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Antimicrobial Susceptibility of S. aureus to Antibiotics Used in the Treatment of Diabetic Patients with Foot Ulcers at Vihiga County Referral Hospital, Kenya

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Abstract
S. aureus though the most important gram-positive bacteria that has in recent times become resistant to most of the therapeutic agents that have been developed, scientists continue to disagree on the most effective antibiotic for the treatment of *S.aureus*. Despite these findings, Vihiga County referral hospital lacks a policy for antimicrobial susceptibility testing making antimicrobial susceptibility of *S. aureus* isolated from diabetic patients with foot ulcers in Vihiga County unknown. This study investigated antimicrobial susceptibility of *S. aureus* to antibiotics used in the treatment of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya. Pus specimens from foot ulcers of diabetic patients were swabbed aseptically for *S. aureus* screening. All the confirmed *S. aureus* strains were subsequently tested for antimicrobial susceptibility based on the Kirby-Bauer disk diffusion method using antimicrobial discs (penicillin G, ceftriaxone and gentamycin) on Mueller Hinton Agar (MHA). Results indicated that *S. aureus* isolated from all the 94 diabetic patients were resistant to penicillin G, 33 were susceptible to Ceftriaxone and 36 were susceptible to Gentamycin. The study concluded that gentamycin was the most effective antibiotic with 38.3% susceptibility rate followed by ceftriaxone at 35.1%.
Keywords: *S.aureus*, Antimicrobial susceptibility, Diabetic foot ulcers

1. Introduction

The International Consensus on the Diabetic Foot (ICDF) defined a diabetic foot ulcer as a wound below the ankle in a person with diabetes, irrespective of duration (Iversen, 2009). Foot lesions and foot ulcers are the most commonly observed impediments with diabetic patients (Mathangi *et al*., 2013). The diabetic foot has been considered the major complication of diabetes care, and the International Diabetes Federation (IDF) dedicated the year 2005 to foot care of people with diabetes in order to raise awareness of foot disease among people with diabetes (Iversen, 2009). Pervasiveness of these ulcers in diabetic patients is as high as 25% and requires constant monitoring and patients have to visit a hospital frequently which is exorbitant (Mathangi *et al*., 2013). Iversen (2009) asserted that infection can complicate any type of diabetic foot ulcer and is one of the most common causes of hospital admission among people with diabetes.

Infection by Staphylococcus is common in developing countries with variations in antimicrobial susceptibility making it difficult to determine the infection patterns of Staphylococcus in socioeconomically underdeveloped regions (Almeida *et al*., 2014). It has been estimated that approximately 20–30% of the general population are *S. aureus* carriers (plata *et al*., 2009). *S. aureus* infection most common in foot ulcers requires careful management because of its ability to acquire antibiotic resistance (Almeida *et al*., 2014). The discovery of antibiotics in the early 20th century fundamentally transformed human medicine; however, the rise of antibiotic-resistant bacterial strains represents a serious threat to public health (Centers for Disease Control and Prevention, 2013). In recent years, there has been an increasing need for antimicrobial agents active against resistant gram-positive bacteria (Moellering, 2003).

S. aureus though the most important gram-positive bacteria that has in recent times become resistant to most of the therapeutic agents that have been developed as opined by Murugan *et al*. (2008), scientists continue to disagree on the most effective antibiotic for the treatment of *S.aureus*, whereas Ako-Nai *et al*. (2005) advocates for pencillin, Rajaduraipandi *et al*. (2006) advocates for aminoglycoside. Vihiga County referral hospital uses penicillin G (penicillin), ceftriaxone (cephalosporin) and Gentamycin (aminoglycoside) to treat diabetic wounds. However, the hospital lacks a policy for antimicrobial susceptibility testing for diabetic patients with foot ulcers which makes antimicrobial susceptibility of *S. aureus* in Vihiga County uncertain. This study therefore examined susceptibility of *S. aureus* to antibiotics used in the treatment of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya for the purposes of generating knowledge on the susceptibility of *S.aureus* isolated from diabetic foot ulcers.

1.2 Objectives of the Study

1.2.1 General Objective

To investigate antimicrobial susceptibility of *S. aureus* to antibiotics used in the treatment of diabetic patients

with foot ulcers at Vihiga County Referral Hospital, Kenya.

1.2.2 Specific Objectives

The specific objectives were to;

1. Examine susceptibility of *S. aureus* to Pencillin
2. Determine susceptibility of *S. aureus* to cephalosporin
3. Examine susceptibility of *S. aureus* to aminoglycoside

1.3 Research Hypotheses

1. H_0 : *S. aureus* is not susceptible to Penicillin G used in the treatment of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya.
2. H_0 : *S. aureus* isolated from diabetic patients with foot ulcers is not susceptible to cephalosporine used in the treatment of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya.
3. H_0 : *S. aureus* isolated from diabetic patients with foot ulcers is not susceptible to aminoglycoside used in the treatment of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya.

2. Literature Review

2.1 Susceptibility of S. aureus to Pencillins, Cephalosporins and Aminoglycosides

In Vihiga County Referral Hospital penicillin (penicillin G), cephalosporin (ceftriaxone) and aminoglycoside (gentamycin) are the antibiotics used to treat diabetic patients with foot ulcers. Ako-Nai *et al*. (2005) in determining the treatment and antibiotic resistant profile of *S. aureus* in Nigeria used penicillin and tetracycline. The study established penicillin as more effective than tetracycline where out of the 178 staphylococcal isolates evaluated, 68% of *S. aureus* isolates were resistant to penicillin and 71% to tetracycline. Although their analysis established that *S. aureus* was more susceptible to penicillin than tetracycline, they did not compare with cephalosporins and aminoglycosides which are also used to treat diabetic patients with foot ulcers at Vihiga County Referral Hospital.

Rajaduraipandi *et al*. (2006) reported the prevalence and treatment of *S. aureus* for a total of 7172 clinical specimens using penicillin, aminoglycoside, cephalosporins and erythromycin. Out of 906 strains of *S. aureus* isolated from clinical specimens, the results indicated that aminoglycoside, cephalosporins, erythromycin was more effective in treatment followed with penicillin recording 99.6% resistance. This study focused on clinical specimens which was not specific to diabetic patients with foot ulcers.

Mir and Srikanth (2013) conducted a retrospective study from June 2011 to November 2012 in a tertiary care hospital in south India to find out the prevalence rate of *S. aureus* and antibiotic susceptibility pattern. A total of 210 Staphylococcus strains were isolated from various clinical samples, 180 were coagulase positive staphylococcus (CoPS) and 30 were coagulase negative staphylococcus (CoNS). Among 180 CoPS, 58 (32.22%) were penicillin resistant. Although they concluded that there is need for continuous monitoring of the antimicrobial susceptibility pattern of *S. aureus* for the selection of appropriate therapy, they did not compare with cephalosporins and aminoglycosides which are also used to treat diabetic patients with foot ulcers at Vihiga County Referral Hospital.

Farzana and Hameed (2006) conducted a study at Pakistan Institute of Medical Sciences, Islamabad, Pakistan during a period of two years to establish the sensitivity pattern of Gram-positive cocci isolated from the samples brought to Pathology Laboratory against commonly used antibiotics using disc diffusion method, at the hospital's laboratory. Samples comprised of blood, pus and urine, from outdoor patients (OPD) as well as indoor patients from different wards of the hospital. Out of 5069, 1688 were *S. aureus*. Out of these Gram-positive cocci 56% were resistant to penicillin group, 27% were resistant to cephalosporin group, 22% were resistant to aminoglycoside group, 15% were resistant to quinolone group and 31% were resistant to other antibiotics. However, their analysis of the susceptibility rate was not specific to specimens from diabetic patients with foot ulcers.

Scientists have tested the susceptibility of *S. aureus* to pencillins, cephalosporins and aminoglycosides used in the treatment of gram-positive bacteria. However, it is evident that they lack consensus on the antibiotic to which *S. aureus* is more susceptible and most studies used specimens which were not specific to diabetic foot ulcers. This study therefore examined susceptibility of *S. aureus* to pencillins, cephalosporins and aminoglycosides used in the treatment of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya.

3. Materials and Methods

3.1 Study Design

This study employed a hospital based cross- sectional study design of diabetic patients with foot ulcers at Vihiga

County Referral Hospital, Vihiga County, Kenya. The study used inferential data analysis technique based on correlation analysis to determine the relationship between antimicrobial susceptibility of *S. aureus* infection with respect to socio-demographics of the patients. The study population comprised of 225 adult (aged 18 years and above) diabetic patients with foot ulcers who attend Vihiga County Referral Hospital for treatment.

3.2 Sample Size and Sampling Technique

Sample size for the study was determined following Fisher *et al*. (1998) formula as modified by Mugenda and Mugenda (2003) as shown below.

$$n_f = \frac{n}{1+\frac{n}{N}} \quad (1)$$

Where

n_f = desired sample for population less than 10,000;

n = desired sample size for target population of more than 10,000; and

N = estimate of population size in the current study,

Hence the sample size in this study was,

$$n_f = \frac{384}{1+\frac{384}{225}} = 142 \quad (2)$$

To ensure equitable representation of the population in the sample, this Study employed simple random sampling. The sample size was adjusted by adding 10% of the calculated sample size to take care of sampling error. Thus the corrected sample size was 156.

3.3 Laboratory Techniques

Pus specimens from foot ulcers of diabetic patients were swabbed aseptically for *S. aureus* screening. All the confirmed *S. aureus* strains were subsequently tested for antimicrobial susceptibility based on the Kirby-Bauer disk diffusion method using antimicrobial discs (penicillin G, ceftriaxone and gentamycin) on Mueller Hinton Agar (MHA).

3.3.1 Antimicrobial Susceptibility Testing

3.3.4.1 Inoculum preparation

The sample was cultured on nutrient agar plate and a straight wire was used to transfer 3-5 isolated colonies to 5ml of sterile saline and mixed, its turbidity was adjusted using 0.5 McFarland turbidity standards.

3.3.4.2 Inoculation on agar plate

Within 15 minutes of inoculum preparation, a sterile cotton swab was dipped into the inoculum and rotated against the wall of the tube above the liquid to remove excess volume of the inoculum. Entire surface of Mueller Hinton agar was swabbed to ensure even distribution, without re-immersing the swab in the suspension. Inoculated MH plate was allowed to stand for 3 minutes to dry. Antibiotic discs were applied using sterile forceps with gentle pressure to ensure complete contact of disc with MH agar. Plates were incubated within 15 minutes of application of discs as diffusion of antibiotics and bacterial growth commences at the same time, plates were incubated at 37◦c for 18 - 24 hours. Diameter of zones of inhibition was measured using a transparent plastic ruler. Any growth around the disc was considered resistant, measured zones of inhibition were compared with that of Clinical and Laboratory Standards Institute (2015).

3.4 Ethical Considerations

Ethical approval for this study was sought from Maseno University Ethical Review Committee and further permission sought from Vihiga County Referral Hospital management through the Chief Officer Ministry of Health-Vihiga County. Written informed consent was obtained from each participant before enrollment.

4. Results

4.1 Susceptibility of S. aureus to antibiotics used in the treatment of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya.

The study by review of patient's files established that penicillin (penicillin G), cephalosporin (ceftriaxone) and aminoglycoside (gentamycin) were used in the treatment of diabetic foot ulcers. Table 4.3 results indicated that a total of 132 diabetic patients with foot ulcers were exposed to antibiotic treatment where; 112(71.8%) were on penicillin G, 8(5.1%) were on ceftriaxone and 12(7.7%) were on gentamycin.

Table 4.3: Type of antibiotic used

Antibiotic	Frequency	Percentage (%)
Penicillin G	112	71.8
Ceftriaxone	08	5.1
Gentamycin	12	7.7
No antibiotic	24	15.4
Total	156	100

Antibiotics Diabetic Patients were exposed to. Sourced, From "Vihiga County Referral Hospital Diabetic Clinic Patient's files"

To ascertain susceptibility of *S. aureus* to antibiotics used in the treatment of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya, all confirmed *S. aureus* strains were tested for antimicrobial susceptibility based on the Kirby-Bauer Disk diffusion method using antimicrobial discs of Penicillin G (10 µg); Gentamycin (10 µg) and Ceftriaxone (30 µg) on Mueller Hinton Agar (MHA) as shown in figure 4.6 and zones of inhibition diameter measured in mm and compared to Clinical and Laboratory Standards Institute (2015). Table 4.4 test results showed that; *S. aureus* isolated from all the 94 diabetic patients were resistant to penicillin G, 33 were susceptible to Ceftriaxone and 36 were susceptible to Gentamycin in comparison to Clinical and Laboratory Standards Institute (2015) for Interpretation of Zones of inhibition (diameter in mm) in appendix 6.

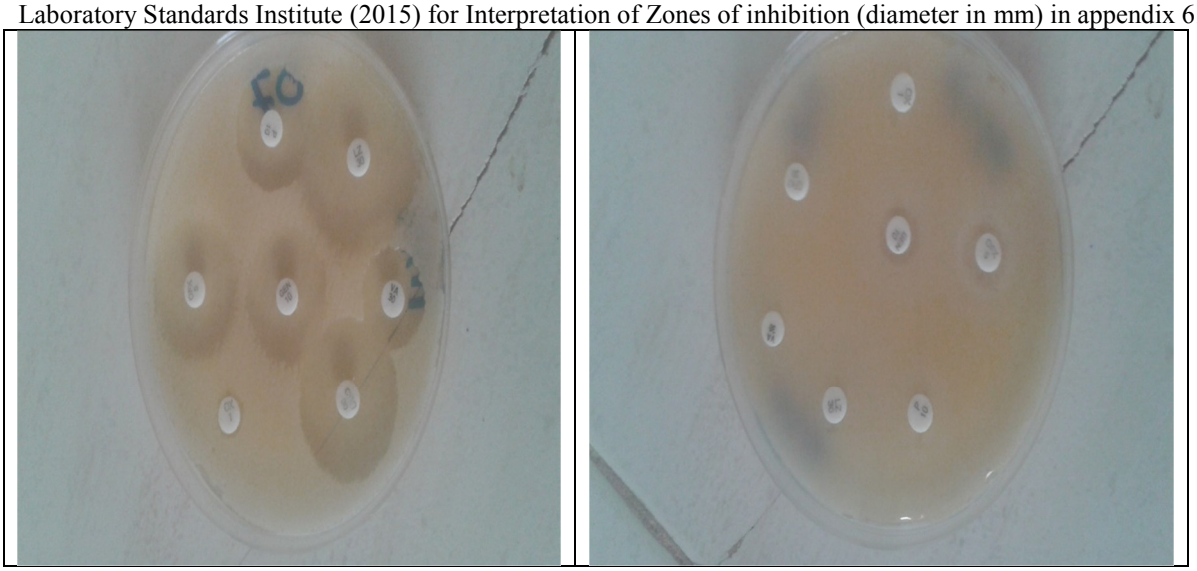

Figure 4.5: Antibiotic Discs on Muller Hinton Agar after Growth Showing Susceptibility test Result.

Table 4.4: Antibiotic susceptibility profile of antibiotcs used in treatment of *S. aureus*

Antibiotic disc	Disc concentration	Susceptible	Intermediate	Resistant
Penicillin G	10µg	0	0	94
Ceftriaxone	30µg	33	0	61
Gentamycin	10µg	36	0	58

Correlation analysis an inferential data analysis technique was conducted to ascertain the relationship between susceptibility of *S. aureus* isolated from diabetic patients with foot ulcers to antibiotics used in the treatment of *S. aureus* at Vihiga County referral hospital and socio-demographic factors (age, gender, marital status and education). Table 4.5 results indicated that there was a relationship between age and susceptibility of *S. aureus* to antibiotics used in treatment of *S. aureus* isolated from diabetic patients at Vihiga County referral hospital. This was indicated by correlation coefficients of ($r = -0.59$) with a p-value of 0.026 for penicillin G, ($r = -0.59$) with a p-value of 0.000 for ceftriaxone and ($r = -0.63$) with a p-value of 0.034 for gentamycin.

Table 4.5: Correlation r (p-values) test results for socio-demographics with Penicillin G, Ceftriaxone and Gentamycin

Socio-demographic	Penicillin G	Ceftriaxone	Gentamycin
Age	-0.59*(0.026)	-0.59*(0.000)	-0.63*(0.034)
Gender	-0.083(0.808)	0.111(0.744)	0.043(0.900)
Marital status	0.289(0.389)	0.293(0.382)	0.179(0.599)
Education	0.13(0.704)	0.36(0.276)	0.08(0.814)

Note. * indicates significant at 5% level of significance, values in parenthesis ( ) indicate p-values and p-value <0.05 implies significant at 5% level of significance

5. Discussion

5.1 Susceptibility of S. aureus to antibiotics used in the treatment of diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya.

Based on the review of patient files, it was evident that the antibiotics used in the treatment of diabetic patients with foot ulcers at Vihiga County referral hospital were pencillin (penicillin G), cephalosporin (ceftriaxone) and aminoglycoside (gemtamycin). The study used both descriptive statistics by use of frequency tables and inferential data analysis technique based on correlation analysis to explain the susceptibility of *S. aureus* to pencillin G, ceftriaxone and gentamycin.

Descriptive statistics analysis indicated that *S. aureus* isolated from diabetic patients with foot ulcers at Vihiga County Referral Hospital, Kenya had a susceptibility rate of 0% (resistant), 35.1% and 38.3% to penicillin G, ceftriaxone and gentamycin respectively. Based on correlation analysis there was significant negative relationship between age and susceptibility of *S. aureus* to antibiotics used in the treatment of diabetic patients with foot ulcers at Vihiga County referral hospital, Kenya. This implied that the susceptibility of *S. aureus* to penicillin G, ceftriaxone and gentamycin decreases as age of a diabetic patient with foot ulcers increases. The findings may be attributed to non-adherence to intake of these antibiotics and not being consistent in clinic visits for treatment. This finding led to the acceptance of null hypothesis for penicillin, implying that *S. aureus* was not susceptible to penicillin G. Although these results contradicts Ako-Nai *et al*. (2005) and Mir and Srikanth (2013) who established that *S. aureus* was susceptible to penicillin to some degree, this study's findings are consistent with the findings of Rajaduraipandi *et al*. (2006) who by using clinical specimens to study the prevalence and treatment of *S. aureus* established 99.6% (100%) resistance or 0% susceptibility of *S. aureus* to penicillin G. For ceftriaxone and gentamycin, the null hypothesis was rejected implying that *S. aureus* was susceptible to ceftriaxone and gentamycin to some degree. The findings are consistent with Farzana and Hameed (2006) findings in their study at Pakistan Institute of Medical Sciences to establish the sensitivity pattern of Gram-positive cocci which showed that *S. aureus* was susceptible to cephalosporins and aminoglycoside to some degree.

6. Summary, Conclusions and Policy Recommendations

6.1 Summary of the Findings

No *S. aureus isolates* were susceptible to penicillin G, 35.1 % were susceptible to ceftriaxone and 38.3% were susceptible to gentamycin. Furthermore susceptibility of *S. aureus* to penicillin G, ceftriaxone and gentamycin was dependant on age. This finding led to the acceptance of null hypothesis for penicillin G, implying that *S. aureus* was not susceptible to penicillin G. 36.2% of

6.2 Conclusion

In conclusion these results indicated that gentamycin was the most effective antibiotic with 38.3% susceptibility rate followed by ceftriaxone at 35.1%.

6.3 Recommendations

i. The County through the County ministry of health should equip the hospital to facilitate conducting of antimicrobial susceptibility tests. This will ensure that the medical practitioners prescribe and administer relevant antibiotics to the patients.
ii. Vihiga County Referral Hospital to reduce the use of penicillin G to which we had 0% susceptibility and embrace the use of ceftriaxone and gentamycin based on sensitivity tests. This will ensure that diabetic patients with foot ulcers get appropriate medication which will reduce infection rate and thus improve the health status of the diabetic patients with foot ulcers.

Declaration

The author hereby declares that the submission is original, not being considered elsewhere and there is no conflict of interest.

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