

Research Article

Antibiotic susceptibility pattern and prevalence of *Staphylococcus aureus* from Patients specimens at Ayub Medical Complex Abbottabad, Pakistan

Sheryar Jamil, Muhammad Azam Khan*, Zahid Ahmad, Sajjad Ali and Iffraz Ali Syed

Department of Microbiology Government Post Graduate College Mandian Abbotabad-Pakistan

*Corresponding author's email: microbiologistazam@gmail.com

Citation

Sheryar Jamil, Muhammad Azam Khan, Zahid Ahmad, Sajjad Ali and Iffraz Ali Syed. Antibiotic susceptibility pattern and prevalence of *Staphylococcus aureus* from Patients specimens at Ayub Medical Complex Abbottabad, Pakistan. Pure and Applied Biology. Vol. 9, Issue 1, pp269-274. <http://dx.doi.org/10.19045/bspab.2020.90031>

Received: 18/07/2019

Revised: 19/09/2019

Accepted: 25/09/2019

Online First: 23/10/2019

Abstract

Staphylococcus aureus causing wide spectrum of infections and has tendency for the development of multidrug resistance. The study was conducted to find out the antibiogram of *S. aureus* from clinical samples at Ayub Teaching institute, Abbottabad since September 2018 to May 2019 and to determine the prevalence of MRSA and MSSA. Total 100 *Staphylococcus aureus* was collected from clinical samples (pus, urine and blood) and identification was done by standard microbiological methods and antibiotic susceptibility testing was done by using disk diffusion method as per CLSI guidelines. Out of these 100 isolates, 44 (44%) were MRSA and 56 (56%) were MSSA. Most of the MRSA isolates were collected from blood 20(45.45%) followed by pus 14(31.81%) and urine 10 (22.7%) correspondingly while the highest prevalence of MSSA was in pus samples 26 (46.42%), and were same in the samples of blood and urine 15 (26.78%). MRSA was sensitive to Amikacin (95%) and Vancomycin (86%) but resistant to Oxacillin (100%) and Imipenem (82%). MSSA was sensitive to Oxacillin (100%) and Doxycyclin (86%) but resistant to Ciprofloxacin and Ofloxacin (64%). All the isolates were sensitive to Amikacin, Doxycyclin and Gentamicin. Frequency of *S.aureus* is common in clinical samples of patients at Abbottabad and these were momentarily resistant to frequently prescribed antibiotics, so, anti-staphylococcal antibiotics correct use is crucial.

Keywords: Methicillin resistant *Staphylococcus aureus*; Methicillin sensitive *Staphylococcus aureus*; Susceptibility; *Staphylococcus aureus* prevalence

Introduction

Staphylococcus aureus is a Gram-positive bacterium that reside upper respiratory tract, mouth, skin and conjunctiva and the major source of both nosocomial and opportunistic infections. It has the potential to develop

resistance against antibiotics by several mechanisms including, inactivation by the enzymes, by variation in target site, and antibiotic trapping [1]. Due to these reasons it results in soft tissue infections, bone infection, skin infection, urinary tract

infections, pneumonia, both in hospital settings and community [2]. This pathogen also form biofilm in wound which is challenging to treat [3]. It produces alpha, beta, gamma and delta hemolysis [4].

Methicillin Resistant strains are frequent cause of hospital acquired infections and are responsible for illness and death [5]. It was first identified in 1960 and was documented b-lactamase resistant and recognized by British scientists in 1961. The first MRSA in US was known in 1968 [6]. Due to misuse of antibiotics the predominance of MRSA has increased in medical care units which has resulted massive incidence of mortality and morbidity through the last two decades. Due to MRSA Approximately ten thousand and nine hundred deceases annually have been described in the US, more than those due to AIDS. Its toxicities can only be cured with certain categories of antibacterial agents. [7]. The incidence of MRSA differs among countries and has been reported all over the world. As compared to northern Europe its incidence in Pakistan and India is higher [8]. In 2007 A study reported 42-51% prevalence of MRSA [9]. A comparable study from two hospitals in Rawalpindi during 2011 presented MRSA value of 53.3% [10]. Its prevalence and antibiotic resistance has created tasks for clinicians and the treatment of common infections have become problematic it takes lengthier time to control the infections triggered by risky bacteria [11]. Antibiogram is better process than molecular techniques as it is very simple, effortlessly accessible, having low cost, and carried out without any trouble. That is why when there is unavailability of molecular methods microbiologists and chemists practice it as a mention guide to clinic particular or public specific resistance pattern [12]. In Europe this method is still used for the epidemiological researches of MRSA [13]. Susceptibility of bacteria to medicines fluctuates from area to area, this research was

carried out to evaluate the occurrence of MRSA and MSSA and *S. aureus* and of antibiotic susceptibility to the repeatedly recommended antibiotics in Ayub Teaching institute.

Materials and methods

Study area

This study was conducted from September 2018 to May 2019 at Ayub Medical Complex, Abbottabad, Pakistan

Samples Collection

A total of 600 medical specimens including pus, urine and blood collected in sterilized bottles and swabs and processed in microbiology laboratory at Ayub Medical Complex Hospital, Abbottabad

Samples culturing

All the samples were cultured on CLED, MacConkey, and Blood agar (Oxoid, England) and incubated for 24hrs at 37°C. 100 *S.aureus* isolates were recognized by performing Standard microbiological techniques such as, Gram staining and biochemical tests.

Antimicrobial susceptibility

To determine antibiotic susceptibility *S.aureus* were Sub cultured on Muller Hinton at 37°C for 24hrs and disc diffusion method was used as per CLSI standards.

Antibiotics used in the study

Levofloxacin(30µg), Ciprofloxacin(30µg), Ofloxacin(30µg), Erythromycin(30µg), Doxycycline(30µg), Gentamicin(30µg), Amikacin(30µg), Cephadrine(30µg) Imipenem(30µg), Meropenem(30µg), Vancomycin(30µg), Amoxicillin(30µg). Oxacillin(cefoxitin 30µg) were used for MRSA screening. The zone size greater or equal to 21 mm was dignified resistant and a zone size greater than 22 mm was dignified susceptible. Isolates were considered as susceptible, or resistant according to CLSI guidelines. As a control *Staphylococcus aureus* ATCC 25923 was taken.

Results

A total of 100 *S. aureus* isolates were explored of which 40(40%) were from pus, 35(35%) from blood and 25 (25%) from urine. Among these 100 samples (n=100) 44(44%) were resistant to oxacilin (MRSA) and 56(56%) were sensitive to oxacilin (MSSA). Out of 44 MRSA isolates 20(45%), 14(31.81%) and 10(22.7%), were isolated from blood, pus and urine respectively. On the other hand out of the total 56(56%) isolates of MSSA the highest prevalence was detected in pus 26 (46.42%) followed by urine 15(26.78%) and blood 15(26.78%) respectively (Table 1).

Antimicrobial susceptibility profile of *Staphylococcus aureus*

Antibiogram of *Staphylococcus aureus* was studied. All isolates were from patients' clinical specimens (mainly pus, urine, blood) and were evaluated according to CLSI guidelines standards. *S. aureus* isolates were sensitive to Amikacin (86%) followed by Doxycyclin (78%), Vancomycin (76%) and Gentamicin (72%). While resistant to Ofloxacin, (76%) followed by Ciprofloxacin (68%) imipenem (62%) and Erythromycin (54%) respectively as presented in the (Table 2). The antimicrobial susceptibility pattern of antibiotics was further explained between both MRSA (resistant to oxacillin) and MSSA (sensitive to oxacillin).

Antimicrobial susceptibility profile of MRSA and MSSA

MRSA isolated from different samples were highly sensitive to Amikacin (95%) followed by Vancomycin (86%), Doxycycline (68%) Gentamicin (59%) and was resistance to Oxacillin (100%) Imipenem (82%) ciprofloxacin (73%), Ofloxacin (71%), Erythromycin (68%), Amoxicillin Levofloxacin (64%) and meropenem (59%) respectively.

MSSA exhibited significant sensitivity to Oxacillin (100%) followed by Doxycycline (86%), Gentamicin (82%), Amikacin (79%),

Vancomycin (68%), Meropenem (61%) and imipenem (54%) and was resistant to Ciprofloxacin and Ofloxacin (64%) and Cephadrine (50%) respectively as presented (Figure 1).

Discussion

The purpose of the current research was to evaluate the antibiogram of *S. aureus* from clinical specimens at Ayub Medical Complex, Abbottabad Pakistan. Maximum *S. aureus* numbers (40%) were from pus specimen. This is similar with a former study done at KNH [14]. A research conducted to determine the antimicrobial profile of *S. aureus* strains isolated from patients in Iran, most of the isolates were from blood specimens (29%) [15]. Other investigation carried out on prevalence and antibiogram of *S. aureus* from clinical isolates in Nigeria revealed a mainstream of the isolates were from urine (76%) [16]. Prominent number of *S. aureus* isolates from pus may be due to exposure of wounds which makes them more predisposed to infections and poor hygiene. *S. aureus* isolates were sensitive to Vancomycin and amikacin this findings are also in agreement with the results of Wankhade (17). In our study, MRSA was tested using Oxacillin screening. MRSA isolates were resistant to Imipenem (82%), Ciprofloxacin (73%), Ofloxacin (71%) and Erythromycin (67%). This is reliable with previous study completed at Ayub Medical Complex [18]. This may be due to the misuse of these antibiotics in daily practice at local hospitals. In our study 14% of MRSA isolates were resistant to Vancomycin. This result is somehow greater to a research in Iran which revealed 5% of the MRSA isolates were resistant to Vancomycin [19]. In the existing study MRSA was most common in blood (45.45 %) parallel finding was also detected earlier in study done in Lahore [20]. Although methicillin resistance and resistance to novel beta lactam antibiotics is due to *mecA* gene and its exhibition [21]. The

study evaluated that furthest potential drug against MRSA is Amikacin (95%) followed by Vancomycin (86%). In the current study on this basis of Oxacillin (0%) sensitivity MRSA and MSSA were distinguished which is almost same as the study completed in KNH [14] and Iran [22]. This study exhibited that MSSA isolates were resistant to Ciprofloxacin (64%), Ofloxacin (64%) and fruitful drug against MSSA is

Oxacillin (100%) followed by Doxycycline (86%), Gentamicin (82%) and Amikacin (79%).

The study demonstrated that the isolates of *S.aureus* were mostly sensitive to Amikacin (86%) followed by Doxycycline (78%), Vancomycin (76%) and Gentamicin (72%). On the other hand the *S.aureus* showed significant amount of resistant against Ofloxacin, Ciprofloxacin and Imipenem.

Table 1. Detail frequency of *S.aureus* isolates among different clinical samples

Specimen	Media	Total sample	Total growth	Growth %	MRSA	% OF MRSA	MSSA	% OF MSSA
Urine	CLED	200	25	25%	10	22.7%	15	26.78%
Blood	MacConkey	200	35	35%	20	45.45%	15	26.78%
Pus	Blood agar	200	40	40%	14	31.81%	26	46.42%
Total		600	100		44		56	

MRSA: Methicillin-Resistant *Staphylococcus aureus*; MSSA: Methicillin-Sensitive *Staphylococcus Aureus*

Table 2. Antimicrobial susceptibility profile of *S.aureus*

S. No.	Antibiotics	Sensitive	Resistant	% Sensitive
1	Levofloxacin	58	42	58%
2	Ciprofloxacin	32	68	32%
3	Ofloxacin	24	76	24%
4	Erythromycin	46	54	46%
5	Doxycycline	78	22	78%
6	Oxacillin	56	44	56%
7	Gentamicin	72	28	72%
8	Amoxicillin	54	46	54%
9	Amikacin	86	14	86%
10	Cephadrine	50	50	50%
11	Imipenem	38	62	38%
12	Meropenem	52	48	52%
13	Vancomycin	76	24	76%

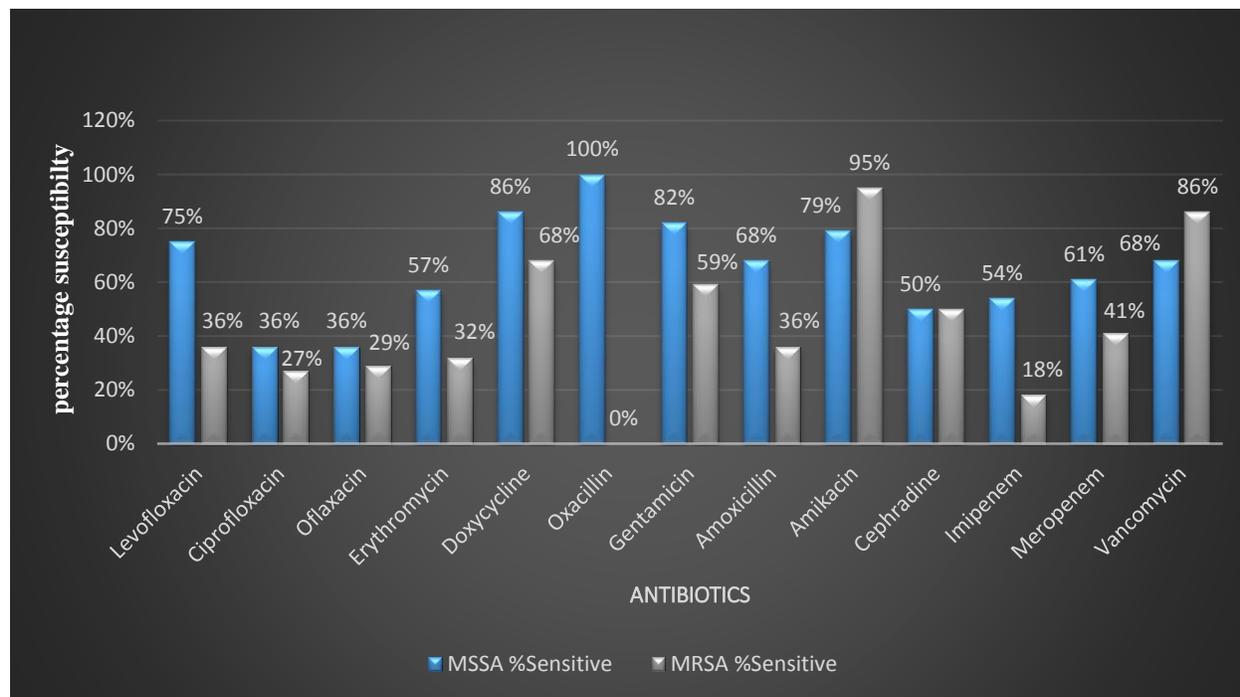


Figure 1. Comparison of Antimicrobial susceptibility profile of MRSA and MSSA
 MRSA: Methicillin-Resistant *Staphylococcus aureus*; MSSA: Methicillin-Sensitive *Staphylococcus Aureus*

Conclusion and recommendations

Present study investigated that *Staphylococcus aureus* is one of the most vital pathogen isolated from most of the clinical specimen like pus, urine and blood. All the isolates were sensitive to Amikacin, Doxycyclin and Gentamicin. Hence endure the choice for *S.aureus*. It had also shown multidrug resistant pattern. So, systematic antimicrobial susceptibility surveillance is vital for area wise observing of the resistant pattern. This will be beneficial to realm the efficacy of antibiotics for healthier patient management. There is need for further exploration to evaluate the genes accountable for resistance and epidemiology of multiple drug resistant *S.aureus* and MRSA. Some information such as date of collection, patient age, gender, clinical information, prior antibiotics use and period of patient visit in the hospital were omitted.

Authors' contributions

Conceived and planned the project and supervised: MA Khan, Performed the experiment: S Jamil, Analyzed the data: S Ali & IA Syed, Helped in proofreading of the manuscript: Z Ahmad.

Acknowledgments

The authors are thankful to the Lab supervisor of Ayub Medical College for providing financial aid to conduct this study. The authors also acknowledge the laboratory staff for their support and collaboration.

References

1. Pantosti A, Sanchini A & Monaco M (2007). Mechanisms of antibiotic resistance in *Staphylococcus aureus*. *J F Microbiol* 2: 323–34
2. Loomba PS, Taneja J & Mishra B (2010) Methicillin and vancomycin resistant *S. aureus* in hospitalized patients. *J Glob Infect Dis* 2: 275–83.
3. Donlan RM & Costerton JW (2002). Biofilms survival mechanisms of clinically relevant microorganisms. *Clin Microbiol Rev* 15(2): 167–193
4. Funke G *et al.* (2011). Manual of Clinical Microbiology, 10th ed. ASM Press,
5. Espedido BA & Gosbell IB (2012). Chromosomal mutations involved in antibiotic resistance in *Staphylococcus aureus*. *Front Biosci* 4: 900–15

6. Enright MC, Robinson DA, Randle G, Feil EJ, Grundmann H & Spratt BG (2002). The evolutionary history of methicillin-resistant *Staphylococcus aureus* (MRSA). *Proc Natl Acad Sci* 99(11): 7687-92.
7. Marlowe EM & Bankowski MJ (2011). Conventional and molecular methods for the detection of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 49(9 Suppl): S53-S6.
8. Anwar MS, Jaffery G, Bhatti KR, Tayib M & Bokhari SR (2004). *Staphylococcus aureus* and MRSA nasal carriage in general population. *J Med Microbiol* 14: 661-664
9. Naqvi ZA, Hashmi K & Kharal SA (2007). Methicillin resistant *Staphylococcus aureus* (MRSA) in burn patients. *Pak J Pharmacol* 24(2): 7-11
10. Khan S, Rasheed F & Zahra R (2014). Genetic polymorphism of agr Locus and antibiotic resistance of *Staphylococcus aureus* at two hospitals in Pakistan. *Pak J Med Sci* 30(1): 172
11. Costelloe C, Metcalfe C, Lovering A, Mant D & Hay AD (2010). Effect of antibiotic prescribing in primary care on antimicrobial resistance in individual patients: systematic review and meta-analysis. *BMJ* 340: c2096
12. Blanc D, Lugeon C, Wenger A, Siegrist H & Francioli P (1994). Quantitative antibiogram typing using inhibition zone diameters compared with ribotyping for epidemiological typing of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 32(10): 2505-9.
13. Marlowe EM & Bankowski MJ (2011). Conventional and molecular methods for the detection of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* 49(9 Suppl): S53-S6
14. Kanaga EL (2014). Antimicrobial susceptibility of bacteria that cause wound sepsis in the paediatric surgical patients at Kenyatta national hospital. <http://erepo.sitor.yuonbi.ac.ke/handle/11295/95412>.
15. Soltani R, Khalili H, Rasoolinejad M & Abdollahi A (2010). Antimicrobial susceptibility pattern of *Staphylococcus aureus* strains isolated from hospitalized patients in Tehran, Iran. *Iran J Pharm Sci* 6: 125-32.
16. Obiazi HAK, Ekundayo AO & Ukwandu NCD (2007). Prevalence and antibiotic susceptibility pattern of *Staphylococcus aureus* from clinical isolates grown at 37 and 44 °C from Irrua, Nigeria. *African J Microbiol Res* 1: 57-60.
17. Wankhade AB, Panda S, Hathiwala R & Keche Y (2017). Study of antibiotic resistance profiling of *Staphylococcus aureus* isolated from clinical specimens of the patients attending a tertiary teaching hospital from Chhattisgarh. *Int J Res Med Sci* 5(11): 4808-4811
18. Hassan AK, Mohammad M, Humera K, Samina N, Ahmed AK, Fridoon JA & Riffat M (2014). Prevalence, antibiotic susceptibility pattern and demographic factors related to methicillin resistant *Staphylococcus aureus* in Lahore, Pakistan. *Int J Microbiol Adv Immunol* 2: 45-48.
19. Arianpoor A, Estaji F, Naderinasab M & Askari E (2015). Antimicrobial susceptibility pattern of *Staphylococcus aureus* Isolates against newly marketed antibiotics. *J Ayub Med Coll Abbottabad* 3: 3-6.
20. Hassan AK, Mohammad M, Humera K, Samina N, Ahmed AK, Fridoon JA & Riffat M (2014). Prevalence, antibiotic susceptibility pattern and demographic factors related to methicillin resistant *Staphylococcus aureus* in Lahore, Pakistan. *Int J Microbiol Adv Immunol* 2: 45-48.
21. Taj R, Muhammadzai I, Ahmad J, Khan A, Syed F & Khan Z (2015). Frequency and antibiotic susceptibility pattern of methicillin resistant *staphylococcus aureus* in Abbottabad city of Pakistan. *KMUJ* (7): 4.
22. Arianpoor A, Estaji F, Naderinasab M & Askari E (2015). Antimicrobial susceptibility pattern of *Staphylococcus aureus* Isolates against newly marketed antibiotics. *J Ayub Med Coll Abbottabad* 3: 3-6.