Short Communication

Sensitivity Pattern of *Staphylococcus Aureus* in a Tertiary Care Hospital in Lahore; Implications and Recommendations

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Abstract

Introduction: Staphylococcus aureus is becoming a major concern worldwide due to its increasing resistance to antibiotics, particularly Methicillin Resistant S. aureus (MRSA). We aimed to investigate the prevalence and antibiotic sensitivity pattern of S. aureus in clinical specimens and to highlight the importance of an Antimicrobial Stewardship Program.

Methods: Two years' data from a tertiary care hospital was analyzed. All S. aureus isolates were tested for their sensitivity by Kirby-Bauer disc diffusion method.

Result: A total of 207 S. aureus isolates were identified, with a high prevalence of MRSA (82.7%). Variable resistance was observed for Ampicillin (97.5%), Ciprofloxacin (85.5%), Cloxacillin (82.7%), and Erythromycin (77.7%), while 100% sensitivity was observed for Linezolid, Vancomycin, and Teicoplanin making them the antibiotics of choice.

Conclusions: Training of doctors on the appropriate use of antibiotics and the restrictions on the sale of antibiotics can be a way forward. Governmental support and legislation can help to overcome the problem.

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Introduction

The Staphylococcus aureus is an important bacterial human pathogen. It is among the normal flora of humans and other animals and continues to be a source of morbidity and mortality worldwide. It causes both purulent and toxic illnesses and it is a significant contributor to Bacteremia, Infective Endocarditis, Osteo-articular infections, Cutaneous, and Soft-tissue infections, Pleuropulmonary infections, and infections brought on by the medical devices and prostheses.[1] The Staphylococci (react Positive) are differentiated from the Streptococci (react Negative) with the help of Catalase Test, whereas S. aureus is separated from other Staphylococci with the help of Coagulase and/or DNase Tests.[2]

The Staphylococcus aureus has become resistant to the first line antimicrobial drugs. Antibiotic-resistant S. aureus, notably Methicillin Resistant Staphylococcus aureus (MRSA) is now a matter of concern in both the community and hospital settings worldwide. Furthermore, MRSA may elude the host's immunity due to its extensive virulence characteristics and the treating physician is left with few treatment options. Currently, MRSA infections can only be treated with a few costly

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and possibly hazardous medications such as Vancomycin, Teicoplanin, and Linezolid.[3] The management of MRSA infections is a challenge worldwide especially in Pakistan where its frequency ranges from 34% with a higher incidence (up to 60.7%) in elderly patients.[4] As a result, appropriate MRSA management is critical to limiting the spread of MRSA.

The purpose of present study is to examine the prevalence of S. aureus especially MRSA isolated from different clinical specimens as well as the pattern of antibiotic sensitivity to various antibiotics at a tertiary care teaching hospital. The study findings will aid in the development of suitable hospital antibiotic stewardship program to lower the risk of S. aureus-associated infections in this locality and the emergence of MRSA.

Methods

It is a descriptive, retrospective study to evaluate the antimicrobial susceptibility / resistance profiles of Staphylococcus aureus at the University of Lahore Teaching Hospital (ULTH). After ethical approval from the Institutional Review Committee, the data was collected from the Laboratory Information System of the Pathology Department at ULTH. All specimens yielding the growth of S aureus were processed further to note their sensitivity/resistance profile against the set of antibiotics tested.

Inclusion Criteria: All specimens received during a two-year period (July 1, 2021 to June 30, 2023) were included.

Exclusion Criteria: Any specimen received twice during the study period was excluded.

According to the established guidelines[5] and the Standard Operating Procedures of the institution, the dehydrated OXOID Blood (CM 0271), MacConkey (CM 0115), CLED (CM 0301), Mueller-Hinton (MH) (CM 0337) and DNAse (CM 0321) Agar Bases were used to make the culture plates of Blood (BAP), Chocolate (CAP), MacConkey, CLED, MH and DNAse Agar plates respectively according to the manufacturer's instruction. All specimens except Blood and Urine received in the Microbiology Section of the Clinical Laboratory ULTH were inoculated on a set of three solid BAP, CAP and MacConkey Agar media. Blood culture specimens were inoculated in the Blood Culture (BacT/Alert) system and after a positive signal, sub-cultures were made. The Urine specimens were inoculated on CLED Agar plates. The sensitivity tests were done by Kirby-Bauer method using the MH Agar plates. The suspected S aureus obtained on Blood Agar or CLED Agar was Gram-stained to ascertain the morphology, and then Catalase Test was performed. All Catalase Positive Gram-Positive Cocci isolates were tested for DNAse presence. S aureus strain ATCC25923 was used as the control strain.

Following identification, Antibiotics Sensitivity of all S aureus isolates was determined against Ampicillin (AMP 10 µg), Cefuroxime (CXM 30 µg), Ciprofloxacin (CIP 5 µg), Clindamycin (DA 10 µg), Cloxacillin/Flucloxacillin using Cefoxitin (FOX 30 µg), Cotrimoxazole (SXT 25 µg), Erythromycin (E 15 µg), Fosfomycin (FOS 50 µg), Fusidic Acid (FD 10 µg), Gentamicin (CN 10 µg), Linezolid (LNZ 10 µg), Nitrofurantoin (F 300 µg), Teicoplanin (TEC 30 µg), and Vancomycin (VA 5µg). The Kirby-Bauer disc diffusion method was used to assess the zones of inhibition of growth after 18-24 hours of incubation at 37°C according to CLSI guidelines[6]. Data obtained from this study was analyzed using IBM SPSS Version 23.

Results

There were no drop outs as it is a retrospective study.

During the period of study, there were 207 isolates of S aureus. The Pus specimens yielded 97 isolates followed by the 42 from Wound Swabs. Other specimens that yielded significant number of S aureus isolated were Blood and Urine specimens (24 and 14 respectively) (Table 1).

There were almost twice as many male patients (64.7%) as compared to the female patients (35.3%). The ages of the patients ranged from 11 years to 80 years old with a mean of 39.4 years and a standard deviation of 16.9 years.

Table 1: *Specimen types with frequency and percentage*

Specimen Type	Frequency	Percentage
Pus	97	46.9
Wound Swab	52	25.1
Blood	24	11.6
Urine	14	6.8
Biological Fluids	7	3.3
Abscess	4	1.9
Tissue Culture	4	1.9
Swab (unspecified)	1	0.5
Ear Swab	1	0.5
Sputum	1	0.5
Throat	1	0.5
Secretion (unspecified)	1	0.5
Total	**207**	**100**

Figure 1 describes the sensitivity profile of the isolates

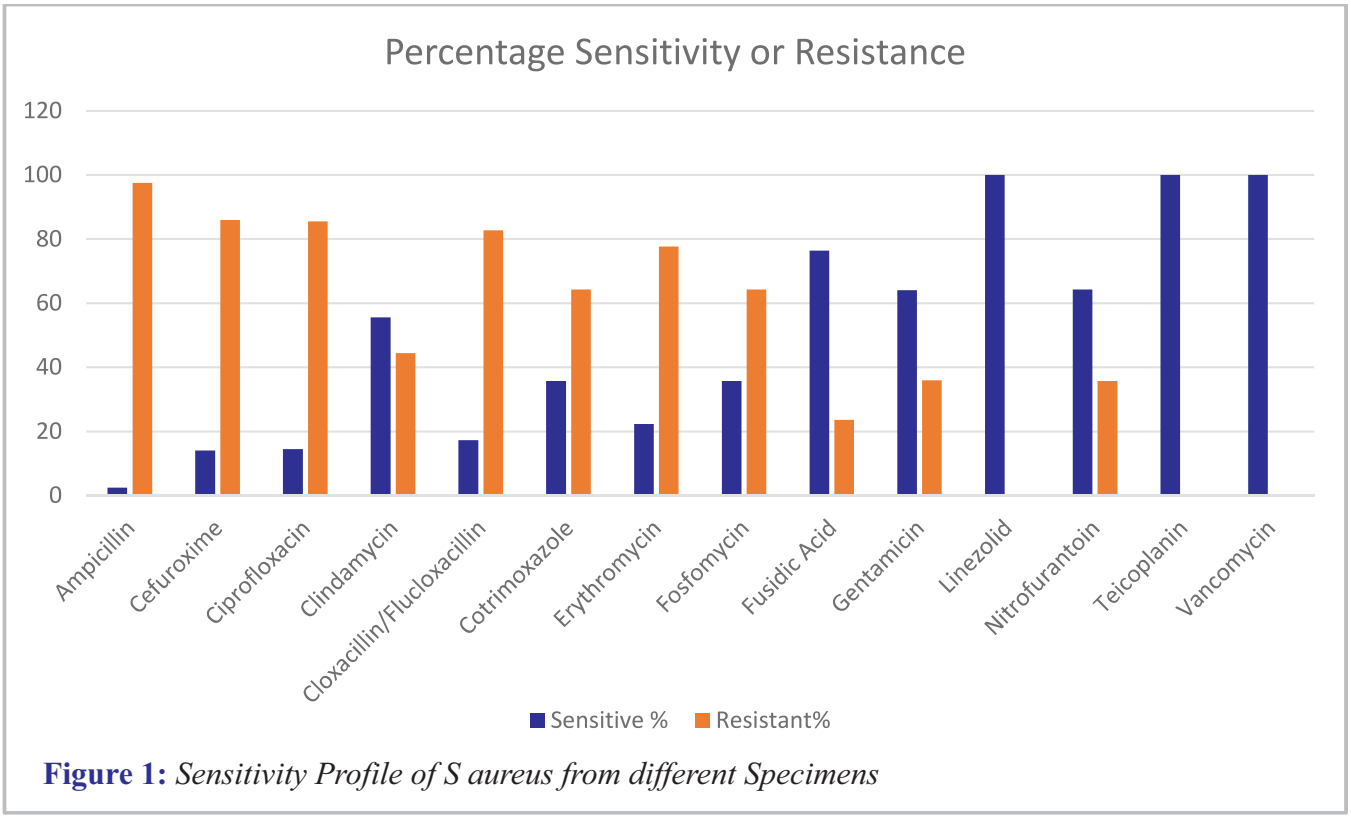

Figure 1: *Sensitivity Profile of S aureus from different Specimens*

studied. All antibiotics were not tested for all isolates as the sensitivity profile was based on the origin of specimens.

The contingency (2x2) table for Ciprofloxacin and Gentamicin using chi square test showed that using Gentamicin in infections caused by S aureus is expected to be more useful than using Ciprofloxacin (p value 0.001).

CN	CIP		P value
	Sensitive	Resistant	
Sensitive	20	72	0.001
	95.2%	60.5%	
Resistant	1	47	
	4.8%	39.5%	

Discussion

The major factors contributing significantly to the morbidity, mortality and cost of treatment in case of infectious diseases include but are not limited to increased (often unjustified) use of antibiotics in human and veterinary medicine, greater ease of movement of the people and increased industrialization.[7] The association of Penicillin Resistant Streptococcus pneumoniae (PRSP) with the increasing rates of resistance of PRSP and the total consumption of antibiotics in a community is an example of this relationship.[8]

The emergence of Methicillin-Resistant S aureus (MRSA) coincided with the use of Methicillin in clinical practice in the early 1960s and the rise in the incidence had been quite steady. The levels had reached 30% in the 1990s. The community acquired MRSA (CA-MRSA) was also reported at the same time although it differed from the Hospital-Acquired MRSA (HA-MRSA) in its sensitivity to other antibiotics such as Clindamycin, Ciprofloxacin, Gentamicin and Cotrimoxazole.[9] The incidence of MRSA in Pakistan had risen to 66% in 2019.[10]

Our results show that the situation has worsened and we are reporting the incidence of MRSA to be 82.7% (based on Cefoxitin testing and reported as sensitive or resistant to Cloxacillin and/or Flucloxacillin) and this is an alarming situation. The gravity of this problem is further aggravated when we look at other commonly used antibiotics for the management of S aureus infections. Ampicillin and other β-Lactamase sensitive Penicillins were excluded from the possible choices of antibiotics for Staphylococcal infections a long ago and our results (97.5% resistance) further strengthen the notion. Similarly, Ciprofloxacin with 85.5%, Cotrimoxazole 64.3%, and Erythromycin 77.7% resistance rates are also no more the empirical choice for Staphylococcal infections. For the management point of view, Vancomycin remains the drug of choice before the culture and sensitivity (C/S) results are available. Later,

the antibiotic(s) selection may be changed according to the C/S results.[11]

In addition to the factors contributing to antibiotics resistance discussed above, another particularly important contributor in Pakistan and many other countries is the "Over the Counter Sale of Antibiotics." In a study conducted in Pakistan, it was reported that almost two third of the antibiotics were dispensed either to the patients or their attendants without a prescription from a registered medical practitioner.[12] In another study, more than half the pharmacists considered themselves to be authorized to dispense antibiotics without a prescription.[10] The situation in other developing countries is no different.[13]

On the other hand, the developed countries might have controlled the use of antibiotics by legislative measures as well as awareness campaigns but the use of antibiotics in the livestock to improve their yield is still a major factor contributing to the emergence of resistance in bacteria and their subsequent species jump to human beings. Last but not the least, the lack of research in the field also contributes to the problem.[14]

Strength of our study is that it is original data of two years from a teaching hospital in Lahore describing the trends in the antibiotics resistance of Staphylococcus aureus isolates. Limitations include the fact that it is limited to one center and the duration of study had to be restricted to two years because of the academic session of the researchers. A multicenter study is proposed so that representative data can be obtained for the population at large.

Conclusion

We wish to conclude by saying that the medical professionals should understand the problem of multidrug resistant bacteria and contribute in whatever way they can to overcome it. A few suggestions would be to ensure a well-informed practitioner who is taught the importance of the use of antibiotics, restriction on the sale of antibiotics without a valid prescription, prohibition of over the counter sale of drugs especially antibiotics, ensuring the production of good quality drugs, implementing effective infection control measures in the hospital, and timely reporting of trends in the resistance profiles in a community. Political support and will is also important and many of these recommendations would need legislation and involvement of regulating bodies like Pakistan Medical & Dental Council and Provincial Healthcare Commissions.

Conflict of Interest

There is no conflict of interest to be disclosed. Any brand name of the antibiotics reported in this publication is used only for clarity and does not amount to endorsing such brand.

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Authors' Contribution

FE, AA, SS, LZ, ZJ: Conception & design, acquisition of data, drafting of article

TN, SI: Analysis & interpretation of data, critically revision for important intellectual content, final approval of the version to be published

MB: Analysis & interpretation of data, drafting of article

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