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Prevalence of Inducible Clindamycin Resistance in Methicillin Resistant *Staphylococcus aureus* Isolated from Different Clinical Samples Received in a Tertiary Care Hospital

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Abstract:
Staphylococcus aureus is recognized as greatest concern associated with both hospital and community acquired infections and if it is methicillin resistant then the severity increases. Erythromycin and clindamycin are considered as treatment of decision. However, protection from erythromycin with phony susceptibility to clindamycin *in vitro* may prompt remedial disappointment. Hence it is mandatory to study the prevalence of inducible clindamycin resistance. Out of the 875 clinical isolated samples, 403 (46.05%) showed presence of *S. aureus*. Out of theses 403 samples, 297 (73.70%) were found to be methicillin resistant *S. aureus* (MRSA) and 106 (26.30 %) were found to be methicillin sensitive *S. aureus* (MSSA). Further MRSA (Methicillin Resistant *S. aureus*) samples were analyzed for erythromycin and clindamycin sensitivity and resistivity. Minimum Inhibitory Concentration (MIC) of clindamycin among D test (Disc test) positive MRSA was also analyzed. The occurrence rate of D test positive MRSA strain was found to be more in Hospital Acquired Infections (HAI) as compared with Community Acquired Infection (CAI). Frequency of D test positive MRSA strains were more in Pus samples as compared with urine and blood samples. Further in case of HAI, D test positive MRSA was predominantly found in patients with Diabetic foot patients with postoperative wound and patients with Necrotizing Fasciitis. Out of samples associated with uropathogenic infection in hospital settings, More D test positive MRSA were found to be associated in patients with catheter installation in without catheter installation. Prolonged hospital stay (>5 days) was observed to be major risk factor for D test positive MRSA. Demographic profile of patients with D test positive MRSA strains revealed the predominance of male in comparison to female.

Keywords: *Staphylococcus aureus*, MRSA, Catheter, D test, Clindamycin, Erythromycin, MIC, VAP, HAI, CAI.

INTRODUCTION

Staphylococcus aureus is ubiquitous organismand known to causes a variety of infections, ranging from skin and soft tissue infections to life threatening endocarditis (Fiebelkorn et al., 2003; Singh et al., 2019). It is reported to be important food borne pathogens which produce staphylococcal enterotoxins (Argudin et al., 2010) and one of the most common bacteria infecting man (Ryan, 2004). This pathogen has been detected from some ready-to-eat foods, such as vegetables salads etc. (Aggarwal et al., 2020; Kumar et al., 2020). In last few years, *S. aureus*is known to cause of hospital acquired and community acquired infection and has emerged as a major problem of public health importance (Gangurde et al., 2014; Upadhyay, 2016a,b). Initially penicillin was drug of choice to treat *S. aureus* infections, but indiscriminate use of penicillin led to production of resistant strains which are now known as Methicillin Resistant *S. aureus* (MRSA) and was first reported in 1961 (Lim et al., 2002). Methicillin resistanceis usually conferred by altered penicillin bindingprotein (PBP-2a) that causes resistance to all β-lactam antimicrobial agents (Fishovitz et al., 2014). Penicillin resistance has led to renewed interest in the use of Macrolide Lincosamide Streptogramin B (MLSB) antibiotics totreat such infections (Drinkovic et al., 2001). However, their widespread use has led to an increase in the number of *Staphylococcus* strains resistant to MLSB antibiotics and lead to the emergence of MLSB resistant strains (Lim et al., 2002; Fokas et al., 2005).

Erythromycin (a macrolide) and clindamycin (a lincosamide) address two specific classes of antimicrobials that exhibit its action by binding to the 50s ribosomal subunit of microorganisms to stops its protein synthesis. Macrolide resistance in *S. aureus* is by differing mechanisms. The insurance from macrolide can develop by efflux part, customarily interceded by *msr*A gene. Another segment is through *erm* gene, which encodes agents that give inducible or constitutive insurance from macrolide, lincosamide streptogramin type B (MLSB) (Laclercq, 2002). This resistance mechanism can be constitutive, where rRNA methylase is continually made (cMLS B) or can be inducible where methylase is conveyed particularly inside seeing an actuating master (iMLS B). Erythromycin is a convincing inducer while clindamycin is a slight inducer. *In vitro*, *S. aureus* separates with constitutive check are impenetrable to both erythromycin and clindamycin; however those with inducible resistance are impenetrable to erythromycin and appear to be sensitive to clindamycin (iMLS B) (Drinkovic et al., 2001; Kumar and Upadhyay, 2016). If clindamycin is used for treatment of such an isolate (iMLS B), determination for constitutive *erm* mutants happens which may prompt clinical disappointment. This inducible MLSB restriction can be recognized by a simple disc test, typically known as D test (Double disc test). For this test, an erythromycin circle is put 15-26mm (edge to edge) from a clindamycin plate in a standard disc diffusion test (Upadhyay, 2016a,b; Kumar et al., 2018; Upadhyay et al., 2019). Following incubation, a smoothing of the zone in the domain between the plates where the two prescriptions have diffused showed that microorganisms have inducible clindamycin resistance (Gupta et al., 2009). Importance of D test in finding clindamycin resistance in MRSA strains is well reviewed (Sedighi et al., 2009). In recent years, the prevalence of MRSA is rising and it has been accounted for a higher death rate than the disease caused by the methicillin-sensitive *S. aureus* (MSSA) strains, and it's becoming problem to treat infection caused by MRSA strains (Hurley, 2002). Hence, it is essential to investigate the predominance of MRSA in population at the different levels to help compelling anticipation and control techniques (Singh et al., 2020). In this way present investigation was taken for detection of erythromycin induced clindamycin resistance among MRSA isolates obtained from various clinical samples.

MATERIALS AND METHODS

a. Study approach and design

It was a prospective laboratory based four months study with cross sectional design. The study was conducted in the Department of Microbiology (SRL Laboratory) of Fortis Hospital, Faridabad during January – April, 2019.

b. Sample size

Sum of 100 clinical isolates of Methicillin resistant *S. aureus* (MRSA) obtained from different clinical samples (like pus, wound swab, aspirates, blood, and sterile fluids) that were received for routine

culture in the Department of Microbiology. The clinical isolates of methicillin resistant *S. aureus* were included in the study however the bacterial strains or isolates other than methicillin resistant *S. aureus* excluded during investigation.

c. Identification and screening of MRSA isolates

The bacterial isolates were identified as *S. aureus* by standard biochemical techniques. The zone of inhibition 21mm or less around the disc of cefoxitin (30μg) indicated Methicillin resistant *S. aureus* (MRSA) and confirmed through Vitek-2 results.

d. Antibiotic susceptibility test

The antibiotic sensitivity testing for MRSA was performed on MuellerHinton agar plates by modified Kirby Bauer's disc diffusion as per guidelines of Clinical and Laboratory Standards Institute (CLSI) interpretation was done only for Erythromycin resistant strains. All the erythromycin sensitive isolates were excluded from the study. There were three different phenotypes were appreciated after testing and interpreted in detail.

i. Methicillin Sensitive* (*MS) phenotype: The MRSA isolate exhibiting resistance to erythromycin (zone size ≤13 mm) while sensitive to clindamycin (zone size ≥21mm) and giving circular zone of inhibition around Clindamycin was labeled as having this phenotype.

ii. Inducible Macrolide Lincosamide Streptogramin (iMLS) phenotype: The MRSA isolates showing resistance to erythromycin (zone size ≤13mm) while being sensitive to clindamycin (zone size ≥21mm) and giving D-shaped zone of inhibition were labeled as iMLS phenotype.

iii. Constitutive Macrolide Lincosamide Streptogramin (cMLS) phenotype: This phenotype was labeled for those MRSA isolates, which showed resistance to both erythromycin (zone size ≤13 mm) and clindamycin (zone size ≤14mm) with circular shape of zone of inhibition if any around clindamycin.

e. Detection of inducible resistance to clindamycin

Inducible resistance to clindamycin was tested by 'D test' (Double disc test) as per Clinical and Laboratory Standards Institute (CLSI) guideline and the observation was recorded, thereafter compared and validated with Vitek-2 results.

RESULTS AND DISCUSSION

Worldwide prevalence of *S. aureus* infections mainly through nasal colonization in the general populationhas been reported to be between 20-40% (Jenney et al., 2014). In the present study also prevalence of *S. aureus* came out to be 46.05% (Table 1). Some previous studies reported prevalence of *S. aureus* from highest of 76.0% to lowest to 2.18 %. The higher incidence of *S. aureus* infection may be due to higher endogenouslevels andprevious colonization, which is reported to be an important risk factor for subsequent infection (Kumar et al., 2015). Frequency of MRSA was found to be 73.70% (Table 2), indicating the upward moving graph of predominance of MRSA in hospital settings. This may be due to overuse of antibiotics for treatment of such infections or through cross contamination with already infected MRSA hospitalized patients (Gupta and Sinha, 2017). Emergence of methicillin resistance in *S. aureus* has left us with very few therapeutic options available to treat such infections (Fridkin et al., 2005).

Table 1: Frequency of *S. aureus* in various clinically isolated specimens

Total number of samples	Samples showing *S. aureus* isolates
875	403 (46.05%)

Table 2: Frequency of MRSA strains among *S. aureus* isolates.

Total *S. aureus* isolates	Methicillin resistant *S. aureus* (MRSA)	Methicillin sensitive *S. aureus* (MSSA)
403	297 (73.70%)	106 (26.30%)

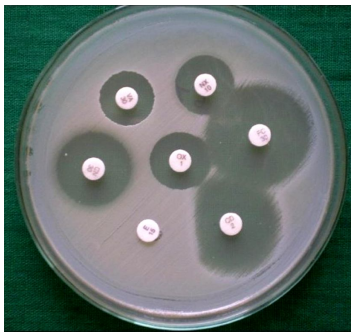

Figure 1: Double disc test (D test) showing flattening of the zone of inhibition around clindamycin disc proximal to erythromycin disc (*D shaped zone of inhibition*).

The clindamycin was an excellent drug for the treatment of MRSA specially an alternative for penicillin allergic patients (Rao, 2000). But its overuse can lead to clindamycin resistance development in MRSA. In the present study only 16.16 % samples were found to be erythromycin and clindamycin sensitive, whereas erythromycin resistant and clindamycin resistant (constitutive MLSB) was 41.07%, D test positive erythromycin sensitive and clindamycin resistant was 33.67% and erythromycin resistant and clindamycin sensitive was 25.25% (D test negative MS phenotype) (Fig. 1, Table 3). The possible mechanism for such resistance is either target site modification mediated by *erm* genes which can be expressed eitherconstitutively (constitutive MLS phenotype) or inducibly (inducible MLS phenotype) or resistance mediated through *msr*A genes *i.e.* efflux of antibiotics (Fiebelkorn et al., 2003). Inducible Clindamycin of MRSA showed due to resistance to erythromycin had been accentuated. The *erm* genes are known to induces resistance to the macrolide, lincosamide and streptogramin B (MLSB) group by a methylation at the 23s rRNA subunit (Lewis and Jorgensen, 2005). Erythromycin induces the production of this methylase, methylation results in impaired binding of clindamycin that share this residue as a common binding site, which is why these strains are resistant (Lewis and Jorgensen, 2005).

Table 3: Association of clindamycin resistance with methicillin resistant strains

Sensitivity pattern of phenotypes	Number of MRSA isolates (n=297)
E-S, CD-S	48 (16.16%)
E-R, CD-R (constitutive MLSB)	122 (41.07%)
E-R, CD-S (D test positive inducible MLSB)	100 (33.67%)
E-R, CD-S (D test negative, MS)	75 (25.25%)

Where: E, Erythromycin; CD, Clindamycin; S, Sensitive; R, Resistant.

As per CLSI guidelines minimum inhibitory concentration (MIC) break points in <0.5µg/ml for clindamycin is considered as sensitive, MIC 1-2µg/ml considered as Intermediate and the MIC value >4µg/ml is considered as resistant (Leonard et al., 2009; Di Gregorio et al., 2015; Mohanty et al., 2019). In present study, the susceptibility of MIC was higher at 0.06µg/ml in which D test positive isolates were 36, followed by 0.08µg/ml which included 34 isolates, 0.5µg/ml which included 21 isolates, 0.01µg/ml which included 9 isolates, MIC at 2µg/ml which included 9 isolates and no D test positive isolates were observed in MIC of 0.001µg/ml, 4µg/ml and 8µg/ml (Table 4). It is appropriate to find MIC values of clindamycin among D test positive MRSA isolates, as MIC value of antimicrobial can help in predicting the efficacy for choice of drugs used in treatment (Tarai et al., 2013). These kinds of studies can help in clinical cure, eradication ofcarrier status of a specific organism, as well as prevention of selection of resistance (Bou, 2007). Earlier very few studies have been conducted to find out the MIC values of clindamycin (Swenson et al., 2007).

Table 4: Minimum inhibitory concentration (MIC) of clindamycin among D test positive MRSA isolates

Clinical isolates	≤0.001	>0.001-0.01	>0.01-0.06	>0.06-0.08	>0.08-0.5	>0.5-2	>2-4	>4-8
D positive MRSA	-	9	36	34	21	-	-	-

In present study, incidence of D test positive MRSA isolates was higher in case of Hospital Acquired Infections (HAI) (68%) as compared with Community Acquired Infection (CAI) *i.e.* (32%) (Table 5). Higher incidence of D-test positive MRSA isolates in HAI was observed by various researchers (Lall and Sahni, 2014). The possible reason for that could be that MRSA is frequently spread by direct contact with an infected wound or from contaminated healthcare providers. Pus sample were found to be predominant for D-test positive MRSA isolates (57.35%) followed by respiratory tract samples (11.76%) and urine samples (33.53%) in the case of HAI (Table 5). Whereas in case of CAI maximum D tests positive MRSA isolates were found in urine samples (Table 5).

Table 5: Occurrence of D test positive inducible MLSB Methicillin resistant *S. aureus* (MRSA)

Sample Source	**Hospital Acquired Infection (HAI) n=68**	**Community Acquired Infection(CAI) n=32**	**Total (%) n=100**
Pus	39 (57.35%)	12 (37.5%)	51 (51.0%)
Urine	16 (33.53%)	14 (43.75%)	30 (30.0%)
Respiratory tract samples (sputum, BAL, ET aspirate)	8 (11.76%)	5 (15.63%)	13 (13%)
Blood	3 (4.41%)	1 (3.13%)	4 (4%)
Body Fluid	2 (2.94%)	0 (0%)	2 (2%)

Where: Chi square value= 4.034; p value= 0.270, the result is not significant at p<0.05.

Some other studies have also highlighted the higher frequency of MRSA isolates in pus samples as compared with other samples (Belbase et al., 2017). The possible reason for this could be faulty injection technique or neglected wounds by unqualified professionals (Ansari et al., 2014). Further analysis of pus samples reveled that, frequency of D test positive MRSA was predominant in patients with Diabetic foot disease (48.71%) followed by patients with postoperative wounds (35.89 %) and necrotizing fasciitis (13.79%) (Table 6). Repeated hospitalization of diabetic patients contribute them to become nasal and skin carriers of MRSA ultimately prone to diabetic foot than post-operative wound and necrotizing fasciitis (Kwon and Armstrong, 2018). Further, MRSA on surgical wards is becoming increasingly common especially in critically ill patients who have spent prolonged periods on the intensive care units.

Table 6: Frequency of D test positive MRSA from pus samples in a hospital setting

HAI- D test positive MRSA isolates	**Diabetic foot**	**Postoperative wound**	**Necrotizing Fasciitis**
39	19 (48.71%)	14 (35.89%)	6 (15.38%)

Similarly, analysis of urine samples pointed that the incidence of D test positive MRSA was more in patients who were catheterized for urine output *i.e.* 81.25% as compared with patients who were not catheterized (18.75%) (Table 7). The reason can be the deposition of a conditioning filmmade up of proteins, electrolytes, and other components of urine along with secreting polysaccharides of microbes on the surface of the catheter forming biofilm. Later on the organisms get detached from biofilm & floats in the urine lead to symptomatic infection (Belbase et al., 2017). Whereas in case of non-catheterized patient there as on can be the partial use of antibiotics, immune-deficiency patients or inappropriate personal hygiene (Lunacek et al., 2014).

Table 7: Association of uropathogenic D test positive MRSA isolates in a hospital setting

D test positive MRSA isolates in urine	Catheterized	Non-Catheterized
16	13 (81.25%)	3 (18.75%)

In modern medicine, the intra-venous devices are required in hospitalized patients. The use of peripheral venous catheters is done for diagnostic as well as treatment purposes. The contrary central venous catheter is used in critically ill patients for the better treatment but unfortunately that may leads to bacteremia (Cuervo et al., 2015). In the present study the D test positive MRSA isolates in patients with peripheral placed catheters was 66.66% and from central venous catheter was 33.33% (Table 8). The reason can be that the peripheral intravenous catheter is a regular procedure in hospitalized patients than central venous catheter. The use of peripheral venous catheter induced infection because of skin microorganism gain access to the bloodstream predominantly from catheter insertion site by extra luminal migration along the catheter (Klevens et al., 2007). Whereas in central venous catheterthere are less number of cases of bacteremia and this is due to the fact that in the hospital expert insert the catheter with all aseptic precaution (Fram et al., 2015).

Table 8: Frequency of MRSA from blood samples in a Hospital Acquired Infections (HAI)

D test positive MRSA isolates in HAI	Central Venous Catheter	Peripheral Venous Catheter
3	1 (33.33%)	2 (66.66%)

Incidence of D test positive MRSA in producing ventilator associatedpneumonia was found to be (66.66%) as compared to pneumonia other than ventilator associated pneumonia was (33.33%) in patients of hospital setting (Table 9). It seems to be novel observation as none of the researchers attempted such study. The reason could be that when patient is on ventilator, at that time lung iscompromised which results in lowered defense mechanism and most of the pathogens can gainentry across mucosal surface of the lung which mightleads to ventilator associated pneumonia (Lollar et al., 2016).

Table 9: Association of rate of D test positive MRSA producing pneumonia in hospital setting

Total no. of D test positive MRSA isolates in HAI	Ventilator Associated Pneumonia (VAP)	Pneumonia other than VAP
3	2 (66.66%)	1 (33.33%)

Out of the various risk factors analyzed, prolonged hospital stay (>5 days) was observed to be major risk factor for D test positive MRSA under different conditions was found to be 37.0% followed by malignancy (25.0%), malnutrition (18.0%), renal failure (17.0%) and Chronic Obstructive Pulmonary Disease (COPD) was (13.0%) (Table 10). The reason could be the fact that prolonged hospitalization leads to long-drawn-out exposure to various nosocomial infections as the immune system is weaker during hospitalization (Abe et al. 2012). Reasons of finding D test positive MRSA for other risk factors may due to the reason that patients are generally immunecompromised during such conditions.

Table 10: Risk factor associated with D test positive MRSA infection in individuals.

Risk factors	No. of MRSA isolates
Prolonged hospital stay (>5days)	37 (37.0%)
Malignancy	25 (25.0%)
Malnutrition	18 (18.0%)
Renal failure	17 (17.0%)
COPD	13 (13.0%)

Finally demographic profile of patients with D test positive MRSA strains was analyzed. In present study, predominance of males was (67.0%) more than females was (33.0%). which signifies the rate of D test positive MRSA exhibited more in male (Table 11). The reason may be because in this study the ratio of males was more as compared to females and usually in rural areas women's avoid minor clinical conditions and the female have XX genotype which makes them less prone to infections and makes their immunity more strong than males (Jacobus et al., 2007). The current study depicts predominance of D test positive MRSA in age group of 20-40 years which was (51.0%) which signifies D test positive MRSA exhibited more in 20-40 years age of patients (Table 11). The more prevalence rate at age group between 20-40 years can be because of more indiscriminate or prolonged use of antibiotics, self-medication, increased travel and mobility in addition to busy life styles with reduced attention to healthcare (Williamson et al., 2013). Also the current study showed increased incidence in rural population (58.0%) as compared to urban population (42.0%) (Table 11) which signifies D test positive MRSA exhibited more in rural patients. The reason for increased incidence in rural population can because there are less health care facilities and usually treated by quacks.

Table 11: Demographic profile of patients with D test positive MRSA strains

Parameters		**No of positive patient**
Gender	**Male**	67 (67.0%)
	Female	33 (33.0%)
Age	**<20**	18 (18.0%)
	20-40	51 (51.0%)
	40-60	19 (19.0%)
	>60	12 (12.0%)
Residential status	**Rural**	58 (58.0%)
	Urban	42 (42.0%)

CONCLUSIONS

The execution of the D-test is a simple and powerful technique for routine antibiotic susceptibility testing, which can predict both inducible and constitutive clindamycin resistance. Early detection of such isolates can help in utilization of clindamycin in infections which are caused by true clindamycin susceptible *Staphylococcus aureus* and hence assist to avoid treatment failures.

Conflicts of Interest

None conflicts of interests.

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