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Original Research Paper

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A STUDY OF INDUCIBLE CLINDAMYCIN RESISTANCE IN STAPHYLOCOCCUS AUREUS ISOLATED FROM VARIOUS CLINICAL SAMPLES

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ABSTRACT Staphylococcus aureus is one of the most common pyogenic bacteria infecting man. The determination of antimicrobial susceptibility of a clinical isolate is often crucial for optimal antimicrobial therapy of infected patients. This study was done to detect Methicillin resistant staphylococcus aureus (MRSA) isolates from a total of 40 staphylococcus aureus isolates and the study was also done to detect the prevalence of inducible clindamycin resistance among Staphylococcus aureus isolates. Staphylococcal resistance to clindamycin may be inducible (iMLS-inducible Macrolide-lincosamide- streptogramin B resistance) or (cMLS- constitutive Macrolide -lincosamide-Streptogramin B resistance). It is noted that treatment of patients harboring iMLS staphylococci with clindamycin leads to the development of constitutive resistance, subsequently leading to therapeutic failure. A total of 40 staphylococcus aureus isolates were collected from various clinical specimens like pus, blood, wound swab, cervical swab, urine and sputum and processed. Staphylococcus aureus were identified using GP ID card and antibiotic susceptibility testing was done using AST-GP628 card of Vitek-2 compact system. Methicillin resistance was detected using cefoxitin disk of Vitek-2 compact system. Staphylococcus aureus isolates collected, from various clinical specimens, 24 (60%) were methicillin resistant staphylococcus aureus (MRSA) and 16 (40%) were methicillin sensitive staphylococcus aureus (MSSA). Double disk approximation test (D-test) revealed 16(40%) isolates were iMLSB, 10(25%) isolates were cMLSB and 04(10%) isolates were MS phenotype (MSP) and 10(25%) isolates were sensitive to both erythromycin and clindamycin. To prevent treatment failure by inducible resistance, D-test must be performed on erythromycin -resistant and/or clindamycin -sensitive isolates.

KEYWORDS : Antibiotic susceptibility testing, MRSA, MSSA, iMLSB, cMLSB, MSP, D-test.

INTRODUCTION

Staphylococcus aureus is one of the most common pyogenic bacteria infecting man. The determination of antimicrobial susceptibility of a clinical isolate is often crucial for optimal antimicrobial therapy of infected patients[1].Emergence of methicillin resistance in staphylococcus aureus has left us with very few therapeutic alternatives available to treat staphylococcal infections[1].

The Macrolide-lincosamide-streptograminB (MLS) family of antibiotics serves as one such alternative, with clindamycin being the preferred agent due to its excellent pharmacokinetic properties like good oral absorption, excellent tissue penetration and no need for dosage adjustment in presence of renal disease[2].

However, resistance to this drug is again a problem. Staphylococcal resistance to clindamycin may be inducible (iMLS-inducible Macrolide-lincosamide- streptogramin B resistance) or (cMLS- constitutive Macrolide-lincosamide-Streptogramin B resistance).

It is noted that treatment of patients harboring iMLS staphylococci with clindamycin leads to the development of constitutive resistance, subsequently leading to therapeutic failure[3].

OBJECTIVES

- To study the antibiotic susceptibility pattern of isolated staphylococcus aureus.
- To detect the inducible clindamycin resistance by Double disk approximation test (D-test).
- To study the incidence of inducible clindamycin resistance among methicillin resistant
- Staphylococcus aureus and methicillin sensitive staphylococcus aureus.

MATERIALS AND METHODS

The present study was conducted in the department of Microbiology, Basaveshwar Teaching and General Hospital, attached to Mahadevappa Rampure Medical College, kalaburagi, for a period of 2 months i.e., December 2021 to January 2022.

A total of 40 staphylococcus aureus isolates were collected from various clinical specimens like pus, blood, wound swab, cervical swab, urine and sputum and processed.

Specimens were processed within two hours of receipt as per standard procedures and guidelines[4].Staphylococcus aureus identification and antibiotic susceptibility testing was done using the GP ID and AST 628 panel of vitek 2 -compact.

Prepare the inoculum according to the guidelines of the system and load the cassette containing the inoculum at the smart carrier station of vitek-2 compact and the card is linked via barcode.

Once the cassette is loaded, the instrument handles all subsequent steps for incubation and reading.

Methicillin resistant S.aureus detected using cefoxitin disk of vitek -2 compact was noted. The detection of inducible clindamycin resistance (ICR) done in the vitek-2 compact was also noted.

All the staphylococcus aureus isolates were initially screened for erythromycin resistance from the antibiotic susceptibility test performed using AST 628 panel of vitek-2 compact.

The isolates that were found to be erythromycin resistant were further studied for inducible clindamycin resistance by conventional method i.e, double disk approximation test (D-

test). An erythromycin (15μg) disk was placed 15-26mm apart from a clindamycin (2μg) disk on a Mueller Hinton agar uniformly streaked with the culture of S.aureus and the plates were incubated aerobically at 37°C for 16-18 hours. Quality control (QC) of the erythromycin and clindamycin disks was performed with S.aureus ATCC 25923, according to the standard disk diffusion QC procedure. Additional Quality control was performed with separate in-house S.aureus isolates that demonstrated positive and negative D-test reactions.[7]

Four different phenotypes were appreciated as follows.
S.aureus isolates which showed resistance to erythromycin (zone size $\leq$13mm) while being sensitive to clindamycin (zone size$\geq$21mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disk were considered as D test positive i.e inducible MLS_B phenotype. (figure 1)

S.aureus isolates exhibiting resistance to erythromycin (zone size$\leq$13mm), while sensitive to clindamycin (zone size$\geq$21mm) and giving circular zone of inhibition around clindamycin disk was considered as D test negative i.e. MS phenotype.(figure 2)

S.aureus isolates which showed resistance to both erythromycin (zone size $\leq$13mm)and clindamycin(zone size$\leq$14mm) were considered as constitutive MLS_B phenotype.(figure 3)

S.aureus isolates which were sensitive to both erythromycin (zone size $\geq$21mm) and clindamycin (zone size $\geq$21mm) with circular zone of inhibition around both the disks. (figure 4).

The results of D-test were compared and analyzed with the inducible clindamycin resistant (ICR) test of the vitek-2 compact.

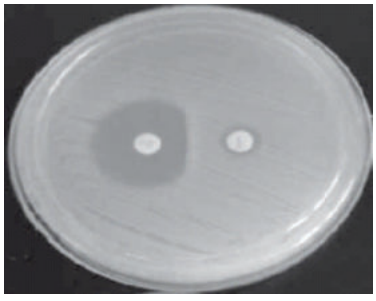


Figure 1: D-test Positive (E-R, CD-S; Inducible MLS_B)

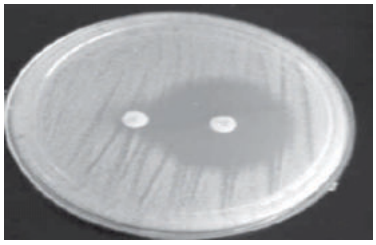


Figure 2: D-test Negative (E-R, CD-S; MS Phenotype)

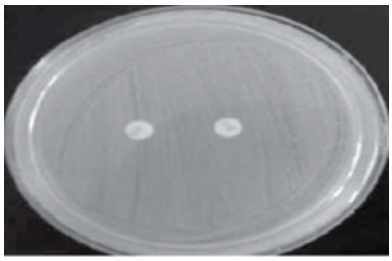


Figure 3: D-test Constitutive MLS_B (E-R, CD-R)

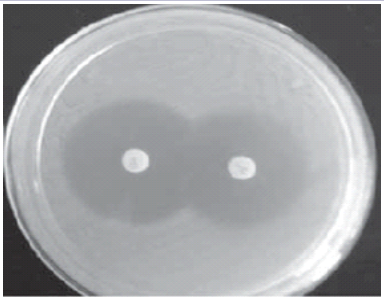
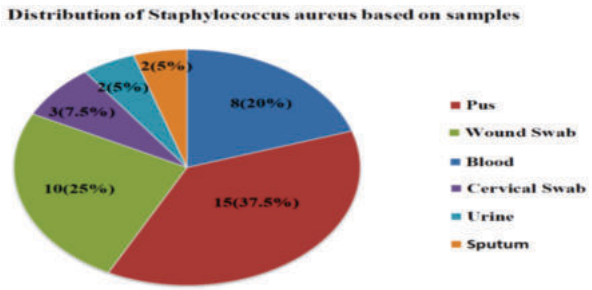


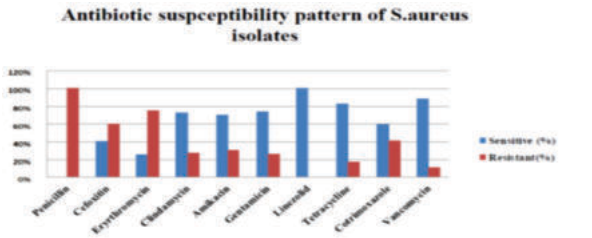
Figure 4: S.aureus sensitive to both Erythromycin and Clindamycin (E-S,CD-S)

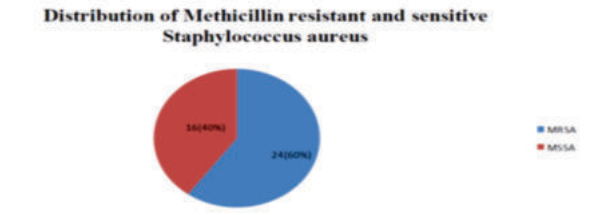
RESULTS

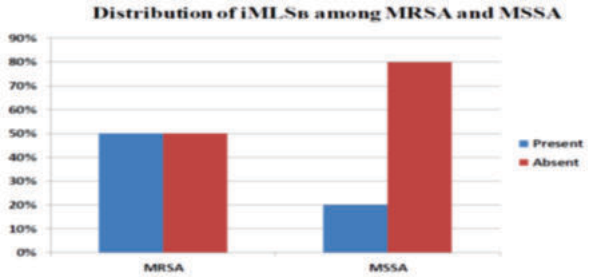
Results were tabulated and analyzed statistically. Of the 40 staphylococcus aureus isolates collected from various clinical specimens, 24 (60%) were methicillin resistant staphylococcus aureus (MRSA) and 16 (40%) were methicillin sensitive staphylococcus aureus (MSSA).

D-test revealed 16(40%) isolates were $iMLS_B$ 10(25%) isolates were $cMLS_B$ and 04 (10%) isolates were MS phenotype (MSP) and 10 (25%) isolates were sensitive to both erythromycin and clindamycin.





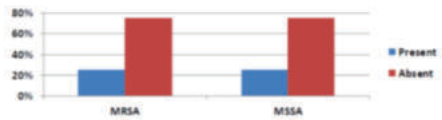




•Among 24 MRSA ,12(50%) were iMLSB positive, 12(50%) were iMLSB negative.

•Among 16 MSSA, 4(20%) were iMLSB positive and 12(80%) were iMLSB negative.

Distribution of cMLSB among MRSA and MSSA



•Among 24 MRSA ,06(25%) were cMLSB positive, 18(75%) were cMLSB negative.
•Among 16 MSSA, 4(25%) were cMLSB positive and 12(75%) were cMLSB negative.

Relation between MRSA, MSSA and MSP

MSP	MRSA	MSSA	TOTAL
Present	3(12.5%)	1(6.25%)	4(10%)
Absent	21(87.5%)	15(93.75%)	36(90%)
Total	24(100%)	16(100%)	40(100%)

•Among 24 MRSA ,03(12.5%) were MSP positive, 21(87.5%) were MSP negative.
•Among 16 MSSA, 1(6.25%) were MSP positive and 15(93.75%) were MSP negative.

DISCUSSION

staphylococcus aureus is the most virulent species of staphylococci encountered[4].Methicillin resistance in staphylococcus was first reported in 1961, and has become a global phenomenon since then[5].The increasing frequency of Methicillin resistant staphylococcus aureus (MRSA) infections and the changing patterns in antimicrobial resistance including multidrug resistance among staphylococcus aureus have led to renewed interest in the use of Macro lide Lincosamide Streptogramin B (MLS_B)antibiotics[6].

Clindamycin is a drug which is useful for treating infections caused by MRSA.However, recent reports indicate that treatment failure may occur in the case of inducible MLS_B ($iMLS_B$)[7].

However if inducible resistance can be reliably detected as a routine basis in clinically significant isolates, clindamycin can be safely and effectively used in patients with true clindamycin susceptible strains[7].

Hence this study was done to detect MRSA isolates from a total of 40 S.aureus isolates and the study was also done to detect the prevalence of inducible clindamycin resistance among S.aureus isolates.

Antibiotic susceptibility pattern of S.aureus isolates.

In the present study, 100% resistance was observed for penicillin which is in correlation with the various studies like Jyoti Kumari *et al* [8]and Qazi MS *et al* [9].

Reports of 100% resistance to penicillin indicate that this drug is no more effective for the treatment of S.aureus infections and should be omitted from the empirical treatment.

In the present study, 70% S.aureus isolates were found to be resistant to erythromycin which correlates with the study of Mahmood *et al* [10] and Tankhiwale *et al* [11].Tsering *et al* [12] reported 95.3% resistance to erythromycin which is contrary to the present study.

Resistance to amikacin was noted in 30% of S.aureus isolates in the present study.similar findings was observed by Kakru *et al* [13] who reported 27% amikacin resistant S.aureus isolates. Where as Kanwal Deep singh Lyall *et al* [14] reported 18.8% resistance to amikacin. In the present study, 26% S.aureus isolates were resistant to Gentamycin.Goel *et a l*[15] also reported resistance of 28%.Higher resistance was observed by Elizabeth *et al* [16]who reported 52% resistance to Gentamycin.

17% of S.aureus strains were tetracycline resistant in the present study.Fule *et al* [17]and Goel *et al* [16] reported 56.9% and 65% tetracycline resistances respectively.

This discrepancy could be attributable to the infrequent use of tetracycline in our hospital. In the present study,41% S.aureus strains were resistant to cotrimoxazole .similar findings was observed by Kakru *et al* [13] and Kanwal Deep singh Lyall *et al* [14] who reported 47% and 35% cotrimoxazole resistant S.aureus isolates.

In the present study, 11% S.aureus strains were resistant to vancomycin. Another study reporting vancomycin resistant strains was kumari *et al* [8] reported 4.6% vancomycin resistant strains.

Fortunately, all s.aureus (both MSSA and MRSA) strains were susceptible to Linezolid, leaving it as the choice of treatment in these cases.several other studies have reported all the staphylococcal isolates being sensitive to Linezolid [8,14,18,19,20]. The resistance to different antimicrobial agents was found to be more among MRSA isolates as compared to MSSA isolates.

Numbers of workers have reported increase in the incidence of MRSA as shown in table No.1

Table 1: Methicillin resistance in S.aureus reported by different workers.

Sl.No	Study Series	Percentage of MRSA
1.	Jyoti Kumari et al(2016)8	30.2%
2.	Farooq S et al(2016)21	61.23%
3.	Saranya Mallamgunta et al(2020)22	43%
4.	Devi Thapa et al(2021)23	50%
5	Present study	60%

our study revealed the prevalence of MRSA at Basaveshwar Teaching and General Hospital to be 60% which is in correlation with the study conducted by Farooq S *et al* (2016)[21].wheras in a study conducted by Jyoti Kumari *et al* (2016)[8],Saranya Mallamgunta *et al* (2020)[22],Devi Thapa *et al* (2021) [23]was 30.2% ,43% and 50% respectively.

Table No.2 various studies across India reporting the prevalence of inducible clindamycin Resistance in S.aureus.

Sl.No	Study Series	Inducible Clindamycin Resistance	
		MRSA (%)	MSSA (%)
1.	Lall et al (2014)24	37.1	6
2.	Swati Tiwari et al (2020)25	76.3	23.7
3.	Saranya Mallam gunta et al (2020)22	16	12
4.	Devi Thapa et al (2021)23	40	34.9
5.	Present study	50	20

Our study reported $iMLS_B$ Phenotypes were found to be higher in MRSA (50%) compared to MSSA Phenotype which were (20%).

This is in correlation with the study of Devi Thapa *et al*(2021)[23] which reported inducible clindamycin resistance in MRSA to be 40% and 34.9% in MSSA ,Lall *et al* [24](37.1% in MRSA and 6% in MSSA) , Saranya Mallamgunta *et al* [22](16% in MRSA and 12% in MSSA), Swati Tiwari *et al* [25] (76.3% in MRSA and 23.7% in MSSA) all of them showed higher percentage of inducible clindamycin resistance in MRSA compared to MSSA Phenotype.

CONCLUSION

Our study which was comprised of a total of 40 staphylococcus aureus isolates from various clinical specimens that were subjected to antibiotic susceptibility test revealed that there is increase in emergence of multidrug resistant staphylococcus aureus in our hospital.

out of 40 staphylococcus aureus isolates 60% were MRSA and 40% were MSSA. Inducible resistance (iMLSB) phenotypes were found to be higher in MRSA isolates compared to MSSA isolates. The prevalence of iMLS may change over time with the emergence of strains with different sensitivity patterns .So periodic surveys should be performed.

The inducible clindamycin test (ICR test) performed by vitek 2 system is reliable in the presence of a positive test. A negative ICR test should be confirmed by CLSI-D test, which is a simple , inexpensive and easy to perform to accurately identify iMLS and true clindamycin susceptible phenotypes which enable us in guiding the clinicians regarding judicious use of clindamycin in staphylococcal infections.

We also recommend that the MIC of vancomycin should be determined and susceptibility should be proven before considering this antibiotic for the treatment of MRSA infections as there is increase in the emergence of vancomycin Resistance Staphyloccus aureus.

Additionally, robust antimicrobial stewardship and strengthened infection control measures are required to prevent the spread and reduce the emergence of antibiotic resistance.

We recommend that whenever clindamycin is intended for treatment of staphylococcal infection, the clinical microbiology laboratory should test the isolated organism for iMLSB by D-test.

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