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RESEARCH ARTICLE

INDUCIBLE CLINDAMYCIN RESISTANCE IN *STAPHYLOCOCCUS AUREUS* AND ITS THERAPEUTIC IMPLICATIONS

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ABSTRACT

Background: *Staphylococcus aureus* is a major cause of hospital-acquired and community-acquired infections worldwide. It causes various types of infections from relatively benign skin infections to life threatening systemic illness. S. aureus can readily develop antibiotic resistance especially with emergence of MRSA strains. The increasing incidence of MRSA has led to the excessive use of Macrolide-Lincosamide-Streptogramin$_B$ (MLS_B) especially clindamycin due to its excellent pharmakinetic properties. Presently, inducible clindamycin resistance is a cause of concern for Clinicians and Microbiologists while treating patients with *S.aureus* infection.

Aim: Hence, the study was undertaken to detect the incidence of inducible clindamycin resistance in clinical isolates of *Staphylococcus aureus* in our tertiary care hospital.

Materials and Methods: 130 clinical isolates of *S.aureus* were studied. Antibiotic susceptibility test was done by Kirby Bauer disc diffusion method. MRSA strains were detected by Cefoxitin (30µg) disc. Inducible Clindamycin resistance was detected by D zone test using erythromycin (15 µg) and Clindamycin (2µg) disc.

Results: 56.2% strains were MRSA. 28.5% *S.aureus* strains produced Inducible Clindamycin resistance and hence were designated as iMLS_B phenotype.

Conclusion: The phenotypic detection of MRSA and Inducible Clindamycin resistance must be done in Clinical Microbiology Laboratory to treat patients effectively with *S.aureus* infection.

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INTRODUCTION

Staphylococcus aureus is a major cause of hospital-acquired and community-acquired infections worldwide. (Lowy FD, 1998, Jevons MP, 1961) It causes various types of infections from relatively benign skin infections to life threatening systemic illness. *Staphylococcus aureus* cause hard-to-treat infections because they can develop resistance to most of the commonly used antibiotics, The emergence of Methicillin resistant *Staphylococcus aureus* (MRSA) in 1961, Vancomycin intermediate *Staphylococcus aureus* (VISA) in 1996 and Vancomycin resistant *Staphylococcus aureus* (VRSA) in 2002, have compounded the problem. The increasing incidence of MRSA has led to the excessive use of Macrolide-Lincosamide-Streptogramin$_B$ (MLS_B) especially clindamycin (Jadhav *et al*., 2011).

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Clindamycin is a semisynthetic derivative of Lincomycin and has excellent pharmakinetic properties. It has excellent tissue penetration except for central nervous system. It has rapid oral absorption and that makes it a good option for outpatient therapy and changeover after intravenous antibiotic therapy (Leclercq, 2002). It does not require dosage adjustment in case of renal impairment and can be used in patients with Penicillin allergy. It is the most efficient antibiotic in treating Staphylococcal skin & soft tissue infections including osteomyelitis. However the increased use of MLS_B antibiotics led to the development of *Staphylococcus aureus* strains with acquired resistance to macrolides (Erythromycin, Azithromycin), lincosamides (Clindamycin, Lincomycin) & Streptogramin$_B$ (MLS_B) antibiotics due to overlapping binding sites in 50S rRNA (Jensen *et al*., 1987). The MLS_B antibiotics are structurally unrelated but they have similar mode of action. They inhibit bacterial protein synthesis by binding to 50S rRNA. (Ciraj *et al*., 2009) Three types of MLS_B resistance can occur i.e. Constitutive (cMLS_B), Inducible (iMLS_B) and MS_B phenotype. In constitutive MLS_B (cMLS_B) resistance, active methylase mRNA is produced in absence of an inducer and

strains show high level cross-resistance to MLS_B drugs. In MS_B phenotype, a fully operational effiux pump that has specificity for 14- and 15- membered macrolides and StreptograminB is responsible for MS_B resistance. In inducible MLS_B ($iMLS_B$) resistance, bacteria produce inactive mRNA which gets activated only in presence of a macrolide (Erythromycin) inducer. Bacterial strains having inducible *erm* gene are resistant to inducer (erythromycin) but appear susceptible to Clindamycin by routine sensitivity test by disc diffusion method leading to therapeutic failure. Accurate antibiotic susceptibility test reports are necessary for effective therapy. Inducible Clindamycin resistance could not be detected by disc diffusion test, broth dilution test, E test or even by automated methods. It can be detected by D test phenotypically (CLSI Guidelines, 2017) and molecular methods by PCR for *erm* gene. The ever changing pattern of antibiotic resistasnce in *Staphylococcus aureus*, has posed a therapeutic challenge to Medical fraternity worldwide Hence, the present study was undertaken to detect the incidence of inducible Clindamycin resistance in clinical isolates of *Staphylococcus aureus* phenotypically in a tertiary care hospital.

MATERIALS AND METHODS

The present study was conducted in Department of Microbiology and was approved by Institutional Ethical Committee (IEC). The study period was 6 months and it was a short term study. The type of study was cross sectional study. 130 *Staphylococcus aureus* strains isolated from clinical samples and characterized by conventional tests (8) only was included in the study. The conventional tests included Gram's staining, catalase test, Hugh-Leifson's oxidative-fermentative test, pigment production, mannitol fermentation test, coagulase test etc. The different clinical samples were received from the Indoor Patient Department (IPD) of our Hospital.

Antibiotic Susceptibility Test: All 130 *Staphylococcus aureus* strains were tested for antibiotic susceptibility test by Kirby Bauer disc diffusion method (Bauer *et al*., 1966) according to Clinical Laboratory Standard Institute (CLSI) Guidelines, 2017. The antibiotic discs used were Penicillin (10 units), Erythromycin (15µg), Clindamycin (2µg), Tetracycline (30µg), Ciprofloxacin (5µg), Gattifloxacin (5µg), Vancomycin (30µg), Linezolid (30µg) etc. For urine sample, additional Nitrofurantoin (300µg) disc was put.

Detection of Methicillin Resistance: It was done by disc diffusion method using Cefoxitin (30µg) disc and zone of inhibition ≤ 21mm (resistant) and ≥ 22 mm (susceptible). Cefoxitin resistance is a surrogate marker of *mec*A gene mediated Methicillin resistance according to CLSI Guidelines, 2017.

Detection of Macrolide Lincosamide and Streptogramin (MLS_B) Resistance

Inducible Clindamycin Resistance was detected by D-test using Erythromycin (15µg) disc & Clindamycin (2 µg) disc keeping them 15mm apart on inoculated Mueller Hinton agar with test strain according to CLSI Guidelines, 2017. A clear D-shaped zone of inhibition around Clindamycin disc facing Erythromycin disc was considered D-test positive. The D^+ phenotype shows blunting of the zone of inhibition but growth of few colonies are present between the edge of zone of inhibition and Clindamycin disc. Both D and D^+ results are considered positive for inducible MLS_B Resistance (Steward *et al*., 2005). Multidrug resistant (MDR) strains were detected as acquired resistance to at least one agent in three or more antimicrobial categories (Magiorakos *et al*., 2012). Statistical analysis was done by calculating percentage and χ^2 (Chi-square) test etc.

OBSERVATIONS AND RESULTS

A total number of 130 *Staphylococcus aureus* strains isolated from different clinical samples and characterized by conventional tests were included in the study.

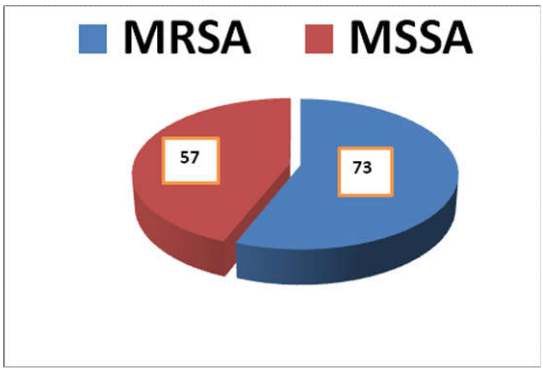


Figure 1. Incidence of MRSA and MSSA strains isolated from clinical specimens (n=130)

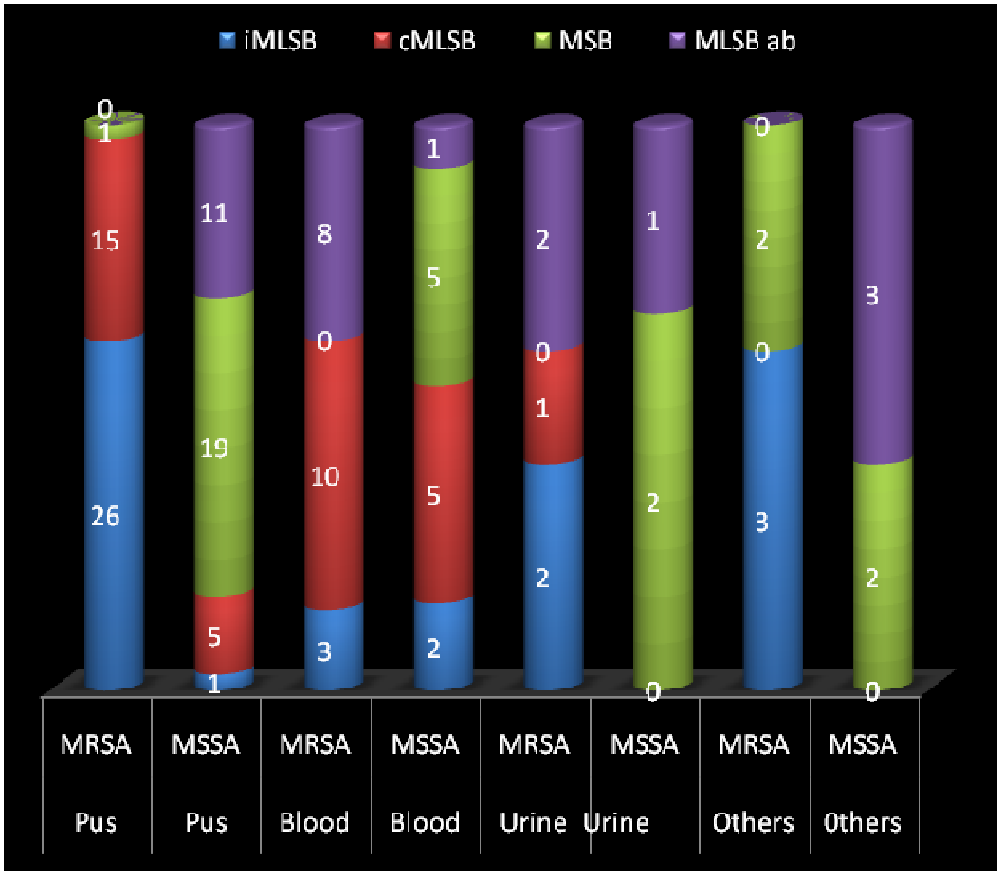
Figure 1 shows the incidence of MRSA and MSSA strains isolated from different clinical specimens. Out of total 130 *Staphylococcus aureus* strains studied, 73 (56.2%) were Methicillin Resistant *Staphylococcus aureus* (MRSA) strains and 57 (43.8%) Methicillin Sensitive *Staphylococcus aureus* (MSSA) strains. All MRSA strains were sensitive to Vancomycin & Linezolid; but resistant to Penicillin.

Photograph1 showing D-zone Test Positive

Table 1 shows the incidence of different types of MLS_B resistance produced by *Staphylococcus aureus* strains isolated from clinical specimens. Out of total 130 *S. aureus* strains studied, 104 (80%) strains produced MLS_B resistance. 26 (20%) strains were negative for MLS_B resistance and out of which 10 (38.5%) strains were MRSA and 16 (61.5%) strains were MSSA. Total 63 (63/104 i.e. 60.6%) strains producing MLS_B phenotypes were MRSA and 41 (41/104 ie. 39.4%) strains producing MLS_B phenotypes were MSSA. 37(28.5%) *Staphylococcus aureus* strains were $iMLS_B$ phenotype, of which 34 (91.9%) strains were MRSA and 3(8.1%) strains were MSSA. Out of 37 $iMLS_B$ phenotype, 31 were D zone

Table 1. Incidence of different types of MLS_B resistance produced by *Staphylococcus aureus* strains studied (n=130)

Staphylococcus aureus (n=130)	$iMLS_B$ phenotype (no.)		$cMLS_B$ phenotype (no.)		MS_B Phenotype (no.)		Negative for MLS_B resistance (no.)	
	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA	MRSA	MSSA
	34	3	26	10	3	28	10	16



Others (10) specimens include: Foley's catheter tip (5), Central line tip (3), Infected tissue (1), Pleural fluid (1)
MLSB ab: indicate MlLSB absent i.e. the strains were negative for MLS_B resistance.

Figure 2. Isolation of different MLS_B phenotypes from different clinical specimens (n=130)

positive and 6were D+ positive. 36 (27.7%) *Staphylococcus aureus* strains belonged to $cMLS_B$ phenotype, of which 26 (72.2%) strains were MRSA and 10 (27.7%) strains were MSSA. Figure 2 shows the isolation of different types of *Staphylococcus aureus* strains from clinical specimens. Out of total 130 *Staphylococcus aureus* strains studied, 78 (60%) strains were isolated from pus and wound swab and 42 (42/78 i.e.53.8%) strains were MRSA. From blood culture, 34 (26.2%) *Staphylococcus aureus* strains were isolated and out of which 21 (21/34 i.e. 61.8%) strains were MRSA. 8(6.2%) *Staphylococcus aureus* strains were isolated from urine specimens and of which 5 (62.5%) strains were MRSA. The single strain isolated from pleural fluid was MRSA. Maximum 26 MRSA strains were $iMLS_B$ phenotype and 15 MRSA strains were $cMLS_B$ phenotype and in both the cases those strains were isolated from pus. It was found that total 37 (28.5%) *Staphylococcus aureus* strains were $iMLS_B$ phenotype and of which 34 (91.9%) were MRSA and 3(8.1%) were MSSA. These 34 MRSA $iMLS_B$ phenotype strains were Multidrug resistant (MDR) strains. Using χ^2 (Chi-square) test, we concluded that null hypothesis was not true and the incidence of $iMLS_B$ phenotype was really more in MRSA strains, compared to MSSA strains.

Out of total 31MS_B phenotype strains only 3(9.7%) were MRSA and 28 (90.3%) were MSSA.

DISCUSSION

It is a cause of great concern about the rapid rise in antibiotic resistance of *Staphylococcus aureus* strains worldwide. In the present study, 100% *Staphylococcus aureus* strains including MRSA strains were resistant to Penicillin which was also reported by Kaur *et al*., 2014. In the present study, 37 (28.5%) *Staphylococcus aureus* strains were inducible clindamycin resistant i.e. $iMLS_B$ phenotype. 34 (26.2%) $iMLS_B$ strains were also MRSA. Our finding correlated well with other workers who have reported 23.6% MRSA strains were $iMLS_B$. (Mallick *et al*., 2009) Similarly, it was found that inducible clindamycin resistant ($iMLS_B$ phenotype) and $cMLS_B$ phenotype are more in MRSA strains than MSSA strains and constituted 26.2% and 20% among MRSA strains respectively compared to 2.3% and 7.7% respectively in MSSA strains. The result is in accordance with few studies (Jadhav *et al*., 2011, Poddar *et al*, 2015, Deotale *et al*., 2010, Prabhu *et al*., 2011). Due to the restricted classes of antibiotics available for treating MRSA infections and known limitations and toxicity of Vancomycin more and

more clinicians are using Clindamycin for serious MRSA infections. If Inducible Clindamycin Resistance is not tested by doing D-test nearly half of the Clindamycin resistant strains would have been missed resulting in therapeutic failure. So presently, for all *Staphylococcus aureus* strains it is necessary to check Inducible Clindamycin Resistance (Mallick *et al*., 2009, Reddy *et al*., 2012). But in the present study, MS_B phenotypes were detected more in MSSA than in MRSA strains i.e. 21.5% were MSSA and only 2.3% were MRSA.

Conclusion

Hence, to conclude, the detection of MRSA, MLS_B resistance especially inducible clindamycin resistance should be done in Clinical Microbiology Laboratory for effective therapeutic outcome for the patients and for implementation of Infection Control Measures to prevent the spread and dissemination of antibiotic resistant *Staphylococcus aureus* and MRSA strains in Health Care Setup.

Scope and Limitations

Scope

The scope of this study is immense as the improper use of antibiotics can be stopped.

Limitations

Molecular studies cannot be included in the study as it is time consuming and require expertise. Moreover longterm multicentric study should be done.

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