



Microbiological profile and Drug Resistant Organism's pattern in Diabetic Foot Ulcer Patients at Tertiary Care Hospital Puducherry

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ABSTRACT

Type II diabetes mellitus is a significant health problem that developed globally. This study was carried out on patients with diabetic foot ulcer (DFU) to assess the bacterial and fungal flora, susceptibility, and drug-resistant isolates and devises an empiric antimicrobial therapy. Clinical data and patient samples were collected from 300 diabetic foot ulcer patients between September 2014, and September 2016 and samples were processed as per CLSI guidelines. Most of the pathogenic isolate recovered according to the Wagner classification system in DFU. The most commonly found isolates in our Study was *Pseudomonas aeruginosa* (22%), *Staphylococcus aureus* (15%), *Escherichia coli* (11%) followed by others. Antimicrobial resistance appears in aerobic, anaerobic as well as candida isolates in our study. Our results show most gram-negative bacteria were sensitive to colistin and tigecycline, and 44% of Gram-negative bacteria were ESBL producers, and among 20% of the gram-negative isolates were Multidrug resistant (MDR) organisms. Proper diagnosis of the causative agents, surveillance monitoring on the susceptibility of the isolates and determining the drugs for the empirical treatment of diabetic foot ulcers will prevent prolonged hospital stay and amputation.

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INTRODUCTION

Foot ulcer infections in diabetic patients are significant complications. 69.2 million peoples were nationwide affected, and globally 415 million people having diabetes ([International Diabetes Federation, 2015](#)). Diabetes is a multifactorial disease

in which various factors act in an elaborate manner ([Walker and Colledge, 2013](#)). The effect of diabetes includes neuropathy, peripheral vascular disease and poor glycolic control. According to the severity of diabetic foot ulcer; there are four classification systems, Wagner's, PEDIS, which are used worldwide ([Chadwick et al., 2013](#)). Wagner Classification System for Foot Ulcers was described in [Table 1](#). Diabetic Patients possess a greater incidence of several common infections, including pulmonary, Urinary Tract infections. It is the most common bacterial infections noticed in patients with diabetes mellitus in clinical practice and the main reason for hospital admittance ([Al-Salihi and Jumaah, 2013](#)). Pathogenic bacteria mostly colonize these ulcers, and infection is facilitated by immunological deficits related to diabetes ([Geerlings, 1999](#)) rapidly progressing to deeper tissues, increasing the presence of necrotic tissue, rendering amputation inevitable ([Lipsky et al., 2005](#)). Diabetic patients fre-

quently require minor or major amputations (15-27%), which contribute to high morbidity among diabetic patients, but is also associated with severe clinical depression and increased mortality rates. Mainly ESBL producing Gram-negative bacilli and MDR Gram-negative bacteria isolates lead to severe infection and also prone to amputation of major or minor below the knee and below great ankle toe, metatarsal (Murali *et al.*, 2014).

Initially, antimicrobials are selected empirically for the treatment of DFU infections. With a declining number of novel antibiotics being developed and impetuous use of available antibiotics, antibiotic resistance has become a universal issue in healthcare institution (Chen *et al.*, 2018). Aim of the present study was to determine microbiological causes of diabetic foot infections and Antibiotic drug-resistant pattern of the isolates.

MATERIALS AND METHODS

Total of 300 samples was collected from diabetic patients with foot ulcers admitted to Sri Lakshmi Narayana Institute of Medical Sciences between September 2014 and September 2016. This study was conducted after obtaining approval from the institutional human ethical committee of SLIMS. After obtaining consent from the patients who are interested in participating were included.

Table 1: Wagner Classification System for Foot Ulcers.

Ulcer Grading	Description
Grade 0	No ulcer but high risk Foot
Grade 1	Superficial Ulcer
Grade 2	Deep Ulcer. No bone Involvement
Grade 3	Deep ulcer with bony Involvement
Grade 4	Localized Gangrene
Grade 5	Extensive Gangrene involving whole Foot

Samples were collected from the patients after the debridement of the ulcer base. Before the sample, sterile normal saline followed by gentle rubbing of the foot wounds and tissue abrasion with 70% alcohol, to avoid contamination. Pus swab and tissue specimen were sent to Microbiology laboratory for sample processing, isolation and identification of microorganisms according to CLSI guidelines. The tissue samples were homogenized and inoculated on blood agar, and MacConkey agar was incubated for 24-48 hours at 37°C under aerobic condition.

Isolation of anaerobic bacteria was incubated in

an anaerobic chamber at 37°C and examined at 48 hours and 96 hours. For isolation of fungal organisms specimen was inoculated in Sabouraud dextrose agar 25-30°C at 48 hours. All isolates were Processes for Grams staining and biochemical tests and antibiotic sensitivity was done using Kirby Bauer's disc diffusion method. The antifungal susceptibility testing of yeast isolates was carried out using the disk diffusion method as per M44-A CLSI guidelines.

RESULTS AND DISCUSSION

Out of 300 patients, 189 (63%) were male, and 111 (37%) were females. During the study period, male's predominance was noted over females shown in Table 2.

Table 2: Gender Distribution of cases.

Gender	Total Cases(300)	Percentage(%)
Male	189	63%
Female	111	37%

Based on the age-wise distribution of the study subjects, most of the patients belonged to the age group of 55-65 years, followed by the others in Table 3.

Table 3: Age wise distribution.

Age wise Distribution	Total(300)	Percentage(%)
35-45	16	5%
45-55	80	27%
55-65	120	40%
65-75	48	16%
75-85	36	12%

Most of the isolates recovered from Wagner classification system II and III in Diabetic foot ulcer (DFU), Wagner's grade 3 ulcer was most common (37%), followed by grade 2 (30%), grade 4 (21%) and then grade 5 (3%) mentioned in Table 4.

Among 300 Diabetic Ulcer patients, 142 (54%) poly microbial and 84 (28%) mono microbial and no growth in 54 (18%) were detected shown in Table 5. Among 245 cases, 384 isolates were isolated from DFU patients. *Pseudomonas aeruginosa* (22%) was the most frequent bacteria followed by *Staphylococcus aureus* (15%), *Escherichia coli* (11%), *Klebsiella pneumoniae* (9%), *Candida albicans* (9%), *Proteus species* (8%) followed by Others in Table 6.

In Table 7 out of all Gram negative bacteria were sensitive to colistin and Tigecycline and 44% of Gram

Table 4: Patient distribution according to the Wagner grade of the Foot ulcer.

Wagner's Ulcer Grading	Total Cases(300)	Percentage(%)
Grade 1	26	9%
Grade 2	90	30%
Grade 3	112	37%
Grade 4	62	21%
Grade 5	10	3%

Table 5: Microbial growth.

Microbial Growth	Total cases(300)	Percentage(%)
Poly microbial	142	54%
Mono microbial	84	28%
No Growth	54	18%

negative bacteria were ESBL producers and among 20% of the gram negative isolates were Multi drug resistant (MDR) organisms. All Gram-positive bacteria were sensitive to Linezolid; it was the most effective antibiotic against all isolates. 33.3 % of *Enterococcus* Sp was resistant to vancomycin, 61 % *Staphylococcus aureus* isolates were MRSA in Table 8.

Of the 24 anaerobic isolates, 8 (33.3%) were resistant to clindamycin, followed by 6 (25%) to penicillin and 6 (25%) to cefoxitin. Imipenem and metronidazole were sensitive to all anaerobes in Table 9. Out of 35 isolates of *C. albicans*, 18 was resistant to fluconazole, 16 were resistant to amphotericin B, 12 was resistant to voriconazole, and 14 were resistance to Itraconazole. Of the 21aa isolates of *Non-Candida albicans* ssp, 11 were resistant to amphotericin B, 9 were resistant to Itraconazole, 6 were resistant to fluconazole and voriconazole described in Table 10.

A foot ulcer is one of the debilitating complications in diabetics. Nearly half of all lower extremity amputations are diabetes-related (Yerat and Rangasamy, 2015). Antibiotic therapy for diabetic foot infections is started empirically following likely causative organism. The definitive treatment is later modified according to bacterial culture and sensitivity report. In this study, the age group of the patients ranged from 35 to 85 years. Most of the patients were in the age group 45–65 years, and most of the diabetic foot ulcer patients are men 63% compared to females 37%. Like our study, males were infected more than female was seen in other studies (Chakraborty and Mukherjee, 2015; Gopi et al., 2017). Most of the iso-

lates in our Study from Wagner grading system II and III in DFU Similarly reported a maximum number of isolates in diabetic foot ulcer patients in Wagner grade II and III (Hefni et al., 2013).

Our study revealed that 54 % of diabetic foot ulcers were polymicrobial infection, (Turhan et al., 2013) reported rates of 16% were polymicrobial. Monomicrobial isolates (28%), reported in our study, another study shows 58% (Akhi et al., 2015). Among 384 bacterial isolates, 304 were aerobic, 24 were anaerobic bacteria, and 56 were candida sp. Many studies support our study results that Gram-negative bacteria showed high prevalence in diabetic-foot infection (Tiwari et al., 2012).

Table 6: List of Microorganisms in Diabetic Foot Ulcer Cases.

Microorganisms	Number of Isolates (n=384)	Percentage (%)
<i>Staphylococcus aureus</i>	56	15%
CONS	30	8%
<i>Enterococcus spp.</i>	12	3%
<i>Pseudomonas aeruginosa</i>	86	22%
<i>E. coli</i>	43	11%
<i>Klebsiella pneumoniae</i>	35	9%
<i>Proteus sp</i>	32	8%
<i>Citrobacter sp</i>	10	3%
Anaerobes	24	6%
<i>Candida albicans</i>	35	9%
<i>Candida tropicalis</i>	15	4%
<i>Candida dubliniensis</i>	6	2%

The most commonly isolated bacteria from diabetic foot infections in the present study was *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *E. coli*, *Klebsiella pneumoniae* and *Proteus sp*. Gram-negative aerobes were the most common pathogens (28.7%), whereas 13.8% of isolates were gram-positive bacteria isolated from diabetic foot infections (Gadepalli et al., 2006). Of the 384 isolates, 24 (6%) were anaerobic organisms. Most common anaerobic organism isolated was 10 (42%) *Pepto streptococcus* spp followed by 8 (33%) *Bacteroides* spp., 4 (16%) *Porphyromonas* spp. and 2 (8%) *Veillonella* spp. In a study from Singapore, most common anaerobic isolates were *Pepto streptococcus* spp. (46%) and *Bacteroides sp* (19%) related to our

Table 7: Resistant Pattern (%) of Gram-Negative Bacteria.

Antibiotics	<i>P.aeruginosa</i> (n=86)	<i>E.coli</i> (n=43)	<i>K.pneumoniae</i> (n=35)	<i>Proteus.sp</i> (n=32)	<i>Citrobacter.sp</i> (n=10)
Amikacin	6(6%)	16(32%)	9(26%)	4(12.5%)	5(50%)
Ciprofloxacin	35(41%)	23(53%)	16(46%)	10(31.25)	4(40%)
Levofloxacin	35(41%)	23(53%)	16(46%)	10(31.25)	4(40%)
Imipenem	25(29%)	4(9%)	6(17%)	0(0%)	0(0%)
Meropenem	25(29%)	4(9%)	6(17%)	0(0%)	0(0%)
Pip/Tazo	38(44%)	4(9%)	8(23%)	0(0%)	0(0%)
Ceftriaxone	NA	32(74%)	20(57%)	2(6%)	2(20%)
Cefotaxime	NA	32(74%)	20(57%)	2(6%)	2(20%)
Ceftazidime	35(41)	32(74%)	20(57%)	2(6%)	2(20%)
Gentamicin	6(6%)	16(32%)	9(26%)	4(12.5%)	5(50%)
Tigecycline	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Colistin	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

Table 8: Resistant Pattern (%) of Gram-Positive Bacteria.

Antibiotics	<i>Staphylococcus aureus</i> (n=56)	CONS (n=30)	<i>Enterococcus.sp</i> (n=12)
Amikacin	15(27%)	9(30%)	0(0%)
Ciprofloxacin	26(46%)	16(53%)	4(33%)
Levofloxacin	26(46%)	16(53%)	4(33%)
Cefoxitin	34(61%)	22(73%)	4(33%)
Oxacillin	34(61%)	22(73%)	4(33%)
Erythromycin	42(75%)	24(80%)	4(33%)
Clindamycin	42(75%)	24(80%)	4(33%)
Penicillin	42(75%)	24(80%)	4(33%)
Linezolid	0(0%)	0(0%)	0(0%)
Tetracycline	8(14%)	4(13%)	0(0%)
Gentamicin	15(27%)	9(30%)	0(0%)
Vancomycin	0(0%)	0(0%)	4(33%)

Table 9: Resistant Pattern (%) of Anaerobic Bacteria.

Antibiotics	<i>Pepto streptococcus spp</i> (n=10)	<i>Bacteroides spp</i> (n=8)	<i>Porphyromonas</i> (n=4)	<i>Veillonella</i> (N=2)
Penicillin	3(30%)	3(37.5%)	0(0%)	0(0%)
Clindamycin	4(40%)	3(37.5%)	1(25%)	0(0%)
Cefoxitin	3(30%)	2(25%)	0(0%)	0(0%)

Table 10: Antifungal Resistant Pattern (%) of Candida species Isolates.

Antifungal Drug	<i>Candida albicans</i> (n=35)	<i>Candida tropicalis</i> (n=15)	<i>Candida dubliniensis</i> (n=6)
Fluconazole	18(51%)	4(27%)	2(33%)
Amphotericin B	16(46%)	7(47%)	4(67%)
Voriconazole	12(34%)	4(27%)	2(33%)
Itraconazole	14(40%)	6(40%)	3(50%)

Study (Ng *et al.*, 2008).

Of the 384 isolates. Most common *Candida* species isolated were 35(62%) *Candida albicans* followed by 15 (27%) *Candida tropicalis* and 6 (11%) *Candida dubliniensis*. Although, among the *Candida* species, *C. albicans* has the highest frequency in the DFU, followed by other species such as *C. tropicalis*, *C. dubliniensis* (Pfaller *et al.*, 1996). Our antimicrobial resistance pattern was similar to the recent studies (Shankar *et al.*, 2005). In our study, 61% of the isolated organism are *Methicillin Resistant Staphylococcus aureus*, and 73 % were *Methicillin resistant Coagulase Negative Staphylococcus (MRCONS)*, and 33%VRE was isolated. Clinical isolates of VRE and MRSA resistant have also been reported (Herrero *et al.*, 2002). For *Staphylococcus* spp. Linezolid and vancomycin were the most effective choice of antibiotics (Akhi *et al.*, 2015).

The Enterobacteriaceae family was resistant to most of the antibiotics tested, except *colistin* and *tigecycline*. High resistance rates to Ciprofloxacin and Levofloxacin in our present study correlates with other studies (Siami *et al.*, 2001). High-Level resistant patterns like VRE, MRSA, and ESBL, our study shows (33%) VRE, (61%) MRSA and (40%) ESBL. Percentage resistance rates are comparable with other studies (Amini *et al.*, 2013). This high antimicrobial resistance among diabetic Foot infecting bacteria may be due to several factors including previous antibiotic usage and its frequency and length of hospitalization stay.

CONCLUSIONS

Our present study shows Gram-negative bacteria are playing a significant role in diabetic foot ulcer infection. Surveillance and monitoring the antibiotic susceptibility of the isolates will be helpful in the empirical treatment of diabetic ulcers. Active infection control team and Clinical pharmacists should continuously monitor the prevalent organisms and prepare their antibiograms, periodically and inform the clinicians. This will help to reduce the cost by unnecessary usage of resistant antibiotics.

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Conflict of Interest

The authors declare that they have no conflict of interest for this study.

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