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Current Antibiotic Trends in *Staphylococcus aureus* with Reference to Methicillin Resistance

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ABSTRACT

The abuse of antimicrobials in the treatment procedures is the sole cause of emerging resistant strains of the common pathogen. Present study was intended to monitor the Methicillin Resistance and the antibiotic resistance pattern among the *Staphylococcus aureus* isolated from clinical specimens. The antibiotic disc diffusion assay was used to study the resistance pattern. The confirmation of MRSA was done using cephoxitin 30 µg disc. The overall isolation rate of *Staphylococcus aureus* from different clinical samples was found to be 65.46% and showed dominance in clinical samples collected from males. Methicillin resistant *Staphylococcus aureus* (MRSA) were detected at the rate of 43.36%. The recovery of MRSA was found to be higher in female gender. The highest resistance of *Staphylococcus aureus* was seen against the penicillin & ampicillin antibiotics; however the vancomycin, chloramphenicol, tetracycline and ofloxacin showed higher sensitivity. The study concludes that, the *S. aureus* acquired resistance to frequently used drugs and becomes major threat in its therapeutic management. Study recommends proper use of antibiotics to avoid the emergence of the drug resistant strains and the searching of new antimicrobials for controlling the resistant strains in urgency.

Keywords: Nosocomial pathogen, drug resistance, MRSA, antibiogram studies.

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INTRODUCTION

The major causes of treatment failure are the emerging drug resistance in the common pathogens that may sometime leads to complications. Hospital acquired infections and community acquired infections both of which are of most concern particularly related to *Staphylococcus aureus.* Drug resistance in a particular species directly reflects the inappropriate use or the misuse of the drugs related to incomplete doses[1.] The *Staphylococcus aureus* is recognized as the most leading nosocomial pathogens in the form of methicillin resistant *S. aureus.* The incidence of MRSA was first time reported in 1961, soon after one year of launching the antibiotic methicillin in the treatment procedures and it continued for two decades as the most leading pathogen[2]**.** The incidence of MRSA in India was reported by Patel *et al*., as 25% in the western part[3], similarly Gopalakrishnan *et al*., reported 50% incidence in southern regions[4]. Another report of National Nosocomial Infection Surveillance System (NNIS) states about 50% of hospital acquired infections in ICUs in the USA are due to the MRSA[5].

The *S. aureus* strains that possess the *mecA* gene for expression of methicillin resistance designated as MRSA. The MRSA strains exhibiting the *mecA* gene are either heterogeneous or homogeneous with reference to their expression of resistance. The expression for heterogeneous infrequently results in minimum inhibitory concentrations that appear to be in borderline and consequently the isolates may be designated as susceptible[6]. In the recent years, the use of cefoxitin as a surrogate marker for detection of *mecA* gene in MRSA isolates have been published in no. of reports [7, 8, 9]. Therefore the present study was undertaken to evaluate the resistivity pattern of *S. aureus* clinical isolates against commercial antibiotics for isolating the MRSA strains and their confirmation by cephoxitin disc diffusion test.

MATERIALS AND METHOD

Collection of isolates:

A total of 472 clinical specimens and carrier screening samples of urine, pus, sputum, vaginal swab, inguinal swab, vaginal fluid, throat swab, blood, pleural and synovial fluid, stool, catheter tip, implant specimens, tissue exudates, conjunctiva swab, ear swab, nasal swab, tracheal swabs and oral thrush were collected for *Staphylococcus aureus* screening. These clinical samples were obtained from various private hospitals, Pathological and Microbiological Laboratories in Nagpur district, India.

Identification of isolates:

All the samples were aseptically handled and proceeded for their phenotypic characterization. The morphotypes were done for all the samples based on the Gram staining method to determine the likely organism present. Subsequently, the clinical specimens and carrier screening samples were inoculated onto Baired Parker agar, blood agar (aerobic with 5% CO_2), Mannitol salt agar and Mac-Conkey agar for preliminary isolation. The colonies obtained from primary isolation media were subjected to morphological, biochemical and cultural characterization. All isolated strains were further tested for the production of free coagulase enzyme using tube coagulase test based on standard methods. *Staphylococcus aureus* MTCC 96 of known coagulase production was included as control strain.

Antibiogram studies:

The antibiotic resistance profile was determined by the disc diffusion technique using different antimicrobial agents, according to the guidelines recommended by Clinical and Laboratory Standards Institute [10]. Fifteen standard antimicrobial discs (Hi Media Laboratories Pvt. Ltd, Mumbai) were used in the present study. These discs were Erythromycin (E10), Ofloxacin (OF 5), Cefpodoxime (CPD 10), Cephalexin (CP 30), Ceftazidime/ Clavunic acid (CAC 30/10), Kanamycin (K 5), Ciprofloxacin (CIP 10), Tetracycline (TE 10), Chloramphenicol (C 10), Trimethoprim (TR 10), Oxacillin (OX 1), Ampicillin (A 10), Penicillin (P 10), Vancomycin (VA 30) and Methicillin (MET 5).

Confirmation of MRSA isolates:

All the previously screened MRSA isolates were confirmed for their methicillin resistance by using cephoxitin disc (30 µg) diffusion test on Mueller Hinton agar. The zone diameters were measured and interpreted [10] as methicillin/ oxacillin resistant (inhibition zone diameter of ≤ 23 mm) and methicillin/ oxacillin sensitive (inhibition zone diameter of ≥29 mm). Many reports have been published regarding the year-wise modifications in the zone size interpretation ranges for cephoxitin disc diffusion assay, including CLSI documents. In past years the zone range was fall in short range. Swenson *et al.*, has recommended cephoxitin disc diffusion assay for the detection of MRSA by using cephoxitin 30 µg potency discs. They interpreted ≤19 mm inhibition zone as methicillin/ oxacillin resistant and ≥ 20 mm as methicillin/ oxacillin sensitive [11].

RESULTS AND DISCUSSION

A total of 309 *Staphylococcus aureus* isolates were obtained by random sampling of suspected 472 clinical specimens of blood =90 (29.12%), pus =82 (26.53%), urine = 60 (19.41 %), sputum

= 27 (8.73 %) and other =50 (16.18%) which includes vaginal swab, inguinal swab, throat swab, pleural and synovial fluid, stool, catheter tip, implant specimen, tissue exudates, conjunctiva, ear, nasal, tracheal swabs and oral thrush (Figure 3). The overall isolation rate of *Staphylococcus aureus* from different clinical samples was found to be 65.46%. The blood specimens showed highest distribution of *S. aureus,* there-after pus & urine, while the miscellaneous samples showed occasional presence of *S. aureus.* Yadav *et al.*, reported the higher rate of isolation (63.26 %) of *S. aureus* from pus samples, wound swabs and aspirates from abscesses, while lower rate of isolation in the specimen of blood, urine, sputum and body fluids like ascetic, pleural fluid and CSF, orderly [12].

The data obtained from the random sampling of *S. aureus* isolates were analyzed for male and female groups. The isolates obtained from blood specimen comprises of 66 (73.33%) *S. aureus* isolates from males and 24 (26.66%) from females. Next to this, specimen pus contains 38 (46.34%) isolates from males and 44 (53.65%) from females. Urine isolates were 15 (25.00%) from males and 45 (75.00%) from females. The sputum sample contains 12 (44.44%) isolates from males and 15 (55.55%) from females. The isolates from miscellaneous samples contains 36 (72.00%) from males and 14 (28.00%) from females. The overall recovery of *S. aureus* isolates from males was 167 (54.04%) and from females it was 142 (45.95%). The distribution rate of *S. aureus* was found to higher in male samples than in female samples (Table 1).

Table 1 Sex-wise distribution of *S. aureus* among the clinical specimens.

Clinical Specimen	Male	Female	No. of Isolates
Pus	38 (46.34%)	44 (53.65%)	82(26.53%)
Blood	66 (73.33%)	24(26.66%)	90(29.12%)
Sputum	12 (44.44%)	15 (55.55%)	27(8.73 %)
Urine	15 (25.00%)	45 (75.00%)	60(19.41%)
Other	36 (72.00%)	14 (28.00%)	50(16.18%)
TOTAL	167(54.04%)	142 (45.95%)	309(65.46%)

The antibiogram studies of *S. aureus* against fifteen commercial antimicrobials showed highest resistance for ampicillin and penicillin antibiotics. About 94.82% isolates (293) of *S. aureus* displayed resistance towards ampicillin and penicillin, while 5.17 % isolates (16) were sensitive. On similar lines Rajaduraipandi *et al.*, reported 71.8% and 35.9% resistance to penicillin and ampicillin, respectively for MRSA isolates **[13].** In present study we noted the higher percentage of resistance for both methicillin resistant (MRSA) and methicillin sensitive (MSSA) *Staphylococcus aureus* isolates. Next to this ceftazidime/clavunic acid combination displayed resistance amongst the 275 (88.99%) isolates, while 34 (11.00 %) isolates were sensitive. The

cefpodoxime antibiotic displayed resistance amongst the 274 (88.67%) isolates, while 23(7.44%) were sensitive & 12 (3.88%) were intermediately sensitive. There- after kanamycin antibiotic exhibited resistance amongst the 222 (71.84 %) isolates, while 24 (7.76 %) were sensitive & 63 (20.38 %) were intermediately sensitive; also the cephalexin antibiotic showed resistance amongst the 205 (66.34%) isolates, while 55 (17.79%) were sensitive and 49 (15.85%) were intermediately sensitive. These six antibiotics showed high degree of resistivity for the *S. aureus* clinical isolates (Figure 1 & Figure 2). These present findings are in agreement with the reports of Sharma *et al.,* who reported the high degree of resistance of MRSA & MSSA to penicillin, erythromycin, cephalexin, norfloxacin and ciprofloxacin; and low level of resistance to antibiotics amikacin, clindamycin, azithromycin and, gentamycin [14]. According to the antibiogram of present data, the resistivity of different antibiotics was graded as Ampicillin/penicillin > ceftazidime/clavunic acid > cefpodoxime > kanamycin > cephalexin > oxacillin > methicillin > vancomycin > erythromycin > ofloxacin > trimethoprim > tetracycline > ciprofloxacin > chloramphenicol.

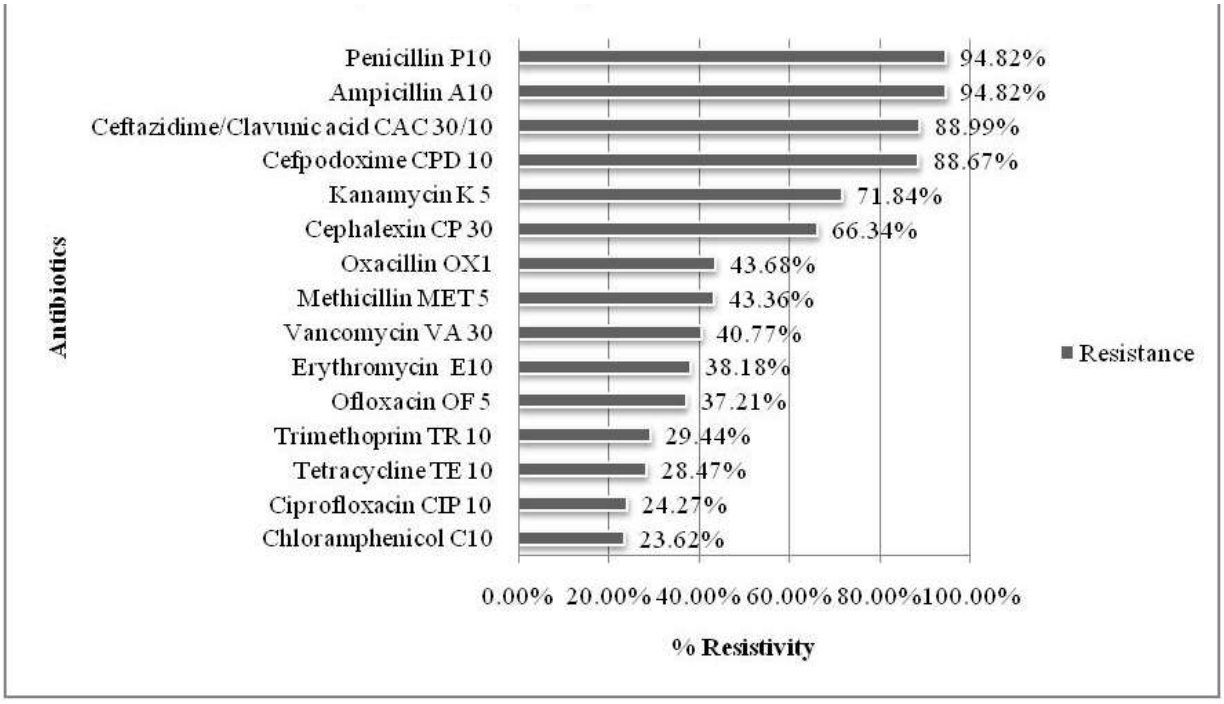

Figure 1: Resistivity biogram of *S.aureus* isolates

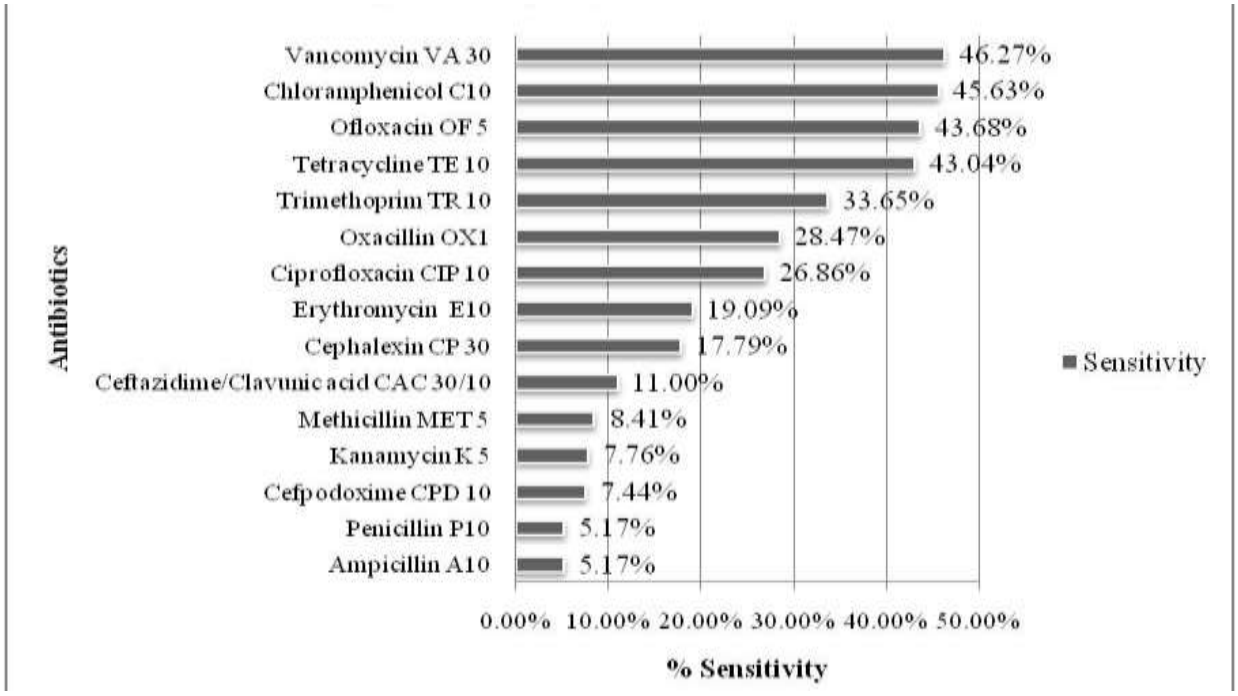

Figure 2: Sensitivity biogram of *S.aureus* isolates

In present studies screened 134 MRSA isolates were confirmed with the cephoxitin 30µg antibiotic disc. According to the guidelines of CLSI, interpretative zone size for methicillin resistance and methicillin sensitivity was set at ≤ 23 mm for methicillin resistant isolates and ≥ 29 mm for methicillin sensitive isolates [10]. In the present investigation, all screened MRSA isolates showed inhibition zones below 17 mm, hence considered as methicillin resistant. Yadhav *et al.* reported the 66.84% prevalence rate of MRSA in south India[12]. Similarly in Gulbarga (Karnataka) 56.7% rate of MRSA prevalence was reported by Chandrashekar *et al.*[15] and in Varanasi 59.3% MRSA rate was find out by Arora *et al.*[16]. In present study, the rate of prevalence of MRSA was found to be 43.36 % in Nagpur region, which is an alarming status, and signifies the potential risk for community health point of view.

The MRSA data of the isolates was analyzed for male & female groups. The blood samples yielded 28 (66.66%) MRSA isolates from males and 14 (33.33%) from females. The pus samples revealed presence of 11 (33.33%) MRSA from males and 22 (66.66%) MRSA from females. The urine samples showed occurrence of 07 (25.00%) MRSA from males and 21 (75.00%) MRSA from females. In sputum samples, 06 (54.54%) and 05 (45.45%) MRSA were detected from males and females, respectively. The miscellaneous samples contains 14 (70.00%) of MRSA from males and 6 (30.00%) from females (Table 2). The overall recovery of MRSA from males was 66 (49.25%) and from females 68 (50.74%), therefore incidence of MRSA was found to be more in females than the males. The similar finding was reported by Sharma and Mall, they

isolated 60.86 % and 39.13 % MRSA from females and males, respectively [17]. Therefore, MRSA has got much importance regarding the outspread control and the therapeutic managements.

Table 2 Sex-wise distribution of MRSA among the clinical specimens.

Clinical Specimen	MALE	FEMALE	No. of Isolates
Pus	11 (33.33%)	22 (66.66%)	33 (40.24%)
Blood	28 (66.66%)	14(33.33%)	42(46.66%)
Sputum	06 (54.54%)	05 (45.45%)	11(40.74%)
Urine	07 (25.00%)	21 (75.00%)	28(46.66%)
Other	14 (70.00%)	06 (30.00%)	20(40.00%)
TOTAL	66 (49.25%)	68(50.74%)	134(43.36%)

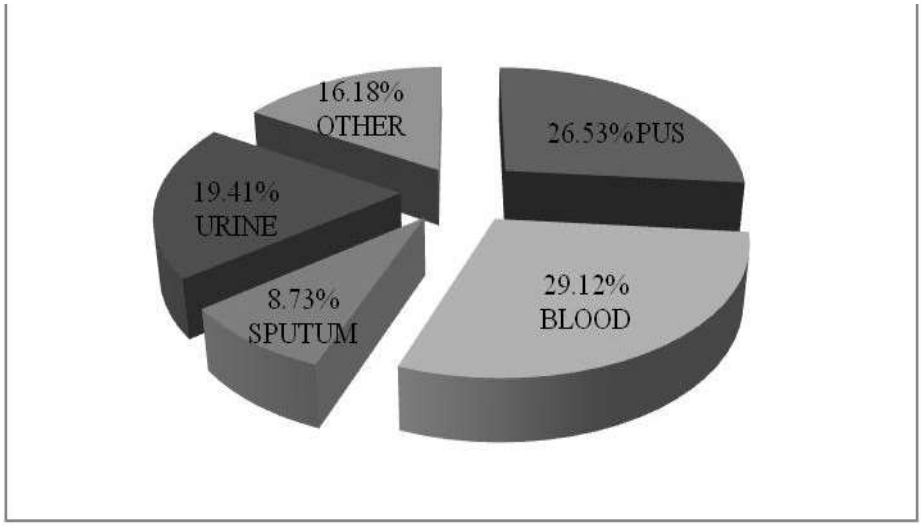

Figure 3: Distribution of *S. aureus* among the clinical specimens

CONCLUSION

As per the data obtained from the present study, it was concluded that the drug resistance in the common microbe *Staphylococcus aureus* has been increased to a great extent and this is an alarming state for physician, medical practitioners & clinicians. The appropriate use of antimicrobials is needed in urgency. This study also suggests the searching new antimicrobials as an alternative for controlling the infections caused by MRSA.

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