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TO STUDY INDUCIBLE CLINDAMYCIN RESISTANCE AMONG CLINICAL ISOLATES OF STAPHYLOCOCCUS AUREUS AT SMS MEDICAL COLLEGE

Medical Microbiology

Dr Meenaxi Saini* PG 3rd year, Department of Microbiology, SMS Medical college ,Jaipur *Corresponding Author

Dr RK Maheshwari Senior Professor, Department of Microbiology, SMS Medical college, Jaipur

Dr RK Mishra Senior Professor, Department of Microbiology, SMS Medical college ,Jaipur

ABSTRACT

Introduction: In every part of the world, Staphylococcus aureus (S. aureus) has been identified as one of the most prevalent pathogens causing nosocomial and community-acquired illnesses. resistance to clindamycin in Staphylococcus species can be either constitutive or inducible. The increasing prevalence of resistance to most antimicrobial agents in staphylococci signifies the need for new effective agents to treat staphylococcal infections. Prevalence of MRSA varies according to countries, regions and also to the hospitals and services in the same hospital. The purpose of this study is to Detect inducible clindamycin resistance and antibiotic susceptibility pattern among Staphylococcus aureus isolates in clinical specimen. **Method:** The study protocol was approved by ethics committee. This prospective study was carried out in the Department of Microbiology, SMS Medical College, Jaipur (Rajasthan) from May 2021 to November 2022.A total of 241 S. aureus were isolated from various clinical specimens were tested. isolated from different clinical samples were subjected to routine antibiotic sensitivity testing by Kirby Bauer's disc diffusion method. Methicillin-resistance was determined Incidence of MLSBc and MLSBi in Staphylococcus aureus isolates by D-test as per CLSI guidelines. **Result:** It was observed that maximum (49.44% and 48.68%) isolation of Staphylococcus aureus CoPS and CoNS were obtained from samples like pus. It was observed that majority of imlsb phenotype (D+ isolate n=30) was obtained in MR-cons. Cmlsb and MS phenotype were observed more in MR-cons (n=39 and 13 respectively). **Conclusion:** The high proportions of MRSA and iMLSB phenotypes among S. aureus emphasize the need for methicillin resistance test and D-test to be incorporated in routine susceptibility testing for effective management of S. aureus.

KEYWORDS

clindamycin resistance, staphylococcus aureus, D-test, MRSA

INTRODUCTION:

In every part of the world, Staphylococcus aureus (S. aureus) has been identified as one of the most prevalent pathogens causing nosocomial and community-acquired illnesses.[1] Because of increasing prevalence of methicillin resistance among Staphylococci this has led to renewed interest in the usage of Macrolide-Lincosamide-Streptogramin B (MLSB) antibiotics to treat S. aureus infections with clindamycin being the preferred agent due to its excellent pharmacokinetic properties.[2] However, widespread use of MLSB antibiotics will lead to an increase in the number of Staphylococcal strains acquiring resistance to MLSB antibiotics.[3]

resistance to clindamycin in Staphylococcus species can be either constitutive or inducible.[4] The most common mechanism for such resistance is target site modification mediated by erm genes, which can be expressed either constitutively (constitutive MLSBphenotype) or inducibly (inducible MLSBphenotype). 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.[3] The increasing prevalence of resistance to most antimicrobial agents in staphylococci signifies the need for new effective agents to treat staphylococcal infections. Prevalence of MRSA varies according to countries, regions and also to the hospitals and services in the same hospital.

The purpose of this study is to Detect inducible clindamycin resistance and antibiotic susceptibility pattern among Staphylococcus aureus isolates in clinical specimen

METHODOLOGY:

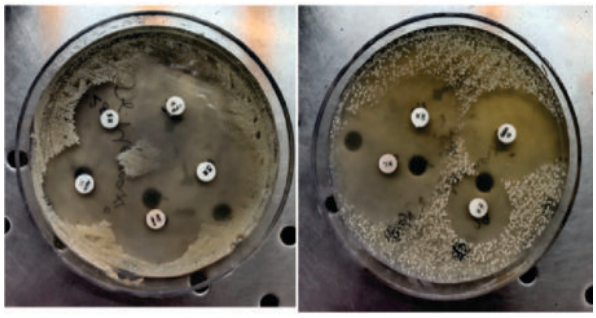
The study protocol was approved by ethics committee. This prospective study was carried out in the Department of Microbiology, SMS Medical College, Jaipur (Rajasthan) from May 2021 to November 2022. total of 241 S. aureus were isolated from various clinical specimens like pus, wound swab, aspirates, blood, body fluids, respiratory tract secretions, sputum, bronchial lavage, central line/neck line/umbilical catheter tips,urine etc. tested. These isolates were confirmed as S. aureus isolates by conventional catalase, tube and slide coagulase. Patients from whom S. aureus was isolated in the absence of clinical disease suggesting colonization were not included in this study. The antibiotic discs tested were amikacin (30 µg), Chloramphenicol (30 µg), Ceftaroline (30 µg), ciprofloxacin (5 µg), clindamycin (2 µg), co-trimoxazole (sulpha/trimethoprim) (23.75/ 1.25 µg), Azithromycin (15 µg), Doxycycline (30 µg), Nitrofurantoin (300 µg), Quinupristin- dalfopristin (15 µg), Linezolid(30 µg), Fusidic acid (30 µg), penicillin-G (10 units), tetracycline (30 µg), vancomycin (Etest for MIC) (HiMedia Pvt. Ltd., India). The zone diameters were interpreted as per CLSI M100-S28 [29]. The CLSI recommends interpreting antibiotic susceptibility results as resistant (R), or intermediate (I), or susceptible (S), but we dichotomously categorized them as susceptible (S) or resistant (R). The isolates showing intermediate susceptibility were also considered resistant. Quality control was maintained using S. aureus ATCC 25923.

Antimicrobial susceptibility testing

All S. aureus isolates were subjected to antimicrobial susceptibility testing using Kirby–Bauer disk diffusion method on Mueller-Hinton agar (MHA) plates

Detection Of Inducible Clindamycin Resistance:

Inducible clindamycin resistance is performed by standard disc diffusion method on Muller Hinton Agar or blood agar purity plate discs containing 15-µg erythromycin and 2-µg clindamycin placed 15–26 mm apart incubated at 35°C± 2°C; ambient air for 16–18 hours.



The results are interpreted as follows:

1. MS phenotype: S. aureus isolate exhibiting resistance to erythromycin (zone size ≤13 mm), while sensitive to clindamycin (zone size ≥21 mm) and giving circular zone of inhibition around clindamycin (D test negative).
2. Inducible MLSB phenotype: iMLSBS. aureus isolates which showed resistance to erythromycin (zone size ≤13 mm) while being sensitive to clindamycin (zone size ≥21 mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc (D test positive).
3. Constitutive MLSB phenotype: cMLSBS. aureus isolates which showed resistance to both Erythromycin(zone size ≤13 mm) and clindamycin (zone size ≤14mm) with circular shape zone of inhibition around clindamycin.

All the generated data were entered and curated by using Microsoft Excel® version 2016 (Microsoft Corporation, USA). All statistical analyses were done in SPSS software© version 20 . Descriptive summaries were presented in text and tables. Descriptive statistics were expressed as percentages. The 2□ ×□2 contingency tables were constructed for the categorical variables and the contingency or association was tested using the chi-square test. The p-value of less than 0.05 was considered statistically significant whenever applicable.

RESULT:

Sample obtained:

It was observed that the maximum (49.44% and 48.68%) isolation of Staphylococcus aureus CoPS and CoNS were obtained from samples like pus, sputum, vaginal swab, tracheal aspirate, ascitic fluid and catheter tip and other body fluids etc, followed by blood (21.35% and 34.21%) ,followed by urine (22.47% and 15%) and CSF(6.74% and 1..32%) respectively. Staphylococcus aureus CoPS and CoNS were isolated from in-patients (73.03% and 69.08%) than out-patients (26.97% and 30.92%)..

Table 1. Distribution of Staphylococcal isolates among different clinical specimens

Specimen	Number of COPS			Number of CONS		
	OPD	IPD	Total	OPD	IPD	Total
Blood	2	17	19	4	48	52
			21.35%			34.21%
Pus/other fluids	17	27	44	33	41	74
			49.44%			48.68%
CSF	0	6	6	0	2	2
			6.74%			1.32%
URINE	5	15	20	10	14	24
			22.47%			15%
Total	24	65	89	47	105	152
	26.97%	73.03%	36.93%	30.92%	69.08%	63.07%

*COPS = Coagulase positive Staphylococci
CONS = Coagulase negative Staphylococci

Age and gender distribution:

The age group most commonly affected by Staphylococcus aureus CoPS and CoNS were 21-40 years (35.96%, 39.47%),closely followed by children <1year (22.84%,24.34%).Staphylococcus aureus CoPS and CoNS isolated were more in males (70.79% and 69.74%) than in females (29.21% and 30.26%).

Table 2 Age-wise distribution of Staphylococcal isolates

Age Group	Number of COPS		Number of CONS	
	No. of Patients	Percentage	No. of Patients	Percentage
<1year	23	25.84%	37	24.34%
1-10 years	10	11.24%	14	9.21%
11-20 years	9	10.11%	12	7.89%
21-40 years	32	35.96%	60	39.47%
41-60 years	10	11.24%	21	13.82%
> 60 years	5	5.62%	8	5.26%
Total	89	36.93%	152	63.07%

Sensitivity pattern of erythromycin and clindamycin among different strains of Staphylococci and distribution of D+ isolates:

It was observed that majority of imlsb phenotype (D+ isolate n=30)was obtained in MR-cons. Cmlsb and MS phenotype were observed more in MR-cons (n=39 and 13 respectively).

Table 3: Sensitivity pattern of erythromycin and clindamycin among different strains of Staphylococci and distribution of D+ isolates

Organism	Total	E-S &CL-S	E-R&CL-S		E-R&CL-R(cMLSB)
			(iMLSB) D+	(MS phenotype) D-	
MRSA	45	14	7	2	22
	18.67%				
MSSA	44	22	5	13	4
	18.26%				
MR CONS	95	13	30	13	39
	39.42%				
MS CONS	57	23	13	14	7

*MRSA= Methicillin resistant S.aureus
MSSA= Methicillin sensitive S.aureus
MR CONS= Methicillin resistant coagulase negative Staphylococci
MS CONS= Methicillin sensitive coagulase negative Staphylococci

Sensitivity pattern of erythromycin and clindamycin in isolates from various clinical specimens :

It shows that maximum numbers of iMLSB phenotype (D+ isolate)is seen in blood specimens from IPD patients which outnumbered the specimens like CSF and other specimens like pus, sputum, vaginal swab, tracheal aspirate, ascitic fluid and catheter tip and other body fluids etc. while constitutive clindamycin resistance and MS phenotype was found to be more common in other specimens from IPD patients.

Table 4 : Sensitivity pattern of erythromycin and clindamycin in isolates from various clinical specimens

Specimen	E-S	E-R	E-R	E-S				
	CL-S	CLS (iMLSB)	CL-R(cMLSB)	CL-R (MS phenotype)				
	OPD	IPD	OPD	IPD	OPD	IPD	OPD	IPD
Blood	0	24	1	13	0	17	0	17
CSF	0	2	0	3	0	0	0	3
Pus,Others	16	19	7	12	13	28	10	13
Urine	1	10	1	5	4	10	4	9

Occurrence of Multidrug resistant and extremely drug resistant case among Staphylococci :

It was observed that occurrence of MDR and XDR is higher in case of Coagulase negative Staphylococci (53.52% and 5.39% respectively) than Coagulase positive Staphylococci (18.26% and 3.32%).Overall prevalence of MDR and XDR cases from all the isolates under study was 71.78% and 8.71% respectively.

DISCUSSION :

In the present study, inducible resistance to Clindamycin (iMLSB) among Methicilln sensitive as well as resistant Staphylococcal strains at our institute was detected by D-test.

In recent years, clindamycin has become an outstanding drug for some Staphylococcal infections, particularly skin and soft tissue infections and as an alternative in penicillin-allergic patients.[5] Also, clindamycin has good oral bioavailability making it a good option for outpatient therapy and changeover after intravenous antibiotics[6]

In this study out Of the total Staphylococcal isolates, 89 (36.93%) were coagulase positive Staphylococcus aureus (COPS) and 152(63.07%) were coagulase negative Staphylococci (CoNS).

Among these,45(18.67%) were Methicillin resistant Staphylococcus aureus (MRSA) and 44 (18.26%) were Methicillin sensitive Staphylococcus aureus (MSSA) however MR-CoNS and MS-CoNS were found to be 95(39.42%) and 57(23.65%) respectively which is lower to other studies Sah et al. (61.4%), Mansouri and Sadeghi (56.8%), and Chudasama et al. (54.78%)[7,8,9]. These variances in MRSA reporting among S. aureus isolates might be related to changes in circulating clones in a different geography, as well as disparities in infection- prevention measures and antibiotic prescribing trends in different hospital settings.

Majority of COPS were isolated from samples of IPD patients like pus and other body fluids 44 (49.44%). Out of 45 MRSA isolated 7(15.56%) exhibited the iMLSB, 22(48.89%)exhibited the constitutive phenotype, 2(4.44%) strains exhibited the MS phenotype while 14 (31.11%) exhibited sensitive phenotype (both erythromycin and clindamycin sensitive).

However in MSSA, 5(11.36%) showed iMLSB, 4(9.09%) showed the constitutive phenotype, 13(29.55%) strains showed the MS phenotype while sensitive phenotype was seen on 22(50%).AM Ciraj() et al has reported 16(20.51%) strains among MRSA are iMLSB phenotype, 10(12.82%) strains among MSSA and 6(7.69%) among MSCNS. So there is high prevalence of iMLSB among MRSA than MSSA.[10] Similar in Mallikarjun K et al [11] treatment of MRSA infections, i.e. Vancomycin, Clindamycin should be considered for management of MRSA that are sensitive to Clindamycin. So, true sensitivity to Clindamycin can be judged only by performing 'D' test for inducible resistance among ERR isolates.

CONCLUSION:

The high proportions of MRSA and iMLSB phenotypes among S. aureus emphasize the need for methicillin resistance test and D-test to be incorporated in routine susceptibility testing for effective management of S. aureus which is simple, inexpensive, reliable method as a routine antibiotic susceptibility testing will help us to differentiate between inducible & constitutive Clindamycin resistance.

This also warrant the need for larger epidemiological AMR surveillances and policy updates to manage S. aureus effectively.

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Conflict of interest: None declared

Ethica lapproval: The study was approved by the Institutional Ethics Committee

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