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BACTERIAL PROFILE IN PATIENTS WITH DIABETIC FOOT INFECTIONS AND ITS ASSOCIATION WITH TNF- α

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Abstract

Diabetes mellitusa (Type 2) is a crucial a health problem, that is rapidly a developed globally. One of the most commonadiabetic complications aisadiabetic foot a infection that occurs from a complicated interplay between a numbers of peril elements such as an europathy, peripheral vascular disease, a foot malformation and a trauma. The study aimed to investigate the bacterial causes of diabetic-foot ulcer and determine the antibiotic aresistance pattern of the bacterialaisolates. In addition to that serum levels of TNF-α was evaluated in diabetic patients (type 2) with foot infections. The study involved 95 wound samples were taken-from diabetic patients (type 2) with foot-infections of both sexes, age ranged between40-80 years. Bacteria isolates and identified by standard laboratory techniques. Antibiotic susceptibility test to commonly used antibiotics was done according to Kirby-Bauer method. Serum levels of TNF-α was measured in diabetic (type 2) patients with foot infections and control group by using ELISA test. *Staphylococcus aureus* most common pathogens isolated from diabetic patients followed by *P. aeruginosa* and *E. coli*. In this study bacterial pathogens showed resistance to nearly all of the antibiotics. Imipenem was the most efficient antibiotics against tested isolates. Higher levels of a TNF-α were detected in the diabetic patientss277.44 pg/ml compared to controls groups (70.13pg/ml). *S. aureus* was the most common isolated bacteria. There are increasing level of TNF-α in diabetic patients with foot infections.

Keywords : Diabetes mellitusa, Staphylococcus aureus.

Introduction

Type (2) diabetes milieus is the most common kind of diabetes, accounting for 90-94% of all diabetes. It usually develops after the age of 40, but it may occur at any age. It is more prevalent among people who are older, with sedentary a life, overweight, or have a family history of the disease (Soni, 2013). Diabetes mellitus (Type 2) or non-insulin dependent diabetes mellitusa (NIDDM) is characterized by impaired beta cell function, insulin resistance which may be combined with relatively reduced insulin secretion and is often associated with other metabolic abnormalities (Al-Tu'ma *et al*., 2011).

Patients with diabetes possess a greater incidence and intensity of several common infections including pulmonary, urinary and soft tissue infections. Foot infections one of the most common bacterial infections noticed in patients with DM in clinical lpractice recorded for up too 20% of diabetes-associated hospital admittance (Salihii and Jumaah, 2013). When the skin is broken, the hidden tissues are subjected to pathogenic organisms colonization (Mutluoglu *et al*., 2011). The resulting wound infection may begin externally, but with delay in medication and weaken body protection mechanisms, it can distributed to the subcutaneous tissues and to even deeper structures. These infections and their squeal are the majorty cause for lower-limb amputation (Raheem *et al*., 2012). Most of DFIs are multimicrobial with a blend of aerobic and an aerobic organisms. The treatment of infection in diabetic patients becomes hard because of antibiotic resistance to the frequently used antibiotics as a result of misuse of particular antibiotics (Kamel *et al*., 2014).

The increased susceptibility of diabetic patients to infections and spoiled wound curative is due to decline vascularity to the lower limb, autonomic dysfunction (Singh and Sridha, 2015), defects in both cell-mediated immunity (CMI) and humorallimmunity; also immune senescence, which happens as a result of aging and which mostly influence CMI (Rajagopalan, 2005). Diabetic foott wounds are markedd by a continuing and unbalanced inflammatory state, an enhanced producing and liberate of pro-inflammatory cytokines such as (IL-1β-, IL-6 and TNF-α) that causes disruption the equilibrium between proinflammatory and anti-inflammatory cytokines (Cory *et al*., 2015). Diabetic patients have also exhibited a remarkable up regulation insserum TNF-α through high blood glucose episodes compared with a comparatively, little change seen in normal patients (Gordin *et al*., 2008).

The aim of this study was to determinee1- the common bacterial causes of diabetic foot infections and determine the antibiotic susceptibility pattern of the

bacterial isolates. 2- Evaluate the serum level of TNF-α inn diabetic patients (type 2) with foot lesions.

Material and Methods

Bacterial Samples Collection and Diagnosis

The study was carried out on 95 diabetic patients (type2) with foot infections of both sexes with age ranged between 40-80ayears who tended to the Merjan medical city and Al-Hilla teaching hospital through a period of six months. Samples involved pus or discharges from the ulcers abase and debridedanecrotic tissues were collected by deep swab. The samples were inoculated onto the blood agar and MacConkey's agar, then incubated aerobically at 37ºC for 48hours. For isolation of anaerobes bacteria, the specimens were incubated in an anaerobic chamber at 37 ºC and examined at 48 hours and 96 hours after incubation. All bacterial isolates were diagnosis by gram′s staining and conventional biochemical tests (Collee *et al.,* 1996).

Antimicrobial Susceptibility Testing

Antibiotics sensitivity testing was conducted by Kirby Bauer′s disc diffusion method and access according to recommended National Committee- for Clinical Laboratory Standards (CLSI) guidelines. The used antibiotics were: Cefotaxime, Cephalexin, Ceftazidime, Ciprofloxacin, Cloxacillin, Carbencillin, pipracillin, Meropenem, Gentamicin, Augmentin, Lincomycin, Kanamycin, Metronidazole (Forbes *et al*.,a2007).

Estimation the Level of TNF- α

Five ml of venous blood was collected in test tube from (66) diabetic patients (type II) with foot lesion and (10) healthy control individual for estimation TNF-α level. The blood was leaved to clot and the serum-was separated-by centrifugation (2500 rpm for 10-min). Sera samples were dispensed into tubes and stored at-20°C until used. The serum level of (TNF- α) in diabetic patients and control group was examined using ELISA test (kits of Boster, USA).

Statistical Analysis

Statistical analysis was conducted. Data were presented as mean and standard deviation or number and percentage as appropriate. The Chi square test was used to analyze the significance of the results. P value <0.05 was considered significant.

Results and Discussion

Isolation and Identification of Bacterial Isolates

A total of 95 hospitalized patients-with diabetic foot infections of both sexes, age ranged between 40-80 years were examined through period of six months. The results of this study showed positive growth from only 80 (84.2%) patients, whereas 15(15.8%) patients no bacteria was isolated (Table1). Similar results was reported by Manisha *et al*. (2012) in his study who found that out of 125-specimens,-108 (86.4%) specimens exhibited bacterial growth were isolated while 17 (13.65%) specimens did not show any growth. The negative culture from infected foot could be attributed to the usage of local antibiotics and antiseptic during wound dressing that decrease the number of isolated bacteria.

Diabetic foot ulcers are common and serious complications of persistent DM. In parallell with a grow prevalence of this disease, the prevalence of -foot infections are elevating worldwide (Raheem *et al*., 2017).

Table 1 : Percentage of positive and negative culture

No. of samples	ve culture+	- ve culture
95	80 (84.2%)	15(15.8)

Single type of bacteria was found in foot infections and polymicrobial infection (i.e. infection with different type of bacteria) was also detected. Many studies support this findings (Akwah *et al*., 2015; Reghu *et al*., 2016). Among 80 bacterial isolates, 68 were aerobic, while 20 were anaerobic bacteria (Table 2). Similar findings were recorded by (Haldar *et al*., 2017; Garg *et al*., 2017).

Many studies support the result of the present study that G-ve bacteria exhibited high prevalence in diabetic-foot infection (Tiwari1*et al*., 2012; Sona *et al*., 2016; Khare *et al*., 2017).

In this study, *S. aureus* was the predominant and commonest pathogen, followed by *P. aeruginosa* and *E. coli.* The bacterial types isolated-from the diabetic foot infections were included in-(table-.2).

Table 2 : Bacterial-species isolated from diabetic foot infections

Type of bacteria	**No.**	**Single**	**Mixed.**	**(%)**
Aerobic	68	80	6	77.3%
S. aureus	17	15	2	19.3%
S. epidermidis	8	8	0	9.1%
Streptococcus pyogenes	6	5	1	6.8%
E.coli	12	10	2	13.6%
Proteus vulgaris	3	3	0	3.4%
K. peumoniea	5	4	1	5.7%
P. aeruginosa	14	12	2	15.9%
Enterobacter Spp.	3	3	0	3.4%
Anaerobic bacilli	20			22.7%
Total	88			100%

Similar results recorded in Jaddue-and Al-Kaisi., (2008)-study, that *S. aureus* was most common bacteria,

while *P. aeruginosa* and *E. coli* were the second common bacteria obtained from diabetic foot infections. Many studies like (Muter *et al*., 2012; Tamalli *et al*., 2015; Smith *et al*., 2016; Ibrahim *et al.,* 2016) support-the result of the this-study that *S. aureus* was the most frequent kind of bacteria seen in diabetic foot infections. *S. aureus* is the most vital isolated pathogen of skin-infections in generic and in manageable -diabetic foot infections.

Diabetic patients usually own persistent non curative foot ulcers by cause of several critical such as elevated plantar pressures, neuropathy and peripheral arterial disease, the risk by high levels of blood sugar in the diabetic patients which destroyed blood vessels, induced them to grow thick and leakage, this make vessels lesser capable to provide the body, exclusively the skin with blood to survive health, the result of low circulation induced ulcers, exclusively those found in the feet, like persist long-standing ulcers, So a board spectrum of bacteria can promote infection in those patients (Jeber and Saeed, 2013). An understanding of the bacterial causes of diabetic foot-infections is essential un-giving antibiotic choice and associated culture result with suitable treatment (Jaddue *et al*., 2008).

Distribution of Diabetic Foot Patients According to the Gender and Age

Regarding the gender, the results revealed that most of diabetic foot patients are men 48 (60%) (Table 3) (P = 0.074). Male predominance was seen in other studies (Chakraborty and Mukherjee, 2015; Karmaker *et al*., 2016; Gangania and Singh, 2016; Gopi *et al*., 2017). This may be as results of higher level of outdoor activity among males compared to females, Also male is subjected more to trauma, in addition to smoking and alcohol drinking which is seen more with male (Tamalli *et al*., 2015).

Table 3 : Distribution of patients according to the gender

Gender	No.	%.
Male	48	60.%
Female	32	40.%
Total	80	100.%

χ^2=3.200, P value=0.074

The results of current study shows that most of diabetic foot patients belongs to age group ranging between 61-70 than other age (table 4) (P = 0.019). This results confirmed by (Dwedar *et al.,* 2015) who found that maximum number of patients belong to age group 60 to 65 years. Mahmood, (2007) also recorded that major of diabetic foot patients with age above sixty years.

The predominance of diabetic foot ulcers in this age range may be due to the fact that patients in this age range have nutritional deficiencies and decreased immunity. In addition, the elderly have-lived for a longer time with diabetes mellitus-than the younger patients by that forming-them more susceptible to the complexity and foot-ulceration (Akwah *et al*., 2015).

Table 4 : Distribution of patients according to the age

Age group	No.	%
40-50	15	18.75%
51-60	23	28.75%
61-70	30	37.5%
71-80	12	15%
Total	80	100%

χ^2=9.900, P value=0.019

Antibiotic Susceptibility Pattern

The sensitivity of the isolated bacteriato traditionly used-antibiotics was detected by Bauer-Kirby method. Majority off *S. aureus* and *S. pygenes* bacteria were-sensitive to imipenem, ceftriaxone, ceftazidime and amoxicillin-clavulanic-acid and gentamycin. All isolates of *S. pygenes* were susceptible to amikacine, but *S. aureus* showed less sensitivity to gentamycin and amikacine (Fig-1). Our finding were compatible with Ratemo, (2014) study who recorded that *S. aureus* was susceptible to imipenem, amoxicillin-clavulanic acid, ceftazidime and ceftriaxone Banoo *et al*. (2012) in his study showed that gram-positive organisms sensitive to amoxicillin/clavulanic acid and they were highly resistant gentamicin, while Gangania and Singh, (2016) stated that cephalosporins and majorty of the aminoglycosides were effective against gram positives bacterial isolates.

Antimicrobial susceptibility results showed that all *K. peumoniea*, *P. vulgaris* and *Enterobacter Spp*. isolates were sensitive to amikacine, while it's has variable susceptibility to ceftriaxone, ceftazidime and gentamycin and full resistance to amoxicillin-clavulanic acid.

E. coli were most sensitive to toceftazidime (90%), ceftriaxone (90%), gentamycine (70%), while the sensitivity to amoxicillin-clavulanic acid was (50%) and to amikacine was (10%). The results off current study in accordance with results of Reghu *et al*. (2016) that thee sensitivity of *P. vulgaris* to amikacin was 100%. Sekhar *et al*. (2014) stated that most gram-negative isolates including ESBL producing strains of *Proteus* were highly sensitive to amikacin.

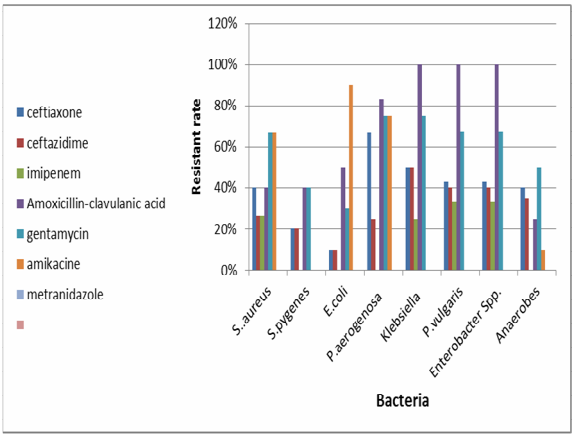

Fig. 1 : The resistant rate of bacterial isolates to the antibiotics

In study done in Iran, Al Benwan *et al.* (2012) stated that imipenem and amikacin were the most efficient antibiotics for gram-negative bacteria, But, Brenyah *et al.* (2014) in his research showed that *Proteus spp.* isolate was sensitive to ceftriaxone, with a sensitivity of 88.9%.

Similarly to our results, *E. coli* was found unsusceptible to the most of tested antibiotics, with the exception of gentamicin and imipenem. Also, it's found that *proteus, E .coli* and *Pseudomonas* showed sensitivity to ceftazidime (Banashankari *et al.*, 2012).

Others studies like Citron *et al.* (2007) revealed that *Enterobacteriaceae* family were susceptible to imipenem, ceftazidime, aminoglycosides, while amoxicillin clavulanate were the least effective antibiotic against gram negative organisms. It is necessary to acknowledge that some gram-negative bacteria from the *Enterobacteriaceae* family possess the capacity to produce highly-active ß-lactamase enzymes, forming them unsusceptible to ß-lactam and cephalosporins antibiotics (Perim *et al.*, 2015).

P. aeruginosa exhibited resistance to amoxicillin-clavulanic acid (83.3%), gentamycin (75), amikacine (75%) and ceftriaxone (66.6%). Our results is confirmed by (Tamalli *et al.*, 2015). Chaudhry *et al.* (2016) stated that *P. aeruginosae* isolates were highly resistant to the most of tested antibiotics.

This increasing multidrug resistant organisms occurrence is a potential peril component in treatment of diabetic foot infections which may promote complexity such as systemic toxicity, gangrene development and amputation of lower parts (Manisha *et al.*, 2012; Mathangi and Prabhakaran, 2013). Its responsible for the increased time of hospitalisation, cost of treatment and mortality of the diabetic-patients (Umadevi *et al.*, 2011).

In present study, most bacterial isolates were sensitive to imipenem (100%), Similar result was recorded by others studies (Perim *et al.*, 2015; Akwah *et al.*, 2015; Haldar *et al.*, 2017) that imipenem was the most active antibiotic against gram positive and gram negative bacteria; therefore, this antibiotic could be useful for usage in empirical treatment. Although in last years, there has been an increasing in the resistance to imipenem and cephalosporins probably due to their misuse of these antibiotics (Gopi *et al.*, 2017).

Antibiotic sensitivity test of anaerobic organisms showed that most isolates were sensitive to imipenem (100%), metronidazole (100%), amikacine (90%), amoxicillin clavulanic acid (75%), ceftazidime (65%), ceftriaxone (60%), gentamycine (50%). Similar findings were recorded by Banoo *et al.* (2012), that all the anaerobes were-sensitive to metronidazole and-imipenem. Haldar *et al.* (2017) also mentioned that imipenem and metronidazole had lowest resistance rates against anaerobic organism.

The increasing rates of antibiotic resistance found in this study may be because of wide spread-usage of broad spectrum antibiotics in ours hospital result in selective survival benefit of pathogens (Ibrahim *et al.*, 2016; Anvarinejad *et al.*, 2015). Also, antibiotic resistance can be partially ascribe to the capacity of these bacteria to make small colony variants after the exposed to environmental stressors or antibiotics. These variants more flexible and considered a survival mechanism used by number bacterial pathogen, such as *S. aureus* to resist changes in the environmental conditions. A late study showed that small colony variants of *S. aureus* were detected at high number in diabetic foot infections (Hassan *et al.*, 2016).

Estimation of TNF- α in Diabetic Foot Patients

The present data in table (5) revealed that the TNF-α mean serum level was-significantly-higher in diabetic foot patients (277.44 pg/mL) as compared with healthy control group (70.13 pg/mL) (P=0.0004).Similar findings were recorded by many studies (Archive *et al.*, 2008; Xu *et al.*, 2013; Yadav *et al.*, 2017) that serum levels of TNF-α were significantly elevated in diabetic foot patients.

Table 5 : Mean serum levelss of TNF-α in diabetic patients-(II) with foot infections and healthy control groups

Groups	No	TNF-α (pg/ml) serum means +SE	Min.	Max.	P1 value
Diabetic patients	66	277.44	125	1000.000	0.0004
Control	10	70.13	70	230	

TNF-α were elevated in serum of diabetic patients with foot infections with peripheral diabetic-neuropathy. In TDM patients proinflamatory-cytokines acting vital role-in the autoimmune pathogenesis of β-cell destroying. Insulin resistance has been associated with abnormal secretion of proinflammatory cytokines like (IL-6) and TNF-α and decreased making of anti-inflammatory mediators like IL-4 and IL-10 (Xiao *et al*., 2014). A high mount of TNF-α,-which suppress angiogenesis and cell reproduction in diabetic lesion and elevated apoptosis extents (Yadav *et al*., 2017).

Conclusion

Diabetic foot infections (DFIs) are important - widely known health concerns. Learning pathogens associated with DFIs and their antimicrobial susceptibility patterns are useful to prepare guidelines for appropriate antimicrobial therapy. Our study showed that *S. aureus* was most common organism detected in diabetic foot1ulcers. Imipenem-was the most efficient antimicrobial agent. We reported higher diabetic foot infection among males than female and also among individual with age ranged between 6-7 years old. Diabetic patients (type 2) with foot infection exhibit increase in the plasma levels of TNF-α compared to control group. These data indicate that the TNF-α system is activated in diabetic foot patients.

Consent

All patients' consents were taken before inclusion in the study.

Ethical Approval

Ethical Committee of the Babylon health directorate approved the study.

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References

Soni, H. (2013). Diabetes mellitus: A review. IJPBR., 4(3): 89-99.

Al-Tu'ma, F.J.; Yassin, A.G.; Tariq, H. and Al-Kayatt, T.H. (2011). Effects of Type-2 Diabetes Mellitus on Serum Leptin, Insulin, Interlukin-8, and Lipid Profile Karbala J. Med.4: (1,2).

Al-Salihi, S.S. and Jumaah, I.A.M. (2013). Bacterial infections of Diabitic ulcer. Diyala J.P. Sci., 9(4).

Mutluoglu, M.; Uzun, G.; Ipcioglu, O.M.; Sildiroglu, O.; Ozcan, O. and Turhan, V. (2011). Can procalcitonin predict bone infection in diabetic persons with infected foot ulcers? A pilot study. Diab Res Clin Pract., 5(23): 1-4.

Raheem, S.G.; Al-Barzinji, R.M. and Al-Dabbagh, A.A. (2012). Serum C-reactive protein level in diabetic foot patients and their relation with bacterial isolates. Diyala J Med., 3: 105-113.

Kamel, N.; Aboulwafa, M.M.; El-tayeb, W.N. and Aboshanab, K. (2014). Antibiotic resistance pattern of aerobic bacteria isolated from patients with diabetic foot ulcers in Egypt. Afri J Micro Res., 8(31): 2947-2954.

Singh, S.K. and Sridhar, G.R. (2015). Infections and diabetes Int J Diabetes Dev Ctries., 35(2): 59–62.

Rajagopalan, S. (2005). Infections in Elderly Patients with Diabetes Mellitus. Clinical Infectious Diseases, 40(7,1): 990–996.

DeClue, C.E. and Shornick, L.P. (2015). The cytokine milieu of diabetic wounds. Diabetes Manag. 5(6): 525–537.

Gordin, D.; Forsblom, C and Ronnback, M. (2008). Acute hyperglycaemia induces an inflammatory response in young patients with Type 1 response. Ann. Med.; 40(8): 627-533.

Collee, J.; Fraser, A.; Marmion, B. and Simons, A. (1996). Mackie and McCartney's Practical Medical Microbiology. (14thed.). Churchill livingistone, U.S.A., P: 561.

CLSI, Clinical and Laboratory Standards Institute (2012). Performance standard for antimicrobial susceptibility testing. Approved Standard-Eleventh Edition. M02-S11. 32(1).

Forbes, B.A.; Sahm, D.F. and Weissfeld, A.S. (2007). Bailey and Scotts' Diagnostic microbiology. 12th ed. Elsevier. China.

Manisha, J.; Mitesh, H.P.; Nidhi, K.S.; Dhara, J.M. and Vegad, M.M. (2012). Spectrum of microbial flora in diabetic foot ulcer and its antibiotic sensitivity pattern in tertiary care hospital in Ahmed Abad, Gujarat. Nation. J. Med., 2(3): 354- 357.

Raheem, S.G.; Al-Barzinji, R.M.; Mansoor, H.Y.; Ali, A. and Al-Dabbagh, A.A. (2017). Procalcitonin and proinflammatory parameters in diabetic foot infection as new predictive factor Citation: AIP Conference Proceedings 1888, 020043

Akwah, L.; Charles, F.N.; Nukenine, N.N.; Tsinda, E.K. and Kamdje, A.H.N. (2015). Bacteriology of Diabetic Foot Ulcers with Reference to Multidrug Resistance Strains at the Yaounde Central Hospital (Cameroon). J. Dis. Med. Plants,1(4): 53-58.

Reghu, R.; Padma, U.D.; Sasankan, V.; Puthur, S.J. and Jose, J. (2016). A Microbiological Study of Diabetic Foot Ulcer in a South Indian Tertiary Care Hospital. Int. J. Pharm. Sci. Rev. Res., 37(1):167-170.

Haldar, J.; Mukherjee, P.; Mukhopadhyay, S. and Maiti, P.K. (2017). Isolation of bacteria from diabetic foot ulcers with special reference to anaerobe isolation by simple two-step combustion technique in candle Jar. Indian J. Med. Res. 145: 97-101.

Garg, R.; Datta, P.; Gupta, V. and Chander, J. (2017). Anaerobic Bacteriological Profile of Infected Diabetic Foot Ulcers with their Antimicrobial Susceptibility Pattern: Need of the Hour. Nation. J. Lab. Med., 6(3): 1-04

Tiwari, S.; Pratyush, D.D.; Dwivedi, A.; Gupta, S.K.; Rai and Singh, M.S. (2012). Microbiological and clinical characteristics of diabetic foot infections in northern India. J. Infect. Dev. Ctries., 6(4): 329-332.

Khare, J.; Srivastava, P.; Khare, J.; Wadhwa, J. and Prasun, D. (2017). Microbiological Profile of Diabetic foot UlcersExperience from a Tertiary care centre in South India. Int. J. Gen. Med. Surg.; 1(2):109.

Sona, S.; Sreeja, P.A.; SwaroopLal, B.L.; SnigdhaAsok, P.N. and Fousiya, K.S. (2016). Bacterial Profile and Antibiotic Resistance in Patients with Diabetic Foot Infections in A Tertiary Care Hospital Palakkad. Inter. J. Pharma Res. Rev.; 5(6): 8-15.

Jaddue, D.A.K. and Al-Kaisi, K.J.I. (2008). Diabetic Foot Infection (Types of Aerobic Bacteria in Iraqi Patients). The Iraqi postgraduate medical J. 7(3).

Muter, B.A.; Jabbar, M.W.J. and Mahdi, M.A. (2012). Bacteriological study of diabetic foot ulcer and it's relation to metabolic control in AL-Nassriah city /Iraq. J.Thi-Qar Sci., 3(2): 40-46.

Tamalli, M.; Gamal, M.A.B. and Alghazal, M.A. (2015). Common aerobic bacterial isolates from diabetics foot ulcer and their antibiotic testing. World J. Pharma. Ceut. Scien., 4(08): 260-266.

Smith, K.; Collier, A.; Townsend, E.M.; O'Donnell, L.E.; Bal, A.M.; Butcher, J.; Mackay, W.G.; Ramage, G. and Williams, C. (2016). One step closer to understanding the role of bacteria in diabetic foot ulcers: characterising the microbiome of ulcers. BMC Microbiology 16: 54.

Ibrahim, A.J.K.; Bhatawadekar, S.M.; Arunima, Peerzada, B.Y.; Modak, M.M. and Lahiri, K.K. (2016). Bacterial Profile of Diabetic Foot Ulcer-Study From Western India. IJHSR., 6(5): 65-71

Jeber, M.A. and Saeed. E.A. (2013). Isolation and Identification of bacterial causes from diabetic foot ulcers Murtada A. Jeber1, Tikrit Journal of Pure Science.,18(3).

Chakraborty, P. and Mukherjee, S. (2015). Spectrum and microbiology of diabetic foot ulcer. Inter. J. Scie. Engin. Tech., 1(5).

Karmaker, M.; Sanyal, S.K.; Sultana, M.; Hossain, M.A. (2016). Association of bacteria in diabetic and non-diabetic foot infection-An investigation in patients from Bangladesh. Journal of Infection and Public Health, 9: 267-277

Gangania and Singh (2016). Bacteriological Profile of Diabetic Foot Infected Patients and their Susceptibility Pattern *Int. J. Pure App. Biosci.* 4(3): 172-178.

Gopi, A.; Samreen, F. and Jain, S. (2017). Foot ulcers at a tertiary care hospital: A clinico-microbiological profile. Indian J. Microbiol. Res., 4(4): 403-407.

Dwedar, R.; Ismail, D.K. and AmrAbdulbaky, A. (2015). Diabetic foot Infection: Microbiological Causes with Special Reference to their Antibiotic Resistance Pattern Egyptian. J. Med. Micro., 24(3): 95-102.

Perim, M.C.; Borges, C; Celeste. S.R.; Orsolin, E.F.; Mendes, R.R.; Mendes, G.O.; Ferreira, R.L.; Carreiro, S.C. and Pranchevicius, M.C. (2015). Aerobic bacterial profile and antibiotic resistance in patients with diabetic foot infections. Rev. Soc. Bras. Med. Trop., 48(5): 546-54..

Mahmood, M.A. (2007). Immunological and Microbiological Profile of Diabetic Patients with Foot Ulcers. Msc thesis. Dentistry college College Basic science.

Ratemo, N.K. (2014). Antimicrobial Susceptibility Pattern of Bacterial isolates from Pus samples at Kenyatta National Hospital, Kenya. M.Sc Thesis. Thesis in science and infectious diseases. Nairobi-Institute of tropical and infectious diseases (united).

Banoo, S.; Shubha, D.S.; Shashidhar, V. and Venkatesh, D. (2012). Bacterial and clinical profile of diabetic foot patients. Trop. Med. Public Health, 5(2): 69-73.

Sekhar, S.M.; Vyas, N.; Unnikrishnan, M.K.; Rodrigues, G.S. and Mukhopadhyay, C. (2014). Antimicrobial Susceptibility Pattern in Diabetic Foot Ulcer: A Pilot Study. Ann. Med. Health Sci. Res., 4(5): 742–745.

Al-Benwan, K.; Al-Mulla, A. and Rotimi, V.O. (2012). A study of the microbiology of diabetic foot infections in a teaching hospital in Kuwait. J Infect Public Health, 5: 1–8.

Brenyah, R.C.; Ephraim, R.K.D.; Eghan Jnr, B.A. and Asamoah, J. (2014). Bacterial Profile of Diabetic Foot Ulcers of Patients Visiting a Specialist Diabetic Clinicat KomfoAnokye Teaching Hospital, Kumasi, Ghana. British Journal of Medicine & Medical Research 4(27): 4501-4510.

Banashankari, G.S.; Rudresh, H.K. and Harsha, A.H. (2012). Prevalence of Gram Negative Bacteria in Diabetic Foot -A Clinico-Microbiological Study. Int. J. Cur. Biomed. Phar. Res., 1(2): 34 – 40.

Citron, D.M.; Goldstein, E.J.C.; Merriam, C.V.; Lipsky, B.A. and Abramson, M.A. (2007). Bacteriology of

Moderate-to-Severe Diabetic Foot Infections and *In Vitro* Activity of Antimicrobial Agents. J. Clinic. Micro.; 45(9): 2819–2828.

Perim, M.C.; Borges J.C.; Celeste, S.R.; Orsolin E.F.; Mendes, R.R.; Mendes, G.O.; Ferreira, R.L.; Carreiro, S.C. and Pranchevicius, M.C. (2015). Aerobic bacterial profile and antibiotic resistance in patients with diabetic foot. Rev. Soc. Bras. Med. Trop., 48(5): 546-54.

Chaudhry, W.N.; Badar, R.; Jamal, M.; Jeong, J.; Jamal Z.J. and Andlee, S. (2016). Clinico-microbiological study and antibiotic resistance profile of mecA and ESBL gene prevalence in patients with diabetic foot infections. Experimental and Their therapeutic medicine; 11:1031-1038.

Mathangi, T. and Prabhakaran, P. (2013). Prevalence of Bacteria Isolated from Type 2 Diabetic Foot Ulcers and the Antibiotic Susceptibility Pattern. 2(10): 329-337.

Umadevi, S.; Kumar, S.; Joseph, N.M.; Easow, J.M.; Kandhakumari, G.; Srirangaraj, S.; Raj, S. and Stephen, S. (2011). Microbiological study of diabetic foot infections. Indian J. Med. Special; 2(1): 12-17.

Anvarinejad, M.; Pouladfar, G.; Japoni, A.; Bolandparvaz, S.; Satiary, Z.; Abbasi, P. and Mardaneh, J. (2015). Isolation and Antibiotic Susceptibility of the Microorganisms Isolated from Diabetic Foot Infections in Nemazee Hospital, Southern Iran. Journal of Pathogens, 7.

Hassan, A.H.; Elamin, H.G.; Elsanousi, S.M.; Sulieman, A.M.E. and Alreshidi, M.M. (2016). The Impact of Individual and Combined Antibiotics against Resistant Bacteria Isolated from Diabetic Foot Infection. J. Micro. Res., 6(4): 69-74

Archive, G.; Adamiec-Mroczek, J. and Oficjalska-Młyńczakfor, J. (2008). Assessment of selected adhesion molecule and proinflammatory cytokine levels in the vitreous body of patients with type 2 diabetes - role of the inflammatory–immune process in the pathogenesis of proliferative diabetic retinopathy. Clinical and Experimental Ophthalmology, 246(12): 1665-1670.

Xu, H.F.; Zhang, C. and Graves, D.T. (2013). Abnormal Cell Responses and Role of TNF- in Impaired Diabetic Wound. BioMed Research International Article ID 754802, 9.

Yadav, S.K.; Arya, A.K.; RichikTripathi, R.; Tripathi, K. (2017). Assessment of Pro-Inflammatory Serum Cytokines in Patients with Type II Diabetes Mellitus with Non Healing Foot Ulcer in North India, Santosh. J Endocrinol Diabetes Obes 5(3): 1108.

Xiao, J.; Li, J.; Lu-Cai, L.; Chakrabarti, S. and Li1, X. (2014). Cytokines and Diabetes Research. Journal of Diabetes Research, 2.