

Inducible Clindamycin Resistance in Methicillin Resistant *Staphylococcus aureus* Blood Culture Isolates in a Tertiary Care Hospital, Ranchi

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Abstract: **Background:** The resistance to antimicrobial agents among Staphylococci is an increasing problem. The management of the infections by it especially methicillin resistant ones is often difficult because methicillin resistant *S. aureus* is usually resistant to multiple antibiotics. This has led to renewed interest in the usage of Macrolide-Lincosamide-Streptogramin B (MLS_B) antibiotics to treat *Staphylococcus aureus* (*S. aureus*) infections as an alternative to vancomycin. In vitro routine tests for clindamycin susceptibility may fail to detect inducible clindamycin resistance due to *erm* genes resulting in treatment failure, thus necessitating the need to detect such resistance by a simple D test on a routine basis. **Materials and Methods:** This retrospective study was conducted over the period of one year from 1st June 2019–1st June 2020 in Microbiology laboratory of Rajendra Institute of Medical Sciences (RIMS), Ranchi to find the incidence of different phenotypes of MLS_B resistance among *S. aureus* from blood culture isolates and their association with methicillin resistance. One hundred thirty five isolates of *S. aureus* were included in the study. Methicillin resistance was detected by cefoxitin disc diffusion method and inducible clindamycin resistance by erythromycin and clindamycin disc approximation test (D-test). **Results:** The overall blood cultures done in 12 months were 1261, in which *Staphylococcus aureus* was isolated from 135 samples. Of the 135 isolates of *S. aureus*, 60% (81/135) were MRSA. Erythromycin and clindamycin resistance was seen in 62.96% (85/135) and 40% (54/135) isolates respectively. Resistance to erythromycin and clindamycin were higher in MRSA as compared to MSSA (erythromycin-resistance: 74.07% Vs 46.29% and clindamycin resistance: 51.85% Vs 22.22%). The overall prevalence of iMLS_B and cMLS_B phenotype was 8.8% (12/135) and 37.03% (50/135) respectively. Both iMLS_B and cMLS_B phenotypes predominated in MRSA strains. **Conclusion:** Our high prevalence of clindamycin resistance in the form of iMLS_B and cMLS_B especially among MRSA emphasizes the need of D-test to be performed routinely in our set up while using clindamycin as an alternative choice to anti-staphylococcal antibiotics like vancomycin and linezolid in the treatment of staphylococcal infections.

Keywords: Inducible clindamycin resistance, methicillin resistant *Staphylococcus aureus*, *Staphylococcus aureus*

1. Introduction

Staphylococcus aureus, one of the most common nosocomial and community-acquired pathogens has now emerged as an ever-increasing problem due to its increasing resistance to several antibiotics. The increasing prevalence of methicillin resistance among Staphylococci is an increasing problem.[1] This has led to renewed interest in the usage of Macrolide-Lincosamide-Streptogramin B (MLS_B) antibiotics to treat *S. aureus* infections with clindamycin being the preferred agent due to its excellent pharmacokinetic properties.[2, 3] However, widespread use of MLS_B antibiotics has led to an increase in the number of Staphylococcal strains acquiring resistance to MLS_B antibiotics.[3, 4] Clindamycin resistance in *Staphylococcus* species can be either constitutive or inducible.[5] The most common mechanism for such resistance is target site modification mediated by *erm* genes, which can be expressed either constitutively (constitutive MLS_B phenotype) or inducibly (inducible MLS_B phenotype). Strains with inducible resistance to clindamycin are difficult to detect in the routine laboratory as they appear erythromycin-resistant and clindamycin sensitive in vitro when not placed adjacent to each other. In such cases, in vivo therapy with clindamycin may select constitutive *erm* mutants leading to clinical therapeutic failure. In case of another mechanism of resistance mediated through *msrA* genes i.e. efflux of antibiotic, Staphylococcal isolates appear erythromycin-resistant and clindamycin-sensitive both in vivo and in vitro and the strain do not typically become clindamycin resistant during therapy.[6]. This study was

conducted to determine the prevalence of inducible and constitutive clindamycin resistance in *S. aureus* blood culture isolates and also to study their association with MRSA in our set up in RIMS, Ranchi.

2. Materials and Methods

From June 2019 to June 2020, all *S. aureus* isolates from blood cultures collected at our hospital were included in the study. All specimens were inoculated on sheep blood agar, MacConkey agar and incubated at 37 °C aerobically for 24 h. The isolates were first identified as *S. aureus* by standard biochemical techniques[7] and then subjected to susceptibility testing by modified Kirby Bauer's disc diffusion method on Mueller Hinton agar plates using erythromycin (15 µg), clindamycin (2 µg), cefoxitin (30 µg) as per CLSI guidelines.[8]. An inhibition zone of 19 mm or less around cefoxitin disc indicates MRSA. Inducible resistance to clindamycin was tested by 'D test' as per CLSI guidelines.[9] Briefly, erythromycin (15 µg) disc was placed at a distance of 15mm (edge to edge) from clindamycin (2 µg) disc on a Mueller-Hinton agar plate, previously inoculated with 0.5 McFarland standard bacterial suspensions. Following overnight incubation at 37°C, flattening of zone (D-shaped) around clindamycin in the area between the two discs, indicated inducible clindamycin resistance [Figure 1].

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Clindamycin resistance was detected as:

- 1) Inducible resistance phenotypes (iMLSB): Resistant to erythromycin and having a clindamycin zone ≥ 21 mm with a D-shaped zone.
- 2) Constitutive resistance phenotypes (cMLSB): resistant to both erythromycin and clindamycin
- 3) MS phenotype: Isolates resistant to erythromycin and susceptible to clindamycin without D-zone [8].
- 4) *S. aureus* ATCC 25923 was used to perform quality control. Separate selected *S. aureus* strains that demonstrated the above phenotype was also used in quality control.

3. Results

The overall blood culture isolates in 12 months were 1261, in which *Staphylococcus aureus* were isolated from 135 samples. Of the 135 isolates of *S. aureus*, 60% (81/135) were MRSA. Erythromycin and clindamycin resistance was seen in 62.96% (85/135) and 40% (54/135) isolates respectively. Resistance to erythromycin and clindamycin were higher in MRSA as compared to MSSA (erythromycin-resistance: 74.07% Vs 46.29% and clindamycin resistance: 51.85% Vs 22.22%). The overall prevalence of iMLSB and cMLSB phenotype was 8.8% (12/135) and 37.03% (50/135) respectively. Both iMLSB and cMLSB phenotypes predominated in MRSA strains (Table 1). Among 85 erythromycin resistant isolates, 10.58% iMLSB, 45.88% cMLSB and 14.11% MS phenotype were MRSA (Table 2).

Table 1: Clindamycin susceptibility patterns among MRSA and MSSA

		MRSA(%) N=81	MSSA(%) N=54	Total(%) N=135
E-S (n=50)	E-S, Cl-S	5(6.1%)	30(56%)	35(25.9%)
	E-S, Cl-R	3(3.7%)	1(1.8%)	4(2.96%)
E-R (n=85)	E-R, Cl-S (iMLSB)	9(11.11%)	3(5.5%)	12(8.8%)
	E-R, Cl-R (cMLSB)	39(48.1%)	11(20.3%)	50(37.03%)
	E-R, Cl-S (MS Phenotype)	12(14.8%)	11(20.37%)	23(17.03%)

Table 2: Clindamycin susceptibility pattern among erythromycin resistant isolates (n = 85)

	MRSA	MSSA	Total
iMLSB	9(10.58%)	3(3.53%)	12(14.11%)
cMLSB	39(45.88%)	11(12.94%)	50(58.8%)
MS Phenotype	12(14.11%)	11(12.94%)	23(27.05%)

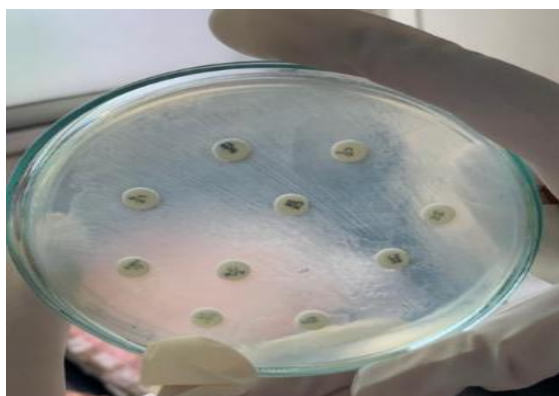


Figure 1: D-test showing inducible clindamycin resistance

4. Discussion

The proportion of MRSA has increased worldwide since last two decades. Its prevalence varies markedly across different countries and among hospitals of the same country [15, 16]. This study showed prevalence rate of 60% which is higher than the study done in eastern part of Nepal [12] India [13] and other part of the world [14]. However similar rates of MRSA were also noted in other studies conducted in a Tertiary Care Hospital, Eastern India by Subasini Majhi [17], Lyall et al., (2013), Majhi et al., (2016) and Sah et al., (2015). These variations could be due to the differences in the circulating clones or due to the variations in infection prevention practices and trends of antibiotics prescription in different hospital set up. Our study revealed 12 (8.8 %) *S. aureus* isolates were D- test positive. It was observed that percentage of inducible clindamycin resistance was higher among MRSA (11.11%) compared to MSSA (5.5%). This finding conforms to many published studies such as Gade et al., (2013), Majhi et al., (2016) and Lall et al., (2014). On the contrary, Sasirekha et al., (2014) and Bottega et al., (2014) had shown a higher percentage of inducible resistance in MSSA compared to MRSA. In our study 62.96% of *S. aureus* isolates were resistant to Erythromycin. Similar high prevalence of resistance to Erythromycin has reported by Mittal et al., (2013) and Sasirekha et al., (2014). Truly clindamycin- sensitive isolates, which exhibit MS phenotype, were present in 20.37% of MSSA and 14.8% of MRSA isolates in our study. This result is similar to Banik et al., (2015) and Phukan et al., (2015). The different patterns of resistance observed in various studies are due to the fact that resistance varies by geographical regions, age groups, antibiotic prescription patterns, methicillin susceptibility and even from hospital to hospital.

5. Conclusion

Since Clindamycin resistance is on higher side among MRSA isolates, it indicates that inducible Clindamycin resistance testing should be done as routine practice in antibiotic susceptibility testing. If not done there is threat of these isolates getting missed and falsely reported sensitive to Clindamycin. Which can lead to treatment failure and ultimately irrational use of other higher antibiotics like Vancomycin, Teicoplanin etc. so there is need to guide the clinicians by delivering appropriate reports to prevent the stage of "NO ANTIBIOTIC ERA".

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