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Antimicrobial Resistance Patterns of Bacteria Isolated from Wounds of Diabetic Patients at Jaramogi Oginga Odinga Teaching & Referral Hospital (JOOTRH), Kenya

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ABSTRACT

The purpose of this study was to determine antimicrobial resistance patterns of bacteria isolated from wounds of diabetes mellitus patients at JOOTRH. A hospital based cross sectional study design was employed with a target population of 168 and sample size of 117 patients involving stratified random sampling. Data was collected using a structured questionnaire and a laboratory form for a period of 6 months. Pus swabs were collected for isolation of bacteria using conventional techniques and serology. Resistance was done using Kirby-Bauer disk diffusion on Mueller Hinton Agar at 37°C for 24 hrs. High susceptibility was established for; *S.aureus* on amikacin and gentamicin, *E.coli* on imipenem and gentamicin, *K. pneumoniae* and *Proteus* species on imipenem and *P.aeroginosa* on ciprofloxacin. Findings provide coherent and effective chemotherapeutic alternatives for managing diabetes patients with wounds and recommends that JOOTRH to adopt susceptibility testing policy for the sake of identifying the most effective treatment regimen for better patient's care.

Keywords: Antimicrobial resistance, bacteria, diabetic patients, wounds.

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I. Introduction

Diabetes mellitus is estimated to consume almost triple the healthcare resources in comparison to other diseases, a contributing factor being the rise in cost for analogue insulins 1 which are increasingly prescribed despite little evidence that they provide significant advantages over cheaper human insulins [1]. For instance, global health expenditure due to diabetes grew from USD 232 billion in 2007 to USD 966 billion in 2021 where Kenya incurred USD 448.6 per person and the global health expenditure is estimated to reach 1.05 trillion by 2045 [2]. Diabetes mellitus is broadly categorized as; type 1, 2 and gestational [3]. Type 1 occurs most frequently in children, type 2 most frequent among adults accounting for 90-95% of all diabetic cases and gestational diabetes occurs during pregnancy [4].

Wounds of diabetic patients frequently acquire infections because of various clinico-demographic causes like hyperglycemia, repressed protection, insufficient blood supply and infected peripheral nerves [5]. There are many factors that impair wound healing process such as inadequate blood supply, contamination, repeated trauma, radioactivity exposure and undernourishment [6]. Infections interfere with the curative sequence of wounds healing by lengthening the inflammatory stage due to bacterial enzymes which destroy important healing elements [7]. Infection also causes amputations [7].

A rise in the occurrence of multidrug resistant bacteria among diabetics in the recent past is due to *Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *Enterobacter* spp (ESKAPE) [8], [9]. Antimicrobial resistance (AMR) is gradually becoming a severe danger to the advances made in health for the realization of the Sustainable Development Goals (SDGs), affecting health security, poverty, economic growth and food security with action being essential through regions to avert and control AMR [10]. According to [10], *Pseudomonas spp., S. aureus, E.coli, Enterococcus spp. K.pneumoniae and Proteus spp*. have been found to be accountable for widespread tissue damage causing reduced blood flow to the wound thus complicating the healing process. The identified bacteria as noted by [11] develop resistance to antibiotics due to development of diverse β-lactamases that counter the activity of penicillins and cephalosporin.

Various scientists have also investigated the antimicrobial resistance for bacterial microbes isolated from diabetic wounds. They include [12] in Sudan; [13] in Libya and [14] in Kenya. It is evident that the effectiveness of antibiotics active against gram positive and gram negative bacteria varies. For instance, [15], [16] noted that gram negative rods

were resistant to gentamycin while [16] noted that gentamycin was one of the most active medication in treating both gram positive and negative bacteria. This is an indication that findings on antimicrobial resistance in a given study area might not be a reflection of expected results from another study area. Therefore, the level of antimicrobial resistance of bacteria isolated from diabetic wounds at JOOTRH remains unknown. Several studies to identify the antimicrobial resistance but contradicting views have been noted with regard to the resistance of antibiotics. This makes it impossible to single out their resistance patterns thus calling for continued research.

II. Literature Review

The innovation of antimicrobials in the 20th century essentially changed human medication; conversely, the rising antimicrobial resistance poses danger to community well-being. Antimicrobial resistance remains a vital risk to the management of the rising array of infections triggered by microorganisms [17]. This reduces the efficacy of antibacterial drugs, makes the management of patients demanding and expensive occasioning persistent sickness and rising deaths on vulnerable patients [17]. Development of antimicrobial resistance is an ordinary occurrence in microorganisms which is enhanced due to pressure brought about by usage and mistreatment of antibiotics in organisms [18].

Recently, there has been an emergent desire for identification of antibiotics more powerful for management of resistant bacteria [18]. This is because the greatest common bacteria have developed resistance to majority of antimicrobials discovered recently [18], [19]. The absence of novel antibiotics in the World to substitute the ineffectual ones brings more urgency to the desire to guard the effectiveness of current medications, advancement and enactment of appropriate approaches to curb the rise and spread of antimicrobial resistance [19].

To screen for the existence of bacteria in wounds of diabetics and their susceptibility to antibiotics in Asia researchers like [19] found varied sensitivity patterns. In India for instance, [20] found gram positive and negative bacteria to be sensitive to ciproflaxcin, impenem, Pefloxacin, Ofloxacin and Chloramphenicol but resistant to Agumentin, trimoxazole, amoxicillin, erythromycin and Gentamycin. On the other hand, [21] in Nepal showed that Amikacin, Gentamycin and Cloxacillin were the most effective. Lack of consensus on the effectiveness of some antibiotics like Gentamycin against bacteria necessitates the need for further susceptibility tests in different regions and populations.

In Africa, antimicrobial resistance poses a great challenge to community health prompting various researchers to determine the resistance profile of antibiotics against bacteria. However, the findings continue to generate divergent views on the drugs efficacy. For example [22] in Libya like [23] in Sudan discovered sensitivity to ciprofloxacin and amikacin but resistance to vancomycin, tetracycline, amoxicillin, methicillin, streptomycin, amoxicillin, and erythromycin. On the other hand, [24] in Nigeria showed that bacteria were highly resistant to ciprofloxacin which contradicts other researchers.

The rising burden of diabetes coupled with antimicrobial resistance continues to worry policy makers in Kenya. Studies conducted to investigate the susceptibility and resistance of bacteria to antibiotics indicate contradicting findings which raises the questions as to which are the most active antimicrobial agents to manage diabetic wounds. For instance, [24], [25] established that bacterial isolates were sensitive to amoxicillin clavulanate, meropenem, clindamycin, ceftriaxone, piperacillin-tazobactam, ciprofloxacin, vancomycin, levofloxacin, linezolid, teicoplanin, imipenem, meropenem, amikacin and levofloxacin but resistant to ampicillin, augmentin, cotrimoxazole, doxycycline and cephalosporins. Reference [25] on the other hand found that bacteria were highly resistant to cephalosporins, amoxicillin clavulanate and imipenem which contravene [26] findings.

III. Materials and Methods

The study employed a hospital based cross-sectional design involving 117 patients. Antimicrobial susceptibility testing was carried out on each identified organism by disc diffusion method on Muller Hinton agar (MHA) as recommended by the Clinical and Laboratory Standards Institute (CLSI) guidelines. 20 ml Mueller Hinton agar was prepared and dispensed aseptically into Petri dishes and allowed to solidify. A sterile straight wire was used to transfer 3 to 5 isolated colonies to 5ml of sterile saline and mixed. Standardized 0.5 McFarland inoculum of test bacteria was inoculated on to the Mueller Hilton agar using sterile swabs. The entire surface of Mueller Hinton agar was swabbed to ensure even distribution, without re-immersing the swab in the suspension.

The isolates were tested against vancomycin (30 μg), gentamicin (10 μg), erythromycin (15 μg), ciprofloxacin (5 μg), ceftriaxone (30 μg), tetracycline (30 μg), amoxicillin clavulanic (20/10 μg), oxacillin (30 μg), ampiclox (10 μg), penicillin G (10 μg), cefipime (5 μg), flucloxacillin (10 μg), amikacin (30 μg), linezolid (30 μg), imipenem (10 μg) and clindamycin (2 μg) which were placed on the inoculated Muller Hinton (MH) plate using sterile forceps and then pre-diffusion allowed for 15 minutes. The plates were then incubated at 37°C for 24 hours. The zones of inhibition were measured to the nearest mm with a transparent meter ruler, recorded and interpreted using [26] guidelines. Clearence around the discs as shown in Fig. 1 indicated susceptibility of the isolated bacteria to the given antibiotic.

Fig. 1. Antimicrobial susceptibility test.

TABLE I: FREQUENCY (%) DISTRIBUTION OF RESISTANCE TO ANTIBIOTICS

Antimicrobial	Number of isolates resistant to antibiotics (%) and MIC Range									
	S. aureus (N=40)	*(MIC Range -mm)*	*E. coli (N=13)*	*(MIC Range - mm)*	*P.aeruginosa (N=24)*	*(MIC Range - mm)*	*K. pneum (N=22)*	*(MIC Range - mm)*	*Proteus (N=18)*	*(MIC Range - mm)*
Amikacin	3 (7.5)	≤ 14	-	≤ 14	3 (12.5)	≤ 14		-	-	-
Ceftriaxone	11(27.5)	≤ 13	-	≤ 13	-	-		-	-	-
Ciprofloxacin	5 (12.5)	≤ 20	1 (7.7)	≤ 20	0 (0.0)	≤ 20	7 (31.8)	≤ 20	-	-
Erythromycin	6 (15.0)	≤ 13	1 (7.7)	≤ 15	10 (41.7)	≤ 15	13 (59.1)	≤ 15	5 (27.8)	≤ 20
Ofloxacin	21(52.5)	≤ 28	0 (0.0)	≤ 28	-	-				
Gentamycin	3 (7.5)	≤ 12	0 (0.0)	≤ 12	3 (12.5)	≤ 12	5 (22.7)	≤ 12	11(61.1)	≤ 15
Linezolid	7 (17.5)	≤ 20	-	-	-	-		-	-	-
Penicillin G	40(100.0)	≤ 28	-	-	-	-		-	-	-
Oxacillin	40(100.0)	≤ 21	-	-	-	-		-	-	-
Tetracycline	27 (67.5)	≤ 14	1 (7.7)	≤ 11	-	-	13 (59.1)	≤ 11	4 (22.2)	≤ 12
Vancomycin	19 (47.5)	≤ 14	-	-	-	-	-	-	-	-
Amox-clav	-	-	-	-	-	-	20 (90.9)	≤ 13	11(61.1)	-
Ampiclox	-	-	-	-	-	-	20 (90.9)	≤ 11	16(88.9)	≤ 13
Cefepime	-	-	-	-	-	-	19 (86.4)	≤ 14	16(88.9)	≤ 11
Clindamycin	-	-	-	-	-	-	13(59.1)	≤ 14	15(83.3)	≤ 14
Imipenem	-	-	-	-	-	-	0 (0.0)	≤ 19	11(61.1)	≤ 14

Note: Frequency outside parentheses while percentage in parentheses () and – indicate microbe not tested for resistance.

IV. RESULTS

The test was based on various antibiotics which included amikacin, ceftriaxone, ciprofloxacin, erythromycin, gentamicin, linezolid, penicillin G, oxacillin, tetracycline, vancomycin, amoxicillin-clavulanate, ampiclox, clindamycin, cefipime and imipenem. Table I results indicated that out of the 40 patients from whom *S.aureus* was isolated, resistance was 3(7.5%) to amikacin, 11(27.5%) to ceftriaxone, 5(12.5%) to ciprofloxacin, 6(15.0%) to erythromycin, 21(52.5%) to ofloxacin, 3(7.5%) to gentamycin, 7(17.5%) to linezolid, 40(100.0%) to penicillin G and oxacillin, 27(67.5%) to tetracycline and 19(47.5%) to vancomycin. Out of the 13 patients from whom *E. coli* was isolated, resistance was 1(7.7%) to ciprofloxacin, erythromycin and tetracycline, 0(0.0%) to gentamycin and imipenem, 9(69.2%) to amoxyclav and ampiclox, 11(84.6%) to cefepime and 3(23.1%) to clindamycin.

The 22 patients from whom *K.pneumoniae* was isolated, resistance was 7(31.8%) to ciprofloxacin, 13(59.1%) to erythromycin, clindamycin and tetracycline, 5(22.7%) to gentamycin, 0(0.0%) to imipenem, 20(90.9%) to amoxyclav and ampiclox and 19(86.4%) to cefepime. For the 24 patients from whom *P.aeruginosa* was isolated, resistance was 3(12.5%) to amikacin and gentamycin, 0(0.0%) to ciprofloxacin, 10(41.7%) to erythromycin, 22(91.7%) clindamycin, 2(8.3%) to imipenem, 14(58.3%) to amoxyclav, 15(62.5%) ampiclox and 7(29.2%) to cefepime while for the 18 patients from whom *Proteus species* was isolated, resistance was 5(27.8%) to ciprofloxacin, 11(61.1%) to erythromycin, clindamycin and tetracycline, 4(22.2%) to gentamycin, 0(0.0%) to imipenem, 16(88.9%) to amoxyclav and ampiclox and 15(83.3%) to cefepime.

V. DISCUSSION

The study findings showed that the resistance patterns of *S.aureus, E.coli, K.pneumoniae, Proteus* species and *P.aeruginosa* varied from one antibiotic to another. *S.aureus* was resistant to amikacin, gentamicin, ciprofloxacin, erythromycin, linezolid, ceftriaxone, vancomycin, ofloxacin, tetracycline, penicillin G and oxacillin. However, amikacin and gentamicin were the most effective antimicrobial agents for treating S.*aureus* followed by ciprofloxacin, erythromycin, linezolid, ceftriaxone and vancomycin respectively. On the other hand ofloxacin, tetracycline, penicillin G and oxacillin showed highest resistance rates thus not most effective antimicrobial agents to treat *S.aureus.* The resistance patterns conform to the findings of [27] who established that in Nepal amikacin and gentamycin were the most effective antibiotics. Although the results are also similar to [27] and [28] findings in Libya and Ethiopia relating to lower resistance to ciprofloxacin and amikacin, there is a contradiction in relation to vancomycin, tetracycline and erythromycin where they established higher resistance. The variance in the resistance patterns of *S.aureus* was driven by the patient's smoking habit, age, marital status, education level, patient setting and regular hospital visit.

E.coli was not resistant to imipenem and gentamicin while it was resistant to ciprofloxacin, tetracycline, erythromycin, clindamycin, amoxicillin clavulanic, ampiclox and cefepime. This implied that imipenem and gentamicin were the most effective antimicrobial agents for treating *E.coli* followed by ciprofloxacin, tetracycline, erythromycin and clindamycin respectively. On the other hand, amoxicillin clavulanic, ampiclox and cefipime showed higher resistance rates thus not effective regimens for treating *E.coli.* The variance in resistance patterns of *E.coli* to the various antibiotics was defined by the patient's age, education level and alcohol drinking. Although the results contradicted [28] findings on resistance of erythromycin in Libya, the resistance patterns conform to the findings of [28], [29] who established that in India, Libya and Sudan respectively ciprofloxacin and imipenem were the most effective antibiotics and [29] who established that clindamycin was the most effective antibiotic at KNH while [30] established that in Nepal gentamycin was effective.

K.pneumoniae was resistant to imipenem, gentamicin, ciprofloxacin, erythromycin, clindamycin, tetracycline, cefipime, amoxicillin clavulanic and ampiclox. This was an indication that imipenem was the most effective antimicrobial

regimen for treating K.*pneumoniae* followed by gentamicin and ciprofloxacin. On the other hand, tetracycline, erythromycin, clindamycin, cefipime, amoxicillin clavulanic and ampiclox showed higher resistance rates thus not effective regimens to treat *K.pneumoniae.* The varying resistance rates of *K.pneumoniae* from one antibiotic to another were attributed to the patient's gender, education level and smoking habit. Although the outcome contradicts [30] and [31] who established that clindamycin was the most effective antibiotic at KNH. The resistance patterns conform to the findings of [32] who established that in India, Libya and Sudan respectively ciprofloxacin and impenem were the most effective antibiotics while [32] established that in Nepal gentamycin was effective.

Proteus species was not resistant to imipenem but resistant to gentamicin, ciprofloxacin, erythromycin, clindamycin, tetracycline, cefipime, amoxicillin clavulanic and ampiclox which indicated that imipenem was the most effective antibiotic for treating *Proteus* species followed by gentamicin and ciprofloxacin respectively. On the other hand, tetracycline, erythromycin, clindamycin, cefipime, amoxicillin clavulanic and ampiclox showed higher resistance rates thus not effective regimens for treating *Proteus* species. The varying resistance rates of *Proteus* species from one antibiotic to another were attributed to the patient's age, gender, and education level. Although the outcome contradicts [33] and [34] who established that clindamycin was the most effective antibiotic at KNH. The resistane patterns conform to the findings of [34] who established that in Nepal gentamycin was effective.

P.aeruginosa showed non-resistance ciprofloxacin but resistance to imipenem, amikacin, gentamicin, cefepime, erythromycin, amoxicillin clavulanic, ampiclox, tetracycline and clindamycin. This revealed that ciprofloxacin, imipenem, amikacin, gentamycin and cefipime were the most effective antibiotics for treating *P.aeruginosa*. However, amoxicillin clavulanic, ampiclox, tetracycline and clindamycin were not as effective given their higher resistance rates. The resistance pattern of P.aeruginosa to antibiotics was dependent on the patient's age, marital status, education level, patient setting, hospital visit, drug uptake. The results contradicted [34] findings who established that clindamycin was the most effective antibiotic at KNH. The resistance patterns conform to the findings of [35] who established that in India, Libya and Sudan respectively ciprofloxacin and impenem were the most effective antibiotics while [36] established that in Nepal gentamycin was effective.

VI. Conclusion

S.aureus was less resistant to amikacin and gentamicin; *E.coli* non-resistant to imipenem and gentamicin*; K. pneumoniae* and *Proteus* spp not resistant to imipenem while *P.aeruginosa* was non-resistant to ciprofloxacin. The resistance pattern of *S.aureus* was mainly dependent on the patient's smoking habit, age, marital status, education level, patient setting and regular hospital visit.

The study recommends that JOOTRH, Kenya to adopt amikacin and gentamicin for *S.aureus*, imipenem and gentamicin for *E.coli*, imipenem for *K. pneumonia and Proteus* spp and ciprofloxacin for *P.aeruginosa* as first line antimicrobial regimens for treatment.

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Conflict of Interest

Authors declare that they do not have any conflict of interest.

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