

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

Mohammad A. Alzohairy

Department of Medical Laboratories, College of Applied Medical Sciences,
Qassim University, Qassim, Saudi Arabia

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 Qassim 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 *Staphylococcus aureus* and 48 h for Coagulase-Negative Staphylococci (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 gymnasias, 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

Sources of isolates	Total No. of isolates	Prevalence of bacterial phenotypes in community				
		Total MLSB (%)	cMLS _B ER-R CL-R constitutive (%)	iMLS _B 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 isolates	Total No.	Bacterial phenotypes				
		Total MLSB No. (%)	cMLS _B 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 MLS_B 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 MLS_B resistance phenotype and a much higher percentage of constitutive MLS_B 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 (Upadhyaya 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 should 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.

REFERENCES

- Ajantha, G.S., R.D. Kulkarni, J. Shetty, C. Shubhada and P. Jain, 2008. Phenotypic detection of inducible clindamycin resistance among *Staphylococcus aureus* isolates by using the lower limit of recommended inter-disk distance. Indian J. Pathol. Microbiol., 51: 376-378.
- Angel, M.R., V. Balaji, J.A. Prakash, K.N. Brahmandathan and M.S. Mathews, 2008. Prevalence of inducible clindamycin resistance in gram positive organisms in a tertiary care centre. Indian J. Med. Microbiol., 26: 262-264.
- Chatterjee, S.S. and M. Otto, 2013. Improved understanding of factors driving methicillin-resistant *Staphylococcus aureus* epidemic waves. Clin. Epidemiol., 5: 205-217.
- Ciraj, A.M., P. Vinod, G. Sreejith and K. Rajani, 2009. Inducible clindamycin resistance among clinical isolates of staphylococci. Indian J. Pathol. Microbiol., 52: 49-51.
- Delialioglu, N., G. Aslan, C. Ozturk, V. Baki and G. Emekdas, 2005. Inducible clindamycin resistance in staphylococci isolated from clinical samples. Jpn. J. Infect Dis., 58: 104-106.
- Deotale, V., D. Mendiratta, U. Raut and P. Narang, 2010. Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. Indian J. Med. Microbiol., 28: 124-126.
- Dinkovic, D., E.R. Fuller, K.P. Shore, D.J. Holland and R. Ellis-Pegler, 2001. Clindamycin treatment of *Staphylococcus aureus* expressing inducible clindamycin resistance. J. Antimicrob. Chemother., 48: 315-316.
- Fiebelkorn, K.R., S.A. Crawford, M.L. McElmeel and J.H. Jorgensen, 2003. Practical disk diffusion method for detection of inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci. J. Clin. Microbiol., 41: 4740-4744.
- Fokas, S., M. Tsironi, M. Kalkani and M. Dionysopoulou, 2005. Prevalence of inducible clindamycin resistance in macrolide-resistant *Staphylococcus* sp. Clin. Microbiol. Infect., 11: 337-340.
- Goyal, R., N.P. Singh, V. Manchanda and M. Mathur, 2004. Detection of clindamycin susceptibility in macrolid resistant phenotypes of *Staphylococcus aureus*. Ind. J. Med. Microbiol., 22: 251-254.
- Huang, H., N.M. Flynn, J.H. King, C. Monchaud, M. Morita and S.H. Cohen, 2006. Comparisons of community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) and hospital-associated MRSA infections in Sacramento, California. J. Clin. Microbiol., 44: 2423-2427.

- Jenssen, W.D., S. Thakker-Varia, D.T. Dubin and M.P. Weinstein, 1987. Prevalence of macrolides-lincosamides-streptogramin B resistance and erm gene classes among clinical strains of staphylococci and streptococci. *Antimicrobial Agents Chemotherapy*, 31: 883-888.
- Juyal, D., A.S. Shamanth, S. Pal, M.K. Sharma, R. Prakash and N. Sharma, 2013. The prevalence of inducible clindamycin resistance among staphylococci in a tertiary care hospital-a study from the Garhwal Hills of Uttarakhand, India. *J. Clin. Diagnostic Res.*, 7: 61-65.
- Lelievre, H., G. Lina, M.E. Jones, C. Olive, F. Forey and M. Roussel-Delvallez *et al.*, 1999. Emergence and spread in French hospitals of methicillin-resistant *Staphylococcus aureus* with increasing susceptibility to gentamicin and other antibiotics. *J. Clin. Microbiol.*, 37: 3452-3457.
- Lim, H.S., H. Lee, K.H. Roh, J.H. Yum, D. Yong, K. Lee and Y. Chong, 2006. Prevalence of inducible clindamycin resistance in staphylococcal isolates at a Korean tertiary care hospital. *Yonsei Med. J.*, 47: 480-484.
- Merino, L., M.J. Torres, A. Cantos, M. Ruiz and J. Aznar, 2005. Detection of inducible clindamycin resistance in cutaneous Staphylococci clinical isolates by phenotypic and genotypic method. *Proceedings of the 15th European Congress of Clinical Microbiology and Infectious Diseases Copenhagen, April 2-5, 2005, Denmark.*
- Mishra, R.P.N., E. Oviedo-Orta, P. Prachi, R. Rappuoli and F. Bagnoli, 2012. Vaccines and antibiotic resistance. *Curr. Opinion Microbiol.*, 15: 596-602.
- O'Sullivan, M.V., Y. Cai, F. Kong, X. Zeng and G.L. Gilbert, 2006. Influence of disk separation distance on accuracy of the disk approximation test for detection of inducible clindamycin resistance in *Staphylococcus* sp. *J. Clin. Microbiol.*, 44: 4072-4076.
- Pal, N., B. Sharma, R. Sharma and L. Vyas, 2010. Detection of inducible clindamycin resistance among Staphylococcal isolates from different clinical specimens in western India. *J. Postgraduate Med.*, 56: 182-185.
- Patel, M.K.B., Wates, S. Amoser, G.A. Cloud and C.J. Hoesley, 2006. Prevalence of inducible clindamycin resistance among community and hospital-associated *Staphylococcus aureus* isolates. *J. Clin. Microbiol.*, 44: 2481-2484.
- Prabhu, K., S. Rao and V. Rao, 2011. Inducible clindamycin resistance in *Staphylococcus aureus* isolated from clinical samples. *J. Laboratory Phys.*, 3: 25-27.
- Rahbar, M. and M. Hajia, 2007. Inducible clindamycin resistance in *Staphylococcus aureus*: A cross-sectional report. *Pak. J. Biol. Sci.*, 10: 189-192.
- Rao, G.G., 2000. Should clindamycin be used in treatment of patients with infections caused by erythromycin-resistant staphylococci? *J. Antimicrob. Chemother.*, 45: 715-716.
- Schreckenberger, P.C., E. Ilendo and K.L. Ristow, 2004. Incidence of constitutive and inducible clindamycin resistance in *Staphylococcus aureus* and coagulase-negative staphylococci in a community and a tertiary care hospital. *J. Clin. Microbiol.*, 42: 2777-2779.
- Seal, J.B., B. Moreira, C.D. Bethel and R.S. Daum, 2003. Antimicrobial resistance in *Staphylococcus aureus* at the University of Chicago hospitals: A 15-year longitudinal assessment in a large university-based hospital. *Infect. Control*, 24: 403-408.
- Shrestha, B., B.M. Pokhrel and T.M. Mohapatra, 2009. Phenotypic characterization of nosocomial isolates of *Staphylococcus aureus* with reference to MRSA. *J. Infect. Dev. Countries*, 3: 554-560.
- Sibery, G.K., T. Tekle, K. Carrol and J. Dick, 2003. Failure of clindamycin treatment of methicillin-resistant *Staphylococcus aureus* expressing inducible clindamycin resistance *in vitro*. *Clin. Infect. Dis.*, 37: 1257-1260.
- Upadhyay, A. and S. Biradar, 2011. The prevalence of inducible clindamycin resistance in *Staphylococcus aureus* in a tertiary care hospital in North-East Karnataka, India. *Health Sci.: Int. J.*, 1: 21-24.
- Weber, J.T., 2005. Community-associated methicillin-resistant *Staphylococcus aureus*. *Clin. Infect. Dis.*, 41: S269-S272.
- Wenzel, R.P. and T.M. Perl, 1995. The significance of nasal carriage of *Staphylococcus aureus* and the incidence of postoperative wound infection. *J. Hosp. Infect.*, 31: 13-24.