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Current Antibiotic Resistance Trend in Clinical Isolates of *Staphylococcus aureus* from a Tertiary Care Hospital

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ABSTRACT

Background: *Staphylococcus aureus (S. aureus)* has remained always an important pathogen of common infections acquired in community and as well as serious nosocomial infections. With advent of penicillins and cephalosporins, infections could be effectively treated, but with the global emergence of Methicillin Resistant *Staphylococcus aureus* strains (MRSA) physicians were again left with limited treatment options. This scenario of increasing resistance is even more intense and challenging for developing countries like Pakistan**.** Hence with this background the study was carried out to establish the frequency of MRSA in clinical specimens and look into the available antibiotic treatment options.

Methods: Samples of pus, blood, urine, body fluids and catheter tips submitted for culture in Microbiology department between August to September 2012, from outdoor and indoor adult patients of Pakistan Institute of Medical Sciences Islamabad, yielding growth of *S. aureus* were included in the study. After identification by standard methods, antibiotic susceptibility of the isolates was performed by Kirby Baeur disc diffusion method. The study was retrospective descriptive and observational.

Results: Total 106 *S. aureus* were isolated. 45.3% of them were MRSA and majorities were from pus samples of hospitalized patients. All MRSA were 100% sensitive to vancomycin, whereas 87.5% to chloramphenicol. To rest of the non – beta lactam drugs, resistance of 80% or more was noted.

Conclusion: *S. aureus* is a common clinical isolate from patients in this region of Pakistan and significant number were MRSA especially from hospitalized patients. Treatment options are limited to vancomycin and chloramphenicol.

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Introduction

Initially staphylococcal infections were treated with beta-lactam antibiotics such as penicillin but due to indiscriminate use of these drugs, the strains developed mechanisms to destroy these beta-lactams by production of beta lactamases. In 1960's betalactamase, stable penicillins were introduced and became the principal drug of choice for staphylococcal infections caused by strains producing beta lactamases. With the course of time, these strains also became resistant to beta lactamase stable penicillins due to chromosomal mutation; such strains are called Methicillin Resistant Staphylococcus aureus (MRSA).

MRSAs have become a nosocomial problem associated with prolonged hospital stay, extended antibiotic courses and the presence of invasive devices such as catheter tips and hence cause life-threatening infections (1). The major factors in transmission of resistant strains are the lack of hand washing practices by hospital staff, irrational use of antibiotics, catheterization and immune-suppression (2-4). As a result of severe but untreatable infections caused by MRSA, the mortality and morbidity rates have increased (5-7).

MRSA is not only a chief concern of industrialized countries but more so of under developed countries such as Pakistan. In 1989, the first MRSA was isolated in Pakistan (8) and since then different workers have reported significantly high rates, as the reasons responsible for emergence and spread are more in countries like Pakistan (9-11).

MRSAs are not only confined within the hospitals, but are also spreading to the community as community acquired MRSA (CA-MRSA) (12).

Hence, with this background the study was carried out to establish the frequency of MRSA in clinical specimens so as to provide treatment and empirical guidelines to treating physicians of the region. It will also help in developing future strategies for implementation of preventive measures for effective control of these tedious strains.

Materials and Methods

Sample collection

Total of 106 different clinical specimens submitted for culture from indoor and outdoor patients and yielding the growth of *S. aureus*, between August to September 2012 at Microbiology laboratory of Pakistan Institute of Medical Sciences Islamabad-Pakistan were included in the study.

Microbiological analysis

Different samples were inoculated on their respective media and suspected colonies of *S. aureus* were identified on the basis of colonial morphology, Gram staining, catalase test, coagulase and DNase tests (13).

Antibiotic Testing and Detection of MRSA

Antibiotic susceptibility test was done by Kirby-Bauer disc diffusion technique on Muller Hinton agar and incubated at 35°C for full 24 hours. The antibiotics tested were the ones which are recommended for treatment of staphylococcal infections. Antibiotic discs (Oxoid UK) used were penicillin (10units), gentamicin (10µg), co-trimoxazole (25µg), ciprofloxacin (10µg),

chloramphenicol (30µg), vancomycin (30µg) and teicoplanin (30µg).

For detection of MRSA, cefoxitin disc (30µg) was used. *S. aureus* having zone diameter of ≤ 22 mm around cefoxitin disc was considered as MRSA following Clinical Laboratory Standard Institute (CLSI) guidelines (14). *S. aureus* ATCC 29213 which is a Methicillin sensitive Staphylococcus aureus (MSSA) was used as quality control organism. For statistical analysis, like percentages etc. SPSS version 16 was used.

Results

Total of 106 samples, were obtained from 70 (66%) male and 36 (34%) female patients.

Isolates were obtained from wide variety of clinical specimens. The highest number of isolates were from pus samples i.e. 63.2% followed by blood (18.9%) and the least were from urine (1.9%) (*Table 1*).

Samples were received from various departments of the hospital, but majority of the patients belonged to the emergency unit 38.6%, followed by surgical ward 27.3% and then medical ward 18.8%. Only a few samples came from other locations *(Figure 1).*

A total number of 48 (45.3%) out of 106 *S. aureus* were identified as MRSA (*Figure 2*).

It has been observed that most of the MRSA isolates were from Surgical wards (39.55%) followed by (29.16%) collectively from various Medical wards and (25%) from Emergency unit *(Table 2, Figure 3).*

Sensitivity Patterns of S. aureus isolates

The sensitivity and resistance patterns of *S. aureus* isolates is shown in Table 3. Vancomycin, teicoplanin and chloramphenicol showed the maximum sensitivity (100%, 100% and 92.5% respectively).

Figure 4 shows comparative sensitivity of MRSA and MSSA. Both types were 100% susceptible to vancomycin and teicoplanin. MSSA showed better sensitivity for all antibiotics other than beta-lactams as compared to MRSA except for co-trimoxazole to which both were only 25% susceptible. After vancomycin and teicoplanin, chloramphenicol showed better susceptibility, as 87.5% of MRSA were sensitive to it. Only less than 20% of MRSA were sensitive to gentamicin and ciprofloxacin.

Table 1. Distribution of Samples with *Staphylococcus aureus* isolates

Type of Sample	n (%)
Pus	67 (63.2)
Blood	20 (18.9)
Fluids	11 (10.4)
Catheter	6 (5.6)
Urine	2 (1.9)
Total	106 (100)

Table 2. Distribution of Samples with *Staphylococcus aureus* isolates

Source (Total S. aureus)	MRSA n (%)
Emergency (41)	12 (25)
Surgical Ward (29)	16 (33.3)
Surgical ICU (3)	3 (6.25)
OPD (3)	1 (2.08)
Medical Ward (20)	8 (16.6)
Medical ICU (2)	2 (4.16)
Private ward (2)	2 (4.16)
CCU (1)	1 (2.08)
Miscellaneous (5)	3 (6.25)
Total Cases (106)	48 (100)

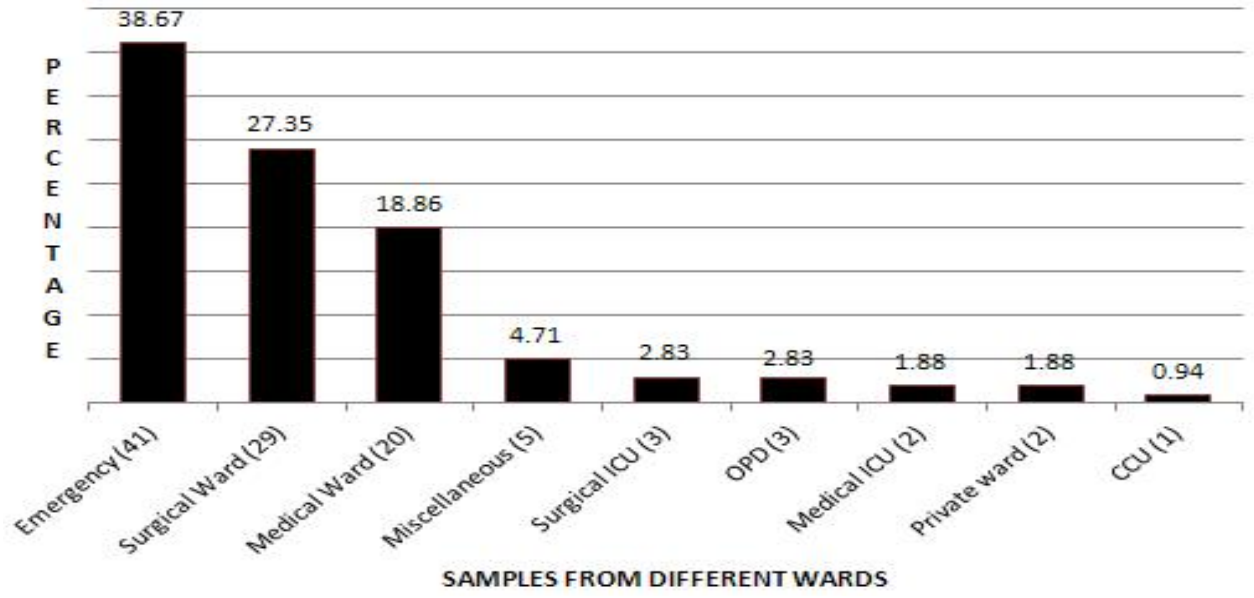

Figure 1. Percentage Distribution of Samples Based on Source

Table 3. Antibiotic susceptibility pattern of *Staphylococcus aureus*

Antibiotics	*S. aureus*	
	Sensitivity n (%)	Resistance n (%)
Penicillin (10 units)	0(0)	106 (100)
Cefoxitin (10µg)	58 (54.7)	48 (45.3)
Gentamicin (10µg)	56 (61.5)	35 (38.5)
Co-trimoxazole (25µg)	36 (37.9)	59 (62.1)
Ciprofloxacin (10µg)	53 (50.5)	52 (49. 5)
Chloramphenicol (30µg)	98 (92.5)	8 (7.5)
Vancomycin (30µg)	106 (100)	0 (0)
Teicoplanin (30µg)	106 (100)	0 (0)

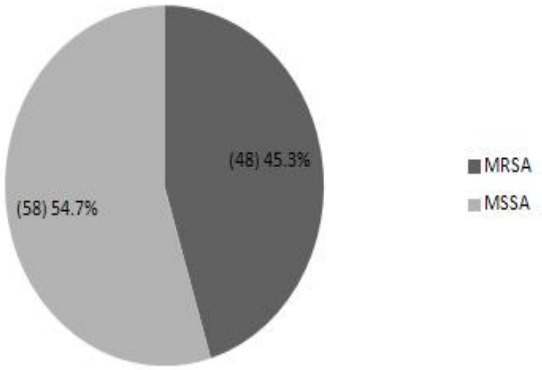

Figure 2. Frequency of MRSA and MSSA

Discussion

S. aureus is one of the commonest organisms causing infection in community and in hospitals. The most liable individuals to be infected are surgical patients, old aged, malnourished persons, Diabetics, newborn babies, and persons with chronic diseases (15, 16).

In duration of 2 months, 106 isolates were obtained and highest numbers of isolates were retrieved from pus (63.2%), indicating pyogenic nature of infections caused by *S. aureus.* The next highest isolation was from blood 18.9%, as 60% of samples were from hospitalized patients. Other workers have also reported highest isolation from pus (9-11).

Highest numbers of MRSA isolates (39.55%) were from surgical patients of

surgical ward and surgical ICU. Its prevalence varies considerably from one region to another and even among the hospitals in the same city, but usually most of the workers are reporting similar findings (16, 17).

In this study, 45.3% of *S. aureus* were MRSA. MRSA strains were first reported in England in 1960 (18). The organism established itself as a major nosocomial pathogen especially in intensive care units of tertiary care hospitals and poses difficulties in eradication. Similar are the findings of this study where maximum number was isolated from hospitalized patients mainly from surgical ward and intensive care unit. Similar were the observations of other workers from the same hospital earlier on in year 2005 (17).

Different studies from various cities of Pakistan have reported MRSA rates ranging from 19.8% to 44% between the years 2000-2012 (19-25). The lowest rate of 19.8% was from Sindh (Pano Akil) (22); whereas cities like Lahore and Kohat had rates of 38.5% (20) and 44% (25) respectively.

The MRSA rate of our study is comparable with most of the cities of Pakistan. The other observation is that rates have not increased markedly over the past decade. Probably the awareness and practices of infection control is reflecting on the results.

MRSA has emerged as a serious public health problem of global concern not only in under developed and developing regions but also in developed countries such as UK (26). The prevalence of MRSA was found to be about ~20% in Germany (27) and another study showed an MRSA rate of ≥ 20% from UK, Northern Ireland and Belgium in 2011 (28).

The real issue with MRSA infections is its treatment because of limited treatment options. MRSA strains which have under gone chromosomal mutation have *mec*A gene and its product PBP2a with a low affinity for beta lactam antibiotics (29). Strains possessing this show resistance to all the currently available beta lactam antibiotics including penicillins, beta-lactam / beta-lactamase inhibitor combinations, cephalosporins, aztreonam and carbapenems.

In the present study the only antibiotic to which all the isolates were sensitive was vancomycin and teicoplanin, followed by chloramphenicol (87.5%), but rest of antibiotics which can be used had resistance of almost more than 80%. Similar are the findings of other regional co-workers as in a study conducted in 2009 by National University of Sciences and Technology, Rawalpindi-Pakistan concluded that vancomycin was 100% susceptible and chloramphenicol 93% (23).

The control of MRSA lies in the treatment of patients with appropriate antibiotics, and controlling the spread of this infection to other patients mainly by implementing infection control measures. Indiscriminate use of antibiotics and availability without prescriptions in the developing countries like Pakistan has contributed to the burden of MRSA. Vancomycin is the drug of choice for MRSA infections (30) and drugs like chloramphenicol has its own limitations like bone marrow depression and effectiveness in MRSA infections. Isolates of this study were all found sensitive to vancomycin, which is also a finding of other local (19-25) and international workers (27, 32). However, vancomycin intermediate Staphylococcus aureus strains (VISA) are being reported and so laboratory methods required for their

detection need to be used in our setups also (33).

Practice of infection control measures like hand washing, isolation of infected patients, detection of carriers amongst health care workers and surveillance has shown to bring down the rate of MRSA by many workers (15, 34) and should be implemented in all health care facilities to control its spread.

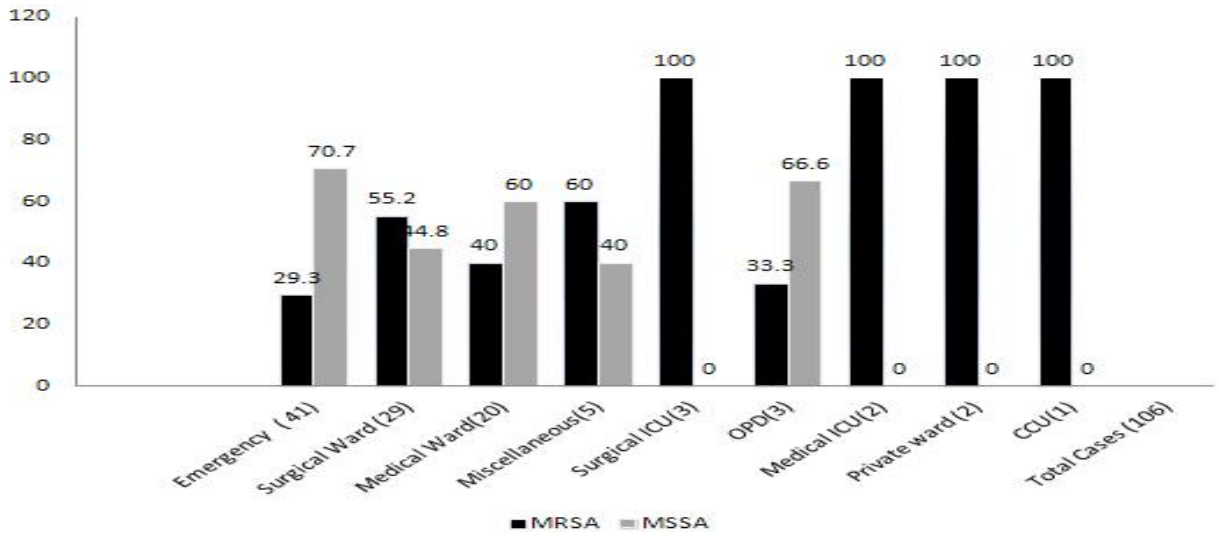

Figure 3. Distribution of MRSA and MSSA According to Source

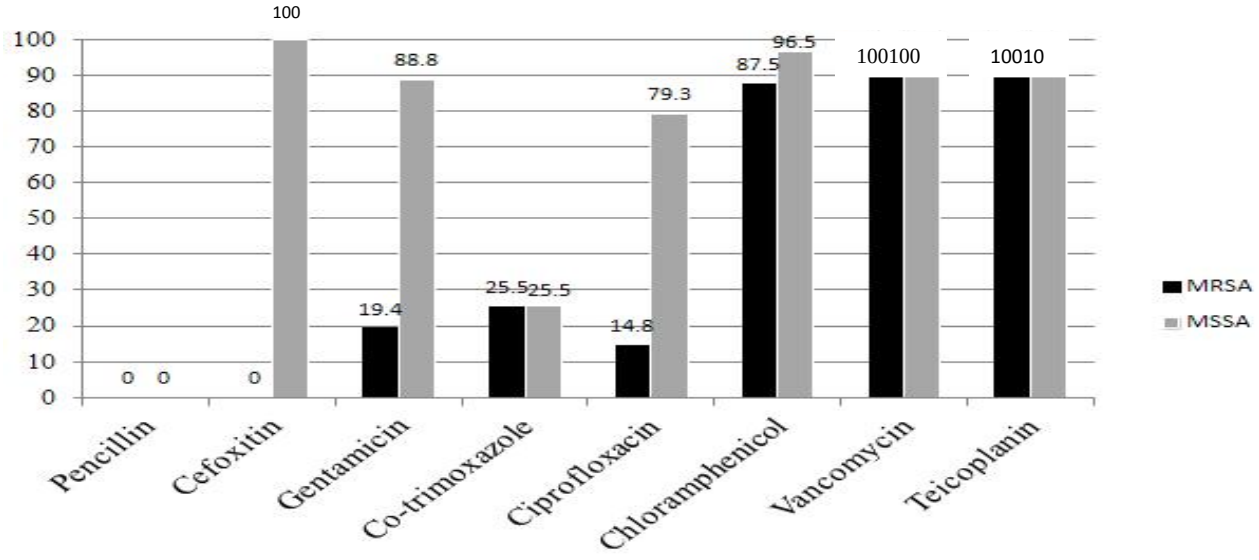

Figure 4. Comparative Percentage Sensitivity of MRSA and MSSA to Anti Staphylococcal Antimicrobials

Conclusion

Staphylococcus is a common clinical isolate from patients and significant number out of them are MRSA especially causing infections in hospitalized patients. The treatment options are limited to expensive carbapenems i.e. vancomycin and teicoplanin.

Practice of standard infection control measures and stopping easy availability of antibiotics over the counter is a dire need of time otherwise a time will come when we would be facing infections with vancomycin resistant Staphylococcus and landing into pre antibiotic era.

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Conflict of Interest

None declared conflicts of interest.

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