Original Article

# Prevalence and antimicrobial susceptibility pattern of methicillin-resistant Staphylococcus aureus isolated from clinical samples and healthcare workers

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## Abstract

**Background:** *Staphylococcus aureus* has been reported as a major cause of community and hospital acquired infections and has serious consequences despite antibiotic therapy. Recent increase of methicillin resistant and multiple drug resistant strains at large hospitals has started to pose great difficulty in selecting antimicrobial agents. This study was carried out to detect prevalence of MRSA infection in patients as well to screen health care workers for MRSA carriage and to detect the difference between their antimicrobial susceptibility patterns which will facilitate in the implementation of appropriate treatment of the patients. **Material and Methods:** The study included two groups, study group and control group. The study group consisted of 200 isolates of *S. aureus* from different clinical samples and 50 anterior nares and hand swabs were collected from health care workers as control group samples. Identification of *S. aureus* strains and antimicrobial sensitivity testing were done as per standard methods. Methicillin resistance was detected by cefoxitin disc diffusion test. **Results:** Prevalence of MRSA was found to be 64.5%. All strains were sensitive to linezolid and vancomycin. All the strains were resistant to penicillin. Maximum sensitivity was observed to clindamycin 65% followed by gentamIcin 58% and tetracycline 56.9%. *S. aureus* isolates, mostly recovered from anterior nares, were found in 44% of controls. 26% were found to be methicillin resistant.
**Key Words:** MRSA, clinical samples, anterior nares, screening, cefoxitin.

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Received Date: 20/10/2015 Revised Date: 15/11/2015 Accepted Date: 18/12/2015

| Access this article online | |
|---|---|
| Quick Response Code: | Website: www.medpulse.in |
| | DOI: 28 December 2015 |

## INTRODUCTION

*Staphylococcus aureus* is a major pathogen causing a diversity of infections including bacteremia, pneumonia, skin, soft tissue and osteo-articular infections[1]. They are well suited to their ecological niche, the skin. Therefore, it is not surprising that despite the availability of potent antimicrobial agents, improved public health conditions and hospital infection control measures, *Staphylococcus aureus* has remained a major human pathogen. Indeed, development of new antibiotic resistance and other epidemiologic conditions have reestablished this microorganism as a major pathogen in human diseases[2]. *Staphylococcus aureus* has been reported as a major cause of community and hospital acquired infections and has serious consequences despite antibiotic therapy[3]. The organism has differential ability to spread and cause outbreaks in hospitals[4]. The prolonged hospital stay, indiscriminate use of antibiotics and the lack of awareness are possible predisposing factors for Methicillin resistant *Staph. Aureus* (MRSA) emergence[5]. Serious endemic and epidemic MRSA infections occur globally as asymptomatically colonized patients and health care workers are the major sources of MRSA in the hospital environment, with the latter being more

How to site this article: Namdev M Suryawanshi, Asha P Pichare. Prevalence and antimicrobial susceptibility pattern of methicillin-resistant Staphylococcus aureus isolated from clinical samples and healthcare workers. *MedPulse – International Medical Journal.* December 2015; 2(12): 966-969. http://www.medpulse.in (accessed 30 December 2015).

commonly identified as links in the transmission of MRSA between patients[6]. Many of the MRSA strains are multidrug resistant and are susceptible only to glycopeptides antibiotics such as vancomycin[7]. Recent increase of methicillin resistant and multiple drug resistant strains at large hospitals has started to pose great difficulty in selecting antimicrobial agents. Sooner the MRSA infection is diagnosed and the susceptibility to antimicrobial agents established, the earlier appropriate therapy and control measures can be initiated. Identification and antimicrobial susceptibility testing are therefore crucial steps in treating, controlling and preventing MRSA infection. Hence, this study was carried out to detect prevalence of MRSA infection in patients as well to screen health care workers for MRSA carriage and to detect the difference between their antimicrobial susceptibility patterns which will facilitate in the implementation of appropriate treatment of the patients.

## MATERIAL AND METHODS

The study included two groups, study group and control group. The study group consisted of 200 isolates of *S. aureus* from different clinical samples like pus, blood, urine, sputum, ear swabs and bronchoalveolar lavage from the patients admitted and/or attending outpatient departments of Medicine, Surgery, Obstetrics and Gynaecology, Pediatrics, Skin and Veneral Disease, Orthopedics, Ear, Nose and Throat and Intensive Care Unit. The control group consisted of 50 samples from anterior nares and hands from health care workers (HCWs). HCWs with history of hospitalization or antibiotic therapy during last three months were not included in the control group. For all the specimens received in the microbiology laboratory, smears were prepared and stained with Gram stain. They were microscopically examined to determine the presence and type of cells along with the number of microorganisms and their relative morphology. Except blood, all other specimens were plated onto culture media blood agar and MacConkey's agar immediately after transporting them to the laboratory and were incubated at 37°C for 24 hours. Blood was inoculated into Brain Heart Infusion (BHI) broth and incubated at 37°C for 6-8 hours before subculturing them onto the respective media. After inoculation, the plates were examined for growth and identified by standard microbiological techniques. Isolates of *Staphylococcus aureus* were identified using standard tests like catalase test, slide and tube coagulase and growth on Mannitol Salt agar. The *S. aureus* isolates were subjected for antibiotic susceptibility testing by employing Kirby Bauer disc diffusion technique as recommended by Clinical and Laboratory Standards Institute (CLSI)[8]. The antibiotics used were: Penicillin G [10 units], Cefoxitin [30 µg], Co-trimoxazole [1.25/23.75 µg], Ciprofloxacin [5µg], Ofloxacin [5 µg], Gatifloxacin [5 µg], Levofloxacin [5 µg], Amikacin [30 µg], Gentamicin [10µg], Tetracycline [30 µg], Erythromycin [15µg], Clindamycin [2µg], Linezolid [30µg], Vancomycin [30 µg] discs. Norfloxacin [10 µg] and Nitrofurantoin [300 µg] discs were used against urinary isolates only. Tetracycline was not used against urinary isolates. All the discs were procured commercially [Hi-media Laboratories Limited]. The diameter of the zone of inhibition was measured and interpreted according to the guidelines of CLSI. Methicillin resistance was detected by cefoxitin disk diffusion test. *Staphylococcus aureus* ATCC 25923 was used as the standard control strain.

## RESULTS

A total of 200 *S. aureus* isolates from clinical samples were collected as study group samples and 50 anterior nares and hand swabs were collected from health care workers as control group samples. Clinical diagnosis of the cases from study group included in the present study were PUO, cellulitis, breast abscess, fracture, osteomyelitis, non-healing ulcer, post-operative wound infection, urinary tract infection, upper and lower respiratory tract infection, acute and chronic suppurative ottitis media. Various samples collected were pus, sputum, blood, urine, BAL and ear swabs. The antimicrobial susceptibility pattern to various groups of antibiotics was also done by Kirby Bauer disc diffusion method for two groups and was compared. In the present study, out of 200 study group *S. aureus* isolates 71 (35.5%) were found to be methicillin sensitive and 129 (64.5%) of the strains were found to be methicillin resistant. Out of 200 isolates, 123 (61.5%) patients were male and 77 (38.5%) were female patients. The male to female ratio in the present study was 6:4.Prevalence of MRSA was found to be more common in males 93 (75.6%) than females 36 (46.75%). The maximum numbers of isolates were in the ages up to 30 years 122 (61%) than ages above 30 years 78 (39%). Maximum numbers of MRSA isolates were also found in ages up to 30 years 83 (68%) than ages above 30 years 46 (59%). In the present study, Maximum strains of *S. aureus* were isolated from pus 132 (66%) and urine 28 (14%), followed by sputum 17 (8.5%), blood 13 (6.5%), BAL 6 (3%) and ear swab 4 (2%). 88 (66.6%) MRSA strains were found from pus samples followed by urine samples 17 (60.7%), blood 11 (84.6%), sputum 9 (52.9%), BAL 3 (50%) and ear swab 1 (25%).

**Table 1:** Distribution of MSSA and MRSA in samples

| Samples | MSSA (%) | MRSA (%) |
|---|---|---|
| Pus (n=132) | 44 (33.3) | 88 (66.7) |
| Urine (n=28) | 11 (39.3) | 17 (60.7) |
| Blood (n=13) | 2 (15.4) | 11 (84.6) |
| Sputum (n=17) | 8 (47.1) | 9 (52.9) |
| BAL (n=6) | 3 (50) | 3 (50) |
| Ear swab (n=4) | 3 (75) | 1 (25) |
| **Total (n=200)** | **71 (35.5)** | **129 (64.5)** |

Out of the total 200 isolates of *S. aureus* from study group samples, all strains were found resistant to penicillin and 100% sensitive to linezolid and vancomycin. Among other antibiotics, maximum sensitivity was observed to clindamycin 130 (65%) followed by gentamicin 116 (58%) and tetracycline 98 (56.9%). Tetracycline was not tested against urinary isolates. Among 28 urinary isolates, 19 (67.8%) were sensitive to nitrofurantoin and clindamycin followed by gentamicin 14 (50%) and norfloxacin 13 (46.4%). Moderate susceptibility was observed to levofloxacin 101 (50.5%), gatifloxacin 90 (45%) and co-trimoxazole 86 (43%).

**Table 2:** Antibiotic Sensitivity Pattern of the Study Group Samples (n= 200)

| Antibiotics | Sensitive | | Resistant | |
|---|---|---|---|---|
| | n | % | n | % |
| Penicillin | 0 | 0 | 200 | 100 |
| Cefoxitin | 71 | 35.5 | 129 | 64.5 |
| Co-trimoxazole | 86 | 43 | 114 | 57 |
| Clindamycin | 130 | 65 | 70 | 35 |
| Erythromycin | 60 | 30 | 140 | 70 |
| Gentamicin | 116 | 58 | 84 | 42 |
| Ciprofloxacin | 65 | 32.5 | 135 | 67.5 |
| Gatifloxacin | 90 | 45 | 110 | 55 |
| Levofloxacin | 101 | 50.5 | 99 | 49.5 |
| Ofloxacin | 65 | 32.5 | 135 | 67.5 |
| Linezolid | 200 | 100 | 0 | 0 |
| Tetracycline* (n=172) | 98 | 56.9 | 74 | 43 |
| Norfloxacin# (n=28) | 13 | 46.4 | 15 | 53.5 |
| Nitrofurantoin# (n=28) | 19 | 67.8 | 9 | 32.1 |
| Vancomycin | 100 | 100 | 0 | 0 |

* This antibiotic was not tested against urine samples, # These antibiotics were tested only for urine samples.

Out of the 50 samples taken from health care workers 25 were anterior nares swabs and 25 were hand swabs. 16 (64%) anterior nares swabs and 6 (24%) hand swabs showed growth of *S. aureus* on mannitol salt agar. Out of 22 (44%) *S. aureus* isolates, 13 (26%) were found to be methicillin resistant. MRSA were detected more commonly in swabs from the anterior nares 11 (44%) than those from hands 2 (8%).

**Table 3:** Antibiotic Sensitivity Pattern of the Control Group Samples (n= 22)

| Antibiotics | Sensitive | | Resistant | |
|---|---|---|---|---|
| | n | % | n | % |
| Penicillin | 0 | 00 | 22 | 100 |
| Cefoxitin | 9 | 40.9 | 13 | 59.1 |
| Co-trimoxazole | 11 | 50 | 11 | 50 |
| Clindamycin | 19 | 86.4 | 3 | 13.6 |
| Erythromycin | 12 | 54.5 | 10 | 45.5 |
| Gentamicin | 19 | 86.4 | 3 | 13.6 |
| Ciprofloxacin | 21 | 95.5 | 1 | 4.5 |
| Gatifloxacin | 21 | 95.5 | 1 | 4.5 |
| Levofloxacin | 21 | 95.5 | 1 | 4.5 |
| Ofloxacin | 21 | 95.5 | 1 | 4.5 |
| Linezolid | 22 | 100 | 0 | 00 |
| Tetracycline | 14 | 63.6 | 8 | 36.4 |
| Vancomycin | 22 | 100 | 0 | 00 |

All the 22 strains were sensitive to linezolid and vancomycin and resistant to penicillin. Among other antibiotics, all the fluroquinolones showed 95.5% susceptibility followed by clindamycin and gentamicin (86.4% each). Out of the 22 strains, inducible clindamycin resistance was observed in 2 (12.5%) strains.

**Table 4:** Antibiotic Sensitivity Pattern of MRSA Isolates (n=129)

| Antibiotics | Sensitive | | Resistant | |
|---|---|---|---|---|
| | N | % | N | % |
| Penicillin | 00 | 00 | 129 | 100 |
| Cefoxitin | 00 | 00 | 129 | 100 |
| Co-trimoxazole | 46 | 35.6 | 83 | 64.3 |
| Clindamycin | 74 | 57.3 | 55 | 42.6 |
| Erythromycin | 29 | 22.4 | 100 | 77.5 |
| Gentamicin | 67 | 51.9 | 62 | 48.0 |
| Ciprofloxacin | 29 | 22.4 | 100 | 77.5 |
| Gatifloxacin | 51 | 39.5 | 78 | 60.4 |
| Levofloxacin | 60 | 46.5 | 69 | 53.4 |
| Ofloxacin | 37 | 28.6 | 92 | 71.3 |
| Linezolid | 129 | 100 | 00 | 00 |
| Tetracycline* (n=112) | 58 | 51.7 | 54 | 48.2 |
| Norfloxacin# (n=17) | 06 | 35.3 | 11 | 64.7 |
| Nitrofurantoin# (n=17) | 11 | 64.7 | 06 | 35.3 |
| Vancomycin | 129 | 100 | 00 | 00 |

*This antibiotic was not tested against urine samples, # These antibiotics were tested only for urine samples.

All the 129 MRSA isolates were 100% sensitive to linezolid and vancomycin. Among other antibiotics, most of the strains showed sensitivity to clindamycin 74 (57.3%) followed by gentamicin 67 (51.9%), tetracycline 58 (51.7%) and levofloxacin 60 (46.5%).

## DISCUSSION

Methicillin Resistant *Staphylococcus aureus* (MRSA) is a major nosocomial pathogen causing significant morbidity and mortality[9]. The epidemiology of MRSA has continued to evolve since its first appearance more than three decades ago. Epidemic strains of these MRSA are

usually resistant to several other antibiotics. During the past 15 years, the appearance and world wide spread of many such clones have caused major therapeutic problems in many hospitals as well as diversion of considerable resources to attempts at controlling their spread. The overall rate of MRSA infection in the present study was 64.5%. This MRSA infection rate is consistent with Anand *et al*[10] and Mehndiratta *et al*[11]. A slightly lower MRSA infection rate was observed by Gupta *et al*[12] and by Anupurba *et al*[13]. Deshmukh *et al*[14] reported higher MRSA infection rate of 72.8%. The rate of MRSA infection was more in males [75.6%] than females [46.7%] in present study which is comparable with Tsering *et al*[15] and Mahmood *et al* (2010)[16]. The increased rate of infection among males could be due to their outdoor occupation where they are more prone for injuries and more exposed to contaminated environment than females. Significantly higher MRSA positive cases were observed from age group less than 30 years (64.3%) than age group more than 30 years (35.7%) in present study. This is in correlation with Tsering *et al*[15] who reported 57.7% MRSA cases in age group less than 30 years. The risk among younger patients is likely related to increased participation in risky activities such as team sports rather than physiologic changes due to aging. In the present study, majority of the MRSA isolates were from pus (68.2%). Slightly higher findings were observed by Tambekar *et al*[17] and Mathur *et al*[18] who reported 71% and 74.3% MRSA isolates respectively. *S. aureus* is a well-known pathogen causing pyogenic lesions. In the present study, 100% resistance was found to penicillin which correlates to the various studies[13,17,19]. Reports of 100% resistance to penicillin indicates this drug is no more effective for the treatment of *S. aureus* infections and should be omitted from the emperical treatment. In the present study, 70% *S. aureus* strains were found to be resistant to erythromycin. Anupurba *et al*[13] reported more than 80% while Tsering *et al*[15] reported 95.8% resistance to erythromycin. 43% *S. aureus* strains were tetracycline resistant in the present study. Fule *et al*[20] and Sen *et al*[21] reported 56.9% and 60.4% tetracycline resistance respectively. This discrepancy could be attributable to the infrequent use of tetracycline in the clinical set – up used for the present study. All MRSA strains were found sensitive to vancomycin and that may be due to the selective use of this drug in ICU patients. In the present study, all the methicillin resistant *S. aureus* strains were resistant to penicillin and all were sensitive to linezolid and vancomycin. All MRSA strains were found sensitive to vancomycin and linezolid in present study. So, as a precautionary measure before starting the patient on these reserved drugs, the clinicians should seek the help of clinical Microbiologist to determine the sensitivity of such strains so that emergence of vancomycin and linezolid resistance can be prevented. Variability in the antibiotic susceptibility pattern has been observed by many workers which possibly reflect the different antibiotic policies and protocols being used in different hospital settings and differences in the geographical locations from where these isolates have been isolated.

**Table 5:** Comparison of antibiotic susceptibility pattern of study and control group isolates

| Antibiotic | Sensitive | | Resistant | | Result |
|---|---|---|---|---|---|
| | Study group | Control group | Study group | Control group | |
| Penicillin | 0 | 0 | 200 | 22 | |
| Cefoxitin | 71 | 9 | 129 | 13 | $X^2$=0.25, df=1, p>0.05, NS |
| Co-trimoxazole | 86 | 11 | 114 | 11 | $X^2$=0.39, df=1, p>0.05, NS |
| Clindamycin | 130 | 19 | 70 | 3 | $X^2$=4.10, df=1, p<0.05, S |
| Erythromycin | 60 | 12 | 140 | 10 | $X^2$=5.45, df=1, p<0.05, S |
| Gentamicin | 116 | 19 | 84 | 3 | $X^2$=6.69, df=1, p<0.05, S |
| Ciprofloxacin | 65 | 21 | 135 | 1 | $X^2$=33.10, df=1, p<0.05, S |
| Gatifloxacin | 90 | 21 | 110 | 1 | $X^2$=20.18, df=1, p<0.05, S |
| Levofloxacin | 101 | 21 | 99 | 1 | $X^2$=16.18, df=1, p<0.05, S |
| Ofloxacin | 65 | 21 | 135 | 1 | $X^2$=33.10, df=1, p<0.05, S |
| Linezolid | 200 | 22 | 00 | 0 | |
| Tetracycline | 98 | 14 | 74 | 8 | $X^2$=0.35, df=1, p>0.05, NS |
| Vancomycin | 200 | 22 | 00 | 0 | |

Statistically significant difference with a p value <0.05 was found for majority of the antibiotics tested against both group isolates. In the present study, increased isolation of MRSA in nasal carriers was observed. Contemporary literature shows highly variable nasal carrier rate ranging from 1.8% to 79.5%. This was an alarming observation as HCWs did appear to be a major source of MRSA in present study, although it would require screening of larger numbers before arriving at any definite conclusions. The high level resistance pattern of MRSA observed in the present study may be due to widespread usage of broad spectrum antibiotics in hospital leading to selective survival advantage of pathogens. Present study shows quite high carrier rate of

MRSA so to prevent spread of MRSA, carriers among hospital personnel should be identified, and treated. Routine and regular surveillance of MRSA and in vitro susceptibility testing, regular monitoring and update of infection control practices and antibiotic policies might change the prevailing trends of antibiotic sensitivities which will reduce the chances of MRSA infections.

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Source of Support: None Declared
Conflict of Interest: None Declared