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Phenotypic Profiling of Selected Bacterial Microbes Isolated from Wounds of Diabetic Patients at Jaramogi Oginga Odinga Teaching & Referral Hospital, Kenya

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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Original Research Article

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ABSTRACT

Aims: To phenotypically profile selected bacteria microbes, determine antimicrobial resistance patterns and genetic diversity of resistant genes encoding extended spectrum β-lactamases in bacteria isolated from wounds of diabetes mellitus patients at Jaramogi Oginga Odinga Teaching and Referral Hospital (JOOTRH), Kenya.

Study Design: A hospital based cross sectional study design.

Place and Duration of Study: Jaramogi Oginga Odinga Teaching and Referral Hospital, Kenya between September, 2019 and February, 2020.

Methodology: Pus swabs were collected for isolation of bacteria using conventional techniques and serology involving 117 patients. Samples collected were inoculated onto MacConkey agar, pseudomonas agar media (cetrimide) and blood agar plates then incubated aerobically at 37°C for 18hours to 24hours. Inoculated plates were examined for growth after 24-48 hours. After attaining pure colonies, Gram staining, colony morphology, and biochemical testing were done. Biochemical

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tests involving Catalase, Coagulase, Indole, Oxidase, Urease, Citrate utilization tests were done to identify specific bacterial species according to microbiological standard procedures.
Results: The results of analysis showed that the prevalence of Gram positive cocci isolated from diabetic wounds was 34.2% while the prevalence for Gram negative rods was 65.8%. The biochemically profiled bacterial microbes were *S. aureus, E. coli, P. aeruginosa*, *K. pneumoniae* and *Proteus* species. *S. aureus* was the most commonly isolated bacteria with a prevalence of 34.2%. *P. aeruginosa* had a prevalence of 24(20.5%), *K. pneumoniae* 22(18.8%), *Proteus* species 18(15.4 %) and *E. coli* 13(11.1%). More importantly, the prevalence basing on chi-square results was significantly associated with education level, age, smoking habit, patient setting, drug uptake and hospital visit at 5% level of significance ($p < 0.05$).
Conclusion: Diabetic patients with wounds at JOOTRH, Kenya are more exposed to Gram negative rods infection. The most prevalent Gram negative rod was *P. aeruginosa* with Its prevalence associated with the patient's education level, smoking habit, patient setting, irregular hospital visit and adherence to taking of prescribe antibiotics regularly. Therefore, JOOTRH, Kenya healthcare providers are urged to initiate wound infection preventive measures that may include awareness campaigns on the importance of wound management, regular hospital visits, stopping smoking and regular uptake of prescribed drugs.

Keywords: Phenotypic profiling; selected bacterial microbes; diabetic patients.

1. INTRODUCTION

Diabetes mellitus (DM) is a chronic metabolic ailment having numerous causes characterized by increased blood sugar with instabilities of carbohydrates, fats and proteins absorption that occur due to deficiencies in the secretion and action of insulin [1]. Diabetes has emerged as the new global epidemic, particularly in the developing world, in which individuals living with the disease continue to increase at a startling rate in comparison to high income countries [2]. Diabetic patients' world over in 2013 stood at 382 million, which is estimated to rise to nearly 592 million by 2035 [3]. The estimated prevalence of diabetes in adults aged 20–79 years has more than tripled, from an estimated 151 million (4.6% of the global population) to 537 million (10.5%) in 2021 and without sufficient action to address the situation, it is predicted that 783 million people will have diabetes by 2045 (12.2% of the population) [4]. Like other parts of the world, Africa has a rising prevalence of diabetes besides other non-communicable diseases. For example, in the year 2010, more than 12 million people were projected as being diabetic in Africa, while the number is estimated to rise to 23.9 million in the year 2030 [5]. In 2021, according to [4], the prevalence of diabetes in Africa among adults aged 20 –79 years was 23.6 million (4.5%) and without sufficient action to address the situation, it is predicted that 54.9 million people will have diabetes by 2045, a prevalence of 5.2%.

More than half of admissions and deaths in Kenyan hospitals are attributed to non – communicable diseases with diabetic patients being the majority [3]. In the year 2021, occurrence of diabetes in Kenya stood at 3.0% a rise from 750,000 individuals in 2015 to 821,500 adults in 2021 [3,2]. Statistics from the Public Health directorate in Kenya as stated by [6] specified that, the number of Kenyans with diabetes is projected to increase to 1.5 million (4.5% of inhabitants) by 2025. Jaramogi Oginga Odinga Teaching & Referral Hospital (JOOTRH) is one of the high volume hospitals located in the western part of Kenya that has over 5 million people [2,7]. County Government of Kisumu ranks diabetes amongst the top most cause of death with about 10 diabetic patients with wounds visiting the referral hospital on a daily basis. The rising incidence of diabetes exposes patients to long term problems such as wounds, cardiac and kidney difficulties [3]. The development of wounds is a serious complication for patients with diabetes [8]. The likelihood of a diabetic individual having a wound can be more than 25% [9,10,11]. Prevalence of diabetic wounds has been estimated at 20.4 % in Netherlands, 4.10% in America and about 4.7 % among the Kenyan population [12]. The prevalence of *S. aureus* in diabetic wounds in Vihiga County is estimated at 62.3% [13].

Amputations in diabetics are normally triggered by long-lasting wounds, well-defined according to [14] as a wound does not show any reduction to 20 % to 40 % size after two to four weeks of optimum management. Infections of these wounds are often polymicrobial where Gram positive bacteria coexist with Gram negative organisms which complicate the long-standing ulcers making them extremely difficult to heal [15,16,17]. Although phenotypic profiling varies in different areas, common bacteria identified include *S. aureus*, Enterobacteriaceae and *P. aeruginosa* which have proved to be multidrug resistant [12,1,3].

Several studies phenotypically profiling bacterial microbes colonizing diabetic wounds have been conducted all over the world which include [9,1,18,19,3] Majority of these studies established that Gram negative bacteria as the most occurring organisms than Gram positive bacteria. [12,1] indicated *Staphylococcus aureus* to be the most predominant pathogen, [3] noted *Enterococcus faecalis* and *Escherichia coli* as the predominant pathogens while [9,19] indicated *Pseudomonas aeruginosa* as the most predominant pathogen. It is also noted that studies conducted in Kenya like [12,20,21] focused on diabetes management, education and foot problems among diabetic patients. This implies uncertainty with regard to the most predominant bacterial microbe colonizing diabetic patient's wounds which might be complicating the treatment of diabetic wounds due to inaccurate diagnosis of the predominant pathogens justifying the study.

1.1 Statement of the Problem and Justification

Wound colonization is more often polymicrobial, encompassing many microorganisms that are possibly pathogenic increasing the risk of wound infection that prolongs healing [22].To reduce the burden of diabetes mellitus complications which pose severe threats to health security an understanding of the predominant bacterial microbe isolated from wounds of diabetes mellitus patients are necessary. Several studies to identify the prevalence have been carried out, however, it has been noted that the studies including those conducted in Kenya showed contradicting views with regard to most phenotypically profiled bacteria colonizing wounds. This makes it impossible to single out the most prevalent bacterial microbe thus calling for continued research. This then justified a study to phenotypically profile selected bacterial microbes isolated from wounds of diabetic patients at Jaramogi Oginga Odinga Teaching & Referral Hospital, Kenya.

Vulnerability to infections by diabetic patients is due to high sugar levels, reduced immune response as well as diminished blood movement to extremities that cause slow wound healing process [23, 24]. For example, 25% of diabetic patients with wounds get contagions with antibiotic resistant bacteria, which dictate amputations [24]. Infections and serious metabolic problems are the major causes of death in Africa where for example, Diabetic ketoacidosis (DKA) accounted for 8 % of hospital admission at Kenyatta National Hospital, 30 % of patients passed on in 48 hours of presentation [25]. Diabetic wounds are observed regularly at tertiary clinics in Kenya and are linked with poor glycemic control, infections, hypertension and dyslipidemia [25].

To isolate the predominant organisms colonizing wounds, [1] in his study conducted in Western India by focusing on diabetic foot ulcers indicated that various Gram positive and negative bacteria were isolated which included *Staphylococcus aureus* (21.8%), *Enterococcus faecalis* (4.6%), *Pseudomonas aeruginosa* (18.2%), *Escherichia coli* (13.6%), *Proteus spp* (9.1%), *and Acinetobacter baumannii* (7.3%), *Klebsiella pneumoniae* (6.4%). It was noted that although Gram negative rods were the most prevalent than Gram positive organisms, *Staphylococcus aureus* remained a predominant pathogen. [3], in their study in South India on pathogens colonizing diabetic foot ulcers established that 54.9% of pathogens isolated were Gram negative bacteria while 38.1% were Gram positive bacteria with *Enterococcus faecalis* and *Escherichia coli* as the most common pathogens isolated at 12.1% each. Unlike [1,19,3] in their studies to isolate the most common bacteria in India found out that *Pseudomonas aeruginosa* and *Klebsiella pneumonia* as the major pathogens respectively. Although the studies focused on the same target population, country they did not consent on which specific Gram positive or negative pathogen that was most predominant. This might be ascribed to the dissimilarity in environmental distribution.

Studies singled out from Iraq such as [9,18] to understand diabetic foot contaminations indicated in the findings that *Psuedomonas aeruginosa* and *Staphylococcus aureus* as the leading microorganism in occurrence respectively. A keen observations of the studies showed that the groups were from different localities that is urban and rural which begs the question whether the place one resides determines the microorganism that can colonize his/her wound.

There is continued debate on the predominance of bacteria colonizing wounds in Africa. Different studies outline conflicting views on the most occuring microorganism. For instance, [26,6,27] who researched in Nigeria, Libya and Ethiopia respectively found Gram positive cocci, specifically *Staphylococcus aureus* to be the most frequent. On the other hand, [28] in Sudan found Gram negative rods and specifically *Pseudomonas aeruginosa* as the pathogen that most commonly inhabit wounds. The divergent results in the different African countries indicate the uncertainty in making conclusions to which pathogen is most prevalent. Thus the need for similar studies in Kenya to establish predominant pathogens isolated from wounds of diabetic patients.

Studies done in Kenya by [29,12,21] at Kenyatta National Hospital show some evidence of pathogens colonizing wounds. For instance, [12] found *Proteus* to be the most common Gram negative, [29] identified *Staphylococcus aureus* to be predominant while [21] got *Pseudomonas* spp as the leading bacteria. Although the research focused on KNH, the differences may be a pointer to other underlying factors that determine the spread of bacteria making it difficult to generalize the results to diabetic patients with wounds at JOOTRH.

From the reviews, several studies to classify the prevalence of Gram positive and negative bacteria colonizing wounds have been undertaken all over the world. Majority concluded that Gram positive pathogens were most predominant as compared to Gram negative pathogens. Studies conducted in Kenya showed divergent views indicating uncertainty in relation to the most common bacteria colonizing wounds. This makes it difficult to make a conclusion as to whether Gram positive or negative bacteria are the most prevalent among diabetic patients with wounds at JOOTRH thus a justification for the study to identify selected Gram positive and negative bacteria isolated from wounds of diabetic patients at Jaramogi Oginga Odinga Teaching & Referral Hospital, Kenya.

2. MATERIALS AND METHODS

Pus swab samples collected from the diabetics wound were inoculated onto MacConkey agar, pseudomonas agar media (cetrimide) and blood agar plates then incubated aerobically at 37°C for 18hours to 24hours. All the inoculated plates were examined for growth after 24 hours to 48 hours. The inoculated plates before and after growth are shown in Figs. 1 and 2, respectively.

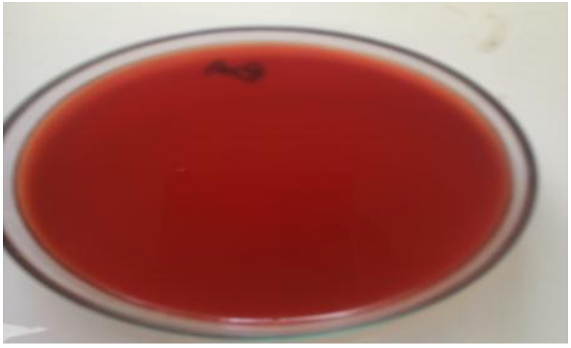

Fig. 1. Inoculated plate before growth

Fig. 2. Inoculated plate after growth

After attaining pure colonies, more procedures were performed by means of the standard microbiological practices, which included Gram staining, colony morphology, and biochemical testing. Biochemical tests were done to identify specific bacterial species. Biochemical tests involving Catalase, Coagulase, Indole, Oxidase, Urease, Citrate utilization tests were done according to microbiological standard procedures.

2.1 Catalase Test

The test involved emulsification of Gram positive colonies in two drops of hydrogen peroxide on a microscope slide. Presence of effervescence indicated colonies for Staphylococcus.

2.2 Coagulase Test

The test distinguished pathogenic *S. aureus* from other Staphylococcus species. 0.3 milliliters of citrated rabbit plasma was put into two test tubes and labeled as control and sample. Tube labeled as sample was inoculated with a colony of Gram positive cocci and mixed by shaking to make a cloudy suspension. On the other hand, the control tube was inoculated with *S. aureus* ATCC 25923 and both tubes incubated at 37°C for 1hour to 4 hours. A clot as depicted in Fig. 3 represented a positive coagulase test.

Fig. 3. A positive coagulase test

2.3 Sugar Fermentation Test

The test was employed to confirm all the Enterobacteriaceae isolates. An inoculum from the pure culture was transferred to a sterile tube having phenol red mannitol broth and incubated at 35-37^{0}C for 18 hours to 24 hrs. Presence of enterobacteriaceae altered the colour of the broth from red to yellow, showing acid-gas production.

2.4 Indole Test

To test the ability of the organism to degrade the amino acid tryptophan and produce indole, motility indole urea medium was inoculated with a small amount of a Gram negative pure culture, incubated at 37°C for 24 to 48 hours using *Escherichia coli* ATCC 35218 indole as a positive control. 1ml of Kovac's reagent was added to the overnight growth. A positive indole test as in Fig. 4 was indicated by the formation of a cherry red ring on top of the medium in 2 to 10 minutes.

2.5 Citrate Utilization Test

The test is used to identify *Klebsiella pneumoniae* that can utilize citrate as the sole carbon and energy source when inoculated on Simmons citrate medium. *Klebsiella pneumoniae* ATCC 700603 was used as positive control. Using a needle, a fresh 16 to 18 hour pure culture was used as an inoculation source. A single isolated colony was picked and lightly streaked on the surface of the slant of Simmons citrate agar, and incubated at 37°C for 18 to 48 hours. Citrate positive growth visible on the slant surface of the medium with an intense Prussian blue indicated the presence of *Klebsiella pneumoniae* as in Fig. 5 while a negative citrate test was shown by no colour change as depicted in Fig. 6.

Before **After**

Fig. 4. Indole test (Before and after addition of Kovac's reagent)

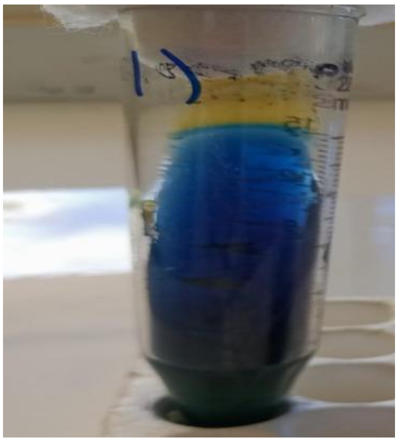

Fig. 5. Citrate utilization positive test

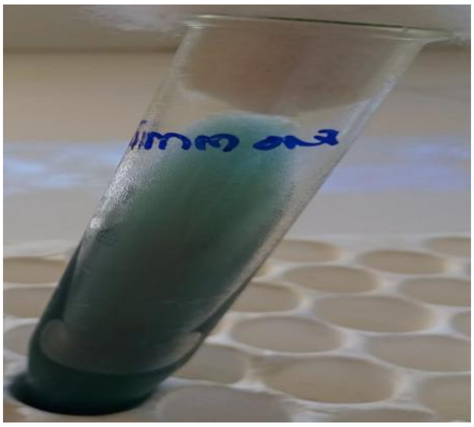

Fig. 6. Citrate utilization negative test

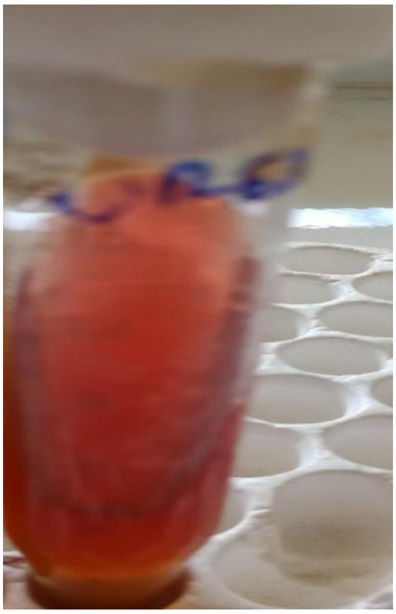

Fig. 7. Urease positive

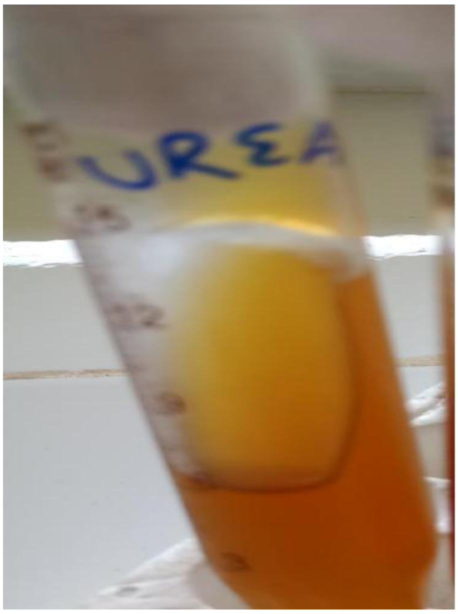

Fig. 8. Urease negative

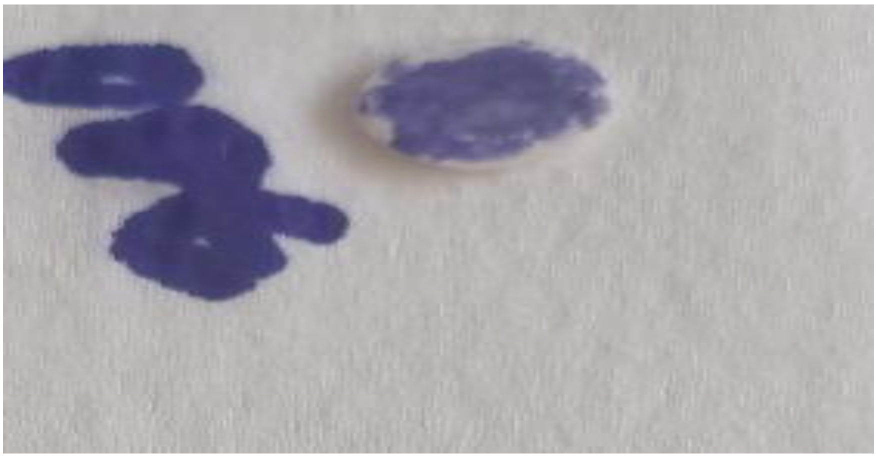

Fig. 9. Oxidase positive

2.6 Urease Test

Proteus species hydrolyzes urea to produce ammonia and CO_2. Ammonia changes the color of Christensen's urea agar slant from light orange to pink. *Proteus mirabillis* ATCC 29906 was used as positive control. An inoculum from an 18 to 24 hour pure culture, was inoculated on the slant surface of christensen's urea agar and incubated at 37^oC. The color change on the slant was observed after 6 hours to 24 hours. Urease positive and negative results were shown by a bright pink color and no colour change on the slant as in Figs. 7 and 8 respectively.

2.7 Oxidase Test

This test is used in identification of *Pseudomonas aeroginosa* which has oxidase enzyme that catalyzes oxidation of cytochrome c, thus turning the impregnated oxidase filter paper from white to deep purple. *Pseudomonas* ATCC 27853 was used as a positive control. Using a loop, a well-isolated colony was picked from a fresh 18 to 24 hour culture bacterial plate and rubbed onto oxidase filter paper for colour change observation. Oxidase positive was shown by colour change from white to dark purple within 5 to 10 seconds as in Fig. 9.

3. RESULTS AND DISCUSSION

3.1 Demographic and Clinical Characteristics

The study analyzed the distribution of patient's based on gender, marital status, level of education and age. The results of analysis as depicted in Table 4 show that out of a Total 117 sampled patients, 58 (49.6%) were male, 59 (50.4%) female, 3 (2.6%) single, 99 (84.6 %) married, 15 (12.8%) widowed, 11 (9.4%) had no education, 50 (42.7%) primary level education, 38 (32.5%) secondary level education and 18 (15.4%) had college/university education. This was an indication that females, married and those patients with none or primary level of education are at a greater risk of developing wounds.

The results of analysis on smoking, drinking, patient setting, sample testing, terminal illness and hospital visit as in Table 1 show that out of a total of 117, non-smokers were 87 (74.4%), smokers 30 (25.6%), non-drinkers 85 (72.6%), alcohol drinkers 32 (27.4%), inpatient 68 (58.1%), outpatient 49 (41.9%), samples not tested 106 (90.6%), samples tested 11 (9.4%), no terminal illness 50 (42.7%), terminal illness 67 (57.3%) where 12 (10.3%) had asthma while 55 (47.0%) had hypertension. 55 (47.0%) do not visit the hospital regularly whereas 62 (53.0%) visit the hospital regularly as prescribed. Test results on duration with diabetes as in Table 2 showed a minimum of 1 year, a maximum of 14 years, a mean of 4.46 years with a standard deviation of 3.29 years while for the period a patient had been on antibiotics since developing a wound had a minimum of 0.1 months, a maximum of 36 months, a mean of 9.99 months and a standard deviation of 8.84. Based on the mean, diabetic patients with wounds have had diabetes for approximately 4.5 years and have been on antibiotics for an average period of approximately 10 months.

The study also computed the measures of central tendency to summarize data for the age variable and measures of dispersion to understand the variability of scores for the patient's age. Results of analysis as depicted in Table 2 showed a minimum age of 22 years and a maximum of 67 years having a mean of 54.41 years and a standard deviation of 8.828. From the mean, it is evident that the average age for diabetic patients with wounds is 54.41 years. However, younger persons are also diabetic with wounds given the minimum age of 22 years. A large standard deviation value shows large variance in the ages of the patients.

3.2 Selected Bacterial Microbes Isolated from Wounds of Diabetic Patients

3.2.1 Morphological profiling of bacterial microbe isolated from diabetic wounds

Descriptive statistics analysis was conducted to phenotypically profile the selected bacterial microbe. The results of analysis as depicted in Table 3 from a total of 117 samples, Gram positive cocci were 40 (34.2%) and Gram negative rods 77 (65.8%). This was an indication that the prevalence of Gram positive cocci isolated from diabetic wounds was 34.2% while the prevalence for Gram negative rods was 65.8%.

3.2.2 Biochemical Profiling of Bacterial Microbe Isolated from Diabetic Wounds

The biochemically profiled bacterial microbes as depicted in Table 4, were *S. aureus, E. coli, P.*

aeruginosa, *K. pneumoniae* and *Proteus* species. *S.aureus* was the most commonly isolated bacteria with a prevalence of 40(34.2%). *P. aeruginosa* had a prevalence of 24(20.5%), *K. pneumoniae* 22(18.8%), *Proteus* species 18(15.4%) and *E. coli* 13(11.1%).

More importantly, the prevalence basing on chi-square results in Table 5 was significantly associated with education level, age, smoking habit, patient setting, drug uptake and hospital visit at 5% level of significance ($p < 0.05$). Patients with lower level of education, those aged between 40-59 years, non-smokers, those on outpatient setting, irregular hospital visits and drug uptake were more exposed to bacterial infection. Out of the 40 patients from whom Gram positive cocci were isolated, 23(57.5%) had primary level education, 11(27.5%) secondary level education, 6(15.0%) college/university education, 3(7.5%) were aged below 40 years, 25(62.5%) between 40-59 years, 12(30.0%) had 60 and above years. 5(12.5%) were smokers, 35(87.5%) non-smokers, 19(47.5%) inpatient, 21(52.5%) outpatient, 16(40.0%) visited the hospital regularly, 24(60.0%) visited the hospital irregularly, 8(20.0%) took prescribed drugs irregularly and 32(80.0%) took prescribed drugs irregularly.

Table 1. Demographic and clinical characteristics of study participants (Categorical Data)

Characteristic	Frequency (n)	Percent (%)
Gender		
Male	58	49.6
Female	59	50.4
Total (N)	117	100.0
Marital Status		
Single	3	2.6
Married	99	84.6
Widowed	15	12.8
Total (N)	117	100.0
Level of Education		
None	11	9.4
Primary	50	42.7
Secondary	38	32.5
College/University	18	15.4
Total (N)	117	100.0
Smoking		
Yes	30	25.6
No	87	74.4
Total (N)	117	100.0
Drinking		
Yes	32	27.4
No	85	72.6
Total (N)	117	100.0
Patient Setting		
In patient	68	58.1
Out patient	49	41.9
Total (N)	117	100.0
Sample Testing		
Yes	11	9.4
No	106	90.6
Total (N)	117	100.0
Terminal Illness		
Yes	67	57.3

Characteristic	Frequency (n)	Percent (%)
No	50	42.7
Specific Terminal Illness		
Asthma	12	10.3
Hypertension	55	47.0
None	50	42.7
Total (N)	117	100.0
Regular Hospital Visit		
Yes	62	53.0
No	55	47.0
Total (N)	117	100.0

Table 2. Clinico-demographic characteristics (Continuous Data)

Characteristic	N	Minimum	Maximum	Mean	Std. Deviation
Age	117	22	67	54.41	8.828
Years with Diabetes	117	1	14	4.46	3.29
Months with Wound	117	1	60	13.00	13.14
Months on Antibiotics	117	0.1	36.0	9.99	8.84

Table 3. Morphological profiling of bacterial microbe isolated from diabetic wounds

Cell Shape	Cell Colour	Gram Stain Reaction	Frequency (n)	Percent (%)
Cocci	Purple/Blue	Positive	40	34.2
Rods	Red/Pink	Negative	77	65.8
Total (N)			117	100.0

Table 4. Biochemical profiling of bacterial microbe isolated from diabetic wounds

Test	Inference	Bacterial Microbe	Frequency (n)	Percent (%)
Coagulase	Clot formation	*S. aureus*	40	34.2
Indole	Cherry red ring on top surface	*E. coli*	13	11.1
Citrate Utilization	Intense Prussian blue colour	*K. pneumoniae*	22	18.8
Urease	Bright pink colour	*Proteus* species	18	15.4
Oxidase	Dark purple colur	*P. aeruginosa*	24	20.5
Total (N)			**117**	**100.0**

On the other hand, out of the 77 patients from whom Gram negative rods were isolated, 11(14.3%) had no education at all, 27(35.1%) primary level education, 27(35.1%) secondary level education, 12(15.6%) college/university education, 0(0.0%) were aged below 40 years, 49(63.6%) between 40-59 years, 28(36.4%) had 60 and above years, 25(32.5%) smokers, 52(67.5%) non-smokers, 49(63.6%) inpatient, 28(36.4%) outpatient, 38(49.4%) visited the hospital regularly, 39(50.6%) visited the hospital irregularly, 29(37.7%) took prescribed drugs irregularly and 48(62.3%) took prescribed drugs irregularly. This implied that Gram negative rods were mostly isolated from non-smokers, those admitted, patients visiting the hospital and taking prescribed drugs irregularly against the doctor's recommendation.

The finding that Gram negative rods were most prevalent than the Gram positive cocci which was associated with education level, smoking habit, patient setting, hospital visit and adherence to taking of prescribed antibiotics regularly with *S.aureus* as the most prevalent pathogen followed by *P. aeruginosa*, *K. pneumonia, Proteus* spp and *E.coli* conforms to the findings of [1], [3], Mukhtar and Saeed [28] in their studies conducted in Western India, South India and Sudan respectively.

Table 5. Prevalence of Gram positive cocci (GP) and Gram negative rods (GN)

	Gender (n)		**Marital Status (n)**				**Education (n)**				**Age (n)**			**Total (N)**
	Male	**Female**	**Single**	**Married**	**Widowed**	**Divorced/ Seperated**	**None**	**Primary**	**Secondary**	**College/University**	**Below 40**	**41-59**	**60 and above**	
GP(n)	19(47.5%)	21(52.5%)	3(7.5%)	32(80.0%)	5(12.5%)	0(0.0%)	0(0.0%)	23(57.5%)	11(27.5%)	6(15.0%)	3(7.5%)	25(62.5%)	12(30.0%)	**40(34.2%)**
GN(n)	39(50.6%)	38(49.4%)	0(0.0%)	67(87.0%)	10(13.0%)	0(0.0%)	11(14.3%)	27(35.1%)	27(35.1%)	12(15.6%)	0(0.0%)	49(63.6%)	28(36.4%)	**77(65.8%)**
Chi-sq [P-value]	**0.104[0.75]**		**5.933[0.06]**				**9.285 [0.03]***				**6.092 [0.04]***			
	Smoking (n)		**Alcohol (n)**		**Patient Setting (n)**		**Hospital Visit (n)**		**Drug Uptake (n)**					**Total (N)**
	Yes	No	Yes	No	Inpatient	Outpatient	Regular	Irregular	Regular	Irregular				
GP(n)	5(12.5%)	35(87.5%)	11(27.5%)	29(72.5%)	19(47.5%)	21(52.5%)	16(40.0%)	24(60.0%)	8(20.0%)	32(80.0%)				**40(34.2%)**
GN(n)	25(32.5%)	52(67.5%)	21(27.3%)	56(72.7%)	49(63.6%)	28(36.4%)	38(49.4%)	39(50.6%)	29(37.7%)	48(62.3%)				**77(65.8%)**
Chi-sq [P-value]	**5.505 [0.02]***		**0.001[0.10]**		**3.916 [0.04]***		**4.746 [0.02]***		**12.642 [0.00]***					
Total														**117(100.0%)**

*Note. n refers to total diabetes mellitus patients with wounds infected with bacteria. * indicates significance at 5% level significance*

4. CONCLUSION

The prevalence of Gram negative rods isolated from diabetic wounds was 65.8% and Gram positive cocci 34.2% while the prevalence for Gram negative rods was 65.8%. *Staphylococcus aureus* was the only Gram positive bacteria isolated and it accounted for 34.2% prevalence followed by *P. aeruginosa* Gram negative at 20.5%. In conclusion, diabetic patients with wounds at JOOTRH, Kenya are more exposed to Gram negative rods infection. The most prevalent Gram negative rod was *P. aeruginosa.* Its prevalence was associated with the patient's education level, smoking habit, patient setting, irregular hospital visit and adherence to taking of prescribe antibiotics regularly. Therefore, JOOTRH, Kenya healthcare providers to initiate wound infection preventive measures that may include awareness campaigns on the importance of wound management, regular hospital visits, stopping to smoke and regular uptake of prescribed drugs.

CONSENT

All authors declare that 'written informed consent was obtained from the patient for publication of this case report and accompanying images. A copy of the written consent is available for review by the Editorial office/Chief Editor/Editorial Board members of this journal.

ETHICAL APPROVAL

All authors hereby declare that all experiments have been examined and approved by the appropriate ethics committee and have therefore been performed in accordance with the ethical standards laid down in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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