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THE BACTERIA PROFILES OF WOUNDS IN DIABETIC PATIENTS HOSPITALIZED IN NORTHERN KWAZULU-NATAL, SOUTH AFRICA

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ABSTRACT
Diabetic wound infections still remain a health concern such that correct identification of bacteria is essential in monitoring the spread of the infections as well as in the administration of the correct treatment. This study therefore focuses on isolating and identifying bacteria present in diabetic wounds of hospitalized patients in northern KwaZulu-Natal and assessing their distribution.The wound specimen were collected and swabbed onto selective and differential media. The bacteria identities were presumptively ascertained through biochemical characterization (Gram-stain, catalase test, oxidase test and API) and then confirmed through 16S rDNA sequencing.A total of 42 isolates were recovered from 83% of the patients sampled from the three participating hospitals (X, Y, and Z). Gram-negative bacilli from *Enterobacteriaceae*were predominant followed by *Staphylococci spp* and *Enterococcus faecalis*with 43% polymicrobial cases from hospital Z and 29% from hospital X. Distribution of some opportunistic pathogens and nosocomially-acquired pathogens were also observed across the patients with five bacterial identities distributed among hospital X and Z. The adverse effects associated with the recovered bacteria in diabetic wounds pose a serious health concern and preventive measure should be taken.

Keywords: Diabetes mellitus, wounds, bacteria, infection

LES BACTÉRIES ADULTE DES PLAIES CHEZ LES PATIENTS DIABÉTIQUES HOSPITALISÉS DANS LE NORD DU KWAZULU-NATAL, AFRIQUE DU SUD

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Résumé
Les infections de plaiesdiabétiquesdemeurent un problème de santé telsquel'identificationcorrecte des bactériesestessentielledans la surveillance de la propagation des infections ainsiquedansl'administration de l'untraitement correct. Cetteétudeportedoncsurl'isolation et l'identification des bactériesprésentesdans les plaiesdiabétiques de patients hospitalisésdans le Nord du KwaZulu-Natal et l'évaluation de leur distribution. Spécimen de la blessureontétérecueilliesetfrottéesur des médias et du différentiel. Les bactériesontétéidentitésprésuméesrévélée par caractérisationbiochimique (coloration de Gram, catalase, oxydaseet test test API) et ensuiteconfirmé par séquençage de l'ADNR 16s. Un total de 42 isolatsontétéretrouvésdans 83 % des patients échantillonnésdans les troishôpitaux participants (X, Y et Z). Les bacilles à Gram négatifd'Enterobacteriaceaeétaientprédominantessuivies par les staphylocoques et spp Enterococcus faecalis avec 43 % des cas de l'hôpitalpolymicrobial Z et de 29 % de l'hôpital X. La répartition de certainspathogènesopportunistes et nosocomially-pathogènesacquisontétéégalementobservésdans les patients avec cinqidentitésbactériennerépartis entre l'Hôpital X et Z. Les effetsindésirablesassociés à la récupération de bactériesdans les plaiesdiabétiquesposent un grave problème de santé et de préventiondoiventêtreprises.

Mots-clés: diabètesucré, blessures, les bactéries, les infections

INTRODUCTION

Literature abounds with reports of bacterial flora on human skin [1, 2], this predispose patients to an increased risk of being infected by bacteria that are free-living on the skin [3]. However, the type and quantity of the microorganisms serves as an indication of the wound infection [4]. Diabetic wound infection is one of the main chronic complication of diabetes with life-threatening adverse effects in healthcare [3, 5].The increased blood glucose impairs the blood flow, leukocyte function, and chemotaxis of the neutrophils and macrophages [3, 6]. Other factors such as surgical procedures, hospitalization and prolonged antibiotic therapy may predispose patients to infection [7]. Infection is driven by the pathogenicity and virulence of the bacteria [7-9], as some bacteria become more virulent in the presence of high glucose [8]. Diabetic wound infections are normally polymicrobial [9], and this can further compromise the host cell function [4].

Accurate identification of polymicrobial bacterial species present in the wound siteis important in determining the cause and predicting the outcome of an infection [10]. Routine analysis of wound specimen normally involves the use of traditional culture methods such as selective and differential agar media to culture the anaerobic and aerobic bacteria [4]. The organisms are classified by means of similarities and differences based on their phenotypic characteristics such as cell appearance, cell shape, size, pigmentation [11-13]. Gram staining, biochemical tests (catalase and oxidase) and controlled growth conditions are required for definitive grouping of bacteria [12]. Biochemical tests demonstrate the ability of test organisms to degrade specific substrates such as carbohydrates, amino acids, and other organic molecules. Other biochemical tests involve the ability of an organism to grow in the presence of a single nutrient source [13]. The major role played by routine analysis of bacteria in wound care is the appropriate use of antimicrobial agents however, it is essential to correctly identify the microbes to help eliminate healthcare burdens [14].

It has become more difficult to identify polymicrobial bacterial species present in an infection through culture methods [10]. However, with the aid of molecular diagnostic techniques, identification has shown that most chronic wounds are polymicrobial [11]. Culture-based techniques alone often fail to identify fastidious bacteria that are important in diagnosis and they may underestimate microbial diversity [11] while culture-independent methods are able to detect bacterial species that were omitted by culture-based techniques [16]. The ability to characterize bacteria using 16S ribosomal RNA (rRNA)-based phylogenies has enabled a much faster way to identify bacteria and elucidate the role of bacterial pathogens in the development of infectious diseases [16]. The 16S rDNA sequencing surveys only a portion of the microbial genome that encodes the 16S rRNA subunit [17]. This molecular technique determines the nucleotide sequence of ribosomal RNA from various bacteria in order to assess their relative position in the evolutionary order [18], thereby grouping bacterial isolates into taxonomic and phylogenetic groups based on their genetic composition [17]. The significance of 16S rRNA is that it is present in all prokaryotic cells with conserved and variable sequence regions evolving at different rates therefore making it suitable for bacteria identification [19].

The assessment of the bacteria present in wounds is essential, it provides antibiotic therapy guide that can help manage and prevent amputations thereby improving the quality of life [20].Tothe best of our knowledge, South Africa (and indeed the Northern KwaZulu-Natal region) has been minimally represented in similar studies. It is hoped that this study will provide the necessary and essential information in this particular field.

MATERIALS AND METHODS

Specimen Collection

This study was carried out after the approval (UZREC 171110-030 PGM 2015/195) from the ethical committee of the University of Zululand was obtained. The full cooperation of the patients was duly obtained. The wound specimens of 18 hospitalized diabetic patients (diagnosed by medical doctors to be diabetic; 22% male and 78% female) were collected from three different rural-based Northern KZN hospitals in 2015-2016. Hospital X is a district healthcare facility which provides services to the rural community while Hospital Y is a district healthcare center that provides health care service to even some neighboring healthcare institutions and Hospital Z is a regional hospital, providing healthcare service that are of high safety standards and cost effective. The demographic data of patients such as age, gender, and ethnic group were recorded prior to sampling. The medical doctors were responsible for swabbing the wounds after washing them with sterile saline and sterile cotton pads. Sterile swabs were introduced at the base of the wound and then subsequently inserted in Amies transport media to maintain the specimen during transportation to the University of Zululand's biochemistry laboratory [5].

Specimen Isolation

The spread plate method described by Ørskov[21] was used to inoculate the specimen from the swabs

onto the primary media containing plates namely nutrient agar, mannitol salt agar and MacConkey agar exclusively. The plates were incubated at 37°C for 24-48hours, after which successive quadrant streak technique was used to purify the colonies. Pure colonies were kept on nutrient agar plates at 4°C and glycerol stocks at -80°C [5].

Identification of the Isolated Bacteria

Isolates were primarily identified using Gram-staining, [22] morphological characterization (colony shape, size, pigmentation)according to the methods of [13]. Standard biochemical tests such as catalase [23], oxidase [24] were carried out followed by the presumptive identification of bacteria using Analytical Profile Index (API) test kits namely; API 20 Staph, API 20 Strep, API 20E, API 20NE according to the manufacturer's instructions (Biomerieux S.A). The confirmation of the bacteria identities was done using PCR by amplifying the 16S rDNA and analyzing the sequenced products through BLAST Search (NCBI) [25]. Universal PCR primer sequences were used (table 1).

TABLE 1: 16S PRIMERS SEQUENCES

Name of Primers	Target	Sequence (5′ to 3′)
16S-27F	**16S rDNA sequence**	**AGAGTTTGATCMTGGCTCAG**
16S-1492R	**16S rDNA sequence**	**CGGTTACCTTGTTACGACTT**

Data Analysis

Variants were analysed using Graphpad prism version 6, determining the one way ANOVA, two way ANOVA, means and standard deviations.

RESULTS

Data Collection

A Total of 7 patients from hospital X, 4 patients from hospital Y and 7 patients from Hospital Z participated in the study. The classification of the patients sampled is asshown in table 2.

TABLE 2: GENERAL CLASSIFICATION OF THE PATIENTS

Variables	**Mean (%)**
Age (years)	**66.6**
Male gender	**22.2**
Female gender	**77.8**
Wound site (Lower limb)	**94.4**
(Other body parts)	**5.6**

Isolation and Presumptive identification

A total of 42 isolates were recovered from 15 (83%) patients; no isolates were obtained from the wounds of 3 (17%) patients. Fifteen, six and 21 isolates were recovered from hospital X (36%), hospital Y(14%) and hospital Z(50%) respectively. Figure 1. Shows the overall distribution pattern of the isolates from the three hospitals

The isolates classified according to their microscopic morphology during the Gram-staining (figure 2) revealed that Hospital Y had more bacilli (83%) isolates compared to the other hospitals. Cocci isolates were predominant at hospital Z (28.6%) while a cocco-bacillus was only recovered from hospital Y. Figure 3 shows how much of the Gram-positives were isolated in comparison with Gram-negatives.

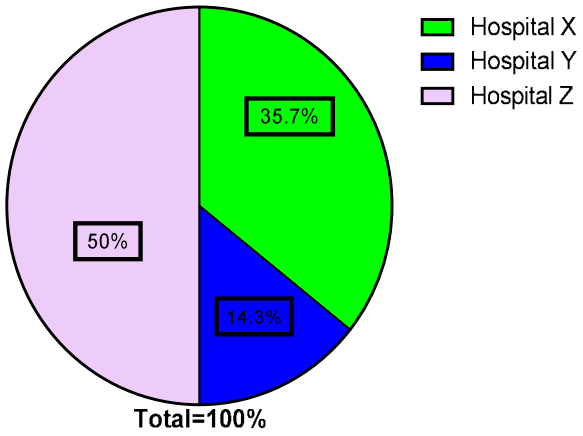
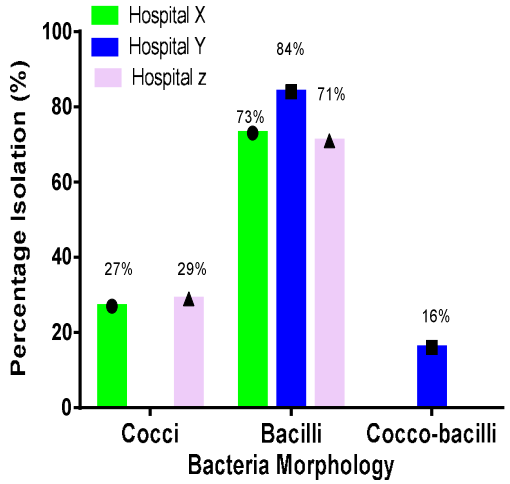


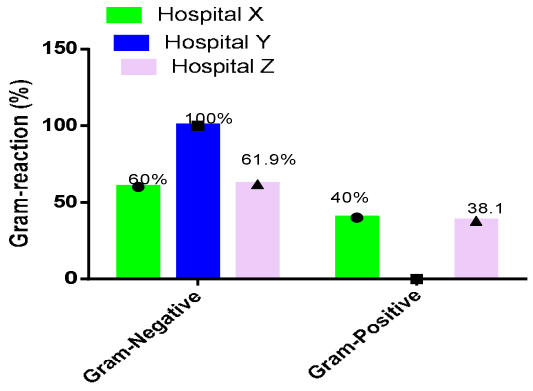
FIGURE 1: THE OVERALL ISOLATION PERCENTAGE ACROSS THE DIFFERENT HOSPITALS.



(Data was subjected to 95% Confidence interval analysis)
FIGURE 2: THE DIFFERENT BACTERIA MORPHOLOGIES ISOLATED FROM THE HOSPITALS

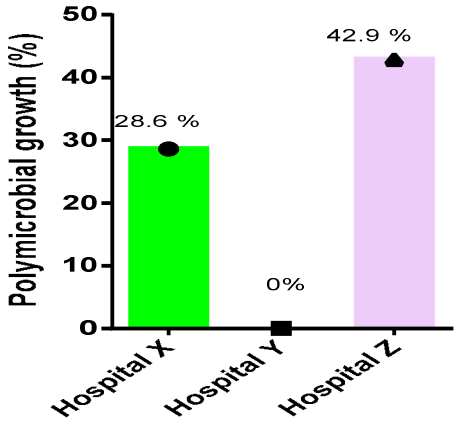
Some wounds were colonized by several types of bacteria, whereby 29% and 43% of the wounds from hospital X and hospital Z were polymicrobial respectively (more than 3 isolates recovered) as indicated in figure 4. No polymicrobial growth was evident from hospital Y patients.

The presumptive identities obtained from API were compared with the 16S rDNA results as shown in table 3 to 5, the observed differences are highlighted in blue. The observed phenotypic differences indicate the anomalies between culture-dependent techniques and 16S rDNA sequencing.

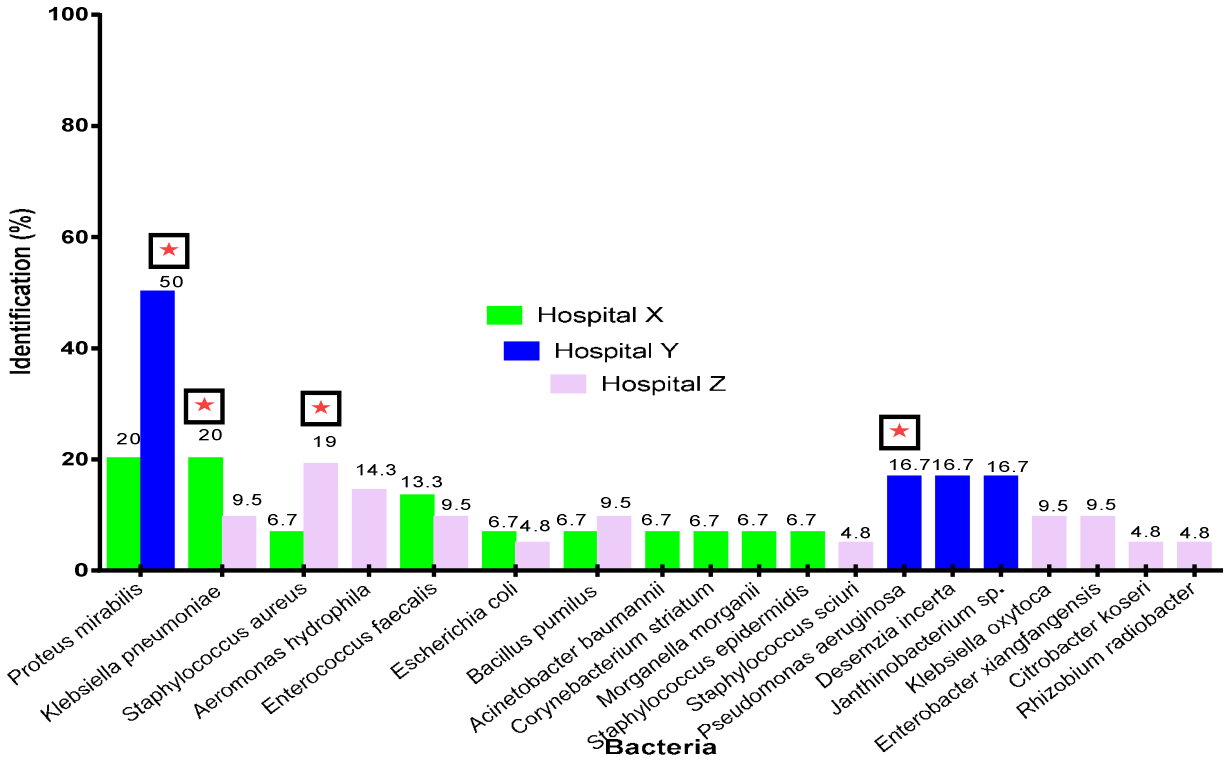


(Data shown to be significantly different through one-way Anova ****, P < 0, 0001)

FIGURE 3: THE GRAM-REACTION OF THE ISOLATES



(Data was subjected to 95% Confidence interval analysis)
FIGURE 4: THE OCCURRENCE OF POLYMICROBIAL GROWTH IN THE DIFFERENT WOUNDS OF HOSPITALIZED PATIENTS



(Data shown to be significantly different through two-way Anova **, P-value = 0.0013)

FIGURE 5: THE PREVALENCE OF BACTERIA ISOLATES RECOVERED FROM THE HOSPITALS UNDER STUDY.

TABLE 3.THE ISOLATES CHARACTERISTICS AND PRESUMPTIVE IDENTITIES FROM HOSPITAL X

Bacterial Isolate	Gram-stain	Morphology	Oxidase test	Catalase test	Presumptive ID	API Identification
*Pat A1	Gram+	cocci	Positive	Positive	*Micrococcus*	*Kocuria varians*
	Gram-	Bacilli	Negative			
Pat A2	Gram -	Bacilli	Negative	Positive	*Enterobacteriaceae*	*Proteus mirabilis*
*Pat B1	Gram-	Coccobacill	Positive	Positive	Non-*E*	*Sphingomonas paucimobilis*
	Gram+	Bacilli				
*Pat B2	Gram-	Bacilli	Positive	Positive	Non-*E*	*Rhizobium radiobacter*
			False Positive	Slow-Positive		
*Pat B4	Gram+	Cocci	Negative	Negative	*Enterococcus*	*Aerococcusviridans*
	Gram-	Bacilli		Slow-Positive		

*Pat B5	Gram+ Gram-	Cocci Bacilli	Positive Negative	Negative Positive	*Enterococcus*	*Globicatellasanguinis*
*Pat B6	Gram -	Bacilli	Positive False- Positive	Positive	Non-*E*	*Burkholderiacepacia*
*Pat C1	Gram -	Bacilli	Positive	Positive	Non-*E*	*Burkholderiacepacia*
*Pat D1	Gram -	Bacilli	Positive Negative	Positive	Non-*E*	*Aeromonashydrophila*
*Pat D2	Gram+	Cocci Bacilli	Negative	Negative Positive	*Enterococcus*	Aerococcus viridans1
*Pat E1	Gram-	Bacilli	Positive Negative	Positive	Non-*E*	*Ochrobactrumanthropi*
Pat F1	Gram +	Cocci	Negative	Positive	*Micrococcus/ Staphylococcus*	*Staphylococcus epidermidis*
Pat F2	Gram+	Cocci	Negative	Positive	*Micrococcus/ Staphylococcus*	*Staphylococcus xylosus*
*Pat F3	Gram +	Cocci pairs/ chains	Negative	Negative	*Enterococcus*	*Streptococcus porcinus*
*Pat F4	Gram+	Cocci cluster	Negative	Negative	*Enterococcus*	*Aerococcus viridans1*
Pat G	-	-	-	-	-	-

Key: Non-E denotes non –*Enterobacteriaceae*, * denotes some anomalies among the biochemical tests, presumptive ID and the Blast report, - denotes no growth

TABLE 4: THE ISOLATES CHARACTERISTICS AND PRESUMPTIVE IDENTITIES FROM HOSPITAL Y

Bacterial Isolate	Gram stain	Morphology	Oxidase test	Catalase test	Presumptive ID	API Identification
Pat A1	Gram-	Bacilli	Positive	Positive	Non-*E*	*Pseudomonas aeruginosa*
Pat A2	Gram-	Bacilli	Negative	Positive	*Enterobacteriaceae*	*Proteus mirabilis*

Pat B1	**-**	**-**	**-**	**-**	**-**	**-**
***Pat C1**	**Gram+**	**Cocci**	**Negative**	**Negative**		***Enterococcus durans***
		Coccobacilli				
***Pat C2**	**Gram+**	**Cocci**	**Negative**	**Negative**	**Non-*E***	***Enterococcus faecium***
	Gram-	**Bacilli**	**Positive**	**Positive**		
Pat D1	**Gram-**	**Bacilli**	**Negative**	**Positive**	***Enterobacteriaceae***	***Proteus mirabilis***
***Pat D2**	**Gram+**	**Cocci**	**Positive**	**Positive**		***Kocuria varians***
	Gram-	**Bacilli**	**Negative**			

Key:Non-E denotes non –*Enterobacteriaceae*, * denotes some anomalies among the biochemical tests, presumptive ID and the Blast report, - denotesno growth

TABLE 5: THE ISOLATES CHARACTERISTICS AND PRESUMPTIVE IDENTITIES FROM HOSPITAL Z

Bacterial Isolate	Gram-stain	Morphology	Oxidase test	Catalase test	Presumptive ID	API Identification
***Pat A1**	**Gram-**	**Bacilli**	**Negative**	**Positive**	***Enterobacteriaceae***	***Proteus vulgaris***
	Gram+	**Cocci**				
***Pat A2**	**Gram-**	**Bacilli**	**Positive**	**Positive**	**Non- *E***	***Vibro alginolyticus***
			Negative			
***Pat A4**	**Gram+**	**Cocci**	**Negative**	**Negative**	***Enterococcus***	***Aerococcus viridans 1***
				Positive		
***Pat A5**	**Gram+**	**Cocci**	**Negative**	**Negative**	***Enterococcus***	***Streptococcus porcinus***
***Pat B1**	**Gram-**	**Bacilli**	**Positive**	**Positive**	***Pseudomonas***	***Pseudomonas aeruginosa***
	Gram+					
***Pat B2**	**Gram-**	**Bacilli**	**Positive**	**Positive**	***Pseudomonas***	***Pseudomonas aeruginosa***
	Gram+					
Pat C1	**Gram-**	**Bacilli**	**Negative**	**Positive**	***Enterobacteriaceae***	***Citrobacter koseri***
***Pat C2**	**Gram+**	**Cocci**	**Negative**	**Positive**	***Staphylococcus***	***Staphylococcus xylosus***
	Gram-	**Bacilli**				
Pat D1	**Gram+**	**Cocci**	**Negative**	**Positive**	***Staphylococcus***	***Staphylococcus spp***

***Pat D2**	**Gram-**	**cocci** **Bacilli**	**Negative**	**Positive**	***Enterococcus***	***Aerococcus viridans***
Pat E1	**Gram+**	**Cocci**	**Negative**	**Positive**	***Micrococcus/ Staphylococcus***	***Staphylococcus aureus***
Pat E2	**Gram+**	**Cocci**	**Negative**	**Positive**	***Staphylococcus***	***Staphylococcus aureus***
Pat E3	**Gram-**	**Bacilli**	**Positive**	**Positive**	**Non-*E***	***Aeromonas hydrophila/ caviae***
***Pat E4**	**Gram-**	**Bacilli**	**Positive** **False-Positive**	**Negative** **Positive**	**Non-*E***	***Aeromonas hydrophila/ caviae***
Pat E5	**Gram-**	**Bacilli**	**Negative**	**Negative**	***Enterobacteriaceae***	***Klebsiellaoxytoca***
***Pat F1**	**Gram-**	**Bacilli**	**Positive** **Negative**	**Positive**	**Non-*E***	***Aeromonas hydrophila***
Pat F2	**Gram+**	**Cocci**	**Negative**	**Positive**	***Micrococcus/ Staphylococcus***	***Staphylococcus aureus***
Pat F3	**Gram-**	**Bacilli**	**Positive**	**Positive**	**Non-*E***	***Rhizobium radiobacter***
Pat F4	**Gram-**	**Bacilli**	**Positive**	**Positive**	**Non-*E***	***Aeromonas hydrophila***
***Pat F5**	**Gram-**	**Bacilli**	**Positive** **False-Positve**	**Negative**	**Non-*E***	***Aeromonas hydrophila***
***Pat F6**	**Gram-**	**Bacilli**	**Positive** **Negative**	**Positive**	**Non-*E***	***Aeromonas hydrophila***
Pat G 1	**-**	**-**	**-**	**-**	**-**	**-**

Key: ŋ/s denotesnot sequenced, Non-E denotes non –*Enterobacteriaceae*, * denotes some anomalies among the biochemical tests, presumptive ID and the Blast report, - denotesno growth

The Prevalence and Distribution Patterns of the Bacteria Species

The Gram-negative bacilli from *Enterobacteriaceae* such as the *Proteus mirabilis* (20%) and *Klebsiella pneumonia* (20%) were the predominant bacteria species from hospital X in comparison to hospital Z, where *Staphylococcus aureus* (19%)was mostly recovered and *Proteus mirabilis* (50%) from hospital Ywas common, as shown in figure 5. A few skin commensals such as *Corynebacterium striatum, Staphylococcus epidermidis* were also recovered. More species diversity was observed in the wounds of the patients from hospital Z, two species of *Klebsiella* were recovered (*Klebsiella pneumoniae* and *Klebsiella oxytoca*). Table 6 presents the frequency distribution of bacteria across the hospitals that participated in the study.

TABLE 6: THE DISTRIBUTION OF BACTERIA ACROSS THE DIFFERENT HOSPITALS

Bacteria Identities	Distribution (%)		
	Hospital X	Hospital Y	Hospital Z
Staphylococcus aureus	25	0	75
Enterococcus faecalis	50	0	50
Bacillus pumilus	33.3	0	66.7
Proteus mirabilis	50	50	0
Escherichia coli	50	0	50
Klebsiella pneumonia	60	0	40

A total of six species were identified to be distributed among the different hospitals; *Proteus mirabilis* between hospital X and Y while five of the identities; *Klebsiella pneumonia*, *Bacillus pumilus, Enterococcus faecalis, Escherichia coli and Staphylococcus aureus* were distributed among patients from Hospital X and Z as shown by Table 6.

DISCUSSION

Diabetic wound infections are a global challenge especially in developing countries, compromising the quality of life [20]. In this study, bacteria were recovered from the wounds of 83% of the sampled diabetic patients, indicating the high prevalence of bacteria in the wounds of diabetic patients, as in agreement with Dunyach-Remy *et al.*,[26]. The recovery of bacteria in diabetic wounds is one of the signs of infection along with clinical symptoms such as erythema, pain, tenderness and pus [9].The wounds were in the lower limbs in 94% of the cases and this in literature has been attributed to the vascular permeability that causes impaired blood supply to the peripheries during a diabetic state [27] and can result to limb amputations [7].The wounds were also noted mostly in the elderly (> 60 year) of which whose immune system is already compromised due to ageing [28] and diabetes thereby increasing the risk of bacterial infections [29]

Biochemical tests are solely based on phenotypic properties of bacteria which are shared by most species [12, 13, 17], as a result misidentification is common, which can also account for the anomalies observed in table 3- 5 whereby culture-based methods of identification (API) misinterpreted some of the results which were confirmed to be different by the 16S rDNA. In relation to this, several studies have reported that antimicrobial therapy may affect the bacterial cell wall without killing the bacteria leading to altered cell morphology thus misidentification is common especially in the Gram-stain [30, 31]. Gram-viable bacteria stain opposite from their true Gram-reaction therefore, limiting the use of the Gram-stain in bacteria identification [30]. Catalase and oxidase tests play a crucial role in enzyme-based methods of identification however, some bacteria contain enzymes different from catalase or cytochrome oxidase c that alter these particular reactions thereby giving false results [14, 17], therefore the 16S rDNA results were the considered results in this study because bacteria was accurately identified. The 16S rDNA technique is able to identify even the unculturable strains, therefore, giving a better understanding of bacteria etiology in infections [16].

The Gram-negative bacilli were most recovered from the patients' wounds in all three hospitals (figure 3), supporting what has been reported by Kamel *et al.,* [5] and Akhi *et al.,* [20] that most diabetic wounds are colonized by Gram-negative bacilli. The wounds were monomicrobial in 76% of the cases, which closely associates them with mild diabetic wound infections [4]. Polymicrobial wounds on the other hand are inclined to severe infections [20] and were noted in 24% of the cases in the study. In severe infections there is an increased risk of biofilm formations which in turn delay wound healing, due to the impaired host defense[32], decreased uptake of treatment drug by biofilms and microbial synergy between less invasive and virulent bacteria [34], leading to longer hospital stays and in extreme cases which may affect the quality of life [32].

The results of the study have shown microbial diversity in diabetic wounds, ranging from skin commensals, opportunistic pathogens, true pathogens and nosocomial-acquired microorganisms which all play a role in the wound etiology [35, 55]. Severe wound infections have been reported to be linked to

facultative anaerobic and aerobic bacteria such as *S. aureus, S epidermidis, Enterococci* spp, *Pseudomonas* spp, *Escherichia* coli [20], which were also recovered in some patients in this study. *Proteus mirabilis* being the most predominant isolate in the study is associated with both nosocomial and community acquired infections [37] and can cause infections in the different body sites [38]. It occurs in moist environments and is a common pathogen implicated in wounds and immuno-compromised hosts along with *E. coli, Enterobacter spp and Klebsiella* spp which were also recovered in the study [39-40]. Through its virulence factors such as fimbriae and flagella it can adhere onto epithelial tissue and cause infection [37].

The factors contributing to the severity of diabetic wound infection includes virulence and pathogenicity which can be attributed to some of the isolates recovered in this study such as *P. aeruginosa* and *S. aureus* that have been reported to produce virulence factors that are so destructive in the wound healing process. *P. aeruginosa* possesses virulence factors such as exoproteases, siderophores, exotoxins, hydrogen cyanide and pyocyanin that attack host defenses and impair wound healing [35] while *S. aureus* possess factors such as coagulase, catalase and clumping factors that play a role in infection mainly occurring in immuno-compromised individuals such as diabetic individuals [9]. *S. aureus* has a role in deepening and spreading infections in body tissue by damaging the host cell membranes and causing cell lysis [26], which can be also attributed to diabetic wounds.

Staphylococcus sciuri (coagulase-negative) among the recovered identities in this study, has been implicated in hospital and community acquired infections [41]. The two species of *Klebsiella* identified in this study are frequently accountable for nosocomial infection in humans and they greatly impact on immunocompromised hosts [36], emphasizing the threat that they pose on public health. Less has been reported about the virulence of *K. pneumoniae* [35], however, three distinct phylagroups (Kp I, Kp II, Kp III) have been defined and all three are implicated in human infections [36].

Conclusion

The presence of bacteria alone is not indicative of infection, however, most bacteria recovered in the study have been reported to have debilitating effects in wounds and in immunocompromised hosts, therefore their recovery alone in diabetic patients' wounds is a serious health concern, such that necessary measures should be taken to curb their spread especially in the hospital setting.

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Disclosure Policy

There is no conflict of interest regarding the publication of this paper.

REFERENCES

1. Kloos W.E. and Musselwhite M.S., 1975. Distribution and persistence of *Staphylococus*and *Micrococcus* species and other aerobic bacteria on human skin. *Applied Microbiology*, **30(3)**: 381-395.
2. Christensen G.J.M. and Bruggemann H., 2014. Bacterial skin commensals and their role as host guardians. *Beneficial Microbes*, **5(2)**: 201-215.
3. HirschT.,Spielmann M., Zuhaili B., Koehler T., Fossum M., Steinau H-U., Yao F., Steinstraesser L., Onderdonk A.B. and Eriksson E., 2008. Enhanced susceptibility to infections in a diabetic wound healing model. *BMC Surgery*, **8(5)**: 1-8.
4. Bowler P.G., Duerden B.I. and Armstrong D.G., 2001. Wound microbiology and associated approaches to wound management. *Clinical Microbiology Reviews*, **14(2)**: 224-269.
5. Kamel N.A., Abouelwafa M.M., El tayeb W.N. and Aboshanab K.M., 2014. Antibacterial resistance pattern of aerobic bacteria isolated from patients with diabetic foot ulcers in Egypt.*African Journal of Microbiology Research*, **8(31)**: 2947-2954.
6. Collins N., Toiba R., 2010. The importance of glycemic control in wound healing. *Ostomy Wound Management*, 18-23.
7. Siddiqui A.R. and Bernstein J.M., 2010. Chronic wound infection: facts and controversies. *Clinics in dermatology*, **28**: 519-526.
8. Sahay B.K., 2013. Infections in diabetes mellitus. The Association of Physician of India Textbook of Medicine, chapter 48: 218-220. (www.apiindia.org) [Accessed date: 07/10/2016).
9. Bessa L.J., Fazii P., Di Giulio M., Cellini L., 2013. Bacterial isolates from infected wounds and their antibiotic susceptibility pattern: some remarks about wound infection. *International Wound Journal*, **12**: 47-52.
10. Rogers G.B., Carrol M.P. and Bruce K.D., 2009. Studying bacterial infections through culture-independent approaches. *Journal of Medical Microbiology*, **58**: 1401-1418.
11. Oates A., Bowling F.L, Boulton A.J.M. and McBain A.J., 2012. Molecular and culture-based assessment of the microbial diversity of diabetic chronic foot wounds and contralateral skin site. *Journal of Clinical Microbiology*, **5(7)**: 2263-2271.
12. Perry J.D. and Freydiere A.M., 2007. The application of chromogenic media in clinical

microbiology. *Journal of Applied Microbiology*, **103**: 2046-2055.
13. Kiser K.M., Payne W.C., Taff T.A., 2011. Clinical Laboratory Microbiology a Practical Approach (Chapter 9). *Pearson Clinical Laboratory Science Series*.
14. Rhoads D.D., Walcott R.D., Sun Y., Dowd S.E., 2012. Comparison of culture and molecular identification of bacteria in chronic wounds. *International Journal of Molecular Sciences*, **13**: 2535-2550.
15. Dowd S.E., Walcott R.D., Kennedy J., Jones C., Cox S.B., 2011. Molecular diagnostic and personalized medicine in wound care: assessment of outcomes. *Journal of Wound Care*, **20(5)**: 232-239.
16. Wigneswaran V., Amador C.I., JelsbakL.,Sternberg C., Jelsbak L., 2016. Utilization and control of ecological interactions in polymicrobial interaction and community-based microbial cell factories. *Faculty Reviews*, **421**: 1-7.
17. Janda J.M. and Abbott S.L., 2002. Bacterial identification for publication: when is enough enough. *Journal of Clinical Microbiology*, **40(6)**: 1887-1891.
18. Sontakke S., Cadenas M.B., Maggi R.G., Diniz P.P.V.P and Breitschwerdt E.B., 2009.Use of broad range 16S rDNA PCR in clinical microbiology. *Journal of Microbiological Methods*, 76:217-225.
19. Srinvasan R., Karoz U., Volegova M., Mackichan J., Kato-Maeda M., Miller S., Nadarajan R., Brodies E.L., Lynch S.V., 2015. Use of 16S rRNA gene for identification of a broad range clinically relevant bacterial pathogens. *PLOS ONE*, **10(2)**: 1-22.
20. Akhi M.T., Ghotaslou R., Asgharzadeh M., Varshochi M., Pirzadeh T., Memar M.Y., Bialvaei A.Z., Sofla H.S.Y. and Alizadeh N., 2015. Bacterial etiology and antibiotic susceptibility pattern of diabetic foot infection in Tabriz, Iran. *GMS Hygiene and Infection control*, **10**: 1-6.
21. Ørskov J., 1922. Method for the Isolation of bacteria in pure culture from single cells and procedure for the direct tracing of bacterial growth on a solid medium. The Journal of Bacteriology, **7 (6)**: 537-549.
22. Gram C., 1884. Ueber die isolirteFärbung der Schizomycetin in Schnitt-und Trockenpräparaten. *Fortschritte der Medicin*, **2**: 185-189.
23. McLeod. J. W. and GordonJ., 1923. Catalase. *Journal of Pathology and Bacteriology*, **26(326)**: 1-3.
24. Gordan J. and McLeod J.W., 1928. The practical application of the direct oxidase reaction in bacteriology, *The Journal of Pathology*,**31(2)**: 185-190.
25. Altschul S.F., Madden T.L., Schaffer A.A., Zhang J., Zhang Z., Miller W. and Lipman D.J, 1997. Gapped blast and PSI-blast: a review generation of protein database search programs. Nucleic Acid Research, **25(17)**: 3389-3402.
26. Dunyach-Remy C., Essebe C.N., Sotto A. and Lavigne J-P., 2016. *Staphylococcus aureus* toxins and diabetic foot ulcers: role in pathogenesis and interest in diagnosis. *Toxins*, **8(209)**: 1-20.
27. Noor S., Zubair M., Ahmad J., 2015. Diabetic foot ulcer- a review on pathophysiology, classification and microbial etiology. *Diabetes and Metabolic Syndrome: Clinical Research and Reviews*, **9**: 192-199.
28. Murphy E.C. and Frick I-M., 2012. Gram-positive anaerobic cocci- commensals and opportunistic pathogens. *FEMS Microbiology Reviews*, **37**: 520-553.
29. Perim M.C., Borges J. da C., Celeste S.R.C., Orsolin E. de F., Mendes R.R., Mendes G.O., Ferreira R.L., Carriero S.C., and Pranchevicius M.C. da S., 2015. Aerobic bacterial profile and antibiotic resistance in patients with diabetic foot infection. *Revista da SociadadeBrasileira de Medicina Tropical*, **48(5)**: 546-554.
30. Stone R.B. and Steele J.C.H., 2009. Impact of reporting Gram stain results from blood cultures on the selection of antimicrobial agents. *American Journal for Clinical Pathology*, **132**: 5-6.
31. Thairu Y., Abdullahi I. and Usman Y., 2014. Laboratory perspective of Gram staining and its significance in investigations of infectious diseases. *Sub-Saharan Africa Journal of Medicine*, **1(4)**: 168-174.
32. Han A., Zenilman J.M., Melendez J.H., Shirtliff M.E., Agostinho A., James G., Stewart P.S., Mongodin E.F., Rao D., Rickard A.H. and Lazarus G.S., 2011. The importance of a multifaceted approach to characterizing the microbial flora of chronic wounds. *Wound Repair and Regeneration*, **19**: 532-541.
33. Vickery K., Hu H., Jacombs A.S., Bradshaw D.A., Deva A.K., 2013. A review of bacterial biofilms and their role in device-associated infection. *Healthcare Infection*, **18**: 61-66.
34. Landis S.J., 2008. Chronic wound infection and antimicrobial use. *Advance in Skin and Wound Care*, **21**: 531-540.
35. Leung K.P., D'Arpa P., Seth A.K., Geringer M.R., Jett M., Xu W., Hong S.J., Galiano R.D., Chen T., and Mustoe T., 2014. Dermal wound transcriptomic response to infection with *Pseudomonas aeruginosa* versus *Klebsiella pneumoniae*in a rabbit ear wound model. *BMC Clinical Pathology*, **14(20)**: 1-14.
36. Holt K.E., Jenney A., Zadoks R.N., Baker S., Whitehouse C.A., Dance D., Jenney A., Connor T.R., Hsu L.Y., Severin J., Brisse S., Cao H., Wilksch J., Gorrie C., Schultz M.B., Edwards D.J., Nguyen K.V., Nguyen T.V., Dao T.T., Mensink M., Minh V.L., Nhu N.T.K., Schultz C., Kuntaman K., Newton P.N., Moore C.E., Strugnell R.A., Thomson N.R., 2015.Genomic analysis of diversity, population structure, virulence and antimicrobial resistance in *Klebsiella pneumoniae,* an urgent threat to Public Health. *PNAS*, 3574-3581.
37. Rozalski A., Torzewska A., Moryl M., Kwil I., Maszewska A., Siwinska M., Staczek P.,2012. *Proteus* sp- an opportunistic bacterial pathogen-classification, swarming growth, clinical significance and virulence factors. Folia Biologicaet Oecologica, **8**: 1-17.
38. Feglo P.K., Gbedem S.Y., Quay S.N.A., Adu-Sarkodie Y., Opoku-Okrah C., 2010. Occurrence,

species distribution and antibiotic resistance of *Proteus* isolate: A case study at the KomfoAnokye Teaching Hospital (KATH) in Ghana. *International Journal of Pharma Sciences and Research*, **1(9)**: 347-352.

39. Mordi R.M. and Momoh M.I., 2009. Incidence of *Proteus* species in wound infections and their sensitivity pattern in the University of Benin Teaching Hospital. *African Journal of Biotechnology*, **8(5)**: 725-730.
40. Kwiecinska-Pirog J., Skowron K., Zniszczol K., and Gospodarek E., 2013. The assessment of *Proteus mirabilis* susceptibility to ceftazidime and ciprofloxacin and the impact of these antibiotics at sub-inhibitory concentration on *Proteus mirabilis* biofilm. *Biomed Research International*, **2013**: 1-8.
41. Nemeghaire S., Argudin M.A., Febler A.T., Hauschild T., Schwarz S., Butaye P., 2014. The ecological importance of the *Staphylococcus sciuri* species group as a reservoir for resistance and virulence genes. *Veterinary Microbiology*, **171(3-4)**: 342-356.