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ANTIBIOTIC SUSCEPTIBILITY PATTERN OF METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM SKIN AND SOFT TISSUES FROM PAKISTAN

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ABSTRACT

Class of microbes that invades and causes inflammation of various tissues including dermis, subcutaneous tissues and epidermis tissues are the main reason of infections of skin. SSTIs can be categorized as complicated, non-complicated, purulent, and non-purulent on the evidence of clinical symptoms and severity. *S. aureus* is the of the leading cause of skin infections. One of the major causes of Hospital acquires and Community acquired skin infections is MRSA due to its resistance to lactam antibiotics. Establishment of antimicrobial activity of different bacterial isolates obtained from skin and pus swabs. 173 isolates were analyzed for bacterial group and antimicrobial activity was determined. Amplification of *mecA* gene was performed by Polymerase chain reaction. Out of 173 samples, 37 were MRSA isolates of which 29 (78.3%) were resistant to Amikacin and 33(89.1%) were sensitive to Linezolid. MRSA has become an emerging pathogen in skin infections from mild to severe due to its resistance to beta-lactam antibiotics. The study demonstrates the distribution of different bacterial isolates in the infections of skin and soft tissues. *S. aureus* is a concern and immediate biosecurity measures are recommended to limit its spread within hospital settings.

Keywords: *Staphylococcus aureus*, methicillin resistant. Skin and soft tissues

INTRODUCTION:

The body's entire surface is covered in skin. Being the largest body's organ, skin acts as the tough and flexible barrier and protects body from harmful invasions **[1]**. The skin is an important first line of defense that protects body from bacterial pathogens such as those in external environment and opportunistic skin microbes The skin is populated with microbial flora. The major symptoms of infectious skin diseases may vary based on the type of pathogen involved, the layers of skin, the affected structures, and the condition of the patient SSTIs range vary from moderate to severe and clinical symptoms represent etiology and pathogenic invasion **[2]**. Microbes that invade and cause inflammation of various tissues including dermis, subcutaneous tissues and epidermis tissues are the main reason of inflammation of skin **[1]**. SSTIs are classified into many categories including simple, complicated, suppurative or nonsuppurative. SSTIs can be of many types including mild, moderate, purulent, non-purulent. It has been reported that the trauma-related infections, ecthyma folliculitis, erysipelas, abscesses, carbuncles, and cellulitis all are the types of common simple SSTIs **[3]**. Other infection cases such as deep tissue infections, perirectal abscesses and burns all comes under the classification of the complicated infections **[4]**. MRSA may infect the skin, mucous membranes, serous membranes, internal organs, and mammary glands of humans as well as other animals (cattle, birds, horses, dogs, pigs, and cats), causing serious illness that is primarily connected to multiple treatment resistance **[5]**. Methicillin was launched as a consequence of the expansion of *S. aureus* strains that shows resistant towards the penicillin as a consequence of the development of a β-lactamase because it was not broken down by β-lactamases, right after the isolation of the methicillin-resistant strain **[6]**. MRSA strains are inherently harmful due to their widespread β-lactam resistance, which is typically linked to the other classes' resistance. Methicillin is resistant due to to *mecA* gene which codes a protein penicillin binding protein. *mecA* belongs to SCCmec **[7]**.

MATERIAL AND METHODS

2.1 Research Design

A cross-sectional study was conducted from March to September 2022.

2.2 Study site and Specimens

All the specimens received at the Microbiology laboratory of Tertiary care hospital of Pakistan following physicians order for investigative procedure were included in study. The samples were collected as per standard microbiological techniques. Samples obtained in clean, leak-proof and screw-capped container with no

visible signs of contamination and labeled properly with demographic information of the patients were included in the study. Molecular analysis of samples was carried out at Macrogen.

2.3 Samples for the Study

A total of 174 different clinical specimens including pus, wound swab and ear swab were collected at Microbiology laboratory of the hospital and processed as per standard microbiological protocol for routine culture and antibiotic susceptibility testing.

2.4 Isolation and Identification of S. aureus

All the samples were inoculated directly onto blood agar and MacConkey agar. *S. aureus* colonies were identified based on colony characteristics. Golden-yellow colored, convex, opaque colonies were indicative of *S. aureus*. Furthermore, confirmation was done by biochemical testes including catalase, coagulase, DNase tests.

2.5 Antibiotic Susceptibility Test

Antibiotic susceptibility tests of all the isolates towards various antibiotics were performed by Kirby-Bauer disc diffusion method as recommended by Clinical Laboratory of Tertiary care hospital. The antibiotic discs tetracycline, ciprofloxacin, gentamicin, clindamycin, Amoxicillin, Amikacin, Doxycycline, Linezolid, cotrimoxazole, erythromycin and cefoxitin were placed. The plates were incubated at 37 °C overnight. After incubation, the zone diameter organism was reported as resistant, intermediate or sensitive. Based on susceptibility pattern of Isolates, bacteria resistant to three or more than three classes of antibiotics were considered to be multidrug resistant (MDR).

2.6 Detection of mecA Gene by Polymerase Chain Reaction (PCR)

DNA of MRSA was isolated by using phenol-chloroform extraction method. The forward primer 5'-ACTGCTATCCACCCTCAAAC-3'and reverse primer 5'-CTGGTGAAGTTGTAATCTGG-3' were used for amplification of mecA gene. The amplification of *mecA* gene is been done by using above mention forward and reverse primers.25microlitre of reaction mixture is used which consisted of 21microlitre of Qiuagen reaction mix, including (0.5microlitres of 10pmole) of both forward and reverse primers and DNA template (3microlitre). 35 cycles were run at different temperature profiles. Denaturation at 94°C for one minute and annealing at 55 degrees Celsius for one minute and extension will also be done for 1minute at 72°C temperature and the final extension will be done for 7minutes at also 72°C temperature. The PCR amplification products were separated by electrophoresis through 2.5% agarose gel and visualized by staining with ethidium bromide. The 155 bp amplicon was detected against 1kb DNA ladder.

2.7 Data Analysis

All the data were analyzed for comparison of antibiotic susceptibility, *mecA* gene was done for MRSA.

3. RESULTS

3.1 Growth pattern and Distribution of Bacteria

Out of 173 samples processed growth was observed. The highest growth was observed in pus was 51%., 40.5% wound swab and 81% of ear swab as shown in **Figure 1**.

Total n=173 bacterial pathogens were identified that caused skin and soft tissue infections, which include (9.8%) *S. aureus*, 11% Coagulase negative *Staphylococcus aureus*, 21% Methicillin resistant *Staphylococcus aureus*, 11% *E. coli*, 17% *Acinetobacter spp*, 3.4% *Proteus spp*, 16% *Pseudomonas aeruginosa* and 11% *Kliebsella Pneumoniae* as shown in **Figure 2**.

3.2 Distribution of MRSA in Different Clinical Samples and Antibiotic Susceptibility Patterns

Among 173 samples 21% (37) were MRSA. Higher number of isolates were found in pus.

Out of 173 samples antimicrobial susceptibility profile of MRSA showed 78.3% resistance to Amikacin, 89.3% to Amoxicillin, 75.5% to cefactor while 8.1% were sensitive to these three. It showed 75.6% resistance to cefoxitin while 2.7% sensitive, 86.4% resistance to cefuroxime, 81% to clarithromycin while 10.8% sensitive to these two. 29.7% resistance to cotrimoxazole and 37.8% sensitive, 27% to doxycycline and 56.7% sensitive, 35% resistant to clindamycin and 67.5% sensitive, 54% resistance to gentamicin and levofloxacin while 27% and 13.5% sensitive, 48.6% resistance to Tetracycline and oxacillin while 27% sensitive to tetracycline, 67.5% resistance to ciprofloxacin and 5.4% sensitive, 2.7% resistance to ceftazidime and 89.1% sensitive to linezolid as shown in **Figure 3**.

3.3 Detection of mecA Gene in MRSA

Isolates of MRSA were selected for PCR. Among 37 isolates sample 1770 was analyzed which possessed *mecA* gene detected in 155 bp as shown in **Figure 4**.

3.4 Determination of MAR (Multiple Antibiotic Resistance) Index

Multiple antibiotic resistance (MAR) index was determined for each isolate by using the formula MAR = a/b, where a represents the number of antibiotics to which the test isolate depicted resistance and b represents the total number of antibiotics to which the test isolate has been evaluated for susceptibility **(Table 1)**.

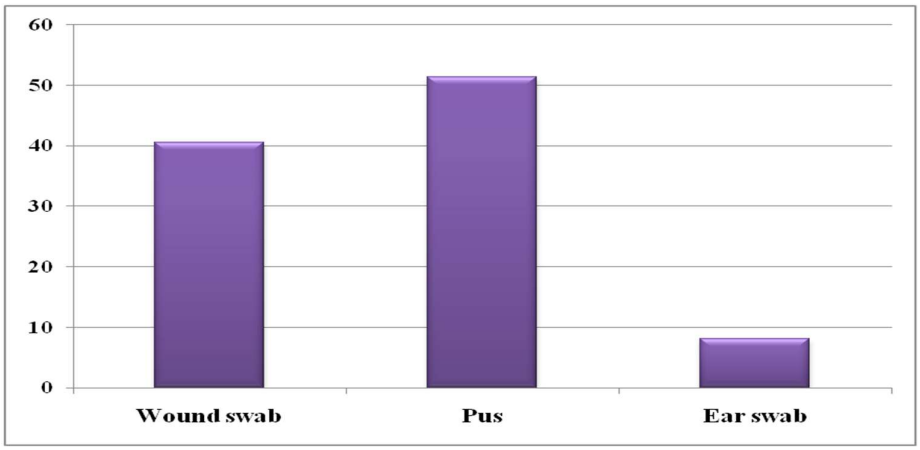

Figure 1: Graph showing distribution of Methicillin resistant *S. aureus*

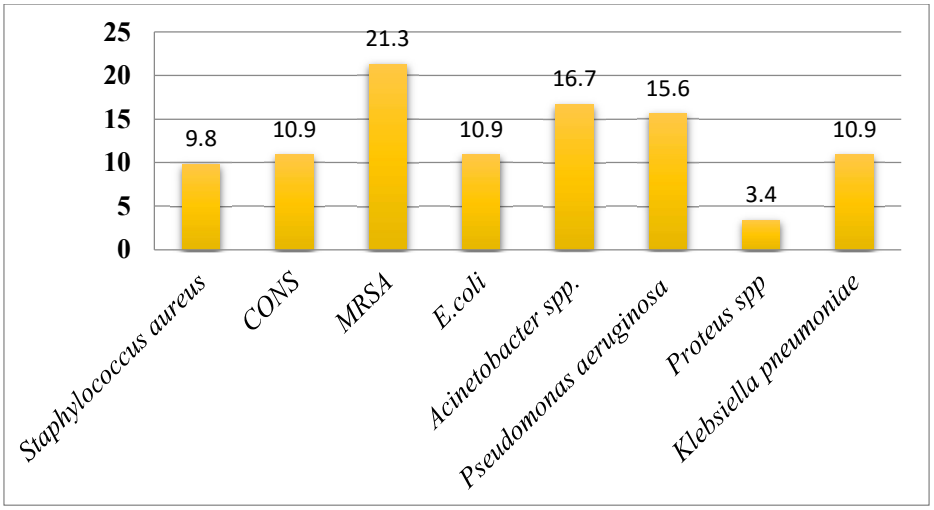

Figure 2: Graph showing bacterial strains isolated from skin infections

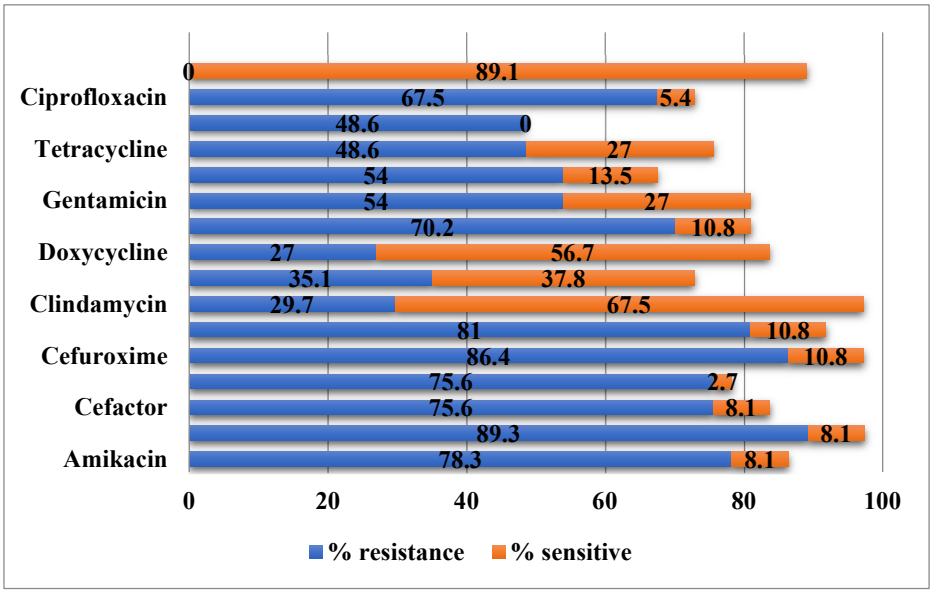

Figure 3: Graph representing Antibiotic susceptibility pattern of MRSA(n=37)

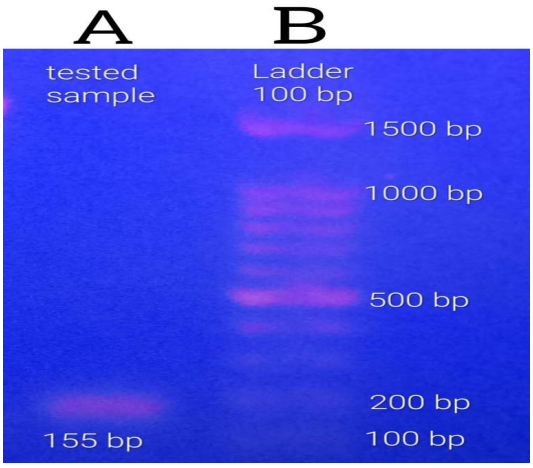

Figure 4: Gel showing PCR results of sequencing of *mecA* gene Lane 1 to 4 showed the amplified product of using gene specific primers of *mecA* gene on MRSA DNA, Plus DNA ladder range from 100 to 10000 bp. Thermo-scientific Gene ruler DNA ladder was used

Table 1: MAR indices of MRSA species (n=37)

MAR Index	Number (%)
0.02	1(2.7)
0.1	0
0.2	1(2.7)
0.3	1(2.7)
0.4	2(5.4)
0.5	2(5.4)
0.6	2(5.4)
0.7	2(5.4)
0.8	3(8.1)
0.9	0
1.0	0

DISCUSSION

The current studies states that one of the important causative agents of skin infections is *Staphylococcus aureus.* Pathogen invasion results in infections of the Skin and soft tissue infections, which can range from moderate to severe **[8]**. Skin infections can be categorized as complicated and non-complicated according to the severity of the infection **[9]**. Skin infections include cellulitis, folliculitis, erysipelas, impetigo, carbuncles, furuncles, and many more. One of the most crucial causes of skin infection is MRSA, in many previous studies including community-acquired and hospital-acquired **[5]**.

From a clinical perspective, antibiotic resistance is a significant issue that doctors must deal with because overdosage of antibiotics confers resistance. By producing penicillin-binding protein MRSA exhibits resistance towards many antibiotics including all beta-lactam antibiotics. Resistance to beta-lactam antibiotics occurs by different mechanisms either due to enzyme hydrolyzing or due to the *mecA* gene. The resistance against methicillin is because of the presence of PBP2a which is encoded by Methicillin resistance gene

called *mecA* gene. The inhibition of bacterial cell wall is caused by beta lactam antibiotics **[10]**.

Many studies have reported that the cause of infections in skin and soft tissues is *Staphylococcus aureus*. Some studies reported that majority of the skin infections can be treated with medication but nowadays skin infections can be so critical that they can take the patient to ICU. It has been reported in many studies that the infections of soft tissues and skin can be cured with intravenous antibiotics and among 1033 patients 73.5% were cured **[11]**. The prevalence of infections of Community acquired and Hospital acquired skin and soft tissues caused by MRSA are increasing. MRSA caused most of the diabetic foot infections.

In our study 173 culture plates were screened for bacterial growth. Among 173 samples 42% were gram-positive species and 57.5% were gram-negative species. Number of infections caused by the gram-negative studies in previous studies is higher than the infection caused by the gram-positive species. In the given study the high number of infections are reported is of MRSA (21.3%). These results suggest that the gram-negative bacteria are now one of the leading cause of infections of skin. *Acinetobacter* is the most important, in our study *Acinetobacter* is also predominant **[12]**.

Among 37 MRSA isolates, all contained the *mecA* gene. Mutations in bacterial genome are the main cause of antibiotic susceptibility. In our study, the highest percentage of MRSA was resistant to Amoxicillin similar to the previous study by **[13]**.

Among 37 MRSA isolates, sample 1770 was selected for PCR which possessed the *mecA* gene viewed on gel electrophoresis. The tested sample had a size of 155kb.

In our study, we determined the MAR Index which is multiple antibiotic resistance in which the total percentage of the isolates that having MAR index more than 0.2 was 76.34% whereas in our study percentage of isolated that has MAR index more than 0.2 was 35.1% this difference might be due to difference in sample number (102).

In our study out of 37 samples of MRSA 33(89.3%) were resistant to Amoxicillin where as in another study 31.1% were resistant to Amoxicillin. In a previous study, greater than 90% of *Staphylococcus aureus* species were susceptible to Linezolid similar to our study in which 88.2% were susceptible to Linezolid.

Acenitobacter baumannii was isolated from 43 of 350 samples in a prior investigation. 53.5% of the isolates had antimicrobial resistance to amikacin, 83.7% to tetracycline, 86% to ceftazidime, 90.7% to trimethoprim-sulfamethoxazole, and 93% to imipenem, cefepime, meropenem, and

ampicillin-sulbactam. All of the isolates were ciprofloxacin resistant, although only 29 of the 173 samples from our investigation were Acinetobacter. According to antimicrobial resistance patterns of isolates, 44.8% were Doxycycline-resistant, 24.1% were Amikacin- and Gentamicin-resistant, 6.8% were tetracycline-resistant, and 58.6% were Ciprofloxacin-resistant **[14]**.

According to a study, CoNS had the following levels of antibiotic resistance. Amoxicillin (74.8%), Oxacillin (70.3%), Ceftriaxone (30.4%), Ciprofloxacin (35.2%), Amoxicillin+clavulanate (32.8%), Ofloxacin (33.6%), Erythromycin (58.3%), Clindamycin (16.3%), Daptomycin (42.5%), Kanamycin (52.2%), Fusidic acid (41.7%), Doxycycline (24.7%), Vancomycin (2.6%) (15).

CONCLUSIONS

Skin and soft tissue infections are predominantly increasing. *S. aureus* is one of the major causes of Community acquired and Hospital acquired skin diseases. Skin infection can be classified into many different categories according to different perspectives. The reason is methicillin, which confers resistance by encoding penicillin-binding protein which stops the formation of bacterial cell wall. This study concluded 21.3% prevalence of methicillin resistant *Staphylococcus aureus* in different clinical specimens mainly pus specimens, collected from a tertiary care hospital of Lahore. Methicillin resistant *Staphylococcus aureus* isolates showed resistance against amoxicillin and sensitivity towards clindamycin. *mecA* gene presence was detected by polymerase chain reaction and the NCBI database was used to predict the sequence and compared to the other strains. MAR indices which are also named as multi antibiotic resistance index is also calculated of methicillin resistant *Staphylococcus aureus* samples. The proportion of isolates that have MAR index more than 0.2 is 35%. Isolates with MAR index of 0.02 is appear to be only 1%.

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