CLINICOMICROBIOLOGICAL PROFILE AND ANTIBIOTIC RESISTANCE ANALYSIS OF DIABETIC FOOT ULCER FROM A TERTIARY CARE HOSPITAL

Ramesh Arunagiri[1], Raja Sundaramurthy[2], Arunkumar Viswanathan[3], Vithiya Ganesan[4], Rajendran Thiruvannamalai[5]

[1]Associate Professor, Department of Microbiology, Velammal Medical College Hospital and Research Institute, Madurai.
[2]Assistant Professor, Department of Microbiology, Velammal Medical College Hospital and Research Institute, Madurai.
[3]Final Year MBBS Student, Department of Microbiology, Velammal Medical College Hospital and Research Institute, Madurai.
[4]Assistant Professor, Department of Microbiology, Velammal Medical College Hospital and Research Institute, Madurai.
[5]Assistant Professor, Department of Microbiology, Velammal Medical College Hospital and Research Institute, Madurai.

ABSTRACT

BACKGROUND

Diabetic foot ulcer (DFU) is a common complication of diabetes mellitus accounting for 30% of hospital admissions among diabetics. If not treated appropriately DFU infection complicates as amputation, sepsis and even mortality. Knowledge about local microbiological profile with their resistance pattern are essential for every institute to aid in formulating antibiotic policy, thus reducing the economic burden and improve the quality of life.

MATERIALS AND METHODS

A total of 100 in-patients with DFU of Grade 2 and above as per Wagner's classification were included in our study. After obtaining the informed consent exudates, tissue biopsy or swabs were collected in a sterile container and further processed in microbiology lab.

RESULTS

All were male participants with mean age of 56.32 years; 74% had mean diabetic duration of 8.2 years. Common predisposing factors were neuropathy, trauma; 54% had single and 46% had multiple ulcers. Even though many on regular treatment for diabetes, DFU as a complication is increasing. Total of 80% (80/100) were culture positive yielding 92 isolates, of which Gram negative organisms (72/92) were predominant; 15% (12/80) were polymicrobial. Pseudomonas aeruginosa was the commonest isolate in both single and multiple ulcer emphasising that non-fermenting Gram negative bacilli are in a raising trend. Among our isolates, 40% were Methicillin resistant Staphylococcus aureus, 47.3% were carbapenem resistant Enterobacteriaceae and 80% of Acinetobacters were multidrug resistant.

CONCLUSION

Local microbiological profile, resistance pattern, proper wound care will significantly diminish the complications of DFU and also aid in formulating the Institutional antibiotic policy.

KEYWORDS

Diabetic Foot Ulcer; Microbiological Profile; Antibiotic Resistance.

HOW TO CITE THIS ARTICLE: Arunagiri R, Sundaramurthy R, Viswanathan A, et al. Clinicomicrobiological profile and antibiotic resistance analysis of diabetic foot ulcer from a tertiary care hospital. J. Evolution Med. Dent. Sci. 2017;6(71):5059-5064, DOI: 10.14260/Jemds/2017/1100

BACKGROUND

Diabetes mellitus (DM), a common metabolic disorder affecting various age groups is a major health issue, which remains an important cause of morbidity and mortality in various parts of the globe,[1] which is more pronounced in India.[2] Prevalence of DM is rapidly rising at an alarming rate all over the globe, especially in India which accounts for 69.2 million people with DM.[3] Burden of disease along with its complications makes the entire issue more complex. Common complications of DM include cardiovascular diseases, stroke, renal failure, leg amputation due to ulcer, blindness and nerve damage.[4] About 25% of diabetics develop diabetic foot ulcer (DFU) in their lifetime,[5] which accounts for more than 30% of hospital admissions among diabetics.[6] Complications of diabetic foot in turn leads to lengthy hospital stay, amputation, sepsis and even mortality.[4,7,8] Infections of diabetic foot ulcer is multifactorial. Bacterial causes of DFU are highly variable and their pattern varies with geographical region, underlying complications and patient population under study.[7,9] Moreover, antibiotic resistance, economic burden, compromise in quality of life due to amputation are common outcomes of this condition.[10]

Management of DFU is an equally complex and a difficult task to execute due to various factors. In addition to this, polymicrobial aetiology and multidrug resistance strains narrow down the choice of antibiotics used to treat the condition adding complexity to the entire issue.[11] So knowledge about the local microbiological profile of DFU with their resistance pattern and common predisposing factors are the essential factors for every institute to have the well-established antibiotic policy against DFU, which in turn reduces the economic burden and improve the quality of life.

Financial or Other, Competing Interest: None.
Submission 29-07-2017, Peer Review 22-08-2017,
Acceptance 28-08-2017, Published 04-09-2017.
Corresponding Author:
Dr. Raja Sundaramurthy,
Assistant Professor,
Department of Microbiology,
Velammal Medical College Hospital and
Research Institute (VMCH & RI),
Anuppanadi, Madurai-625009, Tamilnadu.
E-mail: rajacmc87@gmail.com
DOI: 10.14260/jemds/2017/1100

Our study aimed at isolating the aerobic bacterial organisms from DFU patients along with their resistance pattern, which will be helpful to the clinician to initiate an appropriate empirical therapy.

MATERIALS AND METHODS

This descriptive study was carried out in Velammal Medical College and Research Institute, Madurai, Tamilnadu for a period of 6 months (September 2016 to February 2017) after obtaining the Institutional Ethics Committee approval (IEC Ref No.: VMCIEC/12/2016). Informed consent was obtained from each patient. A total of 100 consecutive in-patients with the clinical diagnosis of diabetic foot ulcer of Grade 2 and above as per Wagner's classification were included in the study. Non-diabetic foot ulcer patients, Wagner's classification Grade 1 diabetic foot ulcers, outpatient department DFU patients, past history of amputation and those not willing for participation were excluded from the study.

Sample Collection

After obtaining an informed consent from the participant, a structured proforma was used to fill the demographic, clinical and microbiological investigation details. Ulcer was cleaned with sterile saline. Exudate from the ulcer or tissue biopsy was collected in a sterile container. Alternatively, if swabs were used to collect the sample, two sterile swabs, one for gram staining and another for culture was used. The collected samples were transported to the microbiology lab as early as possible for further processing.

Processing

Gram staining was done on the sample and the findings were recorded. The sample was inoculated into Blood agar and MacConkey agar and incubated at 37ºC for 24 hours. After overnight incubation, plates were examined for growth. Gram staining of the culture was done and the observations were recorded. Final identification of the organisms were based on the conventional techniques.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed on Mueller-Hinton agar by Kirby-Bauer disc diffusion method as per CLSI guidelines. Antibiotic discs and Va E strip were commercially obtained from Hi-Media Laboratories. Following antibiotic discs and Va E strip were used in our study.

For Gram positive isolates- Penicillin (P), Cefoxitin (CX), Clindamycin (CD), Erythromycin (E), Gentamicin (G), Amikacin (AK), Ciprofloxacin (CIP), Cotrimoxazole (COT), Netilmicin (NET), Doxycycline (DO), Linezolid (LZ) and Vancomycin (VA).

For Gram negative isolates- Ceftriaxone (CTR), Ceftazidime (CAZ), Cefepime (CPM), Cotrimoxazole (COT), Ciprofloxacin (CIP), Levofloxacin (LE), Amikacin (AK), Gentamicin (G), Netilmicin (NET), Doxycycline (DO), Piperacillin-Tazobactam (PIT), Imipenem (IMP) and Colistin (CL) were used.

RESULTS

A total of 100 patients of diabetes with at least Grade 2 Wagner's classification of foot ulcers were enrolled in our study. All of them were males and known case of type 2 diabetes mellitus. Age range of study population was 34 to 74 years with the mean age of 56.32 years.

Among the study population, major group (74%) of DFU patients were with the diabetic duration of 1 - 10 years with the mean diabetic duration of 8.2 years followed by 16% were with 11 - 20 years diabetic, 8% were with 21 - 30 years diabetic and 2% does not know their diabetic status on presentation.

Among the study population, 48% were from rural setting and 52% were from urban setting. Most common predisposing factor for DFU among the study population was neuropathy 48% followed by trauma 30%, systemic hypertension and retinopathy 8%, nephropathy 4% and coronary artery disease 2%.

In our study population, 62% were on regular treatment. Among this 74% people were on oral hypoglycaemic agents, 12% were on insulin, another 14% were on both insulin and oral hypoglycaemic; 2% of the study population was not on any treatment.

On analysing the ulcer, 54% had single ulcer and 46% had multiple ulcers. All the subjects had ulcer in the sole of the foot, especially on the base of the toe and the heel. All ulcers were grade 2 as per Wagner's classification.

Among 100 samples collected 80% were culture positive. These 80 samples yielded 92 isolates. Among them 72 were Gram negative organisms and 20 isolates were Gram positive organisms.

A total of 15% (12/80) of cases revealed polymicrobial isolates. The commonest combinations were 8 cases Klebsiella pneumoniae with Proteus mirabilis, 2 cases were Klebsiella pneumoniae with Acinetobacter and 2 were Escherichia coli with Beta Haemolytic Streptococci.

Among the Gram negative isolates n= 72, 38 (52.7%) were Enterobacteriaceae (Figure 1) and 34 (47.2%) were non-fermenting Gram negative bacilli (NFGNB).

Among the NFGNB, Pseudomonas aeruginosa was the commonest isolate 70.5% (24/34) followed by Acinetobacter baumannii 29.4% (10/34). In the Gram positive isolates, Staphylococcus aureus was the predominant isolate (Figure 2).

Pseudomonas aeruginosa (24/92= 26%) was the commonest pathogen isolated in both single as well as multiple ulcer followed by Klebsiella pneumoniae (16/92= 17.4%), Staphylococcus aureus (10/92= 10.9%) and Acinetobacter baumannii (10/92= 10.9%) (Table 1).

Pseudomonas aeruginosa followed by Klebsiella pneumoniae and Acinetobacter baumannii were the common isolates in single ulcer DFU patients, whereas Pseudomonas aeruginosa followed by Escherichia coli and Staphylococcus aureus were the common isolates among the multiple ulcers DFU patients.

Among the Gram negatives, 18 isolates of the total 38 (47.3%) were Carbapenem resistant Enterobacteriaceae strains (CRE). Among the NFGNB, 80% of Acinetobacter baumannii were multidrug resistant. A total of 40% of Staphylococcus aureus isolates were MRSA (Table 2 and 3).

Organisms	Number of Isolates (Percentage)
Pseudomonas aeruginosa	24 (26%)
Klebsiella pneumoniae	16 (17.4%)
Staphylococcus aureus	10 (10.9%)
Acinetobacter baumannii	10 (10.9%)
Escherichia coli	8 (8.7%)
Proteus vulgaris	6 (6.5%)
Proteus mirabilis	6 (6.5%)
Beta-haemolytic Streptococci	4 (4.3%)
Enterococcus faecalis	2 (2.17%)
Enterococcus faecium	2 (2.17%)
Coagulase Negative Staphylococcus	2 (2.17%)
Enterobacter species	2 (2.17%)

Table 1. Organisms Isolation among Study Population

Isolates (Numbers)	CAZ	CTR	CPM	CIP	COT	AK	G	NET	LE	DO	PIT	IPM	CL
Klebsiella pneumoniae	10	10	8	10	8	12	14	2	2	4	10	8	0
Escherichia coli	4	4	4	4	8	2	4	3	4	8	4	4	0
Pseudomonas aeruginosa	2		2	0		4	6	2			2	2	0
Acinetobacter baumannii	8	8	8	8	10	8	8	8	4	8	6	6	0
Proteus mirabilis	4	4	4	3	3	4	4	4			2	2	
Proteus vulgaris	3	3	3	3	3	4	4	4			3	3	
Enterobacter species	1	1	1	0	1	1	1	0	1	1	1	1	0

Table 2. Resistance Pattern- Gram Negative Isolates

*Ceftazidime (CAZ), Ceftriaxone (CTR), Cefepime (CPM), Cotrimoxazole (COT), Ciprofloxacin (CIP), Levofloxacin (LE), Amikacin (AK), Gentamicin (G), Netilmicin (NET), Doxycycline (DO), Piperacillin-Tazobactam (PIT), Imipenem (IMP), Colistin (CL)

Isolates (Numbers)	P	CX	E	CD	CIP	COT	DO	AK	G	NET	LZ	VA E Strip
Staphylococcus aureus (10)	10	4	6	2	10	2	2	6	2	0	0	0
Beta-haemolytic Streptococci (4)	0		0	0			0				0	0
Coagulase negative Staphylococcus (2)	2	2	2	1	2	1	1	2	2	2	0	1
Enterococcus faecalis (2)	1								1		0	0
Enterococcus faecium (2)	2								2		0	0

Table 3. Resistance Pattern- Gram Positive Isolates

*Penicillin (P), Cefoxitin (CX), Clindamycin (CD), Erythromycin (E), Gentamicin (G), Amikacin (AK), Ciprofloxacin (CIP), Cotrimoxazole (COT), Netilmicin (NET, Doxycycline (DO), Linezolid (LZ), Vancomycin (VA).

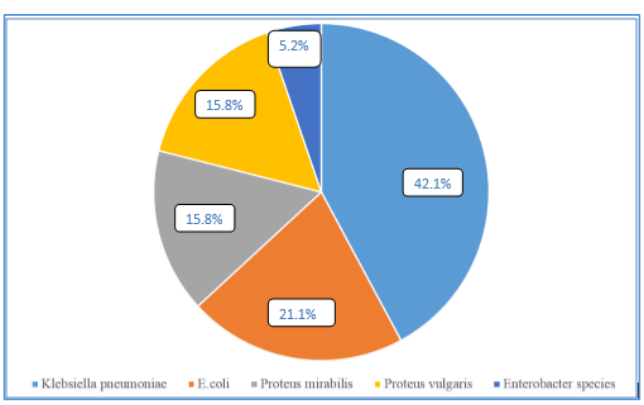

Figure 1. Enterobacteriaceae Isolation among DFU

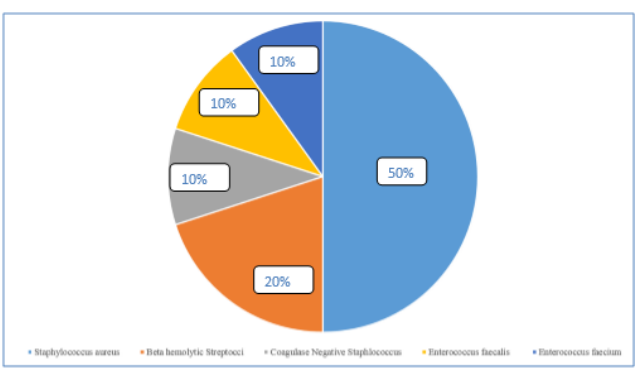

Figure 2. Gram Positive Isolation among DFU Patients

	Isolation (%) of the 38 Isolates
Klebsiella pneumoniae	42.1
E. coli	21.1
Proteus mirabilis	15.8
Proteus vulgaris	15.8
Enterobacter species	5.2

	Isolation (%) of the 20 Isolates
Staphylococcus aureus	50
Beta-haemolytic streptococci	20
Coagulase Negative Staphylococcus	10
Enterococcus faecalis	10
Enterococcus faecium	10

DISCUSSION

Chronic non-healing foot ulcer is one of the most common complications encountered by diabetic patients which may occur due to several factors like neuropathy, vasculopathy and trauma accounting for up to 30% of diabetes-related hospital admissions.[12–14] Such chronic long-standing ulcers in diabetic patients are more prone for bacterial infections that spread rapidly which may end up in significant morbidity, increasing economic burden to the patients in the form of prolonged hospital stay, laboratory and drug costs, lower extremity amputations, sepsis and even mortality.[15–18] Knowledge about microbiological profile of DFU with their sensitivity pattern, targeted therapy and high standard of care of ulcer are essential to diminish the detrimental consequences associated with diabetic foot ulcers and to significantly improve the outcome.

A total number of 100 diabetic patients with Wagner's classification grade 2 were enrolled in our study. All of them were males between 34 to 74 years with the mean age of 56.32 years. Our findings were in concordance with studies carried out by Patil SV et al,[19] Ramani et al[20] and Viswanathan et al[21] who also reported that DFU was most common among males and late 50's age group. Male predominance may be due to the factors that males spend more time in outdoor works and exposure to traumas may be higher in these groups. Common prevalence among late 50's might be due to altered immune responses, occurrence of neuropathy and vasculopathy were more evident in these groups as the disease progress.

In our study, 74% of the DFU were found out in patients with diabetes duration of 1 - 10 years with the mean of 8.2 years. Our study findings were similar to the findings of Viswanathan et al[21] and Patil SV et al[19] who also reported that mean diabetic duration was 9 years. Our study results depict that occurrence of DFU starts even with diabetic duration of 1 year. Infections among these DFUs increase proportionately with duration of diabetes as all the diabetic foot yielded growth in the patients with diabetic duration of > 10 years.

Among the study population, 48% were from rural and 52% were from urban setting. Though the diabetes is prevalent among the urban population, no significant difference was found in the DFU prevalence between urban and rural diabetic population.

Most common predisposing factor for DFU was neuropathy (48%) followed by trauma (30%), which was similar to the results of Ramani et al[20] and EM Shankar et al,[22] but discordant with the findings of Reiber et al[23] and Patil SV et al[19] who reported that history of trauma was the main predisposing factor. Discordance may be due to the fact that our patients not able to recollect the trauma history and neuropathy in diabetes also showing raising trend. Loss of sensation from neuropathy results in failure to perceive damage caused by trauma- such as penetration of pointed objects, excessive heat or friction from bad fitting shoes which in turn leads to ulcer. Though, 62% of our study population were on regular treatment, occurrence of DFU as a complication has shown increasing trend.

On analysing the ulcer type, 54% had single ulcer and 46% had multiple ulcers. Ulcers were most commonly present either in base of the toes or heel, which was in concordance with the study report of Shanmugam P et al.[24]

Among the 100 samples collected, 80% (80/100) were culture positive which yielded 92 isolates in total. Of these, 85% (68/80) were monomicrobial aetiology and 15% (12/80) were polymicrobial which was almost similar to the results of Scot E Dowd et al[25] and Sharma VK et al,[26] who reported 16% and 19% of polymicrobial infections respectively.

Of the 92 isolates, 72 isolates were Gram negative organisms and 20 isolates were Gram positive organisms which emphasises that Gram negative organisms are the predominant aetiology of DFU than Gram positive organisms which was similar to the results of Shankar et al,[22] Gadepalli et al[7] and Sharma VK et al.[26]

Among the Gram negative isolates, though Enterobacteriaceae (52.7%) were predominant, non-fermenting Gram negative bacilli (NFGNB) are showing the raising trend. Among the Enterobacteriaceae Klebsiella pneumoniae was the commonest isolate (42.1%) and among the NFGNB Pseudomonas aeruginosa was the commonest isolate (70.5%), which was in concordance with the study report of Sharma VK et al.[26] Also our study reveals that Pseudomonas aeruginosa was the commonest pathogen isolated in single as well as multiple ulcer followed by Klebsiella pneumoniae was the second common isolate in single ulcer. Escherichia coli was the second common isolate in multiple ulcers.

Among the Gram positive isolates, Staphylococcus aureus (50%) was the common isolate followed by Streptococci (20%). Coagulase negative Staphylococcus (10%), Enterococcus faecalis and Enterococcus faecium (10% each) show the increasing trend.

Antibiotic resistance pattern revealed 40% of Staphylococcus aureus isolates were MRSA. Among the Enterobacteriaceae, 47.3% were Carbapenem resistant Enterobacteriaceae strains (CRE). Among the NFGNB 80% of Acinetobacter baumannii were multidrug resistant, which is the alarming note has to be effectively managed.

CONCLUSION

Our study reveals that DFU increases proportionately with duration of diabetes and neuropathy was the most common complication. Even though 62% were on regular treatment for diabetes, occurrence of DFU as a complication has shown increasing trend. Total of 80% (80/100) were culture positive, which yielded 92 isolates. DFU was most commonly present either in base of the toes or heel and 15% of the infections were of polymicrobial aetiology. Our study emphasise that Gram negative organisms were the predominant and Pseudomonas aeruginosa was the most common pathogen in both single as well as multiple ulcer. Among our isolates 40% were MRSAs, 47.3% were CREs and 80% of Acinetobacters were MDR. Emergence of CRE, MDR and MRSA add complexity to the whole issue. This is a challenging situation, especially in diabetic population where healing mechanisms are compromised. Awareness about the disease, its complications, multidisciplinary approach, patient compliance to treatment modalities and good family support are essential in combating this metabolic disorder and its complications.

Implications

Our study emphasises that DFU can occur as early as one year after the onset of diabetes even in patients who are on regular treatment; that implies early screening for neuropathy (podiatric care, peripheral nerve conduction studies and advocating appropriate footwear) should be included in the early stages of diabetes. Neuropathy augmented with trauma paves way for injury, ulceration and delayed wound healing, complicates the entire issue. In the absence of proper foot care, infection sets in. In the hospital environment, multidrug pathogen causes havoc and make treatment more complicated and increases the economic burden. So proper screening for neuropathy in early diabetics can prevent this complication in a large number of diabetic populations.

As Gram negative organisms (mainly Pseudomonas and Klebsiella pneumoniae) are the most common organism in our setup and more than 50% showing sensitivity to beta lactam/inhibitor combinations, we recommend our clinician to start with Piperacillin-Tazobactam (PIT) as an initial drug; and we made culture and sensitivity testing as mandatory for all DFU to aid the targeted therapy which in turn prevents the spread of disease, unnecessary antibiotic use and increased health care costs.

Applications

Knowledge about the disease, its complications, local microbiological profile with resistance pattern, targeted therapy with multidisciplinary approach of wound care and good family support are essential in combating this metabolic disorder and its complications which in turn prevents the spread of disease, unnecessary antibiotic use and increased health care costs and also makes clinician more informed treatment decision regarding the use of novel therapeutics.

Key Features

- Study highlights Diabetic foot ulcer (DFU) showed increasing trend, even though many of them were on regular treatment for diabetes.
- Depicts that occurrence of DFU starts even with diabetic duration of 1 year with mean diabetic duration of 8.2 years and most common predisposing factors were neuropathy, trauma.
- Emphasises that 80% of DFUs were culture positive, of which Gram negative organisms were predominant; 15% were of polymicrobial aetiology.
- Pseudomonas aeruginosa was the commonest isolate in single and multiple ulcers emphasising that non-fermenting Gram negative bacilli are in a raising trend.

Depicts the alarming status of antibiotic resistance pattern of 40% Methicillin resistant Staphylococcus aureus, 47.3% Carbapenem resistant Enterobacteriaceae and 80% Acinetobacters were Multidrug Resistant.

ACKNOWLEDGEMENTS

I am very thankful to Dr. Geni VG, Assistant Professor, Microbiology; Dr. Jhansi Charles, HOD and Professor, Microbiology who helped us in every aspect starting from study formulation, reporting and compiling of the results and analysis of manuscript.

REFERENCES

[1] Boutayeb A, Boutayeb S. The burden of non communicable diseases in developing countries. Int J Equity Health 2005;4(1):2.

[2] Upadhyay RP. An overview of the burden of non-communicable diseases in India. Iran J Public Health 2012;41(3):1–8.

[3] Kaveeshwar SA, Cornwall J. The current state of diabetes mellitus in India. Australas Med J 2014;7(1):45–8.

[4] Al-Rubeaan K, Derwish MA, Ouizi S, et al. Diabetic foot complications and their risk factors from a large retrospective cohort study. PLOS ONE 2015;10(5):e0124446.

[5] Singh N, Armstrong DG, Lipsky BA. Preventing foot ulcers in patients with diabetes. JAMA 2005;293(2):217–28.

[6] Aalaa M, Malazy OT, Sanjari M, et al. Nurses' role in diabetic foot prevention and care; a review. J Diabetes Metab Disord 2012;11:24.

[7] Gadepalli R, Dhawan B, Sreenivas V, et al. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care 2006;29(8):1727–32.

[8] Hwang DJ, Lee KM, Park MS, et al. Association between diabetic foot ulcer and diabetic retinopathy. PLOS ONE 2017;12(4):e0175270.

[9] Leone S, Pascale R, Vitale M, et al. Epidemiology of diabetic foot. Infez Med 2012;20(Suppl 1):8-13.

[10] Sriyani KA, Wasalathanthri S, Hettiarachchi P, et al. Predictors of diabetic foot and leg ulcers in a developing country with a rapid increase in the prevalence of diabetes mellitus. PLOS ONE 2013;8(11):e80856.

[11] Hartemann-Heurtier A, Robert J, Jacqueminet S, et al. Diabetic foot ulcer and multidrug-resistant organisms: risk factors and impact. Diabet Med J Br Diabet Assoc 2004;21(7):710–5.

[12] Cavanagh PR, Lipsky BA, Bradbury AW, et al. Treatment for diabetic foot ulcers. Lancet 2005;366(9498):1725–35.

[13] Laing P. The development and complications of diabetic foot ulcers. Am J Surg 1998;176(2A Suppl):11S–9S.

[14] Cheer K, Shearman C, Jude EB. Managing complications of the diabetic foot. BMJ 2009;339:b4905.

[15] Armstrong DG, Lipsky BA. Diabetic foot infections: stepwise medical and surgical management. Int Wound J 2004;1(2):123–32.

[16] Lipsky BA, Berendt AR, Deery HG, et al. Diagnosis and treatment of diabetic foot infections. Clin Infect Dis Off Publ Infect Dis Soc Am 2004;39(7):885–910.

[17] Fosse S, Hartemann-Heurtier A, Jacqueminet S, et al. Incidence and characteristics of lower limb amputations in people with diabetes. Diabet Med J Br Diabet Assoc 2009;26(4):391-6.

[18] Raja NS. Microbiology of diabetic foot infections in a teaching hospital in Malaysia: a retrospective study of 194 cases. J Microbiol Immunol Infect 2007;40(1):39-44.
[19] Patil SV, Mane RR. Bacterial and clinical profile of diabetic foot ulcer using optimal culture techniques. Int J Res Med Sci 2017;5(2):496–502.
[20] Ramani A, Ramani R, Shivananda PG, et al. Bacteriology of diabetic foot ulcers. Indian J Pathol Microbiol 1991;34(2):81–7.
[21] Viswanathan V. Epidemiology of diabetic foot and management of foot problems in India. Int J Low Extrem Wounds 2010;9(3):122–6.
[22] Shankar EM, Mohan V, Premalatha G, et al. Bacterial etiology of diabetic foot infections in South India. Eur J Intern Med 2005;16(8):567-70.
[23] Reiber GE, Lipsky BA, Gibbons GW. The burden of diabetic foot ulcers. Am J Surg 1998;176(2A Suppl):5S–10S.
[24] Shanmugam P, M J, Susan SL. The bacteriology of diabetic foot ulcers, with a special reference to multidrug resistant strains. J Clin Diagn Res 2013;7(3):441–5.
[25] Dowd SE, Wolcott RD, Sun Y, et al. Polymicrobial nature of chronic diabetic foot ulcer biofilm infections determined using bacterial tag encoded FLX amplicon pyrosequencing (bTEFAP). PLoS ONE 2008;3(10):e3326.
[26] Sharma VK, Khadka PB, Joshi A, et al. Common pathogens isolated in diabetic foot infection in bir hospital. Kathmandu Univ Med J 2006;4(3):295–301.