Prevalence of MLSB Phenotypes of *Staphylococcus aureus* isolates in a tertiary care hospital of Delhi

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Abstract

Against the backdrop of the ever-changing Staphylococcal resistance pattern, clindamycin remains a viable therapeutic alternative Variation of Clindamycin drug resistance patterns with geographic area make inducible clindamycin resistance testing imperative for all staphylococcal isolates to avoid therapeutic failure. This was a prospective study conducted over a period of 1.5 years from January 2021 until June 2022. Prevalence of different MLS_B Phenotypes of *Staphylococcus aureus* isolates was determined by standard disc diffusion method as per CLSI guidelines. Pyogenic samples received in the Microbiology lab that yielded *Staphylococcus aureus* were further tested for the presence of clindamycin resistance by disc diffusion method. Out of 6586 total pyogenic and respiratory specimens received in the lab, *Staphylococcus aureus* was yielded in 752 samples. On further testing for the MLS_B phenotypes, 16.3% isolates were found to be $iMLS_B$, 19.28% were $cMLS_B$, 43.1% were of MS_B type. ICR screening will reduce the unessential subjection of the patient to the antibiotic, and would prevent unnecessary adverse effects in the patients.

Introduction

Staphylococcus aureus (*S. aureus*) is a potential pathogen as well as a colonizer of the humans owing to the arsenal of virulence factors including toxins such as TSST-1 (toxic shock syndrome toxin), exfoliative toxins (ETA and ETB), heat stable enterotoxins etc. Manifestation of Staphylococcal infections ranges from local (folliculitis, carbuncles, furuncles, impetigo, wound infections) to systemic (endocarditis, pneumonia, sepsis, osteomyelitis, arthritis). Localised *S. aureus* infections have the potential to become invasive and cause bacteremia at any stage of the infection. The mainstay of treatment for these infections include cell wall inhibitors such as β-lactams, glycopeptides, DNA gyrase-inhibiting quinolones, and ribosomal inhibitors such as macrolides, lincosamides and streptogramins (MLS_B).

MLS_B drugs are a good alternative in treating infections, especially in current times of increasing resistance. Clindamycin in particular is an important antibiotic for skin and soft tissue infections caused by *S. aureus* (especially MRSA *i.e.*, Methicillin resistant *Staphylococcus aureus*) due to its ease of administration (available as oral/parenteral) and its property to neutralise toxins. It switches off production of toxins like TSST responsible for toxic shock syndrome,[1] alpha toxin which is a pore forming cytotoxin leading to infections such as dermonecrosis, keratoconjuctivitis and pneumonia[2] and pVL (Panton-Valentine leukocidin), which is associated with manifestations like necrotising pneumonia, purpura fulminans and skin sepsis.[3] The three antimicrobial classes of MLS_B act by binding to the 50s ribosomal subunit, thus inhibiting protein synthesis in the bacteria.[4] Resistance amongst these can be conferred mainly by three mechanisms – target site modification, antimicrobial inactivation and efflux.

The enzyme erythromycin ribosome methylases plays the most significant role in the resistance, by attaching the adenine residue of 23s rRNA to methyl groups, thus decreasing affinity for MLS_B antibiotics. It is encoded by the *erm* (erythromycin ribosome methylation) gene which is of three main types *i.e.*, *erm* (A), *erm* (B) and *erm* (C); also, genes *erm* (F) and *erm* (Y) may be responsible.

The other mechanisms that contribute to the cross resistance of these MLS_B phenotypes include drug inactivation mediated by *lun* gene and active efflux mechanisms that pumps out antimicrobials from the bacteria, mediated by *msr* gene.[5]

MLS_B drugs can exist as different phenotypes – constitutive, inducible, or MS_B (Figure 1): i) constitutive MLS_B ($cMLS_B$) – defined as those isolates which are clindamycin and erythromycin resistant; ii) inducible MLS_B ($iMLS_B$) – defined as isolates which are clindamycin susceptible and erythromycin resistant. However, a D-shaped zone of inhibition is seen around clindamycin, with flattening towards the erythromycin disc; iii) MS_B – is defined as those isolates which are clindamycin susceptible and erythromycin resistant with a circular zone of inhibition around the two.

Clinical and Laboratory Standards Institute (CLSI) states two methods for detecting Inducible Clindamycin Resistance (ICR), *i.e.,* by disc diffusion and broth microdilution. Detection of inducible clindamycin resistance in particular holds significance in clinical scenarios, wherein the *S. aureus* isolates exhibiting *in vitro* clindamycin susceptibility will not show *in vivo* response on administration of the drug. This leads to unnecessary overuse of the drug in the patient, thus enhancing the risk of emergence of resistant strains of bacteria and putting the patient at increased risk of side effects of the drug. Improper treatment during the initial phase can also put the patient at risk for metastasis of the disease.

Our current study aims at identifying the distribution of MLS_B phenotypes of *S. aureus* isolates for better understanding of

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resistance patterns to crucial antibiotic of clindamycin in the management of infections caused by *S. aureus.*

Material and Methods

This was a prospective study carried out over a period of one and a half year spanning from January 2021 to June 2022 in our tertiary care hospital of Delhi. A total of 6586 samples, including pus aspirates, peritoneal fluid, pleural fluid, synovial fluid, respiratory samples, and genital secretions were received in the microbiology lab of our hospital. The samples were cultured on Blood agar, MacConkey agar and Chocolate agar using standard laboratory protocols. Bacterial identification of the growth was done by conventional methods, using biochemical reactions (Catalase, slide and tube coagulase, Mannitol salt agar).

The samples that yielded growth of *S. aureus* on culture were further subjected to Antimicrobial Susceptibility Testing (AST) by Kirby Bauer disk diffusion method, according to latest CLSI guidelines.[6] For AST 0.5 McFarland of the strain was lawn cultured on Muller Hinton agar, followed by placement of the antimicrobial discs at a distance of 15-20 mm edge to edge from each other and incubation at 35°C±2°, ambient air.

Isolates were classified as Methicillin susceptible or resistant on the basis of zone of inhibition diameters of Cefoxitin. While, presence of clindamycin resistance (constitutive, inducible and MS_B) was determined by performing disk diffusion method, placing Erythromycin (15µg) and Clindamycin (2µg) at a distance of 15-26mm from each other. Zone cut-offs for the antibiotics have been descried in the Table 1. Isolates with intermediate zone diameters were considered as resistant for ICR analysis. Presence of D-zone *i.e.*, flattening of the zone of inhibition adjacent to the erythromycin disc was interpreted as inducible clindamycin resistance, as shown in Figure 1a.

Results

Out of the total 6586 pyogenic and respiratory samples received, *S. aureus* was isolated from 11.4% (752/6586) samples. Majority of these samples were received from the patients admitted in surgical wards. The organism was isolated more commonly from the male population (54.9%) as compared to the females (45.07%). Isolation of *S. aureus* was more common from adult patient population (71.8%) in comparison to the paediatric population (28.9%).

Of the total *S. aureus* isolates 335 (44.54%) were MSSA (Methicillin sensitive *Staphylococcus aureus*), while 417 (55.45%) were MRSA (Methicillin resistant *Staphylococcus aureus*. All the strains of this gram-positive organism were tested for different MLS_B phenotypes *i.e.*, inducible, constitutive and MS_B. Inducible clindamycin resistance was found in 16.35% of the isolates; constitutive clindamycin resistance was observed in 19.28% of the observed isolates, while MS_B phenotypes were observed in 43.08%. Percentage distribution of various MLS_B phenotypes has been described in Table 2.

Distribution of MSSA and MRSA were also observed among the MLS_B phenotypes (Table 3). On application of Fischer's exact test, no significant association was observed between methicillin susceptibility of the isolates and the constitutive and MS_B phe-

Figure 1. Identification of various MLSB phenotypes of *Staphylococcal aureus* isolates from clinical samples (n=752): a) inducible MLS_B ($iMLS_B$); b) constitutive MLS_B ($cMLS_B$).

Table 1. Antimicrobial susceptibility break points (CLSI 2022).

Antibiotic	Susceptible	Intermediate	Resistant
Erythromycin (15 µg)	≥23 mm	14-22 mm	≤13 mm
Clindamycin (2 µg)	≥21 mm	15-20 mm	≤14 mm
Cefoxitin (30 µg)	≥22 mm	-	≤21 mm

Table 2. Distribution of various MLSB phenotypes among *Staphylococcal aureus* isolates from clinical samples (n=752).

Erythromycin susceptibility	Clindamycin susceptibility	D Test	Phenotype	No. of isolates	Percentage
Susceptible	Susceptible	Negative	-	160	21.27
Resistant	Resistant	Negative	cMLSB	145	19.28
Resistant	Susceptible	Positive	iMLSB	123	16.35
Resistant	Susceptible	Negative	MSB	324	43.08

notypes, as the *p* value was found to be 0.0556.

Association of methicillin susceptibility was established in the isolates displaying inducible clindamycin resistance. Of the total 123 isolates showing inducible clindamycin resistance, 29.2% were Methicillin susceptible while the rest 71% were found to be Methicillin resistant (Figure 2). No significant association was observed between ICR phenotype and Methicillin susceptibility ($p \geq 0.05$).

Discussion

S. aureus is the most common aetiological agent of pyogenic infections. Drugs such as Trimethoprim-Sulfamethoxazole, Tetracyclines (Minocycline and Doxycycline) and Clindamycin have gained importance in present scenario of increasing drug resistance in staphylococcal isolates.[7]

Clindamycin, belongs to the Lincosamide group of antibiotics and possesses activity against gram-positive as well as anaerobic bacteria. Its properties such as good tissue penetration, cost, spectrum and, oral bioavailability make clindamycin conducive to treating infections. It is thus, used for skin and soft tissue infections, with particular significance in cases of CA-MRSA infections, wherein an oral treatment regimen can suffice for the patient. This Lincosamide antibiotic is also effective in treating conditions such as pleural empyema, osteomyelitis and septic arthritis.

Though clindamycin has several properties to its advantage, there are a few challenges that a clinician faces while using the drug. Pseudomembranous colitis due to *Clostridioides difficile* is observed in 0.1-10% of the patients using clindamycin persistently[1] and likelihood of failure if the strain possesses *erm* gene are the two main disadvantage to clindamycin use. Clindamycin resistance can either be induced or can be rendered constitutively based on the phenotype.

In our study, constitutive resistance to the MLS_B drugs was found to be more (19.3%) in comparison to the inducible phenotype. ICR rates were found to be 16.35%, which were considerably higher in MRSA isolates (70.8%) than the MSSA strains. Not many studies have commented upon the reason justifying the higher prevalence of ICR in MRSA, but one possible explanation is more positivity rate for *ermA* in MRSA than MSSA.[8] This is indicative of increased chances of treatment failure with clindamycin in resistant infections. Table 4 compares the distribution of MLS_B phenotypes in various geographical regions of our country and beyond.

The presence of MS_B phenotype in our study was higher in comparison to the other two variants. Similar finding was observed in the other areas of Delhi.[10] Therefore, Clindamycin can be used empirically by clinicians for indicated infections with lesser chances of it turning out to be ineffective.

Table 4 shows the Geographical distribution of MLS_B phenotypes in various geographical regions. In our study higher prevalence of $cMLS_B$ than that of $iMLS_B$ was observed, which was found to be in concordance with other studies conducted in the regions of Kolkata, Shimla and Nepal.[4,6,7] Conversely higher prevalence of $iMLS_B$ than $cMLS_B$ was observed in other regions of Delhi and Wardha.[5,8] The varying

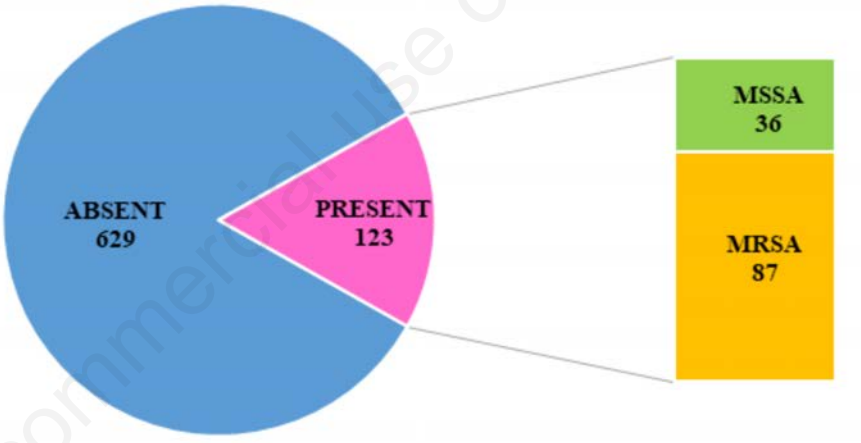

Figure 2. Distribution of MSSA and MRSA among *Staphylococcus aureus* isolates exhibiting inducible clindamycin resistance (n=752).

Table 3. MSSA & MRSA distribution amongst the constitutive and MSB phenotypes.

MLS_B Phenotype	MSSA (%)	MRSA (%)
Constitutive	59 (40.7)	86 (59.4)
MSB	171 (52.8)	153 (47.2)

Table 4. Geographical distribution of MLSB phenotypes in various geographical regions.

Study	Year	Region	No. of isolates (n)	$iMLS_B$ (%)	$cMLS_B$ (%)	MSB (%)
Kumar *et al.*[9]	2010	Kolkata, India	195	16.9	23.1	16.9
Lall and Sahni *et al.*[10]	2014	Delhi, India	305	43.1	21.4	54.3
Mokta *et al.*[11]	2015	Shimla, India	350	13.71	17.14	8.28
Deotale *et al.*[12]	2017	Wardha, India	247	14.5	3.6	14.17
Adhikari *et al.*[13]	2017	Nepal	147	21	53.4	25.17
Our study	2022	East Delhi, India	752	16.35	19.28	43.08

geographical prevalence of different resistance patterns emphasizes upon the importance of Clindamycin testing in all isolates.

It was observed that the prevalence of clindamycin resistance (both $cMLS_B$ and $iMLS_B$) was more in MRSA isolates in comparison to the MSSA isolates that was consistent with the findings of other studies.[11,14,15]

Against the backdrop of the ever-changing Staphylococcal resistance pattern, clindamycin remains a viable therapeutic alternative. Our study may prove useful in better understanding of varying distribution of different MLS_B phenotypes of *S.aureus* in recent times. Variation of Clindamycin drug resistance patterns with methicillin susceptibility, geographic area and even intercity[16] differences make ICR testing imperative for all staphylococcal isolates to avoid therapeutic failure.

References

1. Clindamycin: An overview - UpToDate. Accessed 2023 May 11. Available from: https://www.uptodate.com/contents/clindamycin-an-overview?search=clindamycin%20in%20staphylococcal%20infections&source=search_result&selectedTitle=3~150&usage_type=default&display_rank=3#H12
2. Alpha Toxin – an overview | ScienceDirect Topics. Accessed 2023 May 11. Available from: https://www.sciencedirect.com/topics/medicine-and-dentistry/alpha-toxin
3. Morgan M. Staphylococcus aureus, Panton-Valentine leukocidin, and necrotising pneumonia. BMJ 2005; 331:793-4.
4. Saribas Z, Tunckanat F, Pinar A. Prevalence of erm genes encoding macrolide-lincosamide-streptogramin (MLS) resistance among clinical isolates of Staphylococcus aureus in a Turkish university hospital. Clin Microbiol Infect 2006;12:797-9.
5. Ghanbari F, Ghajavand H, Havaei R, et al. Distribution of erm genes among Staphylococcus aureus isolates with inducible resistance to clindamycin in Isfahan, Iran. Adv Biomed Res 2016;5:62.
6. CLSI-31-2021.pdf. Accessed 2022 Oct 9. Available from: https://www.treata.academy/wp-content/uploads/2021/03/CLSI-31-2021.pdf
7. Moellering, Jr. RC. Current treatment options for community-acquired methicillin-resistant Staphylococcus aureus infection. Clin Infect Dis 2008;46: 1032-7.
8. Nahar L, Hagiya H, Nada T, et al. Prevalence of Inducible Macrolide, Lincosamide, and Streptogramin B (inducible MLSB) Resistance in Clindamycin-Susceptible Staphylococcus aureus at Okayama University Hospital. Acta Med Okayama 2023;77.
9. Kumar S, Bandyopadhyay M, Bhattacharya K, et al. Inducible clindamycin resistance in staphylococcus isolates from a tertiary care hospital in Eastern India. Ann Trop Med Public Health 2012;5:468.
10. Lall M, Sahni AK. Prevalence of inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. Med J Armed Forces India 2014;70:43-7.
11. Mokta KK, Verma S, Chauhan D, et al. Inducible clindamycin resistance among clinical isolates of Staphylococcus aureus from sub himalayan region of India. J Clin Diagn Res JCDR 2015;9:DC20-3.
12. Deotale V, Mendiratta D, Raut U, Narang P. Inducible clindamycin resistance in Staphylococcus aureus isolated from clinical samples. Indian J Med Microbiol 2010;28:124-6.
13. 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;17:483.
14. Supriyarajvi, Gupta A, Tina G, Sharma BP. Detection of inducible clindamycin resistance among Staphylococcal isolates from various clinical specimens in a tertiary care institute in north west region of Rajasthan, India. Int J Curr Microbiol Appl Sci 2015;4:741-9.
15. Molecular Characterisation of Methicillin-Resistant Staphylococcus aureus Isolated from Patients at a Tertiary Care Hospital in Hyderabad, South India. Indian J Med Microbiol 2020;38:183-92.
16. Schreckenberger PC, Ilendo E, Ristow KL. 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 2004;42:2777-9.