Detection of Methicillin resistance of Staphylococcus aureus among Minia University Hospital Patients

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Abstract
The increasing prevalence of methicillin resistant *Staphylococcus aureus* represents a significant threat to public health mainly due to antibiotics abuse. This study aimed to detection prevalence of resistant isolates of *S. aureus* to different antimicrobials from plastic surgery department. Methods: In this study, incidence of *Staphylococcus aureus* infection in Minia University hospital was determined by testing a random sample of 52 patients from wounds samples of plastic surgery department. Then, the disc diffusion method was used to determine the patterns of antimicrobial resistance and efficacy of commercial antibiotics in treatment of *Staphylococcus aureus* infection. Results: Prevalence of *Staphylococcus aureus* infection was 42.6% among our patients. Antibiotic resistance rates were alarmingly high, up to 82% in oxacillin, 77% in cefotriaxone, and 54% in amikacin and 50% in cefoxitin. Discussion: This Study showed unprecedented antimicrobial resistance rates of *Staphylococcus aureus*. New alternative treatment regimens should be considered and investigated to replace the current ineffective therapies.

Keywords: *Staphylococcus aureus*, public health, antimicrobials

Introduction
*Staphylococcus aureus* (*S. aureus*) is a facultative anaerobic Gram-positive pathogen that has been recognized as an important cause of human disease for more than 100 years[1]. Almost 30% of the human population is asymptomatically colonized with commensal *S. aureus* and it is one of the most common nosocomial and community-acquired infections[2, 3].

*S. aureus* causes many diseases including pneumonia, mastitis, meningitis, wound infections, sepsis, abscess formation, osteomyelitis, endocarditis, toxic shock syndrome (TSST) and food poisoning [4]. Resistance against methicillin, lincomamides, macrolides, aminoglycosides and a combination of these antibiotics have been frequently reported in staphylococci[5].

Penicillin was considered the drug of choice for *S. aureus* infections until *S. aureus* developed penicillin resistance by production of beta-lactamase[6].

In 1961, in an attempt to combat emerged penicillin resistant *S. aureus* (PRSA) isolates, methicillin was introduced. But within 1 year of its clinical use, first Methicillin resistant *S. aureus* (MRSA) strains were reported[7, 8].

Methicillin resistance is generally caused by *mec* A gene that encodes polypeptide penicillin binding protein (PBP2a), the enzyme that is responsible for crosslinking the peptidoglycans of bacterial cell wall. It has a low affinity for β-lactams, thereby leading to resistance to this category of antibiotics [9]. Glycopeptides, especially vancomycin, are generally used against methicillin-resistant *S. aureus* (MRSA) since the last few decades of 20th century. Antibiotics abuse has been led to the emergence of vancomycin resistance *S. aureus* strains [10].

Material and Methods
Subjects
This study was conducted from April 2019 till December 2019. This study included 52 patients referred to outpatient of plastic surgery department of Minia University hospital. Samples were obtained from patients suffering from burn wounds with clinical symptoms and signs of burn wound infection. Full personal and medical histories were taken from the
patients including; gender, age, cause of admission and history of antimicrobial agents intake.

**Collection and transport of samples**
Wounds swabs samples were transferred by sterile cotton swabs containing transport media and endotracheal secretions transferred by sterile cups. All samples were transferred within 1 hour to Microbiology and Immunology Department, and processed as soon as possible.

**Isolation and identification of S. aureus**
Fifty two wounds swabs samples were cultured on mannitol salt agar (Oxoid, England) to isolate S. aureus isolates. Gram stained smears were prepared for all samples and isolated colonies. The slides were examined microscopically. Catalase test and coagulase test were used to differentiate between staphylococci and other Gram positive organisms. After full identification, S. aureus isolates were incubated in nutrient broth and then preserved on glycerol and then frozen at -20°C. Glycerol was supplied by Al-Nasr Company (Egypt).

**Antimicrobial susceptibility testing:**
Antibiotic susceptibility test was done by disc diffusion method to all isolates. Results interpretation was done according to CLSI guidelines 2019(11). The following antimicrobial discs were used: Amikacin (30μg), Oxacillin (1μg), Cefoxitin (30μg) and Cefotriaxone (30μg). All were from Oxoid company.

Using a sterile loop, 3-5 well isolated colonies from the pure culture of the organism were emulsified in sterile saline.
In a good light, the turbidity of the suspension was matched to the turbidity of 0.5 Mcfarland standards by visually comparing the inoculum tube and the 0.5 McFarland standards against a card with a white background and contrasting black lines. A sterile swab was dipped in the inoculum suspension; excess fluid was removed by squeezing the swab against the inner side of the tube and then rubbed over plate of Muller-Hinton (MH) agar in three different directions rotating the plate 60° to ensure even distribution. Using a sterile forceps, antibiotic disks were placed on the inoculated plate and adequate gap was left in between each disk. After overnight incubation, results were reported by measuring the zone of inhibition size in mm. Antimicrobial susceptibility results interpretation was done according to CLSI guidelines.

**Results**

**Demographic characteristics of the studied subjects:**
Our study included 29 men and 23 women with ages ranged from 1 year to 80 years (mean age 30 years). The percentage of patients that had history of antibiotics administration was 84%. The percentage of S. aureus isolates from total wounds samples was 42.6% (22/52).

**Antimicrobial susceptibility testing results:**
The MIC results were available for all isolates. The antimicrobial resistance rates were detected to all S. aureus isolates. The rates of resistance to oxacillin was 82% (18/22), to cefotriaxone was 77% (17/22), to amikacin was 54% (12/22) and to cefoxitin was 50% (11/22).

**Discussion**
Staphylococcus represents a broad genus of Gram-positive bacteria that colonizes on the skin and mucous membranes of humans and most mammals. S. aureus is the most problematic pathogen of the genus as it causes numerous acute and chronic infections12. S. aureus has evolved resistance mechanisms against antibiotics. Multi drugs resistant isolates developed even against last new therapeutics and resistance is associated with therapeutic failure and increased mortality13. The present study included 22 isolates of S. aureus from 52 patients who admitted to plastic surgery department of Minia University Hospital.

In this study prevalence of males was 62.93% (14/22) and females represented 37.07% (8/22) unlike results observed by Karki et al., that were 42.7% (47/110) males and 57.3% (63/110) were from female14. The highest resistance was to oxacillin, 82% (18/22). This result was less than results observed by Gitau et al who reported resistance 91.97% (867/944)15. Cefotriaxone had also high resistance percentage that reached up to 77% (17/22). This percentage is different from that obtained by Onwubiko et al., as it was 28.6% out of 150 S. aureus isolates16. Resistance to amikacin was 54% (12/22) and to Cefoxitin was 50% (11/22) while in 2020, Kot et al., found that amikacin
resistant percentage was 14.2% out of 112 isolates[17].

The percentage of antibiotics expression genes and resistance rates of antibiotics in this study were different. It may be probably due to to be multifactorial reasons such as marked sociodemographic differences between patient population, antibiotic exposure, differences in the hospital environment as well as in infection prevention and control (IPC) practices drug abuse and standard precautions excution.

References