Research Journal of Medical Sciences 9 (1): 13-17, 2015
ISSN: 1815-9346


# Macrolide-Lincosamide-Streptogramin-B ($MLS_B$) Resistance Phenotypes in *Staphylococcus* Isolates from Non-Healthcare Community Surfaces

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**Abstract:** The present study was performed to investigate the phenotypic resistance of all isolated species of *Staphylococcus* to macrolide-lincosamide streptogramin-B through double disc test. One hundred eighty one strains of staphylococci, consisting 59 *Staphylococcus aureus* and 122 Coagulase-Negative Staphylococci (CNS) were collected between March and April 2013 from environmental sources such as gymnasia, shopping malls and ATM key in the Qasssim region and study was conducted at College of Applied Medical Sciences, Qassim University, Saudi Arabia. All isolates were subjected to *in vitro* anti-microbial susceptibility testing by using Kirby Bauer Method as per Clinical and Laboratory Standards Institute guidelines. The prevalence rate of total $MLS_B$ resistance phenotype was observed in 29.8% of Methicillin-Resistant (MR) and Methicillin-Sensitive (MS) *Staphylococcus* species. The constitutive ($cMLS_B$), inducible ($iMLS_B$) and MS phenotypes prevalence was in 16.9, 16.9 and 22%, respectively among *Staphylococcus aureus*. While prevalence of constitutive ($cMLS_B$), inducible ($iMLS_B$) and MS phenotypes in coagulase negative *Staphylococcus* was 2.4, 3.2 and 16.3%, respectively. The high prevalence of $cMLS_B$ and inducible Clindamycin $iMLS_B$ resistance phenotypes in Methicillin resistance staphylococci as compared to Methicillin sensitive isolates was noticed in Qassim Province of Saudi Arabia. Therefore, we recommend that routine screening of staphylococci isolates by double-disc test is critical and necessary before the treatment so that therapeutic failure may be avoided.

**Key words:** MRSA, $MLS_B$, community, currency, Qassim

## INTRODUCTION

The emergence of drug resistance and infection caused by resistant bacterial pathogens are major problem for clinicians worldwide (Chatterjee and Otto, 2013; Mishra *et al*., 2012). Community-Associated Methicillin-Resistant *Staphylococcus aureus* (CA-MRSA) infections are an emerging problem in the developing and developed world. CA-MRSA isolates were first recognized by distinct resistance profiles against antimicrobial drugs that lacked resistance to older antimicrobial drugs (Lelievre *et al*., 1999; Seal *et al*., 2003; Weber, 2005). Carriage of *S. aureus* in the nose appears to play a key role in the pathogenesis of infection and also shows connection with increased risk of infectious complications (Wenzel and Perl, 1995). This poses a risk for the emergence of new carriers among the members of communities and hospitals. The arbitrary use of Macrolide-Lincosamide-Streptogramin B ($MLS_B$) antibiotics has led to an increased number of staphylococcal strains acquiring resistance to $MLS_B$ antibiotics (Ajantha *et al*., 2008; Deotale *et al*., 2010). The one of the common mechanism for clindamycin, (lincosamide) resistance is target site modification mediated by *erm* genes which can be expressed either as constitutive (constitutive $MLS_B$ phenotype) or inducible expression (inducible $MLS_B$ phenotype) (Prabhu *et al*., 2011; Lim *et al*., 2006). Earlier study confirmed that clinical failures in CA-MRSA infection after clindamycin used to treat strains with inducible clindamycin resistance (Sibery *et al*., 2003). Since, the existing knowledge on species distribution and recognition of risk factors of CA-MRSA is vital in treatment and control strategies. Therefore, the study was conducted to assess the erythromycin-resistant *Staphylococcus* isolates and the isolates were also tested for constitutive ($cMLS_B$) and inducible clindamycin resistance ($iMLS_B$) by using double Disk test (D-test).

## MATERIALS AND METHODS

**Sample collection sites and identification of *Staphylococcus* species:** One hundred eighty one staphylococci were isolated between March and April 2013 from different environmental sources such as gymnasia (68), shopping malls (66), paper currency's (38)

and ATM key boards (09) in Qassim region. The study was conducted at College of Applied Medical Sciences, Qassim University, Saudi Arabia. Bacterial isolates were characterized to species level by gram stain, growth on mannitol salt agar (BBL, France), catalase and coagulase production (Slidex Staph Plus, Biomerieux, France).

**Methicillin resistance detection and confirmation by Chrom agar plate and PBP2a methods:** Initially Methicillin resistance was detected by Agar Screen test inoculum was prepared by suspending organisms from 24 h culture in sterile saline and adjusting the turbidity to 0.5 McFarland. A sterile cotton swab was dipped into the bacterial suspension; spot inoculated on Mueller-Hinton agar plate (supplemented with 4% NaCl containing 6 μg of oxacillin per mL). The plates were incubated at 35°C for 24 h for *Staphylocccus aureus* and 48°h for Coagulase-Negative Staphylocccl (CNS). Growth of even a single colony is indicative of resistance.

Further, confirmation of MRSA and MRCoNS by Chrom ID (OXOID) was performed. The methicillin resistance *Staphylococcus* only grew on the Hi Chrome agar plate while the methicillin-sensitive was inhibited on the same agar plate. Cultures showing bright blue color growth were considered as methicillin resistance positive isolates while all others were recorded as methicillin sensitive. For confirmation of methicillin-resistance in *Staphylococcus*, the Oxoid PBP2' Latex Agglutination test (OLA) was used. This assay was used according to the manufacturer's instructions to confirm the presence of Penicillin-Binding Protein2' (PBP2') the protein encoded by the *mecA* gene. *S. aureus* ATCC 25923 (mecA negative) and ATCC 43300 (mecA positive) strains were used for the quality control.

**Detection and interpretation of Macrolide-Lincosamide-Streptogramin-B ($MLS_B$) phenotypes through double-disc Diffusion (D-test) test:** Double-disc diffusion testing (D-test) was performed for each isolate as per earlier prescribed method (Fiebelkorn *et al*., 2003). Each isolate was suspended in 0.85% sodium chloride solution and the turbidity was matched with 0.5 McFarland standards. The isolates were then inoculated on MH agar plate. Clindamycin (CLI, 2 μg) and Erythromycin (ER, 15 μg) disks were placed manually 15 mm apart edge to edge. Following overnight incubation at 37°C, flattening of zone (D shaped) around Clindamycin in the area between the two discs, indicated inducible Clindamycin resistance. Three different phenotypes were recorded after testing and interpreted as:

**Inducible $MLS_B$ phenotype:** Staphylococcal isolates showing resistance to erythromycin (zone size <13 mm) and sensitive to clindamycin (Zone size >21 mm) with D-shaped flattening in the zone of inhibition at the interface of erythromycin disc were labeled as inducible MLSB phenotype.

**Constitutive $MLS_B$ phenotype:** Staphylococcal isolates that showed resistance to both Erythromycin (Zone size <13 mm) and Clindamycin (Zone size <14 mm) with circular shape of zone of inhibition.

**MS phenotype:** Isolates showing circular zone of inhibition around Clindamycin (Zone size >21 mm) and resistant to Erythromycin (Zone size <13 mm) were labeled as MS phenotype.

## RESULTS

Based on identification methods used a total of 181 *Staphylococcal* isolates were isolated from community surface areas such as shopping malls, gym, currency and ATM key boards.

**$MLS_B$ phenotypes on collected samples:** The study demonstrated that the D shape of the Clindamycin zone adjacent to an Erythromycin disc in a conventional disc diffusion test can serve to detect *S. aureus* or Coagulase negative *Staphylococcus* strains with inducible resistance to Clindamycin. The results on isolates are listed in Table 1 and 2. This study revealed a relatively higher distribution of inducible Clindamycin resistance among the Methicillin resistant *Staphylococcus* isolates.

Out of the 181 *Staphylococcus* isolates, 68 (37.5%) were recovered from gymnasia, 66 (36.4%) from shopping mall, 38 (20.9%) from currency notes and 9 (4.9%) from ATM key boards. From all the 59 *S. aureus*, 27 (45.7%) were resistant to Erythromycin. These isolates were subjected to D-test where 10 isolates showed resistance to both Erythromycin and Clindamycin indicating constitutive MLSB phenotypes, 4 isolates showed resistance to Erythromycin and inducible Clindamycin (positive D-test) indicating inducible MLSB phenotype. Whereas 13 isolates showed resistance to Erythromycin and susceptible to Clindamycin (D-test negative) indicating MS phenotypes.

Out of the 122 Coagulase negative *Staphylococcus*, 27 (22.1%) isolates were found to be resistant to Erythromycin. These isolates were subjected to D-test and found 03 isolates showing resistance to both Erythromycin and Clindamycin indicating constitutive

Table 1: Prevalence of Clindamycin and Erythromycin susceptible phenotypes in *Staphylococci* isolated from community

| | | Prevalence of bacterial phenotypes in community | | | | |
|---|---|---|---|---|---|---|
| Sources of isolates | Total No. of isolates | Total MLSB (%) | cMLSB ER-R CL-R constitutive (%) | iMLSB ER-R CL-S inducible+ (%) | MS phenotype ER-R CL-S susceptible, D (%) | ER-S CL-S non-phenotypes (%) |
| Gymnasia | 68 | 23 (33.8) | 7 (10.2) | 3 (4.4) | 13 (19.1) | 45 (66.1) |
| Shopping mall | 66 | 16 (24.2) | 4 (6.0) | 2 (3.0) | 10 (15.1) | 50 (75.7) |
| Currency | 38 | 10 (26.3) | 1 (2.6) | 1 (2.6) | 8 (21.0) | 28 (73.7) |
| ATM, key pads | 09 | 05 (55.5) | 1 (11.1) | 2 (22.2) | 02 (22.2) | 04 (44.4) |
| Total | 181 | 54 (29.8) | 13 (7.1) | 8 (4.4) | 33 (18.2) | 127 (70.1) |

Table 2: Susceptibility to Clindamycin and Erythromycin among coagulase positive and coagulase negative *Staphylococcus* isolates from community

| | | Bacterial phenotypes | | | | |
|---|---|---|---|---|---|---|
| Bacterial isolates | Total No. | Total MLSB No. (%) | cMLSB ER-R CL-R constitutive (%) | Imlsb ER-R CL-S inducible, D+ (%) | MS phenotype ER-R CL-S susceptible, D (%) | ER-S CL-S non-phenotypes (%) |
| MRSA | 16 | 10 (62.5) | 3 (18.7) | 3(18.7) | 4 (25.0) | 6 (37.5) |
| MSSA | 43 | 17 (39.5) | 7 (16.2) | 1 (2.3) | 9 (20.9) | 26 (60.4) |
| MRCoNs | 37 | 19 (51.3) | 2 (5.4) | 3 (8.1) | 14 (37.8) | 18 (48.6) |
| MSCoNs | 85 | 8 (9.4) | 1 (1.1) | 1 (1.1) | 6 (7.0) | 77 (90.5) |
| Total | 181 | 54 (29.8) | 13 (7.1) | 8 (4.4) | 33 (18.2) | 127 (70.1) |

MRSA: Methicillin Resistance *Staphylococcus aureus*; MSSA: Methicillin Sensitive *Staphylococcus aureus*; MRCNS: Methicillin Resistance Coagulase Negative *Staphylococcus*; MSCNS: Methicillin Sensitive Coagulase Negative *Staphylococcus*; ER: Erythromycin; CL: Clindamycin; R: Resistance; S: Susceptible

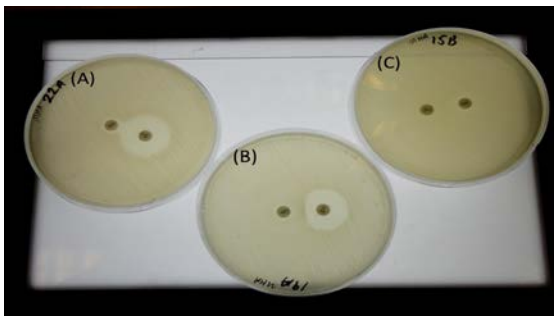


Fig. 1: D-test, Erythromycin and Clindamycin disks were placed in adjacent positions: A) MS phenotype; B) Inducible $MLS_B$ phenotype; C) Constitutive $MLS_B$ phenotype

MLSB phenotypes, 04 isolates showing resistance to Erythromycin and inducible Clindamycin (positive D-test) indicating inducible MLSB phenotype and the rest 20 isolates showing resistance to Erythromycin and susceptible to Clindamycin (D test negative) indicating MS phenotypes. These findings indicated a very low incidence (4.4%) of the inducible MLSB resistance phenotype and a much higher percentage of constitutive MLSB phenotypes among the various staphylococcal isolates (16.9% of *S. aureus* and 2.4% of CNS isolates) (Fig. 1).

## DISCUSSION

The manifestation of resistance to multiple antibiotics among Gram-positive cocci is major difficulty for the clinicians and treatment failure. Present findings indicated a very low incidence (4.4%, 8/181) of the inducible $MLS_B$ resistance phenotype and a much higher percentage (7.2%, 13/181) of constitutive $MLS_B$ phenotypes among the various staphylococcal isolate (16.9% of *S. aureus* and 2.4% of coagulase negative *Staphylococcus*, CoNS isolates) (Table 1 and 2). However, study from different part of the world shows different type of resistance pattern. Earlier study based on Chicago (Schreckenberger *et al*., 2004) reported that inducible $MLS_B$ resistance in 83% of the isolates which is discordant with present finding whereas another study based on India has observed 33.6% and the incidence pattern was more among the CoNS isolates (Juyal *et al*., 2013). Another finding revealed that out of 414 *S. aureus* isolates, 150 were methicillin resistant *Staphylococcus aureus*. In addition, there were 264 methicillin sensitive *Staphylococcus aureus* isolates, only 7.95% of which were inducibly clindamycin resistant (Upadhya and Biradar, 2011). From the literature, it is very clear that the resistance patterns were different in the different part of the world but the exact reason behind this not fully understood. Even though Clindamycin is one of the appropriate antimicrobial agent in the treatment of CA-MRSA infections but numerous Erythromycin-resistant MRSA isolates have been noticed as inducible Clindamycin resistance that may finally lead to the treatment failures. Earlier finding has found high percentage of Erythromycin-resistant *S. aureus* isolates (28.42%). Furthermore, among them 37.52% isolates tested positive for inducible Clindamycin resistance by D-test whereas rest of the isolates were negative, out of which 16.66% were shown to have constitutive Clindamycin resistance and 29.62% has shown that true sensitivity to

Clindamycin (MS phenotype) (Prabhu *et al*., 2011). However, in the present study, it was found that prevalence to $MLS_B$ erythromycin resistance was much higher in 62.5% MRSA as compared to MSSA 39.5%. Furthermore, Out of 16 MRSA, 3 (18.7%) isolates were positive for D-test, 03 (18.7%) were constitutive $MLS_B$ and 4 (25.0%) were D-test negative. However, the occurrence of constitutive $MLS_B$ (18.7%) found in the current study was low as compared to earlier investigations (Fokas *et al*., 2005) and was close to other report (Shrestha *et al*., 2009). In 43 (72.8%) MSSA isolates, 1 (2.3%) were inducible Clindamycin resistant, 7 (16.2%) were constitutive $MLS_B$ and 9 (20.9%) were found to be D-test negative. It was noticed that 3 (8.1%) inducible clindamycin resistant phenotype among Methicillin Resistance Coagulase Negative *Staphylococcus* (MR-CoNS) isolates and 1 (1.1%) among Methicillin Sensitive Coagulase Negative *Staphylococcus* (MS-CoNS) isolates. The 2 (5.4%) isolates were constitutive $MLS_B$ in MR-CoNS and 1 (1.1%) in MS-CoNS isolates whereas 14 (37.8%) MR-CoNS and 6 (7.0%) MS-CoNS were D-test negative. There have been a numerous reports showed that macrolide resistance in staphylococci. Some earlier investigators have reported a higher level of incidence of $iMLS_B$ resistance (Goyal *et al*., 2004; O'Sullivan *et al*., 2006; Merino *et al*., 2005; Angel *et al*., 2008; Ciraj *et al*., 2009; Patel *et al*., 2006) while other investigators showed that lower incidence of the $iMLS_B$ resistance (Rahbar and Hajia, 2007; Jenssen *et al*., 1987; Delialioglu *et al*., 2005; Huang *et al*., 2006). This study also found that $iMLS_B$ resistance rate was low, i.e, *S. aureus* (6.77%) and CNS (3.27%) as compared to earlier finding (Pal *et al*., 2010). The exact reason of differences is not known fully but it might be due to $iMLS_B$ resistance varies from one geographical region to the other, inducible clindamycin resistance as well as age group. However, further detailed investigation should be made to know the exact mechanism of the difference of resistant patterns. There has been no scarcity of reports on the clinical failure due to antibiotics resistance using clindamycin against *S. aureus* strain (Rao, 2000; Dinkovic *et al*., 2001).

## CONCLUSION

In view of therapeutic implication public health laboratories shoud be aware of the local prevalence of $iMLS_B$ isolates. D-test should be made mandatory because is simple, inexpensive, easy to perform, reproducible and necessary to help in guiding therapy and avoid the treatment failures.

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