Prevalence and Susceptibility Pattern of Methicillin-Resistant Staphylococcus aureus (MRSA) in Rural Kerala: A Tertiary Care Hospital Study

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A B S T R A C T

Infections with Staphylococcus aureus continue to be a major problem in the world. Prompt diagnosis is essential to avert the further complications of this infection particularly Methicillin-resistant Staphylococcus aureus (MRSA) when compared to Methicillin-sensitive Staphylococcus aureus (MSSA). This retrospective study was done from January 2016 to June 2018 in a tertiary care hospital to know the prevalence of MRSA infections in both the inpatients as well as out patients. Of the 403 total isolates of S. aureus the overall prevalence of MRSA in our study was 42.9%, majority of them were accounted by skin and soft tissue infections (SSTI) followed by urinary tract infections, blood stream infections and respiratory infections. The study showed an increased prevalence of MRSA among the inpatients when compared to the outpatients. A single intervention will unlikely to have a significant impact on attenuating MRSA infection rates. Multifactorial bundled approach is the need of the hour in containing the MRSA infections along with robust antimicrobial stewardship and prompt infection control measures and judicious use of the drugs of choice for MRSA infection which have to be preserved for the future use.

Keywords
Prevalence, Antibiotic, sensitive, Resistant, MRSA, MSSA

Introduction

Hospital-associated infections and community-acquired infections caused by Staphylococcus aureus continue to be a dangerous threat for the outcome of the patient. Methicillin resistant was reported to S. aureus soon after its introduction in October 1960 (Jevons, 1961). Between regions and between hospitals in the same region the prevalence of MRSA varies (Gadepalli et al., 2009) and it is endemic worldwide in hospitals (Goetghebeur et al., 2006). One of the leading causes of healthcare associated infection is MRSA (Dantes et al., 2011; Kaye et al., 2009). MRSA has been estimated to cause over 80,000 “invasive” infections and over 11,000 deaths in the United States according to Centers for Disease Control and Prevention (CDC) in 2011 (Dantes et al., 2011). The health care cost attributable to MRSA infection is enormous. In order to prevent the enormous economic burden and health care cost to the
individuals with MRSA infection each and every hospital should have the prevalence rates for MRSA infections. This study was carried out to know the prevalence rates of MRSA infections in a tertiary care hospital which will help in taking the appropriate measures in controlling and spreading of MRSA infections in the hospital and will act as a baseline data in deciding the appropriateness of the control measures taken.

**Materials and Methods**

**Patients and hospital setting**

This study was done at a tertiary referral hospital with 750 beds. In this retrospective study, all the susceptibility data for *S. aureus* from January 2016 to June 2018 were analyzed. The study population was involved outpatient or inpatient (including Intensive Care Unit) patients.

**Sample collection and laboratory analysis**

Using standard precautions different samples from the various departments of the hospital were collected and transported to the microbiology laboratory for the isolation and susceptibility testing. The antibiotic susceptibility testing was performed by the Kirby Bauer’s’ disc diffusion technique, using Clinical and Laboratory Standards Institute (CLSI) recommendations. Cefoxitin (30 μg) was used for methicillin resistance.

The other antibiotics tested included penicillin (10 units), gentamicin (10 μg), co-trimoxazole (1.25/23.75 μg), ciprofloxacin (5 μg), erythromycin (15 μg), clindamycin (2 μg), vancomycin (30 μg) and linezolid (30 μg). Discs from Hi-media were used by the laboratory. Inoculum was prepared by making a direct saline suspension of isolated colonies selected from an 18- to 24-h blood agar plate. Turbidity of the suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard and five discs were applied on a 100mm Mueller Hinton agar plate as per CLSI guidelines. *S. aureus* ATCC 25923 was used as the quality control strain for disc diffusion.

**Variables measured**

The following information age, gender, specimen, source of referral (Outpatient, inpatient, IUC), along with the antibiotic susceptibility data for *S. aureus* was collected in a structured excel sheet.

**Definitions**

According to CLSI guidelines 2016 (Clinical and Laboratory Standards Institute, 2016)

**MSSA**– zone diameter ≥ 22 using cefoxitin (30μg) disc, Kirby Bauer’s’ disc diffusion technique

**MRSA**– zone diameter ≤ 21 using cefoxitin (30μg) disc, Kirby Bauer’s’ disc diffusion technique

**Statistical analysis**

SPSS version 16.0 for Windows (SPSS, Inc., Chicago, IL) was used for all data analysis.

Continuous variables are expressed as means and range. Simple proportions were calculated to estimate the hospital-based prevalence of MRSA and MSSA.

**Qualitative data**

For assessing difference between proportions, Pearson’s chi-square test was used as a test of significance. When expected value in a cell was less than 5 in a 2x2 table, Fisher's exact test was used as a test of significance. When expected value in a cell was less than 5 in
other tables, likelihood ratio test was used a test of significance. P values less than 0.05 were considered statistically significant.

**Results and Discussion**

Of the 403 total isolates of *S. aureus* detected from January 2016 to June 2018, 125 (31%) were detected in 2016, 205 (50.9%) were detected in 2017 and rest 73 (18.1%) in 2018. The majority of the isolates were obtained from males 241 (58.9%) rest were from female patients. Mean age of the patient was 47 years. The sample was collected from 1-day old baby to 89-year-old adult patient. Thus, specimen was collected from wide age range of the patient. Majority of patients were referred from General Surgery department, followed by General Medicine, Orthopedics, Pediatrics, Urology, rest all other department had referred less than 5% of patients (Figure 1).

The prevalence of MRSA was 43.2%, 43.9% and 39.7%, for year 2016, 2017 and 2018 respectively (Table 2). The overall period prevalence was 42.9%. The prevalence of MRSA was high in inpatients compared to the outpatients for all the specimens (Table 3). All patients from different intensive care units and operation theater were considered as in patient for analysis purpose.

Antibiotic susceptibility testing data for azithromycin (15µg), amikacin (30µg), ciprofloxacin (5µg), clindamycin(2µg), co-trimoxazole (23.75/1.25 µg), erythromycin (15µg), gentamycin (10µg), levofloxacin (5µg), linezolid (30µg), norfloxacin (10µg), nitrofurantoin (300 µg), ofloxacin (5µg), penicillin (10 units), tetracycline (30µg), tigecycline (15µg), teicoplanin (30µg) and vancomycin (30µg) were compiled. There was no resistance documented against tigecyclin, teicoplanin, vancomycin and linezolid. Resistance to antibiotics amongst the MRSA isolates was more than that in methicillin sensitive *S. aureus* (MSSA) (*P*<0.05) (Table 4).

| Source                        | No. of *S. aureus* isolates | Percent |
|-------------------------------|-----------------------------|---------|
| In Patient (IP)               | 276                         | 68.5    |
| Out Patient (OP)              | 108                         | 26.8    |
| Medical Intensive Care Unit (MICU) | 7                          | 1.7     |
| Surgical Intensive Care Unit (SICU) | 5                          | 1.2     |
| Neonatal Intensive Care Unit (NICU) | 4                          | 1       |
| Operation Theatre (OT)        | 2                           | 0.5     |
| Pediatric Intensive Care Unit (PICU) | 1                          | 0.2     |
| Total                         | 403                         | 100     |

| Type of the Specimen          | No. of Specimen | Percent |
|-------------------------------|-----------------|---------|
| Pus                           | 306             | 75.9    |
| Urine                         | 52              | 12.9    |
| Blood                         | 26              | 6.5     |
| Sputum                        | 19              | 4.7     |
| Total                         | 403             | 100     |
Table.2 Year wise prevalence of MRSA

| Year | MRSA      | MSSA      | Total |
|------|-----------|-----------|-------|
| 2016 | 54 (43.2) | 71 (56.8) | 125   |
| 2017 | 90 (43.9) | 115 (56.1)| 205   |
| 2018 | 29 (39.7) | 44 (60.3) | 73    |
| Total| 173 (42.9)| 230 (57.1)| 403   |

Table.3 Prevalence of MRSA in different specimen

| Specimen | MRSA  | MSSA  | Total |
|----------|-------|-------|-------|
|          | n (%) | n (%) |       |
| Pus      |       |       |       |
| IP       | 87 (40.5) | 128 (59.5) | 215   |
| OP       | 33 (36.3) | 58 (63.7) | 91    |
| Urine    |       |       |       |
| IP       | 28 (73.7) | 10 (26.3) | 38    |
| OP       | 7 (50.0) | 7 (50.0) | 14    |
| Sputum   |       |       |       |
| IP       | 7 (41.2) | 10 (58.8) | 17    |
| OP       | 0 (0.0) | 2 (100) | 2     |
| Blood    |       |       |       |
| IP       | 11 (44.0) | 14 (56.0) | 25    |
| OP       | 0 (0.0) | 1 (100.0) | 1     |
| Total    | 173 (42.9) | 230 (57.1) | 403   |
## Table 4 Antibiotic susceptibility results of *Staphylococcus aureus* 2016-2018

| Antibiotics | Strain | Total Strain type (n) | Sensitive n (%) | Resistant n (%) | Intermediate n (%) | P value |
|-------------|--------|-----------------------|----------------|----------------|-------------------|---------|
| Amikacin    | MRSA   | 173                   | 151 (87.3)     | 21 (13.9)      | 1 (0.57)          | 0.006   |
|             | MSSA   | 230                   | 218 (94.7)     | 9 (4.1)        | 3 (1.3)           |         |
| Azithromycin| MRSA   | 6                     | 1 (16.6)       | 5 (83.3)       |                   | 0.013   |
|             | MSSA   | 12                    | 10 (83.3)      | 2 (20)         |                   |         |
| Ciprofloxacin| MRSA | 171                   | 41 (23.9)      | 128 (74.8)     | 2 (1.1)           | 0       |
|             | MSSA   | 226                   | 100 (44.2)     | 124 (54.8)     | 2 (0.8)           |         |
| Clindamycin | MRSA   | 123                   | 79 (64.2)      | 44 (35.7)      |                   |         |
|             | MSSA   | 186                   | 146 (78.4)     | 40 (21.5)      |                   |         |
| Co-trimoxazole | MRSA | 161                   | 108 (67.0)    | 52 (32.2)      | 1 (0.6)           | 0       |
|             | MSSA   | 211                   | 181 (85.7)     | 30 (14.2)      |                   |         |
| Erythromycin| MRSA   | 115                   | 40 (34.7)      | 75 (65.2)      |                   |         |
|             | MSSA   | 183                   | 112 (61.2)     | 71 (38.7)      |                   |         |
| Gentamycin  | MRSA   | 170                   | 107 (62.9)     | 63 (37.0)      |                   | 0       |
|             | MSSA   | 225                   | 199 (88.4)     | 26 (11.5)      |                   |         |
| Levofloxacin| MRSA   | 172                   | 47 (27.3)      | 125 (72.60)    |                   |         |
|             | MSSA   | 224                   | 117 (52.3)     | 107 (47.7)     |                   |         |
| Linezolid   | MRSA   | 172                   | 172 (100)      | 0              |                   |         |
|             | MSSA   | 227                   | 227 (100)      | 0              |                   |         |
| Nitrofurantoin | MRSA | 35                    | 33 (94.2)      | 2 (5.7)        |                   | 0.203   |
|             | MSSA   | 17                    | 17 (100)       | 0              |                   |         |
| Norfloxacin | MRSA   | 35                    | 2 (5.70)       | 33 (94.2)      |                   | 0.002   |
|             | MSSA   | 17                    | 7 (41.7)       | 10 (58.8)      |                   |         |
| Ofloxacin   | MRSA   | 81                    | 29 (35.8)      | 52 (64.1)      |                   | 0.021   |
|             | MSSA   | 102                   | 54 (52.9)      | 48 (47.0)      |                   |         |
| Penicillin  | MRSA   | 173                   | 0              | 173 (100)      |                   | 0       |
|             | MSSA   | 229                   | 102 (44.5)     | 127 (55.4)     |                   |         |
| Tetracycline| MRSA   | 124                   | 58 (46.7)      | 66 (53.2)      |                   | 0.581   |
|             | MSSA   | 178                   | 89 (50)        | 89 (50)        |                   |         |
| Tigecycline | MRSA   | 127                   | 127 (100)      |                |                   |         |
|             | MSSA   | 198                   | 198 (100)      |                |                   |         |
| Teicoplanin | MRSA   | 138                   | 138 (100)      |                |                   |         |
|             | MSSA   | 213                   | 213 (100)      |                |                   |         |
| Vancomycin  | MRSA   | 173                   | 173 (100)      |                |                   |         |
|             | MSSA   | 230                   | 230 (100)      |                |                   |         |
Throughout the world MRSA has become a widespread pathogen with outbreaks occurring in hospital as well as in community settings (Kil et al., 2008; Boucher et al., 2008; Goetghebeur et al., 2006). A single facility cannot eliminate or control the occurrence of MRSA infection and the prevalence is affected by the measures taken at one facility when compared to the other facility (Lee et al., 2012). Approximately 30% is the colonization rate of S. aureus in the United States (Gorwitz et al., 2008). MRSA colonized patients display a higher risk of developing a MRSA infection (Ramarathnam et al., 2013; Shukla et al., 2009; Gupta et al., 2011; Wenzel et al., 1995; Ziakas et al., 2014). The most frequent pathogen implicated in post-surgical infections was S. aureus (Merre et al., 2007) as well as in a survey conducted in Europe (Sader et al., 2010). In this survey MRSA prevalence ranged from 48.4% in Belgium to 0.4% in Sweden (Sader et al., 2010). There was an increase in the overall incidence of community onset MRSA SSTI (skin and soft tissue infections) in a study spanning over 10 years in US (Tracy et al., 2011).

The overall prevalence of MRSA in our study was 42.9%. Different studies from India have reported the prevalence of MRSA ranging from 40 to 50% (Patel et al., 2010; Gopalakrishnan et al., 2010; Wattal et al., 2010; Varghese et al., 2010; Arora et al., 2010; Sangeeta Joshi et al., 2013). In our study we observed a prevalence of MRSA among the inpatients which was 32%. Among the admitted patients the rate of MRSA colonization in patients ranges from 1.3% to 7.6% (Nixon et al., 2006; Chen et al., 2012; Pofahl et al., 2011) and are at an increased risk of acquiring these infections when they undergo a surgical procedure (Dantes et al., 2011).

The prevalence of MRSA among the outpatients was 11% in our study. Other studies from India had a high prevalence of MRSA amongst the out patients which was 54% (D’Souza et al., 2010). Among the S. aureus isolated from the SSTI, MRSA accounted for 30% in our study whereas two different study from India have documented 35% and 60% of S. aureus isolates respectively from SSTI (Varghese et al., 2010; Sangeeta Joshi et al., 2013). We documented a higher prevalence of resistance to antibiotics in MRSA isolates when compared to MSSA isolates. Significant difference was observed to antibiotics like ciprofloxacin, gentamycin, amikacin, Co-trimoxazole, norfloxacin in our study which correlated with a study from north India.

Antibiotics like glycopeptides, linezolid, should be judiciously used only in MRSA cases. Our study did not document any resistance to these antibiotics. A recent study surveillance of antibiotic resistance in India have also not documented any resistance to glycopeptides and linezolid (Sangeeta Joshi et al., 2013). Our study did not document any resistance to teicoplanin, glycylcylines also which should be preserved for the future use which will be most important in treating the MRSA cases. In all cases of MSSA de-escalation of vancomycin to β-lactams after culture sensitivity report reveals a MSSA isolate which is considered inferior to β-lactams in treating MSSA bacteraemia and endocarditis.

Due to the limited resources the molecular study of the S. aureus isolates was not possible. The minimum inhibitory concentration (MIC) testing was also not possible.

To conclude the study highlighted the multidrug resistance of MRSA isolates when compared to MSSA isolates. As the antibiotics used for treating MRSA infections have not shown any resistance, they showed be cautiously and judiciously used which will make a big difference in the future.
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