The study of inducible clindamycin resistance & antimicrobial susceptibility pattern of the *Staphylococcus aureus* isolates from various clinical samples

T. R. Patil[1,*], S. V. Wankhede[2]

[1]PG Student, [2]Professor and HOD, Dept. of Microbiology, Smt. Kashibai Navale Medical College, Pune, Maharashtra, India

***Corresponding Author:**
Email: tejupatil1206@gmail.com

Abstract

Introduction: *Staphylococcus aureus* is responsible for skin and soft tissue infections, surgical site infections. Macrolide-lincosamide-streptogramin B (MLSB) antibiotics particularly clindamycin is favoured agent to treat skin and soft tissue infections cause by *Staphylococcus aureus*. Broad utilization of these antibiotics has prompted an expansion in resistance against these antimicrobials, requiring the need to recognize such resistance on routine premise utilizing a D test.

Aims and Objectives: To study inducible clindamycin resistance & antimicrobial susceptibility pattern of *S. aureus* isolates.

Materials and Methods: 300 isolates of *S. aureus* from different clinical examples were subjected to antimicrobial susceptibility testing by Kirby Bauer's disc diffusion method. Just erythromycin resistant isolates were subjected for D test to contemplate inducible clindamycin resistance according to CLSI guidelines.

Results: Out of 300 isolates, 171 (57%) S. aureus isolates were erythromycin resistant. Among these isolates 84 (28%) showed MS phenotype, 68 (22.66%) showed inducible resistance & 19 (6.33%) showed constitutive resistance.

Conclusion: A D test can be utilized to identify inducible clindamycin resistance. This test will help for legitimate treatment of the patients yet additionally counteract misuse of anti-microbial agents.

Keywords: *Staphylococcus aureus*, D test, Inducible clindamycin resistance, Erythromycin.

Introduction

Staphylococcus aureus is a part of normal human flora. Roughly 25 to half of people might be permanently or transitorily colonized with S. aureus.[1] It is available in the anterior nares of up to 30% of the population.[2] *Staphylococcus aureus* principally causes diseases like different kinds of skin infections like; Staphylococcal Scalded Skin Syndrome (SSSS), & other infections like Osteomyelitis, Meningitis, Pneumonia, Septicemia, Gastroenteritis.

The decision of antimicrobial agents to treat staphylococcal infection has turned out to be progressively hazardous in light of the rise of multidrug resistant strains. Clindamycin is the favoured operator for S. aureus diseases because of its great pharmacokinetic properties and great infiltration into different tissues However, boundless utilization of Macrolide-Lincosamide-Streptogramin B (MLS B) anti-microbial has prompted an expansion in the quantity of Staphylococcal strains gaining resistance to MLSB anti-microbial agents. Clindamycin resistance in *Staphylococcus species* can be either constitutive or inducible. Strains with inducible resistance to clindamycin are hard to distinguish in the standard research facility.

D test is utilized to recognize inducible clindamycin resistance. Clindamycin isn't a reasonable medication for D test positive isolates yet it is a medication of choice for D test negative isolates. This test manages about inducible clindamycin protection and averts misuse of antibiotics.

Aims and Objectives

1. To study antimicrobial susceptibility pattern of *S. aureus* isolates.
2. To study inducible clindamycin resistance among *S. aureus* isolates by using D test.

Materials and Methods

This investigation was directed at the department of Microbiology of a tertiary health care centre, from Jan 2015 to Dec 2016. An aggregate of 300 clinical specimens were received from patients admitted to different wards, ICU and from OPD. The samples were processed by the routine microbiological procedures.[3] *S. aureus* isolates acquired were distinguished by different biochemical tests.

Antimicrobial susceptibility tests of the *S. aureus* isolates were done by modified Kirby–Bauer disc diffusion method for the accompanying antimicrobial agents as indicated by the Clinical and Laboratory Standards Institutes (CLSI) guidelines[4] -Penicillin G (10 unit), Cefoxitin (30 µg), Ciprofloxacin (5 µg), Erythromycin (15 µg), Clindamycin (2 µg), Tetracycline (30 µg), Co-trimoxazole (1.25/23.75 µg), Cefazolin (30 µg), Vancomycin (30 µg), Teicoplanin (30 µg).

Erythromycin resistant strains were further subjected to 'D test' as per CLSI guidelines.

Three different phenotypes were seen after testing:

1. MS Phenotype - Isolates exhibiting resistance to erythromycin (zone size ≤13mm) while sensitive to clindamycin (zone size ≥21mm) and giving circular zone of inhibition around clindamycin

2. Inducible MLS $_B$ Phenotype - Isolates showing resistance to erythromycin (zone size ≤13mm) while being sensitive to clindamycin (zone size ≥21mm) and giving D shaped zone of inhibition around clindamycin with flattening towards erythromycin disc.
3. Constitutive MLSB Phenotype - Isolates showing resistance to both erythromycin (zone size ≤13mm) and clindamycin (zone size ≤14mm) with circular shape of zone of inhibition if any around clindamycin.

Results

Isolates of *Staphylococcus aureus (S. aureus)* from different clinical examples were incorporated into this study. An aggregate of 300 *S. aureus* clinical isolates were contemplated.

As demonstrated in table 1 and Fig 1, maximum isolates of *S. aureus* were from Indoor patient department (IPD).

Table 2 and Fig 2 demonstrates that *S. aureus* infections are common in 21-30 yrs (22.66%), trailed by 61 and over 61 yrs (15.66%).

Fig 3 demonstrates that *S. aureus* was maximally (37.67%) responsible for abscesses and wound infections.

As showed in Table 3 and Fig 4, *S. aureus* isolates were indicating more resistance for Penicillin G (93.33%) and Ciprofloxacin (71%), while complete susceptibility were seen to vancomycin, teicoplanin. Lower resistance noted to tetracyclines (6.99%) & co-trimoxazole (37.66%)

Table 4 shows that out of total 300 *S. aureus* strains, 129 (43.0%) were sensitive to erythromycin and remaining 171 (57.0%) were resistant to erythromycin. Of these 171 erythromycin resistant strains, 19 showed resistance and 152 isolates showed susceptibility to clindamycin on disc diffusion test. These 152 erythromycin resistant and clindamycin sensitive (on disc diffusion testing) strains were when subjected to D- test, 68 strains showed inducible clindamycin resistance. The remaining 84 strains (erythromycin resistant and clindamycin sensitive) which were D- test negative were referred as MS phenotype. The total 19 strains which were resistant to both the clindamycin and erythromycin are the constitutive clindamycin resistant *S. aureus* isolates.

Discussion

Staphylococci are ubiquitous colonizers of the skin and mucosa and exceedingly effective opportunistic pathogen. This organism can produce wide assortment of diseases. The capacity of *S. aureus* is to create anti-microbial resistance mirrors the phenomenal limit of this organism to adjust and make due in an incredible assortment of situations.[5]

In the present study, 253 (84.33%) *S. aureus* isolates were from inpatient department (IPD) and 47 (15.66%) were from outpatient department (OPD) (Table 1). It corresponds with the findings of Joshi et al,[6] who announced 74.8% *S. aureus* strains from the IPDs, 25.1% from the OPDs in 2008 and 71.6% strains from IPDs and 28.3% strains from OPDs in 2009. A high event of *S. aureus* strains in IPD setting might be because of comfort in spreading of disease among patients through health care workers and instruments.[7]

In this study (Table 2), *S. aureus* infections were more typical in age group 21-30 yrs (22.66%), took after by age group 61 or more 61 yrs (15.66%) and age group 11-20 yrs (15.33%). Ankurkumar et al[8] had additionally observed *S. aureus* infections more typical in 21-30 yrs (30.9%).

In the present study, most extreme number of *S. aureus* isolates were from abscess and wound infections (37.67%) trailed by ENT infections (15.67%) and skin, soft tissue infections (15.33%). Comparable findings were noted by Mehndiratta et al,[9] 39.65% of *S. aureus* isolates were from pus. In the study of Joshi et al,[6] the major part of *S. aureus* strains were isolated from patients with skin and soft tissue infections followed by those suffering from blood stream infections and respiratory infections.

In the present study, most astounding resistance i.e. 93.33% was seen to penicillin, followed by ciprofloxacin (71%). It corresponds with the findings of Duran et al (92.8%),[10] Bouchiat et al[11] (91.3%) for penicillin resistance in *S. aureus*. Bouchiat et al[11] likewise observed comparative outcome for ciprofloxacin. All isolates were sensitive to vancomycin and teicoplanin. Comparative outcomes noted by Datta et al,[12] Vidhani et al,[13] Anupurba et al.[14]

Erythromycin resistance was seen in 57% *S. aureus* isolates while clindamycin resistance was seen in 44.33% isolates (Table 3). Comparable findings were found in a study by Verma et al.[15] Erythromycin and clindamycin resistance was observed to be 52.8% and 46.28% respectively in *S. aureus* isolates. In the present study, inducible clindamycin resistance (iMLSB) was seen in 68 (22.66%) and constitutive clindamycin resistance (cMLSB) in 19 (6.33%) *S. aureus* isolates. MS phenotype was seen in 84 (28%) isolates (Table 4).

The outcomes acquired by Mittal et al[16] were in accordance with this study. It demonstrated iMLSB 23.2%, cMLSB 6.15% and MS phenotype 15%. Shantala et al[17] announced that, out of 230 *S. aureus* isolates they contemplated, 24.9% strains were of iMLSB phenotype, 18.3% were of cMLSB phenotype and 15.7% were of MS phenotype. The events of iMLSB differ broadly by hospital and geographic area.[18] Variation of utilization of the medication in various areas and transformation of inducible phenotype to constitutive phenotype amid treatment prompts high level of variety for constitutive clindamycin resistance between different studies.[19]

The prevalence of inducible clindamycin resistance is low in exhibit contemplate. To forestall increment in inducible clindamycin resistance we suggest:

1. Utilization of D test in routine setup alongside other antimicrobial susceptibility testing.
2. Usage of antimicrobial stewardship program.
3. Strict utilization of infection control approach in the hospitals.

Table 1: IPD & OPD wise distribution of *S. aureus* isolates

	No. of Isolates (%)
IPD	253 (84.33)
OPD	47 (15.66)
Total	300 (100)

Table 2: Age wise distribution of cases of *S. aureus* infections (n=300)

Age distribution years (yrs)	No. of Isolates (%)
0-1 yr	19 (6.33)
2-10 yr	25 (8.33)
11-20 yr	46 (15.33)
21-30 yr	68 (22.66)
31-40 yr	28 (9.33)
41-50 yr	41 (13.66)
51-60 yr	26 (8.66)
61 & above 61	47 (15.66)
Total	300 (100)

Table 3: Antimicrobial sensitivity testing of *S. aureus* isolates:

Antibiotic	Sensitive no. (%)	Resistant no. (%)		
		Intermediate	Resistant	Total
Penicillin G (Pen)	20 (06.66)	-	280 (93.33)	280 (93.33)
Cefoxitin (Cx)	147 (49.0)	-	153 (51.0)	153 (51.0)
Ciprofloxacin (Cip)	87 (29.0)	133 (44.33)	80 (26.66)	213 (71)
Erythromycin (Ery)	129 (43.0)	84 (28)	87 (29.0)	171 (57.0)
Clindamycin (Cd)	167 (55.66)	46 (15.33)	87 (29.0)	133 (44.33)*
Tetracycline (Tcy)	279 (93.0)	8 (02.66)	13 (4.33)	21 (07)
Cotrimoxazole (Sxt)	187 (62.33)	12 (04.0)	101 (33.66)	113 (37.66)
Cefazoline (Czo)	85 (28.33)	-	215 (71.66)	215 (71.66)
Vancomycin (Van)	300 (100)	-	0 (0)	0 (0)
Teicoplanin (Tei)	300 (100)	-	0 (0)	0 (0)

*Includes inducible resistant isolates

Table 4: Erythromycin & Clindamycin resistance in *S. aureus* isolates (n=300)

Clindamycin	Erythromycin no. (%)		Total no. (%)
	Sensitive	Resistant*	
Sensitive	129(43)	84(28)	213(71)
Inducible resistance	0	68 (22.66)	68 (22.66)
Constitutive resistance	0	19 (6.33)	19 (6.33)
Total	129(43)	171 (57)	300 (100)

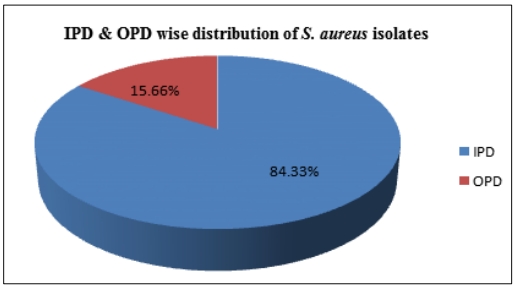

Fig. 1: IPD & OPD wise distribution of *S. aureus* isolates

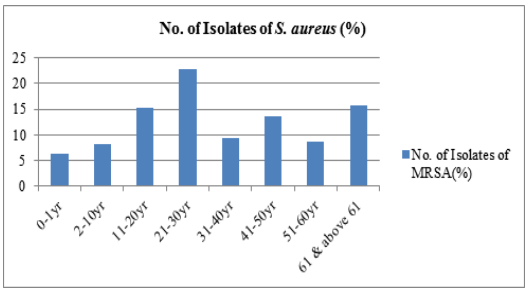

Fig. 2: Age wise distribution of cases of *S. aureus* infections

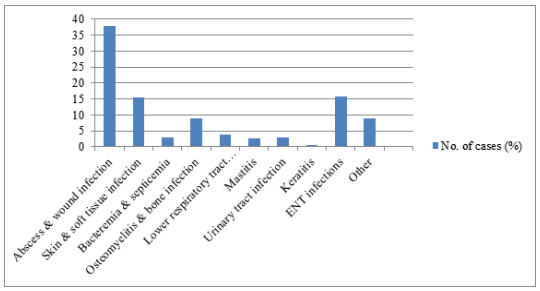

Fig. 3: Infections caused by *S. aureus* (n=300)

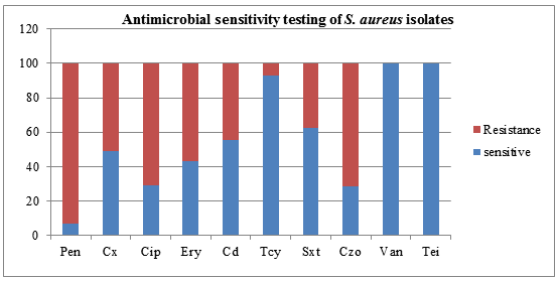

Fig. 4: Antimicrobial sensitivity testing of *S. aureus* isolates

Conclusion

The prevalence of inducible clindamycin resistance was 22.66% in this study. Utilization of D test in routine set up is prescribed to keep up low level of clindamycin resistance. Alongside this infection control approach and antimicrobial stewardship program usage is required.

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