Original Research Article

Demonstration of Virulence Markers and Methicillin Susceptibility of *Staphylococci* in Various Clinical Isolates at SKMC&H, Muzaffarpur, Bihar, India

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Abstract

Introduction: The staphylococci are a diverse group of bacteria that cause diseases ranging from minor skin infections to life-threatening bacteremia. The organism can produce an array of potential virulence factors such as alpha-, beta-, gamma- and delta-toxins, coagulase and slime formation. Hence, the current study was done to demonstrate some virulence factors in coagulase positive (COPS) and coagulase negative staphylococcal (CONS) isolates from various clinical samples and their further correlation with methicillin susceptibility. **Methodology:** This current study was an observational study conducted by Department of Microbiology of Shri Krishna Medical College & Hospital, Muzaffarpur, Bihar during a study period of 6 months from July 2020 to December 2020. A total of 100 staphylococcal isolates from various clinical specimens were included in the study. The isolates subjected to the following tests to demonstrate the virulence markers: Coagulase, Phosphatase, DNase (Deoxyribonucleic), Hemolysis, Slime Formation. Methicillin susceptibility was done by cefoxitin disc diffusion method as per CLSI guidelines. Descriptive statistics was applied to calculate different variables in the table and arrange them in order. Results were statistically analyzed by using SPSS for Windows (version16.0).**Results:** Of the 100 Staphylococcal isolates, 92 were coagulase positive and 8 coagulase negative. Phosphatase was expressed by all. DNAse was observed in 59, hemolysis in 60 and slime formation in 34 isolates. Statistical correlation was carried out between methicillin resistance and various virulence markers. Statistical significance was not observed in Coagulase and DNAse markers. Statistically significant correlation was observed between virulence markers haemolysin and slime formationin both coagulase positive and negative Staphylococci with methicillin resistance. **Conclusion:** Given the number and severity of S. aureus infections it is important to understand the nature and pathogenesis of infections and the current strategies available for therapy and prevention.

Keywords: Virulence Markers, Methicillin Susceptibility, Staphylococci.

Introduction

The staphylococci are a diverse group of bacteria that cause diseases ranging from minor skin infections to life-threatening bacteremia[1]. Staphylococcus aureus has been recognized as a major human pathogen ever since Sir Alexander Ogston first proposed, in the 1880s, that it was the major cause of wound suppuration[2]. The organism can produce an array of potential virulence factors such as alpha-, beta-, gamma- and delta-toxins, coagulase[3] and slime formation. Virulence factors are required for colonization of host tissue and for protection against the host defense. Timely correct expression of the virulence factors is essential for the establishment and maintenance of an infection and represents a highly regulated process[4]. Biofilm producing Staphylococci frequently colonize catheters and medical devices and may cause foreign body related infections[5] enabling it to persist by evading host defenses and antimicrobials[6].

S. aureus strains associated with human infection have variable combinations of pathogenic determinants/virulence factors and either the presence or the expression of given combinations varies depending on the type of infection and genetic susceptibility of the affected host[7]. Although virulence factors have been associated mainly with S. aureus, the coagulase negative staphylococci(CONS) isolated from clinical specimens have been reported to also express these virulence factors[8]. CONS are commonly isolated in clinical specimens and several species are recognized as important agents of nosocomial infections, especially in neonates[9] and have gained substantial interest as pathogens involved in nosocomial, particularly catheter-related infections[10]. The introduction of penicillin and betalactamase stable penicillins, although dramatically improving the management of staphylococcal infection, have also contributed to the emergence of methicillin resistant Staphylococcus aureus (MRSA) strains[11]. MRSA has become a progressively more important human pathogen since its initial description in 1961 and the first documented outbreak of infection in 1968[12]. Numerous clinical studies have indicated, based on mortality rates, that methicillin resistant Staphylococcus aureus (MRSA) strains are more virulent than methicillin- susceptible S. aureus (MSSA) strains[13]. Given the number and severity of S. aureus infections it is important to understand the nature and pathogenesis of infections and the current strategies available for therapy and prevention. Hence, the current study was done to demonstrate some virulence factors in coagulase positive (COPS) and coagulase negative staphylococcal (CONS) isolates from various clinical samples and their further correlation with methicillin susceptibility.

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Methodology

This current study was an observational study conducted by Department of Microbiology of Shri Krishna Medical College & Hospital, Muzaffarpur, Bihar during a study period of 6 months from July 2020 to December 2020. A total of 100 staphylococcal isolates from various clinical specimens were included in the study. The

isolates subjected to the following tests to demonstrate the virulence markers:

Coagulase: Coagulase activity was determined by the method described by Quinn et al (1994). This test was performed as a Tube Coagulase test. Several colonies of each organism were mixed with 0.5 ml of citrated plasma in a sterile test tube. The tube was incubated at 37ºC and examined after 4 and 24 h. Clot formation at either reading was recorded as positive[9].

Phosphatase: 1% aqueous solution of sodium phenolphthalein diphosphate sterilized by filtration. 10 ml of this solution added to 1000ml of nutrient agar cooled to 50ºC and poured into slopes. Phenolphthalein diphosphate agar slopes inoculated and incubated overnight. A few drops of ammonia added. Test read positive when the colonies turn bright pink within a few minutes[14].

DNase(Deoxyribonuclease): This test was carried out by using commercially available DNase agar (Difco). Spot inoculation were done on the DNase agar and incubated at 37ºC. After incubation, 1 N HCl was poured on the agar. Clearing around the bacterial growth was evaluated as positive[9].

Hemolysis: Blood agar was prepared by adding 7% of sterile human blood aseptically to sterile nutrient agar which had been cooled to 45^0 C and mixed thoroughly. To test for the production of haemolysin, the plates were streaked with loopfuls from bacterial cultures and incubated at 37 oC 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 was Brain Heart Infusion Broth (BHIB) 37 g/l, sucrose 50 g/l, agar 10 g/l and Congo red 0.8 g/l. Isolates which produced black colonies with dry crystalline consistency were regarded as slime positive, whereas those showing pink colonies were slime negative[9].

Methicillin susceptibility was done by cefoxitin disc diffusion method as per CLSI guidelines. Statistical analysis: Descriptive statistics was applied to calculate different variables in the table and arrange them in order. The crosstabs procedures applied for two-way and multi-way tables to know the association between various tests and measures. Chi-square test procedures used for tabulation. Results were statistically analyzed by using SPSS for Windows(version16.0).

Results

Of the 100 Staphylococcal isolates, 92 were coagulase positive and 8 coagulase negative. Phosphatase was expressed by all. DNAse was observed in 59, hemolysis in 60 and slime formation in 34 isolates.

The distribution of virulence markers in various isolates among COPS has shown in Table 1. All the haemolytic and slime forming isolates of sputum and urine were methicillin resistant. Correlation of methicillin resistance with various virulence markers among coagulase positive staphylococcal isolates is depicted in Table 2.

Phosphates was demonstrated in all the isolates. DNAse was not demonstrated in any CONS isolates. The distribution of other virulence factors among CONS is shown in Table 3. All the isolates of CONS who were positive for one or another virulence marker showed methicillin resistance.

Statistical correlation was carried out between methicillin resistance and various virulence markers. Statistical significance was not observed in Coagulase and DNAse markers. Statistically significant correlation was observed between virulence markers haemolysin and slime formation in both coagulase positive and negative Staphylococci with methicillin resistance.

Table 1: Distribution of various virulence markers among COPS isolates

Isolates	Haemolysin	DNAse	Slime formation
Exudate (40)	19	27	11
Blood (39)	24	19	18
Sputum (9)	9	9	9
Urine (4)	4	4	4

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

Isolates	Haemolysin	DNAse	Slime formation
Exudate (40)	15	26	10
Blood (39)	21	13	15
Sputum (9)	9	7	9
Urine (4)	4	3	4

Table 3: Distribution of various virulence markers among CONS isolates

Isolates	Haemolysin	Slime formation
Exudate (4)	2	1
Blood (3)	1	1
Urine (1)	1	0

Discussion

In this study, various virulence factors of Staphylococcal isolates from clinical specimens were demonstrated and their further correlation with methicillin susceptibility was observed. A number of biochemical activities are considered to contribute to the virulence of pathogenic staphylococci[9]. Coagulase activity, phosphatase, DNAse, hemolysis, and slime formation of the Staphylococcus spp. were regarded as pathogenicity criterions in laboratory. In our study, coagulase was expressed in 90 isolates and phosphatase in all. Staphylococcal isolates were grouped into coagulase positive and negative for further study. A higher expression of virulence markers were seen in coagulase positive staphylococci. These results were parallel with other studies[15, 16].Citak et al[17] reported that 704 of 851 Staphylococci isolates from milk samples were S. aureus. These findings correlated with our study.

Damage to host cells is in part mediated by staphylococcal haemolysins, which contribute importantly to virulence in S. aureus. Turkyilmaz and Kaya[9] had earlier found a comparable rate of 58.9% in S. aureus while the rate for CONS (28.9%) was comparatively lower. Testing for biofilm formation is another useful marker of the pathogenicity of staphylococci. This is because biofilm colonization by staphylococci facilitates infections that are often difficult to treat and therefore engender high morbidity and mortality[18, 19]. Many workers have reported that bacteria growing in a biofilm can be up to 1,500 times more resistant to germicides than the same bacteria growing in liquid culture[18]. The result is in accordance with Akinkunmi et al.[8] which found slime formation 36% in COPS and 32.8% in CONS.

The impact of methicillin resistance on the mortality of various infections remains controversial. In our study, 75 out of 100 Staphylococcal isolates were methicillin resistant.Statistical analysis of the above data wascarried out. Since phosphatase wasexpressed by all, it could not beconsidered as a significant virulencemarker of methicillin resistance. Statistically significant relation wasseen in virulence factors hemolysis andslime formation when correlated with methicillin resistance.Several studies have attempted to compare the outcome of nosocomially acquiredMSSA and MRSA infections[20]. Three studies haveobserved similar mortality rates in patientswho have MRSA and MSSAbacteremia[21-23]. In contrast, 3 other studies have reported thatmethicillin resistance is a significant and independent risk factor for death inpatients who have episodes of S. aureus bacteremia[24-26].Some authors have observed a higher incidence of bloodstream infection withMRSA as compared with MSSA in humans[27] while somehave reported that nosocomial MRSA isolates produce significantly moreantiphagocytic coagulase[28] than do methicillin- sensitive stains. Jordens et al.[29] reported that enterotoxin A was producedby MRSA but not by MSSA. Therefore, differences in microbial virulence remain controversial, but they might have contributed to the results observed in the study.

Conclusion

Given the number and severity of S. aureus infections it is important to understand the nature and pathogenesis of infections and the current strategies available for therapy and prevention.

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