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METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS;

PREVALENCE AND ANTIMICROBIAL SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN A TERTIARY CARE HOSPITAL.

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ABSTRACT… Introduction: The versatility of Staphylococcus aureus has been transformed as "Methicillin resistant Staphylococcus aureus". The most challenging are the disastrous virulence patterns being expressed due to the selection pressure of antibiotics. For assessing the prevalence of methicillin resistant Staphylococcus aureus; screening by cefoxitin disc (30μg) diffusion method is still a realistic approach among conventional phenotypic methods, being applied in most of the laboratories. This reliable and feasible technique contributes significantly for MRSA detection. **Objective:** To evaluate the prevalence and identify the sensitivity pattern of methicillin resistant Staphylococcus aureus isolates from different clinical specimens in a tertiary care hospital. Study **Design:** Descriptive study. **Setting:** Pathology Department, Microbiology Laboratory, PGMI. **Period:** January 2015 to December 2015. **Materials & Methods:** A total 713 clinical isolates of Staphylococcus aureus were processed. Identification and confirmation of Staphylococcus aureus was done by colony morphology on blood agar, gram stain, catalase, coagulase and DNA-ase tests. Screening for methicillin resistance was done using cefoxitin disc (30μg, OXOID); while different antibiotic discs were used to assess the sensitivity profile by Modified Kirby-Bauer Disc Diffusion method according to CLSI guidelines (2016). **Results:** Out of 713 Staphylococcus aureus isolates, 92 (12.90%) isolates were labelled as methicillin resistant by cefoxitin disc diffusion test. Out of 92 MRSA isolates, 57 (14.65%) were recovered from male patients and 35 (10.80%) from female patients. While, 60 (65.22%) MRSA isolates showed hemolysis on blood agar. Among 92 MRSA isolates, 41 (44.57%) were recovered from pus specimen. Resistance to trimethoprim/sulfamethoxazole was highest (65.22%) after penicillin (100%); while all the MRSA isolates were 100% sensitive to both vancomycin and linezolid. **Conclusion:** The prevalence of MRSA in hospital care settings is of great clinical concern. To combat this public health threat effectively, continuous surveillance of health-care associated infections, along with local antibiotic sensitivity pattern of MRSA; as well as formulation of a definite antibiotic policy is required.

Key words: Cefoxitin, Kirby-Bauer Disc Diffusion Test, Methicillin Resistant Staphylococcus Aureus, Clinical and Laboratory Standards Institute (CLSI).

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INTRODUCTION

The acquirement of antibiotic resistance has led to progression of *Staphylococcus aureus infections* in this antibiotic era.[1] Antimicrobial resistance makes diseases harder and more expensive to treat not only in developed countries but progressing towards financial crisis in undeveloped countries. The reasons are: Poor infection control in hospitals; In-appropriate food handling; Poor sanitary conditions; and most important misuse and/or overuse of antimicrobial drugs in humans and in breeding crops and animals.[2]

Among multidrug resistant infections, methicillin resistant Staphylococcus aureus (MRSA) is the foremost challenging pathogen, accounting for 40–70% of the Staphylococcus aureus infections worldwide.[3]

Methicillin-resistant Staphylococcus aureus (MRSA) infections are known globally both in developed and developing countries as a cause of frequent hospitalizations and invasive infections

associated with remarkable high morbidity, high mortality and increased management costs. In recent years, developing multidrug-resistant strains of *Staphylococcus aureus* (MDRSA) have made treatment of *Staphylococcus aureus* infections more prolonged, troublesome and distressing.[4]

Staphylococcus aureus has emerged as the most violent organism commencing a cluster of ailments including septicemia, pneumonia, wound sepsis, burn infections, septic arthritis, endocarditis, meningitis, urinary tract infections, toxic shock syndrome, food poisoning, scalded skin syndrome, and postsurgical infections.[5]

Methicillin resistance initially appeared among nosocomial isolates of Staphylococcus aureus shortly after its institution; by the late 1960s was endemic in hospitals and labeled as hospital –acquired or hospital associated MRSA (HA-MRSA).[6]

At the mid-1990s, evolutionary modifications and epidemiologic extension in the clones of MRSA occurred and a dominant pathogen known as community-associated MRSA (CA-MRSA), emerged rapidly in the community beyond the confines of health care facilities. CA-MRSA is capable of producing aggressive harmfulness in young, otherwise healthy people.[7]

Methicillin resistance in Staphylococcus aureus has been connected with modifications in the penicillin binding proteins (PBPs) subsequently generating an additional penicillin-binding protein, PBP2a or PBP2'. PBP2a is encoded by the mecA gene which is carried on a large genomic island labeled SCCmec.[8] This study has been designed to assess the prevalence and antimicrobial susceptibility pattern of methicillin resistant Staphylococcus aureus among clinical specimens in a tertiary care hospital in Lahore.

MATERIAL AND METHODS

Study Design
Descriptive study.

Sampling Technique
Convenient Sampling.

Inclusion/Exclusion Criteria
Clinical isolates of Staphylococcus aureus resistant to cefoxitin were only included in the study project.

Sample Collection and Processing
This descriptive study was conducted in Pathology Department of PGMI, Lahore; during the period from January 2015 to December 2015. Clinical specimens like blood, CSF, central venous lines, urine, pus, wound swabs, throat swabs, sputum, aspirates, high vaginal swabs were received from patients admitted in different clinical wards of Lahore General Hospital (LGH). All clinical samples were processed according to standard operating guidelines in microbiology laboratory of Pathology department, PGMI, Lahore.

CULTURE AND IDENTIFICATION
All the specimens were inoculated on blood agar and McConkey agar (prepared as instructions given by the manufacturer). The plates were incubated aerobically at 35°C for 24 hours. Preliminary identification of Staphylococcus aureus isolates was done by observing the colony morphology on blood agar plates, (size, shape, surface, margins, consistency, elevation, color, translucency, and presence or absence of hemolysis). Small to medium sized, round, low convex, butyrous, opaque colonies with regular margins irrespective of color and hemolysis were processed. After finding gram positive cocci in clusters, further biochemical tests like Catalase, Coagulase and DNA-ase were implemented for the confirmation of Staphylococcus aureus.

1. Catalase Test
Catalase test was performed by using 3% hydrogen peroxide to differentiate between Staphylococci and Streptococci.

2. Coagulase Test
Coagulase test was done for detection of coagulase enzyme by slide and tube method. Those isolates which shown negative results by slide coagulase were subjected to tube coagulase

test for confirmation. This test differentiated coagulase producing Staphylococcus aureus from Coagulase Negative Staphylococci, commonly termed as CoNS.

1. DNA-ase Test

DNA-ase test helps in the recognition of Staphylococcus aureus which generates deoxyribonuclease (DNA-ase) enzyme. After overnight incubation, this enzyme hydrolyzes deoxyribonucleic acid (DNA) present in the medium, and due to this DNA-ase enzyme the colonies are encircled by transparent clear areas when flooded with a weak hydrochloric acid solution.

2. Screening

Screening was implemented on all isolates of Staphylococcus aureus by Modified Kirby Bauer disc diffusion method using 30μg cefoxitin disc (Oxoid) on Muller Hinton agar according to the Clinical and Laboratory Standards Institute (CLSI, 2016) guiding principles.[9] For each strain, a bacterial suspension adjusted according to 0.5 McFarland turbidity standards was used. Sensitivity plates were incubated at 35°C and zone of inhibition was determined after 24 hours. A zone diameter of ≤ 21 mm was taken as resistant, and interpretation was done according to CLSI criteria (2016). Methicillin resistant Staphylococcus aureus (MRSA) ATCC 33591 and methicillin sensitive Staphylococcus aureus (MSSA) ATCC 25923 were used as positive and negative controls, respectively. Isolates showing zone ≤ 21 mm were taken as resistant and only MRSA isolates were included in the study project.

SUSCEPTIBILITY TESTING

Antibiotic susceptibility of MRSA isolates was determined by employing modified Kirby-Bauer disc diffusion method according to CLSI guidelines (2016). For each strain of MRSA, a bacterial suspension adjusted to 0.5 McFarland turbidity standards was prepared and inoculated on Mueller Hinton agar (MHA). Antibiotic discs of Penicillin 10U (P), Vancomycin 30μg (VAN), Erythromycin 15 μg (E), Clindamycin 2μg (DA), Linezolid 30μg (LZD), Ciprofloxacin 5μg (CIP), Gentamicin 10μg (CN), Trimethoprim/sulfamethoxazole 25μg (TMP/SXT), were applied; and the plates were incubated at 35°C for 24 hours.[9]

STATISTICAL ANALYSIS

All the data was entered and analyzed by using SPSS Version 20.0. Qualitative variable i.e. gender, hemolysis, type of specimen from different clinical wards and methicillin resistance were presented as frequencies and percentages. Pi charts were used to present the data graphically. Chi - square test was used to determine the significant difference among qualitative variables. Statistically, p-value of < 0.05 was considered significant and $p > 0.05$ was considered insignificant. p- value < 0.001 is taken as highly significant.

RESULTS

During the study period from January 2015 to December 2015; a total of 8465 different clinical samples were received from Lahore General Hospital. Out of all the samples processed, 713 isolates of Staphylococcus aureus were isolated after culture. The overall frequency of MRSA isolates was 92 (12.90%) in 12 months, as shown in Table-I. This table also demonstrates the gender wise distribution of MRSA among total Staphylococcus aureus isolates (n=713). It shows that frequency was more in males (14.65%) as compared to females (10.80%). However, the difference was statistically non-significant ($p > 0.05$).

Gender	No. of Staphylococcus aureus isolates		MRSA	
	No.	%Age	No.	%Age
Male	389	54.56	57	14.65
Female	324	45.44	35	10.80
		Total	92	12.90

Table-I. Distribution of MRSA among total Staphylococcus aureus isolates according to gender (n=713)
Chi-square = 2.33
Probability = 0.127 (no significant difference)

Out of 713 Staphylococcus aureus isolates 38.99% showed no hemolysis on blood agar (Table-II). There was a higher frequency of MRSA isolates showing hemolysis (65.22%) as compared to

those showing no hemolysis (34.78%). However, the difference was statistically non-significant (p> 0.05).

Blood Agar	No. of Staphylococcus aureus isolates (n=713)		MRSA (n=92)	
	No.	%Age	No.	%Age
Hemolytic	435	61.01	60	65.22
Non-Hemolytic	278	38.99	32	34.78

Table-II. Distribution of MRSA among total Staphylococcus aureus isolates according to hemolysis on blood agar
Chi-square = 0.786
Probability = 0.375 (no significant difference)

Figure-1 of our study shows the breakup of MRSA isolates from different clinical specimens (n=92). Among 92 MRSA isolates, 44.57% were recovered from pus specimen. Statistically, the difference was significant (p 0.05) among percentage of MRSA isolates from different clinical specimens.

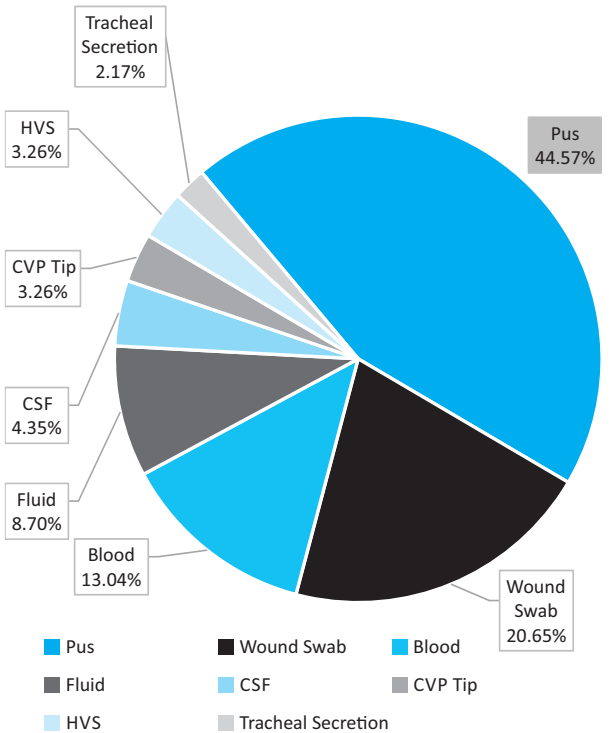

Figure-1. Breakup of methicillin resistant staphylococcus aureus isolates from different clinical specimen (n=92)

Sensitivity pattern of MRSA isolates to various antibiotics (n=92) is described in Table-III of our study. It shows that all the 92 MRSA isolates were (100%) resistant to penicillin, followed by 60 (65.22%) to trimethoprim-sulfamethoxazole, 55 (59.78%) to ciprofloxacin, 52 (56.52%) to erythromycin, 46 (50.00%) to gentamicin, and 40 (43.48%) to clindamycin. All the 92 MRSA isolates in the study were (100%) sensitive to vancomycin and linezolid.

Antibiotics	No. Resistant	Percentage (%)
Penicillin (P)	92	100.00
Vancomycin (VAN)	0	0.00
Erythromycin (E)	52	56.52
Clindamycin (DA)	40	43.48
Linezolid (LZD)	0	0.00
Ciprofloxacin (CIP)	55	59.78
Gentamicin (CN)	46	50.00
Trimethoprim/sulfamethoxazole (TMP/SXT)	60	65.22

Table-III. Resistance pattern of methicillin resistant staphylococcus aureus to different antibiotics (n=92)

DISCUSSION

The massive challenge of antimicrobial resistance has to be tackled effectively; otherwise by 2050, death toll could be staggering one person every three seconds. The critical impact of antimicrobial resistance can be reduced by lowering demand of antibiotics and by decreasing their unnecessary use for animals and in agriculture. The main interventions which are helpful in combating drug resistant infections include: global public awareness; good sanitation and hygiene; infection prevention, control and surveillance; promotion of new, rapid and low-cost techniques for definitive diagnosis; developing and using alternatives to antibiotics and vaccines.[10]

In coming days, we will be in danger of untreatable infections because of resistance to available antibiotics along with continuous decline in the development of antibacterial to an unacceptable level globally. The new antibiotics are required to fight with the emerging threats of resistant strains causing infections both in health care and community settings.[11]

Table-I of our study shows the distribution of MRSA among total Staphylococcus aureus

isolates according to gender. Out of 713 Staphylococcus aureus isolates, 92 (12.90%) turned out to be methicillin resistant. The result shows, the predominance of MRSA in male patients 57 (14.65%) as compared to female patients 35 (10.80%). Statistically, the difference is not significant ($p>0.05$) in our study. Many researchers have reported male predominance in MRSA infection.

According to Debnath and Chikkaswamy, (2015); and Bazzi et al., (2017) in Saudi Arabia high prevalence rate of MRSA is seen in males than in females.[12,13] However, Bhatt et al., (2014) from Nepal reported higher frequency of MRSA in females which is in contrast to our study.[14]

Distribution of MRSA according to hemolysis is shown in Table-II. Out of 92 MRSA; 60 isolates (65.22%) gave hemolysis on blood agar while 32 isolates (34.78%) were non-hemolytic. A study by Boriollo et al., (2017) in Brazil reports 74% of the resistant isolates exhibit hemolytic expression.[15] Similarly, Wolter et al., (2013) in Seattle demonstrated 24% non-hemolytic MRSA isolates which is comparable to our study (34.78%).[16] These MRSA strains have been recovered from many chronic infections (endocarditis, device associated infections, osteomyelitis) and also prevalent in patients of cystic fibrosis esp., children. These slow growing, non-pigmented, and non-hemolytic resistant strains of Staphylococcus aureus are known as Small Colony Variants. These SCVs also highlighted in another study by Kim et al., (2016) in Korea.[17]

Breakup of MRSA isolates among different clinical specimens is revealed in Figure-1; which shows that majority MRSA isolates were recovered from pus (44.57%) and wound swab (20.65%), followed by blood (13.04%), fluid (8.70%), CSF (4.35%), CVP (3.26%), HVS (3.26%) and tracheal secretion (2.17%). Similar studies have been conducted locally as well as abroad which are comparable with our study. Many researchers have reported higher frequency of MRSA isolated from pus and wound swabs.[18,19] Similarly, Dibah et al., (2014) in Iran reported majority of MRSA isolated from sputum and urine specimens.[20] Maximum number of MRSA (57.69%) isolated from endotracheal secretions and CV catheters documented by Mir et al., (2017) from Lahore.[21] These findings are in contrast with our study.

A sensitivity pattern of all the 92 MRSA isolates to different antimicrobial drugs is shown in Table-III. All the isolates were 100% resistant to penicillin, followed by trimethoprim/sulfamethoxazole (65.22%), ciprofloxacin (59.78%), erythromycin (56.52%), gentamicin (50%) and clindamycin (43.48%). Sensitivity to vancomycin and linezolid of all the 92 MRSA isolates was 100% in our study. Similar findings have been reported by different researchers with in the region and abroad. Many other researchers have also reported resistance to ciprofloxacin (70-80%) followed by erythromycin (60-80%), trimethoprim/sulfamethoxazole and/or cotrimoxazole (60-80%) and 50-70% resistance to gentamicin.[14] Resistance to penicillin is documented by Gupta et al (2017) and Mehta et al (2017).[22,23] Similarly, Dat et al (2017) has documented 100% sensitivity to vancomycin and linezolid, as given in our study.[24] All these findings are equally in comparison with our study.

CONCLUSION

Our study showed the current prevalence and drug resistance pattern of MRSA in our hospital, but only vancomycin and linezolid gave 100% sensitivity. Regular surveillance of hospital-associated infection, monitoring of local antibiotic sensitivity pattern and formulation of definitive antimicrobial pattern is required to reduce MRSA prevalence. Moreover, effective infection control practices with emphasis on strict hand washing will be helpful in reducing the burden of MRSA infections in the hospital.

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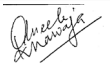
"Unknown"

AUTHORSHIP AND CONTRIBUTION DECLARATION

Sr. #	Author-s Full Name	Contribution to the paper	Author=s Signature
1	Aneela Khawaja	Entire research work, sample collection, methodology, analysis, literature review and write up.	
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