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Research Article

Antimicrobial susceptibility of bacteria that infect diabetic foot ulcers at Kenyatta National Hospital, Kenya

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Background: Diabetic foot ulcers are prone to bacterial infection and this forms the major cause of hospital admission among patients with diabetes. Local bacterial sensitivity patterns to antimicrobials used to treat the infections is necessary in guiding drug selection for prompt management of the diabetic foot infections.

Objective: To determine the etiology and antimicrobial sensitivity patterns of bacteria that infects diabetic foot ulcers at Kenyatta National Hospital.

Methodology: A cross- sectional study was carried out on 75 adult diabetic patients attending Kenyatta National Hospital. The patients were selected by convenient sampling and data obtained via a questionnaire and antimicrobial susceptibility determination of bacteria from the diabetic foot ulcers using disk diffusion method.

Results: A total of 85 bacterial isolates were identified with *Staphylococcus aureus* (37.3%), *Proteus spp (*21.3%) and *Klebsiella spp* (14.7%) as the most prevalent organisms. Among the *Staphylococcus aureus*, 39.3% were methicillin resistant. All the bacteria were sensitive to imipenem. Gram positive and negative bacteria were sensitive to ciprofloxacin and piperacillin-tazobactam, respectively. Varied sensitivities to commonly used antibiotics: amoxicillin-clavulanate, meropenem, clindamycin, ceftriaxone, piperacillin-tazobactam and ciprofloxacin to different isolates are reported.

Conclusion: In Kenyatta National Hospital, diabetic foot ulcers are infected with both gram negative and positive bacteria that are highly sensitive to imipenem. This study recommends the initiation of empirical antibiotic therapy with imipenem for moderate to severe diabetic foot infections as culture and sensitivity tests to determine more specific antimicrobials are awaited.

Key words: Antimicrobial, Antimicrobial susceptibility, Diabetic foot ulcers.

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1. Introduction

Diabetes mellitus (DM) is a common and potentially disabling chronic metabolic disorder characterized by chronic hyperglycemia (Desalu et al, 2011). Poorly managed diabetic patients are prone to complications including cardiovascular disease, kidney disease, neuropathy, blindness, diabetic foot ulcers and even lower-extremity amputations (Deshpande, 2008). Diabetic foot ulcers (DFU) is a major chronic

complication of DM being the main cause of admissions that sometimes may lead to amputations and even mortality (Desalu et al, 2011). It is estimated that about 2.5% of people with diabetes develop foot complications every year.

The use of antimicrobials in the management of DFU infections (DFIs) is common with appropriate and prompt treatment necessary to prevent progression to chronic complications in diabetes. Appropriate selection of the antimicrobial agents, guided by proven effectiveness, is therefore essential. The frequent emergence of antimicrobial resistance makes the choice for empirical treatment more challenging (Ratemo, 2014). It has been established that there is a direct relationship between the total amounts of an antibiotic used in a particular hospital during a certain period and the number of resistant strains that emerge (Al-hamead et al, 2013). There is therefore need for regular isolation of infection causing organisms and determining their antimicrobial susceptibility patterns to commonly used antibiotics at the hospital to guide agent selection in managing DFU infections. This study set out to identify the bacteria infecting DFU and determine their antimicrobial susceptibility patterns at Kenyatta National Hospital (KNH).

2. Methods

2.1 Study site and design

A cross sectional study was conducted on adult diabetic patients attending Kenyatta National Hospital from the medical and surgical wards as well as from the outpatient diabetes clinic between April and August 2016.

2.2 Study population and eligibility criteria

Adult consenting diabetic patients with a diabetic foot ulcer were recruited by convenient sampling. They must have had a diabetic foot ulcer and put on an antimicrobial empirically. Those who had an antimicrobial susceptibility analysis done within the preceding seven days were excluded from the study.

2.3 Sample size and sampling procedure

A corrected sample size of 75 participants based on calculation using Fisher`s formula (Raudys and Jain 1991) and an estimated prevalence of DFUs at KNH of 4.6% (Nyamu et al, 2003). Participants were recruited by convenient sampling.

2.4 Data collection

The participants were interviewed using a structured questionnaire to obtain social- demographic and clinical characteristics. The patient records were used to obtain information on the antimicrobial(s) prescribed for the diabetic foot ulcer.

Wound swabs were collected using sterile swabs and submitted to the University of Nairobi laboratory within one hour for bacteriological analysis. Precautions were taken to avoid cross contamination. The wound swab was well labeled with the patient's study number and date of collection.

Only growth of aerobic bacteria was targeted in this study. Specimens were inoculated onto two Blood Agar media (BA), one MacConkey media (MAC) within 2 hours of collection using streaking method of inoculation. The agar plates were incubated as follows; one BA was incubated in carbon (IV) oxide candle extinction jar for 5% - 10% CO_2 requirement and the second BA and MAC plate aerobically at 37^0 C for 24 hours. The organisms were identified according to Bergey's manual (Holt et al, 1994) and manual of clinical microbiology (Martins et al, 2011). Kirby-Bauer Disc Diffusion sensitivity test carried out in triplicate was used to determine sensitivity patterns shown by zones of inhibition of growth around the discs.

The sensitivity of drugs on KNH formulary and commonly prescribed for DFU were tested inform of commercially prepared disks: amoxicillin-clavulanate (30µg), cefuroxime (30µg), ceftriaxone (30µg), meropenem (10µg), ciprofloxacin (5µg), clindamycin (2µg), ceftazidime (30µg), vancomycin (30µg), imipenem (10µg), amikacin (30µg), and piperacillin/tazobactam (110µg). The diameter of the zones was compared to a standard and categorized as sensitive, intermediate or resistant as per standard procedures(Martins et al, 2011).

2.5 Data analysis

The obtained data was analyzed using the statistical software SPSS version 20. The variables included; antimicrobials prescribed for infected foot ulcers, bacteria isolated from the DFUs and susceptibility pattern of the bacterial isolates. All variables were subjected to descriptive and inferential statistics and results presented as tables and a figure.

2.6 Ethical considerations

Permission to carry out the study was granted by the Kenyatta National Hospital/University of Nairobi Ethics and Review Committee (KNH/UON ERC) - **KNH-ERC/A/128** of 13th April 2016). Informed and signed consent was obtained from the participants. The cost of the sensitivity testing was met by the investigators. The results obtained were incorporated into the patients' treatment plan. Confidentiality was maintained by using a patient study number instead of the names.

3. Results

Social-demographic and Clinical characteristics of Study Participants

The mean participants' age was 55.4 yr (±12.7 sd) with the majority aged above 50 years (70.7 %) and male (n=42, 56.0%). The participants were recruited from the medical wards (n=37, 49.3%), surgical wards (n=7, 9.3%) and the diabetes clinic (n=31, 41.3%). The median duration of suffering from diabetes and foot ulcers was 7yr and 2 months, respectively. (Inter-Quartile Range (1QR) 1.0-4.0)

Isolation of bacteria infecting ulcers

The bacterium found infecting DFU and their frequency are shown in **Table 1** and **Figure 1**. A total of 85 bacteria isolates were isolated with Gram negative

aerobic bacterial infections slightly more at 43 (50.6%) than gram positive aerobic bacteria 42, (49.4%). The most commonly isolated microorganism were *S. aureus* (37.3%), *Proteus spp* (21.3%), *Klebsiella spp* (14.7%), *E. coli* (13.3%), *E. faecalis* (12.0%), *P. aeruginosa* (8.0%) and Coagulase Negative *Staphylococcus* (CONS) (6.0%). Among the *S. aureus*, 39.3% were oxacillin resistant.

Anaerobic bacteria were not isolated in this study.

Table 1: Bacteria isolated from diabetic foot ulcers

Variable	n (%)
Bacterial gram stain	**(Total isolates = 85)**
Gram positive bacteria	42 (49.4)
Gram negative bacteria	43 (50.6)
No growth	7 (9.3)
Bacteria species	
Staphylococcus aureus	28 (37.3)
Oxacillin sensitive *Staphylococcus aureus*	17 (60.7)
Oxacillin resistant *Staphylococcus aureus*	11 (39.3)
Proteus species	16 (21.3)
Klebsiella species	11 (14.7)
Escherichia coli	10 (13.3)
Enterococcus feacalis	9 (12.0)
Coagulase negative *Staphylococcus*(CONS)	5 (6.7)
Pseudomonas aeruginosa	6 (8.0)
Gram positive bacteria	**(Total isolates =42)**
Staphylococcus aureus	28 (66.7)
Enterococcus faecalis	9 (21.4)
Coagulase Negative *Staphylococcus* (CONS)	5 (11.9)
Gram negative bacteria	**(Total isolates =43)**
Proteus species	16 (37.2)
Klebsiella species	11 (25.6)
Escherichia coli	10 (23.3)
Pseudomonas species	6 (14.0)

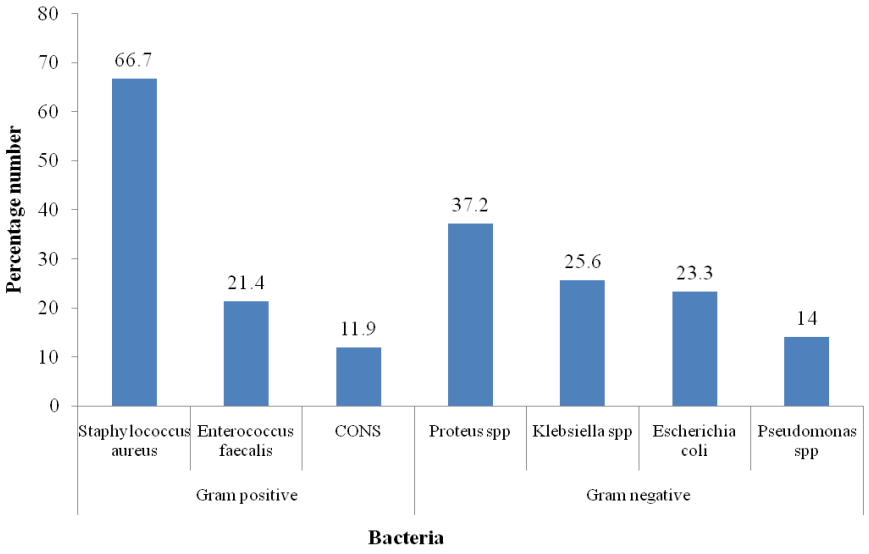

Figure 1. Prevalence of isolated bacteria

Antimicrobial susceptibility of isolated gram positive bacteria

The antimicrobial susceptibility of isolated gram positive bacteria to imipenem, ciprofloxacin, meropenem, clindamycin, ceftazidime, vancomycin, ceftriaxone and amoxicillin-clavulanate, antimicrobials commonly recommended for gram positive infections, is summarized in **Table 2**. *Staphylococcus aureus* was highly sensitive to imipenem (92.9%) and ciprofloxacin (64.3%). The highest resistance was to amoxicillin-clavulanate (50%) and meropenem (50%). About 14% of the *S. aureus* isolates had intermediate susceptibility to amoxicillin-clavulanate. Oxacillin resistant *S. aureus* showed high resistance (52.9%) towards amoxicillin-clavulanate and ceftriaxone. All of the coagulase negative *Staphylococcus* (CONS) isolates showed susceptibility to imipenem (100%) and other antimicrobials tested except clindamycin and meropenem. Most of the *E. faecalis* isolates were sensitive to imipenem (77.8%), ciprofloxacin (66.7%) and amoxicillin-clavulanate (55.6%) but were all resistant to cefuroxime, ceftriaxone, ceftazidime, clindamycin and vancomycin. Around 11% of *E. faecalis* had intermediate sensitivity to meropenem.

Antimicrobial susceptibility of isolated gram negative bacteria.

The antimicrobial susceptibility of the gram negative aerobic bacteria against drugs recommended for their treatment at KNH: imipenem, piperacillin-tazobactam, meropenem, ciprofloxacin, ceftazidime, amikacin, ceftriaxone, clindamycin and cefuroxime is summarized in **Table 3**. *Proteus* species were highly sensitive to piperacillin-tazobactam (93.8%), ciprofloxacin (87.5%) and imipenem (68.8%). The bacteria were however highly resistant to clindamycin (93.8%), cefuroxime and ceftazidime. A third of isolated species had intermediate sensitivity to amikacin.

Most of the *Klebsiella* isolates showed susceptibility to imipenem, piperacillin-tazobactam, amikacin, (81.8%) and meropenem (72.7%) but exhibited resistance towards ceftriaxone (81.8%), clindamycin and cefuroxime (72.7%). Most of the *E. coli* isolates showed highest sensitivity to imipenem (90%) and piperacillin-tazobactam (80%) but showed the highest resistance to cefuroxime and clindamycin (90%).

Table 2: Antimicrobial susceptibility of isolated gram positive bacteria

Antimicrobial	*Staphylococcus aureus* n (%)	Oxacillin-Resistant *Staphylococcus aureus* n (%)	*Enterococcus faecalis* n (%)	Coagulase Negative *Staphylococcus* n (%)
Imipenem	26 (92.9)	16 (94.1)	7 (77.8)	5 (100)
Ciprofloxacin	18 (64.3)	10 (58.8)	6 (66.7)	4 (80)
Cefuroxime	14 (50.0)	9 (52.9)	-	2 (40)
Meropenem	14 (50.0)	8 (47.1)	3 (33.3)	-
Clindamycin	14 (50.0)	10 (58.8)	2 (22.2)	-
Ceftazidime	14 (50.0)	8 (47.1)	2 (22.2)	3 (60)
Vancomycin	14 (50.0)	8 (47.1)	3 (33.3)	3 (60)
Ceftriaxone	12 (42.9)	7 (41.2)	1 (11.1)	2 (40)
Amoxicillin - clavulanate	10 (35.7)	6 (35.3)	5 (55.6)	1 (20)
Total isolates	**28**	**17**	**9**	**5**

Table 3: Antimicrobial susceptibility of isolated gram negative bacteria

Antimicrobial	*Proteus species* n (%)	*Klebsiella species* n (%)	*Escherichia coli* n (%)	*Pseudomonas aeruginosa* n (%)
Piperacillin- tazobactam	15 (93.8)	9 (81.8)	8 (80.0)	5 (83.3)
Ciprofloxacin	14 (87.5)	4 (36.4)	5 (50.0)	1 (16.7)
Imipenem	11 (68.8)	9 (81.8)	9 (90.0)	4 (66.7)
Ceftriaxone	10 (62.5)	2 (18.2)	4 (40.0)	1 (16.7)
Meropenem	10 (62.5)	8 (72.7)	5 (50.0)	2 (33.3)
Cefuroxime	4 (25.0)	1 (9.1)	-	-
Clindamycin	1 (6.3)	2 (18.2)	1 (10.0)	-
Ceftazidime	7 (43.8)	5 (45.5)	5 (50.0)	2 (33.3)
Amikacin	7 (43.8)	9 (81.8)	5 (50.0)	2 (33.3)
Total isolates	**16**	**11**	**10**	**6**

*Pseudomonas aerug*inosa showed high sensitivity to piperacillin-tazobactam (83.3%) and imipenem (66.7%) but absolute resistance to clindamycin, cefuroxime, ciprofloxacin and ceftriaxone. About 33% of the isolates indicated intermediate sensitivity to amikacin. The culture and sensitivity results obtained were used to appropriately guide or adjust treatment for the individual patients.

4.0 Discussion

The observed mean age as well as the median duration of diabetes and DFUs among the participants was similar to previous studies on DFUs at KNH (Nyamu et al, 2003). The studies suggest that DFUs as a complication of diabetes commonly occurs after about seven years since diabetes onset.

Specimens from 7 participants (9.3%) did not show any bacterial growth on appropriate nutrient culture as had also been observed in previous studies (El-tahawy 2000; Turhan et al, 2013; Mohanasoundaram 2012). This implies that either the wounds were not infected or the empirically prescribed antibiotics were effective.

The observed *Staph. aureus* high sensitivity to imipenem and ciprofloxacin has been reported elsewhere (Akhter. 2012; Shabaki 2014). A much lower sensitivity to vancomycin against *S. aureus,* is however reported contrary to other studies (El-tahawy 2000; Yerat 2015; Suresh et al, 2013). Similarly, cefuroxime, meropenem, amoxicillin-clavulanate and ceftazidime showed lower sensitivities. This could be an indication of relatively high levels of *S. aureus* resistance to regularly used drugs at the hospital. However, earlier studies (El-sheikh et al, 2014; Elamenya et al, 2015) reported high *S. aureus* sensitivity to amoxicillin-clavulanate. As in previous studies (Ravisekhar et al, 2006; Turhan et al, 2013) an increase in prevalence of oxacillin resistant *S. aureus* is reported in this study although the isolated species had a high sensitivity to imipenem, ciprofloxacin and clindamycin as reported elsewhere (Dezfulian et al, 2011). They however showed high resistance to amoxicillin-clavulanate and ceftriaxone contrary to other findings (Mathangi and Prabhakaran, 2013). The reported low sensitivity to vancomycin has been reported in other studies (Bengalorkar and Kumar, 2011) contrary to most studies reporting great sensitivity towards MRSA (Mukadam et al, 2011; Sharma, 2006; Akhter, 2012; El-tahawy 2000).

The high sensitivity of *E. faecalis* to imipenem, ciprofloxacin, vancomycin and amoxicillin-clavulanate is inconsistent to other studies (Dezfulian et al, 2011). A study at KNH found *E. faecalis* sensitivity to amoxicillin-clavulanate, cefuroxime, ceftriaxone and ciprofloxacin but resistance to imipenem and ceftazidime (Elamenya et al, 2015). These varying results show the ever changing nature over time of sensitivity patterns of bacteria infecting DFUs even in the same settings.

All of the CONS isolates showed susceptibility to imipenem, ciprofloxacin, ceftadizime, and vancomycin as reported elsewhere (Dezfulian et al, 2011). The isolates were found resistant to clindamycin and meropenem as earlier reported (Xavier, 2014) in addition to resistance to amoxicillin- clavulanate contrary to previous findings (El-sheikh et al, 2014).

Proteus infections, mainly considered a community acquired infection, showed high sensitivity towards piperacillin-tazobactam, ciprofloxacin, imipenem, ceftriaxone and meropenem, consistent with other studies(Sharma, 2006; Yerat, 2015; Akhter, 2012; Suresh et al, 2013). However, they showed intermediate sensitivity to amikacin contrary to other studies (Sharma, 2006; Shalbha et al, 2012) that reported a high sensitivity. High resistance to clindamycin, cefuroxime and ceftazidime as in previous findings at KNH is reported (Ratemo, 2014; Elamenya et al, 2015) .

As in other previous studies, *Klebsiella* species showed highest sensitivity to imipenem, piperacillin-tazobactam, amikacin and meropenem (Hena, 2010; El-sheikh et al, 2014; Ratemo, 2014). Highest resistance was displayed against ceftriaxone, clindamycin and cefuroxime contrary to other findingsin KNH (Suresh et al, 2013; Elamenya et al, 2015).

Escherichia coli showed highest sensitivity to imipenem and piperacillin-tazobactam similar to other findings(Dezfulian et al, 2011; Mathangi and Prabhakaran, 2013; Hena, 2010) but quite low sensitivity to amikacin as compared to other studies (Al-hamead et al, 2013; El-sheikh et al, 2014). They however showed the highest resistance to cefuroxime and clindamycin similar to findings (Ratemo, 2014).

Pseudomonas aeruginosa, a common invasive pathogen in DFUs causing severe tissue damage, exhibited a high resistance to broad spectrum antibiotics: ampicillin, cefoperazone, erythromycin and norfloxacin (Sivanmaliappan and Sevanan, 2011). This study however reports an appreciable sensitivity to piperacillin-tazobactam and imipenem as in other studies (Turhan et al, 2013; Mukadam et al, 2011; Dezfulian et al, 2011). Only cefotaxime and ciprofloxacin showed greater activity as compared to other studies (Mukadam et al, 2011) where most isolates were resistant to ciprofloxacin. In this study, all the 6 isolates were resistant to clindamycin, cefuroxime, ciprofloxacin and ceftriaxone. In this study, amikacin had intermediate sensitivity to *P. aeruginosa* contrary to earlier recommendations that it is the best antimicrobial for the organism(Sharma, 2006; Al-hamead et al, 2013).

5.0 Conclusion

Diabetic foot ulcers at KNH are to a large extent infected with both Gram positive and negative aerobic bacteria. Imipenem was found to be the most effective antimicrobial against the majority of these bacteria. Gram positive bacteria in addition, were sensitive to ciprofloxacin as was piperacillin-tazobactam for Gram negative bacteria. This study recommends that empirical therapy for moderate to severe DFU infections may be initiated with imipenem as culture and sensitivity test results are awaited to determine the most specific antimicrobial. These findings should inform any future development or review of guidelines on antibiotic use in KNH to minimize on challenges faced by diabetic patients with DFU, the entire health care system as well as the entire community reflected in

increased hospital stay, increased health care costs, decreased quality of life and possible mortality.

Conflict of Interest declaration

The authors declare no conflict of interest.

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