *S. aureus* was almost unknown when penicillin was first introduced in 1943 observed the antibacterial activity of the penicillium mould was growing a culture of *S. aureus*. By 1950, 40% of hospital *S. aureus* isolates were penicillin resistant; and by 1960, this had risen to 80% [5].

Patients with affections determined by *Staphylococcus aureus* and which are under standard therapy with antistaphylococcal penicillin or vancomycin, were administered initial low dose gentamicin, in order to increase the treatment's efficiency. By studying the the administration of a standard dose of gentamicin as a part of therapy for *Staphylococcus aureus* bacteremia, but also for native valve infective endocarditis, is proven that it is nephrotoxic and should not be used routinely [9,12].

Testing the sensitivity of *Staphylococcus aureus*, on different kind of antibiotics, is very important as concerning the clinical picture of the disease generated by this pathogen agent. With the help of these tests, we had in view to ameliorate the health estate, especially for the doctors to recommend the most efficient treatment in case of this disease.

Tested antibiotics of *Staphylococcus aureus* show that the most efficient antibiotic is oxacilin (76,66%), followed by rifampicin and ciprofloxacin.

Other efficient antibiotics in the treatment of *Staphylococcus aureus* are eritromycin and linezolid.

The most efficient antibiotic in the treatment of disease caused by *Staphylococcus aureus* has proved to be the penicillin (63.33%).

## REFERENCES

- [1] Andreoli, T.E., Bennet J.C., Carpenter C.C.J., Plum, F., (1999): Cecil Esentialul în Medicină. M.A.S.T. Publishing House, Bucharest, 998 p.
- [2] Bayer, A.S., Bolger, A.F., Taubert, K.A., (1998): Diagnosis and management of infective endocarditis and its complications. *Circulation*, 98(25): 2936-2948.
- [3] Barada, K., Hanaki, H., Ikeda, S., Yamaguchi, Y., Akama, H., Nakae, T., Inamatsu, T., Sunakawa, K. (2007): Trends in the gentamicin and arbekacin susceptibility of methicillin-resistant *Staphylococcus aureus* and the genes encoding aminoglycoside-modifying enzymes. *Journal of Infection and Chemotherapy*, 13(2): 74-78.
- [4] Blot, S.I., Vandewoude, K.H., Hoste, E.A., Colardyn, F.A., (2002): Outcome and attributable mortality in critically ill patients with bacteremia involving methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. *Arch. Intern. Med.*, 162(19): 2229-2235.
- [5] Chambers, H.F., (2001): The changing epidemic-cology of *Staphylococcus aureus*?. *Emerg. Infect. Dis.*, 7(2): 178-182.
- [6] Chang, S., Sievert, D.M., Hageman, J.C., Boulton, M.L., Tenover, F.C., Downes, F.P., Shah, S., Rudrik, J.T., Pupp, G.R., Brown, W.J., Cardo, D., Fridkin, S.K., (2003): Infection with vancomycin-resistant *Staphylococcus aureus* containing the van A resistance gene. *N. Engl. J. Med.*, 348(14): 1342-1347.
- [7] Cincea, R., Popovici, M., Dumitrașcu, V., Ana, D., Chevereșan, A., Ana, I., Șipos, S., Șuta, N., Malița, I., Barac, B., Lengyel, D. M. (2006): *Curs de Farmacologie*. Ediția a II-a, Mirton Publishing House, Timișoara, 279 p..
- [8] Conterno, L.O., Wey, S.B., Castelo, A., (1998): Risk factors for mortality in *Staphylococcus aureus* bacteremia. *Infect. Control. Hosp. Epidemiol.*, 19: 32-37.
- [9] Cosgrove, S.E., Vigiiani, G.A., Campion, M. (2009): Initial low dose gentamicin for *Staphylococcus aureus* bacteremia and endocarditis is nephrotoxic. *Clin. Infect. Dis.*, 48(6): 713-721.
- [10] Drăgan-Bularda, M., (2000): *Microbiologie Generală Pentru Uzul Studenților*. University Press, Cluj Napoca, pp. 115-123.
- [11] Dunca, S., Nimițan, E., Ailiesei, O., Ștefan, M., (2004): *Microbiologie Aplicată*. Demiurg Publishing House, Iași, 263 p..
- [12] Gentry, C.A., Rodvold, K.A., Novak, R.M., Hershov, R.C., Naderer, O.J., (1997): Retrospective evaluation of therapies for *Staphylococcus aureus* endocarditis. *Pharmacothe-rapy*, 17: 990-997.
- [13] Hiramatsu, K., Hanaki, H., Ino, T., Yabuta, K., Oguri, T., Tenover, F.C., (1997): PDF methicillin-resistant *Staphylococcus aureus* clinical strain with reduced vancomycin susceptibility. *J Antimicrob Chemother*, 40(1): 135-136.
- [14] Menichetti, F., (2005): Current and emerging serious Gram-positive infections. *Clin. Microbiol. Infect.*, 11 Suppl. 3: 22-28.
- [15] Odore, R., Colombatti-Valle, V., Re, G., (2000): Efficacy of Chlorhexidine against Some Strains of Cultured and Clinically Isolated Microorganisms. *Veterinary Research Communications*, 24(4): 229-238.
- [16] Popa, M.I., (2004): *Diagnostic de Laborator în Microbiologie*. Medicală Press, Bucharest, 244 p.
- [17] Soriano, A., Martinez, J.A., Mensa, J., Marco, F., Almela, M., Moreno-Martinez, A., (2000): Pathogenic significance of methicillin resistance for patients with *Staphylococcus aureus* bacteremia. *Clin. Infect. Dis.*, 30: 368-73.
- [18] Stroescu, V., (2002): *Farmacologie*. Ediția a V-a, ALL Publishing House, Bucharest, 440 p.
- [19] Vaida, T., (2002): *Diagnostic microbiologic și imunologic în laboratorul clinic*. University of Oradea Press, pp. 118-123.