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Frequency of Methicillin-resistant Staphylococcus and antibiogram profile of Staphylococcus aureus recovered from different clinical samples

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ABSTRACT

Background: Staphylococcus is a ubiquitous bacterium and well-known pathogen causing a variety of infections. The global spread of Methicillin-resistant Staphylococcus aureus **(**MRSA) constitutes one of the most prevailing challenges to the management of infections caused by this bug. Our objective is to determine the frequency of MRSA and antibiogram profile of S. aureus recovered from different clinical samples.

Methods: This cross sectional study was carried out in the Microbiology Laboratory of Shalamar Hospital Lahore. The data of the Staphylococcus isolates including MRSA from pus and swab samples was collected through Electronic Medical Record of the Shalamar Hospital from 1st Jan to 31st Dec 2021**.** S. aureus was identified by standard protocol including Gram stain, catalase, coagulase, and DNase tests. Antimicrobial susceptibility was carried out by modified Kirby Bauer method. MRSA frequency was determined by the result of sensitivity to cefoxitin.

Results: Out of 885 samples submitted for culture, 517 (58.4%) were reported for microbial growth of a known pathogen. The most frequently isolated pathogen was S. aureus (37.9%), followed by E. coli (22.4%), other members of Enterobacteriaceae family (17.8%), Pseudomonas (15.5%), Enterococcus (3.5%), Candida (2.1%), and Streptococcus (0.8%). Amongst S. aureus, MRSA was documented in 46.9% cases. Substantial difference was detected in the susceptibility pattern of Methicillin-sensitive and resistant staphylococci. All staphylococci were resistant to ampicillin while no vancomycin resistance was encountered.

Conclusion: MRSA was seen in the local population with a high frequency and they also showed marked resistance against other commonly used antibiotics. Fortunately no vancomycin-resistant S. aureus was reported.

Key words: Frequency, MRSA, Pus, Staphylococcus, Swab.

Introduction

Though the Staphylococcus genus has several species and subspecies, Staphylococcus aureus is clinically the most important. S. aureus is a ubiquitous organism and colonizes skin and mucosal surfaces of humans as well as a variety of animal species. It colonizes the nasal mucosa of almost 30% people. [1, 2] S. aureus is a well-known bacterium capable of causing numerous different types of infections including localized as well as disseminated and life-threatening illnesses, such as a wound infection, toxic shock syndrome, scalded skin syndrome, food intoxication, endocarditis, osteomyelitis, otitis media, abscesses, pneumonia, brain abscess, meningitis, urinary tract infection, indwelling medical devices-related infections and bacteremia.[2,3] S. aureus is amongst the most frequent pathogens recognized globally as a causative agent both community and hospital-acquired infections contributing significantly to the morbidity and mortality in the industrialized countries.[3,4] It is the commonest pathogen involved in skin infections worldwide.[5] Several studies conducted in various cities of neighboring countries, including China, India and Iran, have reported high prevalence of colonization and infections by S. aureus.[6,7,8] Similarly, many studies carried out in different cities of Pakistan have shown a high frequency of infections due to S. aureus.[9,10,11]

In addition to its high prevalence, S. aureus has the potential to get resistance to multiple antimicrobials.

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Antibiotics obtained from Penicillium moulds (or produced synthetically) were used for treating infections due to S. aureus since long. However, over a period of time and in a gradual manner, it has acquired resistance to penicillin including Methicillin and related drugs. Above 90% strains of S. aureus possess plasmids encoding beta lactamase, an enzyme which inactivates most of the penicillins. The strains of S. aureus resistant to Methicillin and related penicillins are due to the changes in the penicillin binding proteins (PBPs) present in their cell membrane. Such altered PBPs are encoded by genes on the bacterial chromosome known as mecA genes. Such strains are called as Methicillin resistant S. aureus (MRSA). As a result, infections due to such pathogens are difficult to treat. The global prevalence of MRSA infection both acquired and in hospitals (HA-MRSA) and in the community continues to rise.[12] Since 1990s, the spread of community acquired Methicillin resistant S. aureus (CA-MRSA) has altered the epidemiology of MRSA.[13]

The incidence of MRSA has gradually increased with strains causing serious infections. Conveyance of MRSA infected patients has resulted in intercountry dissemination. However, the most common form of transmission is person-to-person within a healthcare center and then to other healthcare facility within the same geographical area. MRSA strains cross-resistant to other beta-lactamase have been increasing, mainly in healthcare settings.[14] HA-MRSA is associated to high mortality and morbidity, as well as extended hospital stays and a significant financial burden on healthcare systems across the globe.[15] Early identification, good infection control protocols, and proper antibiotic practices have been advised to reduce the spread of MRSA. The present study aimed to determine the frequency of MRSA and to find out the antibiogram of isolates so as to provide guidelines to our physicians for appropriate antibiotic therapy to fight against infections caused by such pathogens.

Methods

The data of the bacterial isolates from pus and swab samples was collected through Electronic Medical Record (EMR) system of the Shalamar Hospital Lahore from 1st Jan to 31st Dec 2021**.** During the research time period, a total 885 samples of pus and pus swabs for cultures from both in-patients and out-patients of all genders and ages were processed in the microbiology laboratory. The identification of gram-positive cocci was made by gram stain and other tests including catalase positive (all Staphylococcus species), coagulase and DNase positive (to distinguish Staphylococcus aureus from other Staphylococcus species), novobiocin sensitive (to distinguish from Staphylococcus saprophyticus), and mannitol fermentation positive (to distinguish from Staphylococcus epidermidis). The gram-negative bacterial species were recognized by colony morphology, gram stain, biochemical tests including BioMérieux Analytical Profile Index (API) or using Vitek®2-compact automated microbiology system. Antimicrobial sensitivity of S. aureus was carried out by Kirby-Bauer method for cefoxitin, ciprofloxacin, clindamycin, erythromycin, fosfomycin, gentamicin, linezolid, penicillin, teicoplanin and vancomycin. To find out the frequency of MRSA, the results of sensitivity to cefoxitin disc were recorded. The data analysis involved transcription, data review, content analysis, and interpretation. Statistical analysis was carried out by descriptive statistics using ratio and percentages. Microsoft Excel was used to generate tables and charts. Qualitative variables were expressed as rates and percentages. Institutional Review Board (IRB) approved the study vide Letter No. SMDC-IRB/AL/152/2021 Dated 28.08.2021.

Results

A total of 885 samples of pus and aspirates, wound swabs, and of swabs other than wound, from different mucocutaneous sites were processed in the microbiology laboratory for culture and sensitivity testing during the study period. The types and frequency of samples submitted for culture and sensitivity testing are shown in Table-1.

Table-1: Types and Frequency of samples submitted for culture (n=885)

Type of Pathogen	No. of Samples Submitted	No. of Positive Cultures
1) Pus and aspirates	311	262 (84.2%)
2) Wound swabs	256	202 (78.9%)
3) Pus swabs*	318	53 (16.7%)
Total	**885**	**517 (58.4%)**

*Other than wound swabs (include ear, eye, genital, nasal)

Out of all, 517 (58.4%) were reported for microbial growth of a recognized pathogen. The maximum number of pathogens i.e., 262 out of 311 (84.2%) were recovered from pus/aspirated samples followed by wound swabs i.e., 202 out of 256 (78.9%). The least yield of positive culture i.e., 53 out of 318 (16.7%) was

observed from superficial swab samples of various mucocutaneous sites, including ear, eye, genital, and nasal.

Table-2: Types and Frequency of all pathogens isolated (n=517)

Type of Pathogen	No. of Positive Cultures	Frequency
1. S. aureus	196	37.9%
2. Enterobacteriaceae (n=208)		
▪ E. coli	116	22.4%
▪ Proteus spp.	40	7.7%
▪ Klebsiella spp.	32	6.2%
▪ Citrobacter spp.	16	3.1%
▪ Enterobacter spp.	4	0.8%
3. Pseudomonas spp.	80	15.5%
4. Enterococcus spp.	18	3.5%
5. Candida spp.	11	2.1%
6. Streptococcus spp.	4	0.8%
Total	517	100%

Table-2 shows the types and frequency of all pathogens isolated. Exclusively, S.aureus was the most frequent isolate (37.9%). The other important isolates were E. coli (22.4%), Pseudomonas species (15.5%), Proteus species (7.7%), Klebsiella species (6.2%), Enterococcus species (3.5%), Citrobacter species (3.1%), Candida species (2.1%), Enterobacter species (0.8%) and Streptococcus species (0.8%). Inclusively, however, the frequency of Enterobacteriaceae isolates altogether was 40.2%. The frequency of infections caused by S. aureus varied in different age groups and genders. Overall, the isolation rate of S. aureus was slightly higher in males and male to female ratio was 1.13:1. The frequency was higher in male children and adolescents as well as in males beyond 60 years (Table-3).

Table-3: Gender-wise dispersion of S. aureus isolates in different age groups

Age Group	Gender			
	Male (n=104)		**Female** (n=92)	
	Count	**%**	**Count**	**%**
▪ Up to 19 years (n=50)	31	62	19	38
▪ 20-40 years (n=58)	26	44.8	32	55.2
▪ 41-60 years (n=75)	36	48	39	52
▪ >60 years (n=13)	11	84.6	2	15.4
Total (n=196)	**104**	**53%**	**92**	**47%**

Table-4 shows antibiotic sensitivity of S. aureus. All the isolates exhibited resistance to Ampicillin, whereas all displayed sensitivity to Vancomycin. Besides Vancomycin, the isolates showed maximum sensitivity to Linezolid (95.4%), followed by Teicoplanin (92.9%) and Fosfomycin (81.6%). A large number were resistant to Clindamycin (58.2%) and Gentamicin (54.1%). Still, a much reduced sensitivity was observed for Erythromycin (36.7%) and Ciprofloxacin (31.6%). The frequency of MRSA was 46.9% (Table-4). Antimicrobial susceptibility pattern of MRSA differed considerably with the category of antibiotic tested. MRSA strains were also often resistant not only to other beta-lactam antibiotics but to aminoglycosides, macrolides and fluoroquinolones.

Table-4: Antimicrobial sensitivity of S. aureus (n=196)

Antimicrobial	Resistant (%)	Sensitive (%)
1. Ampicillin	196 (100%)	0 (0%)
2. Cefoxitin (To detect MRSA*)	92 (46.9%)	104 (53.1%)
3. Ciprofloxacin	134 (68.4%)	62 (31.6%)
4. Clindamycin	82 (41.8%)	114 (58.2%)
5. Erythromycin	124 (63.3%)	72 (36.7%)
6. Fosfomycin	36 (18.4%)	160 (81.6%)
7. Gentamicin	90 (45.9%)	106 (54.1%)
8. Linezolid	9 (4.6%)	187 (95.4%)
9. Teicoplanin	14 (7.1%)	182 (92.9%)
10. Vancomycin	0 (0%)	196 (100%)

*Zone diameters of Cefoxitin as per CLSI guidelines: ≤21/≥22mm (Resistant/Sensitive)

Discussion

S. aureus enjoys an unrestrained lifestyle which is enabled by a comprehensive display of virulence features. In contrast to most other bacterial pathogens, which generally depend upon one or a few virulence factors to confirm pathogenicity, S. aureus produces an astonishing array of virulence factors. The successful colonization of S. aureus in various milieus, living hosts, or different inanimate surfaces is possible due to the large number of virulence factors that this bacterium uses.[16] Being ubiquitous, the same bacterium that exists as a commensal and has potential to spread in both community and healthcare settings is also a principle source of various clinical presentations from localized infections such as abscess, cellulites, impetigo, scalded skin syndrome, and wound infection to disseminated and life-threatening illnesses including brain abscess, endocarditis, food poisoning, indwelling medical devices-related infections,

meningitis, necrotizing fasciitis, pneumonia, septicaemia, and toxic shock syndrome. In addition its great pathogenic potential, S. aureus is well recognized for its capability to get resistance to different antibiotics together with Methicillin. MRSA is among the most efficacious contemporary pathogens.

Our study has shown a higher rate of isolation from pus and wound swabs as compared to other swab samples collected from different mucocutaneous sites. This finding was because many of the aspirated pus or wound samples were submitted by surgical ward and burn units. Generally, swabs are not appropriate because of the lesser quantity of specimen. Moreover, these are frequently contaminated with normal skin flora, thus making reading of results problematic. Mixed growths are perplexing to interpret even by the Microbiologist.The microbiologist would report mixed growth with dubious significance and usually requests for a repeat specimen if clinically so warranted. It is essential to emphasize that shallow pus swabs are of low quality compared to aspirated pus collections. It is, however, recommended that when collecting specimens using swabs, the innermost material of the lesion should be collected, avoiding the contamination with superficial microflora.[17]

In the present study the most frequently isolated pathogen was S. aureus. This observation is in conformity with the finding of many analogous studies done in different other regions[18, 19,20,21,22]. The overall frequency of infections caused by S. aureus in our study varied in different age groups and genders. Although the overall isolation rate of S. aureus was slightly higher in males, however, its frequency was relatively higher in young women of reproductive age. These variations could be explained in relations with more physical activities by boys and adolescents, adiposity, reproductive life periods in women, hygienic practice and differences in socio-cultural, trade and working activities of the population.[23, 24]

Various studies have described differences in the prevalence rates of MRSA in diverse parts of the globe.[25, 26, 27] The frequency of MRSA in our study was found to be 46.9 %. Similar studies conducted in our neighboring countries and within Pakistan have also shown comparable results.[28, 29, 30] A report from Rawalpindi region has documented 44% MRSA prevalence .[31] Another study conducted in healthcare setups of Karachi has claimed MRSA rate as 52%[11], whereas a study conducted in Rahim Yar Khan has revealed MRSA as high as 66%.[32] The data on antibiotic resistance from several healthcare centers and medical laboratories is accessible on a website called Pakistan Antibiotic Resistance Network, abbreviated as PARN .[33]

MRSA poses a challenging clinical threat, with steadily high morbidity and mortality because it is also resistant to other beta-lactam antibiotics as well as often resistant to other groups of antimicrobials agents including aminoglycosides, macrolides and quinolones. Resistance to other antimicrobials including vancomycin and teicoplanin has also been recognized[34], thus making MRSA as multidrug-resistant bacterium. The spread of MRSA carries an enormous risk to the patients as well as to the community in term of extraordinary monetary losses. Over the years, we have witnessed a gradual rise of resistance to antibiotics that are currently available. Anti-staphylococcal treatment failure has been associated with reduced susceptibility to vancomycin. This is because of irrational use of such antibiotics in response of wrong diagnoses or incorrect monitoring of the under-line cause of the infection. Luckily we have not reported vancomycin-resistant staphylococci so far. Besides vancomycin at least two most effective drugs namely linezolid and teicoplanin were recognized against MRSA in our healthcare setting. Yet there is a need for proper identification of the pathogen and right treatment using effective antimicrobial agents. Appropriate use of vancomycin could be a therapy to control resistance of Staphylococcus to this drug.

Conclusion

The frequency of MRSA in our healthcare facility is quite high. Besides vancomycin, linezolid and teicoplanin are the alternatives for treating such infections. However, erythromycin and ciprofloxacin are found to be least effective. The rising trend of MRSA impresses upon the dire need of stringent infection control practices, appropriate use of antibiotics and a continuous surveillance program for MRSA.

Limitation and Future Recommendations

Because of the lesser sample collection, the results of this study may not be illustrative of the whole populace of the region. In order to elucidate the actual resistance pattern in our community, we recommend performing a larger-scale investigation.

Conflicts of Interest: None declared.

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