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Research Article

# PREVALENCE OF METHICILLIN RESISTANT AND INDUCIBLE CLINDAMYCIN RESISTANT *STAPHYLOCOCCAL* ISOLATES IN A TERTIARY CARE HOSPITAL

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**Abstract-** Background: *Staphylococcal* infections, especially the infections caused by Methicillin Resistant Strains have become a big concern due to its increasing resistance to several other antibiotics. Macrolide–lincosamide streptogramin B (MLSB) antibiotics are used as an alternative to vancomycin to treat such infections. However, widespread use of these antibiotics has also led large no. of *staphylococcus* strains resistant to them. Methods: This study was conducted for a period of a year from January 2017 to December 2017. Phenotypic detection of Methicillin resistance *Staphylococcal* isolates was detected by cefoxitin disc diffusion method and Inducible Clindamycin resistant was detected by erythromycin and clindamycin disc approximation test (D-test). Results: Among the 217 clinical isolates of *Staphylococcus* spp. 90(41.4%) were found to be Methicillin resistant, 28(21.8%) were inducible MLSB phenotype (MLSBi), 78(60.9%) were constitutive MLSB phenotype (MLSBc) and 22(17%) were Macrolide Streptogramin (MS) phenotype. Conclusion: It emphasizes the need of D-test to be performed as a routine test while using clindamycin as an alternative choice to anti-*Staphylococcal* antibiotics in the treatment of Methicillin resistant *Staphylococcal* infections. There is also a need to monitor the antimicrobial susceptibility pattern and judicious use of antibiotics to reduce the incidence of these infections.

**Keywords**- *Methicillin Resistant, MLSBc, MLSBi, Staphylococcus., Phenotype*

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## Introduction

Methicillin is a derivative of the penicillin group and resistant to penicillinase. It is the first choice for the treatment of *Staphylococcal* infections. Biomarker gene mecA, which is responsible for methicillin resistance, contains genes encoding resistance to several other antimicrobial drugs [1]. The ever-increasing reports showing resistance to Methicillin has led to implement the alternative usage of MLSB antibiotics to treat *Staphylococcal* infections with clindamycin being the preferred agent due to its excellent pharmacokinetic properties. However, widespread use of MLSB antibiotics has led to an increase in the number of *Staphylococcal* strains acquiring resistance to them [2]. Clindamycin resistance in *Staphylococcus* species can be either constitutive or inducible [3]. The most common mechanism for such resistance is target site modification mediated by erm genes, which can be expressed either constitutively (constitutive MLSB phenotype) or inducibly (inducible MLSB phenotype) [4]. 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 [4]. The present study was done to detect the prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA), Methicillin resistance coagulase negative *Staphylococcus* (MRCONS) and the prevalence of inducible clindamycin resistance among *Staphylococcal* spp. isolated in a tertiary care hospital.

## Material and methods

A cross sectional study was carried out in the Department of Microbiology, Gujarat Adani Institute of Medical Sciences, Bhuj, for a period of a year *i.e*., from January 2017 to December 2017. All the *Staphylococcus* spp. isolated in routine culture and sensitivity from various clinical specimens and which were non duplicate was included in the study. The isolated microorganisms were identified by standard conventional methods (colony morphology, gram staining, catalase test and coagulase test) [5]. Antimicrobial susceptibility test was done by using Kirby Bauer disk diffusion method as per guidelines from Clinical and Laboratory Standard Institute (CLSI) [6]. Methicillin resistance strains were detected by using cefoxitin disc diffusion method. All strains were tested with 30-µg cefoxitin discs (Hi-Media) on Mueller-Hinton agar (MHA) plates. The zone of inhibition was determined after 16-18 h incubation at 35°C. Zone size was interpreted according to CLSI [6] criteria: Susceptible: ≥ 22 mm; Resistant: ≤ 21 mm. Detection of inducible clindamycin was tested by D-test as per CLSI guidelines [6], briefly, all the isolates were tested with 15-µg erythromycin and 2-µg clindamycin disks spaced 15-26 mm apart on MHA plates.

**Three different phenotypes were interpreted as**:

- Inducible resistance phenotypes: resistant to erythromycin and having clindamycin zone ≥21 mm with a D-shaped zone.
- Constitutive resistance phenotypes: resistant to both erythromycin and clindamycin.
- MS phenotype: isolates resistant to erythromycin and susceptible to clindamycin without D-zone [7].
- The quality control of antibiotic discs used was performed with S. aureus ATCC 25923.

## Results

A total of 217 *Staphylococci* spp. were isolated from different clinical specimens such as pus, urine, blood, wound swab etc. Among the 217 *Staphylococcal* isolates, 86 (39.6%) were *Staphylococcus aureus* and 131(60.3%) were Coagulase negative *Staphylococci*.

Table-1 *D-test result analysis for different Staphylococcal spp*.

| Phenotypes | MRSA(n =23) | MSSA(n=63) | MRCONS(n=67) | MSCONS(n=64) |
|---|---|---|---|---|
| ER-R,CL-R (MLSBc) | 12(9.7%) | 7(5.6%) | 39 (31.7%) | 20(16.2%) |
| ER-R, CL-S (MLSBi), D-test: +ve | 7 (5.4%) | 4(3.1%) | 11(8.5%) | 6(4.6%) |
| ER-R,CL-S (MS), D-test: -ve | 2(1.6%) | 3(2.4%) | 7(5.6%) | 10(8.1%) |

*ER = Erythromycin, CL = Clindamycin, R = Resistant, S = Sensitive*

Methicillin resistant strains were observed in 90 (41.4%) out of which 23(25.5%) of them are MRSA and 67(74.4%) were MRCONS. Methicillin sensitive *Staphylococcus aureus* (MSSA) was found in 63(29%) strains and 64(29.4%) were Methicillin sensitive Coagulase negative *Staphylococci* (MSCONS). Out of all the *Staphylococcal* isolates 128(58.9%) were erythromycin resistant strains. D-Test result analysis have shown in Table no.1,78(60.9%) were MLSBc phenotype *i.e*., resistant to both erythromycin and clindamycin, 28(21.8%) were MLSBi phenotype *i.e*., resistance to erythromycin and clindamycin sensitive (D-Test +ve) and 22(17%) were MS phenotype *i.e*., resistance to erythromycin and clindamycin sensitive (D-test-ve). Based on the D-Test 21.8% erythromycin resistant *Staphylococcal* isolates were MLSBi and reported as Clindamycin resistant which otherwise would not have been detected in routine disk diffusion test.

## Discussion

The overall burden of *Staphylococcal* disease particularly that caused by methicillin resistant *S. aureus* strains (MRSA), is increasing in many countries in both healthcare and community settings [8-13]. In Indian scenario, Verma *et. al*., has reported a rapid increase in MRSA prevalence, from 12% to 80.89%, over seven years, in a Tertiary Care Centre at Indore [14]. As per current Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group's report, the prevalence of MRSA varies from 22% to 68% in Indian hospitals [15]. In our study, 23(26.7%) of all *S. aureus* infections are caused by MRSA. Susceptibility test profiles revealed a higher level of resistance to commonly prescribed antimicrobial agents among MRSA. All isolates were sensitive to vancomycin and linezolid. These results were comparable to studies carried out by Rajaduraimandi and his colleagues in the clinical samples from various hospitals and laboratory in Southern India [16]. Besides *S. aureus*, the Coagulase-Negative *Staphylococci* (CONS) are now recognized as important causes of human infection and are the most frequently isolated bacteria in the clinical microbiology laboratory [17]. The prevalence of MRCONS (51.1%) in our study is lower than the other studies done by Singhal R. *et. al*., (72.3%)[18] and Jain, Agarwal and Bansal (66.0%)[19], from India, however the prevalence rate of our study is higher than the study reported by Rashmi and Mahantesh,40% [20]. Clindamycin is effective against both the methicillin resistant and the methicillin sensitive *Staphylococcal* infections. The increased frequency of the *Staphylococcal* infections, along with the changing drug susceptibility patterns, have led to a renewed interest in the CL usage, but the possibility of an inducible resistance to CL remains a major concern and this could limit the use of this drug. The frequency of MLSBi ranges from 7% -94% [21, 22]. The present study reveals that inducible clindamycin resistant was seen in 30.4%, 6.3%, 16.4%, and 9.3% of the MRSA, MSSA, MRCONS and MSCONS. The findings of our study were comparable with reports by other authors. Baragundi Mahesh C. *et. al*. has reported MLSBi in 24.4%,12.04% MSSA, 16.39% MRCONS and 3.2% MSCONS [23]. Similarly, a study done by Yilmaz G. *et. al*., demonstrated MLSBi in 24.4%, 14.8% MSSA, 25.7% MRCONS and 19.9% MSCONS [24].

## Conclusion

*Staphylococcal* infection is one of the leading global issues especially the infections caused by the antibiotic resistant strains of *staphylococcus* spp. Since these are resistant to the commonly used antibiotics, there is a need for the development and enforcement of infection control policies in all the hospital set up. The prevalence of MLSBi and MLSBc also limits the therapeutic options for methicillin resistant strains to antibiotics like lincosamide and vancomycin. So, monitoring the antimicrobial susceptibility pattern, judicious use of antibiotics and surveillance can reduce the incidence of these infections.

**Application of research**: By the detection of various antibiotic resistant strains and their antibiotic sensitivity pattern it can be a helping hand to the physicians for proper treatment and effective steps can be taken to curtail this resistant pathogens.

**Research Category**: Medical microbiology

**Abbreviations**:
MLSB: Macrolide–lincosamide streptogramin B
D-test: disc approximation test
MLSBi: inducible MLSB
MLSBc: constitutive MLSB
MS: Macrolide Streptogramin
MRSA: Methicillin resistant *Staphylococcus aureus*
*S. aureus*: *Staphylococcus aureus*
MRCONS: Methicillin resistance coagulase negative *Staphylococcus*
CONS: Coagulase negative *Staphylococci*
MHA: Mueller–Hinton agar
CLSI: Clinical and Laboratory Standard Institute
MSSA: Methicillin sensitive *Staphylococcus aureus*
MSCONS: Methicillin sensitive Coagulase negative *Staphylococci*
ER: Erythromycin, CL: Clindamycin, R: Resistant, S: Sensitive

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