THE FREQUENCY OF THE TYPES OF HEMOLYSIS AND THE FREQUENCY OF COAGULASE POSITIVE AND COAGULASE NEGATIVE STRAINS ON THE BAIRD-PARKER MEDIUM OF STAPHYLOCOCULUI AUREUS

Raluca POPOVICI 1#*, Corina BALDEA 1

University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea, Romania University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru St., 410048 Oradea, Romania

REVIEW, RESEARCH ARTICLE

Abstract

The chromogenic media are used mainly for the usual bacteriologic diagnosis, having as purpose the reducing of time and of the consumables used, by the classic methodology. The chromogenic media are used most frequently either for the diagnosis of S. aureus subsp. aureus, or for the differentiation of the resistant methicillin strains of S. aureus subsp. aureus (MRSA). Identification is made based on the morphotinctorial, cultural characteristics and of some tests of pathogenicity. For the epidemiologic purpose, the diagnosis is completed with phage types. On the smears of cultures you can see gram positive cocci with characteristic establishment, in bulk. On agar blood S. aureus produces characteristic colonies easily recognizable. They are colonies of S type, large, with diameter of 1-3 mm, round, bulged, smooth, with regular margins, hemolytic and pigmented. The young colonies are colorless. The pigmentation of the mature colonies is made in the presence of oxygen and at the room temperature. The most frequent pigment is the gold one (80%), followed by the white pigment and very rarely the citrine one

Keywords: colonies, cultural, morphotinctorial

#Correspondingauthor:<u>rugeraluca@yahoo.com</u>,corina68a@yahoo.com

INTRODUCTION

Staphylococcus aureus colonizes usually the skin and the mucous. It is present in 20-40% of the healthy persons in the nasal vestibule and in the medical staff in proportion of 90%. Staphylococcus aureus is a germ especially resistant, for this reason it disseminates fast in the hospital medium. It resists over 30 minutes at 60°C. It crosses the digestive tube, being present alive in the excrements. In the dry biological products and in dust the staphylococci can be isolated even after a few months.

They are Gram positive cocci with diameter of 0,5-1,5µm, placed in bulk, immobile, aerobe, facultative anaerobe, catalase positive, non sporulating, usually not encapsulated, mesophylls (are developed between 10-42°C), with optimum development on 37°C. They grow on usual media, on which they form round, convex colonies, pigmented with yellow-gold. On agar blood they produce a complete hemolysis.

They tolerate concentrations of over 5% NaCl, and some species are even halophiles (they tolerate concentrations of 10-15% NaCl).

The cellular wall of S. aureus is made of the basal layer of murein, characteristic to the gram positive bacteria, to which are connected on the exterior the teichoic acids. The majority of S. aureus strains have on their surface an enzyme, connected with the cellular wall, named "clumping factor" connected or coagulase that transforms the fibrinogen in fibrin. You should not mistake it for "free coagulase", secreted in the exterior of the bacterial cell and which is characteristic to the S. aureus species. Through the clumping factor S. aureus is fixed to the fibrinogen from the injured tissues, of the medical implants, and of the catheters on which it was deposited before fibrinogen. On the main strains of S. aureus, peptidoglycan is covered by the protein A. This has the property to connect in a nonspecific way the antibodies by the Fc fragment, which confers the staphylococci anti opsonization properties.

MATERIAL AND METHODS

For the performing of the study we used also the archive, registered in the specific program of the computer from the laboratory of S.C. Diaser, Oradea, in the computerized data base of the unit, respectively.

Necessary materials for the performing of the examination:

• A recipient of collection (collection recipient with collecting spoon) with transport medium

- Wooden spatula
- Latex gloves

For the collection of fecal matter it has to be collected a sample of fecal matter of 5-10g introduced in the collection recipient of fecal matter with transport medium. If the stool is liquid, it will be collected 5 ml. It is recommended to be chosen a liquid, mucous and bloody portion, if there is one. Don't collect quantities larger than 10g because it will reduce the chances of isolating the pathogen bacteria.

RESULTS AND DISCUSSIONS

Staphylococcus aureus secrets in the medium a series of enzymes and toxins,

responsible in part for the clinical manifestations of staphylococci infections.

Coagulase is the marker of virulence of *S. aureus.* 96% from the Staphylococcus aureus strains elaborate a free coagulase, which, following the reaction with a globulin factor from the plasma, forms staphylothrombin, which in its turn catalyzes the conversion of fibrinogen in insoluble fibrin.

The fibrinolysin lyses the layer of fibrin that is formed around a staphylococci abscess, thus being a factor of diffusion of tissues.

Dezoxyribonuclease hydrolases the DNA and is a factor of diffusion. It has a value of diagnosis for Staphylococcus aureus because it is present only in very few strains of SCN.

Hemolysines or hemotoxins. There are 4 hemolysines known: α , β , γ and δ . More important in the human pathology are alphatoxin (hemolysine) that produces a lysis of erythrocytes and injures the thrombocytes and beta-toxin which, deteriorating the sphingomyelin, it is toxic for erythrocytes but also for other types of cells.

Leukocidin, secreted especially by the strains isolated from furuncles, lyses the neutrophil polymorphonuclear leucocytes and macrophages and thus confers an increased resistance to phagocytosis. Complete hemolysis – incomplete a - absence of hemolysis.



Fig.1. Frequency of the hemolysis types

In regard to the frequency of the hemolysis types, a part of the strains have produced a complete hemolysis, of 20,5%,

following a descending of incomplete hemolysis, $\alpha,~8,6\%$ and a significant increased percentage

2022

of the absence of hemolysis, being represented by 70,9%.

On the Baird-Parker medium, after an incubation of 24 hours, on 37°C, the strains that have synthetized the free coagulase, have formed colonies of average dimensions, of bright black color, surrounded by a clear halo. The strains of staphylococci that did not synthetize the free coagulase did not produce these characteristic modifications, the colonies being of bright black color and without a halo around them.

CONCLUSIONS

The causing of the disease by S. aureus is a very complex process and probably involves a large number of factors, connected with cells but also secreted. In this review, we examined many of the important secreted factors of virulence of S. aureus.

From these studies we can see clearly that the major effects of toxins are the inactivation of the cells of host immunity system, either the releasing of cytokine mediated by superantigen, or by direct cytotoxicity, as it is the case of the hemolysin action. Beside all these there are many other things to learn about the way the toxins function.

REFERENCES

1. ARUP Laboratories. Test Directory: Hemosiderin, Urine. www.aruplab.com 2010. Ref Type: Internet Communication.

2. Buiuc D., Neguţ M. 2009 – Tractate of clinical microbiology – 3^{rd} edition, Ed. Medicală, Bucharest.

3. BENNETT J.B., DOLIN R., BLASER M. J. 2019 - Principles and Practice of Infectious Diseases, vol 2, Ninth edition, Churchill Livingstone Elsevier.

4. Buiuc D. 2003 – Medical microbiology: guide for the study and practice of medicine, Ed. "Gr. T. Popa" laşi.

5. Cepoi V., Azoicăi D. 2012 – Guide of management of nosocomial infections. Ed. Arte, Bucharest.

6. Constantiniu S., Ionescu G. 2005 – Acinetobacter type in human pathology. Bacteriology, Virusology, Parasitology, Epidemiology, pp. 50:1-2, 157-173.

7. Crisan A., Nicoara E. 2015 - Course of Infectious diseases, Ed. de Vest, Timișoara.

8. CORNELISSEN C. N. HOBBS M. M. 2020 – Microbiology, fourth edition, Lippincott Illustrated reviews.

9. Campfield T, Braden G, 2010. Urinary Oxalate Excretion by Very Low Birth Weight Infants Receiving Parenteral Nutrition. In Pediatrics,pp. 84(5):860-3.

10. CAROLL K.C., PFALLER M.A., LANDRY M.L., McADAM A.J., PATEL R. RICHTER S.S., WAENOCK D.W, 2019 - Manual of Clinical Microbiology, 2 volume, (ASM Books), 12th edition. 11. Dumitraşcu V., Laboratory Medicine. Biochemistry of urine, EdituraOrizonturiUniversitare, Timişoara, 2002.

12. Earnest DL. Enteric Hyperoxaluria. In Adv Intern Med, 1979.LaboratorSynevo. Specific references to the work technology used in 2015. Ref Type: Catalogue. pp.24:407-27 (review).

13. Dumitraşcu V. and collab. 2007 -

Pharmacology – antimicrobial medicine, Ed. de Vest, Timişoara.

14. Dumitraşcu V. and collab., 2007 –

Pharmacology in clinical laboratory, Ed. de Vest, Timişoara.

15. Garrity G.M., Bell J.A., and Timothy G.I., 2004 – Taxonomic outline of the Prokaryotes, Bergey's Manual of Systematic Bacteriology – II-ndedn. Bergey Manual Trust, Springer, New York.

16. Heymann D.L., 2012 - Manual of management of transmissible diseases, Ed. Amaltea, Bucharest.

17. Holtmann H., Nitschke J., 2017 – Basics Medizinische Mikrobiologie, Hygiene und Infektiologie, 4 Auflage, Elsevier GmbH Deutschland.

18. Inglis T.J.J. – Microbiology and Infection. Churchill Livingstone, 2007.

19. Ionescu G., 2006 – Phenotypic and molecular characterization of Acinetobacter strains isolated from the hospitals. PhD thesis.

20. Jehl F., Chomarat M., Weber M., Gerard A., 2004 – From antibiogram to prescription, Ed. Ştiinţelor Medicale, Bucharest.

21. Lennette E.H., Balows A., Hausler W.J., Truant J.P., 2002 – Manual of Clinical Microbiology, 4th ed., American Society for Microbiology, Washington, D.C..

22. Licker M., Moldovan R. and collab., 2002 – Resistance to antibiotics, history and actuality, Ed. Eurostampa, Timişoara.

Licker Monica, Moldovan Roxana and collab. -Course of special microbiology - vol. I, 2008– bacteriology. Ed. Eurostampa, Timişoara.

24. Licker M., Nicoară E. and collab., 2011 – Guide for the prevention of the bacterial multi resistance. Ed. Eurobit, Timișora.

25. Lorian V., 2000 – Antibiotics in clinical medicine, 2nd Ed., Williams and Wilkins, Baltimore.

26. Mandell G. L., Douglas R. G., Bennett J. E, 2002 – Principles and practice of infectious disease – Antimicrobial therapy, Churchill Livingstone, New York.

27. Mandell G. L., Douglas R. G., Bennett J. E, 2010 – Principles and practice of infectious disease – 7 th Ed., Churchill Livingstone, New York, Edinburgh, Melbourne.

28. Engemann JJ, Carmeli Y, Cosgrove SE, et al, 2003. Adverse clinical and economic outcomes attributable to methicillin resistance among patients with Staphylococcus Aureus surgical site infection. Clin Infect Dis, pp. 36(5):592-598.

29. Francis JS, Doherty MC, Lopatin U, et al,2005. Severe community-onset pneumonia in healthy adults caused by methicillin-resistant Staphylococcus Aureus carrying the PantonValentine leukocidin genes. Clin Infect Dis, pp.40(1):100-107.

30. Fridkin SK, Hageman JC, Morrison M, et al, 2005. Methicillin-resistant Staphylococcus Aureus disease in three communities. N Engl J Med, pp. 352(14):14361444.

31. Głuszek, J., 2000. The effect of glucose intake on urine saturation with calcium oxalate, calcium

phosphate, uric acid and sodium urate, International Urology and Nephrology, pp. 20 (6), 657-663. 32. GOERING VG, DOCKRELL HM,

32. GOERING VG, DOCKRELL HM, ZUCKERMAN M, CHIODINI PL 2019 – Mims Medical Microbiology and Immunology, Elsevier, sixth edition.