THE OXIDASE TEST FOR STAPHYLOCOCUS AUREUS

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REVIEW, RESEARCH ARTICLE

Abstract

In Romania more than 50% of the isolated strains of *Staphylococcus Aureus* from blood cultures are methicillin resistant and this percentage has increased in the last years. The process of nutrition is developed in the cellular metabolism by reactions of deterioration of the macromolecular compounds in compounds with reduced molecular mass that can be transported inside the cell, exergonic reactions that take place in the cellular catabolism. Simultaneously with the catabolism reactions, using simple compounds and energy, the living cell synthetizes in the anabolism cellular compounds essential for growth. The life of the microbial cell is possible as long as the two processes are developed simultaneously. The microorganisms met in the food industry are chemosynthetitic microorganisms and they are achieving the energy by deteriorating the organic compounds, releasing the potential energy of the nutritive underlayer that it develops, leading most of the times to its specific alteration. The possible pathogenic microorganisms can grow on the food products rich in nutrients and can develop toxins in some conditions but by the ingestion of the contaminated food there are conditions of food poisoning or toxicosis occurrence.

Keywords: microorganisms, blood cultures, cellular catabolism

INTRODUCTION

The bacteria of the Staphylococcus type are pathogenic agents of the human being and also of many mammals. Based on their capacity to coagulate the sanguine plasma (reaction of coagulase) they were divided in two groups, coagulase-positive staphylococci that include the most pathogenic species of Staphylococcus aureus, and coagulase-negative staphylococci including many species, 17 of them being indigene to human being, the rest being non-human pathogens. The coagulase-negative staphylococci were considered for a long time commensals of the skin and mucous, their important role as pathogenic agents being recognized only lately. *Staphylococcus aureus* is one of the most pyogenic bacteria, capable to produce infection with any localization in the body, beginning from simple cutaneous staphylococci and up to systematic infections with severe evolution. The strains of *Staphylococcus aureus* are considered the species associated with infections of the skin but also with generalized infections.

The increasing incidence of the infections caused by these bacteria can be rendered to their affinity to foreign materials that are integrating in the modern medicine. The more and more utilization of the prosthetic devices, the intravascular catheters and of other invasive technologies in sick, immunosuppressed patients, took the staphylococci at the head of the nosocomial pathogenic agents, which leads to a considerable morbidity and medical costs in excess in the patients with compromised immunity. Although previously it was only considered an inoffensive commensal microorganism on the human skin, Staphylococcus epidermidis is seen in the present as an important opportunist pathogen. The strains of Staphylococcus epidermidis are the most frequent cause of bacteremia related to foreign bodies and medical devices from the interior of the body, complicated by the formation of the biofilm, which is a key-factor in the virulence of this species and has a huge impact on the pathogenesis and on the therapy of these nosocomial infections.

The antimicrobial resistance represents a primary problem on the global level, being caused, largely, by the inadequate and uncontrolled utilization of the antimicrobial products, including the polypharmacy, the administration of suboptimal doses, the insufficient period of treatment and the wrong diagnosis that leads to the unfit choosing of the antimicrobial product. The resistant microorganisms are transmitted among the patients and the resistance factors are transferred between the bacteria, both appearing more frequently in the units

providing medical services. In Europe the antibiotics resistance is in continuous growth, The European Medicines Agency relates that annually over 380 000 Europeans suffer of infections caused by the bacteria resistant to medicines and approximately 25 000 persons from UE die of this cause.

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.

■ A recipient of collection (coprosampler with collecting spoon) with transport medium
■ Wooden spatula
■ Latex gloves

In regard to the collection, it has to be done as close to the beginning of the diseases as possible and before the beginning of any antimicrobial treatment.

• Collection from a spontaneous stool – it is preferred and is indicated in all forms of acute diarrhea when the emission of fecal matters is frequent.
• For bacterial and parasital examinations the collection is made with the "spoon" of the coprosampler, aiming at the liquid and, especially the mucous and/or bloody parts, if they exist. The volume of the collection has to be minimum of 5 ml or 3-5 cm^3, if the stool is formed.
• For isolations or virologic exams there are 5-10 cm^3 of fecal matters collected or minimum 5 ml, if the stool is not formed.

RESULTS AND DISCUSSIONS

Another essential test is the test for oxidase that accomplishes the differentiation of the staphylococci of medical interest from the similar genres, *Micrococcus*, *Dermacoccus,* respectively etc, which are oxidase positive. The principle that is the basis of this test is represented by the reaction by which it is oxidized tetrametyl-p-phenylenediamine in a compound of purple color, under the action of cytochrome oxidase. The cytochrome oxidase, known also with the name of indophenol oxidase is a hemeprotein from the respiratory chain of transport of electrons coupled with the oxidative phosphorylation and which, as a consequence, is absent in the strictly anaerobe bacteria. In regard to the Gram-positive cocci it is tested in the culture of 18-24 hours on agar with 9% ram blood. The cultures made on other media can be tested only after three days and the reaction is becoming positive in a period of approximately 10 minutes.

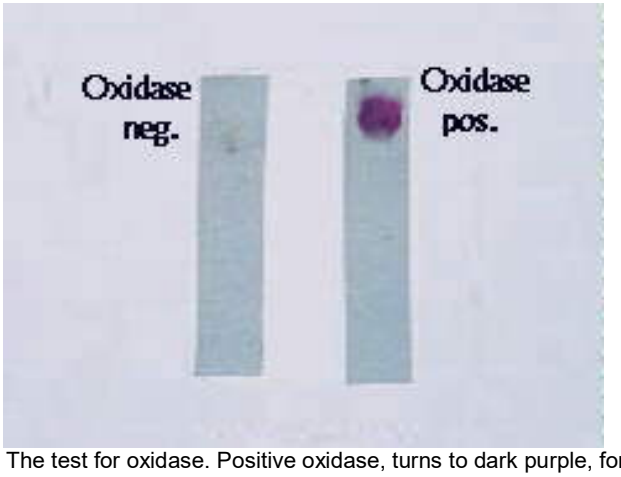

Fig.1 The test for oxidase. Positive oxidase, turns to dark purple, for *S. aureus*

The test used for underlying the activity of the catalase helps reaching the differential diagnosis compared to other species of pathogen cocci, especially streptococci and pneumococci. The staphylococci are catalase positive, while the streptococci and pneumococci don't have a catalase.

This tests can be performed by the fast technique on the blade where it is placed a drop of a solution 30% of Hydrogen peroxide, then is collected a loop well loaded of the tested culture, on agar slants and is mixed in the peroxide drop.

The gas bubbles that appear immediately indicate the presence of catalase. It is not indicated the collection of colonies from the agar blood media because the erythrocytes possess catalase and falsify the reaction.

It is recommended the utilization of a strain of enterococci and a strain of staphylococcus aureus as negative and positive controls of reaction, in parallel with the tested strain.

In the study called "Utilization of a quantitative test of oxidase for the characterization of the oxidative metabolism in bacteria" has underlined the fact that it was possible the quantification of the terminal oxidase reaction using the latent cells of the bacteria and was demonstrated the utility of this reaction for taxonomic purposes. The values of Q(O2) for the TMPD oxidase presented a perfect correlation with the Kovacs oxidase test and, moreover, it was possible to define the quantity of the point that separates the oxidase positive bacteria compared to those oxidase negative. The oxidase negative bacteria presented a value of the TMPD Q(O2) oxidase (after the correction for endogen by decreasing) smaller or equal to 33 and had an uncorrected TMPD/endogen report smaller or equal to 5. The values of the TMPD Q(O2) oxidase were also correlated with the data obtained for the Oxferm Hugh-Leifson test. In general, the bacteria that presented a respiratory mechanism had larger values of the TMPD oxidase, while the fermentative organisms had a decreased activity of the TMPD oxidase. All the exceptions from this rule are noted. This quantitative study has demonstrated also that the organisms that (i) lack the cytochrome of type c or (ii) lack a system of transport of electrons that includes cytochrome, as would be the bacteria of lactic acid, have presented decreased or insignificant values or TMPD Q(O2) oxidase. From the 79 bacterial species (36 genuses) examined, it seems that this quantitative oxidative test has a taxonomic value that can differentiate the oxidative relations between the bacteria on the levels of subspecies, species and genuses.

CONCLUSIONS

The test of oxidase is a biochemical reaction that analyzes the presence of cytochrome oxidase, an enzyme called sometimes indophenol oxidase.

In the presence of an organism that includes the cytochrome oxidase enzyme, the colorless reduced reactive becomes a colored oxidized product.

The final phase of the bacterial respiration involves a series of components incorporated in the membrane, known collectively under the name of chain of electrons transport. The last step from the chain can involve the utilization of the cytochrome oxidase enzyme that catalyzes the oxidation of the cytochrome c while reducing the oxygen in order to form water.

The test of oxidase uses frequently a reactive, tetramethyl-p-phenylenediamine dihydrochloride as artificial donor of electrons for cytochrome c. When the reactive is oxidized by cytochrome c, it passes from colorless to a dark blue or purple compound, blue indophenol.

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