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Detection of Inducible Clindamycin Resistance and Methicillin Resistance in *Staphylococcus species* from Various Clinical Samples

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Abstract: ***Staphylococcus species is an important cause of nosocomial and community acquired infections worldwide. Clindamycin is an alternative agents used to treat Staphylococcal infections. Accurate identification of clindamycin resistance is important to prevent therapeutic failure. Unfortunately, inducible CL resistance is not detected by standard susceptibility tests. The aim of the present study was to detect the prevalence of inducible clindamycin and methicillin resistance among clinical isolates of Staphylococcalspecies via antibiotic sensitivity test. Total 129 Staphylococcal isolates were tested for antimicrobial susceptibility testing by as per guidelines. For detection of MRSA cefoxitin disc and for inducible clindamycin resistance, D test was performed. Out of 129 samples, 101 wereStaphylococcus aureus and 28 were Coagulase negative Staphylococcus(CoNS).Out of which 61.3% were MRSA and 38.6% were MSSA. Inducible clindamycin resistant was detected in 30.6 % and MS phenotype in 42.7%. D-test should be routinely performed for every Staphylococcus isolates otherwise clindamycin resistance may misinterpreted as clindamycin sensitive resulting in therapeutic failure.***

Keywords: Staphylococcus aureus, Clindamycin resistance, MRSA

1. Introduction

Staphylococcus species are most common pathogen responsible for various nosocomial and community acquired infections [1]. 30% of normal healthy population asymptomaticallycolonized *Staphylococcus aureus*[2].They can produce a wide spectrum of disease starting from superficial skin infection,invasive disease to toxin mediated life threatening conditions [3]. Foreign materials such as indwelling catheters,implanted joints and sutures are very much susceptible to *Staphylococcus epidermidis* which are commonly colonized over them and act as their point of entry of the infection. *Staphylococcus epidermidis* are resistant to various antibiotics due to formation of biofilms. They are also served as reservoir for antibiotic resistant genes which can be transferred to other bacteria [4]. Other than *Staphylococcus aureus*, species of Staphylococcus group are collectively referred as Coagulase negative Staphylococcus (CoNS). A special strain of Staphylococcus emerge as antibiotic resistant refer as Methicillin resistant Staphylococcus aureus (MRSA). This strain expressed a modified penicillin binding protein (PBP-2a) encoded by *mec A*gene and is present in 4 forms of *Staphylococcus*cassette causes resistance to all ß-lactam antimicrobial agents. As Methicillin is an unstable drug, Cefoxitin is used for sensitivity testing. Cefoxitin resistance correlates with the presence of *mecA*gene present in all MRSA strain [5].

Methicillin resistance *Staphylococcus aureus* (MRSA) is an increasing problem day by day [6].Clindamycin is an excellent pharmacokinetics agents and useful as alternative treatment option for patients who are allergic to Penicillin for treatment of localised as well as systemic infections caused by drug resistant *Staphylococcus aureus*[7]. Due to indiscriminate use of Macrolides, Lincosamide and group B Streptogramins which have a common binding sitecross resistance resulting in therapeutic failure of Clindamycin. It is also an alternative choice for MRSA due to its excellent pharmacokinetics properties[8]. Clindamycin resistance in *Staphylococcus species* may be constitutive or inducible .Most common mechanism is target site modification by *erm* genes. It may express by either constitutively or inducible.In routine laboratory practise it is difficult to detect inducible clindamycin resistance if the disc is not placed adjacent to each other with maintenance of proper distance. Then in vitro the result will be erythromycin resistant and clindamycin sensitive but in vitro therapy clindamycin may select *erm* mutants leading to clinical therapeutic failure [9].

This study was conducted to investigate the inducible Clindamycin resistance and Methicillin resistance in *Staphylococcus* species from different clinical samples via Antibiotic Sensitivity Test (AST) with various antibiotics.

2. Material and methods

The present prospective study was conducted at Microbiology department of a teaching hospital Nadia, West Bengal, during April to June 2019.A total 129 *Staphylococcus species* were isolated from various type of clinical specimen such as pus, wound swab, aspirares, blood, urine and sterile fluids were tested. All samples were inoculated into blood agar and Mac Conkey agar and overnight incubation done at 37^0C .Then colony morphology were studied by gram stain and all gram

positive cocci were tested for catalase (3%) test and identified as *Staphylococcus species*. Further slide and tube coagulase was performed to differentiate between Staphylococcus aureus and Coagulase negative Staphylococcus (CONS). All the isolates were further tested by standard biochemical techniques [10].The antibiotic susceptibility test was performed in Mueller –Hinton agar plate and evaluation done by Clinical and laboratory standard institute guideline (CLSI)[11]. The isolates were tested for cefoxitin (30µg), clindamycin (2 µg), erythromycin (15µg), linezolide(30µg), mupirocin(5µg), flurazolidone (50µg).The inhibition zone of 22mm or less around cefoxitin disc indicates MRSA.

Inducible clindamycin resistance was tested by 'D test' as per CLSI guideline. Test was performed in Mueller –Hinton agar plate which was inoculated with 0.5 McFarland standard bacterial suspensions.Then placement of erythromycin disc (15µg) at a distance of 15 mm(edge to edge) from clindamycin (2 µg) was done.Plate was incubated at 37^0C overnight.Flattening of zone (D shaped around clindamycin in the area between two discs indicated inducible clindamycin resistance[11] [Fig-1].

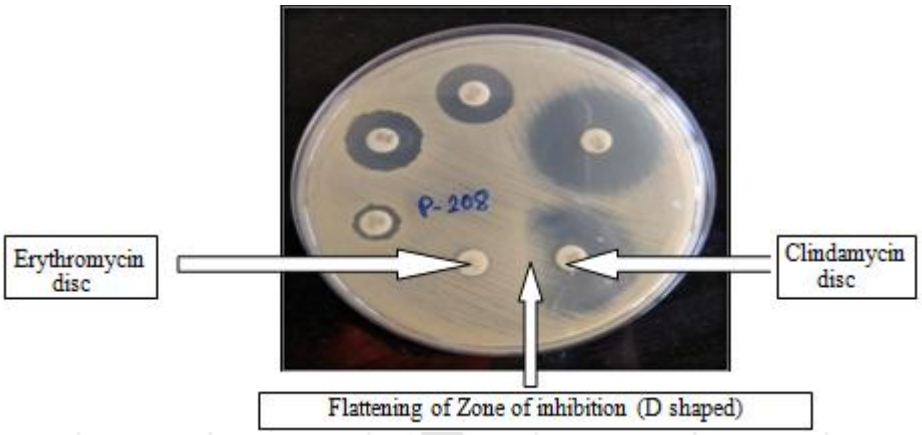


Figure 1: Inducible clindamycin resistant (Positive D test)

3. Result

Out of total 1938 samples, 129 (6.65 %) clinical isolates of *Staphylococcus species*were obtainedduringthe study period. Among these 129 samples, 11 (8.5%) samples were from urine, 31(24%) samples were from blood and 87 (67.4 %) samples were from pus. Distribution of Staphylococcus species isolates of various clinical samples are shown in Fig-2.

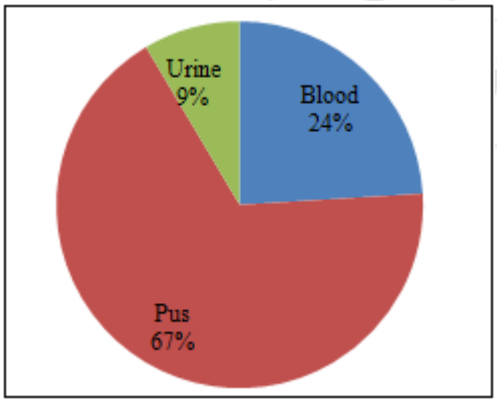


Figure 2: Distribution of isolates in sample

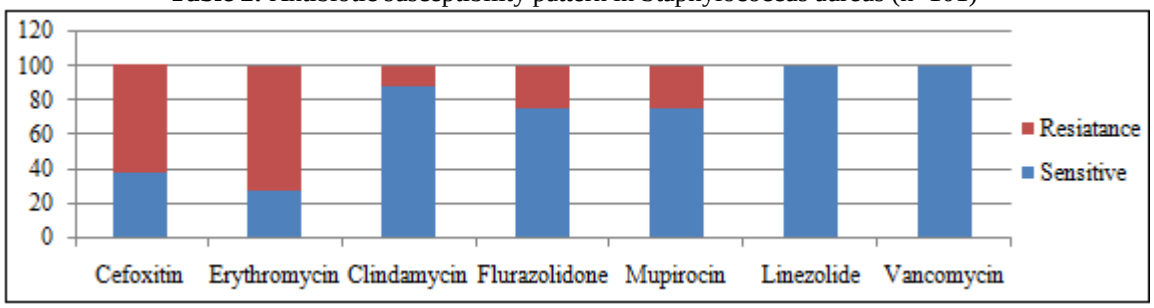
Therefore, from total 129*Staphylococcus species*, 101 (78.29 %) were *Staphylococcus aureus* and 28 (21.7 %) were CoNS.Out of 101 samples of *Staphylococcus aureus*, 62 (61.3 %) were MRSA, 39 (38.6%) were MSSA. Among 28 isolates of CoNS 22 (78.5%) were methicillin sensitive and 6 (21.4%) were methicillin resistant.

Out of 101 isolates, samples yielded (inducible MLS_B phenotype) D-test positive, in *Staphylococcus aureus* were 31 (30.6 %) and D-test negative isolates (MS-phenotype) were 43(42.5%), Constitutive MLS_B phenotype were seen 11(10.8 %). Both erythromycin and clindamycin sensitive were 16 (15.8%). Percentage of inducible resistance was higher [Table-1]. Positive D-test (inducible MLS_B phenotype) was not observed in case of CoNS. Predominantly the isolates from inducible clindamycin resistance were from female patients were 74.1% as compare to male 25.8%

Table 1: Distribution of isolates

Susceptibility Pattrn (PHENOTYPE)	MRSA (%)	MSSA (%)	Total (%)
ERY-S, CL-S	8 (7.9)	8 (7.9)	16 (15.8)
ERY-R, CL-R (Constitutive MLS_B)	11 (10.8)	0	11 (10.8)
ERY-R, CL-S (D+Test positive $iMLS_B$)	19 (18.8)	12(11.8)	31 (30.6)
ERY-R, CL-S (D-Test negative, MS)	24 (23.7)	19 (18.8)	43 (42.5)
TOTAL	62 (61.5)	39 (38.6)	101

Table 2: Antibiotic susceptibility pattern in Staphylococcus aureus (n=101)



The above table showed the antibiogram of gram positive *Staphylococcus aureus* (n=101) .Out of 101 isolates of *Staphylococcus aureus*37.2% were Cefoxitinsensitive and 62.7% were resistant. All isolates were sensitive to vancomycin (100%) and linezolide (100%) followed by clindamycin (88.1%) and flurazolidone (76.2%) and mupirocin (76.2%). It was also observed that linezolide and vancomycin also was effective against MRSA.

4. Discussion

The worldwide remarkable challenge for public health is the emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA). Based on Centers for Disease Control (CDC) reports, 1% of all Staphylococcal infections and 50 % of healthcare-associated Staphylococcal infections are caused by MRSA[12]. It is now the common hospital acquired pathogen in many countries. Infection due to MRSA is significant cause of mortality and morbidity across world. Early detection of MRSA and formulation of effective antibiotic policy has tremendous importance [13].

For determining appropriate therapeutic regimens, accurate detection of antimicrobial resistance in a microbe is an essential factor. This is particularly important considering the increase of resistance and the emergence of multi-drug resistant organisms. The emergence of resistant to multiple antibiotics among gram-positive cocci has left very little therapeutic options for clinicians. The increase in frequency of Staphylococcal infections among patients, and changes in antimicrobial resistance patterns have led to renewed interest in the use of clindamycin therapy [14,15].

Clindamycin (Lincosamide) has long been an attractive option to treat skin, soft tissue and bone infection due to its efficacy against Methicillin Sensitive *Staphylococcus aureus*(MSSA) and Methicillin Resistance *Staphylococcus aureus* (MRSA) for its good bone marrow and tissue penetration and potential antitoxin effects. In fact, it accumulates in abscesses and no renal dosing adjustments areneeded.However, among clinical isolates there has also been a considerable increase in resistance to clindamycin including inducible resistance. The differentiation of inducible MLS_B ($iMLS_B$phenotype) isolates from isolates with (MS phenotype) resistance is a critical issue because of the therapeutic implications of using clindamycin to treat a patient with aninducible clindamycin-resistant *Staphylococcus aureus* isolate[15,16].

In our study majority of the isolates of Staphylococcus aureus were resistant to erythromycin (72.2%) and sensitive to clindamycin (88.1%) which is higher than (15.7% & 28.4%) two studies reported in literature [17,8].

Rate of isolation of MRSA (61.3%) and MSSA (38.6%) in our study is similar with one study conducted by Lyall KDS et al [9]Vivek et al ,Fasihet et al and Cetin et al also reported 32.5 %, 36% and 91% MRSA among Staphylococcus aureus [18,19,20,]. The result indicates non-judicious use of cloxacillin in health care set up.

In our study,inducible clindamycin resistance seen in 30.6% isolates which is resembles with the results of two studies (37.5% and 33.3%)[8,20] and lower rate also found in two studies (10.5% and 13.1%) reported by others.[16,21] Inducible clindamycin resistance among MRSA and MSSA are18.8% and 11.8%. Few studies showed higher inducible resistance in MRSA[17,8] and MSSA[22,23] .These result indicates that inducible clindamycin resistant phenotype may vary in different hospital set up.

Accurate susceptibility data are important for appropriate therapy decisions. The pattern of macrolide resistance in *S. aureus* varies in different regions. Depending upon this the prescription rate will not be uniform in different regions. There is no substantial data regarding clindamycin prescription from India. It is kept as a reserve drug and is usually advocated in severe in-patient MRSA infections depending upon the antimicrobial susceptibility results. Further, the proper use of clindamycin in MRSA, can reduce the use of vancomycin (glycopeptide)[4,8].

Accurate result can be achieved by antimicrobial susceptibility testing including the application of D-test. Thus D-test guide the clinicians in the use of clindamycin, as clindamycin it is not suitable drug for D-test positive isolates.

5. Conclusion

The rate of prevalence of inducible clindamycin resistance may differ from hospital to hospital.Accurate drug susceptibility data is essential to avoid indiscriminate usage of antibiotics on trial and error basis. All *Staphylococcus* isolates should be checked for inducible clindamycin resistance. In case of positive D-test, it can cause therapeutic failure and in case of negative D-test it confirms the susceptibility to clindamycin. Thus, enables us to provide guideline for the judicial use of antibiotic therapy for clinician.MRSA are also checked to find out the effectiveness of the drug and proper use of clindamycin in

MRSA can reduce the use of vancomycin and non-judicious use of glycopeptides.

So it can be concluded from our study that D-test should be routinely performed for every *Staphylococcal*isolates otherwise clindamycin resistance may misinterpreted as clindamycin sensitive resulting in therapeutic failure.

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