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Original Research

To investigate the existence of virulence indicators and the sensitivity of Staphylococci to methicillin in various clinical isolates

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ABSTRACT:
Aim: To investigate the existence of virulence indicators and the sensitivity of Staphylococci to methicillin in various clinical isolates. Material and Methods: This research was an observational study undertaken by the Department of Microbiology. The investigation comprised a total of 120 staphylococcal isolates obtained from different clinical specimens. The isolates were tested to determine the presence of virulence markers. Coagulase, Phosphatase, DNase (Deoxyribonuclease), Hemolysis and SlimeFormation were studied. **Results:** Out of the 120 Staphylococcal isolates, 110 tested positive for coagulase whereas 10 tested negative for coagulase. All individuals expressed phosphatase. 70 isolates exhibited the presence of DNAse, 75 isolates showed hemolysis, and 45 isolates displayed slime formation. All the isolates of sputum and urine that caused haemolysis and produced slime were resistant to methicillin. Phosphates were detected in all of the isolates. No CONS isolates showed evidence of DNAse. All CONS isolates that tested positive for any virulence marker exhibited methicillin resistance. Conclusion: To fully comprehend the nature and development of S. aureus infections, as well as the existing methods for treatment and prevention, it is crucial to consider the quantity and severity of these infections.
Keywords: S. aureus, CONS, Methicillin resistance, Hemolysis

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INTRODUCTION

Staphylococci are a varied collection of bacteria that may cause a range of illnesses, from simple skin infections to potentially fatal bacteremia[1]. Staphylococcus aureus has been recognized as a significant human pathogen since Sir Alexander Ogston first suggested in the 1880s that it was the primary reason for wound suppuration[2]. The organism has the ability to generate a variety of possible virulence factors, including alpha-, beta-, gamma-, and delta-toxins, coagulase, and slime formation. Virulence factors are essential for the establishment of infection in host tissue and for evading the host's immune response. The timely and accurate expression of the virulence factors is crucial for the initiation and continuation of an infection and is a tightly controlled process[3,4]. Staphylococci that produce biofilm often inhabit catheters and medical devices, and may cause infections associated with foreign bodies. This allows them to survive by avoiding the body's defenses and antimicrobial treatments[5,6]. S. aureus strains linked to human infection exhibit diverse combinations of pathogenic determinants/virulence factors, and the presence or expression of these combinations changes depending on the kind of infection and the genetic vulnerability of the afflicted host[7]. While virulence factors have mostly been linked to S. aureus, it has been observed that coagulase negative staphylococci (CONS) isolated from clinical samples also exhibit similar virulence features[8]. Coagulase-negative staphylococci (CONS) are often found in clinical samples and are known to cause significant nosocomial infections, particularly in newborns. They have garnered attention as bacteria responsible for nosocomial infections, particularly those connected to catheters[9,10]. The advent of penicillin and penicillins that are stable against betalactamase enzymes, although significantly enhancing the treatment of staphylococcal infections, have also had

a role in the development of methicillin resistant Staphylococcus aureus (MRSA) strains[11]. MRSA has become a progressively more importanthuman pathogen since its initial description in 1961 and the firstdocumented outbreak of infection in 1968[12]. Numerous clinicalstudies have indicated, based on mortality rates, that methicillinresistant Staphylococcus aureus (MRSA) strains are more virulentthan methicillin- susceptible S. aureus(MSSA) strains[13]. Given thenumber and severity of S. aureus infections it is important tounderstand the nature and pathogenesis of infections and the currentstrategies available for therapy and prevention. Hence, the currentstudy was done to demonstrate some virulence factors in coagulasepositive (COPS) and coagulase negative staphylococcal (CONS)isolates from various clinical samples and their further correlationwith methicillin susceptibility.

MATERIAL AND METHODS

This research was an observational study undertaken by the Department of Microbiology. The investigation comprised a total of 120 staphylococcal isolates obtained from different clinical specimens. The isolates were tested to determine the presence of virulence markers.

Coagulase: The technique published by Quinn et al (1994) was used to assess coagulase activity. This test was conducted as a Tube Coagulase test. Multiple colonies of each organism were combined with 0.5 ml of citrated plasma in a sterile test tube. The tube was placed in an incubator set at a temperature of 37ºC and observed after 4 and 24 hours. Positive clot development was reported at both readings[9].

Phosphatase: The sodium phenolphthalein diphosphate is sterilized by filtering to create a 1% solution in water. 10 milliliters of this solution should be added to 1000 milliliters of nutritional agar that has been chilled to a temperature of 50 degrees Celsius. The mixture should then be poured onto slopes. Phenolphthalein diphosphate agar slopes were inoculated and incubated overnight. Introduce a little amount of ammonia. The test is considered positive when the colonies exhibit a vibrant pink coloration within a short period of time[14].

DNase(Deoxyribonuclease): This test was carried out by usingcommercially available DNase agar (Difco). Spot inoculation wasdone on the DNase agar and incubated at 37ºC. After incubation, 1 NHCl was poured on the agar. Clearing around the bacterial growthwas evaluated as positive[9].

Hemolysis: Blood agar was prepared by adding 7% of sterile humanbloodaseptically to sterile nutrient agar which had been cooled to 45^0C and mixed thoroughly. To test for the production of haemolysin, the plates were streaked with loopfuls from bacterial cultures andincubated at 37^0 C for 24 h. Clear zones around bacterial colonies indicated haemolysin production[8].

Slime Formation: The Congo Red Agar (CRA)method developed by Freeman was used in this study. The composition of medium wasBrain Heart Infusion Broth (BHIB) 37 g/l, sucrose 50 g/l, agar 10 g/land Congo red 0.8 g/l. Isolates which produced black colonies withdry crystalline consistency were regarded as slime positive, whereasthose showing pink colonies were slime negative[9].The methicillin susceptibility was determined using the cefoxitin disc diffusion technique in accordance with the recommendations set by the Clinical and Laboratory Standards Institute (CLSI). Statistical analysis: Descriptive statistics were used to compute various variables in the table and organize them in a systematic manner. The crosstabs techniques are used to determine the relationship between different tests and measurements in two-way and multi-way tables. The chi-square test is used for the purpose of tabulating data. The results were subjected to statistical analysis using SPSS version 22.0.

RESULTS

Out of the 120 Staphylococcal isolates, 110 tested positive for coagulase whereas 10 tested negative for coagulase. All individuals expressed phosphatase. 70 isolates exhibited the presence of DNAse, 75 isolates showed hemolysis, and 45 isolates displayed slime formation. The presence of virulence markers in different isolates among COPS is shown in Table 1. All the isolates of sputum and urine that caused haemolysis and produced slime were resistant to methicillin. The relationship between methicillin resistance and other virulence indicators among coagulase positive staphylococcal isolates is shown in Table 2. Phosphates were detected in all of the isolates. No CONS isolates showed evidence of DNAse. Table 3 shows the distribution of additional virulence variables among CONS. All CONS isolates that tested positive for any virulence marker exhibited methicillin resistance. A statistical correlation analysis was conducted to examine the relationship between methicillin resistance and other virulence indicators. No statistical significance was seen in the Coagulase and DNAse markers. A statistically significant link was found between the virulence markers haemolysin and slime formation in both coagulase positive and negative Staphylococci that were resistant to methicillin.

Table 1: Distribution of various virulence markers among COPS

Isolates	Number =110	Haemolysin=68	DNAse=70	Slime formation=41
Exudate	50	24	33	6
Blood	45	29	22	19
Sputum	10	10	10	10
Urine	5	5	5	5

Table 2: Methicillin resistance among COPS isolates and in various virulence markers expressing groups

Isolates	Number =110	Haemolysin	DNAse	Slime formation
Exudate	50	21	30	5
Blood	45	25	18	18
Sputum	10	10	7	10
Urine	5	5	3	5

Table 3: Distribution of various virulence markers among CONS isolates

Isolates	Number	Haemolysin	Slime formation
Exudate	5	3	2
Blood	3	2	2
Urine	2	2	0

DISCUSSION

This research aimed to illustrate the different virulence factors present in Staphylococcal isolates obtained from clinical specimens. Additionally, the investigation examined the association between these factors and the sensitivity of the isolates to methicillin. Several biochemical processes are believed to contribute to the virulence of pathogenic staphylococci[9]. In the laboratory, the pathogenicity of Staphylococcus spp. was assessed based on criteria such as coagulase activity, phosphatase activity, DNAse activity, hemolysis, and slime production. Our analysis found that coagulase was expressed in 110 isolates, whereas phosphatase was detected in all isolates. The Staphylococcal isolates were classified into two groups, coagulase positive and coagulase negative, for further investigation.A higher expression of virulence markerswas seen in coagulase positive staphylococci. These results wereparallel with other studies[15, 16].Citak et al[17] reported that 704 of851 Staphylococci isolates from milk samples were S. aureus. Thesefindings correlated with our study.

Damage to host cells is in part mediated by staphylococcalhaemolysins, which contribute importantly to virulence in S. aureus.Turkyilmaz and Kaya[9] had earlier found a comparable rate of58.9% in S. aureus while the rate for CONS (28.9%) wascomparatively lower. Testing for biofilm formation is another usefulmarker of the pathogenicity of staphylococci. Thisis because biofilmcolonization by staphylococci facilitates infections that are oftendifficult to treat and therefore engender high morbidity andmortality[18, 19]. Many workers have reported that bacteria growingin a biofilm can be up to 1,500 times more resistant to germicidesthan the same bacteria growing in liquid culture[18]. The result is inaccordance with Akinkunmiet al.[8] which found slime formation36% in COPS and 32.8% in CONS.

The impact of methicillin resistance on the mortality of variousinfections remains controversial. In our study, 90 out of 120Staphylococcal isolates were methicillin resistant.Since phosphatase wasexpressed byall, it could not be considered as a significant virulence marker ofmethicillin resistance. Statistically significant relation was seen invirulence factors hemolysis and slime formation when correlated withmethicillin resistance. Several studies have attempted to compare theoutcome of nosocomial acquired and MRSA infections[20].Three studies have observed similar mortality rates in patients who have MRSA and MSSA bacteremia[21-23].Incontrast,3 other studies have reported that methicillin resistance is a significant and independent risk factor for death in patients who have episodes of S.aureus bacteremia [24-26]. Some authors have observed a higher incidence of bloodstream infection with MRSA as compared with MSSA in humans while some have reported that nosocomialMRSA isolates produce significantly more antiphagocyticcoagulase than do methicillin- sensitive stains[27,28].

CONCLUSION

To fully comprehend the nature and development of S. aureus infections, as well as the existing methods for treatment and prevention, it is crucial to consider the quantity and severity of these infections.

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