academicJournals

Vol. 10(9), pp. 280-284, 7 March, 2016
DOI: 10.5897/AJMR2014.7095
Article Number: 3395FC957520
ISSN 1996-0808

http://www.academicjournals.org/AJMR

African Journal of Microbiology Research

Full Length Research Paper

Status of inducible clindamycin resistance among macrolide resistant *Staphylococcus aureus*

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Received 1 August, 2014; Accepted 18 May, 2015

Clindamycin has long been an option for treating both methicillin sensitive *Staphylococcus aureus* (MSSA) and methicillin resistant *S. aureus* (MRSA) infections. So, it is utmost important to perform the susceptibility test for erythromycin and clindamycin. And, there is concern on use of this antibiotic in the presence of erythromycin resistance because of the possibility of induction of cross-resistance among members of macrolide, lincosamide and streptogramin B (MLS_B) group. During August 2011 to May 2012, a total of 207 isolates of *S. aureus* were isolated and among which 29.47% (61) isolates were confirmed as MRSA by cefoxitin (30 µg) disc. All the isolates were further processed for MLSB resistance test by double disc diffusion test of erythromycin (2 µg) and clindamycin (15 µg) at a distance of 15 and 22 mm between them. This study result show 12.56% (26) and 14.49% (30) of inducible macrolide-lincosamide-streptogramin B phenotype ($iMLS_B$) resistance type at 22 and 15 mm disc distance, respectively, showing 15 mm disc distance is potential than 22 mm and 17.39% (36) of $cMLS_B$ resistance type. Similarly, both iMLSB and cMLSB are greater in MRSA than MSSA and constitutes 18.05 (11) and 36.06% (22), respectively. Thus, this study concludes that D-test should be used as a mandatory method and is more potential in 15 mm disc apart.

Key words: *Staphylococcus aureus,* methicillin resistant *S. aureus* (MRSA), methicillin sensitive *S. aureus* (MSSA), inducible macrolide-lincosamide-streptogramin B phenotype ($iMLS_B$), cMLSB, D-test.

INTRODUCTION

Staphylococcus aureus acquisting *mecA* gene which encodes PBR-2a with low affinity for β-lactams, is methicillin resistant *S. aureus* (MRSA) (Brumfitt and Hamilton, 1989), which is the major cause of nosocomial and community acquired infection (Frank et al., 1999). Changing pattern in antimicrobial resistance and increasing incidence of MRSA infection have led to treating such infection with MLS antibiotics (Jadhav et al., 2011). However, their wide use resulted in increasing number of Staphylococci strains resistant to MLS_B

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antibiotics (Saiman et al., 2003). Macrolide, lincosamide and Streptogramin B (MLS_B) antibiotics are chemically distinct but have a similar mode of action (Gadepalli et al., 2006; Leclercq and Courvalin, 1991). The MLS family of antibiotics has three different mechanisms of resistance such as; target site modification, enzymatic antibiotic inactivation and macrolide efflux pumps (Jadhav et al., 2011).

As the methicilin-resistant *S. aureus*are emerge, the clindamycin has become an excellent drug for some staphylococcal infections, particularly skin and soft tissue infections and as an alternative in penicillin-allergic patients (Drinkovic et al., 2001). Clindamycin, is among the limited choice of antimicrobials effective against MRSA, has good oral bioavailability making it a good option for outpatient therapy and changeover after intravenous antibiotics (Jadhav et al., 2011; Leclercq, 2002). There is concern about use of this antibiotic in the presence of Erythromycin resistance because of the possibility of induction of cross-resistance among members of the macrolide, lincosamide, strepto-gramin B (MLS_B) group (Hussain et al., 2000). Clindamycin has long been an option for treating both MSSA and MRSA infections. So, it is utmost important to perform the susceptibility test for erythromycin and clindamycin as *S. aureus* possesses two types (constitutive and inducible) of clindamycin resistance pattern. This resistance mechanism can be constitutive where rRNA methylase is always produced ($cMLS_B$) or can be inducible where methylase is produced only in the presence of an inducing agent ($iMLS_B$). MRSA has adapted to survive treatment with beta-lactam antibiotic such as penicillins, cephalosporins including methicillin, dicloxacillin, nafcillin and oxacillin. MRSA is especially troublesome in hospital-associated (nosocomial) infection (Boucher and Corey, 2008; Creechs et al., 2005; Eveillard et al., 2004).

It is very important that microbiologists keep a close eye on the developing patterns of drug resistance to be able to guide therapy effectively. Inducible resistance to clindamycin could limit the effectiveness of this drug. Demonstration of $iMLS_B$ phenotype in isolates that are susceptible to clindamycin and resistant to erythromycin is possible by using double disk diffusion agar inhibitory assay or simply D-test (Jadhav et al., 2011; Gadepalli et al., 2006; Steward et al., 2005; Reddy and Reddy, 2012). In this study, we have attempted to characterize MLSBi resistance in both hospital and community associated *S. aureus* isolates, including MRSA and MSSA, at KIST medical college and hospital.

MATERIALS AND METHODS

The present study was conducted from August 2011 to May 2012. During the study, a total of 4230 clinical samples were processed and among which 207 isolates of *S. aureus* were isolated. Clinical samples include pus, blood, wound swab, body exudates, tips and urine.

S. aureus isolates were identified using the standard conventional methods (Frank et al., 1999; Saiman et al., 2003; Fiebelkorn et al., 2003). Antimicrobial susceptibility testing were done by Kirby Bauer's disc diffusion method on Muller-Hinton agar plates using Penicillin (10 U), Ampicillin (10 µg), Cloxacillin (5 µg), Erythromycin (15 µg), Clindamycin (2 µg), Cotrimoxazole (1.25/23.75 µg), Ciprofloxacin (5 µg), Ofloxacin (5 µg), Cefotaxime (30 µg), Chloramphenicol (30 µg) as first line antibiotics and Amikacin (30 µg), Gentamicin (10 µg), Ceftazidime (30 µg), Amoxycillin/Clavulanic acid (20/10 µg) and Vancomycin (30 µg) as second line anitibiotics. *S. aureus* (ATCC 25923) was used as quality control for disc diffusion test as recommended by CLSI (2011).

The organisms which showed resistant to Ampicillin, Penicillin and Cloxacillin were subjected to test with Cefoxitin (30 µg) to confirm MRSA. The isolates with resistant to at least two classes of first line antibiotics were regarded as MDR (Sahm et al., 2001; Simner et al., 2011). MRSA isolates were preserved in nutrient agar containing 20% glycerol at -7°C until further investigation.

Isolates were plated on a Muller Hinton Agar plate at a Mac Farland concentration of 0.5 to eventually cover the agar surface.Clindamycin and Erythromycin disks, containing 2 and 15µg each respectively were placed in the center of the plate separated by a distance of 15 and 22 mm from the centre of discs. Plates were incubated at 37°C for 24 h. Inducible resistance to Clindamycin was defined as blunting of the clear circular area of no growth around the Clindamycin disc on the side adjacent to the Erythromycin disc and was designated D-test positive. Absence of a blunted zone of inhibition was designated D-test negative. Three different phenotypes were interpreted as follows (Deotale et al., 2010; Kloos and Banerman, 1999). *S. aureus* ATCC 29213 (D-test negative) and *S. aureus* ATCC 25923 (D-test positive) were used as quality control.

RESULTS

Among 207 isolates of *S. aureus* isolated from different clinical specimens, 29.47% (61) were confirmed as MRSA distributing higher percentage in IPD than OPD (35.71 vs. 23.85%), in age group 51-60 years (42.11%), in female (29.91 vs. 28.89%) and in nephrology ward (31.44%). All MRSA were highly resistant to penicillin (100%), ampicillin (98.36%), ceftazidime (88.53%) and erythromycin (88.53%) while all MRSA were sensitive to vancomycin showing all MRSA isolates were MDR MRSA.

The overall prevalence of $iMLS_B$ resistant phenotype was found to be 14.49% (Table 1) among *S. aureus,* however, 4 more isolates of *S. aureus* were found to be $iMLS_B$ when placed in 15 mm distance than 22 mm distance and higher in MRSA (18.03%) than MSSA (13.01%) (Table 2). Among 30 isolates of $iMLS_B$, *S. aureus* were found to be the highest in female (18.80%), age group 31-40 years and OPD patients (14.68%).

DISCUSSION

An important distinctive feature of *S. aureus* strains is the susceptibility to methicillin; hence, strains are categorised as MSSA or MRSA which was first reported in 1960s in the hospital setting. Most MRSA strains are multidrug-resistant, being commonly resistant to macrolides,

Table 1. Distribution of MLS_B resistance in *S. aureus.*

	Inducible clindamycin test	
	15 mm; n(%)	**22 mm; n(%)**
$cMLS_B$	36 (17.39)	36 (17.39)
Er/Cl* sensitive	103 (49.76)	103 (49.76)
MS_B resistance	38 (18.36)	42 (20.29)
$iMLS_B$	30 (14.49)	26 (12.56)
Total	207 (100.00)	207 (100.00)

Table 2. Distribution of MLS_B resistance among MSSA and MRSA isolates.

Resistant and sensitive phenotypes	Ery	Cld	D-test	MRSA	MSSA
				No. (%)	**No. (%)**
Inducible MLS_B ($iMLS_B$)	R	S	D+	11 (18.03)	19 (13.01)
Constitutive MLS_B ($cMLS_B$)	R	R		22 (36.06)	14 (9.59)
MS_B resistant	R	S	D-	21 (34.43)	17 (11.64)
Ery/Cl * sensitive	S	S		7 (11.48)	96 (65.76)
Total				61 (100.00)	146 (100.000)

aminoglycosides and fluoroquinolones (Pantosti et al., 2007). The emergence of resistance to multiple antibiotics among staphylococci has left very few therapeutic options for clinicians. A therapeutic decision is not possible without the relevant clinical and microbiological data (Frank et al., 2002; Levin et al., 2005). Newer antibacterial agents as tigecycline, dalbavancin, oritavancin and ceftobiprole are now available for staphylococcal infections; however, it is possible that these antibiotics will also gain resistance towards the pathogens in due course of time. So, a wise decision would be to conserve those antibiotics which are still highly effective against staphylococci; clindamycin is one of such drugs due to its pharmacokinetic properties.

Though detection of *mecA* gene is considered as the gold standard for revealing methicillin resistant gene (Arbique et al., 2001; Fatholahzadeh et al., 2008), however in the present study, phenotypic method (test with cefoxitin disc; 30 µg/ml) as described by CLSI (2011) was employed. The results of the study revealed that MRSA were detected in 29.47% which is in accordance with the findings disseminated by other studies (Fatholahzadeh et al., 2008; Mdani et al., 2001; Vaez et al., 2011) and various regions in Nepal as well (Kumari et al., 2008; Sanjana et al., 2010; Shrestha et al., 2009). Some of the previous studies found the percentage of MRSA in different area ranging 15.4-44.90% (Kumari et al., 2008; Sanjana et al., 2010; Shrestha et al., 2009; Subedi and Brahmadathan, 2005). A study at a tertiary care hospital of Nepal has reported 42.42% MRSA in 2008 (Mishra, 2008). All these studies have depicted the alarming condition due to MRSA isolates which is still in increasing trend. The prevalence is still higher in the well developed countries where it ranged from 50-60% by mutated strains of *S. aureus* (Vazquez, 2006). But in the developing countries like Nepal, the higher prevalence of MRSA may have contended the fact that the inappropriate use of antibiotics for community as well as hospital acquired infections has resulted in the increment of the pressure to select MRSA and other resistant bacteria (Kumari et al., 2008; Sanjana et al., 2010; Subedi and Brahmadathan, 2005).

Increasing frequency of MRSA infections and changing patterns in antimicrobial resistance have led to renewed interest in the use of macrolide lincosamidestreptogramin B (MLS_B) antibiotics to treat such infections. However, their widespread use has led to an increase in the number of *Staphylococcus* strains resistant to MLS_B antibiotics (Saiman et al., 2003) and as MRSA infections have become increasingly common in the community setting, the development of empirical antimicrobial therapeutic strategies for staphylococcal infections has become more problematic. The increasing frequency of MRSA with *in vitro* inducible clindamycin resistance raises a concern of clindamycin treatment failures and this is where the D test becomes significant (Frank et al., 2002; Levin et al., 2005).

In this study, 14.49% of *S. aureus* isolates were inducible macrolide-lincosamide-streptogramin B phenotype ($iMLS_B$) and 17.39% were of $cMLS_B$. The results are in accordance with a previous study in Nepal in which 18.2% of $iMLS_B$ were reported (Shrestha et al., 2009). This study also correlates with the study done earlier which reported 34% of $iMLS_B$, 19% $cMLS_B$ and 30% of MS phenotypes (Mohanasoundaram, 2011). This study showed that the S-phenotype is mostly associated

with MSSA than MRSA which is supported by a previous study (Reddy and Reddy, 2012). Similarly, this study showed $cMLS_B$ phenotype is higher among MRSA (36.06%) which is lower than the report of 44.2% $cMLS_B$ among MRSA from Turkish hospital (Yilmaz et al., 2007). The D-test results of staphylococci isolates showed four phenotypes; including D-positive, D-negative, MS (R) and S phenotype. Most of the MRSA showed MS-phenotype followed by D-negative while the most of the MSSA showed S-phenotype followed by $iMLS_B$ phenotype in this study, which is supported by other studies (Jadhav et al., 2011; Yilmaz et al., 2007; Chelae et al., 2009). MRSA exhibit $iMLS_B$ predominantely than MSSA, the result being in accordance with a few studies reported before (Jadhav et al., 2011; Gadepalli et al., 2006; Yilmaz et al., 2007; Chelae et al., 2009; Rahabar and Hajia, 2007).

CLSI has recommended using D-test in which 15 µg Ery and 2 µg Cld should be placed 15-26 mm apart from edge-edge (Clinical and Laboratory Standards Institute, 2011). This study evaluated the efficacy of two inter disc distances for $iMLS_B$ phenotype detection, by placing at 15 and 22 mm from edge to edge of Ery and Cld discs. Four phenotypes failed to be detected as $iMLS_B$ at 22 mm distance than at 15 mm distance in this study which is supported by the study done in India reporting 7 more isolates were detected as $iMLS_B$ strains at 15 mm distance previously reported as D-test negative at 22 mm distance concluding low interdisc distance induces production of methylase by inducible agents (Ajantha et al., 2008).

Due to the restricted range of antibiotics available for the treatment of methicillin-resistant staphylococcal infections and the known limitations of vancomycin, clindamycin should be considered for the management of serious soft tissue infections. In addition, such testing can provide information about resistant to MLS phenotype group of antibiotics and can be useful for surveillance studies related to MLS resistance in staphylococci. If D-test is not performed, nearly half of the erythromycin resistant and clindamycin sensitive *S. aureus* isolates might have been missed and resulting in therapeutic failure with clindamycin. So before declaring the clindamycin sensitivity among the clinical isolates of *S. aureus*, it is necessary to check for inducible resistance (Jadhav et al., 2011; Gadepalli et al., 2006; Reddy and Reddy, 2012; Fiebelkorn et al., 2003; Shrestha et al., 2009; Mohanasoundaram, 2011; Chelae et al., 2009; Rahabar and Hajia, 2007; Ajantha et al., 2008; Delialioglu et al., 2005; Mshana et al., 2009; Rodrigues et al., 2007; Zorgani et al., 2010). Negative D-test among the erythromycin resistant isolates confirm the sensitivity to clindamycin and possible to choose clindamycin as drug of choice in the treatment of staphylococcal infections (Leclercq, 2002). By consistently performing the D-test, the diagnostic laboratory can properly guide the clinician and clindamycin could be a valuable weapon against the staphylococci. It would be better to implement the D-test for iMLSb detection on a routine basis in the hospital laboratory.

Conclusion

In this study, the prevalence of inducible clindamycin resistance was high among macrolide resistant *S. aureus* isolates. Since the results of this study represent the scenario of a single hospital and might not be representative of the rest of the country, it is recommended that D-test for iMLSb detection should be carried out in the hospital laboratory on a routine basis throughout the country. The use of highly advanced molecular methods for such results would be more promising in such studies.

Conflict of Interests

The authors have not declared any conflict of interests.

ACKNOWLEDGEMENTS

The authors thank the staff of KIST Medical College for their technical help. Authors are also extremely grateful to the Medical Superintendents, doctors, nurses, staff and patients of the hospital for their kind support during the study.

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