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Research Article

Prevalence and Antimicrobial Resistance Pattern of Methicillin Resistant *Staphylococcus aureus* (MRSA) Strains Isolated from Clinical Specimen in Udaipur, Rajasthan

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Abstract

Background and Objective: MRSA strains and their resistance are increasing worldwide. The aim of this study was to find the prevalence of MRSA strains isolated from clinical specimens and to evaluate their resistance pattern. Development of intrinsic (homogenous and heterogenous) and extrinsic (borderline) resistance in *S. aureus* to methicillin with decrease susceptibility to AMAs (anti microbial agents). Additionally we compared the phenotypic and genotypic methods for detection of methicillin resistance.

Materials and Methods: In this cross-sectional study, a total of 500 isolates of *S. aureus* were collected from various clinical specimens from January 2017 to January 2018 at teaching hospital (PMCH) in Udaipur, Rajasthan region. All isolates were identified at the species level by standard biochemical tests. The methicillin resistance was evaluated using three methods: PCR for *mecA* gene, agar dilution for determination of oxacillin MIC and disk diffusion test to detect methicillin, oxacillin and cefoxitin resistance. Antimicrobial resistance patterns were determined by disk diffusion method according to CLSI (Clinical and Laboratory Standards Institute) guidelines.

Results: The results identified 210 (42%) out of 500 isolates as MRSA. Most of the MRSA strains (65.4%), intermediate or borderline (BORSA) 06(2%) susceptible (MSSA) 284 (56%) were isolated from patients. All isolates were susceptible to vancomycin, mupirocin and linezolid. Among other antibiotics co-trimoxazole was more active against MRSA isolates. Using PCR as reference method all the phenotypic tests showed 100% specificity. 193(92%) were homogenously resistant and 17 (8%) were heterogenic resistant.

Keywords: Methicillin Resistant Staphylococcus aureus (MRSA); Methicillin Sensitive Staphylococcus aureus (MSSA); Borderline Oxacillin Resistant Staphylococcus aureus (BORSA); CLSI (Clinical And Laboratory Standards Institute); MIC (Minimum Inhibitory Concentration); Amas (Anti Microbial Agents)

Introduction

Staphylococcus aureus is one of the most common pathogens causing a variety of infections ranging from relatively benign skin infections to life threatening systemic illness such as pneumonia, endocarditis, septic arthritis, subcutaneous or visceral abscesses [1].

Before the introducion of penicillin in the late 1940s, Staphylococcal septicemia was associated with an extremely high mortality rate. Penicillin dramatically improved the prognosis of this infection [2]. However, penicillin resistant strains were discovered shortly and penicillin became ineffective both in the hospital and community settings [3,4]. Clinically, infections caused by HA-MRSA strains are associated with higher mortality and morbidity with BORSA [5]. Some CA-MRSA strains express additional virulence factors that enable them to causes more serious diseases [6].

Some CA-MRSA strains express additional virulence factors that enable them to causes more serious diseases [6]. Currently, MRSA strains account for many of staphylococcal infections and reports of MRSA strains are increasing worldwide [7]. There are also several reports from Iran showing the prevalence of methicillin resistance among clinical isolates of *S. aureus* [8-9]. A meta-analysis study recently carried out in Iran by Askari., *et al.* showed that the average prevalence rate of MRSA isolates among clinical specimens were 52.7% [10]. Understanding the prevalence, antibiotic resistance patterns and information on accurate and reliable detection methods of MRSA as mentioned in CLSI guideline are necessary

for appropriate antibiotic treatment and effective infection control. The current study was performed to find the prevalence and evaluate the antimicrobial resistance profile of MRSA strains isolated from clinical specimens in Udaipur, Rajasthan. Additionally we compared the phenotypic and genotypic methods for detection of methicillin resistance in *S. aureus* strains.

The World Health Organization (WHO) recommends continuous surveillance as a means of controlling the spread of diseases caused by resistant pathogens. Such systems of tracking diseases are lacking in most developing countries like India [11].

Materials and Methods

Study location

The present study was a hospital-based cross-sectional study conducted on both in and out patients attending the Pacific Medical College and Hospital from January 2017 to January, 2018. This hospital is a multi-speciality hospital located in Udaipur. It serves patients from all over the district and adjacent area.

Ethical considerations

Ethical clearance for this study was obtained from the Institute ethics committee (IEC) human studies. All participants were issued consent forms and only those who consented were enrolled into the study.

Specimen Collection

A total of 500 consented participants who presented with various infectious were included into the study. Specimens such as pus, ear discharges, urine, genital and throat swabs, CSF, ET secretion, body fluids, blood, were collected aseptically and process within 2hour duration in Microbiology laboratory.

Laboratory analyses

Isolation and identification of *S. aureus*

Each sample was inoculated onto mannitol salt agar (MSA), blood agar (BA) and nutrient agar (NA) the plates were incubated at 37°C for 24 hours. Plates were observed for characteristic yellow colonies on MSA and beta (β) hemolysis on BA. Isolates were examined by Gram staining, catalase and coagulase tests. Methicillin resistance was assessed by determining the resistance profile of *S. aureus* isolates to oxacillin according CLSI guidelines.

Determination of methicillin resistance

Methicillin resistance was evaluated using three methods:

1. Disk diffusion test using 30 µg cefoxitin disk (≤ 21mm indicated MRSA), 1 µg oxacillin disk (≤ 10 mm indicated MRSA), and 5 µg methicillin disk (≤ 9mm indicated MRSA).
2. Polymerase chain reaction (PCR) for the detection of *mecA* gene (positive indicated MRSA) [12,13].

Antibiotic disks were obtained from Himedia (Himedia Laboratories, Pvt. Ltd., Mumbai, India). All tests were compared for sensitivity and specificity with PCR for *mecA* gene as reference method. Sensitivity was calculated by dividing the number of *mecA*-positive isolates detected as resistant using phenotypic methods by the total number of *mecA*-positive strains (ether susceptible or resistant). Specificity was calculated through dividing the number of *mecA* negative isolates classified as sensitive based on phenotypic criteria by the total number of *mecA*-negative isolates [14].

Antibiotic sensitivity testing and interpretation

The antimicrobial susceptibility testing of all *S. aureus* isolates to different antibiotics were carried out according to the guidelines of Clinical and Laboratory Standards Institute (CLSI) by Kirby-Bauer disc diffusion method [15]. The 10 antibiotics tested were Oxacillin (OX-1µg), penicillin (P-10µg), Ciprofloxacin(CIP, 5µg), Cefotaxime (30µg, CT), Tetracycline (30µl), Cephalexin (30µg, CP), Cotrimoxazole (23µg, COT), Gentamicin (GM-10µg), Erythromycin (EM-15µg), amoxicillin-clavulanic acid (AMC-30µg), Lincomycin (LN- 15µg), Pefloxacin (5µg, PF), Amikacin (AK-30µg), Roxythromycin (15µg, RXT), Ofloxacillin (OFX-5µg) and Vancomycin(VA-30µg).

PCR amplification of *mecA* gene

Total bacterial DNA was extracted from *S. aureus* using DNP™ Genomic DNA Extraction Kit DNeasy Blood and Tissue Kit by qiagen. Oligonucleotide primers [15]: 5'- AAAATCGATGGTAAAGGTTGGC-3' (forward) and 5'- AGTTCTGCAGTACCGGATTTGC-3' (revers) were synthesized by QIAGEN's GeneReader NGS System. PCR was performed in a 20 × µL Accu Power™ PCR Pre Mix (Bioneer) with 10 pmol of each primers under the following conditions: initial denaturation at 95°C for 5 min, followed by 34 cycles of 95°C for 1 min, 55°C for 1 min and 72°C for 1 min, and a final incubation at 72°C for 5 min. The amplified DNA fragments (PCR product: 533 bp) were separated on 1% (w/v) agarose gel, stained with ethidium bromide and visualized under ultraviolet light. *S. aureus* ATCC 29213 and ATCC 33591were used as *mecA* negative and positive controls respectively [16,29].

Statistical analysis

Chi-Square test at a 95% confidence level. $P < 0.05$ was considered statistically significant and compare the prevalence of MRSA and MSSA.

Results

Prevalence of MRSA

Out of 2190 clinical samples analysed, *S. aureus* was isolated From 500 (22.83%). MRSA identified from 500 *S. aureus* sample were 210 (42%), BORSA 6 (1.2%), MSSA 284 (56.8%) (Figure1).

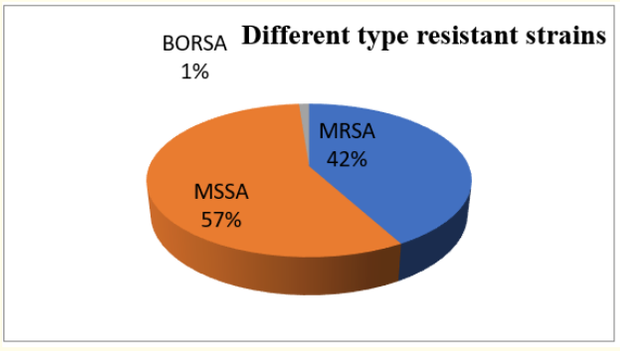

Figure 1: Isolation of *S. aureus* and evaluation of resistance pattern.

Incidence of borderline resistant strains

Ampicillin/Sulbactam test for BORSA (Borderline *Staphylococcus aureus*) a total of 6 borderline susceptible strains with oxacillin disk diffusion tests. They were tested with Ampicillin/Sulbactam susceptibility by disk diffusion method. All the strains were susceptible to Ampicillin/Sulbactam. These strains were not considered oxacillin/methicillin resistant but were taken as borderline resistant strains, due to effect of hyperproduction of β lactamase and not due to intrinsic methicillin resistance and were considered as methicillin sensitive *Staphylococcus aureus*. Overall incidence of susceptible, resistant and borderline resistant strains.

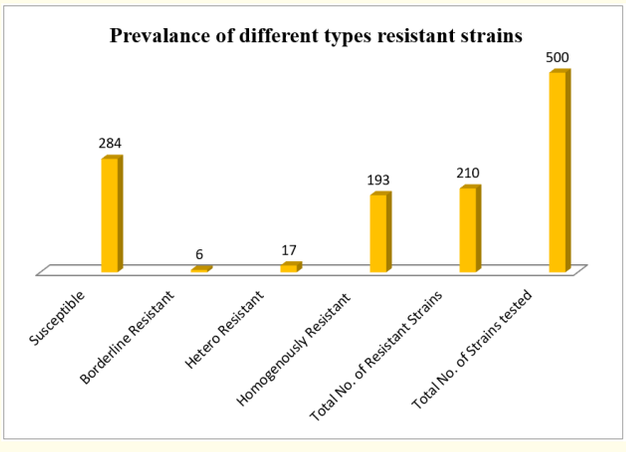

Figure 2

Incidence of homogenous and heterogenous resistant strains

Homogenously resistant 193 (92%) Heteroresistant 17 (8%), 210 Out of 500 strains.

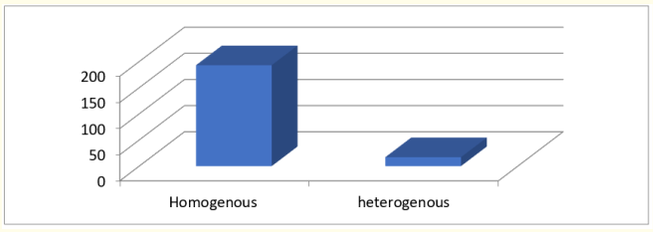

Figure 3

Incidance of borderline homogenous and heterogenous resistant strains

The overall incidence of borderline resistance is 2% (6 out of 500). 5 (83.33%) strains were homogenous resistant and 1 (16.66%) heterogenous resistant out of 6 samples.

Incidance of MRSA strains in different samples

155 (47%) strains of MRSA were detected from 330 S. aureus isolates from Pus, making (74%) of total MRSA. 30 (27%) strains of MRSA were detected from 110 S. aureus isolated from blood culture making (14%) of total MRSA. Rest 12% Were isolated from other samples.

SAMPLE	No. of *Staphylococcus aureus*	No. of MRSA
Pus	330	155
Blood Culture	110	30
Throat Swab	20	08
Sputum	15	06
Urine	05	01
CSF	01	00
Vaginal Swab	06	03
Fluid	02	01
Ear Swab	11	06
Total	500	210

Table 1

Antibiotic Susceptibility Pattern of MRSA Strains

Total No. of strains tested 210. More than 80% of MRSA showed resistance to cotrimoxazole (86%), Roxythromycin (83%) and Gentamicin (83%). and approximately, 79% of strain are susceptible to pefloxacin, ofloxacin and ciprofloxacin. The strains are susceptible to pefloxacin, ofloxacin and ciprofloxacin in descending order

to ciprofloxacin (23%), Pefloxacin (21%) and ofloxacin (19%). All strains were susceptible to Vancomycin.

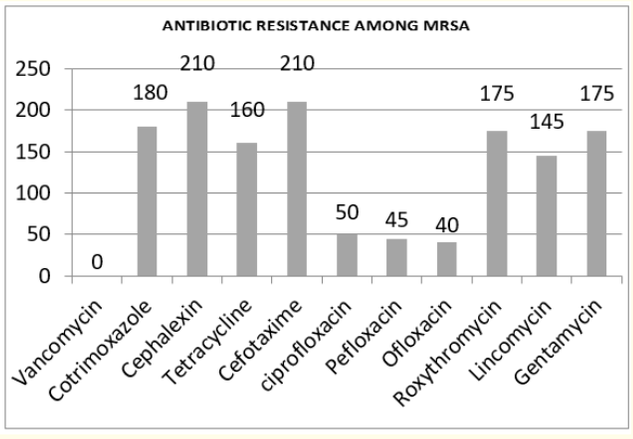

Figure 4

Discussion

Incidence of MRSA

In the present study, 210 (43%) out of 500 were found to be methicillin resistant. The incidence of MRSA reported by other workers is shown in Table- In 1960 first 3 strains of MRSA were identified in London. In 1965, incidence of MRSA in New Delhi was 11.84%. In 1965, incidence of MRSA was 6% in Melbourne. In Melbourne, 53% of *Staphylococcus aureus* isolates from blood culture was resistant to methicillin in 1981.

In 1991, 35% MRSA in Malaysia In 1992, the incidence of MRSA was 44% in Paris. In China, incidence of MRSA was 50.7% in 1994. IN 1995, high incidence of rate of MRSA (87%) was noted in Mumbai Vancomycin has been widely and successfully used for the treatment of MRSA. More recently teicoplanin has been released for clinical use in Europe because of a novel pharmacokinetic profile and easier administration, but a few failure cases have been reported following treatment with Teicoplanin.

PCR testing revealed the presence of *mecA* gene in all Isolates which were determined as methicillin resistant by the phenotypic methods.

In other study carried out by Anita Chakravarty, and colleagues in 1998 showed 45% pattern of MRSA.44 In the present study overall incidence of MRSA is 42% from maximum from pus isolates. Comparison of susceptibility tests was as follows.

Author	Year	Disk Diffusion Test (%) MRSA
Anita Chakraworti	1988	45%
Rohini Kelkar	1990-91 1994	45% 67%
Thomas B. Pulimood	1996	24%
Present Study	2017-2018	42%

Table 2

The present study correlates well with study of Rohini., et al. having app same incidence rate of MRSA isolates from pus samples. All the MRSA strains were uniform susceptible to Vancomycin. So Vancomycin is the drugs of choice for MRSA, 45 Emergence of Vancomycin resistance have been reported in the world. K. Hiramatsu in 1996 has reported a MRSA strain with reduced Vancomycin susceptibility. In 1997, 2 MRSA having reduced Vancomycin susceptibility have been reported in USA. The mechanism for Vancomycin resistance in staphylococci has not yet been identified though it is different from Vancomycin resistance in enterococci. A few biochemical and phenotypic changes have been noted from a study of a few clinical and lab.

1. Increased production of one or more proteins with no. mass of 39k.
2. Modification in PBP2, a high molecular weight Penicillin binding protein.
3. Alteration in structure of peptidoglycan cross linking bridges.
4. Decreased capsule and phage type ability.
5. Increased in the cell wall thickness and size.
6. Decreased in coagulase activity.
7. Decreased in lysostaphin sensitivity.

Conclusion

MRSA are resistant to a number of antibiotics commonly used in our institution, but uniformly susceptible to Vancomycin. It indicates Vancomycinis the drug of choice for MRSA infection in our institution. In future we have limited choices of drug to treat MDR pathogens which commonly associated with the infection in hospital acquired and also occasionally find in community. A proper microbiologist suggested antimicrobial guideline essential for prevention further resistance development in microbes. Now we have limited option of antibiotics in which some antibiotics have more side effects. So we need a good and proper antibiotic guideline for proper treatment.

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