ANTIBIOTIC SUSCEPTIBILITY OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN ORTHOPAEDIC PATIENTS IN RURAL SETTINGS

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ABSTRACT:

Introduction: Throughout the world, Methicillin Resistant Staphylococcus aureus (MRSA) infection is a well-documented public health issue. Due to extensive use of antibiotics including beta lactams and others, resistance to antimicrobials has grown over time. Due to the genetic modifications in micro-organisms including MRSA, multiple new strains of resistant bacteria have emerged.

Materials and Methods: 50 patients with infected wounds who came to the outpatient department of AVBRH hospital Sawangi, Wardha. Pus culture sensitivity swabs of all the patients were sent and based on the reports of antibiotic susceptibility patients were classified. Specimen were taken mostly from surgical wounds, abscess pus and trauma patients.

Results: Out of 50 patients 32(64%) patients were found to harbour S.aureus. and out of those 32 patients 23 had MRSA. Patients of age 10-30 years (12 patients) were found to have more chances of infection with MRSA as compared to 30-60 age group (10 patients) and older age group (1 patient). The results also showed that out of 50 patients, 29 were sensitive to vancomycin, 31 patients were sensitive to amikacin, 17 to clindamycin, 16 to ciprofloxacin.

Conclusion: The study concluded that majority of patients with infected wounds harbour MRSA and most of the patients with MRSA infection were susceptible to vancomycin (21 patients 91.30%). Therefore it is advisable to use susceptible antibiotics in infection control practices.

KEYWORDS: Antibiotic susceptibility, MRSA, Staphylococcus Aureus

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INTRODUCTION

Multi drug resistance in staphylococcus strains was initially described in 1960s. In the last decade these strains have grown rapidly as a cause of hospital acquired infections and have caused potentially life threatening conditions such as pneumonias, endocarditis, septic arthritis and toxic shock syndromes¹. Many identifiable risk factors have been reported in the literature regarding the infection of MRSA such as immune-suppression, diabetes mellitus, young age, long term use of steroids, non-healing ulcers².

Initially MRSA was described in hospital acquired infections hence it was named as Health care associated MRSA (HA-MRSA). There have been 2 genetically distinct varieties of MRSA described namely the community associated MRSA and livestock associated MRSA³. Also the evolving resistance even to the latest group of antibiotics namely the linezolid group has worsened the crisis associated with MRSA^{4,5}.

As compared to the methicillin sensitive MRSA, the hospital acquired variant of MRSA is associated with increased morbidity and mortality of the infected patient and has more socioeconomic burden over him⁶. The EAARS (European antimicrobial resistance surveillance system) has reported the prevalence of hospital acquired MRSA between 1% and 24% in acute and long term hospital stay⁷. In Indian scenario, the prevalence of MRSA has increased from 12 percent in 1992 to 80.8 percent in 1999⁸.

In general practice the 2 distinct variety of MRSA namely the CA-MRSA and the HA-MRSA differ each other in susceptibility to antibiotics. The CA-MRSA is generally resistant to Beta lactam antibiotics and susceptible to fluoroquinolones, cotrimoxazole, and chloramphenicol whereas the HA-MRSA is resistant to fluoroquinolones and clindamycin and is usually sensitive to vancomycin⁹. But a distinct differentiation between HA-MRSA and CA-MRSA has not been possible yet. One differentiation has been found out regarding the PVL (Panton-Valentine Leukocidin) gene which generally encodes for pores forming cytotoxin, is commonly found in CA-MRSA and rarely found in HA-MRSA.

Methicillin resistance in staph is created by PBP2a protein which is encoded by MEC A gene which

provides resistance to MRSA from most of the B-Lactam antibiotics¹⁰. In order to fight against MRSA and its drug resistance, further investigations and surveillance is needed. In this study the antibiotic susceptibility of MRSA to antibiotics in rural orthopaedic patients with control measures for infection have been discussed.

MATERIAL AND METHODS:

Study Design: This study was conducted at Acharya VinobhaBhave Rural Hospital (AVBRH), Sawangi, Wardha. Specimens collected from the patients with soft tissue and skin infection purulent wound discharges were then sent to the microbiology laboratory for further processing. Information in addition to the specimens like age, onset of discharge, sex, any history of antibiotic use and any other comorbidities like diabetes and/or hypertension or any other medical condition was taken. The study was conducted in the year January 2019-July 2020.

Inclusion Criteria:

Patient included who had –

- i) active discharge and/or pus from the surgical site infection,
- ii) abscess in the skin

Exclusion Criteria:

- i) patients not giving informed consent,
- ii) patients with gangrenous changes in wounds and
- iii) mangled extremities.

Sample:

50 samples were taken from the abscess, infected surgical site wound which were collected using sterile swab sticks and sent in sterile culture bottles. Levine's technique was used for the preparation of the sites from where the samples were to be collected¹¹. This technique included rotating of the wound swab over 1-2 cm of the wound. Purulent discharges, pus collected from the surgical site wound and abscess were inoculated and cultured in the nutrient agar (NA) and mannitol salt agar (MSA) respectively and incubated for one day at 37 degree Celsius in aerobic environment.

Golden yellow color bacterial colonies on MSA were visible typical of *S.aureus*, which were then gram stained and subjected to catalase test & DNase test. Gram positive bacteria, fermenting mannitol appearing as grape like cluster that have catalase positive activity were then cultured in DNase agar and incubated for one day at 37 degree Celsius. HCL (1N) was introduced in the DNase agar plates. It was seen that the DNA was hydrolyzed which is the ability of *S.aureus*. Nutrient agar components include 0.5% of peptone extract, 0.3% beef extract, 1.5% agar, 0.5% NaCl, distilled water and pH is adjusted to neutral (7.4) at 25 degree Celsius.

Antimicrobial testing for sensitivity was also performed. As per the Clinical and Laboratory Standards Institute (CLSI) guidelines, a Kirby-Bauer disk diffusion method using the Muller Hinton Agar (MHA) plate technique was used¹². Antimicrobial disks with multiple drug classes were used for oxacillin, vancomycin and gentamycin. These were incubated for one day at 37 degree Celsius in MHA along with 2 percent NaCl. For each antimicrobial testing an inhibition

zone diameter was measured and labelled as resistant (R) and sensitive (S) after comparison

with *mecA* negative & *mecA* positive *S.aureus*. These zones were interpreted as per CLSI guidelines. Hospital acquired infections were defined as infections occurring for more than three calendar days after admissions in the hospital.

Ethical clearance was obtained and every participant was explained about the study and informed consent was taken. For underage patients parents and/or legal guardians were asked to sign consent forms. Patients confidentiality along with the reports was maintained until the study was completed.

RESULTS:

Out of 50 specimen collected, 38(76%) are males and 12 (24%) are females. Range of age group included in the study was 0 years to 90 years. Age groups between 30 year to 60 year were more in number. Of the 50 samples collected 30 (60%) were *S.aureus* sensitive. 15(65.21%) of males were MRSA sensitive and 7 (100%) were MSSA sensitive (Table-1).

Table.1: Association of organisms with respect to age group and gender

Variables		MRSA (number and %)	MSSA (number and %)	Others (number and %)	Total
Sex	Males	15(65.21%)	7(100%)	16(80%)	38(76%)
	Females	8(34.79%)	0(0%)	4 (20%)	12(24%)
	Total	23(100%)	7(100%)	20(100%)	50(100%)
Age group	0-30	12(52.17)	2(28.57)	6(30%)	20(40%)
	30-60	10(43.47%)	4(57.14%)	12(60%)	26(52%)
	60-90	1(4.36%)	1(14.29%)	2(10%)	4(8%)
	Total	23(100%)	7(100%)	20(100%)	50(100%)

In association with antibiotic sensitivity, 21 patients (91.30%) & 7(100%) of MRSA & MSSA are sensitive too Vancomycin, 19 (82.60%) & 5 (71.42%) of MRSA & MSSA were sensitive to Amikacin, 18(78.23%) & 6 (85.71%) of MRSA & MSSA are sensitive to Clindamycin, 14 (60.8%) & 6 (85.71%) of MRSA & MSSA are sensitive to Linezolid and 6 (26.08%) & 3 (42.45%) of MRSA & MSSA are sensitive to Ciprofloxacin (Table-2).

The antibiotic sensitivity test for MRSA resulted 2

(8.70%) resistant to Vancomycin, 4 (17.4%) resistant to Amikacin, 5 (21.7%) resistant to Clindamycin, 9 (39.2%) are resistant too Linezolid, 17(73.92%) are resistant to Ciprofloxacin (Table2).

The antibiotic sensitivity test for MSSA resulted 2 (28.58%) resistant to Amikacin, 1 (14.29%) resistant to Clindamycin, 1 (14.29%) are resistant too Linezolid, 4 (57.5%) are resistant to Ciprofloxacin (Table-2).

Table 2: Antimicrobial sensitivity of MRSA and MSSA to different antibiotics

	Vancomycin	Amikacin	Clindamycin	Linezolid	Ciprofloxacin
MRSA susceptibility	21/23 (91.30)	19/23 (82.60%)	18/23 (78.23%)	14/23 (60.8%)	6/23 (26.08%)
MSSA susceptibility	7/7 (100%)	5/7 (71.42%)	6/7 (85.71%)	6/7 (85.71%)	3/7 (42.45)
Others susceptibility	10/20 (50%)	9/20 (45%)	8/20 (40%)	11/20 (55%)	14/20 (70%)

DISCUSSION:

Staphylococcal cassette chromosome mec (SCCmec) contains an exogenous gene (mecA) which has been traced from the evolution of methicillin resistant *S. aureus* which is under control of a repressor and a transducer namely MecI and MecRI which are the signalling proteins or regulatory proteins in the blaZ system¹³. Penicillin-binding protein (PBP2a) which is a peptidoglycan transpeptidase and is coded by mecA gene which confers resistance to all β -lactam antibiotics like penicillins, cephalosporins & carbapenems. A variant of SCCmec type II & III have higher resistance potential due presence of more resistance genes. The acronym MRSA – or Oxacillin- resistant *S.aureus* (ORSA) is because of the presence of PBP2a or mecA positivity which can be typed using the methicillin or oxacillin¹⁴.

S. aureus isolation rate from the 50 samples was 30/50 samples according to our data. Sex of the patients was not associated with the presence of *S. aureus*. Nature of patient sampled included surgical wound and diabetic patients. Due to long hospital stay these patients are at increased risk of developing bacterial diseases invasively and this results in colonization of MRSA.

The isolation rate from wound and abscess discharges was the highest (100%) and from the pus and blood samples was about 62.1 percent and 40 percent, respectively¹⁵. Eyob Yohannes Garoy et al conducted a study in which they isolated 82 *S.aureus* samples out of which 59 (72 percent) were MRSA, 16 (19.5 percent) were MSSA, while the remaining 7 (8.5 percent) were MISA¹⁵.

Prevalence of MRSA has been associated to many factors including different study design, specimen types, procedures in the laboratory, population and

the duration of study. For example, some of the studies depend upon the phenotypic MRSA detection procedures while the other procedures depend upon DNA (MLST- multilocus sequence typing), sequencing of genome, micro arrays, convention technique – PCR) which being gold standard for mecA. PCR technique which relies on detection of the genotype reports lower MRSA prevalence¹⁶. There are other studies which have demonstrated that cefoxitin disk diffusion test have better performance & the oxacillin assays have acceptable specificity, sensitivity, negative and positive predictive value (NPV—PPV). In major southern districts of Tamil Nadu, 250 strains of methicillin resistant *S.aureus* were isolated from the 90 strains. Clinical and carrier subjects of all strains are sensitive to vancomycin¹⁴. CA-MRSA isolates are increasing in India recently. D' Souza et al in their study studied 412 confirmed cases of MRSA and out of which 54 % were true CA-MRSA having the SCCmec and SCCmec genes¹⁷. Arora et al in their study in north India found that the prevalence of MRSA was 46% and MRSA isolates are more resistant to other antibiotics than MSSA¹⁸.

LIMITATION:

The data presented in this study is from where information from AMR is limited. The true case load of hospitals is still unknown. The data collected and interpreted results should not be applied to the entire country. The difference between HA-MRSA & CA-MRSA is not clear. Thus, the source of infection is not certain. Also some parameters like place of residency, the days of hospital stay of the patient, any history of antibiotics intake and any history of antimicrobial usage. Even after so many limitations, this study provides important information regarding MRSA

and patterns of AMR associated with it. Thus, this data implies an important quality care for the patients, antibiotic selection, good practice of infection control.

CONCLUSION:

To summarize, there is prevalence of MRSA in the hospital with increase MRSA positivity in strains of *S.aureus* to antibiotics. Although the drug of choice for MRSA infection still is Vancomycin. Their should be monitoring of the patterns of MRSA susceptible to the antibiotics, helping in formulation for antibiotic policy which might be helpful in near future for decreasing the prevalence of MRSA infection. Their should be a tracking system to gather the data on emerging trend of infection which can be used later for designing infection control practices and the use of antimicrobial agents.

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