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Bacterial Profile of Diabetic Foot Ulcers of Patients Visiting a Specialist Diabetic Clinic at Komfo Anokye Teaching Hospital, Kumasi, Ghana

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Authors' contributions

This work was carried out in collaboration between all authors. Authors RCB, JA and RKDE designed the study, performed the statistical analysis, wrote the protocol, and wrote the first draft of the manuscript. Authors BAEJ, RKDE and JA managed the analyses of the study.

Authors RKDE, RCB and JA managed the literature searches. All authors read and approved the final manuscript.

Original Research Article

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ABSTRACT

Aims: Diabetic foot ulcers (DFU's) pose socio-economic challenges and are a major cause of hospital admissions and morbidity often causing suffering and poor quality of life for diabetics especially in developing world. The aim of this study was to determine the bacterial profile and antibiotic susceptibility and resistance pattern of foot ulcers of diabetics at Komfo Anokye Teaching Hospital (KATH).

Study Design: Descriptive cross-sectional. Twenty seven (27) diabetics with foot ulcers comprising 15 males and 12 females attending the diabetic clinic at KATH were recruited

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for this study.

Place and Duration of Study: The study was conducted at the diabetic clinic of the Komfo Anokye Teaching Hospital (KATH) between November 2006 and April 2007.

Methodology: Demographic parameters of the participants were recorded and wound swabs were obtained and cultured on blood and MacConkey agar. Organisms isolated were identified and tested for their antimicrobial sensitivity patterns using Kirby-Bauer method.

Results: The mean age of the participants, duration of diabetes and FBS were 58.2 ± 12.0 years and 6.5 ± 2 years and 12.3 ± 4.0 mmol/L respectively. Two (2) patients had their toes amputated. Twenty nine (29) isolates were detected from the 27 ulcer specimens out of which 28 (97%) isolates were gram negative organisms. *Proteus spp* (31%) and *Escherichia coli* (24%) were the most common gram negative pathogens isolated in this study and *Staphylococcus aureus* was the only gram positive organism isolated. Ciprofloxacin (100%), ceftazidime (100%), Ceftriaxone (88.3%), gentamycin (80%) and cefotaxime (80%) were most sensitive to the isolates whereas ampicillin (0%), tetracycline (0%) and chloramphenicol (0%) were the most resistant.

Conclusions: Gram negative organisms' highly sensitive to ciprofloxacin, ceftazidime and Ceftriaxone are the most common pathogens in DFU's in KATH.

Keywords: Diabetes mellitus; foot ulcer; antibiotics; blood sugar; bacteria.

1. INTRODUCTION

Diabetes mellitus is an endocrine disorder characterized by an inability to auto-regulate the plasma levels of glucose leading to several neurologic, micro and macro vascular complications such as vascular disorders, neuropathy, nephropathy and retinopathy. It affects approximately 170 million people worldwide, [1], and by 2030 these numbers are projected to double [2,3].

Diabetic foot ulcers (DFU's) has been identified as a key economic, social and medical burden and a primary cause of hospital admissions for people with diabetes in the developing world and is a major cause of morbidity often causing suffering, and poor quality of life for these patients. DFU's are estimated to occur in 15% of all patients with diabetes [4] and precede 84% of all diabetes-related lower leg amputations [5]. Cardiovascular complications have also been reported in diabetics with a history DFU's [6].

Generally, it is estimated that about 15% of diabetics worldwide will at some stage develop DFU [7]. Customarily, routine procedures like administering frequent glycaemic controls, surgical removal of dead infected tissue, pressure offloading and maintenance of adequate blood supply, are performed in addition to the evaluation of the different microorganisms infecting the wound [8]. The profiles of microbes affecting DFU have been widely studied [9-11]. However, this study seems to be the first of its kind in Ghana. Determining the bacterial profile of diabetic foot ulcers in Komfo Anokye Teaching Hospital (KATH) will be a good source of information for clinicians taking care of diabetics. Knowledge of the commonest bacteria isolated from patients with diabetic foot ulcers will be essential information to ensure better management of these ulcers. This will ensure a reduction in the number of amputations and other complications associated with DFU. This study was thus designed to evaluate the bacterial profile, antibiotic susceptibility and resistance pattern of organisms isolated from DFU of patients attending the KATH diabetic clinic.

2. MATERIALS AND METHODS

2.1 Study Area

This descriptive cross sectional study was undertaken at the Komfo Anokye Teaching Hospital (KATH), Kumasi the capital of the Ashanti region of Ghana, the main referral medical facility serving the northern parts of the country between November 2006 and April 2007.

We used purposive sampling to recruit 27 confirmed diabetics made up of 15 males and 12 females from the diabetic clinic at KATH. The participants were selected with the help of the resident specialist on duty in the clinic. Demographic and clinical information were obtained from the folders of the selected participants. Diabetics with ulcers on their lower limbs and who are not on any antimicrobial therapy were recruited whereas those on antimicrobials for the treatment of infections or have a history of antimicrobial use over the past one week were excluded. Pregnant women, patients with ulcers due to skin diseases, patients using drugs containing steroids and unconfirmed diabetics with elevated sugar levels were excluded.

2.1.1 Ethical consideration

Ethical clearance was obtained from the ethics and research committees of the KATH and the School of Medical Sciences (CHPRE/SMS/KNUST) and informed written consent was also obtained from the eligible participants. All procedures followed were in accordance with the ethical standards of the Ministry of Health, Ghana as well as the Helsinki Declaration of 1975.

2.1.2 Collection of blood and wound swab samples

After an overnight fast (8-12 hours) about 2 ml of venous blood was collected into fluoride oxalate tubes, centrifuged at 1500 g for 5 minutes and stored at -80°C until assayed. Wound swabs were taken from ulcers on the lower limbs using sterile dry cotton swabs before antiseptic dressing. The swabs were immersed into containers of Amies transport media and transported to the laboratory.

2.2.3 Fasting blood sugar (FBS)

We used the glucose oxidase/peroxidase method (Trinder, 1969) to estimate the glucose concentration in the plasma of participants. The colour developed at the end of the reaction was measured with a spectrophotometer [(Spectronic-20), 820 Linden Avenue, Rochester, NY 14625, USA] at a wavelength of 500 nm.

2.2.4 Wound culture and sensitivity

The cotton swab with the culture material was inoculated onto blood and MacConkay agar and incubated at 35- 37°C in a carbon dioxide enriched medium overnight. These were then sub cultured at 24, 48 and 72 hours in an IPF 400 Precision incubator (Mettler, Germany).

2.2.5 Identification of bacterial isolates

Bacterial colonies were identified based on their colonial morphology (color, growth size, and growth pattern). Standard biochemical tests including citrate, urease, indole, catalase, and coagulase tests were used for further identification of isolates.

2.2.6 Antimicrobial susceptibility test

The Kirby Bauer method [12] was used to determine the susceptibility of the isolates to selected antimicrobial agents. Antibiotic-impregnated paper discs (Medical wire and Equipment Co. Ltd., Potley Corsham, England) containing the following antibiotics: gentamicin (GEN, 10 µg), tetracycline (TET, 30 µg), cefotaxime (CFT, 15 µg), ceftazidime (CTZ, 15 µg), ceftriaxone (CFX, 15 µg) cotrimoxazole (COT, 25 µg), ampicillin (AMP, 10 µg), cefuroxime (CRX, 30 µg) and ciprofloxacin (CIP, 30 µg), chloramphenicol (CHL, 30 µg), amikacin (AMK, 10 µg), erythromycin (30 µg, ERY) were used.

Fresh isolates of pure colonies were emulsified in peptone water using a sterile straight wire loop, and the turbidity was adjusted to the equivalent of 0.5 McFarland's standard. A portion of the emulsified suspension was obtained using a sterile cotton swab, and a three dimensional streak was made on a Mueller Hinton agar plate. A sterile cotton swab was then used to obtain a portion of the emulsified suspension to make a three – dimensional streak on a Mueller-Hinton agar plate. Based on the organism's gram reaction an appropriate antibiotic disc was placed on the plated agar within 15 minutes of seeding and then incubated at 37°C overnight (18 – 24 hour). A caliper was used to determine the zone of inhibition which was then compared to a standard chart to determine susceptibility categorized as sensitive or resistant as previously described by [13]. A Gram negative-organism *Escherichia coli* (NCTC 10418) and *Staphylococcus aureus* [National collection of type cultures (NCTC) 6571] a Gram-positive organism, were used as controls.

2.2.7 Statistical analysis

GraphPad Prism version 5.00 for windows and Excel were used for statistical analysis (GraphPad software, San Diego California USA, www.graphpad.com).

3. RESULTS

The mean age was 58.2±12.0 years. Fifty-six percent (56%) of the patients were males. The mean duration of diabetes and FBS were 6.5±2 years and 12.3±4.0 mmol/l respectively. Two (2) out of the 27 patients had their toes amputated and 50% were hypertensive (Table 1).

A total of 29 isolates were detected from the 27 ulcer specimens. Twenty-eight (97%) isolates were gram negative. All the organisms isolated were aerobes and facultative anaerobes. Seven (7) different organisms were isolated namely *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Proteus species*, *Klebsiella species*, *Escherichia coli*, Coliform species and *Enterobacter species* (Table 2).

Table 1. General demographic, clinical and biochemical characteristics of study participants

Variable	Percentage/Mean
Age (mean, yrs)	58.2±12.0
Gender (n, %)	
Males	15 (55.6)
Females	12 (44.4)
HTN (%)	13(50)
Time since initial diagnosis (yrs)	6.5±2
FBS (mmol/l)	12.3±4.0
<i>Type of Medication</i>	
OHA (n, %)	20 (74.1)
Insulin (n, %)	4 (14.8)
OHA + insulin (n, %)	3 (11.1)
Amputations (n, %)	2

FBS=fasting blood sugar; OHA=oral hypoglycemic agents, HTN=hypertension

Table 2. Profile of bacteria isolated from foot ulcers of study participants

Characteristics	Organisms isolated	N (%)
Gram negative aerobe (n=28)	<i>Proteus</i> species	9 (31)
	<i>Escherichia coli</i>	7 (24)
	<i>Klebsiella</i> species	5 (17.2)
	<i>Pseudomonas aeruginosa</i>	5 (17.2)
	<i>Coliforms</i>	1 (3.5)
	<i>Enterobacter</i> species	1 (3.5)
Gram positive aerobe (n=1)	<i>Staphylococcus aureus</i>	1 (3.5)

Proteus spp. the predominant isolate was sensitive to ceftriaxone, cefotaxime and ciprofloxacin each with a sensitivity of 88.9%. *E. coli* and *Pseudomonas aeruginosa* showed a sensitivity of 85.71% and 100% respectively to cefazidime. Ciprofloxacin was the most effective antibiotics for *Klebsiella pneumoniae* with 100% sensitivity, followed by gentamycin, ceftriaxone, and cefotaxime each with a sensitivity of 80%. All the gram negative organisms showed resistance to ampicillin, tetracycline, and chloramphenicol (Table 3).

Table 3. Antimicrobial sensitivity pattern of gram negative organisms

AST	<i>Proteus sp</i> %	<i>E. coli</i> %	<i>Klebsiella spp</i> %	<i>Pseudomonas spp</i> %	<i>Coliforms</i> %
AMP	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
TET	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
GEN	4(44.4)	0(0.0)	4(80.0)	2(40.0)	5(100.0)
COT	2(22.2)	0(0.0)	2(40.0)	0(0.0)	0(0)
CHL	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0)
CTR	8(88.9)	4(57.1)	4(80.0)	1(20.0)	1(100.0)
CRX	4(44.4)	2(28.6)	3(60.0)	0(0.0)	0(0.0)
CTX	9(88.9)	4(57.1)	4(80.0)	1(20.0)	1(100.0)
CFZ	3(33.3)	6(85.7)	1(20.0)	5(100.0)	1(100.0)
CIP	8(88.9)	4(57.1)	5(100.0)	1(20.0)	1(100.0)

AST=antimicrobial sensitivity test; GEN=gentamicin, TET=tetracycline, CFT=cefotaxime, CTZ=ceftazidime, CFX=ceftriaxone; COT=cotrimoxazole; AMP=ampicillin; CRX=cefuroxime CIP=ciprofloxacin; CHL=chloramphenicol; AMK=amikacin, ERY=erythromycin

4. DISCUSSION

Despite improved treatment regimen, a significant number of diabetic foot ulcers do not heal and eventually lead to significant morbidity and amputations [9,14,15]. Important local factors determining the healing rate of ulcers are pressure at the site of the ulcer, adequacy of blood supply, and infection. It is generally accepted that for optimal healing, infection needs to be treated. This descriptive cross-sectional study evaluated the bacterial and antimicrobial sensitivity profile of isolates from DFU's. This was achieved by culturing wound samples, performing susceptibility test on the isolates and estimating the FBS of the participants. We identified *Proteus spp.* as the most prevalent isolate and ceftriazone was the most sensitive drug for this population.

Males predominated in this study (Table 1) which is in agreement with observations made in other studies [8,16,17]. This finding gives credence to established reports that male sex is a risk factor in the development of diabetic foot ulcers [8,16,17]. The 6.5±2 years mean duration of diabetes recorded in this study corroborates reports in other studies and confirms the fact that foot ulcers are rampant in diabetics who have had with the condition for five or more years

Hyperglycaemia has been identified as a factor that lowers the immunity of diabetics through the inactivation of lymphocytes and thus glucose levels above 10 mmol/L promotes the development of these lesions [18]. Observations made in this study support this assertion.

Majority of the pathogens isolated in this study were gram negative organisms (96.5%) whereas the rest were gram positive organisms (Table 2). This is consistent with findings made in other studies [16,19,20] though percentages recorded in this study were comparatively higher. This however, contradicts other reports which identified gram positive organisms as the predominant cause of DFU's [21,22]. *Proteus spp.* and *E. coli* were the most common gram negative pathogens isolated in this study (Table 2) which is contrary to observations made by [23] and [24] but in agreement with the reports of [25,26] and [27]. Consistent with the findings of Esmat and Al Islam (2012) and Demetriou et al. [19] we identified *Staphylococcus aureus* as the most prevalent gram positive isolate from DFU's in this study.

A number of studies have reported multiple drug resistance involving ampicillin, cotrimoxazole, tetracycline and chloramphenicol among diabetics with DFU [23,28]. Observations from our study confirmed these reports on multiple drug resistance among isolates from diabetic foot ulcers. These supposedly prescribed drugs are now mostly obtained over the counter and that could account for their high resistance among our participants.

Many of the microbes isolated from the diabetic ulcers were susceptible to ceftriazone, ciprofloxacin, ceftazidime, gentamycin and cefotaxime (Table 3). This is consistent with observations made in other studies [28] which identified a similar pattern among diabetics. The susceptibility of these antibiotics implies that these still remain the choice drugs for the treatment of DFU's among diabetics in the catchment area of the KATH. Factors such as frequent of admission to hospital, chronic nature of the ulcer and inappropriate antibiotic therapy has been identified as the main reasons why multi drug resistance is common in diabetics. The interplay of these factors and the knowledge of common isolates of DFU and their susceptibility pattern will help improve the management of diabetics and forestall complications such as septicaemia amputation and even death.

This study has numerous limitations which could affect the generalization of our findings. The small sample size due to the difficulty in getting eligible participants because of the selection criteria means that though we identified pathogens susceptible even to the commonest antimicrobials, our findings cannot be generalized to cover the entire country, Ghana. Furthermore the statistical accuracy cannot be vouched for due to this limitation. Other limitations of this study include the use of FBS to determine glycemic control, the use of patients' records and interviews to determine the duration of diabetes and the fact that anaerobic organisms were not isolated due to our inability to use anaerobic methods in the culturing of the wound specimen.

5. CONCLUSION

The first study in Ghana to examine the microbial profile and sensitivity pattern of DFU identified *Proteus spp* and *Staphylococcus aureus* as the most predominant organisms among diabetics at the KATH. Ceftriaxone, ciprofloxacin, ceftazidime, gentamycin and cefotaxime were most sensitive to the isolates whereas ampicillin, cotrimoxazole, tetracycline, and chloramphenicol were the antimicrobials most resistant. It is essential therefore to educate patients on the need to seek prompt treatment and avoid the use of over-the-counter antimicrobials in the management of diabetic foot ulcers. Furthermore, future studies should employ a larger sample size to enhance the statistical accuracy of the findings and also establish the genetic profile responsible for the hundred percent resistances of isolates to ampicillin, tetracycline and chloramphenicol.

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COMPETING INTERESTS

The authors declare no conflicting interest. The authors are responsible for the content and writing of the paper.

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