ORIGINAL ARTICLE e-ISSN: 2349-0659 p-ISSN; 2350-0964

Detection and Prevalence of Inducible Clindamycin Resistance in Clinical Isolates of *Staphylococcus aureus*: Experience from Tertiary Care Hospital in Jaipur

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Abstract

Background: *Staphylococcus aureus* is a frequent cause of bacterial infections in both developed and developing countries. Emerging resistance to methicillin in this organism has left us with very few therapeutic alternatives to treat the infections caused by them. **Objective:** We aimed to determine the prevalence of inducible clindamycin resistance in clinical isolates of *S. aureus* and antimicrobial susceptibility pattern of *S. aureus* isolates due to the increasing prevalence of resistance to most antimicrobial agents in staphylococci signifies the need for new effective agents to treat staphylococcal infections. **Methods:** The study was carried out in the Department of Microbiology at Mahatma Gandhi Hospital, Sitapura, Jaipur, Rajasthan. All *S. aureus* isolates (non-repetitive) from different clinical samples received in clinical microbiology laboratory from in and outpatients in Mahatma Gandhi Medical College and Hospital, Jaipur, Rajasthan, were included in the study. **Results:** Out of 157 erythromycin-resistant *S. aureus*, 74 (47.133%) show MS phenotype, 48 (30.57%) show inducible clindamycin resistance, and 35 (22.29%) show constitutive resistance. All 48 *S. aureus* isolates which showed inducible clindamycin resistances (D-test positive) were further subjected to antimicrobial susceptibility testing.In the present study, 100% sensitivity was observed by vancomycin, linezolid and tigecycline, followed by tetracycline, 89.58% and gentamicin, 83.33% while 100% resistivity were observed by levofloxacin. **Conclusion:** We can conclude that there is high percentage of inducible clindamycin resistance among *Staphylococcus* isolates. If D-test would not have been performed, many inducible clindamycin-resistant *S. aureus* could have been easily misidentified as clindamycin susceptible, leading to therapeutic failure. Thus, simple and reliable D-test can be incorporated into routine in clinical microbiology laboratory.

Keywords: Clindamycin, D-test, Methicillin-resistant *Staphylococcus aureus*, *Staphylococcus*, Susceptibility

Asian Pac. J. Health Sci., (2022); DOI: 10.21276/apjhs.2022.9.3.20

Introduction

Staphylococcus aureus is one among the most common pyogenic bacteria infecting man, causing both hospital- and community-acquired infections.[1] *S. aureus* is a frequent cause of bacterial infections in both developed and developing countries. Emerging resistance to methicillin in this organism has left us with very few therapeutic alternatives to treat the infections caused by them. Clindamycin in macrolide-lincosamide-streptogramin B (MLSB) family of antibiotics serves as one such alternative for treating both methicillin-susceptible *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) infections, due to its excellent pharmacokinetic properties.[2] However, widespread use of this antibiotic has led to a large number of staphylococcal strains resistant to it.[3] Resistance to MLSB antibiotics occurs by many different mechanisms. The most common mechanism for such resistance is target site modification mediated by erm genes, which can be expressed either constitutively ($cMLS_B$ phenotype) or inducible ($iMLS_B$ phenotype). The erm gene codes for methylase enzyme which methylates and alters the target site of MLSB antibiotics, that is, the 23S ribosomal RNA.[4] It is very difficult to detect the inducible clindamycin resistance 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, that is, efflux of antibiotic, staphylococcal isolates appear erythromycin resistant and clindamycin sensitive both *in vivo* and *in vitro* and the strain does not typically become clindamycin resistant during therapy.[5] Thus to avoid clinical therapeutic failure in the resistance case mediated by erm gene, it is very important to detect inducible clindamycin resistance phenotypes *in vitro* which can be made by erythromycin-clindamycin disc approximation test (D-test).[6]

The increasing prevalence of resistance to most antimicrobial agents in staphylococci signifies the need for new effective agents to treat staphylococcal infections. Therefore, all erythromycin-resistant *S. aureus* should be tested for inducible clindamycin resistance to prevent clindamycin treatment failures and to report prevalent resistant phenotypes, which vary widely. The aim of the present research work is to find out the prevalence of inducible clindamycin resistance in *S. aureus* isolates and to analyze the antimicrobial susceptibility pattern of *S. aureus* isolates.

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How to cite this article: Shrigaur A, Mamoria VP, Rajni E, Sharma R. Detection and Prevalence of Inducible Clindamycin Resistance in Clinical Isolates of *Staphylococcus aureus*: Experience from Tertiary Care Hospital in Jaipur. Asian Pac. J. Health Sci., 2022;9(3):96-101.

Source of support: Nil

Conflicts of interest: None

Received: 19/02/2022 **Revised:** 07/03/2022 **Accepted:** 14/03/2022

Materials and Methods

Collection of Specimen

Urine, pus, discharge from skin and soft-tissue infection, sputum, ear swab, throat swab, and blood were received in laboratory between June 2019 and May 2020 for bacterial culture and sensitivity from various outdoor patient departments (OPDs) and indoor patient departments (IPDs) wards of Mahatma Gandhi Hospital (MGH), Sitapura, Jaipur, Rajasthan. The specimens were then transferred to the laboratory as quickly as possible, usually within 1 h after collection and processed as soon as possible. When the processing was delayed, they were stored at 4°C.

Processing of Specimen

Direct microscopy of all specimens were done except urine. Then, all the specimens were plated on nutrient agar, blood agar plate and Mannitol Salt Agar were incubated for 24 h at 37°C aerobically. After 24 h of incubation, *S. aureus* isolates were identified on the basis of colony characteristics, Gram's staining, and biochemical reaction catalase and coagulase (slide and tube coagulase).

Ethics Approval

To carry out the research, necessary institutional permissions were obtained from the relevant units, and the ethics committee permission was obtained from the ethics committee. This study was approved by the Institutional Ethics Committee, Mahatma Gandhi Medical College and Hospital, Jaipur, held on August 31, 2019, and approval number MGMCH/IEC/JPR/2019.

Disk Diffusion Test

Disk diffusion (Kirby–Bauer disk diffusion) method for antimicrobial susceptibility test[5]

Modified Kirby–Bauer disk diffusion test method is a reference method which could be used as a routine technique to test the sensitivity of the isolate in the clinical laboratory.

E-Test (Epsilometer Test)

The E-test gradient technology is based on a combination of the concepts of dilution and diffusion principles for susceptibility testing.

Inoculum Preparation

Emulsify several well-isolated colonies of MRSA from a pure overnight culture into a suitable suspension medium. Fastidious organisms should be suspended in broth and used within 15 min. Compare the turbidity to the appropriate 0.5 McFarland standard.

Inoculation

Dip a sterile cotton swab to the inoculum suspension and press against the inside wall of the tube to remove excess fluid carefully streak the entire agar surface evenly in three directions. Allow excess moisture to be fully absorbed and ensure that the surface is completely dry before applying E-test strips. When the inoculum and inoculation are optimal and even confluent growth will be obtained.

Interpretation

MIC breakpoint for defining susceptibility categories as provided could be used for interpreting E-test minimum inhibitory concentration (MIC) values. E-test strips of vancomycin which is one of the glycopeptides are used to check the susceptibility. MIC values of *S. aureus* are interpreted as S (Susceptible), I (Intermediate), or R (Resistant) by comparing the breakpoint values of vancomycin antibiotic, as susceptible (≤2), intermediate (4–8), and resistant (≥16), respectively.[5]

D-Test (Disk Approximation Test)

The strains of *S. aureus* that are erythromycin resistant but clindamycin sensitive were further subjected to D-test (Disk Approximation Test) by inoculating 0.5 McFarland bacterial suspensions on the Mueller-Hinton agar plates with the help of sterile swabs and placing the erythromycin (E – 15 μg) and clindamycin (CD – 2 μg) disks side by side with edge-to-edge distance of 15 mm. Plates were analyzed after 18 h of incubation at 35°C. Standard disk diffusion procedure for inoculum preparation was followed. Inducible resistance to clindamycin will be defined as blunting of the clear circular area of no growth around the clindamycin disk on the side adjacent to the erythromycin disk and was designated D-test positive. Absence of a blunted zone of inhibition will be designated as D-test negative.[5]

Results

The study was conducted at the department of microbiology, during the period 1 year from June 2019 to May 2020. Samples were obtained from various OPDs and IPDs wards of MGH, Sitapura, Jaipur, Rajasthan. Out of total 16,479 clinical samples, 224 *S. aureus* isolates were obtained which includes 69 pus samples, 68 swabs (including pus swab, vaginal swab, and wound swab), 44 blood samples, 12 urine, nine ET, three sputum, 10 fluids (including cerebrospinal fluid, ascitic fluid, pleural fluid, and peritoneal fluid), seven tissue samples, and two central line tips were processed in the department of microbiology, during the study period [Graph 1 and Figure 1]. The highest prevalence of *S. aureus* was observed in males 136 (60.71%) as compared to females 88 (39.28%) in the age group of 51–60 followed by 21–30 and 11–20 age group [Table 1]. All 224 *S. aureus* isolates were subjected to antimicrobial susceptibility testing by Kirby–Bauer disk diffusion method using following antibiotics, as shown in Graph 2. Among all 224 *S. aureus* isolates, 100% sensitivity were observed by vancomycin,

Table 1: Gender- and age-wise distribution of study subjects

Age	*Male*	*Female*	*Total*
0–10 years	14	06	20
11–20 years	22	13	35
21–30 years	15	27	42
31–40 years	18	15	33
41–50 years	17	08	25
51–60 years	33	10	43
>60 years	17	09	26
Total	136 (60.71%)	88 (39.28%)	224

Table 2: Prevalence of methicillin-resistant *S. aureus*

Total no. of isolates	*MRSA*	*MSSA*
224	167 (74.55%)	57 (25.44%)

S. aureus: Staphylococcus aureus, MERA: Methicillin-resistant *Staphylococcus aureus*, MSSA: Methicillin-susceptible *Staphylococcus aureus*

Table 3: No. of isolates which are erythromycin resistant, but clindamycin sensitive

Total	*Erythromycin sensitive (%)*	*Erythromycin resistant but clindamycin sensitive (%)*
224	67 (29.91)	157 (70.80)

Table 4: Prevalence of MLSB resistance in *S. aureus*

ERSA	*MS phenotype %*	*iMSLB resistance %*	*cMLSB resistance%*
157	74 (47.133)	48 (30.57)	35 (22.29)

MLSB: Macrolide-lincosamide-streptogramin B, ERSA: Erythromycin-resistant *Staphylococcus aureus,* cMSLB: Constitutive macrolide-lincosamide-streptogramin B, iMSLB: Inducible macrolide-lincosamide-streptogramin B

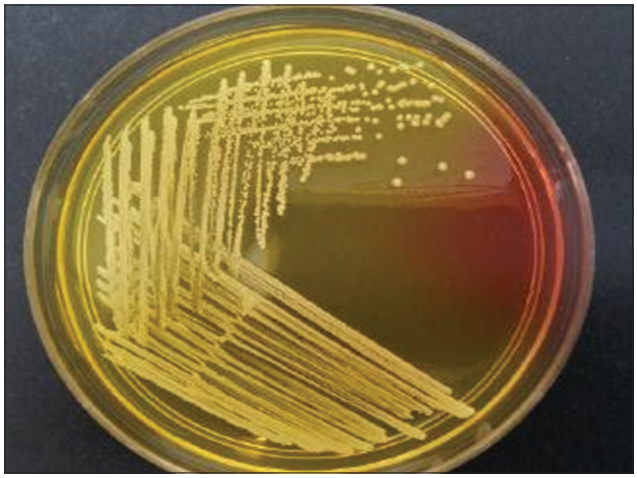


Figure 1: *Staphylococcus aureus* on Mannitol Salt Agar

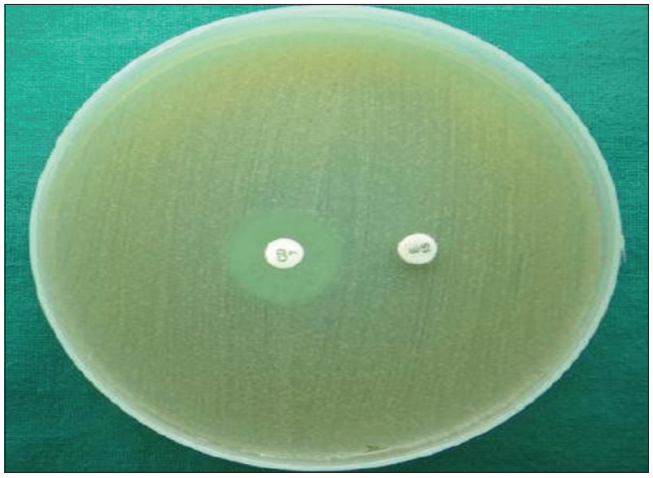


Figure 2: MS phenotype

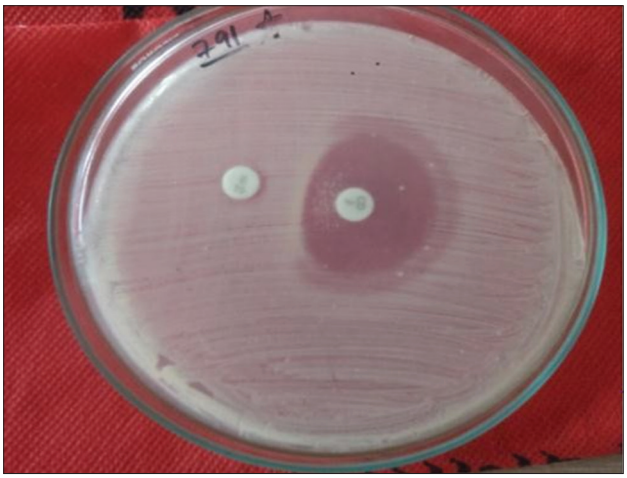


Figure 3: Inducible macrolide-lincosamide-streptogramin B (D-test)

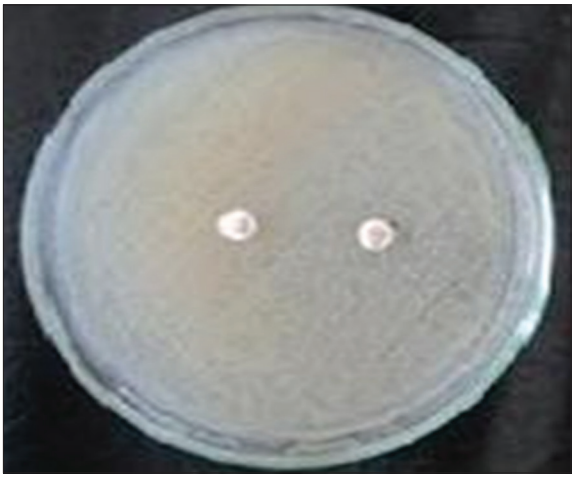
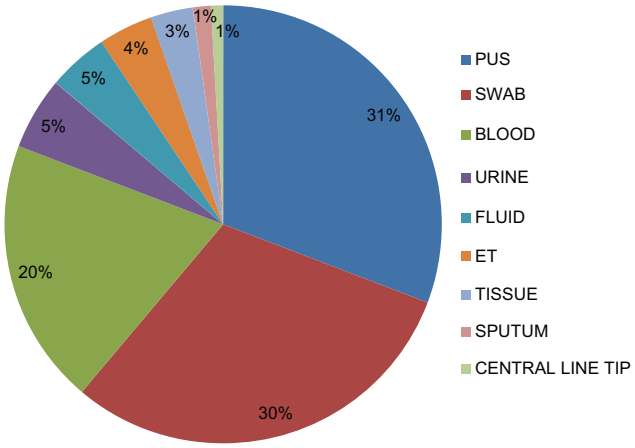
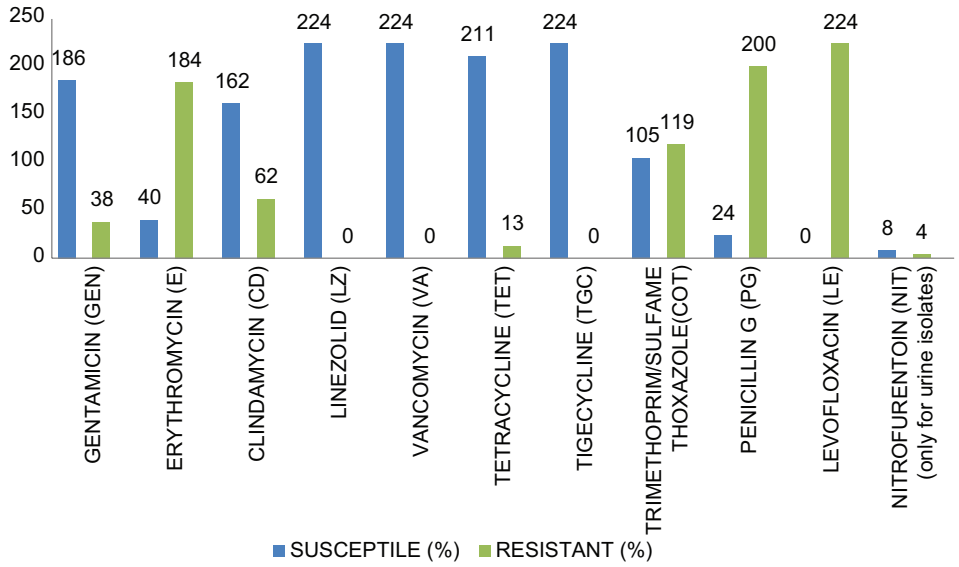


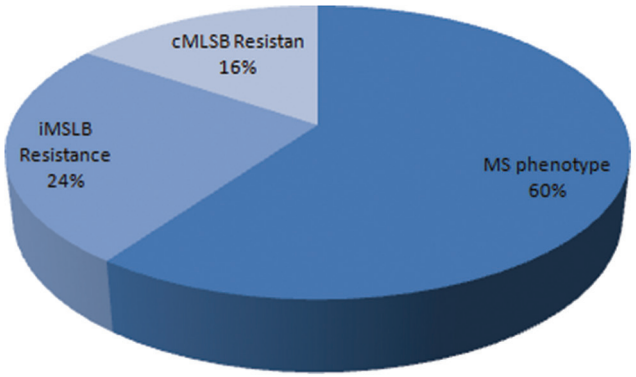
Figure 4: Constitutive macrolide-lincosamide-streptogramin B (no zone)



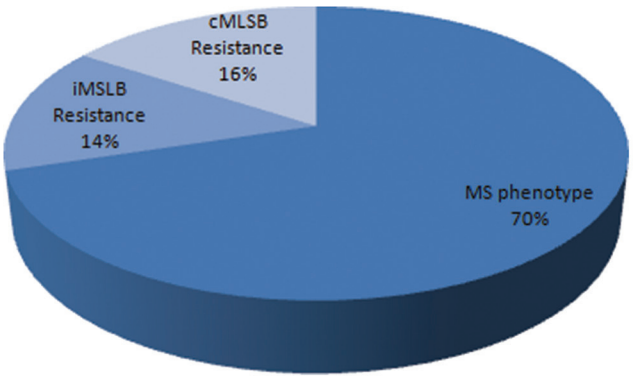
Graph 1: *Staphylococcus aureus* on Mannitol Salt Agar



Graph 2: *Staphylococcus aureus* on Mannitol Salt Agar



Graph 3: *Staphylococcus aureus* on Mannitol Salt Agar



Graph 4: *Staphylococcus aureus* on Mannitol Salt Agar

linezolid, and tigecycline followed by tetracycline (94.19%) and gentamicin (83.03%). About 100% resistivity were observed by levofloxacin followed by penicillin G (89.86%) and trimethoprim/sulfamethoxazole (53.12%).

In the present study, among the total of 224 *Staphylococcus aureus* isolates, highest prevalence of *Methicillin resistant Staphylococcus aureus* as compared to *Methicillin sensitive Staphylococcus aureus* [Table 2]. A high percentage of erythromycin resistant was observed in the present study. Out of 224 isolates of *S. aureus,* 157 (70.80%) show erythromycin resistance but clindamycin sensitive and 67 (29.91%) were sensitive to erythromycin [Table 3]. In the present study, out of 157 erythromycin resistant but clindamycin sensitive *Staphylococcus aureus* isolates were obtained, which were further subjected to D-test in which 74 (47.133%) shows MS phenotype , 48(30.57%) shows inducible clindamycin resistance and 35 (22.29%) shows constitutive resistance [Table 4 and Figure 2-4]. Out of 224 isolates, 167 (74.55%) are MRSA and 57 (25.44%) are MSSA. Out of 167 MRSA, 100 (59.88%) show MS phenotype, 41 (24.55%) show inducible clindamycin resistance, and 26 (15.56%) show constitutive resistance [Graph 3] while among 57 MSSA, 40 (70.17%) show MS phenotype, 8 (14.03%) show inducible clindamycin resistance, and 9 (15.78%) show constitutive resistance [Graph 4].

Among all 48 *S. aureus* strains with inducible clindamycin resistance, 100% sensitivity were observed by vancomycin, linezolid, and tigecycline followed by tetracycline (89.58%) and gentamicin (83.33%). About 100% resistivity were observed by levofloxacin, followed by penicillin G (83.33%) and trimethoprim/sulfamethoxazole (22.91%) [Table 5].

Discussion

Clindamycin is a protein synthesis inhibitory agent that has the ability to suppress the expression of virulence factors in *S. aureus* at sub-inhibitory concentrations.[7] Therefore, recent guidelines recommend the use of clindamycin for the treatment of toxin-mediated infections (e.g., toxic shock syndrome and necrotizing pneumonia).[8] This modulation of virulence expression by clindamycin occurs in clindamycin-susceptible *S. aureus* strains but is abolished in constitutive clindamycin-resistant strains.[9] There has been increasing awareness that inducible clindamycin resistance in *S. aureus* and coagulase-negative *Staphylococcus*

Table 5: Antibiotic susceptibility pattern of inducible clindamycin-resistant (D-test positive) *Staphylococcus aureus* isolates

S. No.	*Name of the antibiotic tested*	*Susceptible%*		*Resistant%*	
1	Gentamicin (GEN)	40	83.33	8	16.66
2	Erythromycin (E)	0	0	48	100
3	Clindamycin (CD)	48	100	0	0
4	Linezolid (LZ)	48	100	0	0
5	Vancomycin (VA)	48	100	0	0
6	Tetracycline (TET)	43	89.58	5	10.41
7	Tigecycline (TGC)	48	100	0	0
8	Trimethoprim/ sulfamethoxazole (COT)	37	77.08	11	22.91
9	Penicillin G (PG)	8	16.66	40	83.33
10	Levofloxacin (LE)	0	0	48	100
11	Nitrofurantoin (NIT) (only for urine isolates)	0	0	0	0

species may not be detected by three standard tests.[10] In the present study, majority of *S. aureus* isolates were isolated from pus samples. These findings were in correlation with the findings of Adhikari *et al*. who reported 71 (57%) isolates from pus samples, 32 (25.80%) from blood, 7 (5.2%) isolated from urine, 6 (4.8%) from sputum, and 9 (7.2%) were isolated from miscellaneous samples that included ear discharge, abdominal drain fluid, throat swab, conjunctival swab, and wound discharges.[6] The present study is similar to Pappu *et al*. who reported that 59 (54%) were isolated from pus samples, 31 (27.8%) from blood, 7 (6.2%) from urine, 5 (4.8%) from sputum, and 8 (7.2%) were isolated from miscellaneous samples including ear discharge, abdominal drain fluid, throat swab, conjunctival swab, and wound discharges.[2]

The highest prevalence of *S. aureus* was observed in males 136 (60.71%) as compared to females 88 (39.28%) in the age group of 51–60 followed by 21–30 and 11–20 age group. Our findings matches with the study done by Adhikari *et al*. who reported the highest prevalence of males (150) than females (120) out of the total 270 *S. aureus* isolates.[6] Among all 224 *S. aureus* isolates, 100% sensitivity were observed by vancomycin, linezolid, and tigecycline. Similar findings were observed by Pappu *et al*. with 100% sensitivity to vancomycin and linezolid, with moderate sensitivity (71.14%) to gentamicin.[2]

In the present study, the highest prevalence of MRSA as compared to MSSA, that is, MRSA 167 (74.55%) and MSSA 57 (25.44%), respectively, among the total of 224 *S. aureus* isolates which are similar to the study done by Yilmaz *et al*. in which MRSA comprised 118 (73%) and MSSA comprised 43 (27%) of the total 161 isolates of *S. aureus* isolates.[11] A high percentage of erythromycin resistant was observed in the present study. Similar type of observation was done in the study done by Pappu *et al*. where a high percentage (70%) of erythromycin-resistant *S. aureus* isolates.[2] Similarly, the study done by Rajak *et al*. who concluded that among a total of 125 *S. aureus*, majority were resistant to erythromycin (70.4%).[12] Out of 167 MRSA, 100 (59.88%) show MS phenotype, 41 (24.55%) show inducible clindamycin resistance, and 26 (15.56%) show constitutive resistance. Adhikari *et al*.[6] in his study concluded that on antibiotic susceptibility testing, 54.4% (147) isolates were erythromycin resistant which were then subjected to D-Test, where 21% of isolates showed iMLBs (D-test positive), 53.4% of isolates showed cMLBs, and 25.2% showed MS phenotype. *In vivo* antibiotic therapy with clindamycin may select constitutive erm gene mutants which may lead to clinical failure, thus necessitating the need to detect such resistance by a simple D-test on a routine basis, so as to ensure safe and effective use of clindamycin, only in those patients with truly susceptible strains.[13] Among all 48 *S. aureus* strains with inducible clindamycin resistance, 100% sensitivity were observed by vancomycin, linezolid, and tigecycline, similar type of observation was done by Pappu *et al*. where 100% sensitivity was observed for vancomycin and linezolid, with moderate sensitivity (71.41%) to gentamicin and least sensitivity to ciprofloxacin (20.95%).[2] In another study done by Rajak *et al*., similar observations were done with 100% sensitivity for vancomycin and linezolid, moderate sensitivity to gentamicin (70%).[12]

Conclusion

From the present study, we can conclude that there is high percentage of inducible clindamycin resistance among *Staphylococcus* isolates. If D-test would not have been performed, many inducible clindamycin-resistant *S. aureus* could have been easily misidentified as clindamycin susceptible, leading to therapeutic failure. Thus, simple and reliable D-test can be incorporated into routine in clinical microbiology laboratory. This will enable us in guiding the clinicians regarding the judicious use of clindamycin in skin and soft-tissue infections as clindamycin is not a suitable drug of choice for D-test positive isolates, while it can definitely prove to be a drug of choice in case of D-test negative isolates.

Acknowledgment

We thank to Mahatma Gandhi University of Medical Sciences and Technology for providing technical and other facilities for completing this research work.

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