Prevalence of Constitutive and Inducible Clindamycin Resistance among Methicillin-Resistant *Staphylococcus aureus* Isolates in a Tertiary Care Hospital, Kashmir Valley

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A R T I C L E   I N F O

A B S T R A C T

Introduction: The increased frequency of Methicillin-resistant *Staphylococcus aureus* infections has led to renewed interest in the macrolide-lincosamide streptogramin B (MLS) group of antibiotics. Resistance to these antibiotics may be constitutive or inducible. Isolates resistant to erythromycin may show false *in vitro* susceptibility to clindamycin, leading to therapeutic failures. This study investigated the utility of the D-Test for detecting inducible clindamycin resistance in methicillin-resistant *S. aureus* isolates and determining the prevalence of various phenotypes in our region. **Methods:** For detecting inducible clindamycin resistance, a D-test using erythromycin and clindamycin as per CLSI guidelines was performed, and four different phenotypes were interpreted as methicillin-sensitive (MS) phenotype (D-test negative), inducible MLSB (iMLSB) phenotype (D-test positive), constitutive MLSB phenotype and sensitive to both. **Results**: Of the 987 isolates tested, 400 (40.53%) were MRSA. The prevalence of iMLSB, cMLSB phenotype, MS phenotype and sensitive phenotype in MRSA isolates was 42.5%, 10.5%, 28% and 19%, respectively. The iMLSB and cMLSB phenotypes were higher in males (24.75%, 6.25%) than females (*P*-value = 0.137). The majority of MRSA isolates originated from pus (83%). All *S. aureus* isolates showed 100% sensitivity to vancomycin and linezolid. **Conclusion**: This study emphasizes the prevalence of inducible clindamycin resistance in MRSA in our setup. Incorporating the D-test into the routine Kirby–Bauer disk diffusion method in clinical microbiology laboratories will help clinicians make judicious use of clindamycin, minimizing treatment failure.

INTRODUCTION

Infections due to *Staphylococcus aureus* present a significant health problem despite the availability of numerous effective antibiotics. *Staphylococcus aureus* is a pluripotent pathogen, causing disease through both toxin-mediated and non-toxin-mediated mechanisms. Staphylococci are members of the Micrococcaceae family, among which *S. aureus* is the primary cause of skin and soft tissue infections, endovascular infections, pneumonia, septic arthritis, endocarditis, osteomyelitis, foreign body infections, and sepsis. In addition, it is also capable of producing toxin-mediated diseases such as toxic shock syndrome, staphylococcal scalded skin syndrome, and food poisoning [1].

The pathogenicity and virulence of *S. aureus* are associated with its capacity to produce several virulence factors. These factors include enterotoxins, toxic shock syndrome toxin-1, cytolytic toxins, α, and β hemolysins, exfoliative toxins, panton-valentine leucocidin (PVL), protein A, and several enzymes [2]. *Staphylococcus aureus* can adhere to the host tissue, which plays a vital role in colonization and pathogenesis. Protein A, a surface protein, fibronectin-binding protein, clumping factors, and collagen-binding proteins help *S. aureus* adhere to the host tissue extracellular matrix component [3].

Methicillin-resistant *S. aureus* (MRSA) was first reported in 1961, two years after the antibiotic was introduced to treat penicillin-resistant *S. aureus* strains. In the following decades, MRSA spread worldwide and is now detected in most hospitals and healthcare facilities [4]. The *S. aureus* resistance to methicillin is caused by the presence of the *mecA* gene, encoding for an additional 78-kDa penicillin-binding protein 2a (PBP2a or PBP2').

MRSA is considered community-acquired (CA-MRSA) if the isolates are recovered within 48 hours of

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hospitalization. The CA-MRSA occurs in individuals in the community who are generally healthy and not receiving healthcare in a hospital or on an outpatient basis [5].

Healthcare-associated MRSA (HA-MRSA) isolates are primarily obtained from people attending the healthcare setting. These patients are old and have one or more comorbid conditions. HA-MRSA strains tend to cause pneumonia, bacteremia, and invasive infections [6]. The prevalence of MRSA infections differs worldwide between 13% and 74% [7]. MRSA is now endemic in India. The incidence of MRSA varies from 25% in western India [8] to 50% in the south [9, 10], and in Kashmir is 25.2% [11].

Recent trends in the epidemiology of MRSA indicate that these strains are no longer limited to healthcare facilities, and new strains appear in the communities. In India, the importance of MRSA as a problem was recognized relatively late [12]. The MRSA prevalence is not uniform and varies in different parts of India. This variation in prevalence may be due to factors like study design, population, and geographical distribution, differential clonal expression, drug pressure in the community, health care facilities available in the hospital, implementation, and monitoring by infection control committee, and rationale antibiotic usage which varies from hospital to hospital. MRSA is a significant cause of nosocomial infection worldwide. Serious endemic and epidemic MRSA infections occur globally, as infected and colonized patients in health care settings are the reservoirs.

Clindamycin is a good alternative for treating methicillin-resistant and susceptible *Staphylococcal* infections [13, 14] and is of particular importance as an alternative antibiotic in penicillin-allergic patients [15]. However, one crucial issue in clindamycin treatment is the risk of clinical failure during therapy. Clindamycin resistance can develop in *Staphylococcal* isolates with inducible phenotype, and from such isolates, spontaneous constitutively resistant mutants have arisen both *in vitro* testing and *in vivo* during clindamycin therapy [16]. The erythromycin resistance methylase (*erm*) genes encode enzymes that confer inducible or constitutive resistance to MLSB agents. Constitutively resistant isolates are resistant to all MLSB antibiotics and readily detected by standard susceptibility testing methods. Inducible resistance is expressed in the presence of potent inducers of methylase synthesis, such as 14 membered (e.g., erythromycin) and 15 membered (e.g., azithromycin) macrolides. The 16 membered macrolides (e.g., spiramycin), lincosamide (e.g., clindamycin), and streptogramin B antibiotics may appear active when susceptibility is tested by the standard method since they are only weak inducers of methylase synthesis. However, inducible resistance can be detected by the disc diffusion induction test (D-test) [17].

Reporting *Staphylococci* as susceptible to clindamycin without checking for inducible clindamycin resistance may thus result in inappropriate clindamycin therapy. On the other hand, negative results for inducible clindamycin resistance confirm clindamycin susceptibility and provide an excellent therapeutic option. Hence, there is a need to identify the mechanisms that confer resistance to MLSB antibiotics concerning clindamycin therapy for *Staphylococcal* infections.

The prevalence of inducible resistance should be known, as it varies by geographical location, bacterial species, methicillin susceptibility, and hospital. In the context of the increase in resistance and emergence of multidrug-resistant organisms, accurate antimicrobial susceptibility data of an isolate is crucial for appropriate treatment decisions.

It is recommended that all erythromycin-resistant *S. aureus* should be tested for inducible clindamycin resistance to prevent clindamycin treatment failures and to report the prevalence of resistant phenotypes, which varies widely.

This study was undertaken to determine the prevalence of various MLSB phenotypes in *S. aureus* isolated from various clinical specimens in a tertiary care center in Kashmir, J&K, India, and to study the utility of D-Test for detecting inducible clindamycin resistance in methicillin-resistant *S. aureus* isolates.

MATERIAL AND METHODS

This descriptive cross-sectional study was conducted in the Department of Microbiology, Government Medical College, Srinagar, from October 2017 to April 2019. Ethical clearance was obtained from the institutional ethical committee of the college (GMC-IEC-2017) after due process.

Processing of isolates. Non-duplicate consecutive isolates of methicillin-resistant *S. aureus* were obtained from patients' clinical samples like blood, pus, body fluids, and wound swabs.

Isolation and identification of *S. aureus*. All specimens were processed as per the standard operating procedures [18]. *Staphylococcus aureus* was identified by colony morphology on 5% sheep blood agar. Cream to golden yellow colonies with or without hemolysis was identified by Gram staining, catalase, and coagulase tests (slide and tube coagulase) using standard microbiological techniques [18].

Antimicrobial Susceptibility testing. Antimicrobial Susceptibility testing was performed by Kirby Bauer's disk diffusion method on Mueller-Hinton agar plates. A 0.5 Mc Farland standard inoculum was prepared from a pure colony, and lawn culture was made on a Mueller-Hinton agar plate. Antibiotic discs, including penicillin (10U), cefoxitin (30 µg), erythromycin (15 µg), clindamycin (2 µg), gentamicin (10 µg), ciprofloxacin (5 µg), vancomycin (30 µg) and linezolid (30 µg) were

placed on it. Susceptibility results were interpreted as per the CLSI guidelines [19].

Quality Control (QC). *Staphylococcus aureus* ATCC 25923 was used to perform quality control. Additional QC was performed with separate in-house selected *S. aureus* strains that demonstrated positive and negative D-test reactions.

For detecting methicillin resistance, cefoxitin was used as a surrogate marker. Isolates with cefoxitin zone size ≥ 22mm were defined as methicillin-susceptible, and those with <21mm as methicillin-resistant [19].

Detection of inducible clindamycin resistance. Inducible clindamycin resistance was examined as per the CLSI guidelines [19]. An erythromycin disk (15 µg) was spaced at a distance of 15-26 mm from the clindamycin disk (2 µg), plates were examined after 18 h of incubation at 35°C, and interpretation of the test was made as follows (Figures 1 and 2). A) Isolates susceptible to erythromycin as well as clindamycin, B) MS phenotypes, isolates resistant to erythromycin (zone size ≤ 13 mm) but sensitive to clindamycin (zone size ≥ 21 mm). No flattening of the zone of inhibition around clindamycin was observed. Isolate is reported as erythromycin-resistant but susceptible to clindamycin, C) cMLSB phenotype, the isolates resistant to both the erythromycin (zone size ≤ 13 mm) and clindamycin (zone size ≤ 14 mm), D) iMLSB phenotype, isolates is resistant to erythromycin (zone size ≤ 13 mm) but sensitive to clindamycin (zone size ≥ 21 mm). However, flattening the inhibition zone around clindamycin towards the erythromycin disk will produce a "D" shaped blunting. In this case, the isolate is defined as resistant to both erythromycin and clindamycin.
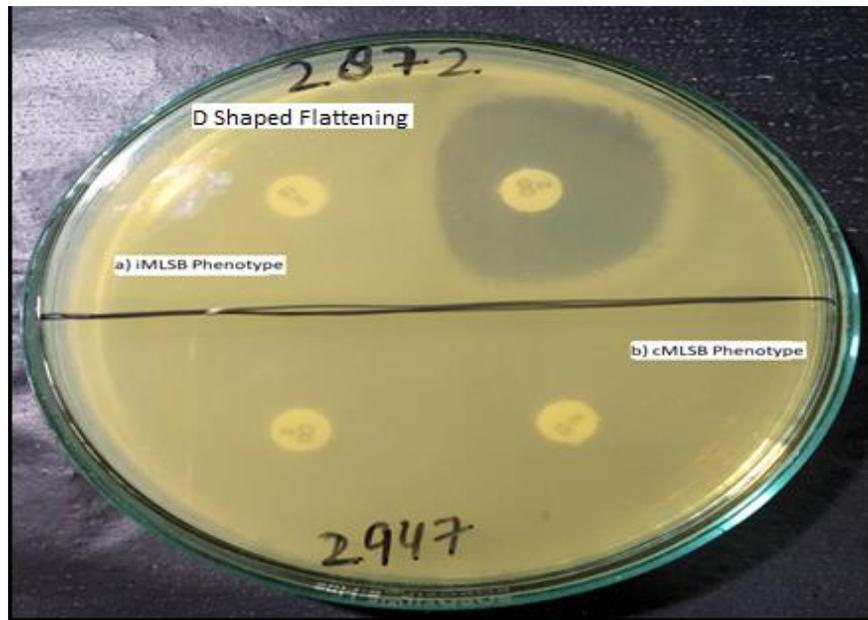
Fig.1. a) Inducible Clindamycin Resistance iMLSB Phenotype (D-test)
b) Constitutive Clindamycin Resistance cMLSB

Data collection and statistical analysis. The patient's age, gender, type of infection, and other details, were collected on a predefined proforma. Data collected was typed in a Microsoft Excel sheet, and variables were summarized as frequency and percentage. Categorical, continuous variables were summarized as mean and ± SD. Data analysis was done using Epi Info 7.0.

RESULTS

During the study period, 987 *S. aureus* isolates were obtained, including 400 (40.53%) methicillin-resistant *S. aureus* (MRSA) and 587 (59.47%) methicillin-sensitive *S. aureus* (MSSA) isolates (Table 1).

**Table 1**. Prevalence of MRSA and MSSA isolates

Total No. of Samples	MRSA	MSSA
987	400 (40.53%)	587 (59.47%)

The male/female distribution of MRSA isolates isolated in our study is shown in table 2, with the majority (n=237, 59.25%) belonging to male patients. The patients' age in which MRSA samples were detected ranged from 5 days to 79 years, with mean±SD 34.9±16.2 years. Most

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samples belonged to the age group 31-40 (26.50%), followed by the 41-50 age group (19.25%).

The distribution of MRSA isolates according to sample type is shown in table 3. Most samples originated from pus (n= 332, 83%), followed by blood (n=40, 10%).
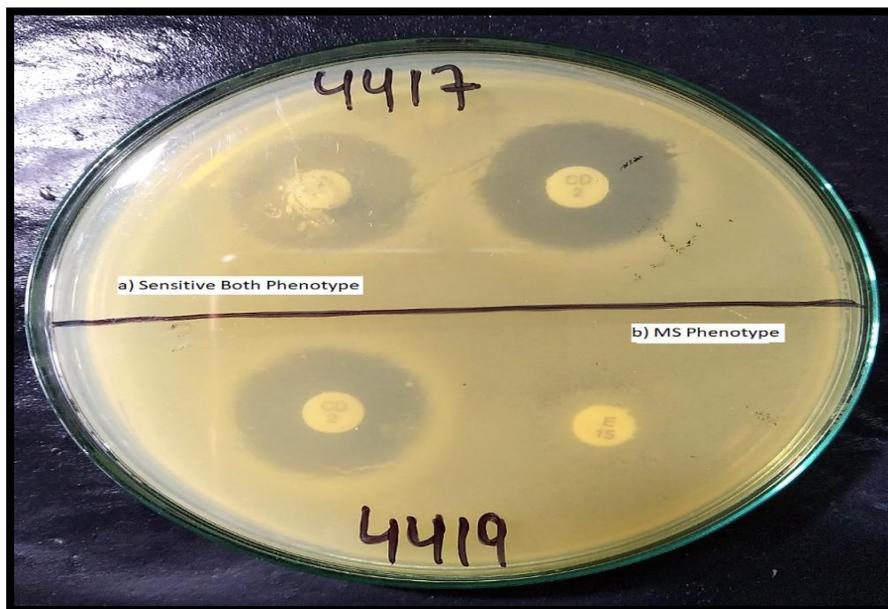
Fig. 2. a) Sensitive to Erythromycin and Clindamycin
b) MS Phenotype (Resistant to Erythromycin and sensitive to Clindamycin)

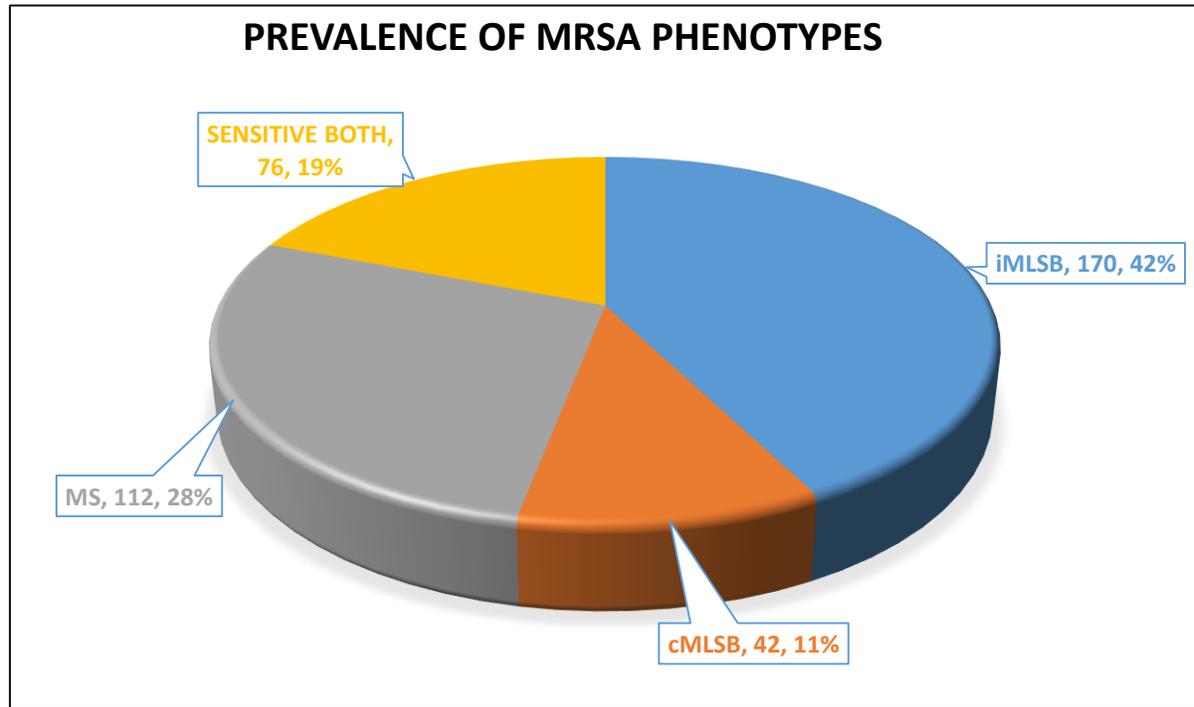**Table 2**. Distribution of MRSA isolates based on gender

Gender	No. of Samples	Percentage
Male	237	59.25%
Female	163	40.75%

Table 3. Distribution of MRSA isolates according to the clinical samples

Sample type	No. of samples	Percentage
Pus	332	83.00%
Blood	40	10.00%
Bal	5	1.25%
Wound swab	7	1.75%
Bone material	1	0.25%
Central line tip	2	0.50%
Et aspirate	2	0.50%
Ett tip	4	1.00%
Infected implant	2	0.50%
Milk secretion	1	0.25%
Nasal secretion	1	0.25%
Plueral tap	1	0.25%
Synovial fluid	1	0.25%
Bile	1	0.25%

The 400 MRSA isolates subjected to D-test to detect inducible clindamycin resistance revealed four phenotypes, with the iMLSB phenotype comprising the majority of isolates (n=170, 42.5%) followed by the MS phenotype (n=112, 28%) (Fig. 3).

PREVALENCE OF MRSA PHENOTYPES

SENSITIVE BOTH, 76, 19%

iMLSB, 170, 42%

MS, 112, 28%

cMLSB, 42, 11%

Fig. 3. Prevalence of MRSA phenotypes

The phenotype distribution concerning sample type is shown in table 4. Most iMLSB isolates were obtained from pus 148 (37%), followed by blood 12 (3%). The prevalence of various MRSA phenotypes from pus samples compared to other samples combined differed significantly (*P*-value < 0.05). In our study, penicillin and cefoxitin showed 100% resistance, followed by erythromycin (81%). The antibiotic resistance pattern of all antibiotics is shown in figure 4.

Table 4. Phenotypes distribution concerning the sample type

Clinical samples	Phenotypes			
	iMLSB	cMLSB	MS	Sensitive both
Pus	148 (37%)	28 (7%)	93 (23.25%)	63 (15.75%)
Blood	12 (3%)	9 (2.25%)	13 (3.25%)	6 (1.5%)
Wound swab	2 (0.5%)	1 (0.25%)	3 (0.75%)	1 (0.25%)
Bal	3 (0.75%)	1 (0.25%)	1 (0.25%)	0 (0.0%)
Ett tip	1 (0.25%)	2 (0.5%)	1 (0.25%)	0 (0.0%)
Central line tip	2 (0.5%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Et aspirate	1 (0.25%)	1 (0.25%)	0 (0.0%)	0 (0.0%)
Infected implant	0 (0.0%)	0 (0.0%)	0 (0.0%)	2 (0.5%)
Bile	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.25%)
Bone material	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.25%)
Milk secretion	1 (0.25%)	0 (0.0%)	0 (0.0%)	0 (0.0%)
Nasal secretion	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.25%)
Plueral tap	0 (0.0%)	0 (0.0%)	1 (0.25%)	0 (0.0%)
Synovial fluid	0 (0.0%)	0 (0.0%)	0 (0.0%)	1 (0.25%)

DISCUSSION

In the present study, the prevalence of MRSA isolates during the study period in our lab was 40.53%. The prevalence of MRSA isolates in similar studies in India were 36.9%. [20], 37.8% [21], 37.96 [22] and 36.88% [23] which is in accordance with our results. Some studies have shown higher rates ranging from 49.8% to 64.9% [24, 25, 26, 27]. Studies from Ethiopia [28] and Jordan [29] have reported higher rates of 82.27% and 77.5%, respectively. The differences in the prevalence of MRSA

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among different countries and between different regions in a country could be due to differences in the study design, population, and geographical distribution. The variation may be due to differential clonal expansion and drug pressure in the community, health care facilities available in the hospital, implementation and monitoring

by infection control committee, and rationale antibiotic usage, which varies from hospital to hospital. Further, it emphasizes the importance of local surveillance in generating relevant local resistance data that can guide empiric therapy [24, 30].
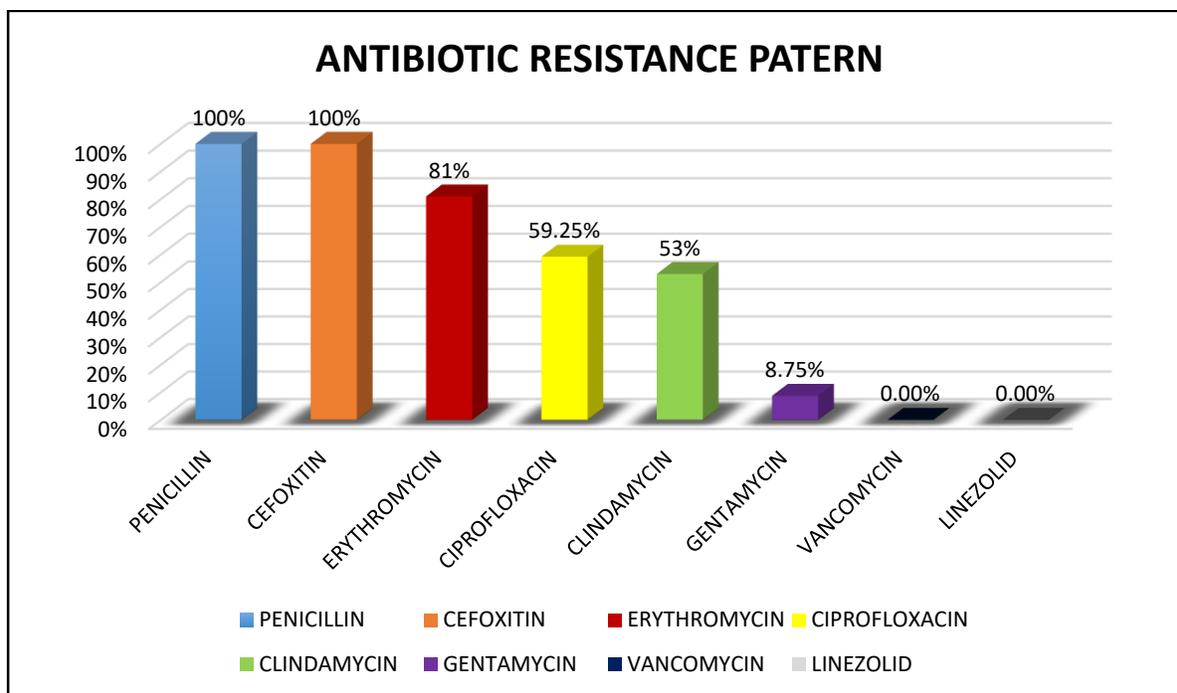
ANTIBIOTIC RESISTANCE PATERN

Values shown: PENICILLIN 100%, CEFOXITIN 100%, ERYTHROMYCIN 81%, CIPROFLOXACIN 59.25%, CLINDAMYCIN 53%, GENTAMYCIN 8.75%, VANCOMYCIN 0.00%, LINEZOLID 0.00%

Legend: PENICILLIN, CEFOXITIN, ERYTHROMYCIN, CIPROFLOXACIN, CLINDAMYCIN, GENTAMYCIN, VANCOMYCIN, LINEZOLID

Fig. 4. Antibiotic Resistance Pattern of MRSA Isolates.

The prevalence of the iMLSB phenotype in our study was 42.5%, showing that more than one-third of isolates were resistant to clindamycin which would have been easily missed and reported as clindamycin susceptible in regular Kirby–Bauer disk diffusion susceptibility testing and could have resulted in therapeutic failure. The iMLSB phenotype rates in similar studies in India were 46.34% [22], 42.1% [31], 44.8% [32], 43.6% and 39.7% [33] which is in accordance with our results. Some studies have shown lower rates ranging from 27.1% to 32.53% [20, 35, 40, 41]. In our study, cMLSB phenotype prevalence was 10.5%. The cMLSB phenotype rates in similar studies in India were 11.11% [24], 12.8% [38], 9.6% [23] which is in accordance with our results. Higher rates of cMLSB phenotype were reported in some studies ranging from 46.6% to 64.8% [20, 21, 39]. Also, actual clindamycin-sensitive isolates exhibiting efflux pump-mediated resistance to macrolides (MS phenotype) had a 28% prevalence. The MS phenotype rates in similar studies in India were 25.39% [36], 22.8% [40], and 22.5% [26], in agreement with our results. The MS phenotype prevalence reported by other studies are 16.7% [20], 8% [21], 20% [39] and 44.6% [41]. This fact implies that clindamycin can be safely and effectively instituted as a therapeutic drug in such clinical scenarios despite macrolide resistance. All erythromycin-resistant isolates

need not necessarily be resistant to clindamycin. Conversely, labelling all erythromycin-resistant isolates as clindamycin resistant and not reporting clindamycin resistance in the presence of the iMLSB phenotype will prevent the use of clindamycin as an effective therapy in situations where it is most likely to respond [41].

The different patterns of resistance observed in various studies are due to geographical regions, patient population studied, age groups, antibiotic prescription patterns, and hospital characteristics [26, 43]. There is a high variation for constitutive clindamycin resistance between various studies, as it depends on the overuse of the drug and conversion of inducible phenotype to constitutive phenotype during treatment [44].

The prevalence of iMLSB and cMLSB phenotypes was higher in males (24.75%, 6.25%) than in females but was not statistically significant (*P* 0.137), which might be due to the high recovery rate of MRSA in male patients. The high MRSA recovery rate in male patients is because men are mainly involved in occupations most likely lead to trauma compared to women [34].

In our study, the highest iMLSB and cMLSB phenotypes rates (12% and 3.5%) were in the 31-40 age group, showing no significant difference from other age groups (*P* 0.807). A higher rate of MRSA isolation

(26.5%) in this age group may be the reason for the higher prevalence of iMLSB and cMLSB phenotypes. Other similar studies have reported higher MRSA rates in ages 31-40 [11, 34, 45].

The majority of sample types (83%) from which MRSA was isolated in our study were pus. The prevalence of iMLSB and cMLSB phenotypes was also highest in pus samples (37%, 7%). The prevalence of iMLSB and cMLSB phenotypes from pus samples showed a significant difference from other samples combined ($P <$ 0.05). Various studies from India [11, 20, 26, 45, 46] and Iran [47] have also observed that pus comprised most samples with high iMLSB and cMLSB resistance phenotypes. This may be due to *S. aureus* being the primary cause of skin and soft tissue infections. Skin and soft-tissue infections (SSTIs) constitute approximately 90% of CA-MRSA cases, and 90% of these are abscesses and/or cellulitis with purulent drainage [48].

We tested penicillin, cefoxitin, erythromycin, clindamycin, gentamicin, ciprofloxacin, vancomycin, linezolid, penicillin, and penicillin and cefoxitin showed 100% resistance, followed by erythromycin (81%). The isolates showed 100% sensitivity to linezolid and vancomycin, followed by gentamycin (91.25%). A similar study in the Kashmir region showed that MRSA isolates were 100% resistant to penicillin, followed by erythromycin (78%) [11]. Also, studies from various parts of India [20, 25, 46, 49] indicated similar antibiotic sensitivity patterns. Here, we detected 100% susceptibility to vancomycin; a similar study from the Kashmir region [50] found that *S. aureus* isolates were 100% susceptible to linezolid and vancomycin, followed by ciprofloxacin (60.43%). The remarkable susceptibility to linezolid, vancomycin, and gentamicin may be due to the lesser use of these antibiotics due to limited availability [26].

Antibiotic sensitivity testing for any clinical isolate is often crucial in determining the course of treatment, especially in multidrug-resistant pathogens. The emergence of methicillin-resistant *S. aureus* has left us with few therapeutic options to treat staphylococcal infections. Clindamycin, a Lincosamide, has excellent oral bioactivity making it an excellent alternative to intravenous drugs. It distributes evenly throughout the body and penetrates quickly into the tissues. Once orally administered, it is metabolized quickly and excreted in urine and bile [51, 52]. Clindamycin is frequently used to treat skin and bone infections because of its tolerability, cost, oral form, and excellent tissue penetration, and the fact that it accumulates in abscesses, and no renal dosing adjustments are needed [53].

Newer antibiotics like vancomycin, linezolid, and quinupristin-dalfopristin have been advocated in managing the methicillin-resistant *S. aureus*, but recent reports of resistance to these agents have raised genuine concerns over how long these uniform susceptibilities will remain [54, 55, 56]. This has led to renewed interest in

using macrolide-lincosamide-streptogramin B (MLSB) antibiotics to treat *S. aureus* infections, with clindamycin being the preferred agent.

In conclusion, this study emphasizes the prevalence of inducible clindamycin resistance in MRSA in the Kashmir valley. The emergence of MRSA has led to a resurgence in interest in clindamycin therapy for *S. aureus* infection. Clindamycin is a valuable therapeutic option for various MRSA infections, including musculoskeletal infections, skin and soft tissue infections, and even pneumonia with empyema. However, the use of clindamycin for these infections has been somewhat hampered by concern over possible inducible resistance to clindamycin and its impact on clinical outcomes.

The actual sensitivity to clindamycin can only be judged after performing the D-test on erythromycin-resistant isolates. The use of the D-test in routine laboratories will help us advise the clinicians regarding clindamycin use in superficial skin and soft tissue infections, as clindamycin should not be used for clindamycin-induced resistant *Staphylococcus,* i.e., D-test positive. At the same time, it can be the drug of choice in case of D-test negative isolates.

Thus, the simple and reliable D-test can be incorporated into the routine Kirby–Bauer disk diffusion method in clinical microbiology laboratories. This test enables clinicians in the judicious use of clindamycin, as clindamycin is not a suitable drug for the D-test-positive *S. aureus* isolates. Therefore, the clindamycin treatment failure could be minimized.

ACKNOWLEDGMENT

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest associated with this manuscript.

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