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Assessment of the Agglutination Test for the Identification of Staphylococcus Aureus Isolates

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Abstract

Background: Staphylococcus aureus are among the most important and several pathogens in human infections.

Objectives: The objectives of this study are to evaluate the efficacy of the agglutination test in the detection of Staphylococcus aureus in the laboratory and to identify the factors associated with staphylococcal infections.

Methods: It is a prospective study of 100 isolates of staphylococci in a period of nine months from May 2021 to January 2022 in the University Hospital of Befelatanana.

Results: Among of the 100 isolates of staphylococci, 49 (49%) were represented by Staphylococcus aureus. Concerning the prediction performance of the agglutination test, it has a sensitivity of 93.8%, a specificity of 100%, a positive predictive value of 100% and a negative predictive value of 94.7%. Concerning the associated factors, the men (63%) (p=0.002), the patients aged 40 to 59 years (63%) (p=0.3; NS) and with suppuration (75%) (p=0.004) were the most affected by Staphylococcus aureus. Moreover, Staphylococcus aureus was often identified in pus samples (72.4%) (p=0.0009).

Conclusion: In brief, agglutination test is a good test and can replace the standard gold test for the detection of Staphylococcus aureus.

Keywords: Agglutination; Staphylococcus aureus; suppuration

Introduction

Staphylococcus, group of spherical bacteria, the best-known species of which are universally present in great numbers on the mucous membranes and skin of humans and other warm-blooded animals. The term staphylococcus, generally used for all the species, refers to the cells' habit of aggregating in grapelike clusters [1]. They are observed in multiple clinical situations, in community acquired infections and nosocomial infections [2]. Among staphylococci, Staphylococcus aureus (S. aureus) is one of the major human pathogens in which it is responsible for toxic shocks, food borne diseases and especially a wide spectrum of suppurative infections [3].

The laboratory of medical biology plays a very important role in the diagnosis of infections, especially bacterial infections. Indeed, the laboratory highlights the infectious agent and can determine its sensitivity to antibiotics. There are several methods for identifying S. aureus strains in the laboratory. In the laboratory of the University Hospital of Befelatanana,

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we used simultaneously the baird-parker culture medium, the coagulase test and the agglutination test for the identification of S. aureus strains. The identification on baird-parker culture medium represents the gold standard test [4-7]. Baird-Parker and coagulase tests require incubation at 37°C in the oven for 24 hours. However, the agglutination test gives a quick result compared to the 2 previous tests because it is obtained after 20 seconds. In this study, we tried to compare the results of Baird-Parker and agglutination tests to see if the agglutination test is as reliable as the Baird-Parker test which is the gold standard test. If the agglutination test is reliable, we can stop doing the Baird-Parker test, which is difficult to perform.

Thus, the aims of this study are to evaluate the efficacy of the agglutination test in the detection of S. aureus in the laboratory and to identify the factors associated with Staphylococcal infections in order to improve the care of the patient.

Materials and methods

a. Type and period of study

It is a prospective study of 100 isolates of staphylococci in a period of nine months from May 2021 to January2022 in the University Hospital of Befelatanana.

b. Procedures

In the beginning, the isolates were identified according to appearance, odor and color of bacterial colonies. Then, the identification of Gram-positive cocci on microscopic examination and the positivity of catalase confirms the diagnosis of staphylococcal infection. For the diagnosis of S. aureus infection, we performed 2 tests simultaneously. The first test is the inoculation of the strain on the baird-parker culture medium. The second is the agglutination test. Baird-parker require incubation at 37°C in an oven for 24 hours. The identification on baird-parker culture medium represents the gold standard test [4-7]. But this test requires several conditions that make it more difficult to perform compared to the agglutination tests. Indeed, a petri dish by strain is necessary for the test. In addition, we need to buy a box of baird-parker culture medium that is quite expensive. Finally, the egg yolk tellurite emulsion must be used, which will be mixed with the baird-parker agar during the preparation of the culture media. The egg yolk tellurite emulsion is necessary for the good growth of S. aureus strains. Indeed, strains of staphylococci will reduce tellurite that will become a black tellurium causing a black staining of staphylococci colonies. Similarly, there is proteolysis of egg yolk proteins, resulting in a clear (transparent) halo around the colony. The presence of this halo is specific to isolate of S. aureus (figure1 and figure 2).

Concerning the agglutination test, staphytect Plus is a latex slide agglutination test for the differentiation of S. aureus by detection of clumping factor, Protein A and certain polysaccharides found in methicillin resistant S. aureus

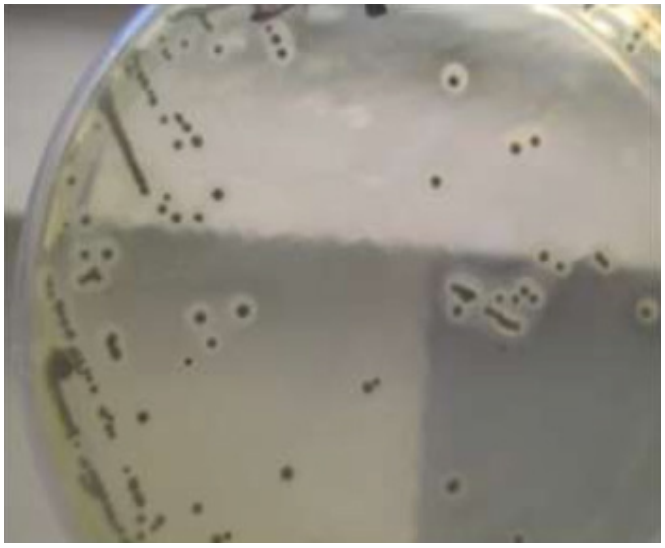

Figure 1: Positive Baird-Parker test.

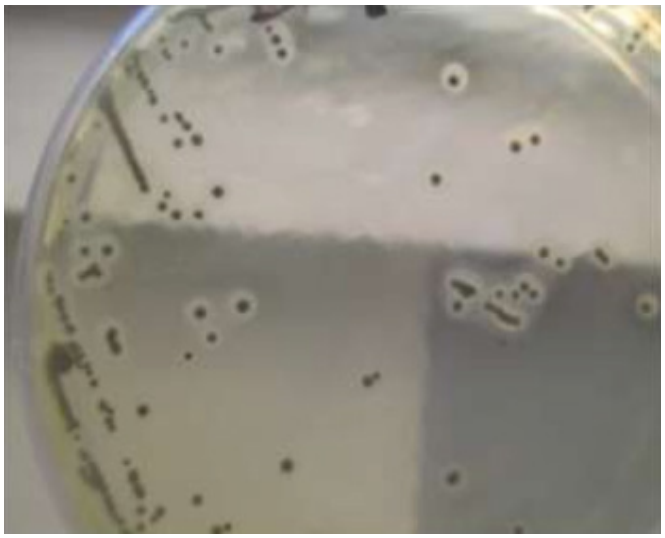

Figure 2: Negative Baird-Parker test.``

(MRSA) from those staphylococci that do not possess these properties. This test is very easy to do. First, we dispense one drop of test latex onto one of the circles on the reaction card and one drop of control latex onto another circle. After, we pick up and rock the card for up to 20 seconds and observe for agglutination under normal lighting conditions. The result is positive if agglutination of the blue test latex particles occurs within 20 seconds. It identifies the isolate as S. aureus (figure 3). A negative result is obtained if no agglutination occurs and a smooth blue suspension remains after 20 seconds in the test circle. It identifies the isolate as a non-aureus Staphylococcus (figure 4).

c. Study parameters

Study parameters were represented by age, gender, clinical information, sample types, Baird-Parker test, agglutination test and performance test results.

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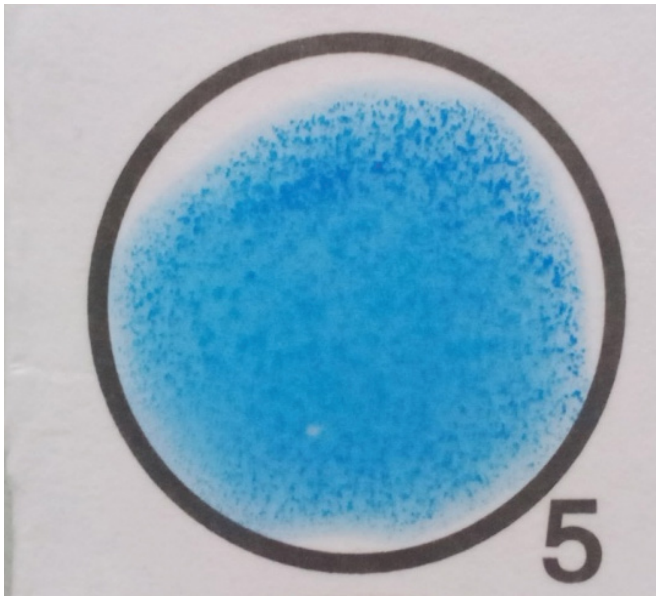

Figure 3: Positive agglutination test.

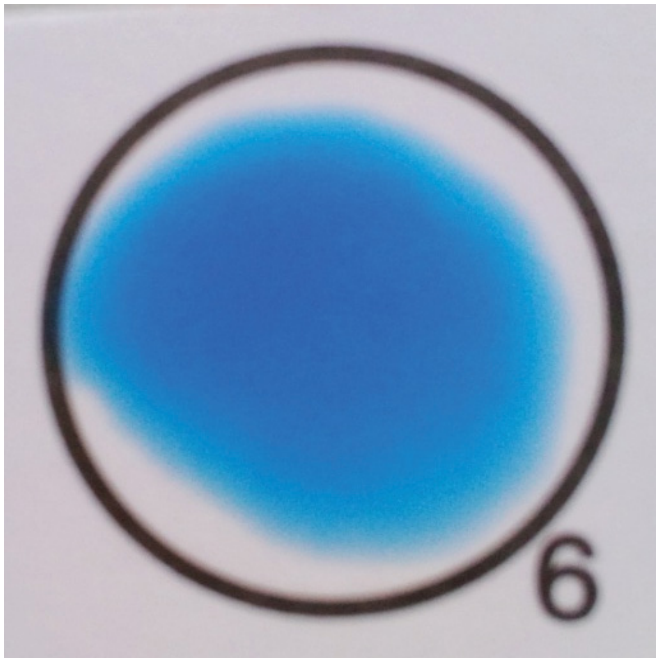

Figure 4: Negative agglutination test.

d. Ethical considerations

The authorization of this study by an ethics committee was not necessary because we analyzed isolates of bacteria. Nevertheless, the authorization of the director of the establishment was obtained before the data were collected in the registers. Likewise, the seizure was done anonymously to maintain confidentiality.

e. Data analysis

The entry and processing of data were performed on Epi-info 3.5.2 software. Proportions are presented as numbers (percent) and were compared using the chi-square test. Results were considered significant if p was 0.05 or less, with a 95% confidence interval.

Results

a. Distribution of staphylococcal isolates

Among of the 100 isolates of staphylococci, 49 (49%) were represented by S. aureus (figure 5).

b. Prediction performance of the agglutination test

Concerning the prediction performance of the agglutination test, it has a sensitivity of 93.8%, a specificity of 100%, a positive predictive value (PPV) of 100% and a negative predictive value (NPV) of 94.7% (table 1).

c. Factors associated with staphylococcal infections

Concerning the associated factors, the men (63%) (p=0.002), the patients aged 40 to 59 years (63%) (p=0.3; NS) and with suppuration (75%) (p=0.004) were the most affected by S. aureus. Moreover, S. aureus species was often identified in pus samples (72.4%) (p=0.0009) (table 2).

Discussion

In this study, almost half of identified staphylococcal isolates were represented by S. aureus. Indeed, S. aureus is a major bacterial human pathogen that causes a wide variety of clinical manifestations [8]. Infections are common both in community-acquired as well as hospital-acquired settings and treatment remains challenging to manage due to the emergence of multi-drug resistant strains such as MRSA [9-10]. S. aureus is found in the environment and is also found in normal human flora, located on the skin and mucous membranes (most often the nasal area) of most healthy individuals [8]. S. aureus does not normally cause infection on healthy skin; however, if it is allowed to enter the bloodstream or internal tissues, these bacteria may cause a variety of potentially serious infections

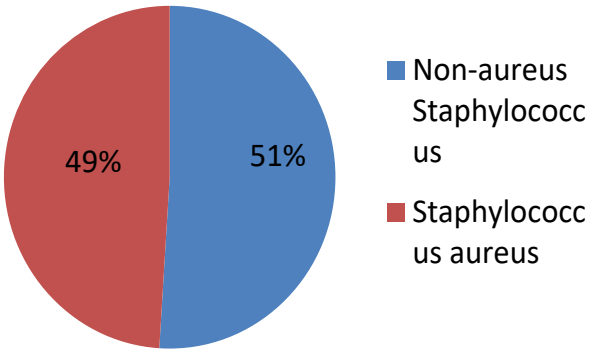

Figure 5: Distribution of staphylococcal isolates

Table 1: Prediction performance of the agglutination test

Test	Sensitivy	Specificity	PPV	NPV
Agglutination	93.80%	100%	100%	94.70%

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Table 2: Factors associated with staphylococcal infections

	Non-*aureus Staphylococcus*		*Staphylococcus aureus*		N	p-value
	n	%	n	%		
Age (years)						
< 20	22	62.9	13	37.1	35	0.3
20-39	9	50	9	50	18	
40-59	10	37	17	63	27	
≥ 60	10	50	10	50	20	
Gender						
Female	31	67.4	15	32.6	46	0.002
Male	20	37	34	63	54	
Clinical information						
Genitourinary disorders	5	100	0	0	5	0.004
Fever	29	53.7	25	46.3	54	
Pus	7	25	21	75	28	
Other clinical signs	10	76.9	3	23.1	13	
Sample types						
Genitourinary sample	19	79.2	5	20.8	24	0.0009
Blood sample	24	51.1	23	48.9	47	
Pus sample	8	27.6	21	72.4	29	

[8]. Transmission is typically from direct contact. However, some infections involve other transmission methods [11]. Thus, a good identification of staphylococci allows better management of the patient.

The evaluation of agglutination test against Baird-Parker test for the identification of S. aureus isolates showed 100% specificity and 100% PPV. Thus, there is never a false positive in the identification of strains of S. aureus when we use agglutination test. On the other hand, the agglutination test is not 100% sensitive and the negative predictive value is lowered. Indeed, when the bacterial colony is mixed with the agglutination reagent, the agglutination could not appear because the colony of S. aureus can be mixed with other bacterial colonies of different species. This situation may explain the reduced sensitivity of the agglutination test giving false negatives. In addition, agglutinations may be very small and not visible to the naked eye giving a false negative result. As a suggestion, the technician must take great care when picking up a colony of Staphylococus from the petri dish to avoid mixing this colony with other colonies of different species. If the colonies overlap, the Staphylococcus colony must be reisolated on a new petri dish and incubated for 24 hours at 37°C. After 24 hours, it is very easy to have a well-isolated Staphylococcus colony giving a good agglutination test result. Similarly, the technician should observe agglutination in a well-lit area or use a lamp to improve vision. By following these recommendations, the agglutination test can well replace the Baird-Parker test. In addition, the agglutination test requires only 20 seconds compared to 24 hours for the Baird-Parker test. This speed of the agglutination test makes it possible to carry out the antibiogram quickly and to treat the patient quickly. Thus, the care of the patient will be improved.

Concerning the associated factors, the patients aged 40 to 59 years were the most affected by bacterial infection with S. aureus but without significant difference. Thus, all age groups can be concerned. However, the literature confirms that very young children and elderly or ill patients in hospitals and nursing homes are particularly susceptible to MRSA infection, which is difficult to treat because of its resistance to most antibiotics [1]. Regarding gender, men were significantly more affected by S. aureus infection than women. Our study in 2019 was found the same result [12]. Indeed, more men work than women. Thus, they are more exposed to the risk of infection. Likewise, their toxic habits can make them more vulnerable such as tobacco and alcohol. Concerning the clinical information and the types of samples, S. aureus infections were very common in patients with suppuration with a very significant difference. Other studies have also found a high prevalence of S. aureus infections in the suppurations [13-14]. Indeed, staphylococcus is a ubiquitous bacterium frequently found on the skin. It is one of the main etiological agents of superficial and deep suppurative infections [15].

In short, the agglutination test can replace the Baird-Parker test for the identification of S. aureus isolates which are the

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most frequently identified bacteria representing almost half of the staphylococci. Similarly, the agglutination test is quick and improves patient care.

Conclusion

This study showed that S. aureus infections were the most frequent and the agglutination test can replace the Baird-Parker test for the identification of these isolates. Common in men and patients with suppuration, isolates of S. aureus can be identified easily by the agglutination test provided the bacterial colonies collected are pure and the observation of agglutination is made in a place well lighted.

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Competing interests

Authors have declared that no competing interests exist.

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