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DIFFERENTIAL DIAGNOSIS BETWEEN STAPHYLOCOCCI AND STREPTOCOCCI BY THE LATEX AGGLUTINATION TEST

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Abstract

The bacteria of the Staphylococci type are cocci gram-positive, isolated disposed, in pairs, in short chains and clusters, irregular, with variations in dimensions and the capacity to retain, the gram, immobile and non sporulated. Most of the species are catalase-positive. The streptococci are spherical or oval cocci, gram positive, placed in chains under pair, immobile and non sporulated. Nutritive demanding, discretionary anaerobe, some carboxiphilic, are better developed at reduced tensions of the oxygen, even anaerobe. They are catalase and oxidase negative and don't reduce the nitrates. They ferment the glucose with production of lactic acid and always without gas. The Staphylococci are not nutritive demanding, are cultivated on the majority of usual mediums and tolerate concentrations of over 5% NaCl, and some species are catalase-positive and discretionary anaerobe, but they grow better aerobe. Once the isolated was characterized preliminary "cocci gram positive" with the tendency to form chains and catalase negative, they have to be submitted to the physiological and serological tests of identification.

Keywords: catalase, oxidase, ferments, anaerobe

INTRODUCTION

The Staphylococi are omnipresent germs. They are found on the level of the teguments and mucous of the human being (especially on the level of the nasopharynx, of the gastro-intestinal and urogenital tube), and in other mammals and birds. The human species of coagulase-negative (SCN) staphylococci colonize especially the nostrils and teguments. *S. aureus* colonizes transitionally the tegument areas or the wet mucous. The colonizing of the newborn with *S. aureus* begins on the level of the umbilical blunt, of the tegument surfaces, of the perineum area. Afterwards, the transmitting of short or long duration to the old child and to the adult was frequently found on the level of the nasopharynx. Approximately 15-30% of the healthy adults are nasopharynx bearers of *S. aureus*. In the medical staff and in the patients with a long hospitalization, the rate of the transmission reaches 40-70% and even 90%, which explains the numerous infections associated to the medical nursing (named also nosocomial infections) with *S. aureus*.

The cellular wall of *S. aureus* is characteristic to the gram-positive bacteria. The majority of the strains of *S. aureus* have on their surface an enzyme connected to the cellular wall, named "clumping factor" or connected coagulase that transforms the fibrinogen in fibrin. It should be mistaken for the "free coagulase", secreted in the exterior of the bacterial cell and which is characteristic to the *S. aureus* species. In the majority of the strains of *S. aureus*, the peptidoglycan is covered by the protein A, which has the property to connect in a nonspecific way the antibodies by the Fc fragment. The capsule, present only in some strains, increases the antiphagocytic properties of the staphylococci, being a factor of virulence.

The *Streptococcus* type, very complex, was classified using multiple criteria that consider the hemolysis, antigenic structure, clinical aspects etc. – Depending on the type of hemolysis on blood agar they are divided in: β-hemolytic streptococci, produce colonies surrounded by a clear area of complete hemolysis, characteristic for *Streptococcus pyogenes* and other pathogen species. α-hemolytic, produce a partial hemolysis with the appearance of a greed coloration of the medium (viridans hemolysis), characteristic to the viridans streptococci and pneumococci α'-hemolytic, produce incomplete hemolysis, with nonlysate erythrocytes non hemolytic (γ-hemolytic). - Lancefield antigenic classification. *Streptococci* were divided based on the antigenic structure – depending on the C glycan from the cellular wall, in serologic groups written with the letters A-H and K-W, - non groupable streptococci – those without group antigen: *Streptococcus pneumoniae* and streptococci viridans. From the clinical point of view the streptococci are classified in: - *Streptococcus pyogenes* (β-hemolytic streptococcus of group A) – is the main human pathogen from the streptococci, being associated to some infections with location, invasive, generalized, and to some allergic complications resulting after repeated infections - *Streptococcus agalactiae* - is part of the group B and is isolated from the vaginal flora. It is involved in the meningitis and septicemias of the newborn. – the streptococci of group C, G and F colonize sometimes the nasopharynx, being the cause of some sinusitis, bacterimias or endocarditis. – non enterococci streptococci of group D (*Streptococcus bovis*) are part of the normal flora of the intestine and are the cause of some endocarditis. They can produce bacterimias in patients with carcinoma of colon. - *Streptococcus pneumoniae* – the non-enclosed version of this species is present in the normal flora of the upper respiratory tube. The enclosed, pathogen versions are the major cause of meningitis in children and of pneumonia.

MATERIAL AND METHODS

It were processed the samples from the patients from Sante laboratory, from Oradea. The species of *Staphylococcus* and *Streptococcus* isolated were identified based on the morphologic, cultural aspect, of the production of coagulase, the presence of hemolysin, the enzymatic activity and the production of acid following the fermenting of some sugars. The API galleries used for the definitive identification of *Staphylococcus* and *Streptococcus* strains were ID 32 STAPH and STREP. For the identification of the *Staphylococcus* and *Streptococcus* strains we used also the Pastorex Staph-Plus kit: the reaction is of latex-agglutination on the slide that shoes simultaneously the presence of "clumping factor" (the factor of affinity for fibrinogen), protein A, and the latex test for streptococci, the test uses the latex suspension specific for groups: A,B,C,D,F,G, for the identification of correspondent Ag group specific it is needed previously an enzymatic extraction.

1. The latex kit of identification of *Streptococci*

Equipment and reagents

Sterile loops

Thermostat

The latex kit

Preparing and preservation of reagents

The latex kit is kept at 2-8°C, before the utilization is kept to heat at the room temperature.

Preparing the biologic material.

The pure young culture on non-selective medium.

Execution of the procedure

With a sterile loop are sampled 2-6 colonies, then discharged in 0.4 ml of extraction enzymes and is hatched 10 min at 35-37°C, is disposed on the agglutination plate a drop of extract and a drop of latex reactive.

2. The latex kit of identification of *Staphylococci*

Principle

The test uses a latex suspension specific sensitized that will agglutinate with those colonies of Staphylococci that include: clumping factor or/and protein A and capsular polysaccharides of *Staphylococcus Aureus*.

Necessary materials

The reactive test contains:

- Sensitized latex particles
- Sodium azide as preservation medium
- The negative control includes non-sensitized latex particles
- Cards for agglutination
- Plates with test strains

- Pipettes
- Sterile loops

Procedure

- Is left the kit to accommodate to the room temperature
- Is homogenized the latex reactive
- Is dispersed 1 drop of reactive on the agglutination card
- Are transferred 2-3 suspect colonies and is mixed the mixture
- Is followed the appearance or the absence of the agglutination in 20 seconds

RESULTS AND DISCUSSIONS

In staphylococci strains, the presence of the connected coagulase was showed with a kit that allows the testing of the strains as a screening test for the detecting of this enzyme.

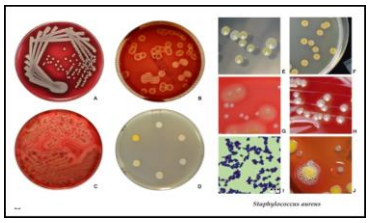

Fig. no.1 *Staphylococcus Aureus*

Fig. no. 2. *Staphylococcus Aureus*

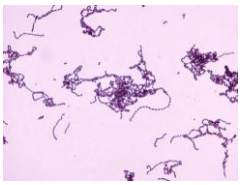

Fig. no. 3 *Streptococcus*.

Fig. no. 4 Kit agglutination of streptococci

The positive reaction indicates the presence of the factor of aggregation by the appearance of some small clots, as a consequence of the precipitation of the plasmatic fibrinogen in fibrin, under the action of this enzyme for 2-3 minutes. The negative reaction is represented by the absence of such clots.

By the precision of the results, a strain of *S. aureus SSP*. *Aureus* was used as a positive control and a strain of *S. epidermidis* as a negative control. The results were the following: - *S. aureus SSP*. *aureus*: 60 possessed strains factor de aggregation ,72,04%; - *S. intermedius*: 73 possessed strains factor de aggregation, 62,27%;

The direct detecting of the streptococcus antigen by the latex kit of agglutination has as ascendant result for the streptococci of group A, 65%, 20% for the streptococci of group G, respectively, the rest of 15% for the streptococci of group B,D,C,F.

DISCUSSIONS

The clinical importance and significance of a staphylococcus coagulase-negative is supported by the isolation of this microorganism in a dominant ratio or in pure culture and by the repeated isolations of the same strains, from blood, urine or other samples. In the case of the systemic nosocomial infections that appeared in the wards with risk, the interpretation of the involvement of some staphylococcus coagulase negative in the infectious syndrome can be oriented by the correlation between the isolated bacteria from the catheter and the presence of the same bacteria in blood cultures.

The microscopic aspect, as a series of fenotypical types, under the aspect of the colonies on non selective mediums, beside a restrained set of enzymatic tests, allow the preliminary identification of the species most frequently isolated from staphylococcus infections.

On the staphylococcus strains, the presence of the connected coagulase was accomplished with the help of a kit that allows the testing of the strains as a test of screening for the detection of this enzyme. The positive reaction indicates the presence of the factor of aggregation by the appearance of small clots, following the precipitation of the plasmatic fibrinogen in fibrin, under the action of this enzyme in a period of 2-3 minutes. The negative reaction is represented by the absence of such clots. The staphylococcus strains that synthetize the connected coagulase, and the protein A produced a positive reaction, because this enzyme, in contact with the sensitized latex particles with IgG and fibrinogen, has agglutinated this mixture.

In the study "Evaluation of RapiDEC Staph for identification of *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Staphylococcus saprophyticus*", accomplished by W M Janda, K Ristow, and D Novak, is specified the fact that, RapiDEC Staph is a test for the presumptive identification of the main human staphylococci species, *Staphylococcus aureus*, *S. epidermidis*, and *S. saprophyticus*. The test includes control cupules for the detection of fluorogenic of coagulase and chromogenic substrates for alkaline phosphatase and beta-galactosidase. These tests identify *S. aureus*, *S. epidermidis*, and *S. saprophyticus*, respectively. The positive results with both chromogenic substrates offer a presumptive identification of *S. xylosus* or *S. intermedius* (*S. xylosus*-*S. intermedius*).

The cupules of testing are inoculated with a suspension of the body, and the reactions are read after an incubation of 2h. RapiDEC-Staph was evaluated with 303 clinical strains and of stock. The identifications were compared with those obtained by the test of coagulation of the tube, a test of coagulation of the diapositives of latex (StaphAUREX), another system of commercial identification (Staph-TRAC) and additional conventional tests. RapiDEC-Staph identified 100% correct from 130 *S. aureus* strains, 70,3% of 74 *S. epidermidis* strains, and 81,3% of 32 S. saprophyticus strains. Four of the five isolations *S. xylosus* were named *S. xylosus-S. intermedius*. Non identified s. epidermidis and s. saprophyticus strains were named "*Staphylococcus spp*. " among 62 other coagulase-negative staphylococci, 4 were wrongly identified as s. epidermidis and 7 were wrongly identified as *s. saprophyticus*. While the sensitivity and specificity of the test of fluorogenic coagulation for *S. aureus* were of 100%, the incapacity to detect the activity of the alkaline phosphatase in more isolations *S. epidermidis* has determined less correct identification by the RapiDEC-Staph test for these species.

Once the isolated was characterized preliminary “cocci gram positive with the tendency to form chains and catalase negative”, have to be submitted to physiological tests and serologic tests of identification. The introduction of streptococci in the algorithm of identification is made based on the aspect of the hemolysis: the β hemolytic streptococci versus non β hemolytic streptococci.

The reagents of agglutination from latex are used on a wide scale in the microbial diagnosis, the identification and serotesting. *Streptococcus pneumoniae* (pneumococcus) is a major cause of morbidity and mortality on the global level. The world organization of health recommended the latex agglutination as an alternative method to “golden standard” of testing for pneumococci, serotyping.

The study “Detection of group B streptococcal antigen in early-onset and late-onset group B streptococcal disease with the Wellcogen Strep B latex agglutination test”, accomplished by D L Ingram, D M Suggs, A W Pearson, showed the fact that the test of agglutination of latex Wellcogen strep B (Wellcome Diagnostics, Dartford, England) was evaluated as a method of detecting of the streptococcus antigen from Group B in urine, cephalorachidian fluid and serum from newborn with early debut (less or equal with 7 days of age) and streptococcus disease Group B with late start. The urine was the best source of antigen that was detected in 100% of six newborn with early streptococcus disease Group B, which had urine available in the first 12 hours of disease and in 88% of the 17 newborn infected with streptococcus from Group B with urine available in the first 48 hours of disease. The antigen was not detected in any group of patients

without streptococci disease of group B, with the exception of the urine of a patient with meningitis *Proteus Mirabilis*. The latex test Wellcogen strep B from the tested lot compares favorably with a test of agglutination from latex available non-commercial.

CONCLUSIONS

A gradual diagnosis, consisting of the performing of multiple tests of differentiation whose results, corroborated, allow the differentiation of the species of staphylococci with an acceptable degree of precision, is the solution agreed in the present by most of the laboratories.

The fast identification of the species of *S. Aureus* can be made with the help of the tests of agglutination. The sensitized reagents with fibrinogen and immunoglobulin G pairs the connected coagulase, respectively protein A from the wall of *S. Aureus*, which has as macroscopic expression the agglutination of the mixture of reactive and the culture with *S. Aureus*. The precision of this test is between 70% and over 90%.

Reactions of reverse agglutination that use as support particles for specific antibodies of group, either staphylococci with protein A, or particles of latex. Numerous types of kits are sold for the agglutination on the slide. Comparing to the classic test of precipitation, the tests of agglutination on the slide were faster, economical for a sensitivity and specificity at least equal.

The kit with the antiserums A,B,C,F,G allows the identification of most of the β hemolytic streptococci involved in the urinary infections. The rare strains that don't react with these serums can be characterized biochemically following to be confirmed by the reference laboratory.

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