ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF STAPHYLOCCUS AUREUS STRAINS IN ISLAMABAD, PAKISTAN

Yasir Rasheed, Kaleem Imdad, Raheela Yasmin*, Ambreen Gul*, Aneela Jamil**, Ummara Aslam

COMSATS University Islamabad Pakistan, *HITEC-IMS Taxila/National University of Medical Sciences (NUMS) Pakistan, **Rawalpindi Medical College, Rawalpindi Pakistan

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 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 ciproxin 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 patients1-3. S. aureus is provided with diverse virulence factors, which include both structural and secreted products which contribute to the pathogenesis of infection4.

In the last decade, resistance and reduced susceptibility to antibiotic agents has become a major medical concern5. 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 producing6. 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 19617.

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 infection8. 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 far9.

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
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 indicated 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 37°C 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 *staphylococcus aureus* was further confirmed through gram staining, catalase and DNase test as they are positive for all of these tests.

After confirmation *staphylococcus 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 37°C 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 (MIC >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 innoculated on N-agar from the broth, 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, samples from urine were positive. S. aureus colony was purified on N-agar. Nasal fluid samples from nasal cavity, samples from blood and samples from urine respectively. Seventeen samples were S. aureus positive. S. aureus was purified on N-agar.

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 designated according to CLSI guidelines. Table-III shows the total MRSA and MSSA samples and their origin.

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%               |

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.

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%) |

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 | Females | Male | Female | Male | Female |
| Wound         | 63    | 21      | 40.47% | 47.62%   | 59.52% | 52.38% | -    | -    | -    | -    |
| Nasal fluid   | 17    | 8       | 55.55% | 62.5%    | 44.44% | 37.5%  | -    | -    | -    | -    |
| Blood         | 9     | 5       | 75%    | 60%      | 40%    | 40%    | -    | -    | -    | -    |
| Urine         | 8     | 3       | 40%    | 33.33%   | 60%    | 66.66% | -    | -    | -    | -    |
| Total         | 97    | 37      | 45%    | 51.35%   | 55%    | 48.86% | 48.2%| 52%    |

Table-IV: Vancomycin resistance in S. aureus.

| Antibiotic | Resistant | Intermediate | Susceptible |
|------------|-----------|--------------|-------------|
| Vancomycin | 1/97(1.03%) | 0            | 96/97 (98.96%) |
to check their antibiotic disc resistance pattern. Table-I shows percentage of resistance and susceptibility against the antibiotic disc, figure shows 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. 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. 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).

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. 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.

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\(^8\), no resistant VRSA strain was detected\(^9\). But latest studies have reported VRSA in Karachi and Peshawar\(^21\).\(^22\).

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.

**REFERENCES**

1. Vidhani S, Mehdiniratta P. Study of methicillin resistant *S. aureus* (MRSA) isolates from high risk patients. 2001; 19(2): 13-16.
2. Alam SMS, Kalam MA, Munna MS, Munshi SK, Noor RJAPJoTD. Isolation of pathogenic microorganisms from burn patients admitted in Dhaka Medical College and Hospital and demonstration of their drug-resistance traits 2014; 4(5): 402-07.
3. Salas M, Wernerck M, Fernández L, Iglesias B, Gutiérrez D, Álvarez A, et al. Characterization of Clinical MRSA Isolates from Northern Spain and Assessment of Their Susceptibility to Phage-Derived Antimicrobials. 2020; 9(8): 447.
4. Plata K, Rosato AE, Wegryzn GJABP. Staphylococcus aureus as an infectious agent: overview of biochemistry and molecular genetics of its pathogenicity. Acta Biochim Pol 2009; 56(4): 1-5.
5. Chessa D, Ganau G, Mazzarello VJTJoIiDC. An overview of staphylococcus epidermidis and staphylococcus aureus with a focus on developing countries. J Infect Dev Ctries 2015; 9(06): 547-50.
6. Lima LM, da Silva BNW, Barbosa G, Barreiro EJB. β-lactam antibiotics: An overview from a medicinal chemistry perspective. Eur J Med Chem 2020; 1(119): 112829-32.
7. Brown DF, Edwards DL, Hawkey PM, Morrison D, Ridgway GL, Towner KJ, et al. Guidelines for the laboratory diagnosis and susceptibility testing of methicillin-resistant staphylococcus aureus (MRSA). J Antimicrob Chemother 2005; 56(5): 1000-18.
8. Hiramatsu K, Hanaki H, Ino T, Yabuta K, Oguri T, Tenover FJ. Methicillin-resistant staphylococcus aureus clinical strain with reduced vancomycin susceptibility. J Antimicrob Chemother 1997; 40(1): 135-36.
9. Hiramatsu K. Vancomycin-resistant staphylococcus aureus: a new model of antibiotic resistance. Lancet Infec Dis 2001; 1(3): 147-55.
10. Wilcox M, Al-Obeid S, Gales A, Kozlov R, Martinez-Orozco JA, Rossi F, et al. Reporting elevated vancomycin minimum inhibitory concentration in methicillin-resistant staphylococcus aureus: consensus inter working group. Future Microbiol 2019; 14(4): 345-52.
11. Vali L, Dashti AA, Mathew F, Udo EE. Characterization of heterogeneous MRSA and MSSA with reduced susceptibility to chlorhexidine in Kuwaiti hospitals. Front Microbiol 2017; 8(1): 1359-62.
12. Emre A, Seyman D, Turker M, Adiguzel Z, Gunay V, Tekeli A, et al. Cases with skin and soft tissue infections caused by community-acquired methicillin-resistant staphylococcus aureus/top-lum kokemli metisilen direnci staphylococcus aureus etkenli deri ve yumusak doku infeksiyonu olgulari. KLMIK J 2020; 33(2): 180-85.
13. Ullah A, Qasim M, Rahman H, Khan J, Haroon M, Muhammad N, et al. High frequency of methicillin-resistant staphylococcus aureus in Peshawar Region of Pakistan. Springerplus 2016; 5(1): 600-05.
14. Sunagar R, Hegde NR, Archana GJ, Sinha AY, Nagamani K, Iislor SJJogar. Prevalence and genotype distribution of methicillin-resistant Staphylococcus aureus (MRSA) in India. J Glob Antimicrob Resist 2016; 7(1): 46-52.
15. Bukhari SZ, Ahmed S, Zia NJJoAMCA. Antimicrobial susceptibility pattern of Staphylococcus aureus on clinical isolates and efficacy of laboratory tests to diagnose MRSA: a multi-centre study. J Ayub Med Coll Abbottabad 2011; 23(1): 139-42.
16. Onwubiko NE, Sadiq NM. Antibiotic sensitivity pattern of staphylococcus aureus from clinical isolates in a tertiary health institution in Kano, Northwestern Nigeria. Pan African Medical J 2011; 8(1): 1-7.
17. Onile B, Odugbemi T, Nwofor C. Antibiotic susceptibility of bacterial agents of septicemia in Ilorin. Nig Med Pract 1985; 9(4): 16-18.
18. Obiazi H, Ekundayo A. Prevalence and antibiotic susceptibility pattern of staphylococcus aureus from clinical isolates grown at 37 and 44°C from Iruua, Nigeria. African J Microbiol Res 2007; 1(5): 57-60.
19. Naik D, Teelu A. A study on antimicrobial susceptibility pattern in clinical isolates of staphylococcus aureus in Eritrea. Pan African Med J 2009; 3(1): 1-5.
20. Kaleem F, Usman J, Sattar A, Hassan A, Omair M, et al. Current status of vancomycin susceptibility against methicillin resistant staphylococcus aureus (MRSA) strains: A study at two tertiary care hospitals of Pakistan. African J Microbiol Res 2012; 6(33): 6243-46.
21. Siddiqui T, Muhammad IN, Khan MN, Naz S, Bashir L MRSA: Prevalence and susceptibility pattern in health care setups of Karachi. Professional Med J 2017; 30(Suppl-6): 2417-21.
22. Ahmad S, Ahmed S, Sabir MS, Khan H, Rehman M, Niaz ZJ. Frequency and comparison among antibiotic resistant staphylococcus aureus strains in selected hospitals of Peshawar, Pakistan. J Pak Med Assoc 2020; 70(7): 1199-202.
23. Jayaweer J, Karunanarathne M, Kumbukgolla WW. The importance of timely introduction of vancomycin therapy against methicillin-resistant staphylococcus aureus (MRSA) bacteremia and severity of MRSA bacteremia at Teaching Hospital, Anuradhapura, Sri Lanka. Inter J One Health 2017; 3(1): 7-11.