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ORIGINAL ARTICLE

ASSESSMENT OF DIFFERENT TESTS FOR IDENTIFICATION OF STAPHYLOCOCCUS AUREUS FROM CLINICAL SAMPLES: A COMPARATIVE STUDY

Sushil Kumar Sharma[1], Neeru Garg[2], Raj Kumar Wasan[3], Rohit Wadhwa[4], Vijeta Chadha[5], Prabhjot Kaur[6]

[1]Reader, Department of Microbiology, Maharaja Ganga Singh Dental College, Ganga Nagar, [2]Reader, Department of Microbiology, Surendra Dental College, Ganga Nagar, [3]Reader, Department of Microbiology, Desh Bhagat Dental College, Punjab, [4]PG student, Department of Conservative Dentistry, Desh Bhagat Dental College, Punjab, [5]PG student, Department of Periodontics, Desh Bhagat Dental College, Punjab, [6] Senior lecturer, Department of Oral Pathology, Desh Bhagat Dental College, Punjab

ABSTRACT:

Background: Staphylococcus aureus is a frequently isolated pathogen and may be cultured early in infancy in cystic fibrosis (CF) patients. Previous reports have shown that a significant number of clinical isolates of methicillin-resistant S. aureus (MRSA) gave negative results by one of newer tests (Staphaurex;). Hence; we planned the present study to assess current versions of Staphaurex and compared them with other diagnostic tests. These diagnostic tests include free-coagulase test, bound-coagulase test. **Materials & methods:** The present study included assessment of 150 consecutive clinical samples of S. aureus strains. For the determination of the methicillin susceptibility, a disk diffusion method on Mueller-Hinton agar using a 5-mg methicillin disk was used. Testing of a total of 200 staphylococcal isolates was done. In the end, the clinical samples yielded 100 S. aureus isolates derived from 60 patients. Following tests were used for the identification of S.aureus: Free-coagulase (tube) test, Bound-coagulase (agar) test, Staphaurex and Staphaurex Plus and Pastorex Staphplus. Strains with variable outcomes when the results of the different tests were compared were retested by all procedures mentioned previously and were subsequently studied further with the aid of the additional tests mentioned below. All the tests and procedures were performed as per manufacturer's condition. All the results were compiled and assessed by SPSS software. **Results:** 100 percent sensitivity was observed only in the Staphaurex Plus group. Inability of tests to identify some MRSA strains correctly resulted in the difference with the free-coagulase test, a bound-coagulase test, and the former Staphaurex test. **Conclusion:** Out of all the above mentioned tests, Staphaurex Plus exhibits maximum sensitivity.

Key words: Methicillin resistant, Staphylococcus aureus, Staphaurex

Corresponding author: Dr Sushil Kumar Sharma, Reader, Department of Microbiology, Maharaja Ganga Singh Dental College, Ganga Nagar, India

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INTRODUCTION

Staphylococcus aureus is a frequently isolated pathogen and may be cultured early in infancy in cystic fibrosis (CF) patients. The incidence of methicillin-resistant Staphylococcus aureus (MRSA) infection in CF patients has increased dramatically, and the presence of MRSA among CF patients contributes to lung disease.[1, 2] CF patients with MRSA present a significant increase in hospitalization rates and treatment with oral, inhaled, and intravenous antibiotics, a greater decline in lung function, as measured by forced expiratory volume in 1 s (FEV1), a higher risk of failing to recover to baseline after pulmonary exacerbations, and an increase in mortality.[3, 4]

Previous reports have shown that a significant number of clinical isolates of methicillin-resistant S. aureus (MRSA) gave negative results by one of newer tests (Staphaurex;).[5]

Hence; we planned the present study to assess current versions of Staphaurex and compared them with other diagnostic tests. These diagnostic tests include free-coagulase test, bound-coagulase test.

MATERIALS & METHODS

The present study was conducted in the department of microbiology of the institute and included assessment of 150 consecutive clinical samples of S. aureus strains. Ethical approval was taken from the institutional ethical

committee in written after explaining in detail the entire research protocol. All the specimens were processed and yielded colonies suspected to be S. aureus isolates. This observation was based on morphological criteria. In addition, testing of strains of MRSA was done. For the determination of the methicillin susceptibility, a disk diffusion method on Mueller-Hinton agar using a 5-mg methicillin disk was used. Testing of a total of 200 staphylococcal isolates was done. In the end, the clinical samples yielded 100 S. aureus isolates derived from 60 patients. Following tests were used for the identification of S.aureus:

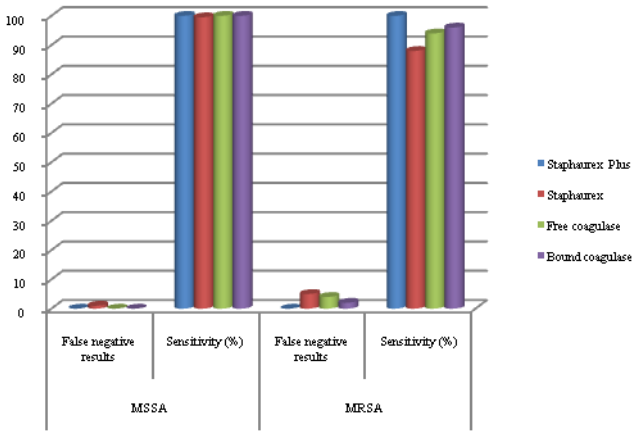
- Free-coagulase (tube) test.
- Bound-coagulase (agar) test
- Staphaurex and Staphaurex Plus
- Pastorex Staphplus

Strains with variable outcomes when the results of the different tests were compared were retested by all procedures mentioned previously and were subsequently studied further with the aid of the additional tests mentioned below. All the tests and procedures were performed as per manufacturer's condition. Typing was performed for all discordant strains and a random sample of strains showing concordant results. All the results were compiled and assessed by SPSS software. Chi-square test and student t test were used for the assessment of level of significance. P-value of less than 0.05 was taken as significant.

RESULTS

100 percent sensitivity was observed only in the Staphaurex Plus group. Inability of tests to identify some MRSA strains correctly resulted in the difference with the free-coagulase test, a bound-coagulase test, and the former Staphaurex test.

Graph 1: Results of different S.aureus identification assays



DISCUSSION

Staphylococcus epidermidis and Staphylococcus aureus are the most common causes of medical device-associated infections, including septicemic loosenings of orthopedic implants.[6, 7] Frequently, the microbiological diagnosis of these infections remains ambiguous, since at least some staphylococci have the capacity to reduce their growth rate considerably.[8] These strains exhibit a small-colony phenotype, and often they are not detectable by conventional microbiological techniques. Moreover, clinical isolates of S. aureus and S. epidermidis adhere to polymer and metal surfaces by the generation of thick, multilayered biofilms consisting of bacteria and extracellular polysaccharides.[9, 10] Hence; we planned the present study to assess current versions of Staphaurex and compared them with other diagnostic tests. These diagnostic tests include free-coagulase test, bound-coagulase test.

In the present study, we observed that staphaurex group exhibited maximum sensitivity. Wu D et al evaluated the epidemiology and molecular features of community-associated methicillin-resistant Staphylococcus aureus and methicillin-sensitive S. aureus (MSSA) from children with skin and soft tissue infections (SSTIs) in Beijing, China, prospective community-acquired S. aureus SSTIs surveillance was conducted at the Beijing Children's Hospital. Susceptibility to 12 antimicrobials was determined by the agar dilution method. Genotypic characteristics of CA-MRSA isolates were tested by SCCmec typing, spa typing, and multilocus sequence typing. Panton-Valentine leukocidin gene was detected. Of 1104 cases, 31.8% (351) were community-acquired S. aureus. CA-MRSA accounted for 4% (14) of S. aureus. Among 14 CA-MRSA and 120 MSSA isolates tested, 100% and 91.7% were multidrug resistant, respectively. ST59-MRSA-IVa-t437 (42.9%) was the most common form of CA-MRSA. Spa typing analysis of 120 MSSA isolates was performed, followed by pulsed-field gel electrophoresis and multilocus sequence typing of a selected number of isolates. The most common spa types among MSSA were t084, t091, t034, t127, t002, and t796. No predominant spa type was seen. Of the MSSA isolates that could be classified into spa-CCs, 15.0% had a genetic background observed in CA-MRSA clones. Panton-Valentine Leukocidin (PVL)-positive community-acquired S. aureus strains were more commonly associated with skin abscesses than other SSTIs.[11]

Berglund C et al performed SCCmec typing (I-IV) of all clinical isolates of MRSA (n = 92) from 1987 to 2004 in Orebro County, Sweden, by real-time LightCycler PCR to detect the essential genetic components mecA, mecR1, IS1272, ccrA and ccrB. Forty-one isolates harboured type IV SCCmec, of which ten could be classified further as subtype IVa, and 27 as subtype IVc. No isolates belonged to subtype IVb, but four isolates could not be subtyped, and may be examples of novel type IV SCCmec subtypes. Thirty-five MRSA isolates, assigned to six different pulsotypes by pulsed-field gel electrophoresis, did not belong to SCCmec types I-IV. The Panton-Valentine leukocidin (PVL) genes were identified in two of these pulsotypes. Only SCCmec type IV has been associated previously with the PVL toxin, but the results suggest that new PVL-positive clones with novel SCCmec types may be arising and disseminating in the

community.[12, 13] Personne P et al designed six commercial agglutination tests for the identification of Staphylococcus aureus were compared by using a strain collection which included 512 staphylococci representing 33 species (318 isolates of Staphylococcus aureus [including 144 oxacillin resistant], 46 S. epidermidis isolates, 15 S. haemolyticus isolates, 12 S. saprophyticus isolates, 29 S. schleiferi isolates, 30 S. lugdunensis isolates, and 62 other coagulase-negative staphylococci). This group also included a proportion of strains with unusual phenotypes (e.g., 19 coagulase-negative S. aureus isolates, 26 clumping factor-negative S. aureus isolates, and 4 S. aureus isolates each with a double deficiency). The overall sensitivity for identification of typical and atypical S. aureus was high with the Staphaurex Plus test (Murex Biotech) (99.7%), the Pastorex Staph Plus test (Sanofi Diagnostics Pasteur) (99.7%), and the Slidex Staph Plus test (bioMérieux) (100%). The overall rate of specificity was affected by the unusual inclusion in this study of a high proportion of non-S. aureus species, such as S. lugdunensis and S. schleiferi, which express a clumping factor and therefore produce a positive result with the agglutination tests.[14]

CONCLUSION

From the above results, the authors concluded that Staphaurex Plus exhibits maximum sensitivity.

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