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STUDY ON *STAPHYLOCOCCUS AUREUS* INFECTION METHICILLIN-RESISTANT (MRSA)

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Key words: *Staphylococcus aureus, MRSA identification, MRSA colonization, antibiotic sensitivity*

INTRODUCTION

Antibacterial treatment regimens are constantly changing, this having a major contribution to changing the sensitivity of the bacterial flora to the chemotherapeutics used.

Methicillin resistance of *Staphylococcus aureus* is associated with other resistance mechanisms, which lead to the inactivation of other families of antibiotics.

Experts say that superbugs are common bacteria that, at some point, suffer from unexplained genetic mutations, thus becoming resistant to antibiotics. Perhaps the best known example is methicillin-resistant *Staphylococcus aureus* (MRSA), which no longer responds to treatment with one of the most powerful antibiotics in existence, methicillin.

Bacteria are highly adaptable and have a "danger detection" mechanism, which causes them to undergo transformations as soon as they have been exposed to drug treatment. Most bacteria are destroyed by the concentrated attack of antibiotics, but over time, they show remarkable versatility and begin to oppose this attack.

If there is a single bacterium that has gained resistance, it will spread rapidly and pass this immunity on to future generations. Over time, ineffective bacteria disappear and only those that have learned to withstand the drug assault remain. Basically, doctors are completely disarmed against such enemies. These bacteria enter the bloodstream or the cerebrospinal fluid and, once here, release huge amounts of toxins, which alarm the patient's immune system. When asked to the maximum, the immune system thus attacked, triggers inflammations that can lead to the cessation of the functioning of certain internal organs and, finally, to death.

MATERIAL AND METHODS

This paper is based on statistical analyzes and processing that were performed between July 2019 and March 2020. A retrospective descriptive study was performed in the previously mentioned period, within the medical analysis laboratory SC. HYRON ARS MEDICA S.R.L. from Bacău. The following data were taken from the records made in the Rilab software: name and surname, age, type of analysis requested and also information on pre-existing conditions and treatments recommended by the doctor were taken.

The study was performed in patients who were explained the purpose and protocol of the study and who gave their consent to participate. The study group included 1304 patients. Of these, 320 patients were infected with Staphylococcus spp., Aged between 1 month and 83 years, of which 285 patients were under 20 years of age at the time of the study.

The working methods used were:

- **Bacteriological examination of pharyngeal exudate** (Fig. 1).The sample is inoculated under the hood on Columbia agar with 5% ram blood, by resuming the buffer on one quadrant of the Petri dish and then depleting the inoculum with the bacteriological loop on the other 3 quadrants.

- **Bacteriological examination of nasal exudate**. The microbiological analysis of the nasal secretions aimed to detect the passage of *Staphyloccocus aureus* and *Streptoccocus pyogenes*.

- **STAPH LATEX KIT.** It is a card agglutination test, which allows to differentiate *S. aureus* possessing clumping factor, protein A and capsular polysaccharide specific to methicillin-resistant strains, from other *Staphylococcus* that do not possess them (Fig. 2).

- **Catalase testing**. Catalase is a hemoprotein that breaks down hydrogen peroxide into water and oxygen. The bacteria that produce it protect themselves from the lethal effects of hydrogen peroxide that appeared as the final metabolite in the aerobic metabolism of carbohydrates. Testing is done by suspending the culture on the slide. Positive test: appearance of gas bubbles until effervescence (Fig. 3).

MRSA identification

- On the stained Gram smear from the culture on blood-agar or other non-selective medium, *Staphylococcus aureus* will appear in the form of Gram-positive shells, available in bunches of grapes;

Fig.1. Harvesting buffers without medium, with transport medium and barcode labeled

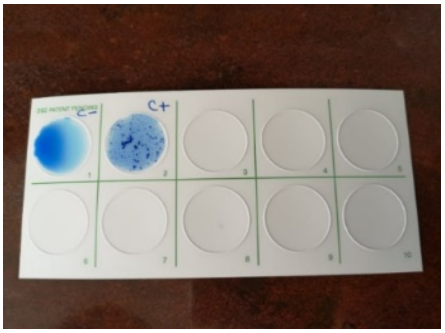


Fig. 2. Negative control (C-) and positive control (C +) STAPH LATEX KIT

Fig. 3. Catalase test

- Catalase test is positive (effervescence on contact with a drop of dilute perhydrol);
- The hemolysis test will show total hemolysis on agar-blood media (Fig. 4);
- The coagulase test is positive (free - the enzyme released in the environment acts on the coagulation or bound factors);
- To the test on the slide, on a drop of distilled water in which a colony of staphylococci is suspended (the suspension must be very dense and uniform), a drop of undiluted, fresh plasma is added. In case of a positive reaction, agglutination occurs in 15-20 seconds. In case of a negative reaction, after 2-3 minutes the absence of agglutination is found and the result must be confirmed by the tube test;
- In the tube test, mix 0.5 ml of oxalate plasma with 0.1 ml of culture for 16-20 hours in broth. Store at 37 ° C and examine at the following intervals: 30 minutes, 60 minutes, 4 hours for coagulation. In the case of a positive reaction, coagulation occurs, regardless of its degree (mass or vein of fibrin). In case of a negative reaction, there is no clot;
- Pathogenic *Staphylococcus* are known to secrete fibrinolysin, which lyses the coagulase-forming plasma clot. The fibrinolysin test highlights the presence of the enzyme, by seeding the staphylococcal strain in an environment with 7 parts nutritious agar and one part oxalated plasma. Incubate for 12-18 hours at 37 ° C. A clear area will be observed around the staphylococcal colonies, due to the melting of the plasma clot produced by the coagulase.

These tests can be performed in different variants, but it is recommended that each laboratory use a reproducible and validated working methodology. If, in rare cases, there are discordant results, of epidemiological interest, it is recommended to send the strain to a reference laboratory.

Colonization with MRSA ("toll") and decontamination

A percentage of at least 20-50% of the population is colonized, at some point, with *Staphylococcus aureus*, some of these colonizations being determined by MRSA strains.

The distinction between MRSA colonization and MRSA infection is relatively simple and can be done as follows:

- In the case of colonization (portage), the tolerance of the human body to the presence of bacteria is highlighted, in the absence of symptoms and biological manifestations of inflammatory reaction.
- In case of infection, there are clinical manifestations - local and / or systemic - caused by the aggressiveness of MRSA and the

reaction of the human body to remove these bacteria.

The purpose of MRSA colonization testing is to:

- identification of persons requiring specific decontamination for MRSA prior to surgery, for example, which may facilitate the occurrence of an MRSA infection;
- isolation of MRSA patients in a hospital in order to reduce the risk of contamination of other patients (directly or through medical staff).

The indication for MRSA toll testing is selective and not generalized and is addressed to:

- patients who are at increased risk of being colonized with MRSA, this category includes:

- patients previously identified as carriers of MRSA;
- patients who may be colonized following contact with other patients with MRSA;
- patients who are prosthetic with medical devices that communicate with the external environment (catheters - central venous, urinary -, digestive tract, etc.);
- dialysis patients.

- patients who once colonized (regardless of the colonized organ) have an increased risk of developing MRSA infections:

- patients with skin lesions: ulcers, wounds, exfoliative dermatitis;
- patients admitted to the ICU, hematology-oncology, transplant, etc .;
- patients who will undergo surgery such as: orthopedic implant, cardiac, thoracic, vascular intervention, etc.

Antibiotic susceptibility testing

The methicillin-resistant or methicillin-sensitive character of a *Staphylococcus aureus* strain is highlighted according to the diameter of the inhibition zone created by the cefoxitin disc (30μg), according to EUCAST:

- Methicillin-sensitive *Staphylococcus aureus* (MSSA) if the diameter is at least 22 mm (Fig. 5);
- Methicillin-resistant *Staphylococcus aureus* (MRSA) if the diameter is less than 22 mm (Fig. 6).

In the case of MRSA strains, other useful tests would be: trimethoprim / sulfamethoxazole (25 mcg), erythromycin (15 mcg), clindamycin (2 mcg), rifampicin (5 mcg), levofloxacin (5 mcg), tetracycline (30 mcg), doxycycline (only for tetracycline-resistant strains and required by MIC), gentamicin (10 mcg), linezolid (10 mcg), nitrofurantoin (100 mcg) (urine isolates only), vancomycin (required by MIC). In the case of micro-tablet testing, only those with the mentioned concentrations are used.

Fig. 4. Hemolysis on blood agar medium in *Staphylococcus aureus*

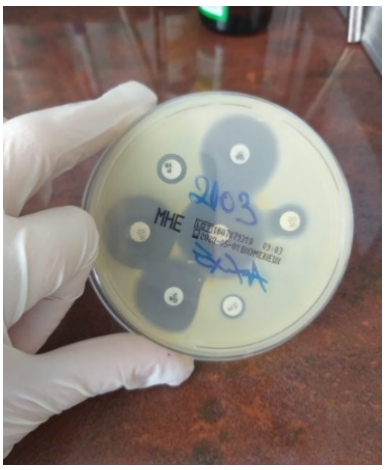


Fig. 5. Strain of *Staphyloccocus aureus* MSSA isolated from pharyngeal exudate

Fig. 6. Stem of *Stapyloccocus aureus* MRSA isolated from the secretion of a postoperative wound

In the case of macrolides and lincosamides, the inducible resistance phenotypes of the double-disc test (test D) will also be monitored: the erythromycin and clindamycin discs are placed at a distance of 12-20 mm measured from the edges of the discs. If the area of inhibition around the clindamycin disc is flattened (positive D test) clindamycin is reported RESISTANT, although the diameter of the area of inhibition is in the sensitivity range (alternative: it is communicated as it results from direct reading, ie clindamycin - sensitive, but a comment is added: there is a risk of therapeutic failure due to resistance during longer treatment with clindamycin).

RESULTS AND DISCUSSIONS

As can be seen in Chart 1, the addressability of patients who requested medical tests that involved sowing on culture media of pathological products, harvested from different areas is 1304 patients. Of these, 984 patients (75%) were not infected and only 320 patients (25%) were confirmed as carriers of *Staphylococcus aureus*.

By performing a monthly statistic (Chart 2) of the period in which this study was conducted, an increased incidence of *Staphylococcus aureus* can be observed in the first 2 months of autumn (September, October), which coincides with the beginning of the year. school. Thus, we can say that school-age people are more susceptible to staph infections. One of the reasons can be considered the poor development of immunity in children and adolescents, possibly also against the background of an increasingly chaotic and unhealthy diet, as well as an inappropriate lifestyle in the case of young adolescents.

Most staphylococcal strains of *S. aureus* species were isolated in females, 278 patients (87%), compared to the male population, which recorded only 42 patients (13%) of the entire group examined (Chart 3).

In terms of age (Chart 4), the highest rate was recorded in patients aged 0-10 years (43%) and between 11-20 years (35%).

There is also a much higher incidence of the presence of *Staphylococcus aureus* in the urban population, 252 patients (79%), compared to the rural population with 68 patients (21%), this upward trend being constantly increasing recently (Chart 5) . This fact can be attributed to the existence of more favorable factors in the urban environment, but probably also to the high degree of addressability of the population to health facilities. It is known that a significant share of *S. aureus* infections comes from the hospital environment, following nosocomial infections.

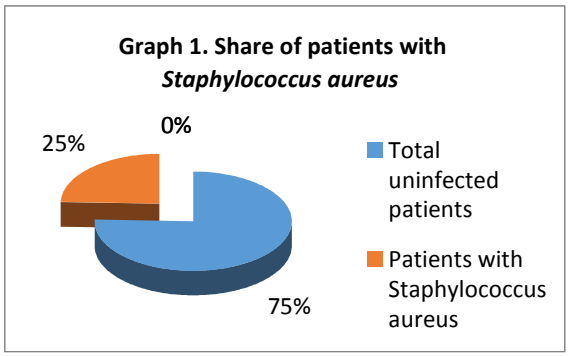
Regarding the location of CA-MRSA (Ghart 6), it is observed that, out of the group of patients studied, 285 (89%) have as an infection point, the skin while other organs were infected in only 35 patients (11%).

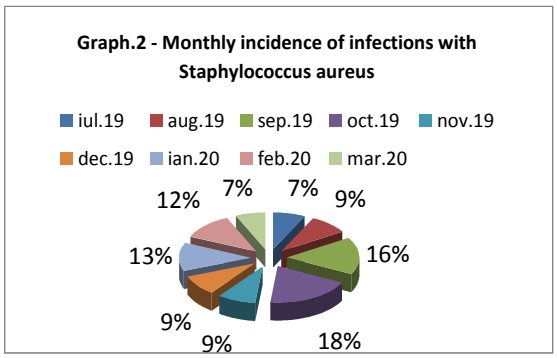
Drawing a parallel between the 127 patients (47%) with strains of MSSA (*Staphylococcus aureus* methicillin-sensitive) and 193 patients (53%) with strains MRSA (*Staphylococcus aureus* methicillin-resistant), a higher incidence is observed in the second case, although the share is somewhat close (Chart 7).

Regarding the correlation between the pathological product harvested and the type of *S. aureus* strain, be it MRSA or MSSA, it can be seen in Chart 8 that the largest share was nasal exudate - 90 patients (28%), pharyngeal exudate - 180 patients (56%) and conjunctival secretions - 26 patients (8%). In other cases, the rate is low: otic secretions - 10 patients (3%), wound secretions - 14 patients (5%).

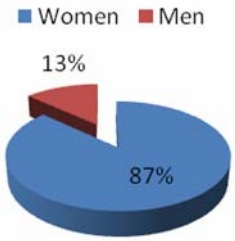
If we were to refer strictly to the type of *S. aureus* strain, as noted in Graphs 9 and 10, the total share is higher in the case of MRSA, but there are differences between the type of biological product harvested.

Depending on a number of risk factors, either local or general, the etiological spectrum of *Staphylococcus* differs relatively widely from case to case. The identification of a possible gateway or pre-existing associated disease may provide us with preliminary data to identify the type of *Staphylococcus* aureus involved.

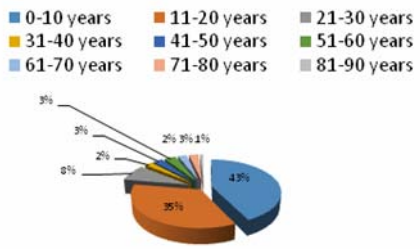




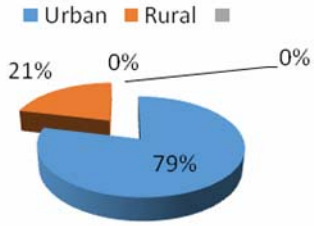
Graph 3. Share of *Staphylococcus aureus* infections by sex



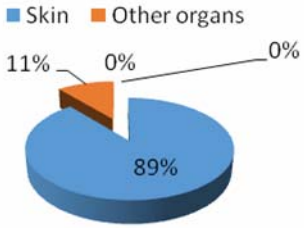
Graph.4- Share of Staphylococcus aureus strain by age



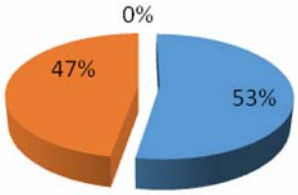
Graph 5. Share of *Staphylococcus aureus* strains according to the environment of origin



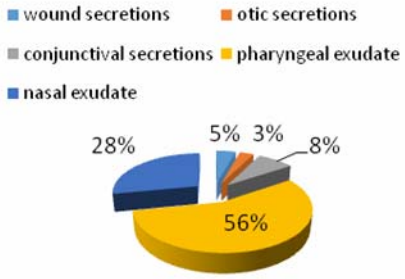
Graph 6. Location of *Staphylococcus aureus* infections



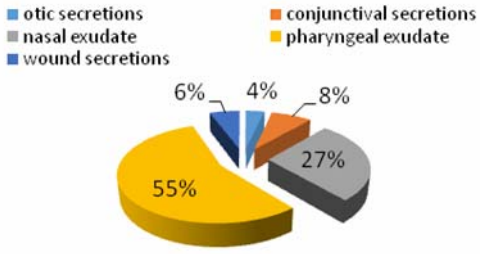
Graph 7. Share of *Staphylococcus aureus* strains by SENSITIVITY / RESISTANCE



Graph 8. Weight of *Staphylococcus aureus* stems according to the pathological product



Graph 9. Share of methicillin-resistant *Staphylococcus aureus* strains on the type of pathological product analyzed wound secretions



Graph 10. Share of methicillin-sensitive *Staphylococcus aureus* strains on the type of pathological product analyzed wound secretions

CONCLUSIONS

The group of 1304 patients included in this study conducted during 9 months, between July 2019 and March 2020, can be considered statistically significant, due to the variation of the selection made.

This statistic was addressed to all age categories, sex, living environment, type of pathological product harvested and took into account different strains of *Staphylococcus aureus*, various locations, risk factors and evolution.

Based on the statistical analysis performed on the group of selected patients, there were statistically significant correlations regarding the association between the diversified categories of patients. It is observed that staphylococcal pathology may have a reserved prognosis.

There has been a significant increase in the percentage of patients in whom *Staphylococcus aureus* manages to enter, infect and spread in the body, despite the body's own defense mechanisms. An important role in this regard is played by nosocomial infections, which are among the main risk factors in terms of the share of infections.

It is possible that in the not too distant future there will be an increasing number of cases, due to the increase in the number of nosocomial infections, especially in the surgical wards, where the highest inducible resistance to clindamycin is noted.

The nasal passage of methicillin-resistant *Staphylococcus aureus* can benefit from decontamination by the use of bacteriophages, a conclusion that can be taken into account especially in intensive care units, to reduce the nasal passage of MRSA encountered in health care staff.

Antimicrobial therapy still has a high degree of subjectivism, in the absence of treatment protocols. However, in recent years, there has been a reshaping of the therapeutic approach, in terms of closer interdisciplinary collaboration.

Further research is needed to elucidate the phenomena of heterogeneous resistance of *Staphylococcus aureus* to methicillin.

ABSTRACT

This paper, which proposed a study on methicillin-resistant *Staphylococcus aureus* (MRSA) infection, is based on analyzes and statistical processing that were performed between July 2019 and March 2020. A retrospective descriptive study was performed in the laboratory of SC medical analysis. HYRON ARS MEDICA S.R.L. from Bacău. The following data were taken from the records made in the Rilab software: name and surname, age, type of analysis requested and also information on pre-existing conditions and treatments recommended by the doctor were taken. The study was performed in patients who were explained the purpose and protocol of the study and who gave their consent to participate. The study group included 1304 patients. Of these, 320 patients were infected with *Staphylococcus* spp., Aged between 1 month and 83 years, of which 285 patients were under 20 years of age at the time of the study. There has been a significant increase in the percentage of patients in whom *Staphylococcus aureus* manages to enter, infect and spread in the body, despite the body's own defense mechanisms. An important role in this regard is played by nosocomial infections, which are among the main risk factors in terms of the share of infections.

REFERENCES

1. BANNERMAN, T.L., 2003 - *Staphylococcus*, *Micrococcus*, and other catalase-positive cocci that grow aerobically. In: Murray, P. R., E. J. Baron, J.H. Jorgensen, M. A. Pfaller, and R. H. Yolken (ed.). Manual of clinical microbiology, 8th ed. American Society for Microbiology, Washington, D.C.
2. COLCIERU, M., JAKAB, E., POPESCU, O., 2010 - Identification of staphylococcal strains from blood cultures with specification of β-lactam resistance phenotypes using the automatic system VITEK® 2 COMPACT. Acta Medica Transilvanica 2 (2): 87-89 (Identificarea tulpinilor stafilococice din hemoculturi cu precizarea fenotipurilor de rezistenţă la β-lactami utilizând sistemul automat VITEK® 2 COMPACT. Acta Medica Transilvanica 2(2):87-89).
3. DAVIES S., 1997 - Detection of methicillin-resistant *Staphylococcus aureus*: the evaluation of rapid agglutination methods, Br. J. Biomed. Sci., 54:13-15.
4. ESSERTS I., and RADEBOLD K., 1980- Rapid and reliable identification of *Staphylococcus aureus* by a latex agglutination test. J. Clin. Microbiol., 641-643.
5. FOURNIER J.M., BOUTONNIER A., and BOUVET A., 1989 - *Staphylococcus aureus* strains which are not identified by rapid agglutination methods are of capsular serotype 5. J. Clin. Microbiol., 27:1372-1374.
6. FOURNIER J.M., BOUVET A., BOUTONNIER A., AUDURIER A., GOLDSTEIN F., PIERRE J., BURE A., LEBRUN L., and HOCHKEPPEL H.K., 1987 - Predominance of capsular polysaccharide type 5 among oxacillin-resistant *Staphylococcus aureus*. J. Clin. Microbiol., 25:1932-1934.
7. ITO, T., KATAYAMA, Y., HIRAMATSU, K., 1999 - Cloning and sequence determination of the entire mec DNA of pre-methicillin-resistant *Staphylococcus aureus*, N315. Antimicrobial Agents and Chemotherapy 43(6):1449-1458.
8. JAKAB, E., 2009 - Genetic mechanisms involved in the heterogeneous methicillin resistance of *Staphylococcus aureus*. Teză de doctorat,

Universitarea Babeş- Bolyai Cluj- Napoca, Facultatea de Biologie şi Geologie: 59.
9. JAKAB, E., POPESCU, O., 2005 - Direct identification of methicillin-resistant Staphylococcus aureus strains by amplification of mecA and nucA genes. Annals of SNBC 10: 331-335 (Identificarea directă a tulpinilor de *Staphylococcus aureus* rezistente la meticilină prin amplificarea genelor mecA şi nucA. Analele SNBC 10:331-335).
10. KLOOS W.E. AND LAMBE D.W., 1991 - *Staphylococcus* Manual of Clinical Microbiology, 5th Ed. Edited by Balows A., Hausler W.J., Herrmann K.L., Isenberg H.D., and Shadomy J.J. American Society for Microbiology, Washington, D.C., Pages 222-237.
11. KLOOS W.E., and SCLEIFER K.H., 1986 - *Staphylococcus* Bergeyís manual of systematic bacteriology. vol. 2, 1013-1035.
12. PRISECARU MARIA, IONUȚ STOICA, DUMITRA RĂDUCANU, 2015 - General microbiology, Alma Mater Bacau Publishing House, 2015, ISBN 978-606-527-466-2.Microbiologie generală, Ed. Alma Mater Bacau, ISBN 978-606-527-466-2.
13. RĂDUCANU DUMITRA, PRISECARU MARIA, 2016 - General microbiology: guide for practical works. – „Alma Mater" Publishing House Bacău, ISBN 978-606-527-532-4 (Microbiologie generală: ghid pentru lucrări practice. – Ed. Alma Mater Bacău, 2016, ISBN 978-606-527-532-4).
14. PRISECARU MARIA, IONUȚ STOICA, 2017 - Medical Microbiology, „Alma Mater" Publishing House Bacău, ISBN 978-606-527-541-6 (Microbiologie medicală, Ed. Alma Mater Bacău, 2017, ISBN 978-606-527-541-6).
15. PRISECARU MARIA, IONUȚ STOICA, 2017 - General and clinical immunology, „Alma Mater" Publishing House Bacău, ISBN 978-606-527-572-0 (Imunologie generală şi clinică, Ed. Alma Mater Bacău, 2017, ISBN 978-606-527-572-0).

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