Microbiology of diabetic foot infections in three district hospital in Malaysia and comparison with South East Asian Countries

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ABSTRACT

Introduction: Diabetic foot infection, a complication that is associated with lower-limb amputation, incurs a huge economic burden to the hospital and health care system of Malaysia. The bacteriological profile of pathogens in diabetic foot infections in Malaysia has been sparsely studied. We investigated the microbiology of diabetic foot infections in patients admitted to the district hospitals on the east coast of Malaysia.

Methods: A retrospective analysis was conducted in three district hospitals (Hospital Kuala Lipis, Hospital Bentong and Hospital Raub) in Malaysia from 1st of January 2016 to 31st December 2016. The clinical specimens were cultured using Clinical and Laboratory Standards Institute (CLSI) guidelines. Antibiotic sensitivity testing to different antibiotics was carried out using the disc diffusion method.

Result: A total of 188 pathogens were isolated from 173 patients, with an average of 1.09 pathogens per lesion. Majority of the pathogens isolated were gram negative pathogens (73.4%). The most commonly isolated pathogens were *Staphylococcus aureus* (17.5%). This was followed by *Klebsiella spp.* (17%), *Pseudomonas spp.* (15.4%) and *Proteus spp.* (13.8%). Gram positive pathogens were sensitive to most of the antibiotics tested except penicillin and fusidic acid. Gram negative pathogens were sensitive to all antibiotics tested except ampicillin and amoxicillin/clavulanic acid. Amikacin provide coverage for all gram negative pathogens in DFI.

Conclusion: For the management of patient with infection in diabetic foot, the choice of antibiotic therapy depends on the sensitivity of the pathogens, the severity of the infection, the patient's allergies history, toxicity and excretion of the antibiotics.

KEY WORDS:
Diabetic foot; ulcer; infection; microbiology; antibiotic; treatment

INTRODUCTION

Diabetes mellitus (DM) is a major non-communicable health problem in the world. Globally, at least 171 million adults were suffering from DM in the year 2000, and this is expected to double to 366 million by the year 2030.[1] In Malaysia, the prevalence of DM for adults above the age of 18 years is 15.2% in 2011 based on the National Health and Morbidity Survey (NHMS) 2011.[2] The prevalence of diabetes in Malaysia has increased by 30% compared with the prevalence of 11.6% in 2006.[2,3] DM is associated with a series of macrovascular and microvascular complications. The complications include diabetic nephropathy, diabetic retinopathy, diabetic foot ulcers, peripheral vascular disease, ischemic heart disease and cerebrovascular disease.[2] It is estimated that 15-25% of diabetic patients have diabetic foot ulcers owing to risk factors such as peripheral vascular disease, impaired immune system and peripheral neuropathy.[4-6] The prevalence of lower limb amputation in diabetic patients is 4.3%, making it a major debilitating condition that subsequently causes significant economic loss to the country.[7]

Diabetic foot infection (DFI), a complication associated with diabetes mellitus, is a major public health problem and it is the main reason many of the diabetic patient admission.[8] For the treatment of DFI, the combination of debridement and antibiotics, coupled with good nutrition and diabetic control is paramount.[9] According to recommendations by the Infectious Disease Society of America (IDSA), empirical antimicrobial treatment should be initiated until the causative pathogens and their antibiotic susceptibility is known.[10] The empirical antimicrobial therapy should be broad spectrum and cover most of the predicted pathogens in DFI. In developed countries, Gram-positive pathogens such as *Staphylococcus aureus* are the leading causative organisms in DFI. Based on a meta-analysis by Zenelaj et al., up to 79% of the pathogens isolated in DFI in developed countries are S.aureus.[11] Nevertheless, recent studies have shown that the pathogens of DFI vary considerably in different parts of the world. Developing countries in Asia have been shown to have more gram-negative pathogens in DFI.[11] Therefore, it is important to obtain the local microbiological profile in DFI and tailor the empirical antimicrobial therapy accordingly so that it is more effective for the treatment of patients.

Previous DFI bacteriology studies in Malaysia have focused on the urban region or have consisted of a small sample

This article was accepted: 15 June 2019
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size.[8,12-13] The aim of this study was to obtain a multicentre microbiological profile of DFI among patients on the east coast of Peninsular Malaysia, focusing on the rural areas in Pahang, Malaysia. For comparison, a non-systematic search was performed to obtain the DFI microbiological profile of all Southeast Asia countries.

MATERIALS AND METHODS

Patient Selection

The medical records of patients who were admitted from 1st of January 2016 to 31st December 2016 to three hospitals in the east coast of peninsular Malaysia, namely Hospital Kuala Lipis, Hospital Bentong and Hospital Raub were reviewed for the suitability of the study. These three hospitals are a cluster of district hospitals under the care of the same orthopaedic surgeons based in Hospital Kuala Lipis. The hospitals were selected because of the completeness of the recorded data and minimal confounding factors caused by various treatments by different specialists. Patients that were admitted for DFI and who have undergone an operative procedure for DFI were included in this study. For patients that were admitted multiple times for the same problem, only the first admission was included in this study. Demographic and clinical characteristics were manually extracted from the medical record.

Microbiological Sample Collection

The sample for the microbiology study were obtained from the intra-operative deep tissue or bone cultures. Other microbiological sample; i.e., pus culture, swab culture, aspiration culture, slough culture and bedside tissue culture were excluded. This is to ensure comparable and representative data. Surgical debridement of DFI was performed by orthopaedic-trained medical officers. All non-viable tissue and slough were removed from the infected wound to obtain the deep tissue or bone samples. The specimens were sent to a microbiology laboratory in the hospital for incubation at 37°C for 24-48 hours in blood agar, MacConkey agar and Chocolate agar. Identification of the isolated pathogens was performed through a conventional method.[13,14]

Antibiotic Susceptibility Test

An antibiotic susceptibility test was performed using a disk diffusion method on Mueller-Hinton agar plates in accordance with the guidelines of the National Committee for Clinical Laboratory Standards.15 The antibiotic disks used were ampicillin (10µg/mL), ampicillin/sulbactam (10µg/mL), amoxicillin (10µg/mL), cefuroxime (30µg/mL), gentamicin (10µg/mL), cefoperazone (75µg/mL), amoxicillin/clavulanic acid (30µg/mL), ceftazidime (30µg/mL), imipenem (10µg/mL), ciprofloxacin (5µg/mL), amikacin (30µg/mL), cefotaxime (30µg/mL), meropenem (10µg/mL), penicillin (10µg/mL), oxacillin (1µg/mL), cefepime (30µg/mL), tetracycline (30µg/mL), ceftriaxone (30µg/mL), erythromycin (15µg/mL), fusidic acid (5µg/mL), vancomycin (30µg/mL), ertapenem (10µg/mL), clindamycin (2µg/mL), peperacillin/tazobactam (10/100µg/mL), cefepime (30µg/mL), tetracycline (30µg/mL), ceftriaxone (30µg/mL) and cefoxitin (1µg/mL).

Comparison with Southeast Asia (SEA) Countries

A comprehensive search of the MEDLINE, EMBASE, Pubmed, Google Scholar and Cochrane computerised literature databases (through June 2017) for studies that investigated the microbiological profile of DFI conducted in Southeast Asia countries were conducted. The subject headings used were diabetes, foot, ulcer, infection, microbiology, bacteriology, antibiotic and sensitivity. All the abstracts retrieved were preliminary screened by two authors independently for suitability. Potential articles with their full manuscripts were then further evaluated by the team for suitability to be included in this study. Only research conducted in Southeast Asia countries and reported cases of bacterial culture in DFI were included in this study.

RESULTS

Demographic

A total of 173 patients with diabetic foot infections were recruited. There were 101 (58.4%) male and 72 (41.6%) female patients, with a male-to-female ratio of 1.4:1. Their age ranged between 23 and 85 years with a mean age of 54.6 years. The age distribution of the recruited patients in this study is shown in Figure 1. The patients were predominantly Malays (80.3%, n=139). This was followed by Indians (11.6%, 20), Chinese (7.5%, 13) and other (0.6%, 1).

Microbiological Profile

A total of 188 pathogens were isolated from the 173 patients, with an average of 1.09 pathogens per lesion. Samples of 56 (32.4%) patients yielded no growth, 66 (38.1%) had mono-microbial infection and 51 (29.5%) patients had poly-microbial infection. Among the 188 pathogens isolated, 138 of them were Gram-negative microorganisms (73.4%) and 50 were Gram-positive microorganisms (26.6%). The organisms isolated from the samples are summarised in Table I.

The isolated pathogens were *Staphylococcus aureus* (17.5%), *Klebsiella spp.* (17%), *Pseudomonas spp.* (15.4%), *Proteus spp.* (13.8%), *Enterobacter spp.* (8%), *Streptococcus spp.* (7.5%), *Escherichia coli* (7.5%), *Acinetobacter spp.* (3.7%), *Citrobacter spp.* (3.2%) and *Morganella morganii* (2.7%). The remaining microorganisms were 1% or less.

Antibiotic Susceptibility

The details of antibiotic susceptibility patterns for Gram-positive and Gram-negative pathogens are shown in Table II and Table III, respectively. There were five pathogens that were multi-drug resistant, of which two were Methicillin-resistant *S.aureus* (MRSA) and the remaining three pathogens were extended spectrum beta-lactamase (ESBL) producing bacteria. All MRSA were sensitive towards vancomycin and all except for one MRSA were sensitive towards rifampicin. All ESBL were sensitive towards amikacin and ertapenem with all except for one were sensitive to imipenem and meropenem.

In terms of antibiotic treatment for Gram-positive pathogens, most of the antibiotics were effective except penicillin and fusidic acid. Up to 80% (n=17) of the S.aureus were resistant to penicillin and 33% (n=7) of them were resistant to fusidic acid.

Table I: Type and number of isolated bacteria from patients with diabetic foot inspection.

Bacteria	Number of isolated bacteria (n)	Percentage of total isolated bacteria (%)	Gram staining
Staphylococcus sp.	33	17.5	Positive
Streptococcus sp.	14	7.5	Positive
Clostridium sp.	2	1.1	Positive
Bacillus sp.	1	0.5	Positive
Klebsiella sp.	32	17	Negative
Pseudomonas sp.	29	15.4	Negative
Proteus sp.	26	13.8	Negative
Enterobacter sp.	15	8.0	Negative
Escherichia coli	14	7.5	Negative
Acinetobacter sp.	7	3.7	Negative
Citrobacter sp.	6	3.2	Negative
Morganella morganii	5	2.7	Negative
Burkholderia sp.	2	1.1	Negative
Serratia sp.	1	0.5	Negative
Hemophilus sp.	1	0.5	Negative
TOTAL	188	100%	

Table II: Antibiotic susceptibility of gram-positive pathogens.

Antibiotic	Staphylococcus sp. (n=21)	Streptococcus sp. (n=9)	Percentage
PENICILLIN	4/21	9/9	43.3%
ERYTHROMYCIN	15/21	8/9	76.6%
CEFOTAXIME	-	9/9	100%
CLINDAMYCIN	15/21	8/9	76.6%
TETRACYCLIN	-	8/9	88.9%
CEFTRIAXONE	-	9/9	100%
VANCOMYCIN	21/21	9/9	100%
CEFOXITIN	17/21	-	80.9%
GENTAMICIN	19/21	-	90.5%
TRIMETOPRIM	18/21	-	85.7%
RIFAMPICIN	20/21	-	95.2%
FUSIDIC ACID	14/21	-	66.7%

Table III: Antibiotic susceptibility of gram-negative pathogens

Antibiotic	Klebsiella (n=22)	Pseudo-monas (n=20)	Proteus (n=19)	Entero-bacter (n=8)	E. Coli (n=6)	Citro-bacter (n=6)	Morga-nella (n=5)	Burkhol-deria (n=2)	Total	Percentage
AMPICILLIN	3/22	-	9/19	0/8	0/6	0/6	0/5	-	12/66	18.2%
AMP-SULBACTAM	21/22	-	18/19	3/8	4/6	5/6	5/5	-	56/66	84.8%
TAZOSIN	21/22	19/20	19/19	8/8	6/6	6/6	5/5	2/2	86/88	97.7%
CEFUROXIME	21/22	-	14/19	3/8	3/6	6/6	-	-	47/61	77.1%
CEFOTAXIME	21/22	-	17/19	7/8	4/6	6/6	5/5	-	60/66	90.9%
CEFTAZIDIME	21/22	19/20	19/19	7/8	4/6	6/6	5/5	2/2	83/88	94.3%
CEFEPIME	21/22	19/20	19/19	8/8	5/6	6/6	5/5	2/2	85/88	96.6%
AMIKACIN	22/22	20/20	19/19	8/8	6/6	6/6	5/5	2/2	88/88	100%
IMIPENEM	22/22	19/20	19/19	8/8	6/6	6/6	5/5	2/2	87/88	98.9%
MEROPENEM	22/22	19/20	19/19	8/8	6/6	6/6	5/5	2/2	87/88	98.9%
ERTAPENEM	22/22	-	19/19	8/8	6/6	6/6	5/5	-	66/66	100%
CIPROFLOXACIN	21/22	19/20	19/19	8/8	6/6	6/6	5/5	2/2	86/88	97.7%
GENTAMICIN	21/22	19/20	18/19	7/8	5/6	6/6	5/5	1/2	82/88	93.2%
AUGMENTIN	19/22	-	16/19	0/8	5/6	5/6	0/5	-	45/66	68.2%

Table IV: Summary of diabetic foot infection studies conducted in Southeast Asia countries

Country	Authors	Centre(s)	Number of patients (n)	Percentage of gram negative pathogens (%)	Percentage of culture with no growth (%)	Percentage of monomicrobial growth (%)
Malaysia	Kow et al.(this study)	Multi-centre (3)	173	73.4	32.4	38.1
Malaysia	Raja et al.[8]	Single-centre	194	52	0	57.2
Malaysia	Ng et al.[12]	Single-centre	33	95	33.3	NS
Malaysia	Abd Wahab et al.[13]	Single-centre	77	71.1	7.8	79.2
Malaysia	Vijaya Kumar et al.[16]	Single-centre	122	71.2	20.95	NS
Brunei	Abd Kadir et al.[17]	Single-centre	75	67	8	52
Singapore	Aziz et al.[18]	Single-centre	100	39.5	0	48
Thailand	Thewjitcharoen et al.[19]	Single-centre	232	58.8	35.1	NS
Philipines	Raymundo et al.[20]	Single-centre	126	68	NS	NS
Indonesia	Pemayun et al.[21]	Single-centre	189	70.8	26	NS

*NS – Not specified

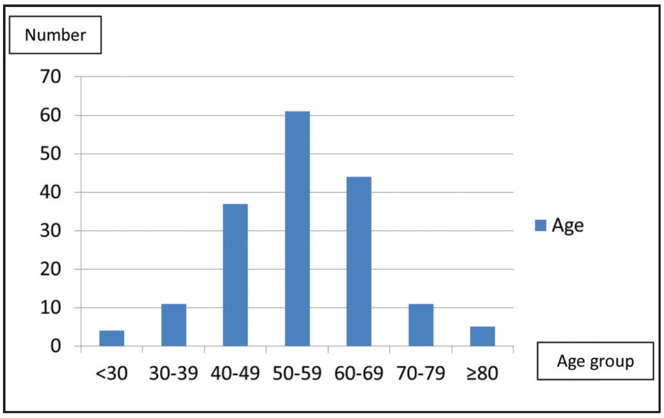

Fig. 1: Age distribution of the participants.

Gram-negative pathogens were least likely to show resistance to amikacin, ertapenem, imipenem and meropenem. Ampicillin/sulbactam, peperacillin/tazobactam, cefotaxime, ceftazidime, cefepime, ciprofloxacin and gentamicin also showed good antimicrobial activity against the Gram-negative pathogens. Ampicillin and amoxicillin/clavulanic acid were the least effective antibiotics against Gram-negative pathogens.

Comparison with Southeast Asia Countries
A total of ten studies (including this study) is included in this review.[8,12,13,16-21] Five of the studies were conducted in Malaysia, and one each in Brunei, Singapore, Thailand, Philippines and Indonesia.[8,12,13,16-21] There were no studies from Vietnam, Myanmar, Cambodia, Laos and Timor Leste. The extracted data from studies in Southeast Asia Countries are summarised in Table IV.

Most of the studies were from a single hospital with the number of patients ranging from 33 to 232.[8,16-21] Aside from the study conducted in Singapore, all the studies reported a higher percentage of gram-negative pathogens in diabetic foot infections with a range of 52% to 73.4%.[8,16-21] In Singapore, the percentage of gram-negative pathogens was only 39.5%.[18]

DISCUSSION

The pathophysiology of diabetic foot infections is complex consequences of host-related complications (immunopathy, neuropathy and arteriopathy) and pathogen-related factors (virulence, antibiotic resistance and microbial load).[22] The combination of insensate foot secondary to neuropathy and deformation predispose the diabetic patients to ulcers formation. The ulcers are colonised by the bacteria and when the overgrowth of the pathogens triggers the deleterious inflammation or tissue destruction, it is termed as an infection.[22] Many different pathogens can cause DFI; it is important for the treating clinician to define the specific causative pathogens to avoid unnecessary, overly broad-spectrum or excessively prolonged antibiotic therapy, which may cause drug-related side effects, incur financial cost and encourage antimicrobial resistance.[10]

To isolate the pathogenic bacteria, we only included the cultures obtained from intra-operative deep tissue and bone samples. This was to avoid the isolation of colonising pathogens through the swab techniques.[23] Some studies have shown that swab and tissue cultures are equally reliable in identifying the causative pathogens in DFI.[24,25] Nevertheless, swab culture may not be reliable in high-grade diabetic foot infections or in the presence of osteomyelitis, as they are associated with high risk of missing pathogens, especially Gram-negative bacteria.[26,27] This may explain the lower percentage of gram-negative pathogens isolated in study by Aziz et al., where culture was obtained from swabbing technique.[18] On top of that, most pathogenic bacteria are found in the biofilm in diabetic foot infection, thus making intra-operative deep tissue and bone samples during surgical debridement a reliable source of identifying the culprit in diabetic foot infection compared to swabbing technique.[29] Biofilm identification and management plays a pivotal role in the treatment of diabetic foot infection. Biofilms in diabetic foot infection are persistent and difficult to eradicate. They response poorly to the antimicrobial agents prescribed and the host's immune response. The exopolymer, which contribute to majority of the biofilm's volume (80-85%), are responsible for blocking the complement activation, inhibit proliferation of lymphocyte, prevent the opsonisation of the phagocytes, and limits the penetration of leukocytes into the biofilm and attenuates leukocytes ability to degranulate and produce reactive oxygen species (ROS).[29] Mature biofilms, persist in chronic wounds indefinitely as long as the wound remains open and they re-emerge two days after the initial debridement. This implies that, after surgical debridement, there is a window of opportunity in which the pathogenic bacteria are susceptible to the empirical antimicrobial agents.[29]

In our study, a high percentage (32.4%) of cultures had no bacterial isolation. This was consistent with other studies by Vijaya Kumar (20.95%) and Ng (33.3%) in other parts of Malaysia as well as Indonesia (26%) and Thailand (35.1%).[12,16,19,21] This may be owing to the fact that empirical antibiotic(s) were often initiated prior to the patient admission to the hospital, reducing the rate of obtaining a positive culture. Of those samples which yielded a bacterial culture, there was a higher percentage of monomicrobial culture (38.1%) than polymicrobial culture (29.5%). This is in consistent with studies by Raja and Abdul Kadir, in which the monomicrobial culture consisted of 57.2% and 52%, respectively.[8,17] The high prevalence of monomicrobial culture may be attributed to the milder infection and low virulence of isolated organisms in this study.[8] It was reported that up to two-thirds of the mild diabetic foot infections are caused by low-virulence organisms such as *S.aureus*, *Streptococcus viridans*, *Staphylococcus epidermidis*, enterococci and other Gram-negative bacteria.[8]

In contrast to European countries, which tend to focus on combating the gram-positive pathogens, especially MRSA in DFI, in developing countries in the SEA regions, gram-negative pathogens predominate in DFI. Nine of the ten studies in SEA regions reported a higher percentage of gram-negative pathogens in DFI, seven of which reported a ratio of more than two-thirds in favour of gram-negative pathogens.

Although the single most commonly isolated pathogens in our study was S.aureus (17.5%), the percentage was significantly lower than that in other studies conducted in developed countries; one study reported the predominance of S.aureus in 50% of the wound specimens.[28] In contrast to studies performed in urban areas or tertiary hospitals, our bacteriology profile closely resembled the study conducted in a rural area by Vijaya Kumar, in which the prevalence of *Klebsiella sp* (17%), *Pseudomonas sp* (15.4%) and *Proteus sp* (13.8%) closely follow *Staphylococcus sp* (17.5%). Patients who presented to the cluster of hospitals were involved in the agricultural field where they are exposed to gram negative bacteria within the soil. Autonomic dysfunction leading to dried and cracked skin predisposed these diabetic patients to colonisation of the bacteria at their feet. Furthermore, immunopathy and vasculopathy further increase the risk of diabetic foot infection in these high-risk patients. Our study revealed a low incidence of multi-drug resistant pathogens, in which there were two MRSA and three ESBL producing bacteria. This was attributed to the compliance with the antibiotic prescribing guideline and infection control measures in place.[8]

Pathogens isolated in this study, both gram-positive and gram-negative, were sensitive to a number of antibiotics. Aside from penicillin, erythromycin and fusidic acid, the other tested antibiotics covered more than 80% of the gram-positive pathogens. Similar to findings by Raja et al., vancomycin was the most effective antibiotic (100%) against gram-positive pathogens.[8] All the antibiotics tested were effective against gram-negative pathogens, except ampicillin, cefuroxime and amoxicillin-clavulanate. The finding is consistent with the results reported by Ng et al, where 83% of the pathogens were resistant to ampicillin.[12] Amikacin was the single most effective antibiotic; all the gram-negative pathogens (100%) were susceptible to it. This was closely followed by carbapenem group; all three antibiotics (imipenem, meropenem and ertapenem) were effective against all but one gram-negative pathogen. In our setting, we advocate the use of ampicillin-sulbactam (84.8%) as an empirical antibiotic for patients with mild and moderate diabetic foot infection and ceftazidime (94.3%) for those with severe DFI upon presentation. Antibiotics such as amikacin and carbapenem should serve as a reserve drug for specific patients to prevent the emergence of multi-drug resistant pathogens.

Our study has several limitations. Due to missing data, we were not able to identify the type of antibiotic taken by the patients prior to presentation to the hospital. Additionally, the three district hospitals involved do not routinely perform anaerobic or fungal sampling for patients with diabetic foot infections. Nevertheless, this is the first comprehensive study with data from multiple district hospitals in Malaysia.

In clinical setting, clinician normally commences an empirical antibiotic to treat the patient with diabetic foot infection. The choice of empirical antibiotic is often based on the patient clinical presentation and the bacteriologic profile of the local setting.[30] Inadequate therapy for infections in critically ill patients has been associated with poor outcomes, including greater morbidity and mortality.[30] Empirical antibiotics are important as initial therapy with the intent to cover the most possible pathogen(s) while waiting for the microbiological results which only available after 72 hours. In this study, we did not access the response of the antimicrobial therapy as suggested by the lab testing as majority of our patients had been discharged prior to obtaining the definitive culture report provided the patients improved clinically after the surgery as evidenced by reducing septic parameters and clean wound. We suggest future study should incorporate both the clinical and biochemical treatment response of the antimicrobial therapy as suggested by the laboratory culture and sensitivity.

CONCLUSION

The management of diabetic foot infections requires a combination of debridement and antibiotic therapy. The choice of antibiotic therapy depends on the sensitivity of the pathogens as well as the severity of the infection, the patient's allergy history, toxicity and excretion of the antibiotics. Prior to identification of the causative pathogens, an empirical antibiotic therapy should be initiated and subsequently changed based on the results of the culture.

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