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# Prevalence and Antibiotic Resistance Pattern of *Staphylococcus aureus* isolates from Clinical samples at a Tertiary Care Hospital, North India

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## Abstract

**Background:** *Staphylococcus aureus* (*S. aureus*) is a well recognized nosocomial and community acquired pathogen which is implicated in causing a wide spectrum of superficial, deep pyogenic infections and toxin mediated illnesses. Localized infections sometimes progress to systemic infections, while 'spontaneous' bacteraemia also occur without an evident septic focus, particularly in chronic debilitated immunocompromised patients. Emergence of drug resistance to penicillins and penicillinase-resistant penicillins (i.e., oxacillin and methicillin) is a globally recognized problem. Methicillin Resistant Staphylococcus aureus isolates are also important with respect to the range of nosocomial infections it causes, leading to an increase in the hospital expenditure and mortality or morbidity rate. The increased prevalence of such resistant strains has narrowed down the list of available therapeutic options. Therefore, information regarding the prevalence of *S. aureus* infections in a health care setting and determining its current antibiotic resistance profile becomes crucial in selecting appropriate treatment regime. Therefore, the current study was done in the department of Microbiology to determine the prevalence of *S. aureus* infections and the antibiotic resistance pattern of *S. aureus* isolates from various clinical specimens at our tertiary care hospital in North India. **Materials and Methods:** A one year prospective study was carried out in the Department of Microbiology, at a tertiary care hospital in North India where non-duplicate strains of *S. aureus* isolated from various clinical specimens received in the Microbiology laboratory were studied. All *S. aureus* isolates were subjected to Antibiotic Susceptibility Testing using Kirby Bauer's disk diffusion method on Mueller Hinton Agar plates in accordance to CLSI guidelines. The antibiotics tested included Penicillin (10U), Amoxicillin-clavulanic acid (20/10µg), Sulphamethoxazole-trimethoprim (1.25/23.75µg), Ciprofloxacin (5µg), Erythromycin (15µg), Clindamycin (2µg), Vancomycin (30µg), Teicoplanin (30µg) and Linezolid (30µg). **Results:** A total of 23,699 clinical specimens were processed in the laboratory while conducting this study, from which 1233 clinical isolates of *S. aureus* were identified and processed further. Among all clinical specimens, pyogenic samples (63.1%) yielded maximum number of *S. aureus* strains followed by blood samples (29.9%) and urine samples (4.8%). *S. aureus* infection was more evident in hospitalized 71.2% patients than in OPD patients 28.8%. Seasonal variation was also seen in isolation of *S. aureus*, with a higher percentage of isolates obtained during summer season than during winter season. On antibiotic susceptibility testing, 49.6% strains were Methicillin Resistant. Majority of the isolates were found resistant to Penicillin (92.1%), followed by Erythromycin (59%). Almost half of the total isolates were resistant to Sulphamethoxazole-Trimethoprim (49.3%) followed by Amoxicillin-Clavulanic acid (47.8%), Ciprofloxacin (43.4%) and Clindamycin (18.4%). Antibiotics to which all isolates showed 100% susceptibility included Vancomycin, Teicoplanin, Linezolid. **Conclusion:** Given the high prevalence of resistance to antibiotics seen in this study, effective treatment of infections caused by multidrug resistant Staphylococcal strains

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may become challenging. Drugs like Vancomycin, Teicoplanin and Linezolid promise to work as miracle drugs against the multidrug resistant MRSA strains but we need to warrant judicious use of these wonder drugs to conserve them for future use.

**Keywords:** Antibiotic Susceptibility Testing, Antimicrobial Resistance, Methicillin Resistant Staphylococcus aureus (MRSA), *Staphylococoous aureus*

# 1. Introduction

*Staphylococcus aureus (S. aureus)* is well recongnised nosocomial and community acquired pathogen of global importance which can cause a wide spectrum of superficial and deep pyogenic infections as well as toxin mediated illnesses[1,2]. Strains capable of invading normal intact skin are rare and most are able to cause infection only if they enter through breaks in the skin. Localized infections sometimes progress to systemic infections, while 'spontaneous' bacteraemias also occur without an evident septic focus, particularly in patients debilitated by such illnesses as chronic hepatic or renal disease, or diabetes mellitus[2]. At the outset, Penicillin was considered as the best therapeutic option for treating life threatening *S. aureus* infections[3]. However, with the emergence of bacterial resistance to Penicillin; consequent upon acquiring plasmids coding for β-lactamase enzymes, semisynthetic, penicillinase-resistant penicillins (i.e., oxacillin and methicillin) became the drugs of choice. However, to our dismal resistance to the penicillinase resistant penicillins emerged early in 1980s and is still enduring to rise world over. Mechanism of resistance to these drugs due to the presence of an altered penicillin-binding protein called PBP2a that results from acquisition of a chromosomal gene called mec A. PBP2a has a low affinity for all β-lactam agents, including cephalosporins. These strains of *S. aureus* expressing mec A gene are known as Methicillin Resistant Staphylococcus aureus (MRSA)[3]. MRSA isolates have established their roots in the healthcare settings globally and are an issue of importance from the perspective of Hospital infection control considering the range of nosocomial infections it causes, leading to an increase in the hospital expenditure and mortality or morbidity rate[4]. In the present scenario, high prevalence rates of MRSA have left us with limited therapeutic options to treat infections caused by these isolates. Therefore, in order to select an appropriate treatment option, it is imperative to know the prevalence of *S. aureus* infections in a health care setting and also the ongoing pattern of resistance to drugs commonly used to treat these infections.

In the light of above mentioned facts, the present study was carried out in the department of microbiology to determine the prevalence and antibiotic resistance profile of *S. aureus* strains isolated from a variety of clinical specimens from patients presenting at our tertiary care hospital in North India.

# 2. Materials and Methods

A one year prospective study was conducted in the Department of Microbiology at a tertiary care hospital in North India from September 2012 to September 2013 where non-duplicate strains of *S. aureus* isolated from various clinical samples received in the Microbiology laboratory were studied.

## 2.1 Specimen Collection and Processing

Samples included pus, blood, urine, high vaginal swabs, expressed prostatic secretions, ear discharge, respiratory secretions and body fluids from OPD and IPD patients. The specimens were processed within 2 hours of receipt as per the standard procedures for specimen management[5]. Samples were inoculated on blood agar and incubated at 37°C for 18 to 24 hours. The growth obtained was confirmed to be *S. aureus* by various biochemical tests like catalase test, slide and tube coagulase test and mannitol fermentation on mannitol salt agar.

# 3. Antibiotic Susceptibility Testing

All the isolates identified as *S. aureus* were then subjected to Antibiotic Susceptibility Test using Kirby Bauer's disk diffusion method on Mueller Hinton Agar plates as per CLSI guidelines[6]. The antibiotics tested included Penicillin (10U), Amoxicillin-clavulanic acid (20/10µg), Ciprofloxacin (5µg), Sulphamethoxazole-trimethoprim

(1.25/23.75µg), Erythromycin (15µg), Clindamycin (2µg), Vancomycin (30µg), Teicoplanin (30µg), Linezolid (30µg). Plates were incubated at 37°c for 18-24 hours. Then the diameter of zone of inhibition around each antibiotic disc was measured and interpreted. Cefoxitin disc (30µg) was used to detect Methicillin resistance. An isolate showing zone of inhibition diameter of 21mm or less around Cefoxitin disc was considered to be a methicillin resistant. Quality Control (QC) for all the antibiotic discs was performed with *Staphylococcus aureus* ATCC 25923 according to the standard disc diffusion QC procedures.

## 4. Result

A total of 23,699 clinical samples which included 8225 blood culture samples, 814 body fluid samples, 693 CSF samples, 1344 respiratory samples, 7206 urine samples and 5417 samples consisting of pus, wound swabs, tissue etc. received in the Microbiology laboratory were processed in the during the study period (Figure 1).

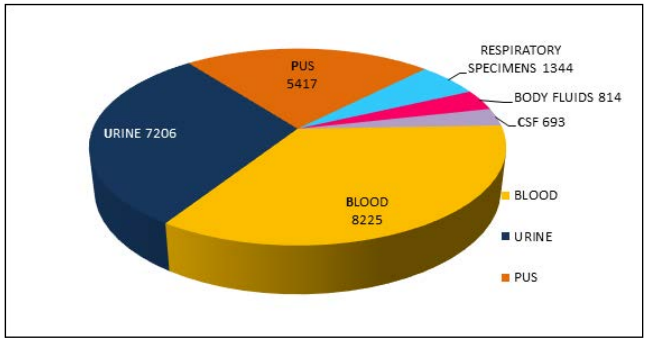


**Figure 1.** Distribution of total samples processed.

Of 23,699 clinical samples processed, only 8172 samples showed growth of one or the other relevant organisms. A total of 1233 clinical isolates of *S. aureus* were identified and included in the study. Out of all clinical specimens, pyogenic samples yielded the maximum number of *S. aureus* isolates (63.1%) followed by blood samples (29.9%) and urine samples (4.8%) (Table 1). *S. aureus* infection was more evident in hospitalized (IPD) 71.2% (878) patients than in patients attending the outpatient department (OPD) 28.8% (355).

Seasonal variation was also seen in isolation of *S. aureus*, with a higher percentage (741/1233, 60.1%) of isolates obtained during summer season (April to September) than during winter season (October to March) (492/1233, 39.9%).

**Table 1.** Distribution of *S. aureus* isolates from different clinical samples

| Type of Sample (n=Total samples processed) | No. of samples showing growth (%) | No. of *Staphylococcus aureus* strains isolated |
|---|---|---|
| Blood (n=8225) | 843 (10.25%) | 369 (29.9%) |
| Body fluids (n=814) | 93 (11.43%) | 11 (0.9%) |
| CSF (n=693) | 15 (2.16%) | 02 (0.2%) |
| Respiratory samples (n=1344) | 531 (39.51%) | 14 (1.2%) |
| Urine (n=7206) | 2591 (35.96%) | 59 (4.8%) |
| Pus, wound swabs, tissue, etc. (n=5417) | 4099 (75.67%) | 778 (63.1%) |
| **Total samples= 23,699** | **Positive growth= 8172** | **Total *S. aureus* = 1233 (100%)** |

Among 1233 *S. aureus* isolates, Methicillin resistance was detected in 49.6% (611) of isolates and rest were Methicillin susceptible 50.4% (622). On performing antibiotic susceptibility testing, majority of the isolates were found resistant to Penicillin (92.1%), followed by Erythromycin (59%). Almost half of the total isolates were resistant to Sulphamethoxazole-Trimethoprim (49.3%) followed by Amoxicillin-Clavulanic acid (47.8%), Ciprofloxacin (43.4%) and Clindamycin (18.4%). Antibiotics to which all isolates showed 100% susceptibility included Vancomycin (100%), Teicoplanin (100%), Linezolid (100%) (Table 2).

**Table 2.** Resistance pattern of *S. aureus* isolates for various antibiotics

| Antibiotic tested | No. of isolates Resistant to antibiotic tested | No. of isolates Susceptible to antibiotic tested |
|---|---|---|
| Penicillin | 1136 (92.1%) | 97 (7.9%) |
| Clindamycin | 227 (18.4%) | 1006 (81.6%) |
| Erythromycin | 727 (59.0%) | 506 (41.0%) |
| Vancomycin | 0 (0.0%) | 1233 (100%) |
| Teicoplanin | 0 (0.0%) | 1233 (100%) |
| Amoxicillin-Clavulanic acid | 589 (47.8%) | 644 (52.2%) |
| Ciprofloxacin | 535 (43.4%) | 698 (56.0%) |
| Linezolid | 0 (0.0%) | 1233 (100%) |
| Sulphamethoxazole-Trimethoprim | 608 (49.3%) | 625 (50.7%) |

## 5. Discussion

In our research we isolated 1233 (15.1%) *S. aureus* strains from 8172 culture-positive clinical samples. This is similar to that reported by Gurung *et al.* and Mukhiya, *et al.*, with growth positivity of around 17%[7,8]. Among all clinical specimens, pyogenic samples (63.1%) yielded maximum number of *S. aureus* strains followed by blood samples (29.9%) and urine samples (4.8%). These findings are comparable to other studies where skin, soft tissues, pus and wound samples grow the maximum number of *S. aureus* strains (65.4% and 78.37% respectively)[7,9]. Pyogenic samples were followed by blood and urine samples in yielding maximum number of *S. aureus* isolates even in these studies.

Parallel to the findings of other researchers[7], who have reported a higher number and percentages of *S. aureus* in IPD patients (55.7%) than OPD patients (44.3%), the number and percentages of *S. aureus* isolates in our study were more in IPD (71.2%) patients than in patients attending the OPD (28.8%).

Seasonal variation was also seen in isolation of *S. aureus*, with a higher percentage of isolates obtained during summer and autumn season (April to September) than during winter season (October to March). There have been various studies that support these findings, who have also reported a peak occurrence of Staphylococcal infections during this time of the year[10-14].

The reason for such distribution may be based upon the fact that bacterial counts are proportionately greater on the skin of the individuals in a high-temperature and high-humidity environment compared with a moderate-temperature, low-humidity environment, leading to more chances of infections[15].

Our study showed that the prevalence rate of MRSA strains was 49.6%, which goes in concordance with the results obtained by other authors who have reported the prevalence of methicillin resistance varying from 48.6% to 52.5% (Table 3)[16-18].

Unlike our study, various researchers have reported a high percentage of methicillin resistance varying from 64% to 75%[19,20]. The prevalence of MRSA strains seems to be low in our hospital setting. However, there are studies that have reported even lower prevalence (31.4% to 40.4%) of Methicillin resistance amongst their isolates[21-23].

The *S. aureus* strains islolated in our study showed maximum resistance to Penicillin (92.1%), followed by Erythromycin (59%), Sulphamethoxazole-Trimethoprim (49.3%), Amoxicillin-Clavulanic acid (47.8%), Ciprofloxacin (43.4%) and Clindamycin. No isolate was found resistant Vancomycin, Teicoplanin and Linezolid. In a similar study carried out by Reddy, *et al.* (2012)[24], maximum resistance in *Staphylococcus aureus* isolates was observed against Penicillin-G (74%) followed by, Ampicillin (72%), Erythromycin (54%), Oxacillin (51.3%), Amoxycillin-clavulanate (40%), Tetracycline (32%), Ciprofloxacin (32%), Clindamycin (16%) and Vancomycin (1.3%).

As evident from our findings, apart from Penicillin, which is of historical importance for the treatment of Staphylococcal infections these days, around 50% of the isolated *S. aureus* strains in our settings are resistant to

**Table 3.** Comparing the percentage of Methicillin Resistant and Methicillin Susceptible *Staphylococcus aureus* isolates obtained in various studies with the present study

| Name of the author | No. of *S. aureus* isolates included in the study | No. of MRSA isolates (%) | No. of MSSA isolates (%) |
|---|---|---|---|
| Gurung, *et al.* (2020) [7] | 52 | 39 (75%) | 13 (25%) |
| Deotale, *et al.* (2010) [16] | 247 | 123 (49.8%) | 124 (50.2%) |
| Mittal, *et al.* (2013) [23] | 260 | 105 (40.4%) | 155 (59.6%) |
| Velvizhi, *et al.* (2011) [19] | 112 | 83 (74%) | 29 (25.9%) |
| Prabhu, *et al.* (2011) [21] | 190 | 60 (31.6%) | 130 (68.4%) |
| Deotale, *et al.* (2010) [16] | 247 | 123 (49.8%) | 124 (50.2%) |
| Fasih, *et al.* (2010)[20] | 2432 | 1562 (64%) | 870 (36%) |
| Angel, *et al.* (2008)[22] | 185 | 58 (31.4%) | 127 (68.6%) |
| Yilmaz, *et al.* (2007) [1] | 883 | 464 (52.5%) | 419 (47.5%) |
| Gadepalli, *et al.* (2006) [17] | 200 | 104 (52%) | 96 (48%) |
| Azap, *et al.* (2005)[18] | 216 | 105 (48.6%) | 111 (51.4%) |
| Present study | 1233 | 611 (49.6%) | 622 (50.4%) |

Penicillinase stable Penicillins, Erythromycin and even the oral drugs like Sulphamethoxazole-Trimethoprim, Amoxicillin-Clavulanic acid and Ciprofloxacin. This makes the use of these antibiotics questionable as empiric therapeutic agents. Three drugs, namely Vancomycin, Teicoplanin and Linezolid, however, seem promising with 100% susceptibility even against MRSA isolates.

## 6. Conclusion

Antimicrobial resistance is a serious problem worldwide that threatens an effective therapeutic management of infections caused by multidrug resistant bacteria. In view of findings observed in the present study, it is clear that an effective treatment of infections caused by multidrug resistant Staphylococcal strains is a challenge. High percentages of resistance seen towards oral drugs like Sulphamethoxazole-Trimethoprim, Amoxicillin-Clavulanic acid and Ciprofloxacin overemphasizes the optimum utilization of antibiotics. Drugs like Vancomycin, Teicoplanin and Linezolid promise to work as miracle drugs against the multidrug resistant MRSA strains but we need to warrant judicious use of these wonder drugs to conserve them for future use.

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