Original Research Paper

Microbiology

A PROSPECTIVE STUDY ON PREVALENCE AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF MRSA ISOLATED FROM ALL CLINICAL SAMPLES IN A TERTIARY CARE HOSPITAL IN NORTHERN KERALA.

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ABSTRACT

Introduction: Methicillin resistant Staphylococcus aureus (MRSA) has become one of the challenging factors in health care settings and community settings. Resistance to non-beta lactam antibiotics also made the situation worsen.

Aim:
- To study the prevalence of MRSA in all clinical samples taken for culture.
- To evaluate the antibiotic susceptibility pattern of MRSA.

Results: A total of 67 MRSA were isolated from 2609 clinical samples. Maximum isolates were from pus 44 (70%). The highest (100%) sensitivity was observed with Vancomycin, linezolid and teicoplanin and lowest (28.4%) with Erthromycin was observed. **Conclusion:** Educating primary health physicians about MRSA is essential to implement effective control measures like Strict adherence to infection control practices, including hand hygiene, preventing the overuse of antibiotics and continues surveillance program for MRSA

INTRODUCTION

Methicillin-resistant Staphylococcus aureus (MRSA) is a leading cause of infection among patients admitted in hospitals[1]. It is a common pathogen of hospital infection with multi-drug resistant characteristics[2]. MRSA have been involved in serious skin infections, necrotizing fasciitis, deep tissue abscesses, and their hematogenous spread can result in bone and joint infections, sepsis and endocarditis[3].The risk factors for emergence of MRSA include carriage of MRSA in nose, axilla, perineum and hands of patients and health care workers (HCWs), longer hospital stay, unsound use of antibiotics, presence of indwelling devices like catheter and cannulas, immunosuppressive conditions etc[4]. Along with these factors diversity in mecA gene pose a major challenge to prevent the spread of this agent in community and hospitals[5]. Knowledge of MRSA prevalence and their antimicrobial profile in a health care set up is necessary to implement control measures for these infections and reduce the usage of second line antimicrobials[5]. Therefore, we planned to study the prevalence and antibiotic susceptibility pattern of MRSA isolated from all clinical samples in a tertiary care hospital in Northern Kerala. As a result of this study, the empirical therapy schedule was recommended to the clinicians and heled to implement stringent hospital infection control program.

MATERIALS & METHODS (Methodology):

This cross-sectional study was conducted for a period of one year from April 2023 in Department of Microbiology, Malabar medical college Kozhikode after obtaining permission of Institutional Ethics Committee with letter no MMCH&RC/IEC/2023/36. The clinical samples received in the microbiology department were included in the study.

Inclusion Criteria

All clinical samples sent to the bacteriology laboratory during the study period of one year were included.

Exclusion Criteria

- Improperly labelled samples and any repeat isolate from the same patient received on more than one occasion
- Methicillin sensitive Staphylococcus aureus,
- Coagulase negative Staphylococcus aureus
- Other organisms are excluded from this study

Methods Including
Method of Sample Collection

Blood samples will be collected under aseptic precautions in blood culture bottles. Urine specimens will be collected in universal containers. Respiratory samples like sputum and endotracheal aspirates will be collected in sterile, wide mouth disposable containers. Pus from superficial wound samples will be collected with swab and from deep wounds will be aspirated and collected in sterile small screw capped bottles. The containers will be labelled with the name of patient, identification number, culture site, date of collection and time of collection.

Sample Processing: The samples will be inoculated on Blood agar and Mac Conkey's agar medium. The media will be incubated at 37^0C for 24-48 hours. The isolates will be identified and its antibiotic susceptibility pattern will be performed by automated method (VITEK-2). MIC breakpoints for 30µg of Cefoxitin disk will be considered as sensitive if MIC value will be ≤ 4 for S.aureus.

Statistical Analysis

Data was entered in MS Excel and statistical analysis was done by using SPSS 20.0. Numerical variables were present as mean ±SD and categorical variables was present as frequency and percentage.

Chisquare test is used to find the association between risk factors and outcome. Statistical significance was defined for $p<0.05$. Test results are presented both graphically and in tabular form.

RESULTS

A total of 6247 samples were received and processed for bacterial culture in the laboratory during the study period. 2609 (41.8%) isolates were obtained from 6247 samples. Out of the 2609 isolates 67 (2.6%) were MRSA.

Figure 1: Sample size

■ positive sample ■ MRSA ■ ■

Out of 67 samples detected for positive MRSA, the maximum 44 was from pus specimen and the lowest was from throat swab and high vaginal swab (Table 1).

Table 1- Distribution of MRSA Based on Type of Specimen

Specimen	N (%)
Pus	44
Blood	10
Urine	5
Sputum	4
Tissue	2
Throat swab	1
High vaginal swab	1
Total	67

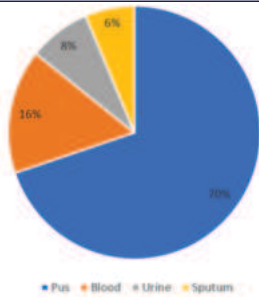**Figure 2: Distribution of MRSA Based on Type of Specimen**

Table 2 - IPD Versus OPD Distribution

Specimen	IPD (n%)	OPD (n%)
Pus	34 (50.7%)	10 (14.9%)
Blood	9 (13.4%)	1 (1.5%)
Urine	4 (5.9%)	1(1.5%)
Sputum	3 (4.4%)	1(1.5%)
Tissue	2(2.9%)	
Throat swab		1(1.5%)
High vaginal swab	1(1.5%)	
Total	53 (79.1%)	14(20.9%)

Majority of MRSA isolates that is 38 (56.7%) were from ward patients. This is followed by 14 (20.9%) from OP patients, 13 (19.4%) from ICU patients, and 2 (2.9%) from PICU patients.
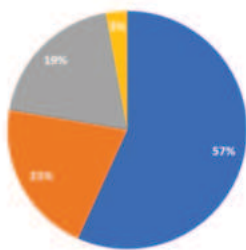

Figure 3: IPD Versus OPD Distribution

MRSA isolated from various age groups are: 0-20yrs (8.9%), 21-40yrs (16.4%), 41-60yrs (31.3%), >60yrs (43.2%). Maximum isolates seen in the age group of >60yrs (43.2%) followed by 41-60yrs of age group (31.3%) [Table3].

MRSA was reported positive in 33 (49.3%) samples in male and 34 (50.7%) in female. It shows more MRSA are more visible in female patients in this study. Out of total 67 positive MRSA patients, 49.3% of male and 50.7% of female patients have MRSA [Table3].

Table 3 - MRSA Isolated From Various Age Groups

Age group (years)	Male (n)	Female (n)	Total (%)
0-20	2 (2.98%)	4 (5.9%)	6 (8.9)
21-40	3(4.5%)	8 (11.9%)	11(16.4)
41-60	13(19.4%)	8 (11.9%)	21 (31.3)
>60	15(22.4%)	14 (20.9%)	29 (43.2)
Total	33 (49.3%)	34 (50.7%)	67 (100)

The sensitivity pattern of all the 67 MRSA isolates to various antibiotics as in the table was done by automated method (VITEK-2). The highest 67 (100%) sensitivity was observed with Vancomycin, linezolid and teicoplanin and lowest 19 (28.4%) with Erthromycin.

Table 4: Susceptibility of MRSA Isolates to Different Antibiotics

Antibiotic	Sensitive (%)	Intermediate (%)	Resistant (%)
Linezolid	100% (67)	-	-
Teicoplanin	100% (67)	-	-
Vancomycin	100% (67)	-	-
Tetracycline	92.5% (62)	-	7.5% (5)
Cotrimoxazole	91% (61)	-	9% (6)
Gentamicin	73% (49)	3% (2)	24% (16)
Clindamycin	56.7% (38)	-	43.3%(29)
Ciprofloxacin	41.8% (28)	6% (4)	52.2%(35)
Erythromycin	28.4% (19)	3% (2)	68.6% (46)

In the hospital Linezolid was the most commonly used antibiotic to treat MRSA patients. Out of 67 patients, Linezolid was administered 21 of them who showed 100% sensitivity to this drug. Gentamicin was the least used antibiotic, only 1 patient was treated with Gentamicin and showed 73% sensitivity. Similarly,18 of the 98 patients were treated with Vancomycin and 11 of the 98 patients were treated with Cotrimoxazole, which showed 100% sensitivity to Vancomycin and 91% sensitivity to Cotrimoxazole. 8 out of 98 patients were treated with Clindamycin and Ciprofloxacin which showed 41.8% and 56.7% sensitivity respectively.

Table 4: Commonly Used Antibiotic to Treat MRSA Patients

Number of patients treated	Antibiotic used	Sensitivity (%)
21	Linezolid	100%
18	Vancomycin	100%
11	Cotrimoxazole	91%
8	Ciprofloxacin	41.8%
8	Clindamycin	56.7%
1	Gentamicin	73%
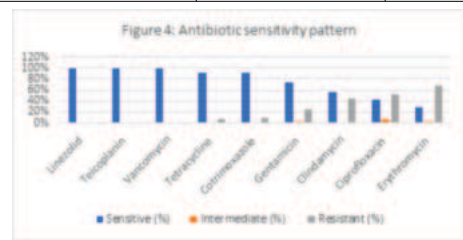
Among the 67 MRSA patients 17 patients were on invasive device like Foley's catheter and 3 patients were on Central line.

DISCUSSION
The MRSA has increased many fold throughout the world, India is no exception for the increase of emergence of this pathogen. This study is focused on studying the susceptibility of MRSA isolated from various clinical samples to Vancomycin and other antibiotics used in the treatment of Staphylococcus aureus.

67 clinical isolates of MRSA from various clinical samples were studied for their susceptibility pattern to drugs including Vancomycin, Teicoplanin, and Linezolid. The prevalence of MRSA of this study was 2.6% which was less than that had been reported by Singh P et al [6]. Different studies have shown variations in the prevalence rates of MRSA, in a study conducted by Dilnessa T et al the prevalence of MRSA was 17.5% [7] and study conducted by M J, Rathinam KK et al the prevalence of MRSA was 37% [8].

The present study depicted that the prevalence of MRSA was isolated from pus (70%) was the highest as compared to other clinical samples and it was compared with the study conducted by Dilnessa T et al the isolation rate of MRSA from pus sample was 55.4% [7]. This was also compared with the study conducted by Singh P et al prevalence of MRSA from pus was 41.3% [6]. Staphylococcus. aureus present on skin as commensal makes the wound more prone for infection.

In our study maximum MRSA isolates was from ward (56.7%). Similar results (54.35%) were seen in a study conducted by Singh P et al in 2022 [6]. The prevalence rate of MRSA from ICU in this study is 19.4% which is lower than the study conducted by Sangwan J et al (24.6%) [5]. The differences in the rate maybe due to good infection control practice in our hospital.

Prevalence of MRSA Among Different Age Groups
Relationship between age and MRSA prevalence was studied it was found that patients in age group of >60yrs, there was maximum prevalence of MRSA (43.2%) which is more than the study conducted by Sangwan J et al in 2019 (34.6%). This is due to various comorbid conditions like Diabetes mellitus and hypertension.

Susceptibility of MRSA to Different Antibiotics
Considering the antibiotic susceptibility pattern of MRSA, all 67 isolates were resistant to antibiotic Penicillin similar to the study of Gupta S et al [9]. In our study all the 67 MRSA isolates showed 100% sensitivity to Vancomycin, Linezolid and Teicoplanin.

There are studies reported about vancomycin resistant MRSA [10] but in this study no vancomycin intermediate Staphylococcus aureus (VISA)

or vancomycin resistant Staphylococcus aureus was found. The present study showed 100% sensitivity to Vancomycin. Similarly, there are also studies reported about Linezolid Resistant Staphylococcus aureus[11] but in our study the Linezolid susceptibility pattern is 100%. A study conducted by Cepeda, J.A in 2004 found that Teicoplanin therapy was clinically and microbiologically as effective as vancomycin therapy for patients admitted with methicillin-resistant staphylococcal infections [12]. It is a good alternative to Vancomycin for the treatment of MRSA infection. In our study all the 67 isolates of MRSA showed 100% sensitivity to Teicoplanin. This may be due to the reduced usage of this drug in our hospital as it is a reserve drug.

Higher sensitivity pattern was observed for gentamicin (93.9%)[13] in a study conducted Garoy EY et al in 2019 but in the present study 73% of MRSA isolates were sensitive to Gentamicin. In a study conducted by Qureshi AH et al[14] the sensitivity rate of Gentamicin was 97.8% which is also higher than the sensitivity rate of our study. This may be due to differences in antibiotic prescription practices between countries.

The present study showed 92.5% of MRSA isolates were sensitive to tetracycline which is more than the study conducted by Qureshi AH et al (88.6%) [15]

In the present study 28.4% and 56.7% of MRSA isolates were sensitive to Erythromycin and Clindamycin respectively which was compared with the study done by Indian Network for Surveillance of Antimicrobial Resistance (INSAR) group, India in 2011 who showed a sensitivity of 29.2% and 53.4% respectively[16].

This study showed 41.8% of MRSA isolates were sensitive to Ciprofloxacin which is lower than the study conducted by Nirwan, P et al (52.73%) [15] which is due to the increased usage of this particular antibiotic in our hospital.

Sensitivity rate of Cotrimoxazole also compared with study conducted by Shugufta Roohi et al (80%) [17]

In our study out of 67 MRSA isolates 21 patients were treated with Linezolid showed 100% sensitivity, in a study conducted by Peter Wilson et al [11] reported Linezolid resistance reported after Linezolid treatment. The development of resistance to linezolid following treatment indicates that use of this antibiotic should be carefully monitored particularly in deep-seated infections.

There was a chance of MRSA if patients have comorbid conditions like usage of invasive devices, in our study out of 67 MRSA patients 17 patients were on invasive devices and this was compared with the study conducted by Gupta S et al [9].

CONCLUSION
The present study suggests teicoplanin and linezolid as alternatives to vancomycin in cases with higher MICs and associated with clinical failure, for the treatment of MRSA infection. Attention should be paid to minimise the risk factors like usage of invasive devices, antibiotic usage and hospitalisation only when indicated. The key measures to MRSA control is early treatment of MRSA infections and the following of good infection control practices. There are only limited drugs available for the treatment of MRSA, irrational use of antibiotics should be avoided and a rational antibiotic policy must be adopted.

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