FREQUENCY AND ANTIBIOTIC SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS IN ABBOTTABAD CITY OF PAKISTAN

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ABSTRACT

OBJECTIVES: to establish the frequency of methicillin resistance among the isolates of Staphylococcus aureus and pattern of antimicrobial susceptibility of methicillin resistant Staphylococcus aureus (MRSA) isolates to the commonly prescribed antibiotics in Abbottabad.

METHODS: This cross-sectional, descriptive study was conducted at Ayub Teaching Hospital, Abbottabad, Pakistan, from 2007 to 2010. Clinical samples from pus, urine and other specimens were inoculated on Nutrient agar, MacConkey agar, and Blood agar and a questionnaire was used to collect the necessary information about the patient. Staphylococcus aureus was identified by standard microbiological procedures. Disc diffusion test and MIC were used to test the susceptibility of S. aureus isolates according to the guidelines of Clinical Laboratory Standards Institute (CLSI, 2009).

RESULTS: Out of 98 S. Aureus isolates, 24 (24.49%) were recognized as MRSA. Out of 24 MRSA isolates, 17 (70.83%) & 5 (20.83%) were isolated from pus and urine respectively. S. aureus was highly sensitive to imipenem but resistant to amoxycillin (100%), and 1st and 3rd generation cephalosporins i.e., ceftazidime (75.51%), cefaclor (65.31%), and cephradine (60.2%). Twenty four (24.49%) S. aureus isolates were found to be MRSA and 74 (75.51%) were methicillin sensitive Staphylococcus aureus (MSSA). Among MSSA, 37.84% were found to be multi drug resistant (MDR). All the 24 MRSA were also found to be MDR.

CONCLUSION: Frequency of MRSA is quite common in patients from Abbottabad and these MRSA are highly resistant to commonly prescribed antibiotics. Due to high resistance of S. aureus to antibiotics, appropriate use of anti-staphylococcal antibiotics is essential.

KEY WORDS: Staphylococcus aureus (MeSH), Methicillin-Resistant Staphylococcus aureus (MeSH), MSSA (MeSH), Multidrug Resistance (MeSH), antibiotics (MeSH).

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INTRODUCTION

Staphylococcus aureus is one of the most important resistant pathogens and is responsible for nosocomial and community acquired infections. Methicillin was the first β-lactamase resistant penicillin introduced in 1960. In 1961 Methicillin resistant S. aureus (MRSA) was first identified by British scientists and the first MRSA in United States was identified in 1968.[1] Highly resistant bacteria, MRSA are responsible for a greater percentage of hospital-acquired infections. The appearance of MRSA in universe creates major therapeutic obstruction to physicians. The prevalence of MRSA is increasing in hospitals because of the pressure exerted by the irrational use of antibiotics. This rise in the frequency of MRSA in hospitals is mainly responsible for morbidity and mortality in hospitals during the last ten years. Around 19,000 deaths per year have been reported in the US due to MRSA, exceeding those due to HIV-AIDS. The MRSA infections can only be treated with limited group of antimicrobial agents, leading to a prolonged hospital stay with increase cost of care.[2]

In Pakistan, 24.6% MRSA was reported in Karachi during 2002-2003,[3] while a recent 2011 study gave a value of 11.5%.[4] About 39% of MRSA were detected in Islamabad during 2004-2005.[5] A similar study by Khan et al gave MRSA value of 53.3% from two hospitals in Rawalpindi during 2011.[6] The prevalence of MRSA in Lahore during 2006-2008 was 41.9% according to Bukhari et al,[7] and the value rose to 50% in 2009 as reported by Chaudary et al.[8]

The increase in the prevalence of antibiotic resistance is one of the major challenges for the healthcare services. Concerning the public health, it is feared

that the treatment of common infections have become difficult and it may take longer time to put an end to the infections caused by resistant bacteria.[9] Antibiogram or susceptibility pattern is a simple method as compared to molecular methods because it is quite inexpensive, easily available and can be determined without any difficulty. Clinicians, microbiologists and pharmacists use it as a reference guide to hospital specific or community specific resistance pattern when sophisticated molecular methods are not available.[10] This method is still considered as a standard for MRSA surveillance in Europe because the microorganism is available to be used for further epidemiological analysis.[2] However, the disadvantage of this method is that it is not reproducible all the time and that antimicrobial susceptibility pattern varies due to plasmids or environmental factors. Furthermore, the discriminatory power of this method is poor.[10] Molecular methods have the advantage of high sensitivity where the sensitivity of the culture method can be improved by broth enrichment, giving results comparable to those of PCR.

As susceptibility of bacteria to antibiotics varies from region to region, this study was conducted to determine the frequency of methicillin resistance among the isolates of S. aureus and the pattern of antimicrobial susceptibility of MRSA isolates to the commonly prescribed antibiotics in Abbottabad.

METHODS

This cross-sectional descriptive study was conducted at Ayub Teaching Hospital, Abbottabad, Khyber Pakhtunkhwa, Pakistan from August 2007 to June 2009. A total of 98 Staphylococcal isolates were isolated from different clinical specimens including pus, urine and other clinical specimens collected from patients admitted in Ayub Teaching Hospital. Samples were aseptically collected in sterilized bottles and submitted to microbiology laboratory. They were cultured on Nutrient agar, MacConkey agar, and Blood agar (Oxoid, England) and incubated at 37°C for 24 h. Standard microbiological procedures were carried out for the identification of bacterial isolates; these include morphological appearance, Gram staining and biochemical tests like catalase, tube and slide coagulase, mannitol fermentation, and deoxyribonuclease activity.[11] Pure cultures of S. aureus were then preserved at -70°C in nutrient broth containing 4% glycerol until use.

The antimicrobial susceptibility of the identified S. aureus isolates was determined by Kirby-Bauer disc diffusion method following the Clinical Laboratory Standard Institute (CLSI, 2011) guidelines. The Oxoid (England) antibiotic discs were used for disc diffusion method. The antibiotics include amoxicillin (30 μg), amoxicillin + clavulanic acid (30 μg), cefuroxime (30 μg), cefaclor (30 μg), cephradine (30 μg), ceftazidime (30 μg), gentamicin (10 μg), cefoperazone + sulbactam (30 μg), ceftriaxone (30 μg), ceftizoxime (30 μg), ciprofloxacin (5 μg), meropenem (10 μg), sulphamethoxazole + trimethoprim (1.25/23.75 μg), imipenem (10 μg), and erythromycin (15 μg). Cefoxitin (30 μg) discs were used for MRSA determination, the zone size of < 21 mm was considered resistant and a zone size of > 22 mm was considered susceptible. Isolates were categorized as susceptible, intermediate, or resistant according to CLSI guidelines. Staphylococcus aureus ATCC 25923 was used as a control for susceptibility testing.

Minimum Inhibitory Concentration (MIC) was determined by agar dilution method.[12] Serial two fold dilutions of antimicrobial agents were prepared and added to molten Muller Hinton agar at near 50°C. Antibiotic stock solutions and dilutions were made in their appropriate solvents. Following formula was used to calculate the appropriate weights of Active Pharmaceutical Ingredients (APIs)

$$W = \frac{1000}{P} \times V \times C$$

Where W = Weight of API in mg, C = final concentration of solution (multiples of 1000) (mg/l), V = volume (ml), and P = Potency of API in μg/mg.

Five antimicrobial categories were selected for screening MDR; they include the following agents, gentamicin

TABLE I: DETAIL FREQUENCY OF S. AUREUS ISOLATES AMONG DIFFERENT SAMPLE TYPES

Sample type	S. aureus	Percentage	MRSA	Percentage2	MSSA	Percentage
Pus	72	73.47	17	70.83	55	74.32
Urine	16	16.33	5	20.83	11	14.87
HVS	5	5.1	0	0	5	6.76
Breast abscess	1	1.02	1	4.17	0	0
Ear swab	1	1.02	0	0	1	1.35
Semen	1	1.02	1	4.17	0	0
Endocervical swab	1	1.02	0	0	1	1.35
Pleural fluid	1	1.02	0	0	1	1.35
Total	98	100%	24	100%	74	100%

MRSA: Methicillin-Resistant Staphylococcus aureus; MSSA: Methicillin-Sensitive Staphylococcus Aureus; HVS: High vaginal swab.

TABLE II : ANTIBIOTIC SUSCEPTIBILITY OF THE METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS ISOLATES

S. No.	Antibiotics	Resistant (%)	Intermediate (%)	Sensitive (%)	MIC50	MIC_{90}
1	AMC	75.00	0.00	25.00	8	256
2	AML	100.00	0.00	0.00	16	256
3	CAZ	79.17	0.00	20.83	64	256
4	CE	83.33	8.33	8.33	32	128
5	CFP	62.50	20.83	16.67	32	64
6	CRO	50.00	50.00	0.00	32	64
7	CEC	83.33	16.67	0.00	64	128
8	CIP	83.33	8.33	8.33	4	128
9	MEM	91.67	0.00	8.33	64	512
10	IPM	0.00	0.00	100.00	8	128
11	CN	45.83	33.33	20.83	8	64
12	ZOX	62.50	8.33	29.17	16	128
13	CXM	70.83	0.00	29.17	8	256
14	SXT	41.67	4.17	54.17	64	256
15	E	83.33	16.67	0.00	16	128
16	FOX	100.00	0.00	0.00	32	128

*AMC, Augmentin; AML, Amoxycillin; CAZ, Ceftazidime; CE, Cephradine; CFP, Cefoperazone+sulbactam; CRO, Ceftriaxone; CEC, Cefaclor; CIP, Ciprofloxacin; MEM, Meropenem; IPM, Imipenem; CN, Gentamicin; ZOX, Ceftizoxime; CXM, Cefuroxime; SXT, Trimethoprim; E, Erythromycin.

TABLE III: ANTIBIOTIC SUSCEPTIBILITY OF THE METHICILLIN SENSITIVE STAPHYLOCOCCUS AUREUS ISOLATES

S. No.	Antibiotics	Resistant (%)	Intermediate (%)	Sensitive (%)	MIC_{50}	MIC_{90}
1	AMC	48.65	0.00	51.35	8	64
2	AML	100.00	0.00	0.00	4	32
3	CAZ	74.32	0.00	25.68	32	256
4	CE	52.70	22.97	24.32	16	512
5	CFP	32.43	27.03	40.54	32	64
6	CRO	32.43	22.97	44.56	32	64
7	CEC	59.46	8.11	32.43	64	256
8	CIP	18.92	45.95	35.14	4	128
9	MEM	50.00	13.51	36.49	64	128
10	IPM	0.00	6.76	93.24	8	128
11	CN	51.35	2.70	45.95	8	64
12	ZOX	32.43	24.32	43.24	8	64
13	CXM	50.00	6.76	43.24	8	256
14	SXT	18.92	12.16	68.92	64	256
15	E	45.95	25.68	28.38	8	128
16	FOX	0.00	0.00	100.00	16	128

* AMC, Augmentin; AML, Amoxycillin; CAZ, Ceftazidime; CE, Cephradine; CFP, Cefoperazone+sulbactam; CRO, Ceftriaxone; CEC, Cefaclor; CIP, Ciprofloxacin; MEM, Meropenem; IPM, Imipenem; CN, Gentamicin; ZOX, Ceftizoxime; CXM, Cefuroxime; SXT, Trimethoprim; E, Erythromycin.

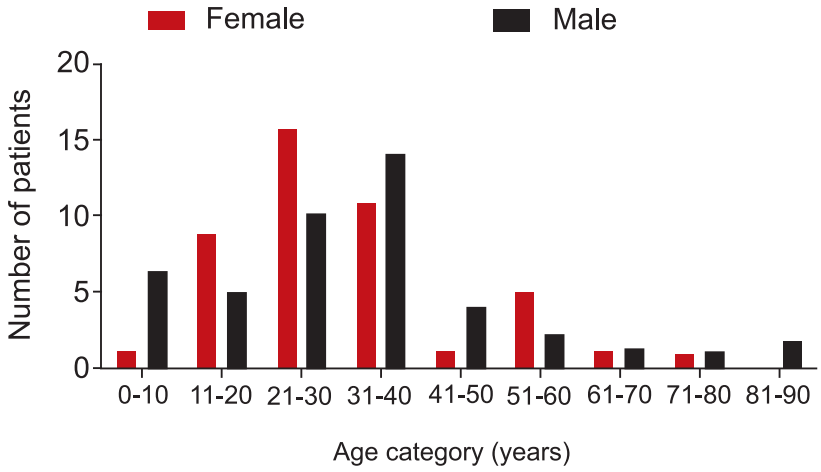

Figure 1: Sexwise distribution of S. Aureus infections among different age groups

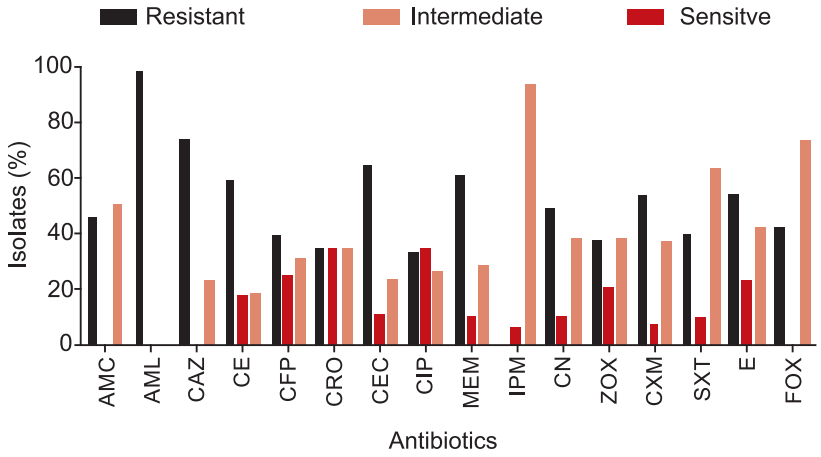

Figure 2: Disc diffusion based antibiogram of S. Aureus

from aminoglycoside, cefoxitin from anti-staphylococcal β-lactams, ciprofloxacin from fluoroquinolones, trimethoprim + sulfamethoxazole from folate pathway inhibitors and erythromycin from macrolides.[13]

RESULTS

In this study, 98 clinical isolates of S. aureus were recognized, of which 72(73.47%) were isolated form pus and 16 (16.33%) were isolated from urine. Out of 24 MRSA isolates, 17 (70.83%) & 5 (20.83%) were isolated from pus and urine respectively (Table 1). Out of total 98 isolates of S. aureus, 53 (54.08%) were obtained from males and 45 (45.91%) from females. The age limit of S. aureus infected patients was 2 months to 82 years with a mean of 32.67 years. The age distribution of total 98 patients with S. aureus infections is indicated in figure 1. About 24.49% isolates resistant to cefoxitin were methicillin resistant S. aureus (MRSA) strains and 75.51% sensitive to cefoxitin were methicillin sensitive S. aureus (MSSA). The resistance pattern of 24 MRSA strains is shown in the Table II. The resistance pattern of antibiotics other than cefoxitin was further elaborated between both cefoxitin sensitive and resistant strains. The resistance pattern among 74 methicillin sensitive S. aureus strains is shown in Table III.

DISCUSSION

Resistance to antibiotics has increased globally during the last ten years and MDR isolates rate has also risen in the clinical setting.[4] S. aureus is the most frequent agent responsible for surgical-site infection and second most common cause of hospital acquired bacteremia.[14] The number of bacterial isolates obtained from males were greater than those obtained from females, with ratio being 1.18:1, respectively. The incidence of S. aureus infections was high in the middle aged patients (21-40 years, both males and females) accounting for 54.08% of total S. aureus infections as indicated in Figure 1. Mubbashir Hussain et al[15] reported 51.79% of S. aureus from male patients and 48.21% from female patients which is in agreement with our findings. This shows that the prevalence of Staph infections was high in males in the Abbottabad region of Khyber Pakhtunkhwa, Pakistan as compared to females.

Our study showed that MRSA rate in Abbottabad is 24.49%. Frequency of MRSA in Kohat during 2012 was 44%.[15] while MRSA rate in Peshawar showed an increase of 54% from the year 2009 to 2011.[16]

Among 74 MSSA, 37.84% were found to be multi-drug resistant (MDR). All the 24 MRSA were also found to be MDR. In total, 53.06% isolates were found to be MDR. Cefoxitin resistant isolates were generally co-resistant to ciprofloxacin and erythromycin (83.33%), gentamicin (45.83%), and co-trimoxazole (41.67%). All these MDR isolates were, however, 100% sensitive to imipenem. Furthermore, there was no such isolate found which was resistant or sensitive to all antimicrobial categories. The most prevalent MDR pattern was resistance to β-lactams, fluoroquinolones, and macrolides. All MRSA strains were 100% sensitive to imipenem. Resistance shown to cephalosporins ranged from 50 to 83.33%. MRSA showed pronounced resistance against amoxycillin (100%), meropenem (91.67%), erythromycin (83.33%), ciprofloxacin (83.33%), and amoxycillin+clavulanic acid (75%). Resistance was high in MRSA as compared to MSSA.

CONCLUSION

This study indicates that the frequency of MRSA is 24.49% in Abbottabad and these MRSA are highly resistant to β-lactams, fluoroquinolones, and macrolides but sensitive to imipenem.

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CONFLICT OF INTEREST

Authors declare no conflict of interest

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AUTHOR'S CONTRIBUTION

Following authors have made substantial contributions to the manuscript as under:

RT: Acquisition of data, drafting the manuscript, final approval of the version to be published

IM: Critical revision, supervision, final approval of the version to be published

JA: Conception and design, critical revision, final approval of the version to be published

AK: Drafting the manuscript, final approval of the version to be published

FS &ZK: Acquisition & analysis of data, final approval of the version to be published

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

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