PREVALENCE OF COAGULASE NEGATIVE STRAINS OF STAPHYLOCOCCUS AUREUS IN CLINICAL SPECIMENS AT THE UNIVERSITY OF BENIN TEACHING HOSPITAL, BENIN CITY, NIGERIA

ABSTRACT

The test for coagulase activity is a simple and non-expensive test for the identification of *S. aureus* strains from clinical samples in resource-poor settings. The results of these tests have been reported to vary with the source of plasma and with some atypicalstrains that test negative with the coagulase test. The study was carried out to determine if misidentification of strains of *S. aureus* exists on account of the reliance on the coagulase tests. Clinical isolates of Staphylococci that tested negative by the coagulase tests from wounds, pus, aspirates, blood cultures andurogenital samples were collected and re-tested by the slide and tube coagulase tests and confirmed to be negative. Each isolate was inoculated onto mannitol sat agar and DNAse agar plates and incubated at 37^{o}C for 18 h. Isolates that fermented mannitol and showed a positive DNAse test were 25/366 (6.8%). The highest number of coagulase-negative *Staphylococcus aureus*(CNSA) occurred within blood culture samples 25.7% and in wound, pus and aspirates, 6.9% and the least proportion, 2.9% from urogenital samples. The generation of CNSA strains is strongly associated with severely-ill patients and the potential for the administration of cell-wall inhibiting antibacterial agents may have important roles to play in the emergence CNSA. The inclusion of DNAse test in the routine identification of staphylococci is advocated especially when an isolate has testedcoagulase-negative.

Key words: *S. aureus*, coagulase test, atypical strains, misidentification,DNAse,

INTRODUCTION

Coagulase-positive isolates of Staphylococci have been referred to as *Staphylococcus aureus*(*S. aureus*).The organism is a gram-positive cluster forming cocci that colonizeup to 60% of healthy individuals [1-4]. Several coagulase positive strains of Staphylococci also colonize a wide variety of primates including those used as pets [5-7]. *S. aureus* has been recognized as an important bacterial agent that is incriminated in several types of human infections that could range from minor soft tissue infectionsto serious sepsis[8-11], deep seatedandlife-threatening conditions[12] including toxin-mediated illnesses [13-15]. *S. aureus*is recognized as a major source of hospital acquired infections, HAIs [16-19].*S. aureus* induced illnesses are some of the most globallyreported that have the tendencies to lead to death[20-21].Most strains of *S. aureus* are capable of producing both types of coagulases, though, occasional strains that produce only the cell-bound coagulase have been reported [22]. Coagulase act on platelets to initiate coagulation of plasma proteins leading to the conversion of fibrinogen to a fibrin clot. [21]. The formation of a fibrin coagulum serves as a protective shield to theorganism against engulfment and phagocytic destruction by the host cells' defense mechanisms, in addition to possessing digestive activity on fibrin that aid the spread of the organism to neighboring tissue cells in conjunction with other endowments of virulent factors of pathogenicity [23]. Atypical strains of *S. aureus* that lack the coagulase gene, coa or where the genes are not expressed or repressed during the log phase of growth by the organism's genetic constitution, misidentification of such strains becomes inevitable. Atypical strains of *S. aureus* irrespective of the loss or repression of the coa gene retain the ability to produce DNase [24].The objective of the study was to determine if coagulase-negative strains *S. aureus* isrecoverable from clinical samples in the study population.

MATERIALS AND METHODS

Consecutive coagulase negative isolates of *S. aureus* that appeared to be significant growth were collected from cultures of wound, pus, aspirates, genital samples and blood cultures.

The isolates were re-tested for coagulase activity by the slide and tube coagulase tests.

Slide Coagulase Test (SCT)

Two separate loopfulsof normal saline were placed in a clean grease-free slide. A thick emulsion of the suspected isolate was made onto each of the slide portions. To one emulsion was added a loopful of citrated human plasma and the other another loopful of normal saline. The emulsions were mixed by gently rotating the slide in a figure '8' fashion. The appearance of granulation or clumping in the emulsion with plasma added within 30 seconds was recorded as positive slide test, if the other emulsion with saline remained smooth or without granulation.

Tube Coagulase Test (TCT)

Every isolate that was SCT negative was further tested by the TCT including all SCT positive isolates to confirm SCT. Each isolate was emulsified into 1.0ml of sterile nutrient broth in a sterile tube and approximately 0.2 mL of citrated human plasma was added. Similarly, a local strain of *S. aureus* was treated as positive control. Both tubes were then incubated at 37^{o}C. The tubes were examined for the presence of a coagulum after 3 hours, if no coagulum was seen, the tubes were further re-incubated and examined at 6 hours and 18 hours for the presence of a coagulum. The presence of a coagulum was recorded as positive TCT with reference to the control.

DNAse Production

Each suspected isolate of Staphylococci that tested negative by both SCT and TCT were further tested by inoculation onto plates ofdeoxyribonuclease agar, DNAse(Oxoid P00128) and mannitol salt agar, MSA (Oxoid CM 0085). A local isolate of *Staphylococcus aureus*that previously tested positive for mannitol fermentation and was also inoculated onto each plate of DNAse agar as positive control organism. The plates were incubated at 37^{o}C 18 h. The DNAseplate was flooded with 1N HCL. Evidence of DNA polymerization was shown by the opacity of the surface of the medium. The production of DNAse by an organism was indicated by a zone of clearing around the colonies of the test organism as in the control organism. This was noted as a positive test.

RESULTS

The total number of strains of coagulase negative *S. aureus* identified were 25 from 366 isolates representing 6.8% as shown in Table 1. The highest number of the strains of *S. aureus* were identified from blood culture samples which was greater than three-folds of the proportion recovered from wound, pus and aspirates that stood (6.9%) for this category of samples. The lowest rate of 2.9% was seen from urogenital samples.

Table 1: Distribution of Coagulase-Negative strains of*S. aureus*within clinical samples andDNAsetest

Nature of Sample	N^{o}. of Cases (%)
Blood cultures (n=35)	9(25.7)
Wound, Pus and Aspirates (n=159)	11(6.9)
Genital/Urinary tract (n=172)	5(2.9)

DISCUSSION

The current study has revealed that as much as 6.8%of Staphylococcus isolates cannot be identified by the use of only the coagulase tests. The distribution of these CNSA strains amongst different categories of specimens revealed that these coagulase-negative strains are more likely to be associated with blood cultures than from other typesof specimens. This may not be unrelated to the fact that these group of patients are by nature very ill and there is always a need to administer antibacterial agents prophylactically pending the outcome of culture and susceptibility tests. The antibacterial agents often chosen are the beta-lactams due to their better tolerability and safety profiles [25-26]. It is important to note that the beta-lactam antibacterial agents act by inhibiting bacterial cell-wall intermediate substrates necessary for transpeptidation steps required for the last cross-linking stage of peptidoglycan synthesis in the bacterial cell-wall [25, 27].Inhibition or repression of cell-wall synthesis therefore, will also ultimately lead to the inhibition of the coagulase gene (coa) or the protein-A that is responsible for the Von-Williebrand factor in the cell-bound coagulase test. This may imply that antibacterial agents that inhibit cell-wall synthesis may be a contributory factor to the generation of CNSA. The recovery rate of the CNSA from wound, pus and aspirates were also markedly lower (6.9%) in comparison to blood culture samples, though with amuch higher recovery rate from urogenital samples. This may infer that CNSA are less likely to be associated with urogenital tract infections and therefore, a lower tendency for prophylactic antibacterial agent administration. The recovery of CNSA from clinical samples is a pointer that theinhibition or repression of the coagulase activity may not be as important for the pathogenesis of*S. aureus*[28]. Although, all isolates from blood culture samples are routinely treated as significant isolates where the chances of contamination have been excluded. The risk however, remains where such isolates are misidentified from specimens, and as a consequence, proper clinical management may not be instituted, thus allowing the organisms to advance the infection process. This is especially more likely when antimicrobial therapy is discontinued and the repression of the coa gene is lifted.

Conclusion

The study highlights the need for inclusion of theDNAse test as a basic test to complement a coagulase-negative test in resource-poor settings to obviate the tendency for misidentification of these strains in clinical specimens

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