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Bacterial Prevalence and Antibiotic Sensitivity in Diabetic Foot Ulcer: Analysis of Diabetes Spectrum

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ABSTRACT

Diabetes is a chronic metabolic disorder that affects a large number of populations globally. Approximately one-fourth of people suffering from diabetes will develop an ulcer over the foot (Diabetic Foot Ulcer- DFU) during their lifetime. Pus samples were collected from the deep base of the ulcer using sterile swabs. The organisms were identified by direct Gram staining, colony morphology and biochemical reactions. Antibiotic susceptibility testing was performed using the Kirby Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines. Total 98 patients with type 2 diabetes (T2D) mellitus were included; the susceptibility to DFU is significantly more common in the males. Patients with more than 60 years of age have a high prevalence of DFU. Eighty patients had a gram -ve bacterial infection and 17 had gram +ve infections. Fungal infection in 12 (Candida non-albicans in 8, *Candida albicans* in 2 and Mucomycosis in 2). The gram -ve bacterial infections were significantly higher as compared to other microorganisms. Among the gram-positive infections *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) infection was common. Gram-negative bacteria showed maximum sensitivity to Amikacin in 51 (63.7%), Meropenem in 45 (65.2%) and Imipenem in 44 (72.1%) patients. Gram-positive bacteria showed sensitivity to Teicoplanin in 5 (83.3%) patients and Vancomycin in 4 (80%). DFU causing fungus, Candida non-albicans showed sensitivity to Amikacin in 7 (87.5%), Meropenem in 6 (75%), Imipenem in 7 (87.5%); *Candida albicans* showed max. sensitivity to Ceftriaxone (100%); Mucomycosis showed sensitivity to Amikacin and Meropenem (each 100%). DFU were predominantly due to Gram-negative bacteria, such as *Escherichia coli*, *Pseudomonas* spp. and *Klebsiella oxytoca*. Amikacin, Ciprofloxacin, Meropenem, Imipenem and Ceftriaxone were most sensitive antibiotics.

Keywords: Diabetic foot ulcer, Gram-positive, Gram-negative, Bacteria, Antibiotic, Resistivity, Sensitivity

INTRODUCTION

Diabetes Mellitus is a chronic metabolic disorder that affects a large number of populations globally and is a major public health problem [1-3]. Approximately one-fourth of people with diabetes will develop an ulcer over the foot (Diabetic Foot Ulcer- DFU) during their lifetime and as many as half of these ulcers will become infected [4,5]. In the people with diabetes mellitus and foot ulcers, several factors, such as inappropriate antibiotic treatment, the chronic nature of the wound, and frequent hospital admission, can influence the presence of multidrug-resistant microorganisms in the foot ulcer [6,7]. Moreover, the specific organism identified in diabetic foot infections can differ not only from patient to patient and hospital to hospital but also from one part of the country to another [6,8,9].

The WHO has projected that the maximum increase in diabetes mellitus will occur in India [10]. India has nearly 33 million diabetic subjects today, which is mainly from the urban population. The scenario is also rapidly changing in rural areas. Diabetes India study confirms that the WHO estimate of 35 million adults with diabetes in India today

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[11]. Ulceration of the foot in diabetes is a common complication [12]. They also have a combined infection involving bone and soft tissue called the fetid foot. The unique anatomy of the foot is the main reason that infection is potentially serious in this location. Dorsal or plantar regions are the most common site of ulcer in diabetic patients followed by the heel and plantar metatarsals [13]. Infection is an underlying cause for the development of ulcers and unless there is aggressive intervention, amputation becomes the result [4]. Fifty percent of all traumatic amputations of the lower extremities are associated with diabetes type 1 and 2. Microbiological profile of diabetic foot ulcers is known to be polymicrobial in nature. It is commonly stated that patients with diabetes mellitus are more susceptible to infection than normal individuals. It further leads to complications and death, more frequently if not treated effectively. There are limitations in assessing the risk of infection and resulting complications for which optimal management requires a multidisciplinary approach. The present study was undertaken to assess the role of aerobic bacteria in the incidence of diabetic foot ulcers, the antimicrobial susceptibility of such isolates, would assist the clinician in instituting therapy to avoid the dreaded complications.

Most diabetic foot infections are real emergencies; therefore, antibiotic therapy should be started immediately, to improve the chances of salvaging the limb. Initial empirical therapy should be based on clinical presentation, gram-staining results and knowledge of the organisms that are most frequently isolated from a particular infection [9,14,15].

The appropriate selection of antibiotics based on the antibiograms of isolates from diabetic foot infections is extremely critical for the proper management of these infections [14,16]. Therefore, the aim of the present study was to evaluate the bacteriological profile of diabetic foot ulcers at our hospital, in order to determine the relative frequencies of bacterial isolates cultured from foot infections and to assess the *in vitro* antibiotic resistance and susceptibility of the isolated bacteria to a variety of commonly used antibiotics.

MATERIALS AND METHODS

Study design and patients

This hospital-based retrospective study includes 98 patients (11 females) with diabetic foot ulcers, who were admitted to the SGPGIMS, Lucknow, India. The study was conducted over a period of 24 months. Demographic and lesion data, including age, sex, duration of diabetic foot, diabetes medications used, features of the lesion and location of the lesion, were recorded for each patient.

Inclusion criteria: Foot ulcer patients who diagnosed or suspected to have diabetes mellitus and confirmed by elevated fasting as well as postprandial blood sugar.

Exclusion criteria: Healthy people who were suspected with foot ulcer having normal fasting and post-prandial blood sugar.

Sample processing

Samples were collected deep from the base of the ulcer using two sterile swabs. One swab was used for gram staining and the other was used for culture. To eliminate the possibility of isolating colonizing bacteria, superficial ulcers were excluded from the study. Direct gram-stained smears were examined under the microscope to evaluate a relative number of microorganisms and their morphological characteristics. Any fungal elements observed were confirmed by KOH preparation. The samples for culture were inoculated onto 5% Sheep blood agar (SBA), Chocolate agar and MacConkey's agar medium and incubated at 37°C for 24 h in 7-10% CO_2 concentration and the plates were examined for growth. Sabouraud's dextrose agar slopes were used for culture of fungus. The organisms were identified by direct Gram staining, colony morphology and biochemical reactions.

Characterization of bacterial isolates

After rinsing the wound area with saline and debriding the wound, swab/tissue samples were collected aseptically from the wound, conditioned in Stuart medium and immediately taken to the microbiology laboratory. The specimens were inoculated on blood and MacConkey agar plates for the isolation of aerobic bacteria. Additionally, thioglycolate broth and mannitol salt agar were inoculated. The media plates and broth were then incubated at 37°C for 24 h. The isolates were identified based on colony morphology, gram-staining results, motility, a catalase test, an oxidase test, a coagulase test and biochemical tests.

Antibiotic susceptibility testing

Antibiotic susceptibility testing was performed using the Kirby Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guideline [17]. The antibiotics tested for Gram-positive bacteria were azithromycin, amoxicillin/clavulanic acid; cefoxitin, cefalexin/cefalotin, erythromycin, imipenem, oxacillin, penicillin, trimethoprim-sulfamethoxazole and vancomycin, while the antibiotics tested for Gram-negative bacteria were amoxicillin/clavulanic acid, amoxicillin, ampicillin, aztreonam, cefotaxime, cefoxitin, gentamicin, imipenem, polymyxin B, norfloxacin and tetracycline. Using the broth macrodilution (tube) method (minimum inhibitory concentration (MIC)), the modified Kirby-Bauer disk diffusion method was validated for vancomycin and polymyxin B susceptibility testing of *Staphylococcus aureus* and *Pseudomonas* spp., respectively. MICs were determined and interpreted according to the criteria of the CLSI [17]. *Staphylococcus* spp. were tested for methicillin resistance using oxacillin and cefoxitin disks as recommended by the National Committee for Clinical Laboratory Standards and

according to the criteria of the CLSI, respectively. Novobiocin disks were used to distinguish *Staphylococcus saprophyticus*, which is resistant to novobiocin in culture, from other coagulase-negative staphylococci (CONS). *Streptococcus pneumoniae* isolates were identified based on standard laboratory procedures, including colony morphology on blood agar and optochin sensitivity tests [7]. *Streptococcus pyogenes* isolates were confirmed with blood agar culture and a bacitracin test, which is used in the presumptive identification of group A, beta-hemolytic streptococci. Multidrug-resistant organisms (MDROs) were defined as bacteria that were resistant to more than one or all classes of antibiotic [17-21].

STATISTICAL ANALYSIS

The statistical analysis was carried out using the SPSS software, version 23.0 and Fisher's Exact Test was used to verify the association between antibiotic use and Gram-negative bacteria resistance. In descriptive statistics, the frequency of isolate distribution and antibiotic resistance was treated as categorical variables. The chi-square or two-sided Fisher's exact test was used to discriminate whether the distributions were significantly different between different groups. The distributed variables were expressed as the Mean ± SD and compared by one-way ANOVA. Variables without a normal distribution were expressed as the median (interquartile range) and compared by Kruskal-Wallis H test. It was considered statistically significant if the two-side p-value is less than 0.05.

RESULTS

We enrolled 98 patients with diabetes and of these 11 were female. The susceptibility to foot ulcer is significantly ($p<0.01$) more in male patients than in female patients. The median age of the patients was 57.50 (23-60) years. We also found that patients with more than 60 years of age have a high prevalence of DFU.

Among the 98 patients, the specimens were culture positive in 97 and one patient had no infection. Eighty patients have a gram-negative bacterial infection and 17 have gram-positive. Out of 97 patients, 12 have a fungal infection, Candida non-albicans in 8, *Candida albicans* in 2 and Mucomycosis in 2 **(Table 1)**. The gram-negative bacterial infection were significantly ($p<0.01$) higher compared to another microorganism. The microorganisms that were isolated from the diabetic foot infections were summarized in **Figure 1**. Out of 80 gram-negative infection, *Escherichia coli*, *Klebsiella oxytoca* and Pseudomonas species are common in gram-negative bacterial infection. In gram-positive infection, *Staphylococcus aureus* and Methicillin-Resistant *Staphylococcus aureus* (MRSA) infection is common **(Table 1)**.

Table 1. Frequency of microorganism isolated from diabetic foot ulcer.

Bacterial Stain		Frequency	Percent
Gram Negative	*Acinetobacter baumannii*	8	10.0
	Enterobacter aerogenes	1	1.3
	Escherichia coli	20	25.0
	Klebsiella pneumoniae	1	1.3
	Klebsiella oxytoca	13	16.3
	Morganella morgani	3	3.8
	Pseudomonas aeruginosa	16	20.0
	Proteus mirabilis	6	7.5
	Pseudomonas species	12	15.0
Gram Positive	*Staphylococcus aureus*	9	52.9
	MRSA	7	41.2
	Staphylococcus epidermidis	1	5.9
Fungi	Candida non-albicans	8	66.7
	Candid albicans	2	16.7
	Mucomycosis	2	16.7

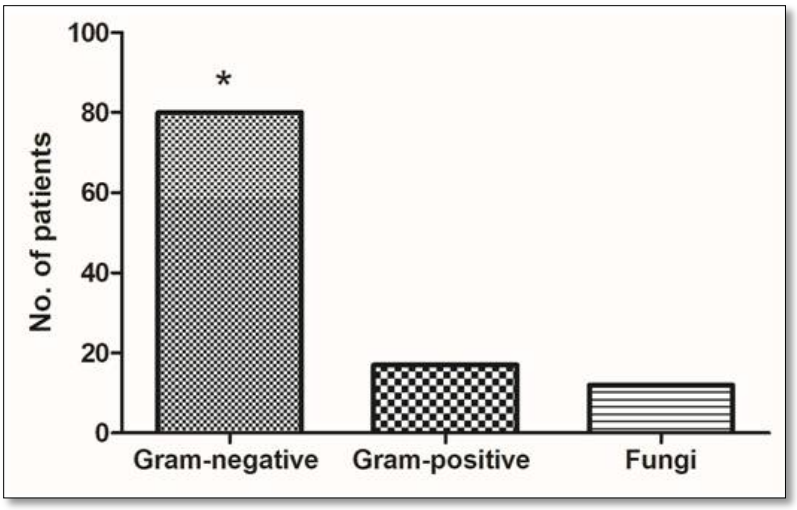

Figure 1. Type of microorganisms isolated from the diabetic foot ulcer.

The antibiotic resistance patterns of the isolated bacteria to commonly used antibiotics, obtained with the Kirby Bauer disk diffusion method, were shown in **Table 2**.

Table 2. Antimicrobial susceptibility pattern of bacteria.

Antibiotics		**Gram-Negative**		**Gram-Positive**	
		Number	**Percent (%)**	**Number**	**Percent (%)**
Amikacin	Sensitive	51	63.7	5	29.4
	Resistance	29	36.3	12	70.6
Ceftazidime	Sensitive	27	36	2	25
	Resistance	48	64	6	75
Ceftriaxone	Sensitive	26	45.6	2	28.6
	Resistance	31	54.4	5	71.4
Ciprofloxacin	Sensitive	27	47.4	3	42.9
	Resistance	30	52.6	4	57.1
Meropenem	Sensitive	45	65.2	4	50
	Resistance	24	34.8	4	50
Imipenem	Sensitive	44	72.1	2	66.7
	Resistance	17	27.9	1	33.3
Erythromycin	Sensitive	6	60	1	11.1
	Resistance	4	40	8	88.9
Teicoplanin	Sensitive	6	100	5	83.3
	Resistance	-	-	1	16.7
Vancomycin	Sensitive	5	100	4	80
	Resistance	-	-	1	20

Gram-negative bacteria showed sensitivity to following antibiotics (Amikacin 51 (63.7%), Ceftazidime 27 (36%), Ceftriaxone 26 (45.6%), Ciprofloxacin 27 (47.4%), Meropenem 45 (65.2%) and Imipenem 44 (72.1%)). Imipenem was the most effective antibiotic against Staphylococcus bacteria. Gram-positive bacteria shows sensitivity to Teicoplanin in five (83.3%) patients and Vancomycin in four (80%). Details of sensitivity were shown in **Table 2**.

Fungus involved in causing diabetic foot ulcers were Candida non-albicans, *Candida albicans* and Mucomycosis. Candida non-albicans showed sensitivity to antibiotics Amikacin in 7 (87.5%), Meropenem in 6 (75%), Imipenem in 7 (87.5%), Ertapenem in 4 (66.7%) patients. *Candida albicans* showed sensitivity to antibiotics Ceftriaxone in 1 (100%), Amikacin in 1 (50%), Ceftazidime in 1 (50%) DFU individuals. Likewise, mucomycosis showed sensitivity to antibiotics Amikacin in 1 (100%) and Meropenem in 2 (100%) patients **(Table 3)**.

Table 3. Antimicrobial susceptibility pattern for studied fungus.

Antibiotics		**Candida non-albicans Number (%)**	***Candida albicans* Number (%)**	**Mucomycosis Number (%)**
Amikacin	Sensitive	7 (87.5)	1 (50.0)	1 (100)
	Resistance	1 (12.5)	1 (50.0)	-
Ceftazidime	Sensitive	3 (42.9)	1 (50.0)	-
	Resistance	4 (57.1)	1 (50.0)	2 (100)
Ceftriaxone	Sensitive	2 (40.0)	1 (100)	-
	Resistance	3 (60.0)	-	2 (100)
Ciprofloxacine	Sensitive	3 (50.0)	1 (50.0)	-
	Resistance	3 (50.0)	1 (50.0)	1 (100)
Meropenem	Sensitive	6 (75.0)	1 (50.0)	2 (100)
	Resistance	2 (25.0)	1 (50.0)	-
Imipenem	Sensitive	7 (87.5)	-	-
	Resistance	1 (12.5)	1 (100)	-
Ertapenem	Sensitive	4 (66.7)	-	-
	Resistance	2 (33.3)	-	-
Sulbactum+Cefoperazone	Sensitive	3 (60.0)	-	-
	Resistance	2 (40.0)	-	-

DISCUSSION

DFU is a global, complex and expensive health problem. The emergence of antimicrobial resistance to selective drug limits the usage of antibiotics to only clinically infected foot ulcers and to use the anticipated spectrum of antimicrobial or else untreated DFU may risk for limb loss [9,22,23].

In the present study, we found that elderly patients (>60 years of age) constituted the majority of patients with foot infections. This may be explained by the fact that foot lesions occur commonly among patients with long-standing diabetes mellitus, particularly the elderly and those with sensory neuropathy [22]. Similar to the previous study we noted that the susceptibility to foot infections is greater in male patients than in female patients [24,25].

Diabetic foot ulcers are colonized by pathogenic bacteria that may predispose a susceptible patient to a lower extremity infection, defined as the invasion and multiplication of microorganisms in body tissues associated with tissue destruction or host inflammatory responses [26].

Our studies have reported that Gram-negative bacteria were predominant. Aerobic Gram-negative bacteria (mainly Enterobacteriaceae and sometimes *Pseudomonas aeruginosa* or other Gram-negative species) are usually isolated in conjunction with Gram-positive cocci in patients with chronic or previously treated infections.

The prognosis of diabetic foot infections remains poor, and the outcomes have been reported to be worse with MDROs than with non-MDROs in patients with diabetic foot

infections. Our study showed that MDROs were common in hospitalized patients with chronic and acute wounds. An increase in the occurrence of chronic wound infections with MDROs in the diabetes mellitus population has been noted over the last decade and has been primarily attributed to MRSA, but antibiotic-resistant Gram-negative organisms, particularly *Pseudomonas aeruginosa*, have also been implicated [27,28]. In our study, few patients underwent some type of amputation. However, almost all patients had chronic wounds caused by monomicrobial infections of Gram-negative bacteria and polymicrobial infections. Moderate to severe infections often necessitate empirical regimens with activity against commonly isolated Gram-negative bacilli, MRSA and perhaps Enterococcus species [29]. Mild infections are often managed with local wound care strategies and/or prophylactic measures. It is important to note that the decisions relating to the antibiotic treatment of wounds are influenced by clinical evidence, the availability of appropriate antibiotic interventions, patient's requirement and practitioner's expertise [30].

The antibiogram-resistogram pattern study of gram-negative bacteria isolated from DFU patients showed that *Escherichia coli*, *Klebsiella oxytoca* and Pseudomonas species are common. On the other hand, Gram-positive bacteria isolated from the foot ulcers of patients with diabetes showed that *Staphylococcus aureus* was the predominant pathogen.

Enterobacter spp. was resistant to the majority of antibiotics tested, which is consistent with the findings of a previous study [31]. Moreover, *Proteus* spp. was resistant to all beta-lactams except imipenem, cefoxitin (a cephamycin) and gentamicin (an aminoglycoside antibiotic). Furthermore, *Escherichia coli* were resistant to the majority of antibiotics tested, except gentamicin and imipenem. Therefore, in our study, gentamicin and imipenem were the most effective antibiotics against almost all bacteria from the Enterobacteriaceae family, which is partially consistent with the results of previous studies [32,33].

We have found that Amikacin and Imipenem are the most effective antibiotic against Gram-negative organisms, including *Pseudomonas aeruginosa*. Differences in the results obtained in many studies show that the patterns of microbial infection are not consistent in patients with DFU; therefore, repeated evaluation of microbial characteristics and the antibiotic sensitivity is necessary for the selection of appropriate antibiotics [6].

In our study, fungal infection caused by Candida non-albicans, *Candida albicans*, Mucomycosis also involve in creating an atmosphere, which cause DFU. *Candida albicans* is the main etiologic Candida species associated with various type of disease including diabetes and related ulcers [34]. Several non-albicans Candida species like *C. glabrata*, *C. parapsilosis*, *C. tropicalis*, *C. krusei* and *C. auris*, etc., are more likely to be antifungal resistant and have the potential to cause outbreaks of diseases [35]. Therefore, above findings highlighted that Amikacin, Ciprofloxacin, Meropenem, Imipenem and Ceftriaxone were most sensitive antibiotics in the cure of DFU caused by microorganisms.

A common risk factor for the development of highly resistant bacteria is the previous use of broad-spectrum antibiotics. In our study, all patients had received antibiotic therapy prior to surgical debridement and this may explain the higher rate of multidrug-resistant bacteria present in the diabetic foot lesions in our study. Patients with DFU are usually hospitalized multiple times and are often exposed to multiple courses of antibiotics [36], which may influence antibiotic resistance. Therefore, the potential presence of such resistant strains emphasizes the importance of obtaining optimal specimens from diabetic foot infections for culture and sensitivity testing [36,37] as well as the need to avoid excessive antibiotic therapy that promotes this resistance.

CONCLUSION

The present study reports the high prevalence of multidrug-resistant pathogens in diabetic foot ulcers. DFU were predominantly due to gram-negative bacteria, such as *Escherichia coli*, *Pseudomonas* spp. and *Klebsiella oxytoca*. Many studies on the bacteriology of DFU have reported results that vary and are often contradictory [38,39]. In such cases, the application of molecular techniques may lead to more accurate microbial characterizations and targeted antibiotic therapy. Therefore, it is necessary to evaluate the different microorganisms infecting the wound on a routine basis and to know the antibiotic susceptibility patterns of the isolates from the infected wound in patients. This knowledge is crucial for planning the treatment of these patients with the appropriate antibiotics, reducing resistance patterns, and minimizing healthcare costs. We hope the data presented in this article can assist the clinicians in determining the multidrug-resistant pathogens in DFU.

DATA AVAILABILITY

All the data created and used to support the findings of this study are included within the article.

CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there are no conflicts of interest.

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REFERENCES

1. Hu FB (2011) Globalization of diabetes: The role of diet, lifestyle and genes. Diabetes Care 34: 1249-1257.

2. Hobizal KB, Wukich DK (2012) Diabetic foot infections: Current concept review. Diabet Foot Ankle 3: 10.
3. Grennan D (2019) Diabetic foot ulcers. JAMA 321: 114.
4. Wu SC, Driver VR, Wrobel JS, Armstrong DG (2007) Foot ulcers in the diabetic patient, prevention and treatment. Vasc Health Risk Manag 3: 65-76.
5. Sumpio BE (2012) Contemporary evaluation and management of the diabetic foot. Scientifica (Cairo) 2012: 435487.
6. Singh N, Armstrong DG, Lipsky BA (2005) Preventing foot ulcers in patients with diabetes. JAMA 293: 217-228.
7. Alexiadou K, Doupis J (2012) Management of diabetic foot ulcers. Diabetes Ther 3: 4.
8. Lipsky BA, Berendt AR, Deery HG, Embil JM, et al. (2004) Diagnosis and treatment of diabetic foot infections. Clin Infect Dis 39: 885-910.
9. Viswanathan V, Thomas N, Tandon N, Asirvatham A, Rajasekar S, et al. (2005) Profile of diabetic foot complications and its associated complications - A multicentric study from India. J Assoc Phys India 53: 933-936.
10. Ramachandran A (2005) Epidemiology of diabetes in India - Three decades of research. J Assoc Phys India 53: 34-38.
11. Jeffcoate WJ, Harding KG (2003) Diabetic foot ulcers. Lancet (London, England) 361: 1545-1551.
12. Rauwerda JA (2000) Foot debridement: Anatomic knowledge is mandatory. Diabetes Metab Res Rev 16: 23-26.
13. Dalla Paola L, Faglia E (2006) Treatment of diabetic foot ulcer: An overview strategy for clinical approach. Curr Diabetes Rev 2: 431-447.
14. Laing P (1994) Diabetic foot ulcers. Am J Surg 167: 31-36.
15. Edmonds M (2006) Diabetic foot ulcers: Practical treatment recommendations. Drugs 66: 913-929.
16. Jorgensen JH, Ferraro MJ (2009) Antimicrobial susceptibility testing: A review of general principles and contemporary practices. Clin Infect Dis 49: 1749-1755.
17. Gardner SE, Hillis SL, Frantz RA (2009) Clinical signs of infection in diabetic foot ulcers with high microbial load. Biol Res Nurs 11: 19-128.
18. Alavi A, Sibbald RG, Mayer D, Goodman L, Botros M, et al. (2014) Diabetic foot ulcers: Part I. Pathophysiology and prevention. J Am Acad Dermatol 70: 1-18.
19. Edmonds ME (1986) The diabetic foot: pathophysiology and treatment. Clin Endocrinol Metab 15: 889-916.
20. Pendsey SP (2010) Understanding diabetic foot. Int J Diabetes Dev Ctries 30: 75-79.
21. Lipsky BA, Pecoraro RE, Ahroni JH (1990) Foot ulceration and infections in elderly diabetics. Clin Geriatr Med 6: 747-769.
22. Lipsky BA (2004) International consensus group on d, treating the infected diabetic f. A report from the international consensus on diagnosing and treating the infected diabetic foot. Diabetes Metab Res Rev 20: 68-77.
23. Sivanmaliappan TS, Sevanan M (2011) Antimicrobial susceptibility patterns of *Pseudomonas aeruginosa* from diabetes patients with foot ulcers. Int J Microbiol 2011: 605195.
24. El-Tahawy AT (2000) Bacteriology of diabetic foot. Saudi Med J 21: 344-347.
25. Hobizal KB, Wukich DK (2012) Diabetic foot infections: Current concept review. Diabetic Foot Ankle 3: 18409.
26. Lipsky BA (2008) New developments in diagnosing and treating diabetic foot infections. Diabetes Metab Res Rev 24: 66-71.
27. Kandemir O, Akbay E, Sahin E, Milcan A, Gen R (2007) Risk factors for infection of the diabetic foot with multi-antibiotic resistant microorganisms. J Infect 54: 439-445.
28. Lipsky BA, Berendt AR, Cornia PB, Pile JC, Peters EJ, et al. (2012) 2012 Infectious Diseases Society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin Infect Dis 54: 132-173.
29. Gottrup F, Apelqvist J, Bjarnsholt T, Cooper R, Moore Z, et al. (2013) EWMA document: Antimicrobials and non-healing wounds. Evidence, controversies and suggestions. J Wound Care 22: 1-89.
30. Banashankari G, Rudresh H, Harsha A (2012) Prevalence of gram negative bacteria in diabetic foot - A clinico-microbiological study. Al Ameen J Med Sci 5: 222-224.
31. Anandi C, Alaguraja D, Natarajan V, Ramanathan M, Subramaniam CS, et al. (2004) Bacteriology of diabetic foot lesions. Indian J Med Microbiol 22: 175-178.

32. Umadevi S, Kumar S, Joseph NM, Easow JM, Kandhakumari G, et al. (2011) Microbiological study of diabetic foot infections. Indian J Med Specialities 2.

33. Guinea J (2014) Global trends in the distribution of Candida species causing candidemia. Clin Microbiol Infect 20: 5-10.

34. Schelenz S, Hagen F, Rhodes JL, Abdolrasouli A (2016) First hospital outbreak of the globally emerging *Candida auris* in a European hospital. Antimicrob Resist Infect Control 5: 35.

35. Gales AC, Reis AO, Jones RN (2001) Contemporary assessment of antimicrobial susceptibility testing methods for polymyxin B and colistin: Review of available interpretative criteria and quality control guidelines. J Clin Microbiol 39: 183-190.

36. Gales AC, Jones RN, Sader HS (2006) Global assessment of the antimicrobial activity of polymyxin B against 54 731 clinical isolates of gram-negative bacilli: Report from the SENTRY antimicrobial surveillance programme (2001-2004). Clin Microb Infect 12: 315-321.

37. Citron DM, Goldstein EJ, Merriam CV, Lipsky BA, Abramson MA (2007) Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. J Clin Microbiol 45: 2819-2828.

38. Abdulrazak A, Bitar ZI, Al-Shamali AA, Mobasher LA (2005) Bacteriological study of diabetic foot infections. J Diabetes Complications 19: 138-141.

39. Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, et al. (2006) A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care 29: 1727-1732.