VOLUME - 12, ISSUE - 02, FEBRUARY - 2023 • PRINT ISSN No. 2277 - 8160 • DOI : 10.36106/gjra

Original Research Paper

Medical Microbiology

DETECTION OF VANCOMYCIN INTERMEDIATE STAPHYLOCOCCUS AUREUS (VISA) OR VANCOMYCIN RESISTANT STAPHYLOCOCCUS AUREUS (VRSA) IN METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS (MRSA) STRAINS ISOLATED FROM A TERTIARY CARE HOSPITAL.

Naveen Kumar. C Associate Professor, Department of Microbiology, PSP Medical College Hospital and Research Institute, Kancheepuram, Tamilnadu.

Dhandapani. E Dhandapani. E, Professor, Department of General Medicine, PSP Medical College Hospital and Research Institute, Kancheepuram, Tamilnadu.

Dhanasekar Ramu* Assistant Professor, School of Allied Health Sciences, VIMS Hospital Campus, Salem, Tamilnadu. *Corresponding Author

ABSTRACT

Introduction: Methicillin Resistant Staphylococcus aureus (MRSA) is a well-recognized public health problem throughout the world now days. The importance and prevalence of MRSA and its antibiotic pattern were occurred frequent modification for the last two decades. In routine antibiogram assay the susceptibility were screened by Disc diffusion method but drug usage and its knowledge may differs from each physicians, leads to VRSA in MRSA, can be prevented by determining drug susceptibility by Minimal Inhibitory concentration (MIC) technique MIC and knowledge about the drug usage. **Aim:** Detection of Vancomycin intermediate Staphylococcus aureus (VISA) or Vancomycin Resistant Staphylococcus aureus (VRSA) in MRSA by performing MIC and Disc diffusion method. **Materials and Methods:** A cross-sectional study was conducted on various samples for Anti-biogram screening in the Microbiology laboratory, tertiary care hospital. To perform Antibiotic susceptibility testing (AST), Disc diffusion method in Mueller Hinton agar was done in Staphylococcus aureus isolates. MIC of Cefoxitin and Vancomycin were performed for all isolates. All the MRSA isolates were checked for susceptibility to common antimicrobial agents such as Cefoxitin (30 µg), Penicillin (10 units), Clindamycin (2 µg), Erythromycin (15 µg), Linezolid (30 µg) and Cotrimoxazole (1.25/23.75 µg). SPSS version 23 used to analyses the data. **Results:** Total 200 S. aureus isolated over the period of 03 months, 78 were Methicillin Resistant (39 %). A 58% were from males and 42% were from females. Cefoxitin MIC50 was 16, Vancomycin MIC50 was 0.5 and Vancomycin MIC90 was 0.25 were obtained. **Conclusion:** Percentage of MRSA out of S. aureus isolates was 39 %. All the MRSA isolates had a Vancomycin MIC ≤1.5 µg/mL. The current study reveals that absence of VISA or VRSA in MRSA strains.

KEYWORDS :

INTRODUCTION:

The MRSA is a serious threat in healthcare associated infections. Several factors enhances the chances to get MRSA infection in patients like diabetes, hospital admission, open wound, surgeries, central line, and chronic skin condition [1,2]. Antibiotic susceptibility was in continuous depletion for last decades, especially in MRSA and its varying regionally. According to worldwide data, Vietnam (7.9%) and Taiwan (3.5%-3.8%) [3], in India the Asian Network for Surveillance of Resistant Pathogens (ANSORP) study shows MRSA infection rates of 38.1%, in hospital settings it is around 29-46% of Community Acquired-MRSA (CA-MRSA) [4, 5]. Penicillin's including Methicillin, Oxacillin, Cloxacillin, Nafcillin, and Dicloxacillin drugs shows MRSA resistance.

Aminoglycosides and Fluoroquinolones - beta lactam agents also become resistant. It forward towards the Multidrug resistant which shows alarming situation to treat MDR-MRSA. The current active drugs were in therapeutic like Fifth generation Cephalosporins and Oxazolidinones (Linezolid), Lipopeptides (Daptomycin) and Glycopeptides (Vancomycin, Teicoplanin). In the year 1997 Japan study shows the first isolate of VRSA [6]. To treat a patient, in routine clinical laboratory drug susceptibility were identified by disc diffusion assay in Mueller Hinton Agar (MHA). Drug concentration E-test can also be used to treat MRSA isolates [7, 8]. Hence the Disc diffusion method standard method for drug delivering process bit it is not much reliable for Vancomycin testing [9].

Other anti MRSA drugs and Vancomycin is the drug of choice based on the MIC [10]. The rapid and easiest method to identify the MIC values by E-test method on agar media after an overnight incubation [11]. Hence it is cost effective it fails to act in the routine microbiological investigation [12-14]. Therefore this current study helps to reveal the VRSA in MRSA by performing E-test, MIC in MRSA isolates.

MATERIALS AND METHODS:

Study Design:

The prevalence study was conducted at a PSP Medical College Hospital and Research Institute, Chennai, from November 2022 to January 2023 period of three months. The study was conducted with well obtained consent and approval of ethical review committee of the institution. All samples like wound, pus aspirates, pus swabs, urine etc., were received in the Microbiology lab for the three month period.

Exclusion Criteria:

- Improper sample collection,
- Insufficient Samples
- Methicillin sensitive *Staphylococcus aureus* (MSSA) – Cefoxitin Sensitive Strains.

Inclusion Criteria:

- Patients with chronic renal disease, chronic liver diseases, trauma cases and ICU.
- Also included paediatric cases,
- Only one isolate per patient was included in the study.
- Only MRSA strains were tested.
- Well Informed consent was obtained from patients before proceeding with the isolates.

Study Procedure:

Clinical samples like fracture wound discharges; surgical site infection, etc. were received from patients. The samples were inoculated to blood agar and MacConkey agar. As per lab Standard Operating procedures manual identification methods were done [15].

The isolates were identified as *Staphylococcus aureus* by Gram staining of the colony smear (Gram positive cocci in clusters), and biochemical reactions (slide coagulase and tube coagulase test both positive). Antibiotic susceptibility testing was done by disc diffusion method in Mueller Hinton agar as per CLSI guidelines [16]. The Himedia discs used were Penicillin (10 units), Cefoxitin (30 µg), Erythromycin (15

µg), Clindamycin (2 µg), Cotrimoxazole (1.25/23.75 µg) and Linezolid (30 µg). D-test (Inducible Clindamycin resistance) was also checked by Erythromycin and Clindamycin discs spaced 15 mm apart as mentioned in CLSI guidelines [17]. Cefoxitin resistant isolates were considered as MRSA. Further MRSA isolates were again subjected to E-test method to determine the MIC tested on agar media for Cefoxitin and Vancomycin together. Here, a dual E-test (Vancomycin (0.19-16.0 mcg/mL) - Cefoxitin (0.5-64 mcg/mL) Ezy MIC™ Strip from Himedia) was used to determine both antibiotics simultaneously. All the MRS isolates were performed dual antibiotic loaded E-test. Quality control of the strip was also done with standard ATCC cultures (*S. aureus* ATCC 25923) recommended by CLSI guidelines.

Statistical Analysis:

IBM SPSS version 23 was used for data analyses. Descriptive statistics were used to determine the frequencies of MRSA isolates from different clinical samples, their susceptibility to various antimicrobial agents, MIC's of Cefoxitin and Vancomycin.

RESULT:

Total 78 (39%) MRSA strains were isolated from 200 different samples received in Microbiology laboratory, among that 58% were from males and 42% were from females in past three month period. The first maximum number of patient's age group was in 41-50 (27/78). The second majority of the age group shows MRSA are 21-30 (11/78). Neonatal, less than 01 year old baby and above 70 years patients shows (01/78) respectively [Table-1]. Totally 78 samples were received from various wards like Surgery, Orthopaedics, Paediatrics, etc [Table-2]. High vaginal swabs of pregnancy women (02/78) at the 35-37 age groups. 72% of Pus swabs were maximum, of total samples (56/78). Orthopaedic department given maximum strains number of isolates (26/70).

Table: 1 – Distribution of Age and Gender

Age group	Male	Female	Total	%
New born	00	01	01	1.28
< 1 year	01	00	01	1.28
1-10 years	03	02	05	6.41
11-20 years	02	03	05	6.41
21-30 years	06	05	11	14.10
31-40 years	05	04	09	11.53
41-50 years	17	10	27	34.61
51-60 years	04	04	08	10.25
61-70 years	07	03	10	12.82
71-80 years	00	01	01	1.28
Total	45	33	78	100.00

Table: 2 – Type of samples received from different departments

Departments	Samples							
	Pus swab	High vaginal swab	Urine	Ear and nasal swab	Blood	Conjunctival swab	Throat swab	Total
Surgery	11	00	01	02	01	00	01	16
Orthopaedics	26	00	01	00	03	00	00	30
Paediatrics	04	00	00	02	02	01	01	10
Dermatology	07	00	01	00	00	00	00	08
Obstetrics and Gynaecology	08	02	00	00	02	00	00	12
ENT	00	00	00	01	00	00	01	02
Total	56	02	03	05	08	01	03	78

Among 200 isolates of *S. aureus*, 78 (39%) were Methicillin Resistant strains and 122 (61%) were Methicillin sensitive (MSSA) strains confirmed by cefoxitin disc diffusion method. The antibiotic susceptibility tests result were elaborated in the [Table-3]. Out of these 78 isolates, high end antibiotics like Vancomycin and Linezolid were susceptible to all isolates. Urine samples were not tested with Erythromycin and Clindamycin due to poorly concentrated. On the same pattern of Linezolid is key antibiotic for Vancomycin Resistant Enterococci (VRE) which causes UTI. So all the three drug were not tested for MRSA susceptibility. Around 60% of pus swabs were sensitivity to both Clindamycin and Cotrimoxazole. Out of the 78 MRSA isolates, 42 were susceptible and 35 were resistant (one urine isolate not checked) to Clindamycin [Fig-1].

Table: 3 - Susceptible Pattern of Antibiogram of MRSA Isolates

Specimens	No. of Isolates	Vancomycin	Linezolid	Cotrimoxazole	Clindamycin*	Erythromycin
Pus	56	56 (100%)	56 (100%)	37 (66%)	35 (63%)	5 (9%)
Blood	8	8(100%)	8(100%)	5 (63%)	5 (63%)	1 (13%)
Urine	3	3(100%)	#	3 (100%)	#	#
Other swabs †	11	11 (100%)	11 (100%)	8 (73%)	3 (27%)	~
*	Out of the 35 isolates which were resistant to Clindamycin, 16 had resistance induced by Erythromycin which was detected by D zone testing.					
~	No Susceptibility and Penicillin drug.					
#	Not Tested - Erythromycin and Clindamycin are poorly concentrated in urine, Linezolid is a key antibiotic for urinary tract infections caused by Vancomycin Resistant Enterococci (VRE).					
†	Includes High vaginal swab, Ear and nasal swab, Conjunctival swab and Throat swab.					

E-test was obtained by Cefoxitin and Vancomycin MIC [Fig-2]. All had Cefoxitin MIC of 8 and above, 49% had an MIC value of >64. MIC50 was 16. Vancomycin MIC was 1.5 and below for all isolates. MIC ranged from 0.19 to 1.5. Vancomycin MIC50 is 0.38 and Vancomycin MIC90 is 0.25. Maximum MIC was 1.5 found in one isolate, which is again in the susceptible range.

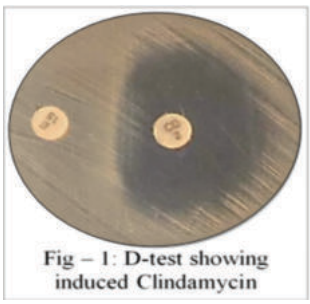

Fig – 1: D-test showing induced Clindamycin

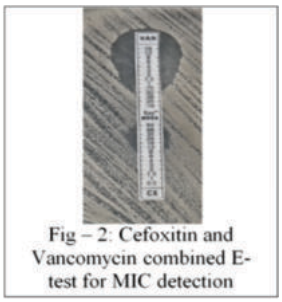

Fig – 2: Cefoxitin and Vancomycin combined E-test for MIC detection

DISCUSSION:

Out of 200 *S. aureus* isolated, 78 were Methicillin Resistant (39%). Overall prevalence of MRSA in India was found to be 30 to 80% [18, 19]. This present study correlates with the world statistics data of MRSA were more common in males (58%) than females (42%). Majority of the samples were pus swabs (56/78). Maximum number of MRSA isolates was from Orthopaedic department (26/78). Regarding the antibiogram of MRSA isolates using disc diffusion method, all were susceptible to Linezolid. 60% of isolates were susceptible to Cotrimoxazole. Erythromycin susceptibility was much lower; only 6 out of 77 isolates (not tested in one sample of urine) were susceptible. Clindamycin also was not checked in urine isolate but 43 isolates from other samples were susceptible. Out of the 35 isolates which were resistant to Clindamycin, 16 had resistance induced by Erythromycin. So out of 78 MRSA isolates, 16 had induced Clindamycin resistance (21%). The

present study data were correlated with the previous study, which showed it as 30% in MRSA [20]. Similarly other studies showed 24.4% and 22.6% in MRSA, respectively [21, 22].

Regarding the MIC detection with E-test, MRSA isolates detected by disc diffusion method were confirmed as the same with MIC method based Cefoxitin MIC of 8 and above. So the Vancomycin MIC was 1.5 and below for all isolates which were in the susceptible range. Vancomycin MIC50 is 0.38 and Vancomycin MIC90 is 0.25 [23, 24]. A 5.6% and 1.7% isolates had MIC in the range of 2.5-3.5 and 4 μg/mL, respectively. MIC50 and MIC90 of these isolates by E-test were 0.75 and 2 μg/mL, respectively. In a study conducted by Chaudhari CN et al., 92% of MRSA strains had a Vancomycin MIC of ≤ 2 μg/mL. In a study by Kumari J et al., Vancomycin MIC as detected by E-test ranged from 0.75-4 μg/mL [25]. A 4.1% of the studied MRSA strains were Vancomycin intermediate (VISA, Vancomycin MIC 4 μg/mL). MIC90 and MIC50 by E-test were 3 μg/mL and 2 μg/mL respectively. They concluded that E-test can be used to determine Vancomycin MIC in the intermediate zone even minor changes in MIC and study "MIC creep". MIC90 and MIC50 were higher compared to the present study. Overall, the prevalence of MRSA according to this study correlates with national and international statistics. Induced Clindamycin resistance, also is routinely tested in the laboratory by the D zone test, as missing of this type of resistance may lead to therapeutic failure by Clindamycin. There were no Vancomycin intermediate or Vancomycin resistant strains obtained in this study which could easily be found out with the E-test method used here.

CONCLUSION:

From the present study, it was concluded that the percentage of MRSA out of *S. aureus* isolates was 39%. Pus swabs were the major sample. All the MRSA isolates had a Vancomycin MIC ≤1.5 μg/mL which was determined by using Vancomycin E-test. All the isolates were in susceptible range. Using an E-test has the advantage of detecting even minor changes in MIC. It is recommended that there should be a continued surveillance to detect the changing patterns of reduced Vancomycin MIC among MRSA isolates from clinical samples to evaluate the clinical outcomes of serious MRSA infections.

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