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Prevalence of Methicillin Resistant *Staphylococcus aureus* (MRSA) in different clinical samples in tertiary hospital Piparia, Vadodara, Gujarat.

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ABSTRACT:

Background: Methicillin resistant *Staphylococcus aureus* (MRSA) is constantly occur and is a major trouble for tertiary care centre. Aims of this study are detection prevalence rate and antibiotics sensitivity of MRSA in our tertiary care centre. **Material & method:** Present study was undertaken from January 2021 to December 2022, at the Clinical Microbiology Laboratory, Dhiraj Hospital, Piparia. The entire clinical specimens were processed as per laboratory standard protocol. Indentified of organisms and antimicrobial susceptibility was done by biochemical reaction and automated VITEK 2 compact system. Out of all isolated organisms only *Staphylococcus aureus* (*S. aureus*) were included in this study. Screening for MRSA was done by cefoxitin disc diffution test, cefoxitin and oxacillin screening done also by automated VITEK 2 compact system (BioMérieux, France). **Result:** During study period, *S. aureus* were isolated from 257 different samples; which include pus and wound swab, blood, sputum, urine and Miscellaneous (Body fluid, endotracheal secretion, tissue, implants), out of this 117 (45.53%) were identified as MRSA. Most of MRSA cases in old age patients (>60 years of age) which was 36.8%. Hospital admitted cases affected mostly with MRSA which was 71.8% with highest in isolates from pus samples (57.3%) and from orthopedics ward (39.3%). Vancomycin, linezolid, and teicoplanin were highest sensitivity against MRSA which were 100%, 93.2% and 94.9% respectively. **Conclusion:** Screening of MRSA and their antibiogram in tertiary care hospital is very essential for early detection of MRSA and for management of the condition.

Keywords: MRSA, Vancomycin, Antibiotic sensitivity, Pus

INTRODUCTION: *Staphylococcus aureus* (*S.aureus*) is fall in family *Micrococcaceae*. They are gram-positive spherical cocci, arranged characteristically in grape like clusters, due to cell division occurring in three planes, with daughter tending to remain in close to each other. Infections caused by *S. aureus* include, skin lesion such as abscesses and carbuncles, and other infections such as osteomyelitis, pyoderma, pneumonia, endocarditis and septicemia [1].

Methicillin resistance develop by mutation in *mecA gene* present in *S. aureus* , which make changes *to* penicillin-binding protein (PBP) present on *S. aureus* cell membrane to PBP2a that is absent in susceptible *S. aureus* strains [2, 3]. That's why β-lactam antibiotics is become ineffective for MRSA and therefore allows MRSA to grow in their presence [3].

After *S. aureus* acquired resistance for penicillin, methicillin usage increase in hospital which emerge of MRSA in 1960 and it increases in world [4, 5]. Clindamycin was used as alternative in treatment of MRSA, but clindamycin resistance cases were also increase in last 10 year [6].

Now a day vancomycin widely used antibiotic for management of MRSA [7]. But resistance towards to vancomycin has been reported in many studies [7]. Further, vancomycin was reported ineffective for MRSA which has minimum inhibitory concentration (MIC) value of vancomycin just below cutoff value [7]. MRSA is resistant to all of available *β*-lactam antibiotics and occurrence of resistance to quinolones, aminoglycosides, and macrolides also increase [8–11].

The prevalence of MRSA continually rises throughout world. Previously, MRSA cases were reported early in only hospital setup but after that community based MRSA cases also increasing rapidly. Now, MRSA strains recategorized in community-associated MRSA (CA-MRSA) strains and hospital-associated MRSA (HA-MRSA) [12-14]. CA-MRSA isolates are usually less resistant than HA-MRSA but resistance increasing rapidly in CA-MRSA also a major concern [15].

In India, MRSA cases rapidly rise with time by time and expand very quickly across the country which was reported by many studies [16-18]. As per, INSAR group, MRSA prevalence was 41% during 2008 to 2009 [19]. In India, MRSA prevalence in *S. aureus* is between 40% to 70%. [20-23]

In European Antimicrobial Resistance Surveillance Network report 2018, MRSA prevalence in *S. aureus* is between 16% to 44%. [24, 25]

MATERIAL AND METHOD: This study was done from January 2021 to December 2022, at the Clinical Microbiology Laboratory, Dhiraj Hospital, Piparia, Vadodara (Gujarat). The patient samples were collected by proper aseptic precaution by recommended methods. All the collected clinical samples were processed using standard operating procedure of laboratory and inoculated into sheep blood agar, mannitol salt agar, and nutrient agar plates, which were then incubated aerobically at 37°C for 24 hours. Next day, identified the microbes based on Gram staining, colony characteristics. After that isolated colonies were processed for various biochemical tests like catalase, slide and tube coagulase test. Final identification and antibiotic susceptibility test was done by VITEK 2 compact system (bioMerieux) as per manufacturer's instructions. For Gram positive organism VITEK® 2 GP card was used and antimicrobial sensitivity done by ready-to-use, flexible VITEK® 2 AST P628 card.

MRSA screening was done by cefoxitin disc diffusion test by using 30µg cefoxitin antibiotics disc (Himedia). After making 0.5 McFarland turbidity standard solutions of *S. aureus* spread this solution on Muller Hinton Agar plate (MHA) with sterile swab. After MHA plates is dried the cefoxitin antibiotics disc were placed in centre and which were then incubated aerobically at 37°C for 24 hours. Next day inhibition zone were measured, interpretation of result done by as per CLSI guidelines 2021.[26] Cefoxitin and oxacillin screening also done by VITEK 2 compact system (bioMerieux) with using ready-to-use, flexible VITEK® 2 AST P628 card [27].

RESULTS: During study period, *S. aureus* was isolated from 257 different samples which were processed between from January 2021 to December 2022. Out of this, 117 (45.53%) were MRSA. [Figure 1] So, prevalence of MRSA was 45.53%

Out of total MRSA isolates, majority cases were isolated from inpatient (84; 71.8%) than outpatient (33; 28.2%). [Figure 2] Most of MRSA were isolated from old age patients (>60 years of age) which was (43; 36.8%) followed by 0–15 years age group patients (29; 24.8%), 46–60 year of age group (21; 17.9%), 31–45 year age group (14; 12.0%), and minimum in 16–30 year age group (10; 8.5%) as mention in [Table 1]. MRSA was isolated from the patients of orthopedic (46; 39.3%) followed by intensive care units (34; 29.1%), surgery (28; 23.9%), pediatrics (04; 3.4%) and medicine (03; 2.6%). [Table 2] Majority isolates of MRSA were detected in pus samples (67; 57.3%) followed by blood (27; 23.1%), urine (9; 7.7%), and sputum (6; 5.1%), samples as mention in [Table 3].

The result antibiotic sensitivity testing of *S. aureus* mention in [Table 4]. Penicillin was resistant in all *S. aureus* isolates. Resistance against erythromycin and clindamycin were 161 (62.6%) and 103 (40.1%) respectively and inducible clindamycin resistance were detected in 53 isolates, which were confirmed by Ready-to-use, flexible VITEK® 2 AST P628. Ciprofloxacin, Co trimoxazole and Gentamicin were resistant in 71.2%, 38.5% and 42.8% respectively. Antibiotic sensitivity for vancomycin, linezolid, and teicoplanin were 100%, 96.9% and 97.7% respectively.

Antibiotic sensitivity pattern of MRSA also studied which were shows that maximum resistant of MRSA isolates to Ciprofloxacin (74.4%), Erythromycin (67.5%), Gentamicin (56.4%), Clindamycin (53%) and Cotrimoxazole (45.3%). Vancomycin, linezolid, and teicoplanin has maximum sensitivity against MSRA isolates which were 100%, 93.2% and 94.9% respectively. [Table 5]

Figure 1: Prevalence of MRSA

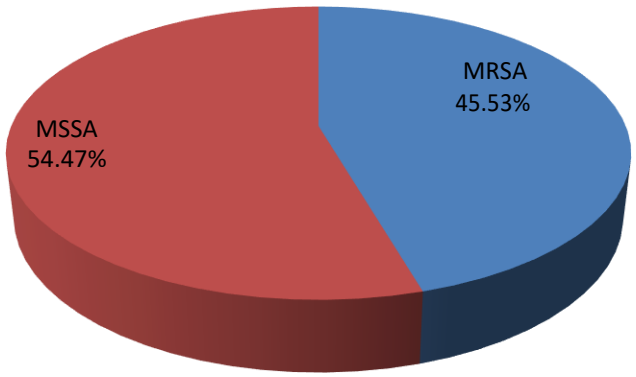

Figure 2: MRSA case distribution in OPD and IPD patients

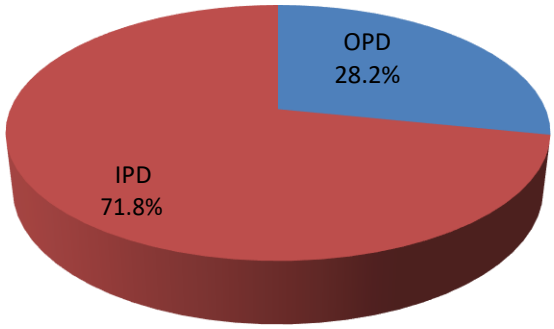

Table 1: Distribution of MRSA in different age group

Age groups	MRSA (%) (n=117)
0-15	29 (24.8%)
16-30	10 (8.5%)
31-46	14 (12.0%)
46-60	21 (17.9%)
>60	43 (36.8%)

Table 2: Distribution of MRSA in different wards

Ward	MRSA (%) (n=117)
Orthopedic	46 (39.3%)
Intensive care unit	34 (29.1%)
Surgery	28 (23.9%)
Pediatrics	4 (3.4%)
Medicine	3 (2.6%)
Other	2 (1.7%)

Table 3: Distribution of MRSA according to clinical samples

Type of sample	MRSA (%) (n=117)
Pus and wound swab	67 (57.3%)
Blood	27 (23.1%)
Urine	9 (7.7%)
Sputum	6 (5.1%)
Miscellaneous (Body fluid, endotracheal secretion, tissue, implants)	8 (6.8%)

Table 4: Antibiotic sensitivity pattern of *Staphylococcus aureus*

Antibiotics	Sensitive (n=257)	Resistant (n=257)
Penicillin	0	257 (100%)
Oxacillin screening	140 (54.5%)	117(45.5%)
Cefoxitin screening	140 (54.5%)	117(45.5%)
Erythromycin	96 (37.4%)	161 (62.6%)
Clindamycin	154 (59.9%)	103* (40.1%)
Ciprofloxacin	74 (28.8.%)	183 (71.2%)
Co trimoxazole	158 (61.5%)	99 (38.5%)
Gentamicin	147 (57.2%)	110 (42.8%)
Linezolid	249 (96.9%)	4 (3.1%)
Teicoplanin	251 (97.7%)	2 (2.3%)
Vancomycin	257 (100%)	0

*103 isolates were resistant to Clindamycin among this 53 isolates had also inducible Clindamycin resistance

Table 5: Antibiotic sensitivity pattern of MRSA

Antibiotics	Sensitive (n=117)	Resistant (n=117)
Erythromycin	38 (32.5%)	79 (67.5%)
Clindamycin	55 (47.0%)	62 (53.0%)

Ciprofloxacin	30 (25.6%)	87 (74.4%)
Co trimoxazole	64 (54.7%)	53 (45.3%)
Gentamicin	51 (43.6%)	66 (56.4%)
Linezolid	109 (93.2%)	8 (6.8%)
Teicoplanin	111 (94.9%)	6 (5.1%)
Vancomycin	257 (100%)	0

DISCUSSION:

MRSA prevalence in our study was 45.53% which was compared with different study. Prevalence of MRSA was between ranges of 33.7% to 69.1%. [Table 6] Many factor responsible for variations of prevalence in different studies like different geographical status, vary in sample sizes and duration of study, vary in clinical samples, methods using in testing, antibiotic using policy vary in different hospital, and status of hospital acquired infection.

Most of MRSA were isolated from old age patients (>60 years of age) and children in our study which is similar result which was reported in Lohan K et al. [37]. [Table 7] Maximum MRSA isolated in hospital admitted patients as compared to OPD cases which could be due to contaminated hospital environment, similar finding were seen in different study likes Kumari N et al.[33], Kandhakumari Gandhi et al.[35], Lohan K et al. [37], Bhatt C et al.[39] and Soumya Kaup et al.[40] [Table 8]

In our study, we found that majority MRSA isolates were from orthopedics followed by intensive care units then surgery, and then rest of wards. Similar patterns were found in Arora S et al. [29], Kumari N et al.[33]and Lohan K et al. [37] [Table 10] Majority isolates of MRSA were detected in pus samples (28; 77.8%), may be due to open wound get exposed to bacteria present in the surrounding conditions and on skin normal flora. Similar findings were found in SH, G et al. [29], Kumari N et al. [33] and Lohan K et al. [37] . [Table 11]

Antibiotics sensitivity pattern of MRSA was compared with different studies done previsioly which was mention in table 12. Among the MRSA, maximum resistance found for Ciprofloxacin, Erythromycin, gentamicin, clindamycin and cotimoxazole. And most of MRSA were found sensitive to vancomycin, linezolid, and teicoplanin which were comparable with different studies.

Table 6: Comparison prevalence of MRSA with different study

Different study	Prevalence of MRSA	Year
Present Study	45.5%	2022
Arora S[28]	46.0%	2010
Chaudhury, N[29]	57.82%	2022
Tiwari HK[30]	69.1%	2009
Adhikari R[31]	35.50%	2017
Lohan K[32]	33.7%	2021
Soumya Kaup[33]	45.42%	2017

Table 7: Age group wise comparison prevalence of MRSA with different study

Different study	Present Study	Lohan K[32]
0-15	29 (24.8%)	22.2%
16-30	10 (8.5%)	12.3%
31-46	14 (12.0%)	14.8%
46-60	21 (17.9%)	16%
>60	43 (36.8%)	34.6%

Table 9: Distribution of MRSA in OPD and IPD

Different study	Present Study	Lohan K[32]	Bhatt, C[34]	Soumya Kaup[33]
OPD	28.2%	24.7%	26.3%	27.52%
IPD	71.8%	75.3%	73.6%	72.48%

Table 10: Comparison prevalence of MRSA with different study according to wards

Different study	Present Study	Arora S[28]	Lohan K[32]
Orthopedic	36.1%	27.82%	23.4%
Intensive care unit	27.8%	-	24.6%
Surgery	25%	9.56%	25.9%
Pediatrics	8.3%	24.34%	4.9%
Medicine	2.8%	17.39%	11.1%

Table 11: Comparison prevalence of MRSA with different study according to clinical samples

Different study	Present Study	Lohan K[32]	Bari [36]
Pus and wound swab	67 (57.3%)	61.70%	45.6
Blood	27 (23.1%)	12.30%	13.6
Urine	9 (7.7%)	23.40%	13.4
Sputum	6 (5.1%)	-	11.7

Table 12: Comparison antibiotics resistant pattern of MRSA with different study

Different study	Present Study	Lohan K[32]	SH, G.[34]	Kandhakumari[35]
Erythromycin	67.5%	76.5%	68%	86.9%
Clindamycin	53.0%	66.7%	52%	50.4%

Ciprofloxacin	74.4%	59.2%	75%	60.7%
Cotrimoxazole	45.3%	53.1%	48%	73.8%
Gentamicin	56.4%	46.9%	75%	-
Linezolid	6.8%	7.4%	0	2.8%
Teicoplanin	5.1%	12.3%	2%	13%
Vancomycin	0	12.3%	0	0

CONCLUSION:

Centre of attention of our study is on continues screening and evaluate antibiotics sensitivity of MRSA in tertiary care hospital. For prevention of MRSA, it is very necessary to regular screening of health care workers, development hand hygiene and aseptic clinical practice and implementation of antibiotic policy in tertiary care. Early detection of MRSA and its susceptibility pattern is essential in management of the condition.

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