INDUCIBLE CLINDAMYCIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES FROM TERTIARY CARE HOSPITAL, BETTIAH, INDIA

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ABSTRACT

BACKGROUND

Staphylococcus aureus is a frequent cause of bacterial infections in both developed and developing countries. The Clinical and Laboratory Standards Institute (CLSI) recommends D-test, which is a phenotypic screening method for inducible Clindamycin resistance.

Therefore, all erythromycin resistant *S. aureus* should be tested for inducible Clindamycin resistance to prevent Clindamycin treatment failures and to report prevalence resistant phenotypes which varies widely.

MATERIALS AND METHODS

This cross-sectional study was conducted for a period of one year from January 2017 to January 2018. We analysed 125 non-duplicate consecutive isolates of *S. aureus* isolated from various clinical specimens like pus, wound swab, aspirates, blood and sterile fluids. A total of 125 *S. aureus* isolates derived from wound infection were evaluated for antimicrobial susceptibility testing by Kirby-Bauer disk diffusion method. Methicillin Resistance was detected using Cefoxitin (30 µg) disk and inducible Clindamycin resistance was determined in all erythromycin resistant isolates by using D-zone test.

RESULTS

Out of 125 *S. aureus* isolates of the MRSA 46 (36.6%) were derived from respective Pus samples 19 (41.53%), the *S. aureus* isolates derived from Wound samples were MRSA 8 (18.03%), the *S. aureus* isolates derived from Blood samples were MRSA 10 (22.40%), the *S. aureus* isolates derived from Miscellaneous samples were MRSA 6 (13.66%) and the *S. aureus* isolates derived from urine samples were MRSA 2 (3.75%). A total of 26 *S. aureus* isolates showed inducible Clindamycin resistance by giving a positive D-zone test; hence, its prevalence was found to be 21.00% (26/125) with percentage distribution of cMLS_B phenotype and MS phenotypes in all *S. aureus* isolates as 19.4% and 24.6% respectively. The susceptible phenotype (E-S and CD-S) predominated in MSSA (39.75%) as compared to MRSA (13.11%). Whereas, the constitutive resistant (cMLS_B) predominated in MRSA (50.27%) as compared to MSSA (15.46%). Both the MS phenotype and the inducible resistant (iMLS_B) phenotypes predominated in MSSA (19.87 and 24.92%, respectively) as compared to MRSA (22.40% and 14.20%, respectively). The antimicrobial susceptibility test result of all the 105 *S. aureus* isolates with iMLS_B phenotype revealed that they were 100% sensitive to Vancomycin and Linezolid with moderate sensitivity (71.14%) to Gentamicin, Cefuroxime and least sensitivity (23.81%) to Doxycycline and 20.95% to Ciprofloxacin.

CONCLUSION

Due to high prevalence of Erythromycin resistance amongst *S. aureus* isolates, we suggest that D-zone test should be routinely done in all laboratories for appropriate prescription of Clindamycin and thereby preventing emergence of inducible resistant strains and treatment failure.

KEY WORDS

Staphylococcus aureus, Inducible Clindamycin Resistance, Wound Infection.

HOW TO CITE THIS ARTICLE: Rajak KC, Poddar CK, Kumar R, et al. Inducible clindamycin resistant staphylococcus aureus isolates from tertiary care hospital, Bettiah, India. J. Evolution Med. Dent. Sci. 2018;7(36):3984-3990, DOI: 10.14260/jemds/2018/890

BACKGROUND

Staphylococcus aureus can produce a wide variety of diseases, from relatively benign skin infections such as folliculitis and furunculosis to deep-seated and life-threatening conditions

'Financial or Other Competing Interest': None.

Submission 28-07-2018, Peer Review 20-08-2018,

Acceptance 27-08-2018, Published 03-09-2018.

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DOI: 10.14260/jemds/2018/890

including cellulitis, deep abscesses, osteomyelitis, pneumonia, sepsis and endocarditis. *Staphylococcus aureus* is a frequent cause of bacterial infections in both developed and developing countries.¹ The MLS_B resistant phenotype in *S. aureus* can be either constitutive MLS_B (cMLS_B) or inducible MLS_B (iMLS_B). Staphylococci that express ribosomal methylase (*erm*) genes may exhibit *in vitro* resistance to Erythromycin, Clindamycin and other drugs of MLS_B group. This resistance is referred to as the cMLS_B phenotype. However, in some Staphylococci, those express *erm* genes require an inducing agent to synthesise methylase for Clindamycin resistance. This type is referred to as iMLS_B phenotype. These organisms are resistant to erythromycin and falsely susceptible to Clindamycin *in vitro*.²

This type of inducible Clindamycin resistance cannot be detected by standard Kirby-Bauer disk diffusion method, broth microdilution testing, automated susceptibility testing devices or Epsilometer test.³ Thus, falsely susceptible Clindamycin will lose its effectiveness *in vivo* and thereby increase the chance of therapeutic failures.⁴

The double disk approximation test (D-test) involves the placement of an Erythromycin disk in proximity to the disk containing Clindamycin. As the Erythromycin diffuses through the agar, the resistance to the clindamycin is induced, resulting in a flattening or blunting of the Clindamycin zone of inhibition adjacent to the Erythromycin disk, giving an "S0" shape to the zone. The Clinical and Laboratory Standards Institute (CLSI) recommends D-test, which is a phenotypic screening method for inducible Clindamycin resistance.⁵ Therefore, all erythromycin resistant *S. aureus* should be tested for inducible Clindamycin resistance to prevent Clindamycin treatment failures and to report prevalent resistant phenotypes, which varies widely. The present study was aimed at determining the constitutive and inducible Clindamycin resistance in *S. aureus* isolated from various clinical specimens at a tertiary care hospital in Bettiah, India.

MATERIALS AND METHODS

This cross-sectional study was conducted for a period of one year from January 2017 to January 2018. We analysed 125 non-duplicate consecutive isolates of *S. aureus* isolated from various clinical specimens like pus, wound swab, aspirates, blood and sterile fluids. Age and sex of the patients were recorded. This study was approved by the Research and Ethical Committees of our Institute and informed consent was obtained from each patient.

Clinical Samples and Bacterial Isolates

During the period of one year, 125 clinical isolates of *S. aureus* were collected from Department of Microbiology and Department of Pharmacology, Government Medical College, Bettiah (West Champaran) Bihar and Associated Hospital. The bacteria which were consecutively isolated from patients in various wards and different specimens such as catheter, blood, wound, discharge, abscess, burn and so on were transported to Department of Microbiology and Department of Pharmacology, Government Medical College, Bettiah (West Champaran) Bihar and Associated Hospital. Identity of isolates were confirmed by standard microbiology tests including Gram staining, catalase, slide and tube coagulase, mannitol fermentation and production of DNase enzyme.⁶

Antibiotic Susceptibility Test

Antibiotic susceptibility testing was performed on Mueller-Hinton agar (HiMedia Laboratories, Mumbai, India) by Kirby-Bauer disk diffusion method as per CLSI guidelines using antibiotic disks (HiMedia Laboratories, Mumbai, India) such as Penicillin (10 units), Gentamicin (10 µg), Tetracycline (30µg), Linezolid (30 µg), Trimethoprim-Sulfamethoxazole (1.25/23.75 µg), Cefoxitin (30 µg), Erythromycin (15 µg), Clindamycin (2 µg) and ciprofloxacin (5 µg). *Staphylococcus aureus* ATCC 25923 was used as standard quality control strain.⁷

Methicillin Resistance Test

Methicillin resistance amongst *S. aureus* was determined using Cefoxitin (30 µg) disk on Mueller-Hinton agar as per CLSI guidelines, and results were read after 18 hours of incubation at 35°C.

The *S. aureus* isolates which showed zone size ≥ 22 mm were considered Methicillin sensitive (MSSA) and those with zone size ≤ 21 mm were considered as Methicillin-resistant *S. aureus* (MRSA). Susceptibility of MRSA strains to Vancomycin was tested by agar dilution method as per CLSI guidelines by inoculating 0.5 McFarland bacterial suspensions on Mueller-Hinton agar (MHA) plates by using sterile swabs. The plates were analysed after 24 hours of incubation at 35°C. Minimal inhibitory concentration (MIC) of Vancomycin of ≤ 2 µg/mL for *S. aureus* was considered as susceptible to Vancomycin.

D-Zone Test (Disk Approximation Test)

The isolates which were resistant to erythromycin were further tested by D-zone test which was performed as per CLSI guidelines by inoculating 0.5 McFarland bacterial suspensions on the Mueller-Hinton agar plates with the help of sterile swabs and placing the Erythromycin (E-15 µg) and Clindamycin (CD-2 µg) disks side-by-side with edge-to-edge distance of 15 mm. Plates were analysed after 18 hours of incubation at 35°C.⁷

Phenotypic Inducible Resistance to Clindamycin by D-Test

Isolates were plated on a Mueller-Hinton agar plate at a McFarland concentration of 0.5 to eventually cover the agar surface. Clindamycin and Erythromycin disks containing 2 µg and 15 µg each, respectively, were placed in the center of the plate separated by a distance of 15 cm between the edges. Plates were incubated at 37°C for 24 hrs. Inducible resistance to Clindamycin was defined as blunting of the clear circular area of no growth around the Clindamycin disk on the side adjacent to the Erythromycin disk and was designated D-test positive. Absence of a blunted zone of inhibition was designated D-test negative.

Three different Phenotypes of Erythromycin Resistant Isolates were interpreted as follows-

1. **The Constitutive MLSB Phenotype (cMLSB):** The *S. aureus* isolates resistant to both E (Zone size ≤ 13 mm) and CD (zone size ≤ 14 mm) with circular shape of zone of inhibition if any around Clindamycin. This suggests selection of erm gene mutants.
2. **The MS Phenotype:** The *S. aureus* isolates which showed resistance to E (Zone size ≤ 13 mm) and a complete circular zone of inhibition around CD (Zone size ≥ 21 mm) indicated negative D zone test. This suggests resistance due to the msrA-coded active efflux pump mechanism.
3. **The Inducible MLSB Phenotype (iMLSB):** The *S. aureus* isolates, which showed resistance to E (Zone size ≤ 13 mm) and susceptibility to CD (zone size ≥ 21 mm) with flattening of zone of inhibition around Clindamycin in the area adjacent to the Erythromycin (D shaped zone), indicated positive D-zone test. This suggests a resistance phenotype due to expression of erm-gene coded methylases.

Statistical Analysis

The collected data was statistically analysed using SPSS Data Editor Software, Chicago, version 20. The statistical association between inducible Clindamycin resistance phenotype and Methicillin-resistant *S. aureus* isolates were evaluated using Chi-square test and $p < 0.05$ was considered as statistically significant.

RESULTS

- Among 125 *S. aureus* isolates included in our study, 71 (57%) were isolated from pus samples, 32 (25.80%) were isolated from blood, 7 (5.2%) were isolated from urine, 6 (4.8%) were isolated from sputum and 9 (7.2%) were isolated from miscellaneous samples as shown in [Table 1].
- Out of 125 *S. aureus* isolates, 46 (36.6%) were Methicillin-resistant (MRSA) and 79 (63.4%) were Methicillin-sensitive *S. aureus* (MSSA). Although, majority of the MRSA isolates (19) were derived from pus samples, the *S. aureus* isolates derived from post-operative wound infection (8) were mostly MRSA. This finding was found to be statistically significant ($p = 0.008903$) [Table 2].
- Out of 125 *S. aureus* isolates of the MRSA, 46 (36.6%) were derived from respectively Pus samples 19 (41.53%), the *S. aureus* isolates derived from Wound samples were MRSA 8 (18.03%), the *S. aureus* isolates derived from Blood samples were MRSA 10 (22.40%), the *S. aureus* isolates derived from Miscellaneous samples were MRSA 6 (13.66%) and the *S. aureus* isolates derived from Urine samples were MRSA 2 (3.75%). This finding was found to be statistically significant ($p = 0.347491$) [Table 3].
- The susceptible phenotype (E-S and CD-S) predominated in MSSA (39.75%) as compared to MRSA (13.11%). Whereas, the constitutive resistant (cMLSB) predominated in MRSA (50.27%) as compared to MSSA (15.46%). Both the MS phenotype and the inducible resistant (iMLSB) phenotypes predominated in MSSA (19.87 and 24.92% respectively) as compared to MRSA (22.40% and 14.20% respectively). This finding was found to be highly significant ($p < 0.00001$) [Table 4].

- Table 5 shows the distribution of *S. aureus* isolates on the basis of the source of collected samples (inpatients or outpatients) and their susceptibility pattern to Erythromycin and Clindamycin disks. Out of 125 *S. aureus* isolates, 73 (58.40%) were derived from samples of outpatients and 52 (41.60%) were derived from samples of inpatients. The frequency of inducible Clindamycin resistant isolates (iMLSB phenotypes) and MS phenotypes predominated in samples of outpatients [(15; 20.89%) and (16; 21.23%), respectively] as compared to inpatients (11; 21.15%) and (11; 20.19%), respectively. This reveals that inducible Clindamycin resistance is both community (outpatients) as well as hospital (inpatients) acquired in our geographic location. However, constitutive resistant isolates (cMLSB phenotypes) predominated in samples of inpatients 18 (28.20%) as compared to samples of outpatients 17 (27.29%). This finding was found to be statistically significant ($p = 0.003089$) [Table 5].
- Out of 125 *S. aureus* isolates, majority were resistant to erythromycin (70%, 88/125). A high percentage of Erythromycin resistant *S. aureus* isolates (70%, 88/125) was detected, of which 86.89% (40/46) were MRSA and 60.25% (48/80) were MSSA. All the erythromycin resistant isolates were subjected to D-zone test and the resulting distribution of *S. aureus* isolates was shown in [Table 6].
- A total of 26 *S. aureus* isolates showed inducible Clindamycin resistance by giving a positive D-zone test; hence, its prevalence was found to be 21.00% (26/125) with percentage distribution of cMLSB phenotype and MS phenotypes in all *S. aureus* isolates as 19.4% and 24.6% respectively [Table 6].
- The antimicrobial susceptibility test result of all the 26 *S. aureus* isolates with iMLSB phenotype revealed that they were 100% sensitive to Vancomycin and Linezolid with moderate sensitivity (71.14%) to Gentamicin, Cefuroxime and least sensitivity to (23.81%) Doxycycline, (20.95%) Ciprofloxacin as shown in [Table 6].

Types of Sample	Samples Number	Samples Percentage
Pus	71	57.00%
Blood	32	25.80%
Urine	7	05.20%
Sputum	6	04.80%
Miscellaneous*	9	07.20%
Total	125	100.00

Table 1. Sample-Wise distribution of *S. aureus* Isolates [n= 125]

[Note*:- Miscellaneous samples include ear discharge, abdominal drain fluid, throat swab, conjunctival swab and wound discharges etc.]

Sample	<i>Staphylococcus aureus</i>			Chi-square (χ^2) and *P-value
	Resistant to Cefoxitin (MRSA) N= 27 (38.25%)	Susceptible to Cefoxitin (MSSA) N= 44 (61.75%)	Total Isolates N= 71 (100%)	
Pus due to any other cause, N (%)	19 (34.23%)	37 (65.77%)	56 (77.89%)	Chi ² =6.8422; DF=1; P=0.008903 statistically significant
Post-operative wound infection, N (%)	8 (52.38%)	8 (47.62%)	16 (22.11 %)	

Table 2. Distribution of *Staphylococcus aureus* Isolates on the basis of Sample and Susceptibility to Cefoxitin (30 µg) Disk

N= Number of isolates MRSA= Methicillin-resistant <i>Staphylococcus aureus</i> ; MSSA= Methicillin-sensitive <i>Staphylococcus aureus</i> . *p-value < 0.05 was considered as statistically significant.				
Sample	<i>Staphylococcus aureus</i>			Chi-square (χ2) and *P-value
	Resistant to Cefoxitin (MRSA) N= 46 (36.6%)	Susceptible to Cefoxitin (MSSA) N= 79 (63.4%)	Total Isolates N= 125 (100%)	
Pus due to any other cause, N (%)	19 (34.23%)	37(65.77%)	56 (44.4%)	Chi²=9.6316 DF=4; P=0.047113
Post-operative wound infection, N (%)	8 (52.38%)	8 (47.62%)	16 (12.6%)	
Blood Culture), N (%)	10 (31.78%)	22(68.22%)	32 (25.8%)	
Miscellaneous* Sample, N (%)	6 (41.67%)	9 (58.33%)	15 (12%)	statistically significant
Urine due to any other cause, N (%)	2 (30.77%)	5 (05.68%)	7 (69.2%)	
Table 3. Distribution of <i>Staphylococcus aureus</i> Isolates on the basis of Sample and Susceptibility to Cefoxitin (30 µg) Disk				
N= Number of isolates. MRSA= Methicillin-resistant <i>Staphylococcus aureus</i> ; MSSA= Methicillin-sensitive <i>Staphylococcus aureus</i> . *n-value < 0.05 was considered as statistically significant.				

Antibiotic Susceptibility Pattern	<i>Staphylococcus aureus</i>			Chi-square (χ^2) and *P-value
	MRSA N= 46 (36.6%)	MSSA N= 79 (63.4%)	Total Isolates N= 125 (100%)	
E-S, CD-S (Susceptible phenotype)	6 (13.11%)	32 (39.75%)	38 (30.00%)	χ^2 = 84.001; df= 3 p= 0.00001
E-R, CD-R (cMLSB phenotype) D-zone test= (-)	23 (50.27%)	12 (15.46%)	35 (28.20%)	
E-R, CD-S (MS phenotype)	10 (22.40%)	16 (19.87%)	26 (20.08%)	
E-R, CD-S (iMLSB phenotype) D-zone test= (+)	7 (14.20%)	20 (24.92)	27 (21.00%)	statistically significant
Table 4. Distribution of <i>Staphylococcus aureus</i> isolates on the basis of their susceptibility to Erythromycin and Clindamycin Disks placed adjacent to Each Other				
N= Number of isolates. MRSA= Methicillin-resistant <i>Staphylococcus aureus</i> ; MSSA= Methicillin sensitive <i>Staphylococcus aureus</i> ; E= Erythromycin (15 µg) disk; CD= Clindamycin (2 µg) disk; S= Sensitive; R= Resistant; cMLSB phenotype= isolates with constitutive resistance to clindamycin; MS phenotype= isolates with susceptibility to Clindamycin (circular zone of inhibition) and negative D-zone test; iMLSB phenotype= isolates with inducible resistance to Clindamycin and positive D zone test. *P-value < 0.05 was considered as statistically significant.				

Antibiotic Susceptibility Pattern	Staphylococcus aureus			Chi-square (χ2) and *P-value
	Outpatient N= 73 (58.40%)	Inpatient N= 52 (41.60%)	Total Isolates N= 125 (100%)	
E-S, CD-S (Susceptible phenotype)	25 (34.59%)	13 (23.56%)	38 (30.00%)	χ2= 13.8692; df= 3 p= 0.003089 statistically significant
E-R, CD-R (cMLSB phenotype);D-zone test= (-)	17 (23.29%)	18 (35.10%)	35 (28.20%)	
E-R, CD-S (MS phenotype)	16 (21.23%)	10 (20.19%)	26 (20.08%)	
E-R, CD-S (iMLSB phenotype) D-zone test= (+)	15 (20.89%)	11 (21.15%)	26 (21.00%)	
Table 5. Distribution of Staphylococcus aureus Isolates on the basis of their Susceptibility to Erythromycin and Clindamycin Disks placed adjacent to Each Other				
N= Number of isolates. E= Erythromycin (15 µg) disk; CD= Clindamycin (2 µg) disk; S= Sensitive; R= Resistant; cMLSB phenotype= isolates with constitutive resistance to Clindamycin; MS phenotype= isolates with susceptibility to Clindamycin (circular zone of inhibition) and negative D-zone test; iMLSB phenotype= isolates with inducible resistance to Clindamycin and positive D-zone test				
*P-value < 0.05 was considered as statistically significant.				

Antibiotic Tested	iMLSB Phenotypes N= 26 (100%)	
	Resistant N (%)	Sensitive N (%)
Vancomycin	0 (00.00%)	26 (100%)
Linezolid	0 (00.00%)	26 (100%)
Ciprofloxacin	21 (79.05%)	5 (20.95%)
Cefoxitin	6 (24.76%)	20 (75.24%)
Gentamicin	5 (22.86%)	21 (71.14%)
Cefuroxime	6 (22.86%)	20 (71.14%)

Amoxyclav	10 (36.19%)	16 (63.81%)
Amoxicillin	1 (33.33%)	2 (67.77%)
Doxycycline	20 (76.19%)	6 (23.81%)
Levofloxacin	10 (40%)	16 (60%)
Table 6. Antibiotic Susceptibility Pattern of inducible Clindamycin resistant <i>Staphylococcus aureus</i> Isolates (iMLSB phenotypes) derived from Infection		
N= Number of Isolates		

DISCUSSION

In emergence of multidrug-resistant *S. aureus*, it is often crucial to determine antimicrobial susceptibility of all clinical isolates for optimal therapy of infected patients.^[8] Due to limited range of antibiotics available for the treatment of Methicillin-resistant staphylococcal infections and the known limitations of Vancomycin and Clindamycin should be considered for the management of serious soft tissue infections with MRSA that are sensitive to Clindamycin.^[9] However, Clindamycin resistance can develop in staphylococcal isolates with inducible phenotype and from such isolates spontaneous constitutively resistant mutants have arisen during Clindamycin therapy.^[10] Reporting *S. aureus* as susceptible to Clindamycin without checking for inducible resistance may result in institution of inappropriate Clindamycin therapy and hence therapeutic failure. On the other hand, negative result for inducible Clindamycin resistance (MS phenotype) confirms true Clindamycin susceptibility and as these isolates do not become resistant to it during therapy Clindamycin can act as a good therapeutic option in such cases.^[11] The true sensitivity to Clindamycin can only be judged after performing D-zone test on the erythromycin resistant *S. aureus* isolates.^[8]

Resistance is induced by the binding of a macrolide to upstream translational attenuator sequences, leading to changes in mRNA secondary structure, exposure of the ribosomal binding site and expression of the multiallele plasmid-borne erythromycin ribosomal methylase (erm) gene that causes the production of the methylase enzymes. These enzymes cause methylation of the A2058 residue, located in the conserved domain V of the 23S rRNA component of the 50S ribosomal subunit, which leads to cross resistance and the formation of the inducible phenotype (iMLSB) of the resistance pattern.^[12,13] Alterations in these 5' upstream sequences including deletions, duplications and other mutations lead to constitutive expression of the methylase gene and constitutive MLSB resistance. Recent evidence also proves that constitutive resistance to Clindamycin in *S. aureus* prevents the inhibition of toxin production and fails to inhibit growth.^[14] Interestingly, the streptogramin B and A combination quinupristin/dalfopristin appears to retain its activity against cMLSB strains of Staphylococci, although the presence of the cMLSB phenotype changes the agent's activity from bactericidal to bacteriostatic.^[15] Treatment of infections by the iMLSB and cMLSB resistant phenotypes with Clindamycin will result in failure. Routine susceptibility testing can easily detect cMLSB phenotypes, but the real challenge lies in correctly identifying those iMLSB strains that are Clindamycin sensitive in vitro, but result in therapeutic failure in vivo. This can be achieved by testing the isolates using the D-test in accordance with CLSI guidelines.^[16] In this test, 14/15 membered Macrolides (strong methylase inducers) and Clindamycin disks are placed 15 mm apart edge-to-edge on a lawn culture of *Staphylococcus* spp. isolates and incubated overnight. This

precludes the prescription of Clindamycin as a therapeutic drug in such scenarios. The benefit of routine D-testing is that we can clearly identify those strains that remain susceptible to Clindamycin despite being resistant to Macrolides. Isolates with the inducible resistance phenotype should be reported as Clindamycin resistant. On the other hand, negative result for inducible Clindamycin resistance confirms Clindamycin susceptibility and serves as a very good therapeutic option.^[15] The true sensitivity to Clindamycin can only be judged after performing D-zone test on the erythromycin resistant *S. aureus* isolates.^[8]

In our study, we have included 125 *S. aureus* isolates derived from pus 19 (41.53%), post-operative wound infection 8 (18.03%), blood samples were 10 (22.40%), Miscellaneous Samples were 6 (13.66%) and urine sample 2 (3.75%) from both outpatients and inpatients of Orthopaedic Department of our Institution. The prevalence of Methicillin resistance amongst all *S. aureus* isolates was found to be 36.6%. The prevalence of MRSA in other studies was found to vary from 12.9% to 49.8% with the geographical area under study.^[17,8]

In the present study, the susceptible phenotypes (susceptible to both Erythromycin and Clindamycin) were found to predominate in MSSA (39.75%) as compared to MRSA (13.11%). A high percentage of Erythromycin resistant *S. aureus* isolates (70%, 88/125) were detected, of which 86.89% (40/46) were MRSA and 60.25% (48/79) were MSSA. All these were tested for D-zone test. Amongst them maximum isolates (40.29%, 35/88) were of cMLSB phenotype (D-zone test negative), followed by constitutive and inducible resistance phenotype. This suggests that majority of the Erythromycin resistant *S. aureus* isolates can still be treated successfully with Clindamycin. In our study the percentage of inducible Clindamycin resistance (iMLSB phenotype, which gave positive D-zone test) amongst Erythromycin resistant isolates was 30% (26/88). This is in agreement to studies from Chandigarh and Bangalore, which reported inducible resistance to be 26.1% and 22.2% respectively among erythromycin resistant isolates. While in two different studies from Karnataka, the iMLSB phenotype was seen to be quite high in 63% and 55.26% isolates respectively among the erythromycin resistant strains of *S. aureus*.^[13,18] In the present study, it was found that the percentage of the constitutive resistant (cMLSB) predominated in MRSA (50.27%) as compared to MSSA (15.46%). Both the MS phenotype and the inducible resistant (iMLSB) phenotypes predominated in MSSA (19.87 and 24.92% respectively) as compared to MRSA (22.40% and 14.20% respectively). Our data show that the MLSBi resistance phenotype is prevalent in clinical laboratories in the Bettiah area; however, the incidence differs from that reported in other Indian cities and Internationally few studies have shown higher percentage of inducible resistance in MSSA as compared to MRSA.^[19,20] The cost benefit of routinely performing the D-test must be evaluated in each

laboratory setting after first determining the incidence of the MLSBi. For example, in of our hospitals, the incidence of the ER-R/I CD-S phenotype in MRSA was low, 14.20%. But other studies from Kolkata, which showed inducible resistance and constitutive resistance in MRSA compared to MSSA (22.6%, 35.5%, and 11.8%, 11.8%, respectively), whereas, MS phenotype predominated in MSSA compared to MRSA (17.6% and 16.1% respectively).^[21] A study from Chandigarh also showed higher inducible and constitutive resistance in MRSA isolates (20% and 46%, respectively) as compared to MSSA isolates (17.3% and 10%, respectively), whereas MS phenotype was predominant among MSSA (37.3%) as compared to MRSA (16%).^[22] Another study from Karnataka also showed higher constitutive and inducible resistance amongst MRSA (15.4% and 38.5%, respectively) as compared to MSSA (0% and 12.9% respectively). However, they did not report any MS phenotype.^[23] A study from Maharashtra also showed higher percentage of inducible resistance amongst MRSA as compared to MSSA (27.6% and 1.6% respectively). However, they also reported higher MS phenotype amongst MRSA (24.3%) than in MSSA (4%), and constitutive resistance of 7.3% in MRSA and none amongst MSSA.^[8]

In our study, higher incidence of inducible Clindamycin resistance was detected among isolates derived from outpatients (community acquired) as compared to inpatients or hospital acquired (58.40% and 41.60%, respectively). This finding was similar to another study, which also reported higher incidence of inducible Clindamycin resistance from community (66.67%) than from hospital (33.33%).^[24] This may be due to the fact that Clindamycin being an oral drug has been increasingly prescribed by the physicians in outdoor clinical settings, thus leading to increased incidence of community-acquired inducible Clindamycin resistance.

In our study, we also looked forward for treatment options for inducible Clindamycin resistant *S. aureus* isolates by detecting their antimicrobial susceptibility to various other antibiotics. It was found that all isolates with iMLSB phenotype were 100% susceptible to Linezolid and Vancomycin followed by moderate susceptibility (71, 14%) to Gentamicin, Cefuroxime and least susceptibility to Doxycycline and Ciprofloxacin (23.81% and 20.95%, respectively). This finding is in concordance to other studies that also found that all the iMLSB isolates were uniformly susceptible to Linezolid and Vancomycin.^[22,24,25]

CONCLUSION

In our study, we also looked forward for treatment options for inducible clindamycin resistant *S. aureus* isolates by detecting their antimicrobial susceptibility to various other antibiotics. It was found that all isolates with iMLSB phenotype were 100% susceptible to Linezolid and Vancomycin followed by moderate susceptibility (71, 14%) to Gentamicin and Cefuroxime and least susceptibility to Doxycycline and Ciprofloxacin (23.81% and 20.95%, respectively). This finding is in concordance to other studies that also found that all the iMLSB isolates were uniformly susceptible to Linezolid and Vancomycin.

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