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Original Research Article

Comparative Study Using Conventional Methods and the Automated VITEK 2 System for Detection of Macrolide, Lincosamide, Streptogramin B Resistance in Coagulase Negative *Staphylococci* & *Staphylococcus aureus* in a Tertiary Health Care Centre in Eastern India

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Abstract

Background: The aim of the study was to investigate the test performance of automated VITEK 2 against the conventional disk diffusion method to detect resistance exhibited by Staphylococcus strains towards macrolides and streptogramins.

Materials and Methods: Blood samples were processed both by BacT/ALERT and manual methods, while all other samples were manually processed for bacterial growth and identification. Bacteria were identified based on colony morphology & biochemical testing and antibiotic sensitivity testing performed following CLSI guidelines. Methicillin resistant strains were identified by Disc diffusion method, while Inducible Clindamycin strains were identified by D-zone test. Both manual and the automated VITEK 2 systems were used to assess the antimicrobial sensitivity pattern among isolates.

Results: The total number of strains that could be correctly identified as inducible clindamycin resistant using both the automated VITEK 2 system and the manual D-zone Test was 82. Of these VITEK 2 failed to detect 4 strains that could be detected only by the disc diffusion method [negative predictive value 100%]. Hence although the VITEK 2 had a sensitivity of 100% positive predictive value was 95.2%. In comparison, the D-zone Test identified all 82 strains

Conclusion: It is vital to perform D-zone test in routine work on the primary AST plate with erythromycin and clindamycin being correctly placed to detect clindamycin resistance, thus enabling the laboratory in giving the report pertaining to iMLSb and cMLSb appropriately. This will help enable missing any inducible clindamycin resistant strain that might be seen using the automated VITEK 2 system.

Keywords: *Staphylococcus aureus*, Coagulase Negative *Staphylococcus*, Inducible MLSB phenotype.

Introduction

A group of antibiotics together known as Macrolide Lincosamide–Streptogramin B [MLSb] even though belong to different classes of antibacterial agents, their mode of action by inhibiting protein synthesis via binding to 50S ribosomal subunit remains the same. [1] Overuse of these antibiotics in treating serious Staphylococcal infections has led to resistance to MLSb group and has disseminated through horizontal gene transfer mechanism amongst Coagulase Negative Staphylococcus aureus [CoNS] and *Staphylococcus aureus*. [2] The methylase enzyme removes a methyl group from an adenine residue in the 23S rRNA component of the 50S subunit of the ribosome. Macrolide, Lincosamide and streptogramin resistant phenotypes has increased due to such mutational changes in the ribosomal target site encoded by erythromycin ribosome methylation gene [*erm* gene]. [3] Available in oral and parenteral formulations, clindamycin has successfully been used to treat MRSA infections as this drug has excellent oral absorption. The detection of methylase enzyme has led to widespread distribution of constitutive and inducible MLSb phenotype in *Staphylococcus aureus* and CoNS. The *mrs* gene encode the active efflux pump and are resistant to macrolides and streptogramin. [4]

Since resistance to clindamycin is sometimes inducible only in the *in vitro* presence of erythromycin, there lies the importance of D-zone test that helps in selection of appropriate drugs only after a relevant antimicrobial susceptibility test is carried out. If Staphylococcal isolates are not subjected to D-zone test, clindamycin sensitive strains might show *in vivo* therapeutic failure. [4]

Automated system like VITEK 2 helps in accurate identification of [Macrolide Lincosamide–Streptogramin B] MLS resistance mechanism. The aim of this study was to get a true picture of MLS resistance in Methicillin Resistant coagulase negative Staphylococcus [MR-CoNS] & Methicillin Sensitive coagulase negative Staphylococcus [MS-CoNS] and Methicillin Sensitive *Staphylococcus aureus* [MSSA] & Methicillin Resistant *Staphylococcus aureus* [MRSA] in this geographical region by both conventional method and the automated VITEK 2 system. [5, 6]

Material & Methods:

This cross-sectional study was carried out in the Central Microbiology laboratory of a Tertiary Health Care Centre in Eastern India where Staphylococcal strains were isolated from various clinical samples like blood, urine, wound swab, pus, catheter tip from patients attending both indoor and outdoor departments with inclusion of demographic details viz: age, gender, ward, surgical intervention, previous use of clindamycin and duration of hospital stay. As the study included clinical samples, all subjects who contributed to having their samples to be included in the study gave their informed consent for the same, and Institutional Ethical clearance was taken prior to conducting the study.

Isolation & Identification:

BacT/ALERT BPA disposable culture bottles [Organon Teknika Corp., Durham, NC] contain 40ml of media and an internal sensor that detects carbon dioxide as an indicator of microbial growth. The media formulation consists of pancreatic digest of casein (1.7% w/v}, papaic digest of soybean meal (0.3% w/v), sodium polyanethol sulfonate [SPS] (0.035% w/v), pyridoxine HCl (0.001% w/v), and other complex amino acid and carbohydrate substrates in purified water. The presence of the microorganisms in the blood culture bottle was indicated by the presence of CO2 produced during metabolism of the bacteria, as indicated by the change in the

colour of the sensor attached to the bottom of each tube to yellow. [7] Subcultures were done from the bottles on Chocolate agar, Blood agar [BA] and MacConkey agar [MA] plates and examined for any growth.

Samples like pus or exudates or pieces of necrotic tissues were collected using swab stick, or otherwise aspirated. Two swabs were used to collect the sample from the depth of the wound. The swabs were transferred to sterile test tubes and transported to the laboratory as soon as possible. Samples were inoculated on BA, MA and Mannitol salt agar and incubated aerobically at 37^0C for 18-24 hours. [8]

Urine samples were inoculated on BA and CLED medium by semi quantitative method using 4 mm [24 SWG] internal diameter standard loop. After overnight incubation at 370C culture plates yielding bacterial counts of ≥ 105 CFU/ml were considered as significant, while counts ranging between 104-105 CFU/ml were of doubtful significance and counts below 104 CFU/ml were taken as insignificant. [8,9]

The isolates were identified as per standard protocol based on colony morphology, Gram's staining findings and a slide coagulase followed by tube coagulase test. Further identification to species level was done using several tests like sugar fermentation tests, presence of urease, ornithine decarboxylase and resistance to novobiocin. [10, 11,12]

Antimicrobial susceptibility testing:

Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion method on Mueller-Hinton agar plates. Antibiotic disks were purchased from HiMedia, Mumbai as mentioned in the **[Table 1]**. Plates were then incubated at 370C foe 18 hours. The zone of inhibition was measured by using a transparent scale and compared with the zone diameter interpretative chart provided by the manufacturer. [13]

Table 1: Details of GP-AST card used in VITEK 2 automated system

VITEK 2 Compact				**Disc Diffusion Method**	
Antibiotic	**Concentration**	**Calling range ≤**	**Calling range ≥**	**Antimicrobial Agent**	**Concentration [µg]**
Benzylpenicillin	0.125, 0.25, 1, 2, 8, 64	0.03	0.5	Amoxycillin	-
Cefoxitin Screen	6	Negative	Positive	Amoxycillin / clavulanic acid	-
Ciprofloxacin	1, 2, 4	0.5	8		-
Clindamycin	0.06, 0.25, 1	0.125	4	Clindamycin	2
Daptomycin	0.5, 1, 2, 4, 16	0.12	8	Erythromycin	15
Erythromycin	0.25, 0.5, 2	0.25	8	Amikacin	-
Gentamicin	8, 16, 64	0.5	16	Gentamicin	10
Inducible Clindamycin Resistance	Clindamycin (CD): 0.5, CD/Erythromycin (E) 0.25/0.5	Negative	Positive	Inducible Clindamycin Resistance	2/15
Levofloxacin	0.25, 2, 8	0.12	8	Levofloxacin	5
Linezolid	0.5, 1, 2	0.5	8	Linezolid	30
Teicoplanin	1, 4, 8, 16	0.5	32	Netilmicin	-
Oxacillin	0.5, 1, 2	0.25	4	Nalidixic acid	-
Trimethoprim/ Sulfamethoxazole	2/38, 8/152, 16/304	10 [0.5/9.5]	320 [16/304]	Trimethoprim/ Sulfamethoxazole	1.25/23.75
Vancomycin	1, 2, 4, 8, 16	0.5	32	Vancomycin	30

Detection of Methicillin resistance in *Staphylococcus* species by disc-diffusion method:

The test was performed on Mueller-Hinton agar with 4% NaCl for oxacillin and plain Mueller-Hinton agar for cefoxitin. By using a sterile swab Mueller-Hinton agar plates were inoculated [lawn culture]. Then a disc of oxacillin 1µg and cefoxitin 30µg were placed on the inoculated medium and incubated for 24 hrs at 350C.Interpretation was done as per CLSI guidelines. *Staphylococcus aureus* ATCC 25923 was used as sensitive control. [13,14,15]

Detection of Inducible Clindamycin resistance, **D-zone test:**

Those strains that were sensitive to both erythromycin and clindamycin were subjected to disk approximation test [D-zone test] where erythromycin [15µg] disc was placed at 15 mm [edge to edge] from clindamycin [2µg] disc on Mueller-Hinton agar plate previously inoculated with test strain adjusted to 0.5 McFarland suspension and incubated at 370C for 16 to18 hrs. Further, three different phenotypes were identified as follows:

MS phenotype [14,15]**:** Staphylococcus isolates exhibiting resistance to erythromycin [zone size ≤ 13 mm] while being sensitive to clindamycin [zone size ≥ 21 mm] and giving circular zone of inhibition around clindamycin was considered to possess that phenotype.

Inducible MLSb phenotype [14,15]**:** Staphylococcus isolates exhibiting resistance to erythromycin [zone size ≤ 13 mm] while being sensitive to clindamycin [zone size ≥ 21 mm] and giving D-shaped zone of inhibition around clindamycin with flattening towards erythromycin disc were supposed to have that phenotype.

Constitutive MLSb phenotype [14,15]: Staphylococcal strains showing resistance to both clindamycin [zone size ≤ 14mm] & erythromycin [zone size ≤ 13 mm] with circular shape of zone of inhibition, if any, around clindamycin.

Automated system:

The VITEK 2 Gram-Positive identification card contain 43 biochemical tests measuring carbon source utilization and enzymatic activities. The battery of biochemical test are as follows: D-amygdalin, phosphatidylinositol phospholipase c, d-xylose, arginine dihydrolase, 1-beta-galactosidase, alpha-glucosidase, ala-phe-pro arylamidase, cyclodextrin, l-aspartate arylamidase, beta galactopyranosidase, alpha-mannosidase, phosphatase, leucine arylamidase, l-proline arylamidase, beta-glucuronidase, alpha-galactosidase, l-Pyrrolydonyl-arylamidase, beta-glucuronidase, Alanine arylamidase, Tyrosine arylamidase, urease, polymixin B resistance, d-galactose, d-ribose, lactose, d-maltose, bacitracin resistance, novobiocin resistance, growth in 6.5% NaCl, d-mannitol, d-mannose, methyl-b-d-glucopyranoside, d-raffinose, salicin, saccharose/sucrose, d-trehalose, arginine dihydrolase 2 and optochin resistance. [16-19]

GP-AST in VITEK 2:

The method used for antibiotic susceptibility testing was doubling dilution technique for MIC based on microdilution method. A suspension of the test organism was made in 3 ml of 0.45% sterile saline filled in unsensitized tube and adjusted with McFarland via insertion into the optical block of the DensiCheck Plus to get an acceptable reading between 0.5 – 0.63. In a second tube containing 3.0ml of saline, 280µl of diluted test organism was transferred. Then this tube was placed in the cassette with a susceptibility card. Each GP-AST card contains selected antimicrobials in varying concentrations, dried with a microbiological culture medium [Table 1]. [20,21]

Statistical analysis: Statistical analysis of data was done using an online application available at

https://www.graphpad.com/quickcalcs/contingency1 to perform the chi-quare test and arrive at the two-tailed p-value. For sensitivity and specificity, the D-zone test results were compared with VITEK 2 AST card test results as a gold standard. Variables measured were the number of true positives [TP], number of true negatives [TN], number of false positives [FP], and number of false negatives [FN]. Sensitivity was calculated as TP/[TP+ FN], specificity as TN/[TN+FP], while the PPV was calculated as TP/[TP+FP] and NPV as TN/[TN+FN]. [22]

Results

This prospective study was undertaken in the Department of Microbiology at a tertiary health care centre in Eastern India. A total of 4024 clinical samples were received in the Department laboratory from December 2020 to November 2021, out of which only 1708 showed growth of various microorganisms. Out of the 1708 samples that showed growth, 1601 had unimicrobial growth and 107 had polymicrobial growth. A total of 1815 strains of various organisms were isolated from both outdoor and indoor patient departments. Staphylococcus species grew in 737 samples, out of which 470 strains were randomly taken up for further study.

281/470 (59.8%) Staphylococcus strains isolated from clinical samples were CoNS which included various species like *Staphylococcus haemolyticus* (33.8%; 95/281), *Staphylococcus epidermidis* (24.2%; 68/281), *Staphylococcus saprophyticus* (14.6%; 41/281), *Staphylococcus lugdunensis* (14.2%; 40/281), *Staphylococcus hominis* (6.7%;19/281), *Staphylococcus warneri* (4.6%; 13/281) and *Staphylococcus capitis* (1.7%; 5/281). All the species identified by conventional method were also detected in automated VITEK 2 system with the exception of 4% strains of *Staphylococcus warneri*, which the automated method identified as *Staphylococcus epidermidis* (Fig 1). The remaining 40.2% (189/470) Gram-positive cocci were identified as *Staphylococcus aureus* based on catalase test followed by slide & tube coagulase, mannitol fermentation, gelatine liquefaction test, DNase and phosphatase test and was in accordance with the automated VITEK 2 system.

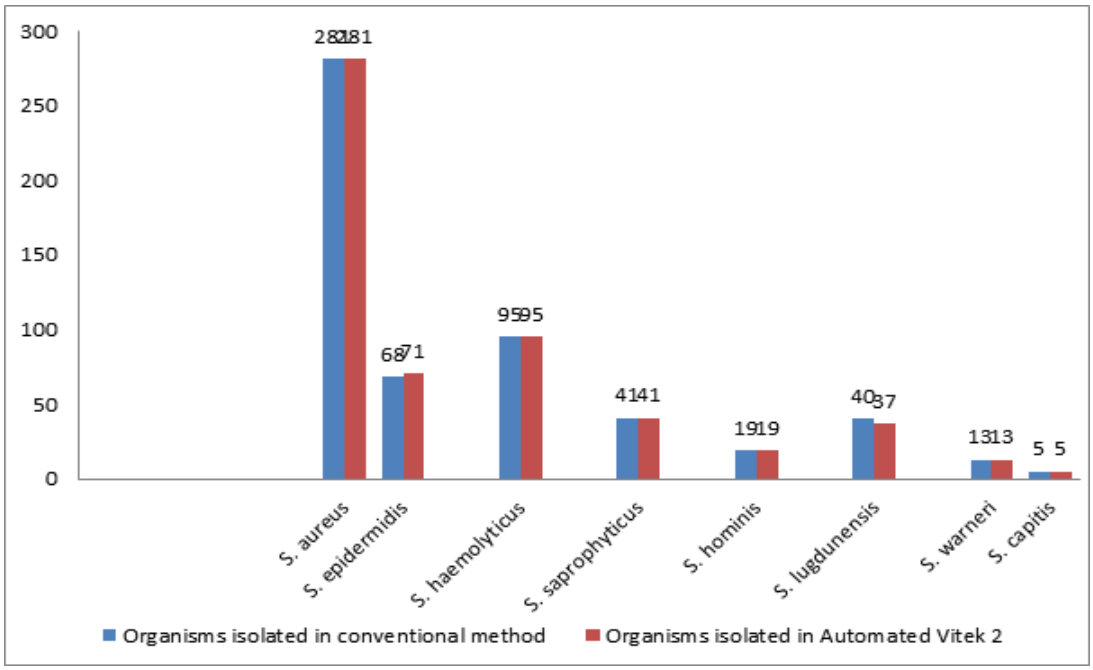

Figure 1: Distribution of CoNS among Staphylococcus isolates from clinical samples.

Of the 189 isolates, 56.1% (106/189) were MRSA and 43.9% (83/189) were MSSA. Likewise, 45.9% (129/281) strains were identified as MR-CoNS whereas 54.0% (152/281) strains were MS-CoNS. The total number of strains that were MRSA and MR-CoNS by Kirby-Bauer disc diffusion method confirmed the test results obtained by VITEK 2. The strains that were found to be moderately sensitive to levofloxacin & ciprofloxacin showed complete resistance in disk diffusion method. On the other hand, strains those were moderately sensitive to vancomycin by disk diffusion method were completely resistant by VITEK 2.

Blood stream infection (BSI) was found to be caused by 180/470 (38.3%) isolates followed by 160/470 (34.0%) isolates causing Urinary tract infection (UTI), 80/470 (17.0%) causing wound infection, 30/470 (6.4%) being isolated from pleural fluid and 20/470 (4.2%) isolated from CVP catheter tips. Of the blood stream isolates *Staphylococcus aureus* (81/180; 45.0%), followed by *Staphylococcus haemolyticus* (54/180; 30.0%) were the commonest isolates; *Staphylococcus aureus* (67/160; 41.8%) followed by *Staphylococcus saprophyticus* (41/160; 25.6%) in case of urine infections; *Staphylococcus aureus* (40/80; 50.0%) followed by *Staphylococcus haemolyticus* & *Staphylococcus lugdunensis* (12/80;15.0% each) in the pus sample isolates and *Staphylococcus haemolyticus* (7/7; 100%) being the main isolate from CVP catheter tip samples (Table 2).

Table 2: Distribution of Staphylococcal strains from different clinical samples

Organisms	Blood Stream Infection (BSI) N=180	Urinary Tract Infection (UTI) N=160	Wound infection N=80	Pleural fluid N=30	CVP tip N=20
Staphylococcus aureus	81 (45.0%)	67 (41.8%)	40 (50.0%)	1 (3.3%)	0 (00%)
Staphylococcus haemolyticus	54 (30.0%)	10 (12.5%)	12 (15.0%)	6 (20.0%)	13 (65.0%)
Staphylococcus epidermidis	30 (16.6%)	26 (16.3%)	6 (15.0%)	5 (16.6%)	1 (5.0%)
Staphylococcus saprophyticus	0 (00%)	41 (25.6%)	0 (00%)	0 (00%)	0 (00%)
Staphylococcus lugdunensis	0 (00%)	7 (4.3%)	12 (15.0%)	15 (50.0%)	6 (30.0%)
Staphylococcus hominis	15 (8.3%)	0	2 (2.5%)	2 (6.6%)	0 (00%)
Staphylococcus warneri	0 (00%)	5 (3.1%)	8 (10%)	0 (00%)	0 (00%)
Staphylococcus capitis	0 (00%)	5 (3.1%)	0	0 (00%)	0 (00%)

Of the 470 staphylococcal isolates, 181(38.5%) were erythromycin resistant and clindamycin sensitive. The distribution of Constitutive Clindamycin and Erythromycin (cMLSb) and Inducible Clindamycin Resistance (iMLSb) phenotypes among MRSA were 20.7% each and 17.9% constituted MS phenotype respectively. On the contrary, 25.3% strains exhibited MS phenotype, followed by 12.1% iMLSb type. MS phenotype was seen in 25.6% MR-CoNS strains in comparison to 17.1% MS-CoNS strains. Further, iMLSb phenotype was seen in

16.3% of MR-CoNS in comparison to 19.0% MS-CoNS isolates (Table 3).

Table 3: Macrolide resistance of the isolates based on disc diffusion method

Phenotype	*Staphylococcus aureus* N=189		Coagulase negative Staphylococcus N= 281	
	MRSA N=106	MSSA N=83	MR-CoNS N=129	MS-CoNS N=152
ER-R, CL-R (cMLSb)	22 (20.7%)	11 (13.3%)	28 (21.7%)	19 (12.5%)
ER-S, CL-S	43 (40.5%)	41 (49.3%)	47 (36.4%)	78 (51.3%)
ER-R, CL-S (iMLSb) D test+	22 (20.7%)	10 (12.1%)	21 (16.3%)	29 (19.0%)
ER-R, CL-S (MS) D test-	19 (17.9%)	21 (25.3%)	33 (25.6%)	26 (17.1%)

The rates of inducible clindamycin resistance among the different staphylococcal isolates are shown in Table 4. The inducible clindamycin resistance was not significant (22/41) among MRSA compared to (10/31) MSSA (p-value=0.0052). Likewise, the differences in the expression of inducible clindamycin resistance in MR-CoNS (521/54) was found to be insignificant, when compared to (29/55) among MS-CoNS, (p-value=0.208).

Table 4: Inducible clindamycin resistance among the isolates based on D-zone test

Organism	Total number of isolates	Inducible Clindamycin resistance (D-zone test positive)		p-value
		Positive	Negative	
MRSA	41	22	19	0.0052
MSSA	31	10	21	
MR-CoNS	54	21	33	0.208
MS-CoNS	55	29	26	

Table 5 shows the antibiotic susceptibility pattern to various antibiotics. Of the MRSA isolates with the iMLSb phenotype, maximum susceptibility was retained to vancomycin (86.4%) followed by levofloxacin (81.8%), netilmicin & linezolid (77.2% each). The iMLSb phenotype showed least sensitivity to nalidixic acid (50.0%). On the other hand, amongst the MR-CoNS, maximum sensitivity to iMLSb phenotype was retained with linezolid (90.9%) followed by vancomycin (85.7%), and netilmicin & levofloxacin (71.4% each). For MS phenotype in MRSA isolates, maximum susceptibility was shown to linezolid (94.7%) and nalidixic acid (78.9%). Least sensitivity was shown to amoxicillin (31.5%). On the other hand, the MS phenotype amongst MR-CoNS were most sensitive to netilmicin (84.8%) and amikacin (66.6%). The sensitivity pattern of MRSA isolates with cMLSb phenotype was maximum with linezolid (86.3%) followed by netilmicin, amikacin & vancomycin (68.2% each). 78.5% & 67.8% of the MR-CoNS isolates with cMLSb phenotype showed maximum sensitivity to linezolid and levofloxacin respectively.

Table 5: Antibiotic susceptibility pattern for all isolates:

Antibiotics	MRSA			MR-CoNS		
	iMLSb N=22	MS phenotype N= 19	cMLSb n=22	iMLSb N=21	MS phenotype N=33	cMLSb n=28
Amoxycillin	14 (63.6%)	06 (31.5%)	05 (22.7%)	07 (33.3%)	13 (39.3%)	06 (21.4%)
Amoxy-clavulanic acid	14 (63.6%)	09 (47.3%)	07 (31.8%)	09 (42.8%)	16 (48.4%)	08 (28.5%)
Gentamicin	17 (77.2%)	09 (47.3%)	13 (59.0%)	10 (47.6%)	16 (48.4%)	07 (25.0%)
Amikacin	17 (77.2%)	09 (47.3%)	15 (68.2%)	11 (52.3%)	22 (66.6%)	08 (28.5%)
Vancomycin	19 (86.4%)	14 (73.6%)	15 (68.2%)	18 (85.7%)	20 (60.1%)	19 (67.8%)
Linezolid	17 (77.2%)	18 (94.7%)	19 (86.3%)	20 (90.9%)	25 (75.7%)	22 (78.5%)
Netilmycin	17 (77.2%)	11 (57.9%)	15 (68.2%)	15 (71.4%)	28 (84.8%)	18 (64.2%)
Nalidixic acid	11 (50.0%)	15 (78.9%)	13 (59.0%)	07 (33.3%)	23 (69.6%)	18 (64.2%)
Ciprofloxacin	14 (63.6%)	10 (52.6%)	07 (31.8%)	13 (61.9%)	17 (51.5%)	15 (53.6%)
Levofloxacin	18 (81.8%)	09 (47.3%)	11 (50.0%)	15 (71.4%)	20 (60.1%)	19 (67.8%)

The result of inducible clindamycin resistance by D-zone test and VITEK 2 are compared in Table 6. The sensitivity and specificity were 100% and 98.9%, with a positive predictive value (PPV) and negative predictive value (NPV) of95.2% and 100% respectively.

Table 6: Comparative analysis of VITEK 2 AST card and D-zone test

D-zone test	No. of isolates positive by VITEK 2		Total
	Positive	Negative	
Positive	78 (TP)	4 (FP)	82
Negative	0 (FN)	388 (TN)	388
Total	78	392	470

Discussion

Clindamycin is the possible drug of choice to treat Staphylococcal strains (including methicillin sensitive and resistant) isolated from skin and soft tissue infection. The concentration of drug required to effectively reach the infected tissue specially the abscess is excellent. It has good oral absorption ability that eliminates the complexity of drug regimen which in turn favourably affects medication adherence. Hence it is a good option for treatment of outdoor patient and sustain patient compliance. There is lot of controversy regarding clinical therapeutic failure with regards to clindamycin that gets inactivated *in vivo* in the presence of the erythromycin, which acts as an inducer molecule leading to inducible clindamycin resistance which fails to be detected by disc diffusion method. Transfer of the resistant genes in between the strains promote constitutive resistance, hence there is a treatment failure. There is a need for CLSI recommended D-zone test to be performed on a routine basis for all Staphylococcal

isolates for detection of inducible resistance for correct interpretation of susceptibility test enabling a guidance for proper therapy. [15,16]

Out of the total of 470, 281 samples (59.8%) were reported as CoNS in our study, *Staphylococcus haemolyticus* (54/180; 33.8%) was the main isolate. A slightly lower number of *Staphylococcus aureus* (81/180; 40.2%) constituted the remaining part of the sample that were processed to detect inducible clindamycin resistance. Other studies with reference to blood culture isolates showed a similar distribution pattern of Gram-positive bacteria amongst which Enterococcus spp. was the predominant isolate (157/752; 20.8%) followed by a CoNS (120/752; 16%) and *Staphylococcus aureus* (61/752; 8.1%). [23]

In our study according to the conventional method, *Staphylococcus haemolyticus* (95/281; 33.8%), *Staphylococcus epidermidis* (68/281; 24.2%), *Staphylococcus saprophyticus* (41/281; 14.6%), *Staphylococcus lugdunensis* (40/281; 14.2%), *Staphylococcus hominis* (19/281; 6.7%), *Staphylococcus warneri* (13/281; 4.6%) and *Staphylococcus capitis* (5/281; 1.7%) were the common isolates. The pattern of isolation of the various species of CoNS by conventional method in a similar study were: *S. cohnii* (23%), *S. xylosus,* (13%), *S. lugdunensis* (11%), *S. epidermidis* (10%), *S. stimulans* (10%), *S. saprophyticus* (3%), *S. schleiferi* (3%) *and S. warnerii* (3%). [24]

All the species identified by conventional method in our study were also detected in automated VITEK 2 system with the exception of 4% strains of *Staphylococcus warneri* which the automated method identified to be *Staphylococcus epidermidis.* Similar findings were reported in other study results, where VITEK 2 correctly identified *S. hamolyticus* (26%), *S. cohnii* (23%), *S. epidermidis* (10%) and *S. saprophyticus* (3%), whereas for the remaining species like *S. xylosus, S. lugdunensis, S. simulans, S. schleiferi* and *S. warneri* the result was doubtful and indistinct. Correct identification was done based on the supplemental test suggested by the manufacturer, by the conventional method only. Conventional method misidentified 3% to be *S. warneri* which the automated system detected as *S. epidermidis*. [24] In the present study, out of 470 isolates, 22.5% MRSA and 27.4% MR-CoNS were identified both by disc diffusion method and VITEK 2 automated system. Similar findings have been reported by other studies as well. [25] Alarmingly higher rate of MRSA (80%) was reported by Mehta et al. [26]

Staphylococcus aureus (45.0% &50.0%) and *Staphylococcus haemolyticus* (30.0% & 15.0%) were the predominant isolate from blood stream infection and wound infections, respectively. 71.3% *Staphylococcus aureus* was isolated from pus samples in other studies. Authors commented that *Staphylococcus aureus* is the common pathogen associated with skin and systemic infection. [27]

Of the MRSA, 12.1% strains exhibiting iMLSb phenotype 20.7% showed constitutive resistance to both erythromycin & clindamycin. A slightly higher number of 26.6% iMLSb phenotype was seen in MR-CoNS. Other findings revealed higher rate of inducible (iMLSb) phenotypes (l23; 39.7%) followed by constitutive (cMLSb) phenotypes (2; 3.5%) and MS phenotypes (33; 56.8%). [28] A slightly lower rate of the distribution of inducible clindamycin resistance phenotype was reported in 19.7% and 4.8% of MRSA and MSSA isolates respectively. [27] The differences in the expression of inducible clindamycin resistance were insignificant (22/41) among MRSA, compared to (10/31) MSSA (p-value=0.0052). Dissimilar results were noted in other test results where both the inducible (19.7% Vs 4.8%) and constitutive (32.5% Vs 10.8%) resistance (P<0.0001 and P<0.0001 respectively). [28] In the present study iMLSb was slightly higher in

MRSA (22/106; 20.7%) as compared to the MR-CoNS (21/129;16.3%). [29]

On analysing the susceptibility pattern of MRSA, it was observed in our study that maximum susceptibility was retained to vancomycin (86.4%) followed by levofloxacin (81.8%) among the iMLSb phenotype followed linezolid (94.7%) and nalidixic acid (78.9%) among the MS phenotype. Likewise, in other studies, of the MRSA isolates iMLBS phenotype was found to retain sensitivity to linezolid (76.92%) followed by 69.23% sensitivity to erythromycin as well as norfloxacin. For MS phenotype in MRSA isolates the best sensitivity was for tetracycline and norfloxacin of75% followed by gentamycin (50%). [15] On the other hand, amongst the MR-CoNS in our study, maximum sensitivity to iMLSb & cMLSb phenotype was retained with linezolid. Similar findings were noted for MR-CoNS with MS phenotype retaining sensitivity to linezolid (89.28%) followed by for gentamycin (64.28%). [15]

Conclusion

It is important to know the local prevalence of iMLSb isolates in different geographic regions. Our study could not detect 4 strains by VITEK 2 that were inducible clindamycin resistance by disc diffusion method (NPV 100%). The total number of strains that could be correctly identified as inducible clindamycin resistance both by machine and manual method were 78; hence the sensitivity was 100% and the PPV was 95.2%. Similar reports were made by other authors, where the NPV was 96.6% and the VITEK 2 AST card could not detect 2 strains isolated from clinical specimen from different wards of the hospital. In a study conducted on 62 strains, the VITEK 2 system was reported to be 98% sensitive. In a study by Pal et al., 2010 sensitivity and specificity of VITEK 2 system was 93% and 100%, respectively.[6] No false positive result was found for inducible clindamycin resistance with the VITEK 2 card. It is vital to perform D-zone test in routine work on the primary AST plate with erythromycin and clindamycin being placed adjacent to each other at appropriate distance to depict the epidemiology of the clindamycin resistance, thus enabling the laboratory in giving the report pertaining to iMLSb and cMLSb at the correct time, the beneficiaries being the patients, since the clinicians get the chance to select a true panel of drugs to avoid therapeutic failure.

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