Original Research Article

Prevalence of Diabetic foot ulcer infections associated with Gram negative bacteria with special reference to drug resistant isolates

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Abstract

Introduction: Diabetic Mellitus type 2 is most common causes are foot ulcer; Infections include abscess, necrotizing, fasciitis, gangrene, arthritis, tendonitis, cellulitis, osteomyelitis and eventually lead to the development of limb amputation.

Aims and Objectives: This study carried out to determine the patterns of infection in diabetic foot ulcers with special reference to the susceptibility and drug resistant isolates.

Materials and Methods: Samples were collected from 199 diabetic foot ulcers patients by using pus swab, tissue, and exudates in sterile containers and processed to using standard microbiological techniques.

Results: A total of 441 bacterial isolates were obtained from 199 patients with diabetic foot ulcers. A maximum number of patients were in the age group of 45 to 64 years and male predominant compared to female. Most of the isolates recovered according to Wagner classification system in DFU (Diabetic foot ulcer) >4cm2, >7.0 HbA1C and blood urea >30 mg/dl and serum creatinine >1.3mg/dl. 55% isolates of non-lactose fermenting Gram negative bacteria were more prevalent than 45% isolates of lactose fermenting Gram negative bacteria. The most commonly found isolate was *Pseudomonas aeruginosa* (39.6%), *Escherichia coli* (17.46%), *Acinetobacter* species (15.41%), *Proteus* species (12.47%), and *Klebsiella* species (9.75%) respectively. Antimicrobial susceptibility of the Gram negative bacteria was also studied. ESBL producing most common bacteria was *Klebsiella* species and *Citrobacter* species in this study than *Escherichia coli* and *Enterobacter* species.

Conclusion: This study shows NLF predominant than LF GNB among the isolates from the diabetic foot ulcers. Antimicrobial susceptibility result of this study suggests that pathogens having susceptible to Imipenem and Piperacillin/tazobactam were sensitive against LF, NLF-GNB and also ESBL producing bacteria. Colistin is the drug of choice multi-drug resistant (MDR) pathogens. Surveillance and monitoring on the antibiotic susceptibility pattern of the isolates will be helpful in determining the drugs for the empirical treatment of diabetic ulcers.

Keywords: Amputation, Diabetic foot ulcer, Antimicrobial susceptibility, Polymicrobial infection.

1. Introduction

Diabetic foot ulcer infections are one of the major complications of diabetes patients. 69.2 million Peoples were nationwide affected and globally 415 million people having diabetes.[1] Almost 25% of diabetic patients experiences diabetic foot ulcer infection during their lifetime.[2] A risk of acquiring foot ulcer is 10 folds greater in diabetic patients as compared to those without having diabetes.[3] Over the last three decades, diabetic foot ulcer infections reported worldwide and the result varies on the commonly encountered pathogen and also the severity of diabetic foot ulcer infection. [4] Diabetes is a multifactorial disease in which various factors act in a complex manner. The effect of diabetes includes neuropathy, peripheral vascular disease, and poor glycolic control. Hyperglycemia impairs neutrophil function, reduces the response of T cell and disorder of immunity. Trauma in patients with one or more of these risk factors precipitates the development of wounds that can be slow to heal and predispose to secondary infection.[5] According to the severity of diabetic foot ulcer, there are four classification systems, Maggit Wagner's, PEDIS (Perfusion, Extent/size, Depth/tissue loss, Infection,

Sensation), University of Texas and SINDBAD (Site, Ischemia, Neuropathy, Bacterial Infection, and Depth) which are used worldwide.[6] Gram negative bacilli are the major pathogen of diabetic foot ulcer patient's.[7] Especially extended spectrum of β-lactamase (ESBL) producing lactose fermenting Gram negative bacilli (LF GNB) and Multi-drug resistant non-lactose fermenting (MDR NLF) Gram negative bacteria isolates which lead to severe infection and also prone to amputation of major or minor below the knee and below ankle (great toe, metatarsal).[8,9] This study carried out to determine the patterns of infection in diabetic foot ulcers with special reference to the susceptibility and drug resistant isolates.

2. Material and Methods

Retrospective study was carried in the department of General surgery, Yenepoya Medical College Hospital (YMCH). YMCH are one of the charitable hospitals, Mangalore with 960 beds. YMCH receives patients from Kerala (Kannur,Calicut, Kasargod, Thrissur, Kottayam, etc) and Karnataka (Davangree, Dakshin Kannada, Hubli, Cithradurga, Udupi, Tumkur, Shimoga, Hassan etc) surrounding areas. All 199 patients with diabetic foot ulcer patients over a 3 year period from January 2013 to December 2015, identified from admitted/In-patients records in medical record department and culture were sent to the microbiology laboratory. This patient's file record designed to provide demographic and clinical information of patients including age, sex, and nature of the specimen, local examination, and diagnosis, details of antimicrobial therapy, discharge summary and patient's request to discharge and advice of the empirical therapy.

Institutional Ethics committee approved to collect data from Medical Record Department, Registration no.-YU 2016/174.

2.1 Patient selection

Diabetic foot ulcer infections patients were included in this study if they infected ulcer, osteomyelitis, necrotizing fasciitis, and gangrene or previous amputation and received treatment either as an inpatient only from January 2013 to December 2015. Each patient included only once in this study.

Antibiotics were used for determined Antimicrobial susceptibility testing- Amikacin (30μg), Imipenem (10μg), aztreonam (30μg), ceftazidime (30μg), cefotaxime (30μg), cefepime (30μg), gentamicin (10μg), piperacillin (100μg), ampicillin (10μg), Piperacillin/tazobactam (100/10μg), cefuroxime (30μg), co-trimoxazole (25μg), ciprofloxacin (5μg), colistin(10μg), and tetracycline (30μg).Pus, swab and Tissue specimen was sent to Microbiology laboratory for processing and identified according to clinical Laboratory Standard Institute (CLSI) guidelines.

2.2 Statistical Analysis

Data expressed as the mean ± standard deviation, and percentages were carried out for data analysis.

3. Result

In our study of 199 patients who attended as inpatients (IP) surgery ward, Yenepoya medical college Hospital from January 2013 to December 2015 over the period of 3 years. 124 Type 2 Diabetes mellitus (DM), 8 Type 1 DM alone and 67 patients having diabetic associated disease were found [Figure 1].

The age group of these patients ranged from 35 to 84 years and the maximum number of patients was in the age group of 45 to 64 years [Figure 2]. The Male mean age (55.57 ± 10.24), 140/199 (70.35%) and female mean age (52.52±10.40), 59/199 (29.64%) were male predominant compared to female. Most of the isolates recovered from Wagner classification system II and III in Diabetic foot ulcer (DFU), >4cm^2 ulcer [Figure 3] also high biochemical parameters such as Glycosylated haemoglobin (HBA1C), blood urea and serum creatinine [Table 1]. A total of 441 positive bacterial isolates were obtained from 199 patients, an average of 2.21 organisms per diabetic foot ulcers. Among 199 DFU patients, 80(40.20%) polymicrobial and 73(36.68%) monomicrobial, single isolates 33(16.58%) and, no growth in 13(6.53%) were detected. Among 198(45%) LF (Lactose fermenting) Gram negative isolates and 243(55%) NLF (Non lactose fermenting) Gram negative bacteria (GNB) isolates. *Pseudomonas aeruginosa* was the most frequent bacteria (n= 175; 39.68%), followed by *Escherichia coli* (n= 77; 17.46%), *Acinetobacter species* (n=68; 15.41%) and *Proteus species* (n=55; 12.47%). *Pseudomonas aeruginosa* were most frequently isolated bacteria among NLF Gram negatives 175/441(72%) and *Acinetobacter species* 68/441(28%). Multidrug resistance (MDR) isolates were most frequently in *Acinetobacter species* 21(30.88) and *Pseudomonas aeruginosa* 2(1.14%) among NLF Gram negative isolated bacteria. Although patients with diabetic foot ulcer infections associated disease which leads to the severity of infections and limb threatening cause foot amputation. In our study minor amputation, 12 Male and 1 Female (below ankle- great toe, metatarsal) were more compared to major amputation, 4 Male and 2 Female (below knee).Antimicrobial susceptibility pattern of the isolates have been tabulated in [Table 2] and geographical distribution of LF and NLF Gram negative bacteria isolates [Table 3]. LF Gram negative isolates distribution bacteria and ESBL activity [Figure 4].
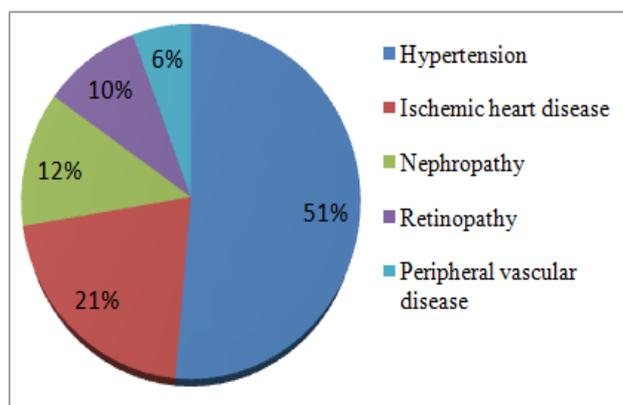
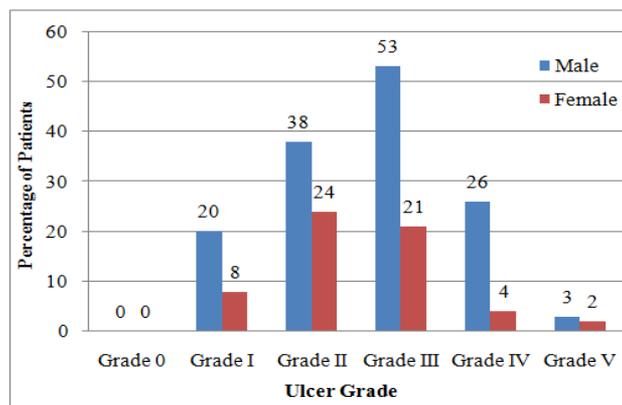Figure 1: Diabetes mellitus type 2 associated diseases

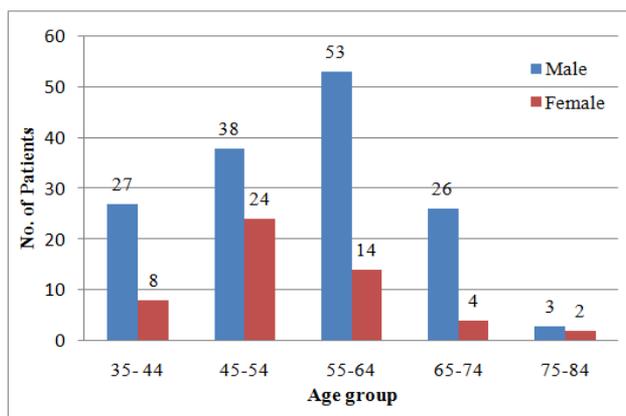Figure 3: Patient distribution according to the Wagner grade of the ulcer

Figure 2: Sex and Age distribution of diabetic foot ulcer patients
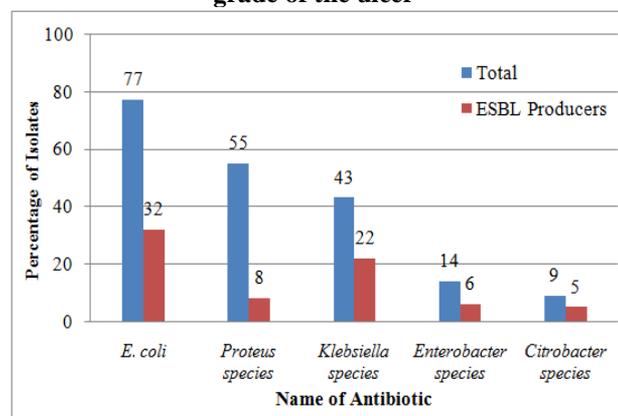

Figure 4: Percentage of ESBL producers in LF Gram Negative bacteria

Table 1: Biochemical Parameters in Diabetic Foot Ulcer Patients

Test	Result Range	Normal Range
HBA1C (Glycosylated Hemoglobin)	7.5 – 19.6%	4.5 – 6.2 %
Blood Urea	43 – 190 mg/dL	19 - 43 mg/dL
Serum Creatinine	1.3 – 11.4 mg%	0.9 – 1.3 mg/dL

Table 2: Antimicrobial susceptibility pattern of LF and NLF GNB

Antibiotics	Lactose fermenting Gram negative bacteria(LF-GNB) Resistance (%)					Non Lactose fermenting GNB Resistance (%)	
	Escherichia coli	*Proteus species*	*Klebsiella species*	*Enterobacter species*	*Citrobacter species*	*Pseudomonas aeruginosa*	*Acinetobacter species*
Amikacin	32	16	45	43	44	25	48
Imipenem	13	5	15	7	0	13	63
Pip/tazo	36	19	36	21	11	22	50
Piperacillin	55	44	51	29	44	45	72
Cipro	64	30	38	14	33	50	72
CTX	54	51	40	50	67	7	76
Cefotaxime	68	49	40	64	44	9	57
Cefuroxime	62	67	53	50	67	7	76
Cefepime	56	33	44	57	78	8	79
Ampicillin	78	51	75	57	33	32	74
Aztreonam	45	40	41	50	33	2	66
Gentamicin	34	35	33	36	22	7	63
Ceftazidime	42	15	51	43	56	25	66
Tetracycline	39	56	33	0	0	2	43

Abberivation - Pip/tazo- Piperacillin/tazobactam; Cipro- ciprofloxacin; CTX- Co-trimoxazole

Table 3: Geographical distribution of LF AND NLF-GNB isolates

Study	*Escherichia coli* %	*Klebsiella Species* %	*Proteus species* %	*Enterobacter species* %	*Citrobacter species* %	*P. aeruginosa* %	*Acinetobacter species* %
Our study	77/441 (17.46)	43/441 (9.75)	55/441 (12.47)	14/441 (3.17)	09/441 (2.04)	175/441 (39.70)	68/441 (15.41)
Ozer *et al*.[14]	36.50	36.50	-	-	-	18.90	2.70
Asha *et al*.[15]	12	17	17			23	6
Viliam *et al*.[16]	15.30	9	0	-	-	24.30	0
Mehta *et al*.[17]	23.60	27.70	8.33	-	-	34.70	15
Shanmugam *et al*.[9]	14.6	8	6.6	1.3	5.3	16	8
Remya *et al*.[18]	12.1	12.8	4.0	1.5	0.7	13.6	3.7
Jyothylekshmy *et al*.[19]	9.1	12.3	4.8	-	4.8	18.2	5.3
Hadadi *et al*.[20]	28.57	4.20	14.28	3.36	3.36	8.40	4.20

4. Discussion

The age group of these patients ranged from 35 to 84 years and the maximum number of patients was in the age group of 45 to 64 years. The Male mean age (55.57 ± 10.24), 140/199 (70.35%) and female mean age (52.52±10.40), 59/199 (29.64%) were male predominant compared to female. A study, Alva KA *et al.* [10], and Bansal *et al*., [11], reported diabetic foot ulcer 56.31% were in the age group of 45 – 64 years. Most of the isolates recovered from Wagner classification system II and III in DFU, >4cm^2 ulcer [Figure 3]. Similarly, Hefni AA *et al*., [12], reported a maximum number of isolates in diabetic foot ulcer patients in Wagner grade II and III. This study revealed that 40.20 % of diabetic foot ulcers were (80/199) polymicrobial infection, Turhan *et al*.[7], Hefni *et al*.,[12], and Murali *et al*., [8], reported rates of 16%, 40%, and 58% respectively. Monomicrobial isolates 73/199(36.68%), Murali *et al*., and Akhi *et al*., [13], reported, 42 % and 58% respectively. Our findings relatively less number of the patient were infected by single pathogens 33/199 (16.58%) compared to other Shanmugam *et al* [9], There were more NLF Gram negative pathogens isolated when compared to LF Gram negative bacteria. Our study shows that both male and female, Glycosylated hemoglobin (HBA1C), mean value 10.19 ± 2.81%, Blood urea, mean value 36.16±23.92 mg/dL, Serum creatinine, mean value 1.36±1.44 mg%.

Antimicrobial susceptibility pattern, Gram negative bacilli, *Escherichia coli* resistance to Ampicillin, cefotaxime, cefuroxime, ciprofloxacin, cefepime, piperacillin and co-trimoxazole, our study shows 78%, 68%, 64%, 62%, 56%, 55%, 54% respectively. Shanmugam *et al* [9], study resistance to ampicillin, cefotaxime, cefuroxime, ciprofloxacin, cefepime, and co-trimoxazole reported 100%, 73%, 73%, 54.5%, 82% respectively. Akhi *et al* [13], the study showed resistance to Ampicillin, ciprofloxacin, cefepime reported that study shows 57%, 86%, and 57% respectively. Another study Zubair *et al* [21], cefotaxime, cefepime, piperacillin resistance reported that 80%, 77%, 93% respectively.

Proteus species resistance to cefuroxime, tetracycline, co-trimoxazole, cefotaxime, piperacillin in our study shows 67%, 56%, 51%, 49%, 44%. Turhan *et al* [7] reported resistance to 93 % cefotaxime, and M. Zubair *et al* [21], study cefotaxime, piperacillin reported resistance 100% and 60% respectively. Chaudhry *et al* [22], resistance to 83.3% cefotaxime reported. Perim *et al* [23], study cefotaxime and tetracycline reported 60% and 50%.

Klebsiella species resistance to ampicillin, cefuroxime, piperacillin, ceftazidime, Amikacin and cefepime in our study shows 75%, 53%, 51%, 51%, and 44 % respectively. Shanmugam *et al* [9], study resistance of ampicillin, cefuroxime, Amikacin, and cefepime reported 100%, 60%, 20%, and 40% respectively. Another study Chaudhry *et al* [22], ceftazidime, Amikacin and cefepime reported 100%, 60% and 100% respectively.

Enterobacter species resistance to 64% cefotaxime, 57% (cefepime and ampicillin), 50% (Co - trimoxazole and aztreonam) and 43% (Amikacin and ceftazidime) in our study. Shanmugam *et al* [9], study resistance to 100% ampicillin and aztreonam reported. Perim *et al* [23], reported 89% cefotaxime, 100% ampicillin, and 78% aztreonam showed resistance.

Citrobacter species resistance shows in our study, 78% cefepime, 67% (cefuroxime and co-trimoxazole), 56% ceftazidime, and 44% (Amikacin, cefotaxime, and piperacillin). Similarly,

Shanmugam *et al* [9], study resistance to 75% cefepime, 100% (cefuroxime, cefotaxime, and co-trimoxazole), 50% Amikacin reported.

Extended spectrum of β- Lactamase (ESBL) screened using ceftazidime/clavulanic acid for LF GNB in our study, highest ESBL producer 5/9(55.5%) *Citrobacter species,* 22/43(51.1%) *Klebsiella species,* 6/14(42.8%) *Enterobacter species,* 32/77(41.5%) *Escherichia coli,* and, 8/55(14.5%) *Proteus species.* ESBL producer was similar to Zubair *et al* [24], and Shanmugam *et al* [9], the study showed ESBL producer 36.3% *E. coli,* 80% *Proteus* species, 40% Klebsiella species, 75% Citrobacter species.

Another study was done by Varaiya *et al* [25], 48.3% *Escherichia coli* and 51.1% *Klebsiella species.*

Pseudomonas aeruginosa resistance to ciprofloxacin, piperacillin, ampicillin, Amikacin and ceftazidime in our study shows that 50%, 45%, 32% 25% and 25% respectively. Shanmugam *et al.,* [9], study resistance to 46% ciprofloxacin, 15% Amikacin, and 61% ceftazidime. Akhi *et al*[13], the study showed resistance to 66.66% ciprofloxacin and 50% piperacillin reported. Perim *et al* [23], study showed resistance to 100% ampicillin. Another study, Chaudhry *et al*[22], showed resistance to 33.3% Amikacin, 100% ceftazidime. Zubair *et al* [24], study resistance to 78.3% piperacillin, 69.6% Amikacin, and 47.8% ceftazidime respectively.

Acinetobacter species resistance all major drugs in our study [Table-2]. Shanmugam *et al* [9], the study showed resistance to 83% ceftazidime, 67% ciprofloxacin, and 50% (cefepime, gentamicin, and tetracycline) reported. Zubair *et al* [24], study resistance to 80% (gentamicin, piperacillin, and cefotaxime), 100% ceftazidime, 20% (cefepime and Amikacin), 60% aztreonam reported.

5. Conclusion

Gram negative bacteria are playing a major role in diabetic foot ulcer infection. NLF predominant than LF GNB among the isolates from the diabetic foot ulcers, susceptible to Imipenem and Piperacillin/ tazobactam was sensitive against LF, NLF-GNB and also Colistin is the drug of choice multi-drug resistant (MDR) pathogens. Surveillance and monitoring on the antibiotic susceptibility pattern of the isolates will be helpful in determining the drugs for the empirical treatment of diabetic ulcers.

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Conflicts of interest: The authors declare that there are no conflicts of interest.

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