ORIGINAL ARTICLE

Microbial Isolates in Diabetic Foot Lesions of Hospitalized Patients at the University Hospital of the West Indies

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ABSTRACT

Objective: *The diabetic foot is a frequent complication of diabetes mellitus. It confers a negative impact on the patients' quality of life and profound burden on the healthcare system. The objectives of this study were to determine the bacteriological profile and antibiotic susceptibility of patients admitted to the University Hospital of the West Indies with diabetic foot ulcer over a 5-year period, and whether methicillin-resistant* Staphylococcus aureus *is a common microbial isolate and if antibiotic resistance played a role on the patients' duration of hospital stay or amputation.*

Methods: *A retrospective analysis was done on the patients admitted from January 2003 to December 2008 with the diagnosis of diabetes mellitus and foot complications. The eligible patients and their medical records were identified by the medical records department. Their demographic data, types of cultures done and results, antibiotic susceptibility and resistance, and treatment regimens were all recorded. Frequency and means were calculated, and statistically significant covariates used as the predictors in univariate and multivariate regression models.*

Results: *Of the 545 cases admitted, 102 had complete data for analysis. Group D* Streptococci *was the most common organism isolated (45.1%) followed by other forms of* Streptococci *and* Pseudomonas aeruginosa*. The majority of cases (80.6%) had two or more bacterial isolates. Gram-negative bacteria (*Proteus *and* Klebsiella*) and anaerobes were also common, 48.0% and 22.5% respectively. There were no cases of methicillin-resistant* Staphylococcus aureus*. Antibiotic resistance was not significant.*

Conclusion: *Gram-positive organisms, especially the* Streptococcus *species, remain an important organism in diabetic foot infections. Current empiric antibiotic regimes used are effective in this tertiary care university hospital.*

Keywords: Antibiotics, diabetic foot, microbial isolates, ulcers.

INTRODUCTION

The term 'diabetic foot' refers to the spectrum of pathological changes that can occur in the lower extremities of patients with diabetes (1). The initial lesion begins with an ulcer that can become secondarily infected and even gangrenous. The aetiology is usually multifactorial and includes a combination of peripheral vascular disease, peripheral neuropathy, poor wound healing, and/or an immunocompromised state resulting from uncontrolled diabetes mellitus (1–4). The development of a diabetic foot is a source of significant morbidity and mortality, as it is the most common cause of lower limb amputations in the diabetic population and is an important source of sepsis (5). In the USA, the diabetic foot and

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its complications are the most common reasons for the hospitalization of patients with diabetes mellitus (6). It is estimated that 15%–20% of patients with diabetes will be hospitalized for a diabetic foot complication (7). The Caribbean has a similar high burden of the disease. One in every eight patients seen in the specialist diabetes clinic at the University Hospital of the West Indies (UHWI) had a foot complication (8). Diabetic gangrene accounted for 42% of all the lower limb amputations carried out in Jamaica (9). In Barbados, the amputation rate has been estimated as 936 per 100 000 (10). The financial burden on the health care system is also quite significant globally. In Trinidad and Tobago, over US$ 13 000 000 was spent to treat patients hospitalized for diabetic foot infections in 1 year (11).

In order to minimize the negative impact of the diabetic foot ulcer, the ideal goal is to prevent its development. However, once an ulcer occurs, one of the treatment goals is to prevent infection, as well as to be able to recognize and treat an infection early with appropriate antibiotics (1, 2, 4). Most infections, when they do occur, are polymicrobial (2). As expected, Gram-positive organisms are the major pathogens isolated. This has been demonstrated in a 1-year review of wound swabs from diabetics with foot ulcers submitted to the Microbiology department at the UHWI (1999–2000) (12). On the other hand, there have been a few studies that reported a predominance of Gram-negative aerobes in diabetic foot ulcers (13, 14). These differences may be partly influenced by a change in the causative organisms over time, geographical location and/or the severity of infection (15).

This study was aimed at evaluating microbial isolates from patients with diabetic foot ulcer over a 5-year period to determine changes, if any, in the type of organisms isolated and their resistance patterns. Any changes would directly affect the type of empiric antibiotics that will be recommended for initial treatment (16). Although methicillin-resistant *Staphylococcus aureus* (MRSA) was not expected to be a significant isolate, a secondary aim of the study was to document its incidence given the increasing emergence of community MRSA infections (17, 18). The study was also aimed at determining if the type of organism itself or its resistance pattern had a direct impact on the patients' outcomes (specifically their duration of hospital stay and amputation), although multiple factors probably contribute.

SUBJECTS AND METHODS

This was a retrospective single-institution study. All the patients with diabetes who were admitted with lower limb ulcers or diagnosed as having a diabetic foot at UHWI between January 2003 and December 2008 were included in the study. Assuming from prior data when approximately 48 subjects per year (12) had wound swabs from diabetic feet, an estimated 250 patients were expected for this study. The patients who had traumatic and decubitus ulcers were excluded. Information on the cultures and sensitivities from the wound swabs, tissue cultures and blood culture was obtained from the medical records. Other data collected included the patients' demographic data, type of diabetes, most recent HbA1c, duration of diabetes, other co-morbid illnesses, outpatient medications, antibiotics used, the duration of intravenous antibiotic administration, number of any amputations, and the duration of hospital stay.

The severity of foot lesions were graded according to Wagner's classification (19, 20):

0—no obvious ulcer, but thick callus, prominent metatarsal heads, claw toes or any bony abnormality
1—superficial ulcer clinically not infected
2—deep ulcer often infected but no bone involvement
3—deep ulcer, abscess formation and bone involvement
4—localized gangrene
5—gangrene of whole foot

As these were hospitalized patients, their feet lesions were expected to be at least grade 2.

Statistical analyses

Continuous data were summarized using means and standard deviations, and categorical data as proportions (with a 95% confidence interval). Pearson coefficients were calculated for the association of resistance patterns and microbial isolates with length of hospital stay. Association of the type of surgical procedure with categorical and non-normally distributed variables was assessed using the Chi-squared test and the Spearman rank correlation analysis, respectively. Statistically significant covariates were used as the predictors in univariate and multivariate regression models. We used the Statistical Package for the Social Sciences (SPSS, Version 17, Chicago, IL, USA) software for the data analyses.

RESULTS

A total of 545 cases with the primary diagnoses of diabetic foot from January 2003 to December 2008 were identified by the medical records department. A total of 230 dockets were located, of which 102 met the inclusion criteria for the study. The data were excluded because the patients were admitted with a different diagnosis (n = 28) or their culture results were missing from the files (n =100).

The majority of the patients admitted were females and half of the patients were 60 years old or higher (Table 1). The average duration of diabetes was 12.6 ± 7.0 years with a mean admitting glucose of 17.1 ± 6.9 mmol/L. Most of the patients did not have recorded glycosylated haemoglobin. Despite these elevated glucose levels, only three patients presented with a hyperglycaemic emergency (two with hyperglycaemic hyperosmolar state, one with diabetic ketoacidosis) and almost half presented with sepsis. There were no documented cases of type 1 diabetes. Hypertension and dyslipidaemia were frequent co-morbidities. Oral hypoglycaemic agents accounted for 89.8% of the outpatients medications, with the sulfonylurea (40.7%) and biguanide groups (44.9%) being the most common drugs used. Peripheral neuropathy and previous diabetic foot were the most common complications (Table 2). Of the patients who were admitted, 37.9% had prior admissions for diabetic foot. The severity of foot lesions on presentation was predominantly Wagner's stage 2 and 3 (combined 66.7%) (Table 3). The vast majority had debridement (99%). Half of the patients had some form of amputations (ray 14.7%, transmetarsal 5.9%, below the knee 25.5%, and above the knee 4.9%). There were no deaths.

The combination of beta-lactam and anti-anaerobic antimicrobials was the most common initial therapy (66%). In 81% this was a beta-lactam + metronidazole and in 19% this was a beta lactam + clindamycin. Approximately 9% received monotherapy with a beta-lactam drug only. Culture directed change in antibiotics occurred in only 13.7% of the patients. The type of cultures sent included wound (45.1%) or tissue (55.9%) and blood (78.4%). Only nine (8.8%) blood cultures grew bacteria (three group D *Streptococcus*, five coagulase-negative *Staphylococcus*, one group C *Streptococcus*, one *Staphylococcus aureus*, one *Acinetobacter*, one *Bacteroides*). Three blood cultures had two organisms isolated. There were 57 tissue cultures done of which 13 grew one organism, 20 grew two organisms, 19 grew three organisms, and 6 grew four organisms. Of the 46 wound cultures done 7 grew one organism, 23 grew two organisms, 9 grew three organisms, and 10 grew four organisms. Table 4 illustrates the specific types of the organisms isolated. The predominant organism grown was group D *Streptococcus*. There were no isolated cases of methicillin-resistant *Staphylococcus aureus*.

Table 5 identifies the different antibiotic sensitivities for the specific organisms isolated. There was no significant antibiotic resistance noted. The Gram-positive organisms were sensitive to the penicillin derivatives,

Table 1: Demographic profile of 102 patients with diabetic ulcers at the University Hospital of the West Indies during the period 2003–2008

	Mean
Age (years)	60.2 ± 14.5
Women (%)	54
Type of diabetes	
• Type 2 (%)	98 (n = 100)
• Steroid induced (%)	2 (n = 2)
Duration of diabetes (years)	12.6 ± 7.0
Admission glucose (mmol/l)	17.1 ± 6.9
Sepsis on admission (%)	47.1
Duration of admission (days)	14.7 ± 11.3
Amputations (%)	52
Pre-existing diabetic complications (%)	54.9
Hypertension (%)	50.4
Dyslipidaemia (%)	20.7
Peripheral artery disease (%)	23.1
Past and current smokers (%)	26
Using insulin (%)	10.1

Means ± SD are stated unless otherwise indicated.

Table 2: Diagnosed diabetic complications in 102 patients with diabetic ulcers at the University Hospital of the West Indies

	N	%
Previous diabetic foot	44	43.1
Peripheral neuropathy	27	26.4
Erectile dysfunction	3	2.9
Diabetic retinopathy		
• non-proliferative	15	14.7
• proliferative	9	8.8
Chronic kidney disease	7	6.9
Cardiovascular disease (stroke, ischaemic heart disease)	11	10.8

Table 3: Wagner's classification of diabetic foot in 102 patients with diabetic ulcers at the University Hospital of the West Indies

Stage	N	%
1 (superficial ulcer not clinically infected)	1	1.0
2 (deep ulcer often infected, no bone involvement)	44	43.1
3 (deep ulcer, abscess formation, bone involvement)	24	23.5
4 (localized gangrene)	16	15.7
5 (gangrene of whole foot)	17	16.7

Table 4: Organisms isolated from wound and tissue cultures in 102 patients with diabetic ulcers at the University Hospital of the West Indies

	%	N
Staphylococcus aureus	13.7	14
Other staphylococci[a]	11.8	12
Group D streptococci	45.1	46
Other Streptococci[b]	21.6	22
Pseudomonas aeruginosa	19.6	20
Escherichia coli	11.8	12
Enterobacter sp.	6.9	7
Proteus sp.	25.5	26
Klebsiella sp.	22.5	23
Morganella sp.	9.8	10
Other Gram negatives[c]	21.6	22
Anaerobes (including *Bacteroides*)	22.5	23
Yeast		1

[a]Other *Staphylococcus* refers to coagulase-negative *Staphylococcus*.
[b]Other *Streptococcus* refers to *Streptococcus viridians*, groups B, C, and *G* streptococci.
[c]Other GNB (Gram-negative bacteria) includes *Alcaligenes*, *Citrobacter*, *Serratia*, *Acinetobacter* and *Providencia* species.

Table 5: Antibiotic sensitivities (%) of the isolated organisms in 102 patients with diabetic ulcers at the University Hospital of the West Indies

Organisms	AC	AM	CX	CA	TZP	CT	CZ	CIP	GM	SXT	MT
Staphylococcus aureus	37	11	30	19	4	–	–	–	–	–	–
Other staphylococci	7	13	13	–	–	–	–	–	–	–	–
Group D streptococci	45	41	–	–	1	4	–	1	3	–	–
Other streptococci	28	26	–	7	–	20	–	–	–	9	–
Pseudomonas aeruginosa	–	–	–	–	28	–	25	4	43	2	–
Escherichia coli	23	–	–	–	–	6	31	6	26	9	–
Enterobacter sp.	10	–	–	–	10	10	10	20	35	–	–
Proteus sp.	31	2	–	–	2	8	16	7	25	10	–
Klebsiella sp.	28	–	–	–	2	5	21	7	21	16	–
Morganella sp.	–	–	–	–	3	7	10	10	38	28	–
Other GNB	9	2	–	–	9	7	16	6	25	22	–
Anaerobes	–	–	–	41	–	–	–	–	3	–	56

AC = amoxicillin/clavulanic acid; AM = ampicillin; CX = cloxacillin; CA = clindamycin; TZP = piperacillin/tazobactam; CT = ceftriaxone; CIP = ciprofloxacin; GM = gentamycin; SXT = cotrimoxazole; CZ = ceftazidime; MT = metronidazole; GNB = Gram-negative bacteria.

whereas the Gram-negative organisms also demonstrated sensitivities to the beta-lactam antibiotics and/or third-generation cephalosporins. Metronidazole and clindamycin were just as effective against the anaerobic bacteria.

The bivariate correlations done between the type of organisms identified, and impact on the subjects duration of admission was not significant (p-values > 0.05). The patients' outcomes (duration of hospital stay, amputation) were not significantly related to their antibiotic sensitivity (p-values > 0.05). In the multivariate analyses, after adjusting for age, gender and the duration of diabetes, there were still no significant relationships with patients' outcomes.

DISCUSSION

In this 5-year retrospective study, the majority of the patients with non-traumatic foot ulcers had a long duration of diabetes with inadequate glycaemic control, and frequently had other microvascular complications. The ulcer grade was mostly type 2 to 3. Aerobic Gram-positive cocci and anaerobic species were the dominant microbes, but there was little if any antibiotic resistance.

As expected, the majority of the patients admitted with infected diabetic foot ulcers had long-standing uncontrolled diabetes mellitus that would be associated with subsequent microvascular complications. It might be partly contributed by the inadequate treatment on oral hypoglycaemic drugs as only 10.2% of the patients were on insulin therapy despite the inadequate control on two or more oral agents. It was difficult to know if the documented initial admission hyperglycaemia was due to poor compliance, infection or inadequately prescribed therapy. There was poor documentation of the patients' glycosylated haemoglobin. Of note, none of the patients had type 1 diabetes mellitus. Whether this reflects inaccurate record keeping or a true occurrence is unknown. In a literature review of similar studies that had been done, the researchers did not indicate the type of diabetes the subjects had (21–24).

The predominant microbes isolated in these patients are the aerobic Gram-positive cocci, especially group D *Streptococci*. These organisms are typically involved because they colonize the skin and become a source of infection when the epidermal layer is broken as in an ulcer. However, numerous studies have shown that *Staphylococcus aureus* as well as the beta-haemolytic *Streptococci* (groups A, B, C, and G) are the most common (21, 22, 25). In our setting, group D *Streptococci* seems to be more common and this was also shown in the previous study done at the UHWI. This finding may indicate that the slight variation is attributable to geographical location. Methicillin-resistant *Staphylococcus aureus* does not appear to have any role in diabetic foot ulcers for our study population.

Proteus sp. and *Klebsiella* sp. dominate in the Gram-negative group. These organisms tend to be seen in the more chronic wounds, but our data analyses did not show increased frequency in the patients who had prior diabetic foot infections. The presence of *Pseudomonas aeruginosa* (19.6%) did not have any documented impact on the patients' hospital stay.

Anaerobic bacteria were also relatively frequent isolates but there was an unusual case where one specimen grew yeast as the only organism. This might have been a contaminant. Fungi are not typically isolated from infected diabetic ulcers but they are usually polymicrobial with a combination of Gram-positive, Gram-negative and anaerobic bacteria (26).

Despite the plethora of the organisms isolated, the most common empiric antibiotic regimes used (amoxicillin/clavulanic acid or cefuroxime or ceftriaxone and metronidazole or clindamycin) have been effective in their treatment. The most prevalent organisms have more than 50% sensitivities to the antibiotic regimes used. The Gram-positive organisms were mostly sensitive to the beta-lactam antibiotics, penicillins and third-generation cephalosporins. All the anaerobic isolates were either sensitive to clindamycin or metronidazole. Even in the patients who had isolates that were not typically sensitive to the initial antibiotics, the overall sensitivity was as expected. For example, *Pseudomonas aeruginosa* was not covered by the empiric antibiotic regime but it was sensitive to antibiotics with good Gram-negative coverage such as ceftazidime and gentamycin. However, empiric antibiotics were not frequently adjusted based on these culture results, yet there was no impact on the patients' overall hospital stay or on the type of surgical intervention. This neutral effect could be because systemic antibiotics play less of a role after the site of infection has been removed by debridement and/or amputation. All of our patients had debridement done on at least one occasion with over 53% requiring various degrees of amputation.

Validated trials on the initial antibiotics to use in cases of diabetic foot infections are lacking. The 2012 Infectious Diseases of America guidelines on the treatment of the diabetic foot do not recommend any specific antibiotic combinations but recommend that the agents given should have activity against at least Gram-positive cocci, with broader coverage against Gram-negative and anaerobic organisms for severe infections (27). The spectrum of coverage and route of administration are dependent on the severity of the infection. The Wagner's classification was used in this study to determine the severity of wound infection but it has many limitations. A single grade might encompass a wide range of infections, and it is skewed towards more severe diseases (28, 29). The determination of infection severity clinically remains subjective.

Other limitations of this study include the small sample size in comparison to the number of the patients admitted for diabetic foot infections over the 5-year period. This might have had an impact on the fact that no significant correlation was seen between the various types of organisms isolated and the patients' length of hospital stay, the presence of peripheral vascular disease or treatment for prior diabetic foot. It would have been interesting to note the frequency of skin grafting in these patients and to document complete wound healing, if any, after their discharge. Mostly wound swabs and not tissue biopsies were done to determine the true invasive/pathogenic microbes. The technique of obtaining the swabs was not documented and may vary depending on the physician. We also cannot eliminate that secular trends have occurred since we did this study. In addition, standard culture techniques are under scrutiny and the use of molecular microbiology methodology, including polymerase chain reaction techniques, to isolate responsible organisms for diabetic foot infections has been suggested (30).

In conclusion, the most frequent microbial organisms isolated from the patients with diabetic foot were Gram-positive organisms, particularly group D *Streptococci*. The ulcers tended to be polymicrobial in nature with Gram-positive, Gram-negative and anaerobic bacteria. Methicillin-resistant *Staphylococcus aureus* is not a problem in our setting. The Gram-positive organisms tended to be sensitive to penicillin, beta-lactam antibiotics and cephalosporins. The Gram-negative organisms were sensitive to third-generation cephalosporins and aminoglycosides. The anaerobic group was equally sensitive to metronidazole and clindamycin. There was no remarkable resistance pattern in these groups, so current antibiotic regimes did not need any modification.

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AUTHOR CONTRIBUTIONS

LAC-H conceived the study, collected the data and interpretations, and wrote the first draft. CAW did the statistical analyses and participated in the data interpretations. ENB participated in the study concept and study design. MSB participated in the study concept and design, data analyses and interpretations, and writing the first draft. All authors revised the report for important intellectual content and approved the final version.

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