ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF STAPHYLOCOCCUS AUREUS STRAINS IN ISLAMABAD, PAKISTAN

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ABSTRACT

Objective: To investigate the prevalence of *S. aureus* in hospitalized patients of Islamabad.
Study Design: Cross-sectional study.
Study Duration: Pakistan Institute of Medical Science, Applied Microbiology and Biotechnology Lab, COMSATS Institute of Information Technology, Islamabad, from Sep 2017 to Sep 2018.
Methodology: A total of 500 samples were collected. The isolates were divided into four study groups according to their source of origin i.e. group 1 (dermal group), group 2 (nasal group), group 3 (blood group) and group 4 (urine group). Gram staining, catalase test and DNA se media analysis were done for validation of *S. aureus*. Disc diffusion test (for antibiotic susceptibility), Oxacillin disc test (to differentiate between methicillin-resistant *Staphylococcus aureus* and *methicillin-susceptible staphylococcus aureus*) and minimal inhibitory concentration (for susceptibility to vancomycin), were performed.
Results: Degree of the prevalence of *staphylococcus aureus* was 21%, 17%, 9% and 8% in group 1, 2, 3 & 4 respectively. The overall prevalence of *staphylococcus aureus* was 19.5% in all isolates. The disc diffusion test showed the descending resistance pattern of isolates i.e. 100, 94, 94, 76, 58, 55, 47, 43, 40 and 37% for penicillin, ciprofloxacin, *Kanamycin, erythromycin, tetracycline, oxazolidinone, sulfamethoxazole, doxycycline, clindamycin,* and *cipoxin* respectively. Minimal inhibitory concentration found only one sample resistant at 2ug/l concentration of Vancomycin. Moreover, Oxacillin disc test showed 52% methicillin-susceptible Staphylococcus aureus while 48.2% methicillin-resistant *staphylococcus aureus* among all isolates.
Conclusion: There is an increase in the frequency of methicillin-resistant *staphylococcus aureus*. Single vancomycin resistant *staphylococcus aureus* strain was also isolated.

Keywords: Antimicrobial, Islamabad, Pakistan, Staphylococcus aureus, Susceptibility.

INTRODUCTION

Staphylococcus aureus (S. aureus) is the leading cause of hospital and community-acquired infections with severe consequences. Blood stream, skin, soft tissues and lower respiratory tracts are targeted in Nosocomial *S. aureus* infections. Major deep-rooted infections, such as endocarditis and osteomyelitis, toxinmediated diseases, such as toxic shock syndrome, scalded skin syndrome and *staphylococcal* foodborne diseases may also be the result of *S. aureus*. The weak immune system, numerous catheter insertions and injections are responsible for infections in hospitalized patients[1-3]. *S. aureus* is provided with diverse virulence factors, which include both structural and secreted products which contribute to the pathogenesis of infection[4].

In the last decade, resistance and reduced susceptibility to antibiotic agents has become a major medical concern[5]. The incidence of bacterial infection was reduced with penicillin discovery in 1940. But then penicillin β-lactam core ring was destroyed by β-lactamase which *Staphylococcus aureus* started producing[6]. β-lactamase resistant methicillin was developed which was effective against *Staphylococcus aureus* until the first strain of *methicillin-resistant S. aureus* (MRSA) were separated in 1961[7].

Vancomycin was considered the most effective medicine for MRSA infections. In Japan, the vancomycin-resistant strain was detected in 1996. In 1958, vancomycin was introduced for medical practice, for treating gram-positive bacterial infection[8]. A specific group of *S. aureus*, known as hetero-VRSA, rapidly develop VRSA *(vancomycin-resistant Staphylococcus aureus strain)*, when exposed to vancomycin. Existence of hetero-VRSA indicates an adverse decrease in the effectiveness of vancomycin in clinical biology. Excess amounts of peptidoglycan cause an increase in the size of the cell wall as the result of which resistance developed against the antibiotic. This is a simple mechanism for all VRSA identified around the globe so far[9].

In Pakistan and particularly Islamabad region, only limited data is available on the susceptibility of MRSA to antibiotics predominantly vancomycin. The current study reveals the prevalence of multidrug-resistant MRSA strains and examines their antibiotic

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sensitivity pattern in a tertiary care hospital of Islamabad.

METHODOLOGY

This cross-sectional study was conducted at the Pakistan Institute of Medical Science Islamabad, from September 2017 to September 2018. Different type of samples including blood, urine, wound secretions and nasal secretions were collected from the patients in Pakistan Institute of Medical Science (PIMS) Islamabad. The samples were taken through gel swab or in blood vales and transported to the Applied Microbiology and Biotechnology Lab, COMSATS Institute of Information Technology, Islamabad in 12-24 hrs. Samples were cultured on the same day. Patients with any co-morbidity were excluded.Written consent was taken. Ethics approval for the study was obtained from the ethics review board committee of the COMSATS Institute of Information Technology, Islamabad (CIIT/Bio/ERB/17/25).

All the blood samples were cultured in blood culture bottles containing broth (Thermo USA). The culturing bottles were incubated in Automated Blood Culture System (ABCS). The presence of bacteria was indicted by machine and bacteria-containing tubes were further cultured on McConkey agar. The samples of nasal secretions and pus samples were cultured on chocolate agar or McConkey agar and incubated at 37C for 18 to 24 hrs. Urine samples were cultured on CLRD media and incubated at 37°C for 24 hrs.

The presence of *S. aureus* was checked through colonial morphology as they give small grey-white colony of 1-2 cm on agar. Presence of s*taphylococcus aureus* was further confirmed through gram staining, catalase and DNAse test as they are positive for all of these tests.

After confirmation s*taphylococcus aureus* colonies were purified on nutrient agar for further study. A single colony of *S. aureus* was picked from nutrient agar and inoculated in test tubes having LB broth and incubated on shaking incubator at 370 C for 24 hrs. Glycerol stocks were produced by homogenizing 50% glycerol solution and bacterial culture (freshly grown) in 1:1 ratio. The bacterial glycerol stocks were preserved at -200 C.

The susceptibility of all positive cultures which yields *S. aureus* were checked on Mueller-Hinton agar by using disc diffusion method as described in the manual of Clinical and Laboratory Standards Institute recommendations. The results were checked after 24h incubation at 37C as sensitivity, intermediate sensitivity and resistant according to zone diameter around each antibiotic disk. Oxacillin disc resistance results were used to confirm MRSA strains.

Minimum inhibitory concentration (MIC) is the minimum concentration of an antimicrobial agent that could inhibit the visible growth of microorganisms after overnight incubation. MICs were determined on the broth dilution method. It included the culturing of similar doses of bacteria in wells of liquid media containing progressively lower concentrations of the antimicrobial agent. MIC was determined based on the growth pattern of bacteria. One gram of vancomycin was added in 200 mL water to make stock. It means 1µL of water contain 5µg vancomycin. 2µL stock was added in 10mL of broth media to form 1µg vancomycin concentration per mL of media. Same like 4µL, 8µL, 16µL and 32µL antibiotic stock were added in 10 mL broth media to form 2µg, 4µg, 8µg and 16µg vancomycin concentrations per mL of media. SPSS-26 was used for Data analysis. Cross tabulation was done and frequency was taken.

RESULTS

A total of 500 samples was collected from PIMS Hospital, Islamabad. Out of which 50 samples were from the blood (25 from female, 25 from male), 300 samples were from wound/pus in which 100 from pimples (60F, 40M), 100 samples from accidental cuts (80M, 20F), 100 samples from operative cuts (73F, 27M), 50 samples were from urine (25F, 25M) and 100 samples were from a nasal fluid (50F, 50M). To measure incidence, there was an immense need to collect a reasonable amount of samples, that's why we collected 500 samples. The samples were collected according to standard procedures and preceded the same day of collection. Table-I shows the number of samples and their origin.

MIC is the smallest concentration of an antibiotic which prevent visible growth of a bacterium. Vancomycin MIC was checked against *S. aureus*. The results were compared according to CLSI (MICs >2 µg/ml should be reported as resistant)[10]. According to results, only one out 97 samples was resistance. Table-IV showing the vancomycin resistance.

Blood samples inoculated in blood culturing tubes were placed in ABCS machine. This machine indicates the presence of staphylococcus aureus bacteria in samples. Nine samples were indicated as positive; those positive samples were cultured on N-agar and again purified on N-agar after colony morphology

conformation. Wound/pus samples were cultured in broth to get bacterial colonies. Bacterial colonies were inoculated on N-agar from the broth[5-6], type of bacterial colonies were grown on N-agar. *S. aureus* colony was purified on N-agar after colony morphology character confirmation. Urine samples were inoculated on CLAD media and about 2-3 type of bacterial colonies were grown[8], samples from urine were positive. *S. aureus* colony was purified on N-agar. Nasal fluid samples were inoculated in broth and then on N-agar[7-8], type of bacterial colonies were found. Seventeen samples were *S. aureus* positive. *S. aureus* was purified on N-agar.

S. aureus presence was confirmed through gram staining, all the *S. aureus* were positive gram staining. Results were reconfirmed through catalase test as all the *S. aureus* bacteria gave positive results for catalase. *S. aureus* colonies were grown at DNase media for further confirmation. All the *S. aureus* bacteria gave positive results for DNase test.

Total 500 samples were divided into four study groups designated as group 1, group 2, group 3 and group 4, based on samples from wound/pus, samples from the nasal cavity, samples from blood and samples from urine respectively.

Table-I: Prevalence of staphylococcus aureus.

Origin of Samples	Total No. of Samples	No. of Samples from Female	No. of Samples from Male	Total Staphylococcus Aureus Positive	No. of Positive Samples in Females	No. of Positive Samples in Males	Prevalence in Female	Prevalence in Male	Overall Prevalence
Wound /Pus	300	143	157	63	21	42	14.68%	26.7%	21%
Nasal Fluid	100	50	50	17	8	9	16%	18%	17%
Blood	50	25	25	9	5	4	20%	16%	18%
Urine	50	25	25	8	3	5	12%	20%	16%

Table-II: Percentage of resistance and susceptibility against the antibiotic disc.

Antibiotics	Resistance	Intermediate	Susceptible
Penicillin	97/97 (100%)	-	-
Ciprofloxin	91/97 (94%)	-	6/97 (6%)
Kanamycin	91/97 (94%)	6/97 (6%)	-
Tetracyclin	56/97 (58%)	6/97 (6%)	35/97 (36%)
Doxycycline	39/97 (40%)	9/97 (9%)	49/97 (51%)
Erythromycin	74/97 (76%)	20/97 (21%)	3/97 (3%)
Cefoxin	36/97 (37%)	13/97 (13%)	48/97 (50%)
Clindamycin	42/97 (43%)	6/97 (6%)	49/97 (51%)
Sulfamethoxazole	46/97 (47%)	5/97 (5%)	46/97 (47%)
Oxazolidinone	53/97 (55%)	17/97 (17%)	27/97 (28%)

(Antibiotic Disc Dose (ug): (Penicillin 10, Ciprofloxin 5, Kanamycin 30, Tetracyclin 30, Doxycyclin 30, Erythromycin15, Cefoxin 30 Clindamycin 10, sulfamethoxazole 25, Oxazolidinone 30).

Table-III: MRSA and MSSA in islamabad.

Sample Origin	S. aureus (positive)	S. aureus (positive)		MRSA		MSSA		Overall MRSA	Overall MSSA
		Males	Females	Males	Females	Males	Female		
Wound	63	42	21	40.47%	47.62%	59.52%	52.38%	-	-
Nasal fluid	17	9	8	55.55%	62.5%	44.44%	37.5%	-	-
Blood	9	4	5	75%	60%	25%	40%	-	-
Urine	8	5	3	40%	33.33%	60%	66.66%	-	-
Total	97	60	37	45%	51.35%	55%	48.86%	48.2%	52%

(MRSA, Methicillin resistant staph aureus, MSSA, Methicillin susceptible staph aureus)

Table-IV: Vancomycin resistance in S. aureus.

Antibiotic	Resistant	Intermediate	Susceptible
Vancomycin	1/97(1.03%)	0	96/97 (98.96%)

48.2% samples were MRSA as they were resistant against oxacillin, and 52% samples were MSSA as they were susceptible to oxacillin. All the results were designed according to CLSI guidelines. Table-III shows the total MRSA and MSSA samples and their origin.

Presence of Staphylococcus aureus was checked and confirmed through different biochemical tests. All the *S. aureus* were grown on Mueller Hinton Agar to check their antibiotic disc resistance pattern. Table-I shows percentage of resistance and susceptibility

against the antibiotic disc, figure shows Antibiotic disc analysis.

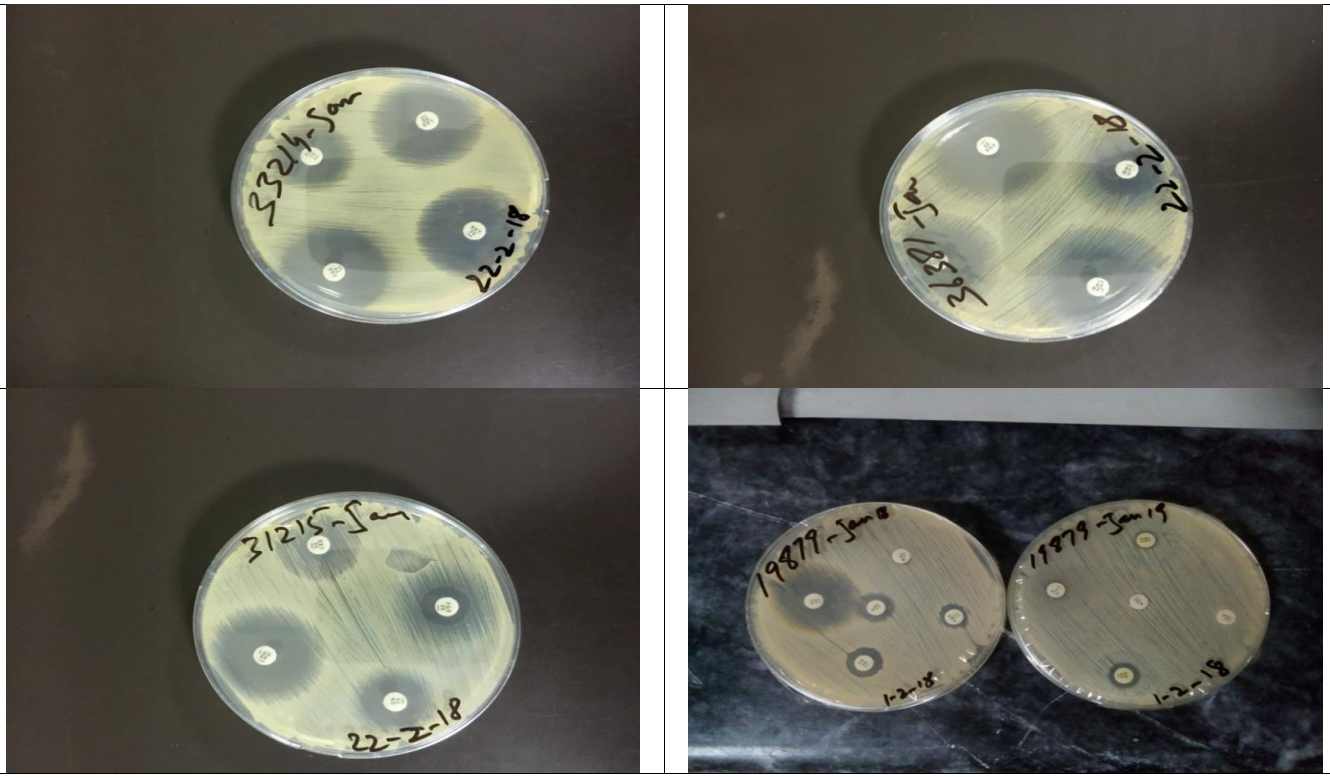

Figure: Antibiotic disc analysis.

DISCUSSION

S. aureus is a chief source of bacteraemia in hospitals and is the most common cause of necrotizing pneumonia, skin, and soft-tissue infections in the community[11,12]. In our study, 48.2% of samples were MRSA and 52% samples were MSSA In 2016, a study in Peshawar reported a 36.1% frequency of MRSA[13]. Furthermore, of 47.42% MRSA, major 67.5% of MRSA was isolated from blood followed by 59% from the nasal fluid. About 36.6% (least) of MRSA was isolated from the urine, 51.35% MRSA isolates were from females (45% from males)[14].

Penicillin was the first-ever antibiotic which was used against any kind of bacteria. It is still in use, although many bacteria are resistance against it. 100% samples of *S. aureus* were resistance against penicillin. Ciprofloxin is used against various bacteria causing skin infections. 6% *S. aureus* was susceptible while 94% was resistance against ciprofloxacin, 75.8% resistance was reported in 2011 study in Lahore[15]. Results against kanamycin showed that 6% of samples were intermediate and 94% were resistant. Tetracycline is also used as an antibiotic against infectious bacteria. Its disc results showed that 36% of samples were susceptible, 6% intermediate and 58% resistant. Doxycycline is mostly used against the bacteria, which cause acne and skin infections. Fifty one percent of samples were susceptible, 9% intermediate and 40% resistant against doxycycline. Twenty one percent samples were intermediate, 3% susceptible and 76% resistant against Erythromycin. Cefoxin results show that 36% of samples were resistant, 13% intermediate and 50% susceptible. 43% isolates were resistant, 6% intermediate and 51% susceptible against Clindamycin, sulfamethoxazole results show that 47% samples were resistant, 5% intermediate and 47% susceptible, while 55% samples were resistant, 17% intermediate and 28% susceptible against oxazolidinone. In other studies, the same procedural disc analyses have been done to identify the susceptibility and resistant pattern of *S. aureus* by using various types of antibiotics available in the market such Penicillin, Ampicillin, Amoxicillin, Streptomycin, Erythromycin, Lincomycin, Tetracycline, Neomycin; Amoxicillin, Clindamycin, SXT/trimethoprim / sulfamethoxazole, Cefoperazone, and Oxacillin[16-19].

This study shows increased vancomycin resistance among MRSA strains in Rawalpindi/Islamabad area. Vancomycin MIC when checked against *S. aureus*

in 97 samples one was resistant as the results were compared according to CLSI. In previous studies conducted in different areas of Pakistan[15], no resistant VRSA strain was detected[20]. But latest studies have reported VRSA in Karachi and Peshawar[21-23].

Although, our sample size is less as compared to the entire population; however, at least it covers the periphery of PIMS hospital and it could be evaluated that MRSA and MSSA are prevalent in the PIMS hospital environment. Hereby, it is recommended that there should be regular periodic reviews of hospital-acquired infections including antimicrobial sensitivity examinations and it would be quite helpful in drawing antibiotic policy for infection control and alleviating the prevalence of multidrug-resistant MRSA and MSSA.

RECOMMENDATION

Prescribing antibiotics other than glycopeptides for MRSA infections will lessen the probabilities of occurrence of VRSA. Good hospital infection control measures are most effective against these infections.

CONCLUSION

There is an increase in the frequency of MRSA among *S. aureus* isolates. Single VRSA strain was also isolated.

CONFLICT OF INTEREST

This study has no conflict of interest to be declared by any author.

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