Antibiotic Resistance of *Staphylococcus aureus* isolated from Diabetic, Surgical, and Gunshot wound types in patients in Teaching Hospital and Polyclinics in Zawia city, Libya

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Abstract

The wound is a break in the skin that exposes the underlying tissues to the outside environment. Loss of skin integrity by wounding provides a moist, warm, and nutritious environment for microbial colonization, proliferation, and infection. Common bacterial skin infections include Staphylococcus aureus (S. aureus). It is a leading cause of

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nosocomial infections (NI) and surgical wound infections; also, the S. aureus developed resistance to many antibiotics in recent years

Objective: *the main aim of this study is to study the prevalence of staphylococcus aureus and antimicrobial resistant of it in surgical site infections at both teaching Hospital and polyclinics in Zawia city.*

Methods: *A cross-sectional study was conducted among 30 patients at teaching hospital and polyclinics in Zawia city from February to March fifteenth 2020. Wound swab samples were collected aseptically using sterile cotton swabs and transported to Alfa Laboratories by using transport media. Isolation of Staphylococcus aureus was done based on cultural and biochemical profiles. Drug susceptibility tests were performed using the disc diffusion technique as per the standard and interpreted based on the Clinical and Laboratory Standards Institute guidelines.*

Results: *The S. aureus infections among the studied groups were 33.3% for the shotgun shot group, 63.6% for the surgical wounds group, and 56.6% for the diabetes foot group.*

The S. aureus resistance for antibiotics was 100% for ampicillin and methicillin, 88% for cifoxitin, and 12% for clindamycin.

Some of the infected samples contain S. aureus that is resistant to 6 out of 8 antibiotics whereas some others were only resistant to 2 out of 8 antibiotics.

Conclusion: *Out of 30 patients suspected of wound infections, 17 cases (56.67%) were infected with S. aureus. Most of these samples were 100% MRSA resistant and showed multiple drug resistance. These findings suggest that new strategies for antibiotic usage are needed in order to reduce antibiotic resistance in bacteria.*

Keywords: *Staphylococcus aureus, wound infection, antibiotic resistance, Zawia Educational Hospital, Zawia Cluster Clinics.*

1. Introduction

Staphylococcus aureus (S. aureus) is a **Gram-positive round-shaped bacterium**. It is a member of the Firmicutes phylum; and is a usual member of the microbiota of the body[1]. It is frequently found in the

upper respiratory tract and on the skin. Direct contact with an infected person, using contaminated objects, or inhaling infected droplets dispersed by sneezing or coughing are the most common ways for the bacteria to spread within the population. The bacteria can also spread through contaminated objects (such as gym equipment, telephones, doorknobs, television remote controls, or elevator buttons).

Skin infections are the most common S. aureus infections even though the bacteria can spread through the bloodstream and infect distant organs. S. aureus skin infections may cause blisters, abscesses, and also redness and swelling in the infected areas[2].

S. aureus presence usually can be detected in the nostrils of about 30% of the healthy adults and on the skin of about 20% of them[23]. The percentages are usually higher for people who are patients in the hospitals or who work there. S. aureus bacteria can be moved by carriers from their noses to other body parts with their hands leading to infecting wounds in these parts [24]. People who are hospitalized or work in the hospitals are more likely to have *S. aureus* infections ranging from mild to life-threatening. The most common staphylococcal infections are skin infections, often causing abscesses almost at any site in the body. However, the bacteria can travel through the bloodstream (called bacteremia) to any part of the body such as heart valves (endocarditis) and bones (osteomyelitis)[23]. The bacteria also tend to accumulate on medical devices in the body, such as artificial heart valves, joints, heart pacemakers, and catheters inserted through the skin into blood vessels.

Many strains have developed resistance to the antibiotics causing the infection to be more difficult to treat[5]. Because antibiotics are widely used in hospitals, hospital staff members commonly carry resistant

strains. When people are infected in health care facilities, the bacteria are usually resistant to several types of antibiotics, including most the antibiotics that are related to penicillin (called beta-lactam antibiotics). Strains of bacteria that are resistant to almost all beta-lactam antibiotics are called methicillin-resistant *Staphylococcus aureus* (MRSA)[6]. Methicillin is a beta-lactam antibiotic related to penicillin. When MRSA strains of S. aureus infections were acquired in the health care facilities they are known as hospital-acquired infections, whereas when the infections of MRSA were acquired outside of the health care facilities they were called community-acquired infection.

2. Materials and methods

This study was conducted from February 1st 2020 to March 15th 2020 in surgical department in Zawia teaching hospital in Zawia city/ Libya. Swab samples were collected from thirty patients with wounds including diabetic foot, gunshots and surgical operations. The swab samples were collected from the depth of wounds using sterile cotton swab under aseptic conditions, then specimens were transferred into sterile nutrient broth in screw capped test tubes and were delivered to laboratory within 1hr. The wound swabs inoculated to blood agar and mannitol agar media which are selective for S. aureus, then the samples were incubated at 37 ℃ for 24hrs.

The antimicrobial susceptibility tests of all S. aureus isolates were done according to the diffusion method from a pure culture. Three to five selected colonies of S. aureus were taken and transferred to tubes containing 5ml sterile nutrient broth and mixed gently until homogenous suspensions were formed. The tubes were then incubated at 37 ℃ until the turbidity become adjusted to 0.5 MCFar land standard. A sterile cotton swab was used to remove the excess suspension by gentle

swabbing of the swabs agonist the surface of the tube. The swab was then used to distribute the bacteria evenly over the entire surface of a Mueller Hinton Plate PH (7.2-7.4).

The inoculated plates were left at room temperature to dry for 3-5 min and a set of eight antibiotic discs were evenly distributed on the surface of Muller Hinton Plates. The set of the antibiotic disks had the following concentrations: Penicillin 10 µg, Methicillin 5 µg, Cefoxitin 30 µg, Doxycycline 30 µg, Gentamicin (CN)10 µg, Ciprofloxacillin 5 µg, Clindamycin (DA) 2 µg, Bactrim(SXT) 25 µg. The plates were incubated for 72 hrs and then examined for bacterial growth. Data were then recorded and analyzed and the results were presented as graphs using Microsoft excel.

3. Results

3.1. Prevalence of *Staphylococcus aureus* infection in wounds

A total of 30 adults participated in this study. Participants with surgical wounds were 11 persons representing (36.67%), Participants with diabetes foot were 16 persons representing (53.33%), and Participants with gunshots were 3 persons representing (10 %). The samples were collected from both Zawia Teaching Hospital and Zawia polyclinics during February 2020 and March 2020. The overall *S. aureus* wound infections were 17 out of 30 representing 56.67%. The prevalence of *Staphylococcus aureus* isolates compared to the total collected samples of each wound type is shown in figure 1. There were 7 samples (63.6 %) of the Surgical wounds isolates were infected by *Staphylococcus aureus* and 9 samples (56.6%) of the diabetic foot isolates were *S.aureus* positive while only one sample (33.3%) gunshot wound was *S. aureus* positive. The overall prevalence of *S. aureus* in all wounds infections presented in this study is alarming**.**

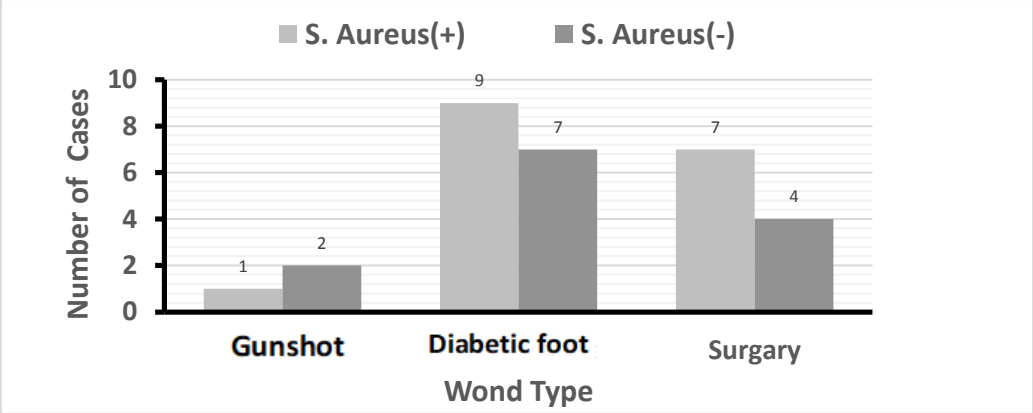

Figure 1: Presence of *S.aureus* in different wound types. The figure shows that whereas only about 33% of the gunshots were contaminated with S. Aureus, the contamination was about 56% for diabetic foot wounds and about 63% for surgical wounds.

3.2. Antibiotic resistance in the *S. aureus* positive samples

The *S. aureus* positive isolates were subjected to three members of penicillin family Methicillin,Penicillin, and Cefoxitin. All the samples in different wound types were (100%) resistant to both Methicillin and Penicillin, whereas their resistance to Cefoxitin was 100% for gunshot, 88.88% for diabetic foot, 85.71% for the surgical wounds as has been illustrated in figure 2.

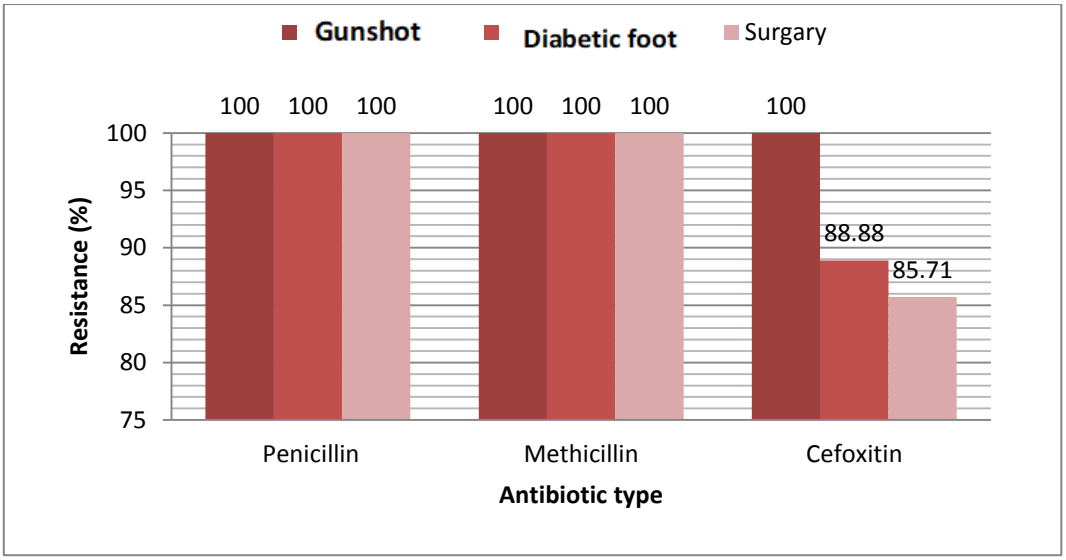

Figure 2: S.aureus resistance to Penicillin family in different wound types. The data showed that both Penicillin and Methicillin were not effective on all samples, whereas Cefoxitin was not effective on gunshot contamination, and its effectiveness was less than 15% for the other two wound types.

One member of each of five other antibiotic classes was also used in this study as has been illustrated in figure 3. The data showed that Clindamycin was the least resisted antibiotic, whereas Gentamicin was the highest resisted antibiotic. The resistance for Doxycycline was 100% for the gunshot, 33.33% for the diabetic foot, and 28.57% for the surgical wounds. The resistance for Clindamycin was 0.00% for the gunshot, 11.11% for the diabetic foot, and 14.28% for the surgical wounds. The resistance for Gentamicin was 100% for the gunshot, 55.55% for the diabetic foot, and 42.85% for the surgical wounds. The resistance for Ciprofloxacin was 0.00% for the gunshot, 55.55% for the diabetic foot, and 14.28% for the surgical wounds. The resistance for Bactrim was

0.00% for the gunshot, 33.33% for the diabetic foot, and 28.57% for the surgical wounds.

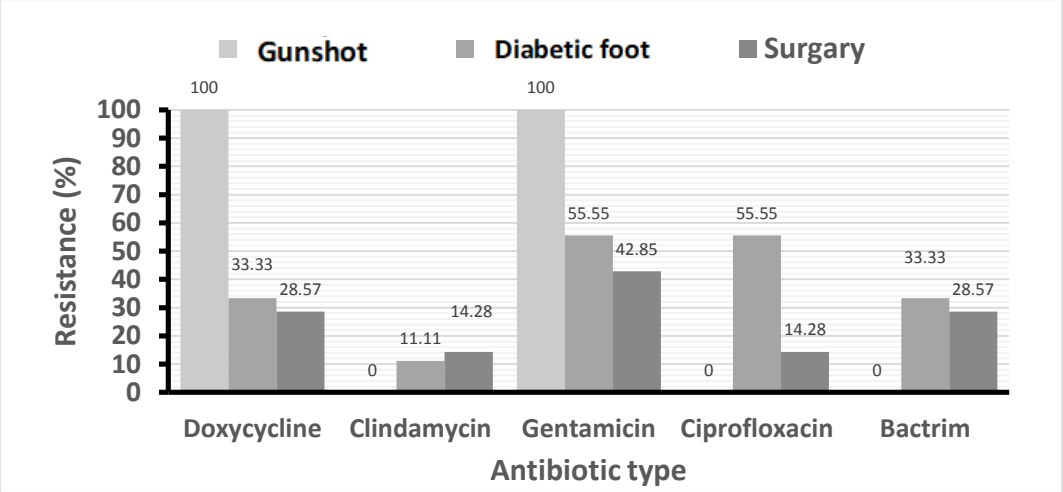

Figure 3: S.aureus resistance to Antibiotics other than Penicillin family in different wound types. The data showed that Clindamycin is the most effective antibiotic and its effectiveness more than 80%, whereas Gentamicin effectiveness was less than 60% in both surgical wound and diabetic foot wound contaminations and it had no effect on gunshot wound contamination.

3.3. Antibiotic resistance in the *S. aureus* positive samples in diabetic foot wounds

The resistance for all the eight antibiotics in the samples infected with *S. aureus* for diabetic foot wounds is illustrated in figure 4. The data showed that these samples were resistant to at least 3 types of antibiotics and this resistance went up to 7 out of 8 types. There were 3 samples resistant to 3 types of antibiotics, two samples resistant to 4 types of

antibiotics, two samples resistant to 6 types of antibiotics, and two samples resistant to 7 antibiotics.

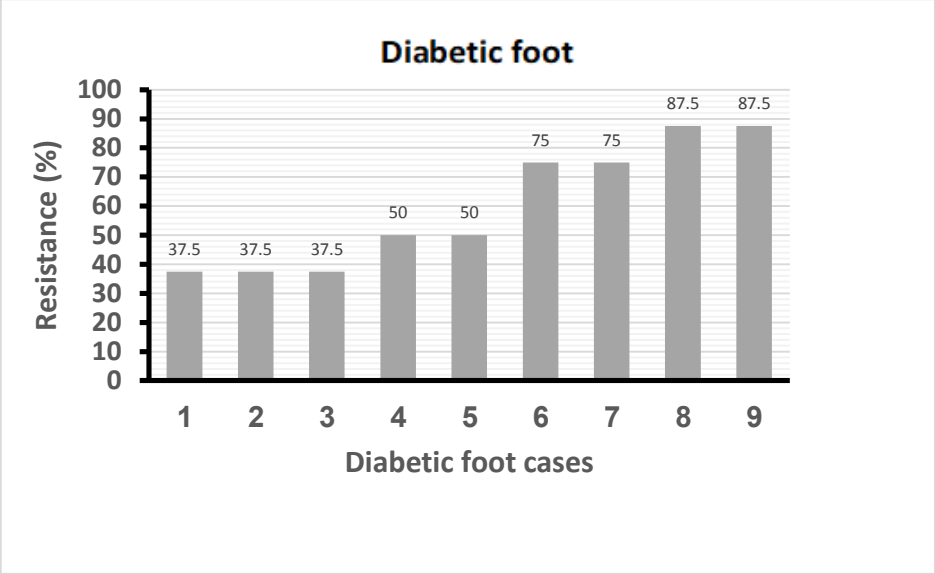

Figure 4: S.aureus resistance to Antibiotics for each case in diabetic foot. The data showed that all the samples were at least resistant to 3 types of antibiotics (37.5%) and this resistance goes up to 7 types of antibiotics (87.5%) in some samples.

3.4. Antibiotic resistance in the *S. aureus* positive samples in surgical wounds

The resistance for all the eight antibiotics in the samples infected with *S. aureus* for surgical wounds is illustrated in figure 5. The data showed that these samples were resistant to at least 3 types of antibiotics and this resistance went up to 6 out of 8 types. There were 4 samples resistant to 3 types of antibiotics, one sample resistant to 4 types of

antibiotics, one sample resistant to 5 types of antibiotics, and one sample resistant to 6 antibiotics.

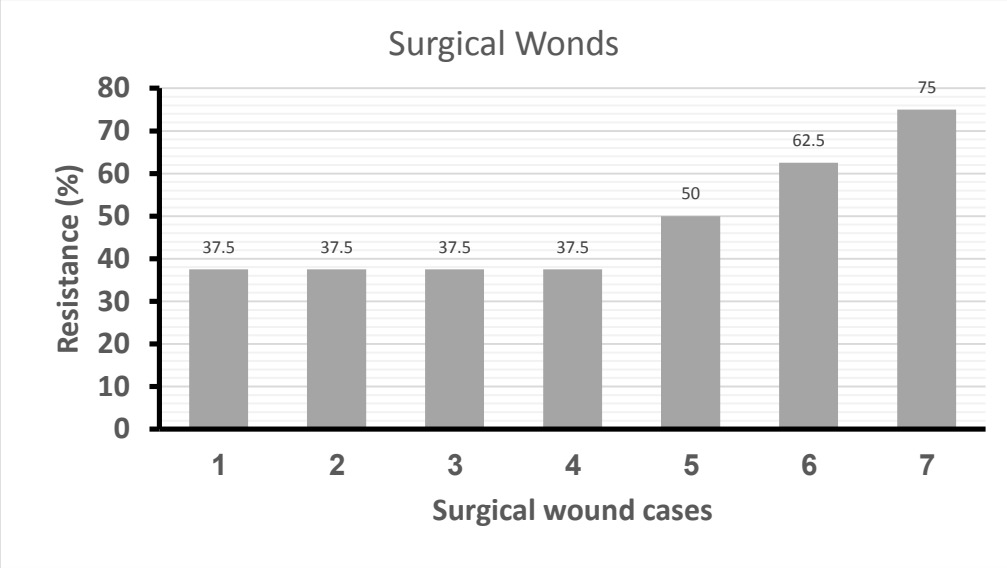

Figure 5: S.aureus resistance to Antibiotics for each case in Surgical wounds. The data showed that all the samples were at least resistant to 3 types of antibiotics (37.5%) and this resistance goes up to 6 types of antibiotics (75%) in one sample.

3.5. The overall antibiotic resistance in all the *S. aureus* positive samples

The resistance for all the eight antibiotics in the samples infected with *S. aureus* for all wound types is illustrated in figure 6. The data showed that these samples were resistant by at least 11.67% for Clindamycin and this resistance went up to 100% for Penicillin and Methicillin. The resistance was more than 50% for 4 types of antibiotics and less than 50% for the other 4 types of antibiotics.

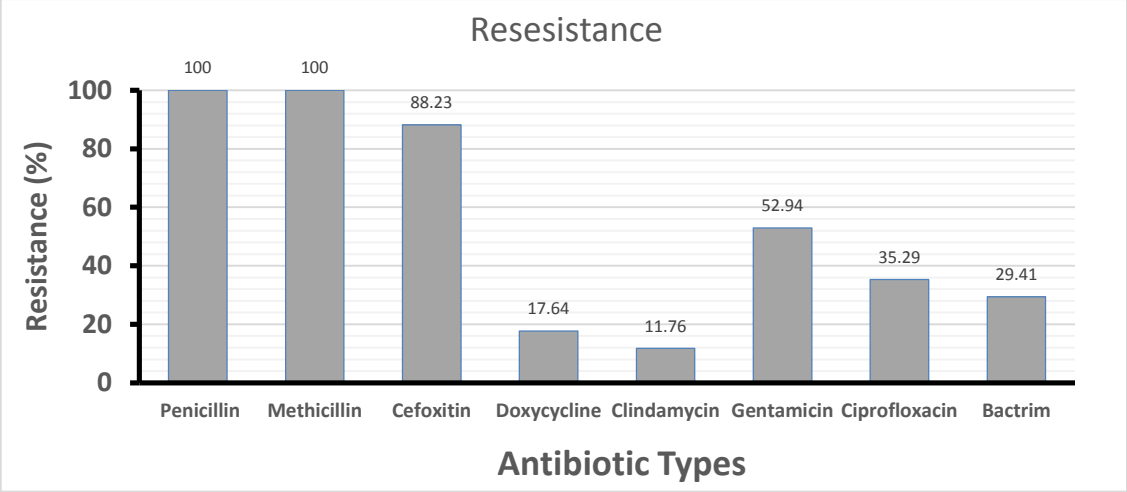

Figure 6: S.aureus resistance to Antibiotics for all contaminated samples. The data showed that the bacteria are 100% resistant to some antibiotics such as Penicillin and Methicillin. This resistance varies for other antibiotics from about 88% for Cifoxitin to about 12% for Clindamycin. None of the Antibiotics is effective 100%.

4. Discussion

In this study there was a relatively high prevalence of S. aurora contamination among the samples that were collected from surgical wound site infections (56.67%) which is alarming, even though it is expected since S. aureus is part of the natural flora in the skin. Similar findings were reported by Orrett and Land where they found that among 2430 isolates of S. aureus collected from various clinical sources in Trinidad and Tobaggo[7]. They found that the contaminations in the isolates from different surgical wounds were (60.1%). Tiemersma *et. al.* conducted a study across the European countries for the period from 1999 to 2002[8]. The study compared a total of 50.759 samples. S. Aureus isolates were collected from 495 hospitals in 26 countries and the prevalence varied from 1% in some Northern European countries to 40% in Southern and Western European countries. The huge variation in S.

aureus contaminations between the north and south in the European countries in their findings might be related to the cold weather in the north and the warm weather in the south. And since the weather in Libya is warmer than the weather in the European countries it is logical to assume that the contamination with S. aureus will be higher in Libya since Orrett and Land also found higher contamination with S. Aureus in in Trinidad and Tobago. This suggestion can also be supported with the study that was carried out in Ethiopia by tiki Anbessa specialized hospital (a tertiary teaching hospital Addis Ababa university) for the period from December 2013 to June 2014 were they found that S. Aureus contaminations in collected samples from wounds of 94 patients reached (57.4%)[9]. Another study was conducted in Libya by Najat Buzaid et al in 2010 in tertiary surgical and trauma hospital in Benghazi Libya found that among a total of 200 samples 62 S. aureus contaminations were representing (31%) of the total[10].

This study showed that the resistance of S. aureus to Methicillin and Penicillin reached 100% for all the wound types, whereas the resistance for Cefoxitin showed differences among the three wound types that were compared. Cefoxitin resistance in the isolates from patients with gunshot was (100%) while it was 88.88% for diabetic foot wounds and 85.71% for surgical wounds. These findings of the S. aureus resistance to these three antibiotics were very high compared to studies conducted by some other researchers. Olowe et.al in Nigeria in 2013 found the resistance to Methicillin among 208S. aureus infected samples were 52.2%, whereas their resistance to Penicillin reached 82.7%[11]. In another study conducted by Ranjan K.P et.al in north India in 2013 for 5259 samples over three years, the resistance to Methicillin was found to be 27.96%[12]. In another study conducted by Chijioke in Nigeria in 2016 out

of 424 patients there were 104 samples found infected with S. aureus, and the resistance to Penicillin reached 81.7% whereas the resistance to Cefoxitin was 42.3%[13]. The findings of yetere FworkTsige et.al in 2020 showed that the resistance to Penicillin for 266 S. aureus samples was 100% whereas the resistance to Cefoxitin was100%[14]. The findings of some of these other studies were not far from our findings, whereas our findings showed much higher resistance for these three antibiotics than the other studies. This comparison suggested that the strains of S. aureus that were collected from wounds in our study have higher resistance which might have been developed against these antibiotics as a result of the excessive use of antibiotics by Libyan patents.

Our study showed that S. aureus isolated from wounds were least resistant to Clindamycin and Gentamicin showed the highest resistance with 100% resistance for gunshot, 33.3% resistance for diabetic foot, and 28.57% resistance for surgical wounds. the resistance for Clindamycin was 0.oo% for gunshot, 11.11% for diabetic foot and 14.28% for surgical wounds .the resistance for Gentamycin was 100% for gunshot, 55.55% for diabetic foot and 42.85% for surgical wounds and was resistant for Ciprofloxacin 0.00% for gunshot, 55.55% for diabetic foot and 14.28% for surgical wounds while the Bactrim was resistance 0.0% for gunshot, 33.33% for diabetic foot and 28.5% for surgical wounds. Similar to study worked by YetereFworkTsige et.al showed high-level resistance to gentamycin 53.8%and 61.5%to Ciprofloxacin, 53.8%to Bactrim, and 4.3% to Clindamycin. Compare to another study worked at Debre Markos Referral hospital in Ethiopia the clinical isolates showed >80% level of resistance to Gentamycin and Bactrim where's <50% level of resistance was observed agonist Clindamycin.

When the resistance percentage to antibiotics for each sample was compared, the lowest resistance was 37.5% which is a resistance to three out of eight antibiotics whereas the highest reached 87.5% which is a resistance to seven out of eight antibiotics. For the gunshot patients, there was only one infected sample and the results were all or none for the antibiotic resistance. In the diabetic wound samples the resistance was higher in some samples that reached seven out of eight whereas the lowest was three out of eight. For the surgical wound infections, the highest was six out of eight whereas the lowest was three out of eight. These data suggested that even though the difference between surgical and diabetic wounds was not big, the diabetic wound S. aureus bacterial infection is slightly more resistant to antibiotics than that of the surgical wounds. Furthermore, S. aureus bacterial infections might be from different stains or at least were developed differently from each other. One would expect similarity in these infections since all the samples were from the same city. Further studies may be required to reveal the reasons behind these differences in antibiotic resistance for S. aureus samples.

Conclusion

Out of 30 patients suspected of wound infections, 17 cases (56.67%) were infected with S. aureus. Most of these samples were 100% MRSA resistant and showed multiple drug resistance. Samples showed variations in antibiotic resistance from three to seven out of eight antibiotics suggesting that they were developed separately from each other. These findings suggest that new strategies for antibiotic usage are needed to reduce the antibiotic resistance in bacteria.

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