Determine Constitutive and Inducible Clindamycin Resistance among Clinical Isolates of Staphylococcus Aureus Isolates from Tertiary Care Hospital, Bettiah, India

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ABSTRACT

Introduction: Clindamycin has been used to treat pneumonia and soft tissue and musculoskeletal infections due to MRSA. One important problem in Clindamycin treatment is the risk of clinical failure during therapy caused by MLSB inducible resistance. The Clinical and Laboratory Standards Institute (CLSI) suggest D-test, which is a phenotypic showing technique for inducible Clindamycin resistance.

Material and Methods: We analyzed antimicrobial susceptibility testing by Kirby Bauer disk diffusion method. Methicillin resistance was detected with cefoxitin (30 µg) disk and inducible clindamycin resistance was unwavering in all erythromycin resistant isolate by using D-zone test.

Results: 100 S. aureus isolate 37 (36.6%) were methicillin resistant (MRSA) and 63 (63.4%) were methicillin-sensitive S. aureus (MSSA). Although, mainstream of the MRSA isolates were imitative from pus samples 15, however, the S. aureus isolates imitative from post-operative wound infection were mainly MRSA 7. A total of 21 S. aureus isolates with iMLSB phenotype shown that they were 100% susceptible to vancomycin and linezolid, with modest sensitivity (71.14%) to gentamicin, cefuroxime and slightest sensitivity to (23.81%) doxycycline, (20.95%) ciprofloxacin.

Conclusion: Outstanding to high happening of erythromycin resistance amongst S. aureus isolates, we recommend that D-zone test have to be regularly done in all laboratories for suitable recommendation of clindamycin and thus preventing appearance of inducible resistant strains and management failure.

Keywords: Staphylococcus Aureus, Wound Infection, Inducible Clindamycin Resistance

INTRODUCTION

Staphylococcus aureus can construct a wide variety of diseases, from relatively benign skin infections such as folliculitis and furunculosis to deepseated and life-threatening conditions, including cellulitis, deep abscesses, osteomyelitis, pneumonia, sepsis, and endocarditis. Staphylococcus aureus is a common cause of bacterial infections in both developed and developing countries.[1-3] The MLS_B resistant phenotype in *S. aureus* be able to be whichever constitutive MLS_B ($cMLS_B$) or inducible MLS_B ($iMLS_B$). Staphylococci that communicate ribosomal methylases (*erm*) genes may demonstrate *in vitro* resistance to erythromycin, clindamycin, and previous drugs of MLS_B group. This resistance is referred to as the $cMLS_B$ phenotype.[4,5]

The double disk estimate test (*D*-test) that involves the handing over of an erythromycin disk in propinquity to the disk containing clindamycin. As the erythromycin diffuses throughout the agar, the resistance to the clindamycin is induced, consequential in destruction or blunting of the clindamycin zone of embarrassment neighbouring to the erythromycin disk, giving a "*S*0" shape to the zone. The Clinical and Laboratory Standards Institute (CLSI) recommends *D*-test, which is a phenotypic transmission method for inducible clindamycin resistance.[6] Therefore, all erythromycin resistant *S. aureus* must be tested for inducible clindamycin resistance to prevent clindamycin handling failures and to report prevalence resistant phenotypes which varies widely. The present study will be designed to establish the constitutive and inducible clindamycin resistance in *S. aureus* isolated from different clinical specimens at a tertiary care hospital in Bihar.

MATERIAL AND METHODS

This cross-sectional learning was conducted for a time of one year from Januay 2018 to June 2019. We analyzed 100 non-duplicate repeated isolates of S. aureus isolated from a variety of medical specimens like aspirates, pus, wound swab, blood, and sterile fluids. Age and gender of the patients were recorded. This study was accepted by the Research and

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Ethical committees of our institution and knowledgeable consent was obtained from each patient.

Clinical samples and bacterial isolates

During of one year period 100 clinical isolates of S. aureus were collected from tertiary care hospital, Bettiah, India. The bacteria which were repeatedly inaccessible from patients in different wards and diverse specimens such as: blood, catheter, wound, discharge, burn, abscess, and so on, were elated to Microbiology Department in tertiary care hospital, Bettiah, India.and were established by standard microbiology tests as well as: Gram staining, slide and tube coagulase, catalase, mannitol fermentation and invention of DNase enzyme.[7]

Antibiotic susceptibility test

Antibiotic susceptibility testing was performed by Kirby Bauer disk diffusion method as per CLSI guidelines using antibiotic disks such as penicillin (10 units), gentamicin (10µg), tetracycline (30µg), linezolid (30µg), trimethoprimsulfamethoxazole (1.25/23.75µg), erythromycin (15µg), cefoxitin (30µg), ciprofloxacin (5µg), clindamycin (2µg). and Staphylococcus aureus ATCC 25923 was used as regular quality control strain.[8]

Methicillin resistance test

Methicillin resistance amongst S. aureus was unconquerable by cefoxitin (30µg) disk under Mueller Hinton agar as per CLSI guidelines, and consequences were examine after 18 hours of incubation at 35°C. The Susceptibility of MRSA strains to vancomycin was experienced by agar dilution technique as per CLSI strategy by inoculating 0.5 McFarland bacterial suspensions on Mueller-Hinton agar (MHA) plates by using sterile swabs. The plates were analyze after 24 hours of incubation at 35°C. Minimum inhibitory concentration (MIC) of vancomycin of ≤ 2µg/mL for S. aureus was considered as susceptible to vancomycin.

D-Zone Test (Disk Approximation Test)

The isolates resistant of erythromycin were supplementary tested by D-zone test which was perform as per CLSI strategy by inoculating 0.5 McFarland bacterial suspensions on the Mueller-Hinton agar plates through the support of sterile swabs and insertion the erythromycin (E-15µg) and clindamycin (CD-2µg) disks side by side surrounded by edge to edge distance of 15mm. Plates were analyzed subsequent to 18 hours of incubation at 35°C.[8]

Phenotypic inducible resistance to clindamycin by D-test:-

Isolates were plate on a Muller Hinton agar plate at a Mc Far land deliberation of 0.5 to finally cover the agar surface. Erythromycin and Clindamycin disks, containing 2 µg and 15µg each correspondingly were located in the center of the plate unconnected by a detachment of 15 cm connecting the edges. Plates were incubate at 37° C for 24 hr. Inducible conflict to Clindamycin was defined as blunting of the clear circular area of no growth approximately the Clindamycin disk on the side neighbouring to the Erythromycin disk and was selected D - test positive. Absence of a blunted zone of embarrassment was designated D - test negative.

Three dissimilar phenotypes of erythromycin resistant isolate were interpret as follows:

The constitutive MLSB phenotype (cMLSB)

The S. aureus isolate resistant to equally E (zone size ≤ 13mm) and CD (zone size ≤ 14mm), with circular shape of zone of embarrassment if any around clindamycin. This suggests assortment of erm gene mutants.

The MS phenotype

The S. aureus segregate which showed resistance to E (zone size ≤ 13mm) and a absolute circular zone of inhibition around CD (zone size ≥ 21mm), indicated negative D zone test. This suggest resistance due to the msrA-coded active efflux pump mechanism.

The inducible MLSB phenotype (iMLSB)

The S. aureus isolate which demonstrate resistance to E (zone size ≤ 13mm) and susceptibility to CD (zone size ≥ 21mm) with pulling down of zone of embarrassment approximately clindamycin in the area bordering 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 statistical involvement between inducible clindamycin resistance phenotype and methicillin resistant S. aureus isolates were evaluate by means of Chi-square test and $p < 0.05$ was considered as statistically significant.

RESULT

Among 100 S. aureus isolates included in our study, 57 (57%) were isolated from pus samples, 26 (25.8%) were isolated from blood, 5 (5.2%) were isolated from Urine, 5 (4.8%) were isolated from sputum, and 7 (7.2%) were isolated from miscellaneous samples as shown in [Table 1]. Out of 100 S. aureus isolates, 37 (36.6%) were methicillin resistant (MRSA) and 63 (63.4%) were MSSA. Although, preponderance of the MRSA isolates were consequent from pus samples 15, however, the S. aureus isolates resulting from post-operative wound infection were frequently MRSA 7. This finding was establish to be statistically significant (P=0.008803). [Table-2]. The susceptible phenotype (E-S and CD-S) predominate in MSSA (39.75%) as compare to MRSA (13.11%). while, the constitutive resistant (cMLSB) predominate in MRSA (50.27%) as compared to MSSA

Types of Sample	Samples Number	Samples Percentage
Pus	57	57.00%
Blood	26	26.00%
Urine	05	05.00%
Sputum	05	04.80%
Miscellaneous*	07	07.20%
Total	100	100

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

Table-1: Sample-wise supply of S.aureus isolates [n=100]

Sample	Staphylococcus aureus			Chi-Square ($\chi 2$) & *p value
	Resistant to cefoxitin (MRSA) N = 22 (38.25%)	Susceptibile to cefoxitin (MSSA) N = 35 (61.75%)	Total isolates N = 57 (100%)	$X^2 = \sum_{i=1}^{r}\sum_{j=1}^{c}\frac{(O_{i,j}-E_{i,j})^2}{E_{i,j}}$.
Pus due to any other cause, N (%)	15 (34.23%)	29(65.77%)	44(77.89%)	$Chi^{2=6.8422}$; DF=1; P=0.008903 statistically significant
Post operative Wound infection, N(%)	7 (52.38%)	6 (47.62%)	13(22.11%)	

N = Number of isolates.
MSSA = Methicillin sensitive Staphylococcus aureus;
MRSA = Methicillin resistant Staphylococcus aureus.
*p value < 0.05 was measured as statistically significant.

Table-2: Allotment of Staphylococcus aureus isolates on the source of sample and susceptibility to cefoxitin (30 µg) disk.

Antibiotic susceptibility pattern	Staphylococcus aureus			Chi-Square ($\chi 2$) & *p value
	MRSA N = 37 (36.6%)	MSSA N = 63 (63.4%)	Total isolates N = 100 (100%)	
E-S, CD-S (Susceptible phenotype)	5 (13.11%)	25 (39.75%)	30 (30.00%)	$\chi^2 = 84.001$; df = 3 p = 0.00001 statistically significant
E-R, CD-R (c MLSB phenotype) D-zone test= (-)	19 (50.27%)	10 (15.46%)	29 (28.20%)	
E-R, CD-S (MS phenotype)	8 (22.40%)	12(19.87%)	20 (20.08%)	
E-R, CD-S (i MLSB phenotype) D-zone test= (+)	5(14.20%)	16(24.92)	21 (21.00%)	

N = Number of isolates. MSSA = Methicillin sensitive Staphylococcus aureus;
MRSA = Methicillin resistant Staphylococcus aureus; E = Erythromycin (15 µg) disk;
CD = Clindamycin (2 µg) disk; R = Resistant; S = Sensitive; cMLSB phenotype = isolates with constitutive conflict to clindamycin; MS phenotype = isolates with resistance to clindamycin (circular zone of embarrassment) 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.

Table-3: Supply of Staphylococcus aureus isolates on the source of their susceptibility to erythromycin and clindamycin disks placed adjoining to each other.

Antibiotic tested	iMLSB phenotypes N = 21 (100%)	
	Resistant N (%)	Sensitive N (%)
Vancomycin	0(00.00%)	21(100%)
Linezolid	0(00.00%)	21(100%)
Ciprofloxacin	17(79.05%)	4 (20.95%)
Cefoxitin	26(24.76%)	79(75.24%)
Gentamicin	5 (22.86%)	16(71.14%)
Cefuroxime	5(22.86%)	16(71.14%)
Amoxyclav	8(36.19%)	13(63.81%)
Amoxicillin	1(33.33%)	2(67.77%)
Doxycycline	16(76.19%)	5 (23.81%)
Levofloxacin	8 (40%)	13 (60%)

N = Number of isolates

Table-4: Antibiotic susceptibility outline of inducible clindamycin resistant Staphylococcus aureus isolates (iMLSB phenotypes) derived from infection.

Figure-1: inducible clindamycin resistant Staphylococcus aureus

(15.46%), Mutually the MS phenotype and the inducible resistant (iMLSB) phenotypes predominate in MSSA (19.87 and 24.92% correspondingly) as compared to MRSA (22.40% and 14.20% correspondingly). This judgment was establish to be highly significant ($p < 0.00001$) [Table 3]. A total of 21 S. aureus isolates showed inducible clindamycin resistance by giving a positive D-zone test, [Figure-1] hence, its prevalence was found to be 21.00% (21/100), with proportion allocation of cMLSB phenotype and MS phenotypes in all S. aureus isolates while 19.4% and 24.6% respectively [Table 4]. The antimicrobial susceptibility test outcome of all the 21 S. aureus isolates by means of

iMLSB phenotype exposed that they were 100% sensitive to vancomycin and linezolid, with restrained sensitivity (71.14%) to gentamicin, cefuroxime and slightest sensitivity to (23.81%) doxycycline, (20.95%) ciprofloxacin as shown in [Table 4].

DISCUSSION

Recent confirmation also proves that constitutive resistance to clindamycin in S. aureus prevents the inhibition of toxin invention and fails to inhibit growth.[9] Treatment of infections beside the iMLSB and cMLSB resistant phenotypes by means of clindamycin will result in collapse. Schedule susceptibility trying can easily perceive cMLSB phenotypes, but the real confront lies in accurately identifying those iMLSB strains that are clindamycin sensitive in vitro but effect in remedial failure in vivo. This container is achieved by testing the isolates by means of the D-test in agreement with CLSI guiding principle.[10] In the nearby study, the predisposed phenotypes (susceptible to both erythromycin and clindamycin) were establish to dominate in MSSA (39.75%) as compared to MRSA (13.11%). A elevated proportion of erythromycin resistant S. aureus isolates (70%, 350/500) were detected of which 86.89% (159/183) were MRSA and 60.25% (191/317) were MSSA. All these were tested for D-zone test. Amongst them maximum isolates (40.29%, 141/350) were of cMLSB phenotype (D-zone test negative), followed with constitutive and inducible conflict phenotype. This suggests that mainstream of the erythromycin resistant S. aureus isolates can immobile be treated successfully with clindamycin. In our study the percentage of inducible clindamycin resistance (iMLSB phenotype, which gave positive D-zone test) amongst erythromycin challenging isolates was 30% (105/350). This is in concurrence to studies from Chandigarh and Bangalore which reported inducible resistance to be 26.1% and 22.2% correspondingly among erythromycin resistant isolates.[11,12] although in two dissimilar studies from Karnataka, the iMLSB phenotype was seen to be moderately high in 63% and 55.26% isolates correspondingly among the erythromycin resistant strains of S. aureus.[13,14]

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 judgment was parallel to another study which also reported higher incidence of inducible clindamycin resistance from community (66.67%) than from hospital (33.33%).[11] This may be outstanding 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 handling options for inducible clindamycin resistant S. aureus isolates by detecting their antimicrobial susceptibility to a variety of other antibiotics. It was establish that all isolates with iMLSB phenotype were 100% susceptible to linezolid and vancomycin, followed by modest susceptibility(71,14%) to gentamicin, cefuroxime and slightest susceptibility to doxycycline, ciprofloxacin (23.81% and 20.95% respectively). This judgment is in concordance to previous studies that also found that all the iMLSB isolates were regularly susceptible to linezolid and vancomycin.[11,12,15]

CONCLUSION

In our study we also looked presumptuous for management options for inducible clindamycin resistant S. aureus isolates by detecting their antimicrobial susceptibility to a variety of other antibiotics. It was initiate that all isolates with iMLSB phenotype were 100% susceptible to linezolid and vancomycin, followed by reasonable susceptibility(71,14%) to gentamicin, cefuroxime and smallest amount susceptibility to doxycycline, ciprofloxacin (23.81% and 20.95% respectively). This judgment is in concordance to previous studies that also found that all the iMLSB isolates were homogeneously susceptible to linezolid and vancomycin.

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