ORIGINAL ARTICLE

Surveillance of Inducible Clindamycin Resistance of *Staphylococcus aureus* in Hyderabad.

Atif A. Patoli*, Bushra B. Patoli*, Nimerta Kumari*, Taj M. Machhi**

*Institute of Microbiology, University of Sindh, Jamshoro. Pakistan
** Department of Medicine, Agha Khan University, Karachi, Pakistan.

Abstract

Objective
To evaluate and analyze the inducible and constitutive clindamycin resistance of *Staphylococcus aureus* from various clinical samples (i.e. blood, wound, nose, and urine in Hyderabad.

Methodology
Staphylococcus aureus isolates from various clinical specimens were included in this study. The sensitivity profiling against test antibiotics and determination of Inducible clindamycin resistance was performed by traditional Kirbeybaur disc diffusion and D-test respectively.

Results
The percentage of erythromycin resistant strains was 37% (n=56), of these ~43% (n=24) displayed constitutive clindamycin resistance while ~57% (n=32) were sensitive to clindamycin. Approximately two-third, 63% (n=20) of Clindamycin-Erythromycin-Discordant strains displayed $iMLS_B$ phenotype. The percentage of $iMLS_B$ phenotype in *Staphylococcus aureus* recovered from male and female was 64% (n=16) and 57.1% (n=4) respectively. OR was 1.33 [CI (95%) = 0.24-7.34]. Statistically non-significant differences (p-value >0.05) for $iMLS_B$ phenotype between genders were seen. $iMLS_B$ phenotype was maximum in case of blood isolates (87.5%) followed by wound isolates (70%). The highest percentage of $iMLS_B$ phenotype was seen in the age group of 51-60 years. Pearson's co-relation co-efficient and p-value were determined to be ($r=0.639$, $p<0.05$), suggesting a significantly positive co-relation between various age groups and $iMLS_B$ phenotype.

Conclusion
The $iMLS_B$ phenotype of *Staphylococcus aureus* is independent of gender and significantly positively co-related with increasing age of the patients.

Key words
S. aureus, Clinical specimen, $iMLS_B$, $cMLS_B$, Clindamycin, Hyderabad.

Corresponding Author: Atif A. Patoli,
Assistant Professor,
Institute of Microbiology,
University of Sindh, Jamshoro, Pakistan.
Email:atifpatoli@gmail.com

Introduction
The growing interest in the resistance of *Staphylococcus aureus* (*S. aureus*) to various antibiotics in the last few decades[1] has led to the use of alternative agents such as macrolide, lincosamide and streptogramin B (MLS) family that act through the inhibition of protein synthesis.[2] Clindamycin is an efficient and economic lincosamide drug used for the treatment of staphylococci infection.[3] Clindamycin has several advantages in the treatment of *S. aureus* infections. It can be administered intravenously and orally with good bioavailability. It penetrates the skin and soft tissue easily, exerts an inhibitory action on toxin production, and is relatively inexpensive. Furthermore it is a useful choice in case of penicillin allergy.[4] In *S. aureus* the resistance against clindamycin can occur through the enzymatic inactivation of lincosamides antibiotic. Such a resistance is mediated by the *inuA* gene, however this is rare.[5] The *S. aureus* also develops resistance against clindamycin through methylation of target site, which is generally due to the cross-resistance developed against erythromycin.[6,7] Soon after the introduction of erythromycin (a Macrolide) into therapy, in 1956, resistance against it was witnessed in staphylococci.[8] Among the two types of resistance mechanisms documented for Erythromycin in *S. aureus*, the enzymatic modification of target site is common.[9] The resistance due to methylation of the target site (i.e. 23S ribosomal RNA) also leads to cross-resistance to other macrolides, lincosamides, and streptogramins B, and is named as MLS_B phenotype.[10] The MLS_B phenotype is encoded by *erm* (erythromycin ribosome methylase) gene,[11] and a variety of *erm* genes have now been reported in a large number of microorganisms.[5]

Studies have also divided the MLS_B phenotype in two categories i.e. $cMLS_B$ (constitutive) and $iMLS_B$ (inducible).[12] The $cMLS_B$ renders the strains stably resistant to macrolides, lincosamides, and streptogramins B while, the $iMLS_B$ is induced by low level of erythromycin.[8] The $iMLS_B$ in *S. aureus* can severely compromise therapy and can result in failure of clindamycin treatment of *S. aureus* infections when non-suitable therapy (e.g. erythromycin) is given.[13] The $iMLS_B$ can complicate therapy when $iMLS_B$ phenotype possibly switches into $cMLS_B$.[9] A D-test can differentiate between $cMLS_B$ and $iMLS_B$ in erythromycin resistant strains. A positive D-test suggests the presence of an *erm* gene that could result in constitutive clindamycin resistance and clinical failure.[9]

Although a great number of studies have consistently investigated the occurrence and prevalence of iMLSB among *S. aureus*.[4,14,15] The prevalence varies by bacterial susceptibility profile, bacterial species, geographic location, and patient age.[16] The data pertaining to Hyderabad regions have never been collected. We therefore wish to investigate the same to ultimately add in the current strategies for empirical therapy against *S. aureus* infections in Hyderabad regions.

Methodology

Chemicals and Media

All the media i.e. Mannitol Salt Agar, Muller Hington Agar and Nutrient broth were purchased from Oxide. Erythromycin and clindamycin impregnated discs (5µg and 2µg, respectively) were from oxide.

Staph aureus isolates

The S. *aureus* isolates used during this study were obtained from Civil Hospital Hyderabad, Diagnostic and Research Laboratory, LUMHS and a few private diagnostic laboratories in Hyderabad. All these isolates were of clinical origin specifically isolated from clinical specimens i.e. blood, wound, nose, and urine. From January 2015 to January 2016 a total of 150 identified *S. aureus* isolates were collected. The study was approved by Board of Advanced Studies and Research [No.DRGS/426] University of Sindh, Jamshoro. Following parameters were used to determine the minimum sample size (n = 87) for $iMLS_B$ phenotype in *S. aureus*; the Z value of standard normal distribution was calculated from 95% (CI) with a margin of error of 5%, and about 6% of previously reported $iMLS_B$.[17]

Antibiotic sensitivity test

The antibiotic sensitivity testing against erythromycin and clindamycin was performed using *Kirby-Bauer* Disc Diffusion method. The liquid cultures of *S. aureus* were prepared in nutrient broth. The overnight culture was diluted to OD_{600} = 0.5. to meet the McFarland's standard required for disc diffusion method. Sterile cotton swab were used to inoculate the diluted culture on Muller Hington Agar and spread evenly. The antibiotic discs (*Oxoid*) were placed on the agar surface. In order to get a flat contact with agar surface a gentle pressure was applied on the discs. The plates were incubated at 37°C for 24 hours. The diameter of the clear zones (zones of inhibition) observed around the antibiotic discs were measured according to Clinical and Laboratory Standard Institute (CLSI).

D-Test for the determination inducible clindamycin resistance.

The $iMLS_B$ phenotype was detected by performing a D-test according to CLSI guideline. Briefly, OD_{600nm}= 0.5McFarland equivalent suspension of organisms was inoculated onto a Mueller Hington agar (MHA) plate, both erythromycin and clindamycin discs were placed 15-26 mm (edge to edge) apart on agar surface. The plates were then incubated at 37°C for about 24 hours. A D-shaped zone formed due to the induction of resistance by erythromycin in clindamycin sensitive strain was recorded as positive D-test.

Statistical Analysis

The data analysis was done using Microsoft excel and online statistic software. The individual as well as collective levels of resistance against erythromycin and clindamycin in *S. aureus* were measured both in terms of absolute and relative values. The percentage of strains expressing $iMLS_B$ phenotype and their relevance to gender and various clinical samples were the variable of interest. The Odds Ratios (OR) and CI with 95% were calculated manually and using a statistic calculator. The *p*-values were calculated using a Fisher's Exact test of independence.

Results

A total of 150 identified *S. aureus* isolates recovered from blood, urine, wound and nose, were included in the current study. One hundred and nine (109) of these were from male patients while 41 were from female patients. Maximum number (n=68) isolates were from nose followed by urine (n=36). All the strains were processed for antimicrobial sensitivity against erythromycin and clindamycin by traditional Kirby baur disc diffusion test. The overall percentage of resistance against erythromycin was found to be 37% (n=56), of these 42.8% (n=24) displayed constitutive clindamycin resistance while 57.2% (n=32) were sensitive to clindamycin (Table 1). These erythromycin-clindamycin (EC) discordant strains were then subjected to a D-Test for the detection of $iMLS_B$ phenotype. The $iMLS_B$ phenotype was detected in 62.5% (n=20) of the EC discordant strains. Categorically higher percentage of $iMLS_B$ phenotype was seen in case of isolates from male patients (64%) than female patients (57.1%) (Figure 1). To evaluate the differences the OR = 1.33 with CI (95%)= [0.24-7.34] was calculated. The analysis using Fisher's Exact test of independence suggested a statistically non-significant level of difference (p=1.000, α=0.05).

Among various clinical specimens, the maximum display of $iMLS_B$ phenotype was witnessed in case of blood isolates (88%) while the minimum was seen in case of nose isolates (20%). The $cMLS_B$ phenotype was most common in case of nose isolates (62%). Figure 2 displays the bar diagram for comparative percentages of $iMLS_B$ and $cMLS_B$ phenotypes among the isolates of various clinical origins.

We also analyzed the data for the display of $iMLS_B$ and $cMLS_B$ phenotype with reference to the age of the patients. The data were categorized in various randomly selected age groups. The magnitude of the age was selected to be 10. Figure 3 displays the percentages of $iMLS_B$ and $cMLS_B$ phenotypes in various age groups. The maximum percentage of $iMLS_B$ and $cMLS_B$ phenotypes were seen in case of age group 51-60 years i.e. (100% and 66.6%, respectively). To determine the co-relation between age of the patient and $iMLS_B$ phenotype (if any) we

performed bivariate co-relation analysis. The Pearson's co-relation co-efficient and p-value were determined to be (r=0.639, *p*=0.00001), suggesting a significantly positive co-relation between various age groups and $iMLS_B$ phenotype.

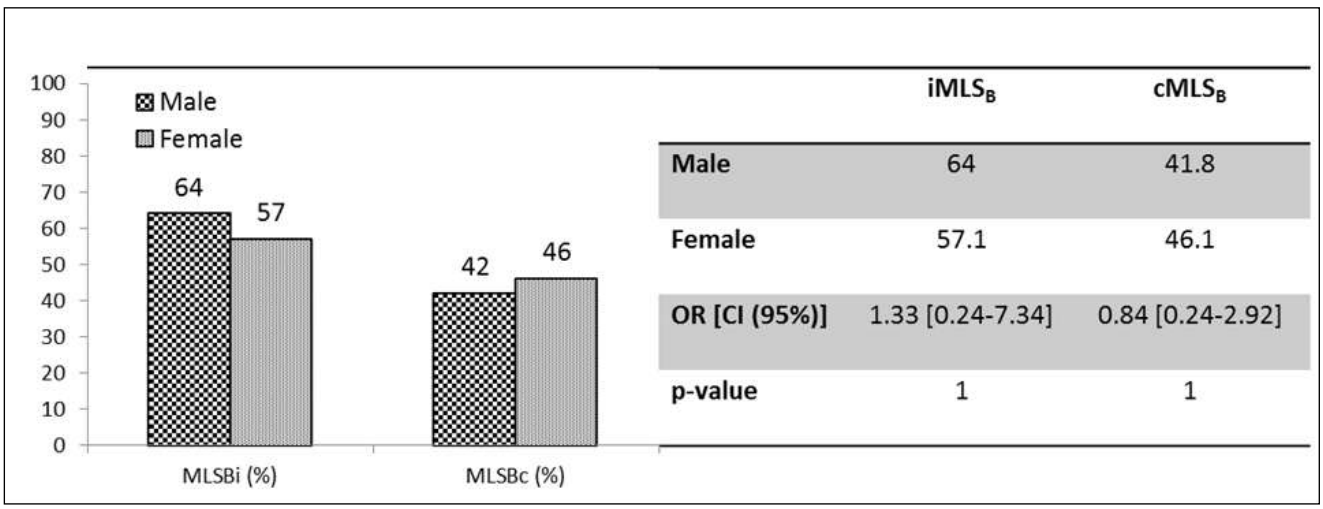
Discussion

Clindamycin is an efficient and economic lincosamide drug used for the treatment of staphylococci infection.[3] Though structurally different the Clindamycin functions similar to Macrolide, other lincosamides and Streptogramin family of antibiotics. All these affect the *S. aureus* growth by interfering the protein synthesis through their binding to the 23S rRNA on the 50S subunit of the bacterial ribosome.[7] The resistance against clindamycin can develop either due to *inuA* gene or can

Table 1: Table showing the absolute and relative values (expressed in percentage) for $iMLS_B$ and $cMLS_B$ phenotypes.

Specimen	*S. aureus* (n)	Ery [R] (n)	Clin [R] (n)	Ery [R]-Cli [R] (n)	EC-Discordant (n)	D - Positive (n)	D - Negative (n)	$iMLS_B$ %	$cMLS_B$ %
Nose	68	21	14	13	8	3	5	37.5	61.9
Wound	27	16	9	6	10	7	3	70	37.5
Blood	19	10	3	2	8	7	1	87.5	20
Urine	36	9	4	3	6	3	3	50	33.3
Total	**150**	**56**	**30**	**24**	**32**	**20**	**12**	**62.5**	**42.8**
Gender									
Male	109	43	22	18	25	16	9	64	41.8
Female	41	13	8	6	7	4	3	57.1	46.1
Total	**150**	**56**	**30**	**24**	**32**	**20**	**12**	**62.5**	**42.8**
Age-groups									
01 -to- 10	1	0	0	0	0	0	0	0	0
11 -to- 20	35	14	8	6	8	5	3	62.5	42.8
21 -to- 30	50	18	9	9	9	6	3	66.6	50
31 -to- 40	40	12	5	4	8	4	4	50	33.3
41 -to- 50	17	9	6	3	6	4	2	66.6	33.3
51 -to- 60	7	3	2	2	1	1	0	100	66.6
Total	**150**	**56**	**30**	**24**	**32**	**20**	**12**	**62.5**	**42.8**

R = Resistant, EC = Erythromycin Clindamycin, Ery = Erythromycin, Clin = Clindamycin.



	$iMLS_B$	$cMLS_B$
Male	64	41.8
Female	57.1	46.1
OR [CI (95%)]	1.33 [0.24-7.34]	0.84 [0.24-2.92]
p-value	1	1

Fig 1. (Left) A bar diagram describing the percentages of $iMLS_B$ and $cMLS_B$ phenotype in *S. aureus* strains isolated from both male and female patients. (Right) Table describing the OR and p-values

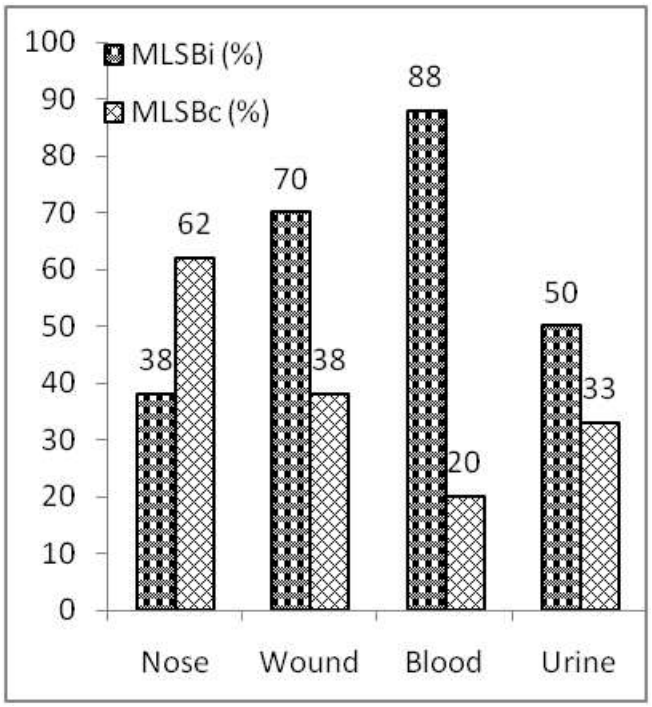


Fig 2. A bar diagram describing the percentages of iMLS$_B$ and cMLS$_B$ phenotype in *S. aureus* strains isolated from various clinical samples.

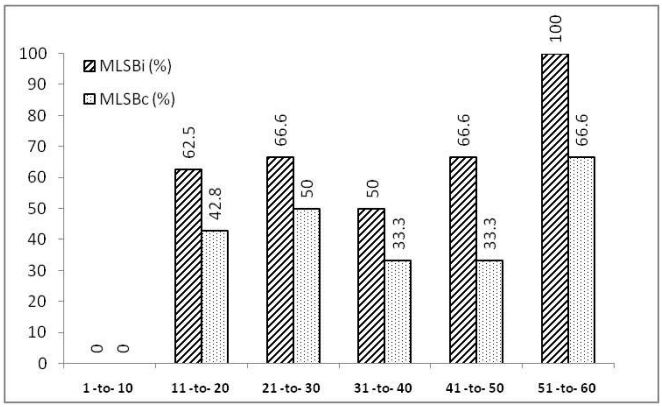


Fig 3. A bar diagram describing the percentages of iMLS$_B$ and cMLS$_B$ phenotype in *S. aureus* strains isolated from patients of various age groups.

be induced due to erythromycin ribosome methylase encoded by *ermA* gene in *S. aureus*. The current study was performed to evaluate the frequency of inducible type of clindamycin resistance in clinical *S. aureus* isolates. Out of 150 *S. aureus* isolates from various clinical specimens 37% (n=56) were resistant to erythromycin. The constitutive clindamycin resistance was seen in 16% of the total *S. aureus* included in this study. A study conducted in 2016 (Israel) reported about 26.8% of the constitutive Clindamycin-resistance.[8] Previously Fasih N. and others.[14] reported about 30% of the constitutive clindamycin resistance in Pakistan. In the current study, we extended our analysis to more specific outcomes. We calculated the percentages of inducible and constitutive types of clindamycin resistance in 56 Erythromycin resistant isolates. Twenty four (42.8%) of these isolates displayed the cMLS$_B$ phenotype while thirty two (57.2%) were sensitive to clindamycin. The potential inducer of inducible clindamycin resistance in *S. aureus* is erythromycin in erythromycin resistant strains. We therefore determined the inducible clindamycin resistance in these strains using a phenotypic D-Test. Out of these 32 EC-discordant strains the iMLS$_B$ was seen in 62% (n=20) of the isolates. Six (6%) and seventy two percent (72%) of the iMLSB has been reported based on a two independent studies conducted on clinical *S. aureus* isolates from Karachi.[14] and Lahore[17]. We calculated the OR and CI(95%) to determine if the iMLS$_B$ is affected by gender or not. The statistical analysis suggested non-significant differences of iMLS$_B$ phenotypes between genders. The data was also analyzed to determine the relationship (if any) between iMLS$_B$ phenotypes and the age of the patients. Various age groups with uniform class magnitude were formed. The frequency of D-positive cases in each group was expressed in relative values. The Pearson's co-relation co-efficient and *p*-value were determined, the results suggested a significantly positive co-relation between various age groups and iMLS$_B$ phenotype. Given the usefulness of clindamycin antibiotic in the treatment of *S. aureus* infections and the scarcity of relevant published literature based on the data in Pakistan, the current findings will play valuable part in the management and rational formulation of antibiotic regime.

Acknowledgment

We would like to acknowledge Institute of Microbiology, University of Sindh, Jamshoro, Pakistan for providing bench space, glassware and equipment.

Funding

This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors.

References

1. Schito GC. The importance of the development of antibiotic resistance in Staphylococcus aureus. *Clin Microbiol Infect* 2006;12 Suppl 1:3-8.
2. Delialioglu N, Aslan G, Ozturk C, Baki V, Sen S, *et al*. Inducible clindamycin resistance in staphylococci isolated from clinical samples. *Jpn J Infect Dis* 2005;58(2):104-6.
3. Oguz VA, Yapar N, Sezak N, Cavus SA, Kurutepe S, *et al*. [The rate of inducible clindamycin resistance and susceptibilities to other antimicrobial agents in staphylococci]. *Mikrobiyol Bul* 2009;43(1):37-44.
4. Afridi FI, Zeb M, Hussain A, Farooqi BJ, Murtuza G. Inducible clindamycin resistance in Staphylococcus species. *J Coll Physicians Surg Pak* 2014;24(7):481-4.
5. Roberts MC, Sutcliffe J, Courvalin P, Jensen LB, Rood J, *et al*. Nomenclature for macrolide and macrolide-lincosamide-streptogramin B resistance determinants. *Antimicrob Agents Chemother* 1999;43(12): 2823-30.
6. Weisblum B. Erythromycin resistance by ribosome modification. *Antimicrob Agents Chemother* 1995;39(3):577-85.
7. Tenson T, Lovmar M, Ehrenberg M. The mechanism of action of macrolides, lincosamides and streptogramin B reveals the nascent peptide exit path in the ribosome. *J Mol Biol* 2003;330(5):1005-14.

8. Leclercq R. Mechanisms of resistance to macrolides and lincosamides: nature of the resistance elements and their clinical implications. *Clin Infect Dis* 2002;34(4):482-92.
9. Levin TP, Suh B, Axelrod P, Truant AL, Fekete T. Potential clindamycin resistance in clindamycin-susceptible, erythromycin-resistant Staphylococcus aureus: report of a clinical failure. *Antimicrob Agents Chemother* 2005;49(3):1222-4.
10. B W. Erythromycin resistance by ribosome modi?cation. *Antimicrob Agents Chemotherapy* 1995;39:577–85.
11. Werckenthin C, Schwarz S. Molecular analysis of the translational attenuator of a constitutively expressed erm (A) gene from Staphylococcus intermedius. *J Antimicrob Chemother* 2000;46(5):785-8.
12. Adhikari RP, Shrestha S, Barakoti A, Amatya R. Inducible clindamycin and methicillin resistant Staphylococcus aureus in a tertiary care hospital, Kathmandu, Nepal. *BMC Infect Dis* 2017;17(1):483.
13. Yilmaz G, Aydin K, Iskender S, Caylan R, Koksal I. Detection and prevalence of inducible clindamycin resistance in staphylococci. *J Med Microbiol* 2007;56(Pt 3):342-5.
14. Fasih N, Irfan S, Zafar A, Khan E, Hasan R. Inducible clindamycin resistance due to expression of erm genes in Staphylococcus aureus: report from a tertiary care Hospital Karachi, Pakistan. *J Pak Med Assoc* 2010;60(9):750-3.
15. Iffat Javeid SM, Aasma Noveen Ajmal. Inducible Clindamycin Resistance in Staphylococcus aureus Isolates Recovered in Specimen from Tertiary Care Hospital. *Pak J Med Health Sci* 2012;6(4):1049-52.
16. Siberry GK, Tekle T, Carroll K, Dick J. Failure of clindamycin treatment of methicillin-resistant Staphylococcus aureus expressing inducible clindamycin resistance in vitro. *Clin Infect Dis* 2003;37(9):1257-60.
17. Iffat Javeid, Sohaila Mushtaq, Aasma Noveen Ajmal; Inducible Clindamycin Resistance in Staphylococcus aureus Isolates Recovered in Specimen from Tertiary Care Hospital. *Pak J Med Health Sci* Oct - Dec 2012;6(4):1049-52
18. Stein M, Komerska J, Prizade M, Sheinberg B, Tasher D, et al. Clindamycin resistance among Staphylococcus aureus strains in Israel: implications for empirical treatment of skin and soft tissue infections. *Int J Infect Dis* 2016;46:18-21.