ISSN: 0975-766X
CODEN: IJPTFI
Available Online through *Research Article*
www.ijptonline.com

CLINICAL AND MICROBIOLOGICAL CHARACTERISTICS OF DIABETIC FOOT INFECTIONS IN TEACHING HOSPITAL AT KANCHEEPURAM DISTRICT

Divya. G*, Pavithra. B, Sathish Kumar. B, Naveena. R, Ashwini. V, Sri Kiruthika.K
Department of Microbiology,
Shri Sathya Sai Medical College & Research Institute, Thiruporur,
Sri Balaji Vidyapeeth, Tamil Nadu, India.
Email: divya.ganesanmicro11@gmail.com

Received on: 15-02-2017 *Accepted on: 25-03-2017*

Abstract:

Background: Diabetic foot infections are the panic complications of the diabetic. There is an increase in rate of amputation and gangrene among diabetics. The present study is conducted to determine the clinico-microbiological characteristics and antimicrobial susceptibility profile of the organisms causing diabetic foot ulcers.

Materials and Methods: A prospective study conducted over a period of 6 months in a tertiary care center at Kancheepuram. Bacterial agents were isolated from 100 wound samples of diabetic patients and their antibiotic susceptibility pattern was determined. Gram-negative bacilli were tested for extended spectrum- β lactamase (ESBL) production by double disc diffusion method. Staphylococcal isolates were tested for susceptibility to Cefoxitin by disc diffusion method.

Results: All patients had ulcers graded 1-3 in the Wagner classification. Majority are male patients (74%). Peripheral neuropathy 31% was the commonest co-morbid condition. *Pseudomonas aeruginosa, Klebsiella pneumonia, Escherichia coli, Staphylococcus aureus* were the common etiological agents isolated. Monomicrobial infection was observed in 66% patients. 1.1% of Methicillin resistance *Staphylococcus aureus* was observed. 51.76 % of gram negative bacteria were found to produce ESBL.

Conclusion: Overall 53.96% of MDR organisms were observed. All the isolates were found to be susceptible to Imipenem, Vancomycin, Piperacillin-tazobatam. Hence combinational regimen modified appropriately to the antibiogram pattern of the isolate from individual patients and location will provide better outcome of the disease.

Key words: Diabetic foot infections; clinco-microbilogical profile; Gram-positive bacteria; Gram-negative bacteria; antibiotic resistance.

Introduction:

Diabetes is a chronic disorder and a significant health problem that affects the mass of people globally [(1).] India has reported 50.8 million of diabetic population, which is expected to shoot up to 87 million by 2030 [(2).]

The diabetic foot is one among the intense complications of the diabetes which prolongs the hospitalization among the diabetic patients [(3).] The wound infection usually begins superficially, but due to impaired defense mechanisms or when the treatment is put off, the infection can stretch out to the subcutaneous tissues and to deeper structures ultimately leading to dreadful complications such as gangrene and amputation [(4).] Diabetic foot occurs due to several pathological complications such as improper foot care, neuropathy, peripheral vascular disease, foot ulceration and infection with or without osteomyelitis [(5).]

Wound Infections are often polymicrobial, with bacterial isolates being *Escherichia coli, Proteus spp., Pseudomonas spp., Staphylococcus aureus* and *Enterococcus spp*., are frequently reported organism in cases of diabetic foot infections [(6).] In addition to that infection by MDR strains such as MRSA and ESBL further worsen the prognosis and grow the likelihood of amputation [(7).] Hence there arises the need for the assessment of infectious microorganisms on a routine basis along with general administration of regular glycemic control, wound care, surgical debridement, pressure-offloading and maintaining adequate blood supply [(8)].

Most of the diabetic foot infections need immediate antibiotic therapy to improve the chances of salvaging the limb. Initial empirical therapy should be based on clinical presentation, gram-staining results, and awareness of the organisms that are most frequently isolated from a particular infection [(9)].

Many studies have reported varied bacteriological pattern of Diabetic Foot Infections (DFIs) over the past 25 year. These discrepancies could partly have been due to the differences in the causative organisms, which had occurred over time, geographical variations, or the type and the severity of the infection, as were reported in the studies [(10)].

Hence the aim of this study was to determine the clinico-microbiological characteristics and antimicrobial susceptibility profile with special reference to MRSA and ESBL production of organisms isolated from patients with diabetic foot ulcers.

Materials and Methods:

Sample Collection:

100 patients with diabetic foot ulcers (DFUs) were included in this study. The study was conducted over a period of 6 months at Shri Sathya Sai Medical College and Research Institute. Samples were collected after explaining the aim of the research and informed consent was obtained from the patient. A clinical history was elicited with regard to patient's age, sex, type of diabetes, duration of diabetes, size of ulcer, duration of ulcer, empirical therapy given and outcome of the patients. Ulcers were graded according to Wagner Classification [(11)].

Sample Processing:

Samples were collected deep from the base of the ulcer using 2 sterile swabs. One swab was used for gram staining and the other was used for culture.

The specimens were cultured onto blood agar, chocolate agar, Mac Conkey's agar and Thioglycollate medium and incubated at 37◦ C overnight and the plates were examined for growth, the next day. The organisms were identified based on the direct gram staining, colony morphology and biochemical reactions.

Antibiotic Susceptibility Testing:

Antibiotic sensitivity testing was done by Kirby-Bauer disc diffusion testing as per CLSI guidelines [(12)]. Antibiotic discs uesd were Cefazolin-CZ (30µg), Cefotaxime-CTX/CE (30µg), Ceftazidime-CAZ/CA (30µg), Cefepime-CPM (30µg), Imipenem-IMP (10µg), Amoxycillin/clavulanic acid-AMC (20/10µg), Amikacin-AK (30µg), Gentamicin-GEN (10µg), Ampicillin-AMP (10µg), Piperacillin/tazobactam-PIT (100/10µg) and Ciprofloxacin-CIP (5µg) for gram negative organisms. Erythromycin-E (15µg), Cefotaxime (30µg), Ceftazidime (30µg), Vancomycin-VA (30µg), Linezolid-LZ (30µg), Gentamicin (10 µg), cefoxitin-CX (30 µg), Amikacin (30µg), Novobiocin-NV (30 µg), High level Gentamicin-HLG (120 µg) and Ciprofloxacin (5µg) for gram positive organisms were procured from (HIMEDIA, Mumbai, India).

MRSA and ESBL production was detected as per CLSI guidelines [(12)].

MRSA detection:

The phenotypic test for the detection of MRSA was done by using a cefoxitin (30 µg) disc.

A zone of inhibition which was less than or equal to 21 mm were considered as Methicillin Resistant *Staphylococcus aureus* (MRSA). Those isolates which produced a zone of inhibition which were equal to or more than 22 mm was

considered as susceptible to Cefoxitin and the organism was reported as Methicillin Sensitive *Staphylococcus aureus* (MSSA).

NCCLS Phenotypic Confirmatory Disc Diffusion Test for ESBL Production (PCDDT):

Ceftazidime (30 µg) and Ceftazidime plus Clavulanic acid (30/10 µg) were placed 20 mm apart on lawn culture of the test isolate on Mueller Hinton agar and incubated. Organism was considered as ESBL producer if there was a ≥ 5mm increase in diameter of Ceftazidime plus Clavulanic disc and that of ceftazidime disc

Result:

A total of 100 type 2 diabetes patients were presented in this study, which includes 74% of male and 26% of female. The age of the patient ranged from 20 years to 90 years with mean age group of 54.4 years. Maximum number of the patients (45%) belongs to 50-60 years. Duration of the diabetes ranged from 1year to more than 20 years. Majority of the lesion was observed in right toe (43%) followed by plantar (28%), dorsal (16%) and left toe (13%). About 35% of the person exhibited chronic lesion, 29% had deep lesion, 24% with superficial lesion and 12% had acute lesion.

All patients had ulcers graded 1-3 in the Wagner classification. Other complications include 31% with neuropathy, 24% with peripheral vascular disease and 16% with hypertension. Retinopathy, nephropathy and Osteomyelitis were presented in 12%, 10% and 7% of the patients respectively. Nearly half (55%) had lesions for ≥ 3 months before presentation at the hospital. The ulcer was non-necrotic in (77%) cases. More than two-thirds (83%) received medical treatment, 17% received surgical treatment, mainly in the form of debridement. None of the patients died during the hospital stay **(TABLE: 1)**. Out of 100 patients, 61 (61%) were positive for culture, while the remaining 39% did not grow any organisms. Among which 40 (66%) had mono-microbial infections and 21 (34%) had poly-microbial infections. A total of 85 bacteria were isolated from 61 positive cultures, of which gram negative bacteria 67 (79.3%) was isolated more frequently than gram positive bacteria 18 (20.7%) **(TABLE: 2).**

Bacterial distributions of diabetic foot ulcer cases were summarized in Table 3. *Pseudomonas aeruginosa* 15 (18%) was the predominant organisms isolated among gram negative followed by

Klebsiella spp, E.coli, Acinetobacter baumannii, Proteus spp and *Citrobacter spp.* On the other hand *Staphylococcus aureus* was the most frequently isolated bacteria among gram positive followed by CONS, Streptococcus and Enterococci **(TABLE: 3).**

Antibiotic sensitivity pattern of the isolates were tabulated in **TABLE 4 & 5.** Among 18 (20.7%) gram positive organisms, 1.1% of MRSA/MRSE was detected (Methicillin resistance *Staphylococcus aureus/* Staphylococcus *epidermidis*). Vancomycin was found to be the most effective antibiotic showing 100% susceptibility. ESBL production was seen in 51.76% gram negative organisms, with highest production was found in *Pseudomonas aeruginosa* (16.47%) followed by *Klebsiella pneumonia* (10.58%). Imipenem was found to be effective against gram negative organisms. Carbapenem resistance was not observed among any of the isolates tested.

Tabel-1: Characteristic of the Patient and Lesion.

S.NO	CHARACTERISTIC OF PATIENT AND LESION	VALUE (n=100)	
		N	%
1	**Age** (Years)	Mean=54.4years	
2	**Sex:**		
	Male	74	74%
	Female	26	26%
3	**Type of Diabetes:**		
	Type-1	-	-
	Type-2	100	100%
4	**Duration of Diabetes**:(years)		
	10-19	56	56%
	< 10	39	39%
	≥ 20	5	5%
5	**Location of foot ulcer:**		
	Toes(right foot)	43	43%
	Plantar	28	28%
	Dorsal portion	16	16%
	Toes(left foot)	13	13%
6	**Size of ulcer:**		
	≤ 4	51	51%
	> 4	49	49%
7	**Nature of ulcer:**		
	Non-necrotic	77	77%
	Necrotic	23	23%
8	**Type of lesion:**		
	Chronic wounds	35	35%

	Deep ulcer	29	29%
	Superficial ulcer	24	24%
	Acute wounds	12	12%
9	**Grade of ulcer:**		
	0	-	-
	1	64	64%
	2	27	27%
	3	9	9%
	4	-	-
	5	-	-
10	**Duration of ulcer**:(months)		
	> 3	55	55%
	≤ 3	45	45%
11	**Diabetic foot ulcer infected with maggots:**		
	Not Infected	87	87%
	Infected	13	13%
12	**Complications:**		
	Neuropathy	31	31%
	Peripheral vascular disease	24	24%
	Hypertension	16	16%
	Retinopathy	12	12%
	Nephropathy	10	10%
	Osteomyelitis	7	7%
13	**Treatment:**		
	Medical	83	83%
	Surgical	17	17%
14	**Discharge status:**		
	Alive	100	100%
	Dead	-	-
15	**Glycemic control at discharge**:		
	Not Control	58	58%
	Control	42	42%

Table: 2. Characteristics Of The Culture And Bacteria Isolated From The Diabetic Foot Lesion:

S.NO	CHARACTERISTICS	VALUE	
		N	%
1	Total no of specimens	100	100%
2	Number of patients with positive culture	61	61%
3	Number of cultures with 1 pathogen is isolated	40	66%

4	Number of culture with 2 or more pathogens isolated	21	34%
5	Total no of pathogen isolated	85	100%
6	Gram positive bacteria	18	20.7%
7	Gram negative bacteria	67	79.3%
8	Mono microbial infection with gram positive bacteria	12	14.1%
9	Mono microbial infection with gram negative bacteria	25	29.4%
10	Poly microbial infection with gram positive bacteria	6	7%
11	Poly microbial infection with gram negative bacteria	42	49.4%

Table: 3. Bacterial Distribution of Diabetic Foot Infection

Grams reaction:	**Organism**	**No of isolates (n=85)**	
		N	**%**
Gram positive	*Staphylococcus aureus*	5	5.8%
	Enterococcus faecalis	5	5.8%
	Staphylococcus epidermidis	5	5.8%
	*MRSA	1	1.1%
	*MRSE	1	1.1%
	Streptococcus pyogenes	1	1.1%
	TOTAL:	**18**	**20.7**%

*MRSA- Methicillin resistance *Staphylococcus aureus,* *MRSE- Methicillin resistance *Staphylococcus epidermidis*

Grams reaction:	Organism	No of isolates(n=85)	
		N	%
Gram negative	*Psedomonas aeruginosa*	15	18%
	Klebsiella pneumonia	13	15.2%
	E.coli	11	13%
	Klebsiella oxytoca	7	8.2%
	Acinetobacter baumannii	6	7%
	Proteus mirabilis	6	7%
	Proteus vulgaris	3	4%
	Citrobacter freundii	2	2.3%
	Citrobacter koseri	2	2.3%

	Citrobacter diversus	2	2.3%
	TOTAL:	**67**	**79.3%**

Table: 4 Antibiotic Sensitivity Pattern of Gram Positive Organisms (% OF Sensitivity).

Name of the organism	Total no of isolates	NV	COT	VA	LZ	CIP	G	AK	CX	CE	CA	E/ HLG*
S. aureus	5	-	2 (40%)	4 (80%)	4 (80%)	3 (60%)	2 (40%)	1 (20%)	3 (60%)	1 (20%)	1 (20%)	-
MRSA	1	-	R	1 (100%)	1 (100%)	1 (100%)	1 (100%)	1 (100%)	R	R	R	-
S. *epidermidis*	5	S	2 (40%)	4 (80%)	5 (100%)	3 (60%)	2 (40%)	2 (40%)	3 (60%)	1 (20%)	1 (20%)	-
MRSE	1	S	R	1 (100%)	1 (100%)	R	1 (100%)	1 (100%)	R	R	R	-
Strep. Pyogens	1	-	1 (100%)	-	-	1 (100%)	1 (100%)	1 (100%)	-	1 (100%)	1 (100%)	1(100%)
E. faecalis	5	-	4 (80%)	5 (100%)	5 (100%)	2 (40%)	4 (80%)	4 (80%)	-	-	-	4* (80%)

Table: 5. Antibiotic Sensitivity Pattern of Gram Negative Organisms (% of Sensitivity).

Name of the organism	Total no of isolates	CIP	AK	G	IPM	CTX	AMC	CPM	CAZ	CZ	PIT
Pseudomonas aureaus	15	11 (73%)	12 (80%)	8 (53%)	14 (93%)	-	1 (7%)	1 (7%)	2 (13%)	1 (7%)	12 (80%)
Kleb pneumoniae	13	9 (69%)	12 (92%)	10 (76%)	10 (76%)	7 (54%)	2 (15%)	4 (31%)	4 (31%)	5 (38%)	12 (92%)
E.coli	11	5	10	6	10	4	2	4	2	2	10

		(45%)	(91%)	(55%)	(91%)	(36%)	(18%)	(36%)	(18%)	(18%)	(91%)
Kleb Oxytoca	7	4 (57%)	5 (71%)	3 (43%)	6 (86%)	2 (29%)	3 (43%)	3 (43%)	3 (43%)	1 (14%)	6 (86%)
Acinetobacter baumannii	6	3 (50%)	5 (83%)	5 (83%)	6 (100%)	3 (50%)	3 (50%)	3 (50%)	1 (17%)	1 (17%)	5 (83%)
Proteus mirabilis	6	4 (67%)	6 (100%)	5 (83%)	6 (100%)	2 (33%)	1 (17%)	2 (33%)	2 (33%)	2 (33%)	5 (83%)
Proteus vulgaris	3	2 (67%)	2 (67%)	1 (33%)	3 (100%)	2 (67%)	2 (67%)	2 (67%)	1 (33%)	2 (67%)	2 (67%)
Citrobacter frendi	2	1 (50%)	2 (100%)	1 (50%)	2 (100%)	2 (100%)	2 (100%)	1 (50%)	2 (100%)	2 (100%)	2 (100%)
Citrobacter koseri	2	1 (50%)	2 (100%)	2 (100%)	2 (100%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	1 (50%)	2 (100%)
Citrobacter diversus	2	2 (100%)	1 (50%)	1 (50%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)	2 (100%)

Discussion:

Diabetes mellitus recognized to be common in Indians of the Asian subcontinent. Currently, 50.8 million Indians have diabetes. These projections indicate that India will have the largest number of diabetic patients by the year 2030 AD [(13)]. Diabetic foot infection is a common cause for the hospital admissions of the diabetic patients and caused by a number of socio cultural practices in India [(14)]. Such practices include barefoot walking, inadequate facilities for diabetic care, low levels of education and poor socioeconomic conditions [(13)].

In the present study, the prevalence of diabetic foot ulcers among the male subjects was found to be higher 74% against female 26%, ranged from 51-60 years of age group. This may be due to higher level of outdoor activity among males compared to females. This is in support with study findings of Lilian Akwah and co workers [(15)], as they indicate male dominate in having diabetes with foot infections when compared to females [(15)].

Maximum numbers of patients (45%) in our study are elderly; belongs to 50-60 years of age. Various other studies also showed high prevalence of diabetic foot among elderly [16,17,18] because elderly are likely to have lived with diabetes mellitus for a longer duration than the younger patients because as the age increases they have nutritional deficiencies and decreased immunity, thereby making them more prone to the complications that predispose to the foot ulceration [19].

Our results showed 100% of our study population had type 2 diabetes. This is in accordance with work of Lilian Akwah and coworkers [15] who had prevalence of 66% of type 2. This may be due to increase in life expectancy, obesity and sedentary life style.

Our study showed most common location of foot ulcer was seen in right foot 43% followed by plantar region 28%, dorsal portion 16% and toes of left foot 13%. This is in concurrence with the study done by Donoso *et al*, Azizul Hasan Aamir *et al* [20][21]

Diabetic patients often undergo chronic long lasting and non healing ulcers due to various underlying factors such as neuropathy, high plantar pressures and peripheral arterial diseases, such factors makes the patients prone to certain bacterial infections that lead to delayed wound healing process [22]. Complications usually begin with unrecognized foot ulcers in a patient with an insensate foot which get infected, leading to significant morbidity and lower extremity amputations [21]. From our study we found that Peripheral neuropathy (31%) was commonly associated risk factor for the development of foot ulcers among the study population. This need to be stressed to the physician especially in primary care setting in order to identify the foot at risk. Similarly to our result Azizul Hasan Aamir *et al.,* [21] Arumugam Suresh *et al.,* [23] also reported high frequency of peripheral neuropathy as a major co-morbid complication among diabetic patients.

Our study shows the predominance of monomicrobial infection in 66% of samples and poly-microbial infection in 34% of samples. This is in accordance with Sajila Nalakath Mukkunnath *et al*., [24], Nadeem Sajjad Raja *et al*., [25], Zubair *et al*., [26] who had 59.3%, 57%, 56% of monomicrobial infection and 30%, 43%, 33% of poly-microbial infection. The low prevalence of poly-microbial infection and low rate of isolated pathogens per lesion may be attributable to the lack of severity of most infections, the low virulence of isolated organisms and proper care of the hospitalized patients in our setting. In our study most predominant isolates were gram negative bacilli accounts for 79.3% followed by 20.7% of gram positive cocci. Studies from Western countries showed high preponderance of gram positive organisms from

Diabetic foot ulcer [10, 27, 28]. Whereas studies from developing countries like India showed dominance of gram negative aerobes [6]. This difference in the prevalence of gram positive and negative organisms among diabetic foot ulcer between Western and Eastern country remains unknown. However, it was suggested that environmental factors and sanitary habits such as use of water for ablution after defecation leading to fecal floral contamination of hands, so they are anticipated to be responsible for increased colonization of gram negative infections in developing country. Similar to our study [8, 15, 29, 30] also reported high incidence of gram negative among diabetic infections.

The most common isolate in both the mixed and single infection was *Pseudomonas aeruginosa* 18%. Considering all other infections *Klebsiella pneumonia* 15.2% was the second common isolate. Among gram positive *Staphylococcus aureus* was the commonest isolate 6.1%, followed by *Enterococcus faecalis* 5.8%. This is in relation to reports of Pappu *et al.,* (3), Zubair *et al.,* [26] and Ramkanth *et al.,* [31].

There is increasing prevalence of MRSA species worldwide, infection with MRSA requires aggressive therapy as infection with this organism may have a worse outcome and leaves treating physician with smaller choice in terms of use of antibiotics [32, 31]. In our study we have reported very less 1.1% of MRSA, MRSE. Recently there are various reports [33,34] on the emergence of Vancomycin resistance among gram positive organisms causing DFUs. However, our results showed fairly highly sensitive group of gram positive isolates towards vancomycin, hence vancomycin can be used as an empirical therapy against infection caused by MRSA in our setting. Whereas, among gram negative organisms tested for the presence of ESBL, nearly half 51.76% of isolates were positive for ESBL production which is high. Similar to our report Gadepalli *et al* (6) have documented 44.7% of ESBL production and Sivaraman Umadevi *et al* [22] stated 56% of ESBL production. ESBL producers are found to be resistance to 3G cephalosporins and aztreonam. Imipenem and Piperacillin/ tazobactam were the drug of choice for control and successful treatment of infection caused by ESBL producers. Our study showed overall 53.96% of MDR isolates. Similarly high frequency of drug resistance was reported earlier [6, 22] among DFUs, this is mainly due to over the counter use of broad spectrum antibiotics and prior treatment with other broad spectrum antibiotics in various other centers.

The main limitations are, our study fail to detect anaerobic bacteria and Candida. The risk factors associated with the prevalence of multidrug resistance and other resistance patterns like AmpC beta lactamases and Metallo Beta lactamases are yet to be analyzed.

Conclusion:

Diabetic foot infections are common among diabetic patients in India and pose a serious health problem. Commonly reported etiologies in our study were *Pseudomonas aeruginosa*, *Staphylococcus aureus* and *Klebsiella pneumonia*. A regimen inclusive of piperacillin-tazobactam, Imipenem and Vancomycin seems to be effective in treating diabetic foot infections especially caused by MRSA and ESBL producers. Hence proper empirical therapy has to be modified appropriately to the antibiogram pattern of the isolate from individual patients and location. This will help in right management and alleviation of the disease.

Acknowledgement: Our sincere thanks to the management and Department of Microbiology, Shri Sathya Sai Medical College and Research Institute for their encouragement and support.

References:

1. Sivanmaliappan TS, Sevanan M. Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* from diabetes patients with foot ulcers. Int J Microbiol 2011; 2011:605195.
2. Shaw JE, Sicree RA, Zimmet PZ Global estimates of the prevalence of diabetes for 2010 and 2030. Diab Res Clin Pract 2010; 87: 4-14.
3. Pappu AK, Sinha A, Johnson A. Microbiological profile of diabetic foot ulcer. Calicut Med Journal 2011; 9(3): 1-4.
4. Abd Al-HameadHefni, Al-Metwally R. Ibrahim, Khaled M. Attia, Mahmoud M. Moawad, Ayman F. El-ramah, Mohamed M. Shahin, Mahmoud Al-Molla and LotfiAbd Al-Satar. Bacteriological study of diabetic foot infection in Egypt. Journal of the Arab Society for Medical Research 2013; 8: 26–32.
5. Shakil S, Khan AU. Infected foot ulcers in male and female diabetic patients: A clinico-bioinformative study. Ann Clin Microbiol Antimicrob. 2010; 9:1-10.
6. Gadepalli R., Dhawan B., Sreenivas V., Kapil A., Ammini A.C., Chaudhry R. A. Clinicomicrobiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care, Vol 29, 1727-32, 2006.
7. Juan Gonzalez Del Castillo, Jose Ignacio BlanesMompo. Treating diabetic foot infection in the emergency department: fundamental considerations. Emergencias, Vol 24, 211-218, 2012
8. Apelqvist J, Bakker K, van Houtum WH, Schaper NC. The development of global consensus guidelines on the management of the diabetic foot. Diabetes Metab Res Rev, Vol 24 Suppl 1, S116-S118, 2008.

9. Gentry LO. Diagnosis and management of diabetic foot ulcer. J Antimicrob Chemother 1993; 32 (supl A):77-89
10. Citron DM, Goldstein EJC, Merriam VC, Lipsky BA. Bacteriology of moderate to severe diabetic foot infections and invitro activity of antimicrobial agents. J Clin Microbiol. 2007; 45 (9):2819–28.
11. Wagner FW: The dysvascular foot: a system of diagnosis and treatment. Foot Ankle 2:64 –122, 1981
12. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Twenty first informational supplement ed. CLSI document M100-S21. 2011: Pennsylvania CLSI (31).
13. Viswanathan V, Epidemiology of DiabeticFoot and Management of Foot Problems in India. The International Journal of LowerExtremity Wounds, 9(3): 122–126, (2010).
14. Viswanthan V, Snehalatha C, Terin M,Ramachandran A. Sociocultural practices that may affect the development of thediabetic foot. IDF Bull, 42; 10-12, (1997).
15. Lilian Akwah, Fokkunang Ntungween Charles,Elias Nukenine Nchinwan,Emmanuel kagning Tsinda,Armel Heere Nwabo Kamdje . Bacteriology of Diabetic Foot Ulcers with Reference to Multidrug Resistance Strains at the Yaounde Central Hospital (Cameroon). Journal of Diseases and Medicinal Plants (2015;1(4) 53-58).
16. C.Manikandan and P.Prabhakaran. Clinical and bacteriological profile of diabetic foot infections in pattukottai area hospitals, Tamilnadu ,India .International journal of current Research and Academic Review, 2015 ; 3 (4) : 166 – 173.
17. V. Jyothylekshmy, Arun s. Menon, Suja Abraham. Epidemiology of diabetic foot complications in a podiatry clinic of a tertiary hospital in South India. Indian Journal of health sciences. 2015; 8 (1) ; 48 - 51.
18. K. Sridhar, E. Vandana Giridhar, K. R.L. Suryakirani. Clinico – Bacteriological profile of diabetic foot infections. Journal of evolution of medical and dental sciences. 2014 ; 3(74) : 15487 – 15491.
19. AYESHA Nageen.A. The Most Prevalent Organism in Diabetic Foot Ulcers and Its Drug Sensitivity and Resistance to Different Standard Antibiotics descriptive and cross sectional study. Journal of the College of Physicians and Surgeons Pakistan 2016, Vol. 26 (4): 293-296
20. Donoso MTV, Rosa EG, Borges EL. Perfi l dos Pacientes com Pé Diabético de um Serviço Público de Saúde. J Nurs UFPE 2013; 7:4740-4746.

21. Azizul Hasan Aamir, Ahamed Nasir, Mohammad Zahid, Khalid Mehmood, Sobia Sabir Ali. Diabetic foot infections and their management in a tertiary care hospital, J Ayub Med Coll Abbottabad 2011; 23 (1): 58-62.
22. Sivaraman Umadevi, Shailesh Kumar, Noyal Mariya Joseph, Joshy M Easow, G Kandhakumari *et al.*, Microbiological Study of Diabetic Foot Infections. Indian Journal of Medical Specialties 2011;2(1):12-17
23. Arumugam Suresh , Gopal Muthu , Ramesh Srivani and Anand Moses. Aerobic bacterial resistance in diabetic foot ulcer from Chennai. International Journal of pharma and bio sciences. 2011 ; (2) : 517 – 528.
24. Sajila Nalaka Mukkunnath, R.Manjunath, Mahesh Desai. A study of the bacteriological profile of diabetic foot ulcer and antibiotic sensitivity pattern.2015 /991.
25. Nadeem Sajjad Raja, Microbiology of diabetic foot infection in a teaching hospital in Malaysia : a reterospective study of 194 cases, 2007, 40:39-44.
26. Zubair M, Malik A, Ahmad J. Clinico-bacteriology and risk factors for the diabetic foot infection with multidrug resistant microorganisms in North India. Biol Med. 2010; 2 (4): 22-34.
27. Dang CN, Prasad YD, Boulton AJ and Jude EB, Methicillin resistant staphylococcus aureus in the diabetic foot clinic: a worsening problem. Diabet Med 20:159–161, (2003)
28. Goldstein EJ, Citron DM, and Nesbit CA, Diabetic foot infections: bacteriology and activity of 10 oral antimicrobial agents against bacteria isolated from consecutive cases. Diabetes Care 19:638–641,(1996).
29. Thokur S . Murali, Shettigar Kavitha, Jain Spoorthi, Deepika V. Bhat, Alevoor S. Bharath Prasad, Zee Upton et al., Characteristics of microbial drug resistance and its correlates in chronic diabetic foot infections. Journal of medical microbiology. 2014; 63 : 1377 – 1385.
30. Vedat Turhon, Mesut Mutluoglu, Ali Aca, Mustafa Hatipoglu, Yalcin Onem, Gunalp uzeen; et al. Increasing incidence of Gram-negative organism in bacterial agents isolated from diabetic foot ulcers. J Infect Dev Ctries. 2013; 7 (10) : 707 – 712.
31. Ramakant, P., Verma, A. K., Misra, R., Prasad, K. N., Chand, G., Mishra, A., Agarwal, G., Agarwal, A. & Mishra, S. K. (2011). Changing microbiological profile of pathogenic bacteria in diabetic foot infections: time for a rethink on which empirical therapy to choose? Diabetologia 54, 58–64.

32. Mantey I, Hill RL, Foster AV, Wilson S, Wade JJ, Edmonds ME. Infection of foot ulcers with Staphylococcus aureus associated with increased mortality in diabetic patients. Commun Dis Public Health 2000;3:288–90.
33. Anahita Dezfulian, Mohammad Mehdi Aslani, Mahvash Oskoui, Parisa Farrokh, Masumeh Azimirad, Hossein Dabiri *et al.,* Identification and Characterization of a High Vancomycin-Resistant *Staphylococcus aureus* Harboring VanA Gene Cluster Isolated from Diabetic Foot Ulcer. Iran J Basic Med Sci 2012; 15(2): 803–806.
34. Teresa Semedo-Lemsaddeka, Carla Mottolaa, Cynthia Alves-Barrocoa, Patrícia Cavaco-Silvab,c, Luís Tavaresa, Manuela Oliveiraa. Characterization of multidrug-resistant diabetic foot ulcer enterococci. Enferm Infecc Microbiol Clin 2016;34(2):114–116.