

# A Comparative Study on Resistance Pattern of Pathogenic *Staphylococcus aureus* against Imipenem, Methicillin, and Vancomycin

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## ABSTRACT

**Objective:** The objective of this study was to evaluate current susceptibility of pathogenic isolates of *Staph. aureus* against imipenem, methicillin and vancomycin. **Methodology:** Antibacterial activity was performed using disc diffusion technique. **Results:** The average zone values were compared by using ANOVA and result found to be significant with p-value <0.001. Highest zone values were found to be  $31.36 \pm 5.14$  for imipenem followed by methicillin  $23.77 \pm 6.53$ . The lowest zone value were found to be for vancomycin i.e;  $14.76 \pm 4.30$ . Imipenem was the found to be the most effective drug as 91.67 % isolates were susceptible to imipenem. While high degree of resistance was observed among isolates against vancomycin (83.33 %) and methicillin (70.83 %). **Conclusion:** Increase in resistance demands the development and rational use of new, safe and effective therapeutic agents.

**Key words:** Antibacterial activity, Bacterial resistance, *Staphylococcus aureus*, Disc diffusion technique

## INTRODUCTION

*Staphylococcus aureus* is the most common pathogen which doctors come across in clinical practice, causing a range of diseases including skin infections, wound infections, nosocomial infections, osteomyelitis, food poisoning, endocarditis, pneumonia and toxic shock syndrome<sup>1</sup>. Infections caused by multiple-resistant Gram-positive organisms continue to occur at an alarming rate worldwide<sup>2</sup>. However widespread indiscriminate prescribing of antimicrobials has resulted in an explosion of microorganisms resistant to all common drugs<sup>3,4</sup>. Resistance development occurs primarily among bacteria already resistant to one or more antimicrobial agents<sup>5</sup>. Methicillin-resistant *S. aureus* (MRSA) is a major cause of hospital-acquired infections that are becoming increasingly difficult to combat because of emerging resistance to all current antibiotic classes<sup>6</sup>.

The problem is not restricted to industrialized

countries. The last decade has seen an alarming increase in MRSA infections in Pakistani hospitals<sup>7</sup>. The hospital wards were blocked by MRSA infections. Even medical and paramedical staffs were at high risk. More than 75% of MRSA were resistant to commonly used anti-staphylococcus antimicrobials<sup>8</sup>. The frequency of MRSA among all nosocomial isolates of *Staphylococcus aureus* has increased to 38% in 2002.<sup>9</sup> Most isolates with reduced susceptibility to vancomycin appear to have developed from preexisting methicillin-resistant *S. aureus* infections<sup>10, 11</sup>. The problem of resistance to antimicrobial drugs is particularly troublesome in developing countries. The underlying problems are largely economic and societal<sup>12</sup>. An urgent need exists for more appropriate selection and use of antimicrobial drugs in the developed as well as in developing countries. The focus in developing countries should be on the availability of safe and effective drugs.

The present study was undertaken to monitor

and compare the resistance pattern of *Staphylococcus aureus* isolates obtained from different patients against imipenem, methicillin and vancomycin.

## MATERIALS & METHODS

### Drugs

Standard Antibiotic discs of methicillin (MET 5), vancomycin (VA 30), and imipenem (IMP 10) from Oxoid England.

### Collection of Bacterial Isolates

Isolates of *Staphylococcus aureus* were procured from the urine & blood samples of the patients randomly at microbiology lab, Fauji Foundation Hospital and Pakistan Institute of Medical Sciences, Islamabad. The sample size was calculated at 5% level of significance and 80% power of test. For *Staphylococcus aureus* these parameters were calculated by using proportions of expected resistance. The estimated sample size was twenty four.

### Determination of Antibacterial Activity

The isolates were identified by colony morphology, catalase and coagulase tests<sup>1</sup>. A single colony was picked from the isolate culture and inoculated in test tube broth. The test tubes were placed in incubator at 37°C for 24 hours. The antibacterial activity or drugs was determined by the Kirby Bauer Technique. Nutrient agar plates were inoculated with the selected isolates @ 200 µl/ plate. The standard drug discs were placed on each plate. The plates were incubated at 37°C for 18-24 hrs. The antibacterial activity was assessed by measurement of zone of inhibition in mm for the respective drug<sup>13</sup>.

### Statistical Analysis

Data was entered and analyzed by using SPSS. 14.0. Quantitative variables zone of inhibition were compared by using ANOVA. Qualitatively sensitivity and resistance for each drug were reported by frequency and percentages. Comparisons for sensitivity and resistance among three drugs were compared by using Chi-square.

## RESULTS

The samples were collected from all the age groups in both the sexes. Out of 100 % (24) *Staphylococcus aureus* isolates samples, 45.83 % (11) *Staphylococcus aureus* were isolated from female patients. And 54.17 % (13) *Staphylococcus aureus* were isolated from male patients. It shows that *Staphylococcus aureus* equally infect both male and female. The ages of patients from which *Staphylococcus aureus* was isolated ranges between 12 and 56 years in both the sexes with a mean age of 32.38 and S.D of 13.44. Table 1 shows that *Staphylococcus aureus* infect all age groups in both the sexes.

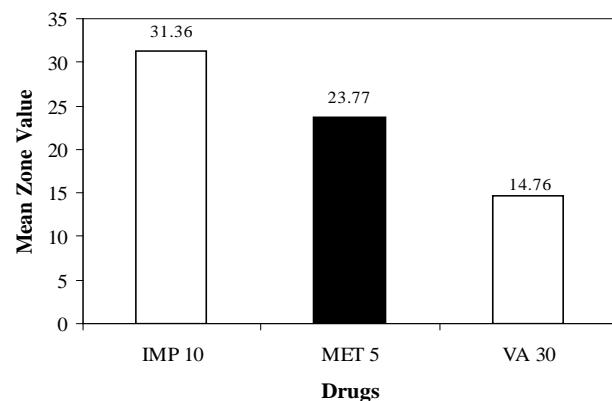


Fig. 1: Average zone values of various drugs against *Staphylococcus aureus*

Inhibition zone appeared for 24, 10 and 20 cases for imipenem, methicillin and vancomycin respectively. Rest of the cases gave completely no response. The average zone values were compared by using ANOVA and result found to be significant with p-value <0.001. The Highest zone value was found to be  $31.36 \pm 5.14$  for imipenem. The lowest value was found to be for vancomycin i.e;  $14.76 \pm 4.30$ . The value for methicillin was found to be  $23.77 \pm 6.53$  (Table 2, Fig. 1). After Post hoc Test, i.e; Tukey's test imipenem was giving significantly higher zone than other two drugs.

Difference was significant among all drugs when a comparison was made for patients who were resistant, sensitive, giving no response and having response but then re-growing, all with p<0.001.

## Resistance Pattern of Pathogenic *Staphylococcus aureus* against Imipenem, Methicillin, and Vancomycin

**Table 1: Distribution of patients and age by status and gender**

Pathogen	Gender	N	%	Mean	S.D	Min.	Max.
<i>Staphylococcus aureus</i>	13	54.17	33.62	13.56	16	56	13
	11	45.83	30.91	13.82	12	52	11
	24	100.00	32.38	13.44	12	56	24

**Table 2: Comparison of zones of inhibition of drugs against *Staph. aureus* via statistical data.**

Drug	N	Mean	Std. Deviation	Std. Error	95% Confidence interval for mean		Min	Max
					Lower bound	Upper bound		
Imipenem	24	31.36	5.14	1.05	29.19	33.53	15.50	35.20
Methicillin	10	23.77	6.53	2.06	19.10	28.44	12.20	33.30
Vancomycin	20	14.76	4.30	0.96	12.75	16.77	9.20	25.50
Total	54	23.81	9.06	1.23	21.33	26.28	9.20	35.20

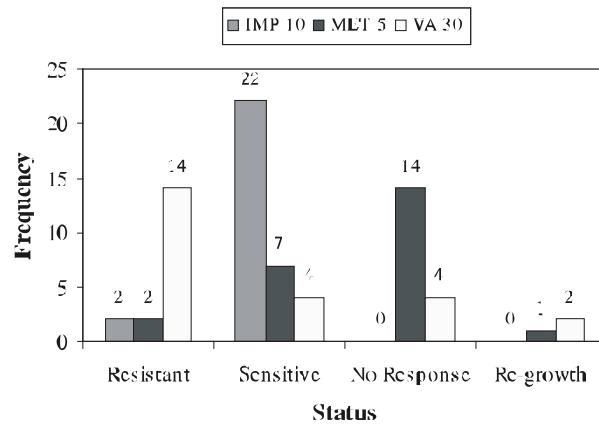
**Table 3: Frequency of resistance and sensitivity of drugs against *Staph. aureus*.**

	Imipenem		Methicillin		Vancomycin	
	No.	%	No.	%	No.	%
Resistant	2	8.33	2	8.33	14	58.33
Sensitive	22	91.67	7	29.17	4	16.67
No Response	0	0.00	14	58.33	4	16.67
Re-growth	0	0.00	1	4.17	2	8.33
Chi-Square	<b>76.44</b>	<b>23.56</b>	<b>19.56</b>	<b>76.44</b>	<b>23.56</b>	
P-Value	<b>0.0000</b>	<b>0.0000</b>	<b>0.0002</b>	<b>0.0000</b>	<b>0.0000</b>	

Significant percentage of vancomycin cases were resistant by cutoff value of <18 for zone value ie; 58.33 %. Among sensitive cases, the results were exactly opposite to that of resistant. 91.67 % (22) cases from imipenem were sensitive. 29.17 % cases from methicillin and 16.67% cases from vancomycin were sensitive to *Staphylococcus aureus*. There were a significant percentage of no responsive cases for methicillin and vancomycin i.e., 58.33 and 16.67 percent respectively, showing complete resistance. Vancomycin was found to have the most resistance i.e. 83.33 %. (Table 3, Fig. 2).

## DISCUSSION

The samples were collected from all the age groups in both the sexes. It was found that *Staphylococcus aureus* equally infect male and female, in all the age groups. All the conditions



**Fig. 2: Resistance status of each drug against *Staphylococcus aureus*.**

during antibacterial testing using Disc diffusion method were standardized. Conditions such as temperature, composition of culture medium, size of

inoculum, time of incubation may interfere in the results of resistance tests to drugs against pathogens<sup>13</sup>.

In the past, the drugs effective to treat Staph. infections were imipenem, vancomycin<sup>9</sup>. In our study imipenem was found to have good inhibitory zones and 91.67 % *Staph. aureus* isolates were susceptible to imipenem. The reason is that Imipenem was a costly antibiotic and not commonly used. Combined administration of vancomycin and imipenem proved effective against methicillin-resistant *Staph. aureus*<sup>11, 14</sup>.

Increase in resistance to methicillin has been reported in previous study<sup>9</sup>. In our study, 70.83 % *Staph. aureus* isolates showed resistance against methicillin. Individuals often take subtherapeutic doses of antibiotics; symptoms may disappear but resistant strains of bacteria are thereby selected and may only become acutely problematic to the individual in a subsequent medical crisis where an infection proves resistant. The threat to the larger population is that reservoirs of drug-resistant bacteria abound.

Reduced susceptibility to vancomycin against *Staphylococcus aureus* strains collected from patient's specimen was observed<sup>11</sup>. Similarly in our study, 83.33 % *Staph. aureus* isolates were found to be resistant to vancomycin. The emerging threat of widespread vancomycin resistance poses a serious public health concern given the fact that vancomycin has long been the preferred treatment of antibiotic-resistant gram-positive organisms<sup>15</sup>. Most isolates with reduced susceptibility to vancomycin appear to have developed from preexisting methicillin-resistant *S. aureus* infections<sup>10</sup>.

The use of antibacterial drugs as growth promoters for animals, as antibiotic prophylaxis in cattle, chicken and aquaculture grew to quantities that drug residues pass on to human through milk, meat and eggs and markedly reduce the effective lifetime of human antibiotics.

## CONCLUSION

One of the primary factors for the current magnitude of the resistance problem is the unabated overuse of older antimicrobials. If this occurs with the newest agents, then their potential benefit will

be limited. Continued development of newer agents, their judicious use and rigorous infection control practices are essential to save lives and limit Gram-positive resistance.

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