

## Detection of inducible clindamycin resistance in staphylococcus aureus isolated from different clinical samples

Komal Kadam<sup>1</sup>, Snehal Patil<sup>1</sup>, Raveendra D. Totad<sup>2</sup>, Sidramappa R. Warad<sup>3\*</sup> and Prasanna Nakate<sup>1</sup>

<sup>1</sup>Department of Microbiology, B.K.L. Walawalkar Rural Medical College & Hospital, Kasarwadi, Sawarde, Kasarwadi-415606, Maharashtra, India, <sup>2</sup>Department of Microbiology, Al Ameen Medical College & Hospital, Athani Road, Vijayapur-586108, Karnataka, India and <sup>3</sup>Department of Dermatology, Al Ameen Medical College & Hospital, Athani Road, Vijayapur-586108, Karnataka, India

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**Abstract:** *Background:* Staphylococcus aureus is one of the most common causes of nosocomial and community-acquired infections in all parts of the world. Prevalence of methicillin resistance in staphylococci has increased and become a serious concern worldwide. Clindamycin resistance is on the rise among clinically important staphylococcal isolates. *Objectives:* Identification and antimicrobial susceptibility testing of Staphylococcus aureus isolates from different clinical samples by using Standard Microbiological procedures as per CLSI guidelines. Detection of Methicillin resistant Staphylococcus aureus was carried out by using Cefoxitin screening test. Inducible clindamycin resistance among clinical isolates of Staphylococcus aureus was tested by using D-test and Vitek -2 compact automated system. *Methods:* Total 107 Staphylococcus aureus isolates from different clinical specimens were processed in the microbiology department at B.K.L. Walawalkar rural medical college. All samples were tested for Cefoxitin screening test and detection of Inducible clindamycin resistance was carried out by using D test and Vitek 2 panel automated system. *Results:* Out of 107 samples of Staphylococcus aureus isolates, cefoxitin screening test result showed 19(17.75%) positive result (MRSA). Percentages of inducible clindamycin resistance were higher amongst MRSA as compared to MSSA. The study revealed 32 (29.90 %) isolates are inducible clindamycin resistance detection result were positive (are Macrolides/ Lincosamides/ Streptogramins MLSB phenotype inducible) by Vitek 2 automated system. *Conclusion:* Detection of inducible clindamycin resistance should be included in the routine antimicrobial susceptibility testing, as it will help in guiding the empirical therapy.

**Keywords:** Methicillin Resistant Staphylococcus Aureus, Cefoxitin Screening Test, Inducible Clindamycin Resistance.

### Introduction

Staphylococcus aureus is one of the most common causes of nosocomial and community-acquired infections in all parts of the world. Prevalence of methicillin resistance in staphylococci has increased and become a serious concern worldwide [1].

It is the leading cause of skin and soft tissue infections such as abscesses, boil and cellulitis. It also causes pneumonia and bone and joint infections. Many common skin infections caused by Staphylococcus aureus are cured within a few weeks. Some severe S. aureus infections usually require hospitalization and intravenous

administration of antibiotics. Staphylococcus aureus most commonly infects others through contaminated hands [2]. With new interest in the use of Macrolide Lincosamide Streptogramins B (MLSB) antibiotics to treat Staphylococcus aureus infections, clindamycin is the preferred drug due to its excellent pharmacokinetic properties [3-4]. However, widespread use of MLSB antibiotics has increased the number of staphylococcal strains that creates resistance to MLSB antibiotics [5].

Clindamycin is an alternative to Staphylococcus aureus infections in penicillin intolerance or methicillin resistance.

Clindamycin is available in oral formulations and has good bioavailability. This medicine is prominently distributed in body fluids, organs and tissues including bone. In recent years, community - acquired methicillin - resistant *Staphylococcus aureus* (CAMRSA), which has rapidly emerged as a cause of skin and soft tissue infections, is often sensitive to multiple antibiotics, including clindamycin. Clindamycin has been shown to inhibit the production of toxins and virulence factors in Gram-positive bacteria by inhibiting protein synthesis. Clindamycin has excellent tissue permeability, effective in abscesses, not effective for the central nervous system and does not require renal adaptation [6].

Clindamycin is used as treatment of choice in case of erythromycin-resistant *Staphylococcus aureus*, causing skin and soft tissue infections. MLSB resistance can be either constitutive (cMLSB) or inducible (iMLSB). *Staphylococci* isolates with constitutive resistance are resistant to both erythromycin and clindamycin, while inducible resistance isolates are resistant to erythromycin but look vulnerable to clindamycin [7]. In the age of automation, the Vitek 2 system provides a panel for detection of inducible clindamycin resistance in combination with other antimicrobial susceptibility tests [8].

### Material and Methods

This study was carried out in the Department of Microbiology at tertiary care centre, Chiplun, Maharashtra during the period of September 2018 to September 2021. Clinical specimens such as pus, blood, urine and wound swab, etc were collected aseptically from suspected patients and processed in the microbiology laboratory with minimal delay. A total of 107 samples from patients of wound infections were collected and processed according to the standard laboratory guidelines.

#### *Inclusion Criteria:*

- Admitted patients with *Staphylococcus aureus* infection, admitted during the period of study.
- *Staphylococcus aureus* isolated from blood, urine and other body fluids.
- Repeated isolation of the same strain of *Staphylococcus aureus* from clinically significant patient.

#### *Exclusion Criteria:*

- Patients with colonisation of *Staphylococcus aureus* with no apparent clinical infection.
- When patients clinical isolate is not attributable to patients' clinical conditions.
- Isolates from improperly collected samples.

All the samples were inoculated on Blood agar, MacConkey agar and Chocolate agar. Bacterial species is identified by using Gram stain morphology, Motility, Catalase test and Coagulase test and Vitek 2 compact automated system.

All the samples were processed in biosafety Cabinet. Sample Preparation Bacterial suspensions were prepared. A sterile swab is used to transfer a sufficient number of colonies (3) of a pure culture and to suspend the microorganism in 3.0 mL of sterile saline (0.45% NaCl) in a clear plastic test tube. Mix suspension use Vortex until have homogenous suspension. Put the tube in DensiCHEK plus then rotate 3600 after that instrument read density acceptable range 0.50-0.63 for bacteria. The turbidity is adjusted accordingly and measured by DensiChek™. If the turbidity is less than range add more bacterial colony. If turbidity is more than range add more saline 0.45%. ID and AST pair setup Tube fixed pipetting (blue for gram +ve) From suspension take 280 microliters from gram+ve. Add the above volume to 3 ml of saline Vitek Tube Compact Cassette. The test suspension tube is placed into the cassette with Reagent card. Sealed test kits are moved into the reader/incubator automatically. Susceptibility Testing (AST) Gram positive antimicrobial susceptibility testing (AST 628). Detection of inducible clindamycin resistance was detected by Vitek 2 panel and All samples were also tested for Cefoxitin screening test for MRSA.

In case of testing an iMLSB resistance strain according to Clinical and Laboratory Standards Institute (CLSI) methods with erythromycin (15 µg) disc was placed at a distance of 15 mm (edge to edge) from

clindamycin (2 µg) disc on a Mueller-Hinton agar plate, previously inoculated with 0.5 McFarland standard bacterial suspensions. After incubation the zone of inhibition around the clindamycin disk is flattened to form a “D” shape (positive D-test), but in the case of MS phenotype, the clindamycin zone remains circular [9].

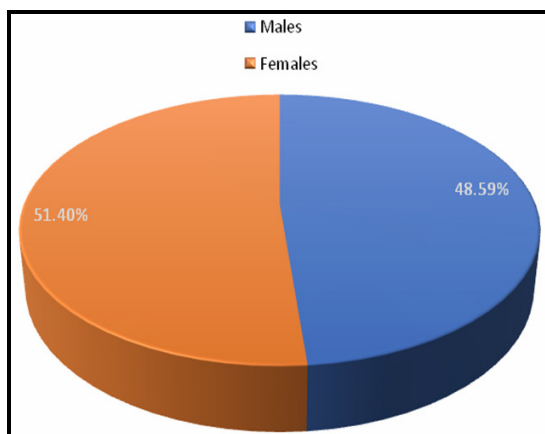
### Results

The present study included 107 Staphylococcus aureus isolates from different clinical specimens obtained from patients with pyogenic wound infections, Respiratory tract infections, Urinary tract infections, Ear infection were included in our study.

**Table-1: Distribution of Staphylococcus aureus isolates from different Clinical samples**

Sr. No.	Samples	No. of samples	Percentage
1	Pus / Wound Swab	76	69.15 %
2	Blood	7	8.41 %
3	Fluid (aspirates from wound)	7	6.54 %
4	Urine	4	4.67 %
5	Tissue	4	3.74 %
6	Sputum	3	2.80 %
7	Ear Discharge	2	1.86 %
8	Biopsy	1	0.93 %
9	Synovial fluid	1	0.93 %
10	Bone Marrow	1	0.93 %
	Total	107	100%

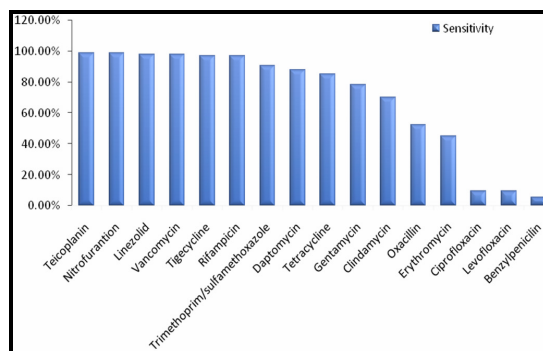
**Graph-1:** Gender wise Distribution of Staphylococcus aureus isolates.



Out of 107 Staphylococcus aureus isolates, frequency of Pus samples was found to 69.5% followed by Blood (8.41%), Fluid (6.54%), Urine (4.67%), Tissue (3.74%), Sputum (2.80%), Ear Discharge (1.86%), Biopsy (0.93%), Synovial fluid (0.93%) and Bone Marrow (0.93%) (Table-1)

Out of 107 isolates, 52 (48.59%) were from male’s patients and 55(51.40%) were from female’s patients (Graph-1).

**Graph 2:** Antimicrobialsusceptibility pattern of staphylococcus aureus isolates from different clinical samples



Antimicrobial Susceptibility pattern of Staphylococcus aureus shows 99.06% of isolates were sensitive to Teicoplanin, followed by Nitrofurantoin (99.06%), Linezolid (98.13%), Vancomycin (98.13%) and Tigecycline (97.19%), Rifampicin (97.19%), Trimethoprim/ sulfamethoxazole (90.65%), Daptomycin (87.85%), Tetracycline (85.04%), Gentamycin (78.5%) Clindamycin (70.09%) (Graph-2).

### Discussion

Staphylococcus aureus is an important human pathogen that causes a variety of infections in both nosocomial and community infections. Gram-positive pathogens are armed with many virulence factors that make it easier for the infection to settle in the host. This organism is known for its ability to develop resistance to various classes of antibiotics [9].

Clinical specimens such as pus, sputum, blood, urine and wound swab, tissue, ear discharge, biopsy, synovial fluid were collected aseptically from suspected patients with pyogenic wound infections, respiratory

tract infection, urinary tract infections and processed in the microbiology laboratory with minimal delay.

A total of 107 samples were included and processed according to standard microbiological procedures and antimicrobial sensitivity testing was carried out as per CLSI guidelines by using Vitek 2 automated system. Out of 107 Staphylococcus aureus isolates, frequency of Pus samples was found to be 76 (69.5%) followed by Blood 7(8.41%), Fluid 7(6.54%), Urine 4 (4.67%), Tissue 4 (3.74%), Sputum 3 (2.80%), Ear Discharge 2 (1.86%), Biopsy 1(0.93%), Synovial fluid 1(0.93) and Bone Marrow 1 (0.93%) [Table 1]. In our study incidence of Staphylococcus aureus isolates infection was more in female patients 55 (51.40 %) as compared to male patients 52 (48.59 %) [Graph1].

In present study, antimicrobial susceptibility pattern of Staphylococcus aureus shows 99.06% of isolates were sensitive to Teicoplanin, followed by Nitrofurantoin (99.06%), Linezolid (98.13%), Vancomycin (98.13%) and Tigecycline (97.19%), Rifampicin (97.19%), Trimethoprim/sulfamethoxazole (90.65%), Daptomycin (87.85%), Tetracycline (85.04%), Gentamycin (78.5%) and Benzylpenicilin (5.6%) [Graph 2]. In present study, antibiotic resistance pattern of Staphylococcus aureus was found against Oxacillin (47.66%) followed by Erythromycin (53.27%), Ciprofloxacin (90.66%) and Levofloxacin (90.66%). Out of 107 samples of Staphylococcus aureus isolates, cefoxitin screening test result shows 19(17.75%) positive result (MRSA), remaining 13 (12.14%) isolates are cefoxitin screening test result negative (MSSA).

Clindamycin, a lincosamide, in a staphylococcal infection is very effective due to its low cost, proven efficacy, availability of oral and parenteral forms, good accumulation in abscesses, excellent tissue penetration. It has been a long-term option for treating staphylococcal skin, soft tissue, and bone infections. No adjustment of renal dose is required. It is effective against both methicillin-resistant Staphylococcus aureus infections and methicillin-sensitive staphylococcal infections. It also directly inhibits the production of

staphylococcal toxins and is a useful if the patient is allergic to penicillin [1].

**Table-2: Association of Inducible clindamycin resistance and methicillin resistance among S. aureus isolates by using D test**

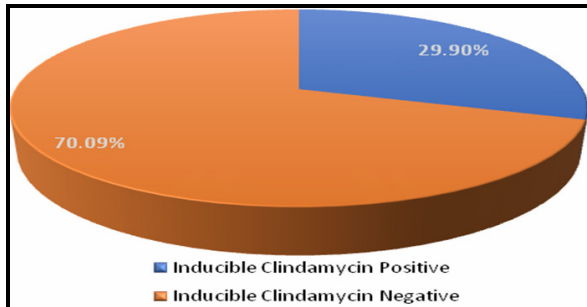
Susceptibility pattern (Phenotype)	MRSA	Percentage
Erythromycin –S, Clindamycin –S	6	31.57%
Erythromycin –R, Clindamycin –R (Constitutive MLSB)	4	21.5%
Erythromycin –R, Clindamycin –S (D positive, inducible MLSB)	6	31.57%
Erythromycin –R, Clindamycin –S (D negative, MS phenotype)	3	15.78%
Total	19	100%

Its excellent oral absorption makes it an important option for outpatient treatment or follow-up after intravenous (IV) therapy (de-escalation) [10]. Clindamycin has been stored as a last resort and is recommended antibiotic for severe MRSA infections, after getting antibiotic susceptibility report. In this study association of MRSA and Inducible clindamycin results by using D test showed 21.5% isolates were cMLSB phenotype, 31.57% were iMLSB phenotype and 15.78% were MS phenotype [Table 2]. Our study is in agreement with study done by Shidiki A et al [11]. In present study we observed that percentages of inducible clindamycin resistance were higher amongst MRSA as compared to MSSA. This finding correlates with study done by Pratibha S, et al [12]. Our study is in agreement with study done by V. Deotale et al. in which inducible clindamycin resistance were higher amongst MRSA as compared to MSSA [4].

Our study is not in agreement with study done by Malikaarjun koppad, et al.in which inducible clindamycin resistance were higher amongst MSSA as compared to MRSA [13]. Out of 107 samples,32 (29.90 %) isolates were inducible clindamycin resistance

positive (are Macrolides/ Lincosamides/ Streptogramins MLSB phenotype inducible) by using Vitek 2 automated system [Graph 3].

**Graph-3:** Distribution of inducible clindamycin resistance in *Staphylococcus aureus* by Vitek 2 automated system



Because of restricted antibiotics for the treatment of MRSA infections and limitations of vancomycin, clindamycin will be considered for serious skin and soft tissue infections in patients with MRSA infection in which clindamycin is sensitive [14]. The Clinical Laboratory Standards Institute (CLSI) recommends testing staphylococcal isolates for inducible clindamycin resistance (ICR) on a regular basis. Vitek 2 is an automated system with a panel that detects

inducible clindamycin resistance. It is simple and less time-consuming than the conventional CLSI reference procedures. Vitek-2 is considered a potentially reliable test method for bacterial identification and antimicrobial testing including inducible clindamycin resistance [15].

### Conclusion

Out of 107 clinical isolates, 76 (69.5%) *Staphylococcus aureus* isolates were from pyogenic wound infections. Percentage of inducible clindamycin resistance were higher amongst MRSA. Detection of inducible clindamycin resistance is a simple, effective and important method that needs to be used on a daily basis as it guides clinicians for empirical treatment for and avoid possible clinical failures. Clindamycin is stored as a last resort and is usually recommended for severe MRSA infections, depending on the outcome of antibiotic susceptibility. Recommendation of the study is to do regular testing for inducible clindamycin resistance in individual settings for the guidance of optimal treatment and prevention of treatment failure.

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\*All correspondences to: Dr. Sidramappa R. Warad, Assistant Professor, Department of Dermatology, Al Ameen Medical College & Hospital, Athani Road, Vijayapur-586108, Karnataka, India. E-mail: drsidram@gmail.com