

## The Antibiotic Resistance of *Staphylococcus Aureus* Isolated from Diabetic Foot Ulcers in a Tertiary Care Hospital

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### Abstract

The antibiotic resistance risk is expanding across the world and treating bacterial infections has become very challenging. The present study aims to identify the antibiotic sensitivity pattern of *Staphylococcus aureus* which is isolated from foot ulcers occurring due to diabetics, and to discover whether they have antibiotic resistance which is chromosomal or plasmid trait. For 89 different isolates antibiotic sensitivity test was conducted. MRSA isolates were discovered to be resistant to antibiotics such as vancomycin (0%), cloxacillin (17.9%), gentamycin (20.68%), amikacin (10.2%), chloramphenicol (23.1%) and  $\beta$ -lactam antibiotics like cefoxitin (100%) and penicillin (100%). Then followed by antibiotic sensitivity tests plasmid curing was done, and the outcomes were compared with those obtained before plasmid curing. The outcomes demonstrated that the *S.aureus* resistance towards Amoxicillin, Amoxycylav, and Cefotaxime was plasmid mediated whereas its resistance shown to Ampicillin and Ciprofloxacin was chromosome mediated one.

**Key words** - *Staphylococcus aureus*, antibiotic resistance, Plasmid curing.

### Introduction

Foot ulcers are common trouble occurring in patients suffering from diabetics. Its occurrence percentage varies among 15% to 25% [1]. There may be complications such as increased infection occurring which may lead to mortality and morbidity varies from (40%–80%) [2]. It is also considered as the most common cause of lower-limb amputations [3]. The

pathophysiology of foot infection caused due to diabetics (DFI) is extremely complicated. The host-related processes (e.g., immunopathy, neuropathy and arteriopathy) and pathogen-related factors (e.g., virulence, antibiotic-resistance, and microbial organization) determines the occurrence and seriousness of the illness [4].

The key reason for lower-limb amputations are the infections occurring in the foot ulcers and the distribution of the infection caused to the soft tissues and to bony structures[5]. Microorganisms are always existing on wounds present in the skin, hence early identification and appropriate therapies are important. As highlighted by the Infectious Diseases Society of America and the International Working Group on the Diabetic Foot (IWGDF) and more recently by the French Society for Infectious Pathology, diagnosis of infection must be on medical aspects but based on microbiological outcomes [6]. However, Diagnosis of foot infection at an early phase in diabetic patients may be challenging, because of the confusing impact of neuropathy and ischemia on local and systemic inflammatory reaction.

In spite of the tendency to produce high rate of mortality, mobility, *S. aureus* occurs as one among the microbiota of nearly one quarter of the total amount of the population. The individuals in who the colonization occurs they are highly expected to develop *S. aureus* mediated illness. To establish *S. aureus* as a part of microbiota of a specific niche, it must interact through certain mechanisms or compared with the other resident flora in the colonized host. This is definitely a difficult process. The microbiota data which are present in enormous amount but it is not sufficient to increase the understanding of the mechanism happening in the molecular level which causes the dense communication among the commensals and the pathogens which are invading like *S. aureus*. There are many proofs which demonstrate that the pathogens are blocked from invading and colonizing in the wounds [7].

Multidrug resistant *Staphylococcus aureus* (MRSA) strains which have been proved resistant to antibiotics such as methicillin and also semi synthetic anti-staphylococcal penicillin such as oxacillin, dicloxacillin, and nafcillin [8]. Often, these are also resistant to beta-lactam antimicrobial agent, which doesn't involve anti-MRSA cephalosporins such as ceftaroline and ceftobiprole. Among the individuals suffering from diabetic foot infection, one of the severe difficulty is the infection caused by MRSA strains [9]. Prolonged hospitalization and high cost of the one's health may be the result of MRSA infection in patients with diabetic foot, the

occurrence of the *S. aureus* strain which acts as the one of the cause for the occurrence of diabetic foot infection and the pattern of the antibiotic resistant of the *S. aureus* strain among the group of individuals suffering from diabetic foot ulcers from South India is the key aim of this study.

## **Materials and Methods**

### **Sample collection and processing**

A potential analysis of individuals suffering from diabetic foot ulcers was conducted from 1 June 2020 to 31 November 2020 was carried out in a private hospital. After superficial pre cleansing of wounds with physiological saline, with the help of a sterile, premoistened swab (Nuova Aptaca SRL, Canelli, Italy) each specimen was collected by rotating the swab across the surface of the wound area of 1 cm in a zig zag way, from middle towards the outer layer of the wound. Then, in a testube containing transport medium (NuovaAptaca SRL) the swab consisting the specimen was kept and it was sent to the Microbiology laboratory in the hospital for further diagnosis of the culture [10].

### **Bacterial isolation**

By swabbing the wound, needle aspiration or tissue biopsies the bacterial culture sample were acquired after the wound debridement. These samples were immediately sent to the departments of bacteriology. The sample which resulted positive for the *S. aureus* in the patients was involved in the analysis. For duration of half a year the individuals with grade 1 ulcers were monitored closely to check with the wound status (infected/non-infected ulcer). A microbial sample was taken after 1 month if the wound is completely healed. The antibiotic treatment was started if the wound status. Surgery or minor amputation was done. Before hand the antibiotic therapy was initiated the specimen and the grade of the ulcer was also kept in clean observation [11].

### **Microbiological examination and characterization**

The taxonomical classification such as Genus, species and susceptibility towards the antibiotics were analysed and inferred according to the previous reports. By using agar diffusion method the susceptibility test for methicillin was tested using cefoxitin disks [12].

### **Antibiotic susceptibility testing**

For a 15 total strains of *Staphylococcus aureus* the antibiotic susceptibility test towards a different antibiotics were performed by agar disc diffusion method. These were inoculated in nutrient broth and incubated at 37°C for a day. Then the sample (approx. 0.1 mL) was spread in surface of MHA plates using spread plate technique which is allowed to dry completely. The antibiotic discs (erythromycin (15 mcg), gentamycin (120 mcg), oxacillin (5 mcg), penicillin-G (10 units/disk), vancomycin (30 mcg), ciprofloxacin (10 mcg), streptomycin (10 mcg), chloramphenicol (30 mcg) and kanamycin (30 mcg)) were kept on the surface of the agar (MHA) and incubated at 37°C temperature for a day. By measuring the zone size the pattern of Antibigram was obtained and determined with standards of NCCLS [13].

### **Isolation and electrophoretic pattern of plasmid DNA**

By alkaline lysis method the isolation of plasmid was done. The gel electrophoresis of plasmid DNA for multiple drug resistant *Staphylococcus aureus* (MRSA) of 10 different strains was performed according to the standardized procedure. A horizontal gel electrophoresis was conducted after the isolation of plasmid DNA, by following the method which was demonstrated by Meyers *et al.* As the standard marker for determining the molecular mass; *E. coli* PDK-9 strain was utilized [14].

### **Curing of the plasmid**

In TSB which consists of acridine orange (dye) all of the  $\beta$ -lactamase positive strains were incubated at temperature of 37°C for a day. The medium containing 0.3% yeast extract, 3% of TSB and 1.5% agar-agar was prepared where the strains were streaked onto this medium after incubation. By using iodometric methods the strains were analysed for the  $\beta$ -lactamase activity one day after incubation. A technique was used for the plasmid curing. Three different types of curing agents, ethidium bromide (75  $\mu$ g/ml, 100  $\mu$ g/ml and 125  $\mu$ g/ml), acridine orange (50  $\mu$ g/ml, 75  $\mu$ g/ml, and 100  $\mu$ g/ml), and sodium dodecyl sulphate (8 %, 10 %, and 12 %) each in three different amount was used for plasmid curing [15].

The antibiotic susceptibility analysis for the plasmid cured isolates were conducted on Muller hinton agar plates, in the presence of antibiotic penicillin (1%) and in absence of penicillin. Incubated for 2 days at temperature 37°C and monitored the growth in the plates and also the

susceptibility analysis was done with the antibiotics which indicated resistance formerly such as vancomycin, erythromycin, streptomycin, kanamycin, and chloramphenicol. The profiling of the plasmid was done and monitored for the occurrence of the plasmid.

## Results

There were 89 various bacterial isolates in 47 wound secretions obtained from ulcers of the foot caused due to diabetics which were resulted 100% positive. The frequent bacterial strain obtained from 22 samples (46.8%) was *S. aureus* (66.7%). *S. aureus* was most frequently occurring organism with 32 different strains, followed by Gram negative rod from the Enterobacteriaceae family (*Pseudomonas aeruginosa* – 12 strains, *Escherichia coli* – 12 strains, *Enterobacter aerogenes* – 11 strains, and *Alcaligenes faecalis* – 9 strains) in the study done in foot ulcers caused by diabetics. As already proven in other studies *Pseudomonas aeruginosa* was the gram negative isolate frequently occurring one. There are many harmful virulence factors produced by *S. aureus* and *P. aeruginosa* which is accountable for keeping the infection in progress and postponing the curing persistent wounds. *S. aureus* produced medically significant infections which is the outcome of activities of virulence factors such as coagulase, catalase, clumping factor A and leucocidines. *Pseudomonas aeruginosa* produces a virulence factors known as elastase which also results in causing infections in the surrounding of the wound. Thus the outcome of our study confirms the most widespread microorganisms identified in the wounds which are infected.

## The antibiotic resistance pattern and plasmid curing

The antimicrobial resistance (AMR) patterns of MRSA and MSSA strains counter to agents containing antimicrobial activity are summarized in table 4. 25% and more MRSA strains were resistant to agents such as ampicillin, ciprofloxacin, cotrimoxazole, erythromycin, clindamycin, azithromycin and tetracycline and small amount of them were identified to be resistant to vancomycin (0%), cloxacillin (17.9%), gentamycin (20.68%) amikacin (10.2%) and chloramphenicol (23.1%). Cefoxitin and penicillin are  $\beta$ -lactum antibiotics which were observed to be not working against MSSA too. The MSSA strains exhibited resistance against antibiotic such as ampicillin (69.2%) and ciprofloxacin (84.2%), the other antibiotic agents exhibited 50% and less resistance to the MSSA strains.

Antibiotic susceptibility pattern of *S. aureus* regarding macrolide lincosamidestreptogramin B, the induction of macrolide lincosamidestreptogramin B (MLSB) in the 52 *S. aureus* strains which constitutes MLSB, MSB and susceptibility was observed in 8 (15.4 %), 7 (13.5%), 17 (32.7%) and 20 (38.5%), respectively. 15.4% (n = 6/39) strains had inducible MLSB resistance out of 39 isolated MRSA strains in 12.8% (n = 5/39) constitutive MLSB was noticed, 38.5 % (n = 15/39) demonstrated resistance against MSB and 33.3% (n = 13/39) was susceptible to erythromycin and clindamycin. 38.5% (n = 5/13) isolates showed susceptibility to both erythromycin and clindamycin out of 13 MSSA strains; Inducible and constitutive MLSB resistance was shown by 2 (15.4 %) strains and 4 (30.8%) strains showed resistances to MSB majority of the MSSA strains showed sensitivity to both erythromycin and clindamycin. The results shows that the constitutive and inducible resistance was identified greatly in isolated MRSA strains in comparison with MSSA strains (Table 5). The ICR and D-test-positive *S. aureus* is displayed in Figure.

With the help of 100 µg/ml and 125 µg/ml ethidium bromide (Table 1), 10 % (w/v) SDS and 50 µg/ml and 75 µg/ml acridine orange the plasmid cured cells were attained, the occurrence of the colonies with cured plasmids were minimal. The most effective, curing agent among the three is demonstrated in the outcomes. The rate of occurrence of the cured cells were 5.55 % (with 50 µg/ml) and 11.76 % (with 75 µg/ml) for acridine orange, 21.05 % (with 100 µg/ml), 17.65 % (with 125 µg/ml) for ethidium bromide and 7.4 % (with 10 % w/v) and 6.67 % (with 10 % w/v) for sodium dodecyl sulfate. These were nil cured cells which resulted when treated with 100 µg/ml acridine orange, 75 µg/ml ethidium bromide and 8 and 12 % SDS. After plasmid curing, our results revealed that strains of *S. aureus* showed resistance to Amoxicillin, Amoxycylav, and Cefotaxime was plasmid mediated one and its resistance towards Ampicillin and Ciprofloxacin was chromosome mediated one. The previous study demonstrates that there was 100% resistance to Ciprofloxacin and 50% to Ampicillin by *S. aureus* strain isolated in patients suffering from otitis media. The study demonstrates that maximum of the resistance shown by isolated staphylococci strains present in wounds and burns against many antibiotics like ciprofloxacin was plasmid mediated. In addition, the appearance and transfer of the genes which are plasmid-borne and resistant in nature are predictable to undergo horizontal gene transfer have endangered most of the end time antibiotic treatments, which is inclusive of quinolones such as Ciprofloxacin.

## Conclusion

The isolates of *S. aureus* strains obtained from the wounds of foot ulcers caused by diabetics is observed to be resistant against multiple drugs which is plasmid mediated one, is revealed in this study. The plasmid encoded resistance towards antibiotic agents is a global challenge, since it spreads from one patient to the other by horizontal transfer hence, therapy for these opportunistic pathogens which causes infection turns as hard challenge. One of the most promising drug vancomycin which is prescribed in all the infection caused by *Staphylococcal* infection turns out to be inefficient because of the emergence of resistant strains, creating the infection to be entirely incurable one. Knowing the widespread type of microbe which occurs in the wounds which are affected by pathogens and the resistance pattern observed is obviously important to pick out the appropriate therapy. To cure the foot ulcers and to enhance the control the effect of pathogen in the wounds, the information's demonstrated in this study along with the discussion can be utilized.

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