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Original Research Article

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A Study of Inducible Clindamycin Resistance in Methicillin Resistant Staphylococcus Aureus in Tertiary Health Care Centre

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ABSTRACT

Background: Staphylococcus aureus is the most frequently encountered pathogen isolated from clinical specimens. Prolonged hospitalization, indiscriminate use of antibiotics, and indwelling medical devices were the cause for the appearance and spread of methicillin resistant staphylococcus aureus (MRSA). MRSA strains have a high effect on patient morbidity and mortality.

Material and methods: A total of 208 Methicillin resistance staphylococcus aureus isolates were taken from various clinical samples and processed in accordance with standard protocol.

Results: In this research, among 601 staphylococcus aureus isolates, 393 (65.40%) were MSSA and 208 (34.60%) were MRSA. Analysis of clindamycin Resistance in 208 MRSA isolates showed 19.7% of inducible clindamycin Resistance.

Conclusion: The pattern of clindamycin resistance to MRSA varies in different regions. When clindamycin is considered for therapy, the kind of resistance (inducible or constitutive clindamycin resistance) which exists to be detected. 'D test' is absolutely necessary in microbiology laboratories. This is because it avoids misinterpretation of clindamycin resistance by clearly delineating inducible clindamycin resistance from constitutive clindamycin resistance.

Keywords: MRSA, Inducible clindamycin resistance, Constitutive Clindamycin resistance

INTRODUCTION

Staphylococcus aureus is the most frequently encountered pathogen isolated from clinical specimens. Staphylococcus aureus has the ability to asymptomatically colonize the normal population either persistently or transiently. 33% of humans are likely to be nasal carriers.[1] Staphylococcus aureus causes variety of human infections ranging from minor skin diseases such as furuncles, cellulitis, abscesses to life threatening infections like toxic shock syndrome, staphylococcal scalded skin syndrome, endocarditis, pneumonia & septicemia.

Penicillin was the drug of choice to which Staphylococcus aureus developed resistance by producing the enzyme betalactamase. So methicillin was introduced in 1959. But methicillin resistant staphylococcus aureus (MRSA) fastly appeared in hospitals in 1961, just 2 years after the first clinical use of methicillin.[2] Alternatively the macrolide-lincosamide streptogramin B group of antibiotics can be used for treating MRSA infection. Clindamycin, a

lincosamide antibiotic has become an attractive option for clinicians because of its bioavailability both in oral & intravenous formulations.

However, fear of appearance of clindamycin resistance during therapy has discouraged some clinicians prescribing it.

The mechanism of inducible clindamycin resistance (iMLSB) is due to target site modification mediated by erm gene which can be expressed by an inducer like erythromycin or constitutively (cMLSB). The overlapping binding sites of macrolides, lincosamides, and streptogramins B in 23S rRNA accounts for the cross resistance to the 3 classes of drugs .[3] The D-test is performed for detecting inducible clindamycin resistance by D-test. If D-test is positive, it suggests the presence of an erm gene that could result in clindamycin resistance.

MATERIAL AND METHODS

Sample collection

This prospective study was conducted at Tertiary Health Care Centre from March 2022 to November 2022 in Department of Microbiology. Total 601 staphylococcus aureus isolates from clinical samples including, pus, sputum, blood, vaginal swab and other body fluid were included in the study.

Samples were received from outpatients and inpatients who attended M. P. Shah Government Medical Collage & Hospital.

Isolation and identification

The received samples were checked for proper labelling with Name, Age, Sex and Indoor Patient/ Outdoor Patients No. of the patient, date and time of collection of the sample and processed immediately. Direct smears were prepared from sample material like pus, sputum, urine and vaginal swab on a clean glass slide. Gram staining was done and examined under microscope.

The findings were recorded. Blood samples sent in Glucose broths were incubated for 18-24 hours and then sub cultured. All the above specimens were inoculated on to the Nutrient agar, blood agar and MacConkey agar, and incubated at 37º C for 18-24 hours aerobically and observed after incubation.

All the suspected colonies were identified by colony morphology, gram staining was done and the organism subjected to various biochemical tests to identify and characterize them. Further confirmation was done by slide and tube coagulase test, and growth on Mannitol Salt Agar. The sensitivity to common antibiotics was done by Kirby Bauer disc diffusion method as recommended by CLSI. Control strains used are staphylococcal aureus ATCC-25923 and MRSA-43300.

Incubation is at 37ºC for 24 hrs after which, the zone of inhibition was measured by using zone measuring scale and interpreted as per the CLSI standards. Transmitted light was used to examine the light growth of methicillin resistant isolates.

Disc diffusion test for detecting Methicillin resistance

Cefoxitin disc diffusion test [4] Zone diameter of 22 mm or more was taken as sensitive and 21 mm or less was considered as resistant. These resistant isolates were considered as MRSA.

'D' test [5, 6, 7] : A 0.5 McFarland suspension of staphylococci was inoculated on Mueller Hinton agar plate. Clindamycin (2µg), and erythromycin (15µg), discs were placed at an edge-to-edge distance of 15 mm, followed by overnight incubation at 37oC.

Description of different types of phenotypes that were looked for:

Inducible Clindamycin resistance: (iMLSB resistance)

Staphylococcal isolates showing resistance to erythromycin (zone size ≤ 13mm) and a clear, D-shaped zone of inhibition round the clindamycin disc was designated as the inducible clindamycin resistance (D phenotype).

MS phenotype

In this phenotype Staphylococcal isolates were erythromycin resistant (zone size ≤ 13mm). But sensitive to clindamycin (zone size ≥ 21mm) showing circular zone of inhibition around it.

Constitutive resistance (cMLSB resistance)

Staphylococcal isolates resistant to erythromycin (zone size ≤ 13mm) and resistant to clindamycin (zone size ≤ 14 mm) were brought under this phenotype.

Susceptible phenotype (S phenotype)

Staphylococcal isolates sensitive to both erythromycin (zone size ≥ 23mm) and clindamycin (zone size ≥21mm) were categorized in this phenotype.

RESULTS

During the course of the research, among 601 staphylococcus aureus isolates, 393 (65.40%) were MSSA and 208 (34.60%) were MRSA, observed as given in Table-1.

Table-1: Prevalence of MRSA & MSSA Isolates in Different Specimens

Total No. Of Samples	MSSA	MRSA
601	393 (65.40%)	208 (34.60%)

Among 208 MRSA isolates, the sample wise distribution was as follows. Pus constituted 136 (65.40%), urine 42 (20.20%), blood 21 (10.10%), sputum 5 (2.40%), vaginal swab 3 (1.40%) and body fluid 1 (0.50%).

The above observation shows that Methicillin resistant staphylococcus aureus was isolated maximally from pus Samples (65.40%) and only few were isolated from blood, sputum, vaginal swab and other body fluids.

The sensitivity pattern of MRSA aureus isolates to different antibiotic groups is given in Table-2.

Table-2: Antibiotic Sensitivity Pattern of MRSA

n=208

Antibiotic Tested	Sensitivity %
TETRACYCLINE 30 µg	31 (14.90%)
LINEZOLID 30 µg	206 (99.03%)
LEVOFLOXACIN 5 µg	122 (58.65%)
ROXYTHROMYCIN 15 µg	92 (44.23%)
COTRIMOXAZOLE 25 µg	90 (43.26%)
CLOXACILLIN 1 µg	IR
AMPICILLIN/SULBACTAM 20 µg	IR
CEPHELEXIN 30 µg	IR
CEFOTAXIME 30 µg	IR
CEFOXITINE 30 µg	IR
CIPROFLOXACIN 5 µg	125 (60.09%)
LINCOMYCIN 2 µg	115(55.28%)
GENTAMYCIN 10 µg	114(54.80%)
ERYTHROMYCINE 30 µg	95 (45.67%)
CLINDAMYCIN 2 µg	119 (57.20%)

*IR: Intrinsic Resistance

Analysis of clindamycin Resistance in 208 MRSA isolates showed 19.7% of inducible clindamycin Resistance, 23.0 % of constitutive clindamycin Resistance, and 45.7 % were sensitive to both erythromycin and clindamycin. MS phenotype was observed 11.5% as given in Table-3 and Figure-1. Above observation shows that, constitutive clindamycin resistance was reported in a higher percentage than inducible clindamycin resistance.

Table-3: Clindamycin Resistant Phenotypes of MRSA by D-Test

n=208

Susceptibility Pattern Of Drug (Phenotype)	%
ERY-R , CLI-S , (D-test +ve; iMLS$_B$)	41 (19.71%)
ERY-R , CLI-R (cMLS$_B$)	48 (23.07%)
ERY-S , CLI-S (S-Phenotype)	95 (45.67%)
ERY-R , CLI-S , (D-test –ve; MS Phenotype)	24 (11.53%)

ERY- R: Erythromycin resistant.
CLI-R: Clindamycin resistant
CLI-S: Clindamycin sensitive
ERY-S: Erythromycin sensitive.
iMLS B- Inducible Clindamycin resistance
cMLS B- Constitutive Clindamycin resistance
S- Phenotype: Susceptible phenotype
MS phenotype- Macrolide Streptogramin (type B) resistance.

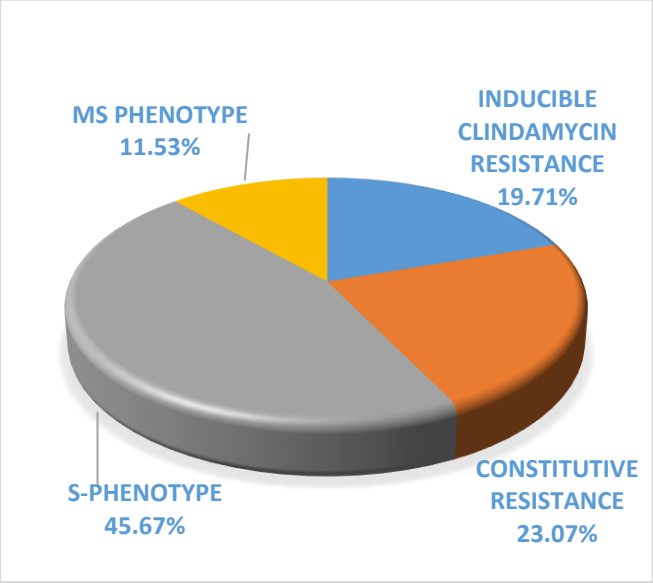

Figure-1: Clindamycin Resistant Phenotypes of MRSA by D-Test

DISCUSSION

MRSA is a major cause of hospital and community acquired infections. Clindamycin is an excellent drug to treat not only serious infections like sepsis, endocarditis, osteomyelitis, pneumonia, and staphylococcal scalded skin syndrome caused by MRSA but also MSSA. It is less expensive compared to newer antibiotics.

As it can be given orally it can be used in outpatient therapy. Drugs like tetracycline and fluoroquinolones are not advised for treating children and pregnant women due to side effects. But clindamycin is a treatment of option for children and it can also be used in penicillin allergic individual. [2, 8]

It is very necessary to distinguish between staphylococci having inducible clindamycin resistance from those with MS Phenotype. Because MS Phenotype in staphylococcal strains does not result in failure of therapy, whereas it occurs in inducible clindamycin resistance. [2]

In the present study 208 samples were processed and results were analysed.

Among 601 staphylococcus aureus isolates, 34.60% were MRSA observed. The above data correlates with the result of Shetty J et al. who has documented 36.90% MRSA.[9] This is in accordance with study of Singh et al has documented 37.80% of MRSA.[10]

Table-4 : Comparison of prevalence of MRSA

Name of the study	MRSA %
Present study	34.60%
Shetty j et al.	36.90%
Singh et al.	37.80%

The present study showed that MRSA is highly sensitive to linezolid 99.03%. Khatoon et al. and Joshi et al. shows 100% of sensitivity with linezolid which shows concurrent result with current study.[11, 12]

Table-5: Comparison of antibiotic sensitivity of Linezolid

Name of the study	% of sensitivity to Linezolid
Present study	99.03%
Khatoon et al.	100%
Joshi et al.	100%

In present study ciprofloxacin sensitivity result was reported 60.09%. Khatoon et al. reported 69.20% of sensitivity to ciprofloxacin which is showing concordant result with present study. It shows similar result with the study of Rostami et al. which shows 54.80% sensitivity to ciprofloxacin .[11, 13]

In this study Gentamycin sensitivity result is 54.80%. Khatoon et al. reported 53.8% sensitivity to gentamycin. Rostami et al. shows 57.3% sensitivity to gentamycin which is similar to present study.[11, 13]

Table-6: Comparison of antibiotic sensitivity of Ciprofloxacin & Gentamycin

Name of the study	% of sensitivity to Ciprofloxacin	% of sensitivity to Gentamycin
Present study	60.09%	54.80%
Khatoon et al.	69.20%	53.80%
Rostami et al.	54.80%	57.30%

Present study showed 19.71%, of inducible clindamycin resistance among MRSA. It shows concordance result with the study done by Seifi et al., 20.45% of inducible clindamycin resistance.[14] khatoon et al. reported 22.40% result of Inducible phenotype.[11] Majhi et al. shows similar result showing 24.80% of inducible phenotype.[15]

Table -7: Comparison of Inducible clindamycin resistance phenotype

Name of the Study	% of Inducible clindamycin resistance
Present study	19.71%
Seifi et al.	20.45%
Khatoon et al.	22.40%
Majhi et al.	24.80%

Constitutive clindamycin resistance phenotype reported 23.07% in present study which is similar with the study of Nashwa et.al who reported 30.30% of constitutive resistance. [16]

Table-8: Comparison of Constitutive clindamycin resistance phenotype

Name of the study	% of Constitutive clindamycin resistance
Present study	23.07%
Nashwa et al.	30.30%

S-phenotype were reported highest of all MRSA in present study (45.67%) showing sensitive to both Erythromycin & Clindamycin. Prabhu et al., and Kavitha et al. has been reported similar results showing maximum constitution of S-phenotype 50.00%, and 58.01% respectively. [17, 18]

Table-9: Comparison of S- phenotype

Name of the study	% of S-phenotype
Present study	45.67%
Prabhu et al.	50.00%
Kavitha et al.	58.01%

In present study MS phenotype found 11.53%. Likewise, Adhikari et al. has reported 10.30% of MS Phenotype. [19]

Table-10 : Comparison of MS- phenotype

Name of the study	% of MS-phenotype
Present study	11.53%
Adhikari et al.	10.30%

CONCLUSIONS

Among 601 staphylococcus aureus isolates, 208 (34.60%) were MRSA. Analysis of clindamycin resistance in 208 MRSA isolates showed 19.71 % of inducible clindamycin resistance. These isolates are seemed to be susceptible to clindamycin in vitro but, treatment failure occurs when this drug is instituted as in vivo therapy. MRSA infection in surgical site is commonly noted. Multidrug resistance to commonly used drugs like ciprofloxacin, amikacin, doxycycline and cotrimoxazole are to be noted with concern.

MRSA is a threat not only to immunocompromised individuals, but also to general public. Moreover emergence of drug-resistance among MRSA is now a major concern. So detection of methicillin resistance in staphylococcus aureus is very important for treating patients and to prevent its spread.

Drugs like clindamycin are needed to stem the severe consequences of MRSA. Use of clindamycin avoids costly, intravenous glycopeptides for treating MRSA. Clindamycin is a treatment of option in children. It can be used in penicillin allergic individual. It has good oral bioavailability. So it can be used by clinicians as outpatient therapy as well as to switchover after intravenous antibiotics in hospitalized patients. The pattern of clindamycin resistance to MRSA varies in different regions. When clindamycin is considered for therapy, the kind of resistance (inducible or constitutive clindamycin resistance) which exists to be detected.

'D test' is absolutely necessary in microbiology laboratories. This is because it avoids misinterpretation of clindamycin resistance by clearly delineating inducible clindamycin resistance from constitutive clindamycin resistance.

So 'D' test is suggested along with routine antibiotic susceptibility testing to detect inducible clindamycin resistance and thus avoid treatment failure. Hence this study was done.

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