

Microbiology of diabetic foot infection in a tertiary care hospital in Government Cuddalore Medical College and Hospital (RMMCH), Cuddalore - A Retrospective Analysis

¹Dr. N. Junior Sundresh, MS, FRCS, FACS, ²K.Vidhya, ³J.Susmitha

¹Professor, ²Pharm D, ³Pharm D

¹Department of General Surgery, ²Department of Pharmacy, ³Department of Pharmacy
¹Government Cuddalore Medical College, ²Annamalai University, ³Annamalai University, Chidambaram

Abstract

Diabetic foot ulcers (DFU) and infection (DFI) are a major diabetes related problem around the world due to the high prevalence of diabetes in the population. Broad-spectrum antibiotic regimens can involve in antibiotic resistance and limit future treatment options. So, to minimize the becoming known of bacterial resistance. There is a necessity to gain knowledge about the microbiological profile and alter the antimicrobial therapy based on the results of cultures. It avoids prolonged treatments.

Objective

The purpose of this study represents evaluate

- Susceptibility pattern of these infection in patients attending Government Cuddalore Medical College and Hospital RMMCH)
- To identify the most frequent DFI pathogens within our geographical region
- Determine and compare the microbiological profile and prevalence of DFI differ according to patient's age, diabetes control and sex.

We retrospectively analysed clinical data and microbiological samples collected from 300 patients with DFU treated at Government Cuddalore Medical College and Hospital (RMMCH).

Method

This was a retrospective study in Diabetic foot ulcer patient admitted in Government Cuddalore Medical College and Hospital (RMMCH), Chidambaram between 2019 and 2022. 300 Diabetic Foot Ulcer meet the inclusion criteria enrolled in this study. There were six antibiotics used for treating Diabetic Foot Ulcer. The common antibiotics were used for Diabetic foot ulcer Ceftriaxone, Meropenem, Ciprofloxacin, Cefepime, Piperacillin/Tazobactam, and Ceftazidime. These drugs used treat Diabetic foot ulcer causing microorganism. These were the most antibiotics that comply with their dose with the guidelines.

Conclusion

In conclusion, diabetic foot infections (DFI) were commonly caused by polymicrobial. *S. aureus* and *P. aeruginosa* were the most common single pathogen. The proportion of anaerobic organisms was low. The antimicrobial susceptibility data suggest that Meropenem and Piperacillin-tazobactam and Ceftazidime are appropriate agents for empirical therapy. In our study, we found a higher prevalence DFI in males with age of 71 years. Prevalence of DFI higher in males than female.

Key words

Microorganism, Prevalence, Antibiotic, Susceptibility.

I. BACKGROUND

Diabetic foot infections are the most serious and frequent or long-term complications in patients with DM. It is most common metabolic disease in the world. The risk of developing a Diabetic foot ulcer in diabetic patients is estimated to be 12 to 25%. In 2014, there was an 423 million adults estimated living with diabetes. 70 percentage of death due to Diabetes before age of 70. Increasing rate of Diabetic Foot Ulcer is higher than 4% per year. It increases hospitalization and healthcare expenditure. The etiology of DFU is multi factorial with diabetic peripheral neuropathy, peripheral arterial disease, and foot deformity responsible for DFU[1]. Other contributing factor to DFU outcomes is diabetic foot infection (DFI), which has been present in 40–70% of all DFU. Most of the individuals have diabetes for more than 20 years, a significant risk factor for the development of a DFU. It leads to increased hospital admissions, worsening outcomes, and increased amputation rates. Infected DFUs are also called as diabetic foot infections (DFI). It carry high morbidity rate and increased risk of mortality rate. Clinically, DFI is identified by signs of inflammation (indurations, erythema, raised temperature, increased pain, and purulent discharge). DFI can be categorized by mild, moderate, severe and mostly polymicrobial.

Gram-positive bacteria, predominantly *Staphylococcus aureus*, and Gram-negative bacteria such as *Pseudomonas aeruginosa*, are the most common pathogens involved in DFI. Other bacteria found in DFI include Streptococci, Enterococci, Enterobacteriaceae, and *Pseudomonas*. The incidence and the prevalence of Methicillin resistant *Staphylococcus aureus* (MRSA) in DFI is considered to be 15-30%. The polymicrobial nature in chronic wounds is the important factor to cause a chronicity and severity of DFU.

Studies had found that *Staphylococcus aureus* is a primary causative pathogen, but others studies found predominantly Gram negative pathogens. These differences caused by presence of various/different causative organisms at various times, geographical regions, socio-economic, personal hygiene, availability to effective health care services, types, severity of the infections, wound depth and sampling technique. It creates an impact and improves treatment outcomes.

Wound care and antibiotics are treatment for DFI. Due to the nature and severity of the infections important to increase the

effectiveness of the treatment and need to administration of prolonged antibiotics cause antimicrobial resistance. To avoid the resistance, need to understand the microbiology of DFI. Microbiological tendency and direct novel therapeutic strategies. There has been a small retrospective study of the microbiology of DFIs in GCMCH (RMMCH). All DFI patients treated at an inpatient at GCMCH during 2019 to 2022 were included.

The objectives were

- Susceptibility pattern of the infection and regarding antimicrobial resistance
- Identify the most frequent DFI pathogens within our geographical region
- Determine and compare the microbial profile and prevalence of DFI differ according to patients age, diabetes control and sex.

We retrospectively analysed clinical data and microbiological samples collected from 300 patients with DFU treated at Government Cuddalore Medical College and Hospital (RMMCH).

II. MATERIALS AND METHODS

Study population

We collected data for this study from Patients with DFUs admitted to the Department of surgery in GCMCH were recruited from 2019 to 2022. During this period, 300 patients with DFU were referred from surgery department. Microbiology samples were taken from 300 Patients. All the diagnoses of the patients were made based on clinical and laboratory examinations. We obtained information from the medical records and collected demographic data, including age, gender, initial cause of ulcers, diabetes duration, glycaemic control and treatment history of diabetes were analysed and reviewed microbiological investigations were taken, before antibiotics administration [2]. Wound sampling was conducted based on Wagner's classification of DFU and samples were taken from dead tissue.

Aerobic and anaerobic microorganism's growth and antibiotic sensitivity testing done from cultured samples. The data recorded bacterial growth condition like no growth, monomicrobial or polymicrobial, predominant organism and antibiotic susceptibility with microorganisms. Microsoft Excel was used to carry out statistics on age, microbiology profile and antimicrobial resistance.

Inclusion criteria

- Patient affected by Diabetic Foot Ulcer.
- Dominant pathogens were detected.
- This pathogen was established by strain more than 50% of the total bacteria strains.
- Both sexes were included

Exclusion Criteria

- Incomplete medical records with unclearly written of the case sheets
- Unclear written of dose and route of administration of antibiotics
- Patient with other co-morbidity were excluded.

Wound grading

DFU classified according to the Wagner's classification System which grades DFU by depth (0, I, II, III) and stage (A, B, C, D) depending on the presence or absence of infection. This validated classification tool was used in all subjects to develop a management plan and determine the outcome of the DFU. Clinical diagnosis of infection confirmed by the presence of the following signs: local swelling or indurations, >0.5 cm of erythema around the wound, local tenderness or pain, local warmth, and purulent discharged was used.

Classification of DFU (Wagner's method)

- Grade 0 -Skin intact but bony deformity led to "foot at risk"
- Grade 1 -Superficial Ulcers
- Grade 2 -Deeper, full thickness extension
- Grade 3 - Deep abscess formation
- Grade 4 - Partial gangrene of forefoot
- Grade 5 - Extensive gangrene

Samples collection and processing

In this study, patients with DFU samples were taken. Before going to sample collection the DFU was cleansed by sterile saline solution. Swab from surface area, soft and deep tissue infections swabs were taken from Dead tissue (degraded wounds). Swabs from surface were collected using Levine's Technique. Deep tissue specimens were collected by the base of the ulcer in the debridement process. A total of 300 microbiological sample were performed.

In this study analysis, 220 microbiological results were included. The study population consist 55 males and 25 females. The age of the patient from 28 to 94. The specimen stored in sterile transport containers. Specimen forward to the microbiology laboratory for aerobic culturing within 20 minutes. Anaerobic culturing not performed in this study. For inoculations, various types of media were used like blood, MacConkey, chocolate, mannitol-salt and thioglycolate broth were used. Plates were incubated until visible growth using aerobic (O₂) at 37°C or 5 days.

MRSA plates were incubated at 32°C. Don't consider lower or upper bacterial growth volume in monomicrobial infection. Result of the 220 microbiological investigations, 65% were culture positive, 38.9% were polymicrobial and 64.1% were monomicrobial. 38.1% male; 37.5% female. Among those infection, result had monomicrobial and the samples taken from foot (42.5%), toe (35.2%), deep tissue (39.3%). A Chi-Square test could not be done with the data due to some patients attained multiple

sample results.

Identification of isolated microorganisms

After 1 day of bacterial culture incubation, monitor all plates for visible growth. Subcultures for thioglycolated broth tubes were done after a 24-hour incubation period by using blood, Macconkey plates. Wound smears from each sample were Gram-stained to identify if Gram-positive or Gram-negative isolates were present and also considered no- growth plates as sterile after five days of incubation.

For confirmation, we performed further biochemical tests for both Gram-positive and Gram-negative isolates using commercially available reagents (API 20E, API strep, and API staph). MRSA confirmation was done by PCR assays. Quality control strains (*Escherichia coli* ATCC1 25922, *Klebsiella pneumonia* ATCC1 700603, *Candida albicans* ATCC1 10231, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 29213, *Enterococcus*.

Antimicrobial susceptibility testing

For the common isolated bacteria (*Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, *Escherichia coli*, *Acinetobacter* sp and *Enterobacter* sp) susceptibility to selected antibiotics were examined. Antimicrobial sensitivity testing was performed on diagnostic sensitivity test plates by Kirby-Bauer method. The method involves resistance of microorganisms to many antimicrobial compounds. Antibiotics and their concentrations used for antimicrobial susceptibility testing follows; Amikacin 30ug, Ampicillin 10ug, Co-Amoxi/clav 30 ug, Ceftazidime 30ug, Ciprofloxacin 5ug, Clindamycin 2ug, Ceftriaxone 30ug, Cefotaxime 30ug, Erythromycin 15ug, Gentamicin 10ug, Imipenem 10mg, Meropenem 10ug, Piperacillin/Tazobactam 110ug, Trimethoprim-Sulfamethoxazole 25ug, and Vancomycin 30ug.

III. STATISTICAL ANALYSIS

Analysed the data using the statistical SPSS software, v22.0 (IBM SPSS Statistics for Windows). using the Student's t-test, chi-square test with F-test, we evaluated differences between continuous and categorical variables. We calculated correlations between variables with the Spearman's rank correlation test. Statistical assessments were two sided and significant at p values <0.05.

IV. RESULTS

We collect 300 patients, of which 224(74.6%) were male with age of 71 years. The most common comorbidity was systemic arterial hypertension (78.4%) and peripheral occlusive arterial disease (57.3%), insulin-dependent diabetes mellitus (39.7%), chronic kidney disease (33.3%), coronary heart disease (15.5%), and smoking (14.9%). Previous use of antimicrobials within 90 days before to hospitalization was observed in 75(25%) patients. Previous amputation was seen in 99(33%) patients. A total of 146 (48.6%) patients had the Diabetic Foot classification III and 127 (42.3%) had grade IV. From the 300 patients, 277 pathogens were isolated and Gram-positive bacteria were 162 (58.4%), Gram-negative bacilli were 75 (27%) of the isolates. 40 (14.4%) of the cultures had no growth of microorganisms.

Prevalence of isolated pathogens in DFI patients (Total = 277). For Gram-positive bacteria, n=162.

Table 1

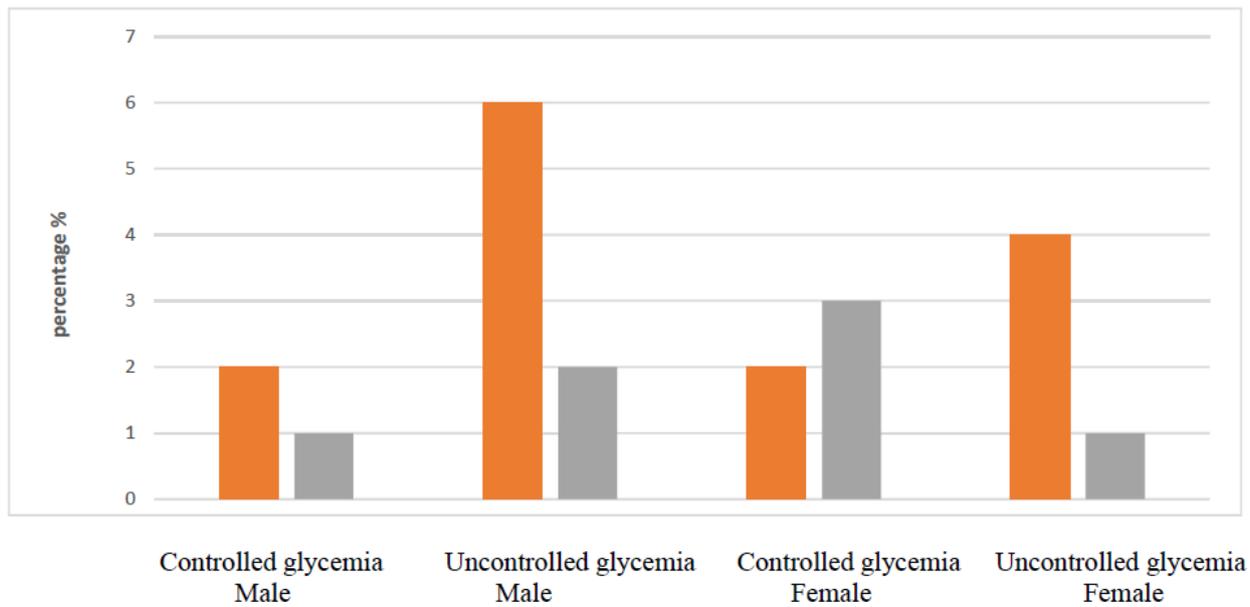
Gram Positive Bacteria	N	%
<i>E. faecalis</i>	30	18.5
<i>S. aureus</i>	67	41.3
Others Coagulase –negative Staphylococci	30	18.5
<i>E. faecium</i>	4	2.46
<i>S. epidermidis</i>	23	14.1
<i>Enterococcus</i> sp.	5	3
<i>S. agalactiae</i>	3	1.8

Prevalence of Gram-negative bacteria, n=75

Table 2

Gram-negative Bacteria	N	%
<i>Pseudomonas aeruginosa</i>	20	26.6
<i>Proteus</i> .sp	12	16
Others Enterobacteria	10	13.3
<i>Klebsiella pneumoniae</i>	7	9.3
<i>Burkholderia cepacia</i>	1	1.3
<i>E. coli</i>	9	12
<i>Enterobacter</i> sp.	8	10.6
<i>Acinetobacter</i> sp.	6	8
<i>Stenotrophomonas maltophilia</i>	4	5.3

Figure 1: Wound grading in patients relative to each sex in the whole study population



Susceptibility pattern of the Gram-positive Bacteria

Table 3

Pathogen (Number)	Susceptibility to oxacillin	
	N	%
S.aureus(67)	60	55
S.epidermidis(23)	3	2.5
Other Coagulase-negative Staphylococci(30)	2	1.6

Table 4

Pathogen (N)	Susceptibility to Ampicillin	
	N	%
Enterococcus faecalis (30)	25	67.5
E. faecium (5)	0	0
Enterococcus sp (5)	4	10.8

Susceptibility pattern of the Gram-negative Bacteria

Pathogen(N)	Susceptibility											
	profloxacin Ce		ftrioxone		Cefepime		Meropenem		Piperacillin-Tazobactam		Ceftazidime	
	N	%	N	%	N	%	N	%	N	%	N	%
P.aeruginosa (20)	10	13.8	-	-	14	19.4	16	22.2	12	16.6	16	22.2
Acinetobacter sp (6)	3	4.1	-	-	3	4.1	2	2.7	2	2.7	3	4.1
Proteus sp (12)	8	11.1	7	9.72	12	16.6	14	19.5	12	16.6	7	9.7
Others	6	8.3	7	9.72	8	11.1	13	18	12	16.6	7	9.7
Enterobacterales (10)	4	13.8	5	6.9	6	8.3	9	12.5	6	8.3	6	8.3
Klebsiella pneumonia (7)	5	12.5	7	9.7	8	11.1	9	12.5	9	12.5	8	11.1
E.Coli(9)	4	5.5	4	5.5	5	6.9	8	11.1	8	11.1	8	9.9

Antimicrobial susceptibility pattern of Gram-negative bacilli from the infected foot ulcers of diabetic patients($n = 20$). Analysis of infected patients result that 250(83.3%) of the 300 patients had polymicrobial infections. In this study, the most common isolates were *S. aureus* in 67 (21%) patients, *P. aeruginosa* in 20 (7.2%) patients and anaerobes in 30(10.8%) patients. *S. aureus* was the most common predominant organism cultured from samples and was cultured from 67 (21%) patients. The majority of these patients were infected by aerobes. The susceptibility pattern of the Gram-positive cocci is shown in the table. In this study *S. aureus* isolates, 55% were oxacillin resistance[3]. The antibiotic resistance of the Gram-negative bacilli is shown in table. meropenem, and piperacillin–tazobactam were active antimicrobial agents. Piperacillin- tazobactam found excellent activity against all anaerobes.

V. DISCUSSION

DFIs are important and progressing problem due to the high prevalence of DM. Etiological agents of DFIs are majorly polymicrobial and a mix of Gram-positive and Gram-negative bacteria and Etiological agents for gram negative bacteria are multi-resistant. The resulted culture had aerobes and anaerobes. In this study, mostly polymicrobial infections, the **rate of anaerobes was lower than aerobes**. In addition to, Gram-positive aerobes are predominant pathogens in DFIs. In this study, ***S. aureus* is the most predominant pathogen and high frequency** in diabetic foot patient. ***E. faecalis* is the most prevalent pathogen, followed by *S. aureus* and coagulase-negative Staphylococci**. Enterococci show low virulence. The high prevalence of *P.aeruginosa* in this study due to previous antimicrobial use, lengthy hospitalization, chronic wounds and surgical procedures. In our study, we found 28.1% of patients with previous antimicrobial use. We also found high proportion of the *Proteus.sp* and Enterobacteriaceae. Meropenem, Ceftazidime and piperacillin–tazobactam were most effective antimicrobial agents against aerobic Gram-negative bacteria[4]. While piperacillin -Tazobactam and Cefepime were active antimicrobial agents against aerobic Gram-positive cocci and anaerobes. Our study shows importance of appropriate selection of antimicrobial therapy based on culture results and the antimicrobial sensitivity. Due to the result ciprofloxacin cannot be recommended for use as an empirical therapy due to the drug was inactive against most pathogens. Ciprofloxacin found to be the lowest susceptibility to Gram-negatives due to the oral antibiotics. For serious and more-extensive chronic infections, initiate with broad-spectrum agents like carbapenems or piperacillin–tazobactam and it is consider as safe. Standard therapy based on culture results, the susceptibility pattern and response to the empirical regimen[5]. Grade IV also severe, where the chance of amputation. Amputation rates of Grade II were higher than rates of Grade III. In susceptibility profile, we found ampicillin-sensitive enterococci in 67.5% and oxacillin-sensitive *S. aureus* in 55%. But in coagulase-negative staphylococci, the sensitivity to oxacillin was only 1.6%[6]. The susceptibility of enterococci to ampicillin is due to the species faecalis and this species has less resistance than species faecium .22.2% susceptibility of *P. aeruginosa* to ceftazidime and Meropenem. Other prevalent Enterobacteriales had great susceptibility to ceftazidime, piperacillin-tazobactam .In the treatment of DFI infections is the duration of antimicrobial treatment is a major issue. We also did not evaluate the duration of treatment, clinical outcomes or adverse events[7].

In conclusion, diabetic foot infections (DFI) were commonly caused by polymicrobial *S.aureus* and *P.aeruginosa* were the most common single pathogen. The proportion of anaerobic organisms was low. The antimicrobial susceptibility data suggest that **Meropenem and piperacillin–tazobactam and Ceftazidime** are appropriate agents for empirical therapy. In our study, we found a **higher prevalence DFI** in males with age of 71years. Prevalence of DFI higher in males than female. Guiding of empirical therapy based on local epidemiology and susceptibility pattern is very important to educate prescribers another healthcare workers in the management of infections. It contributes to improve knowledge about bacterial resistance, and to implement antimicrobial practices. Each hospital should obtain its own microbiologic profile of patients with diabetic foot infections and treat them with the most appropriate empirical antibiotic.

REFERENCES

- [1] Robinson, W.P.; Loretz, L.; Hanesian, C.; Flahive, J.; Bostrom, J.; Lunig, N.; Schanzer, A.; Messina, L. Society for Vascular Surgery Wound, Ischemia, foot Infection (WIFI) score correlates with the intensity of multimodal limb treatment and patient-centered outcomes in patients with threatened limbs managed in a limb preservation center. *J. Vasc. Surg.* 2017, 66, 488–498.e2.
- [2] Van Haelst, S.T.; Teraa, M.; Moll, F.L.; de Borst, G.J.; Verhaar, M.C.; Conte, M.S. Prognostic value of the Society for Vascular Surgery Wound, Ischemia, and foot Infection (WIFI) classification in patients with no-option chronic limb-threatening ischemia. *J. Vasc. Surg.* 2018, 68, 1104–1113.e1.
- [3] Mathioudakis, N.; Hicks, C.; Canner, J.K.; Sherman, R.L.; Hines, K.F.; Lum, Y.W.; Perler, B.A.; Abularrage, C.J. The Society for Vascular Surgery Wound, Ischemia, and foot Infection (WIFI) classification system predicts wound healing but not major amputation in patients with diabetic foot ulcers treated in a multidisciplinary setting. *J. Vasc. Surg.* 2017, 65, 1698–1705.e1.
- [4] Lipsky BA, Itani K, Norden C, Linezolid Diabetic Foot Infections Study G: Treating foot infections in diabetic patients: a randomized, multicenter, open-label trial of linezolid versus ampicillin- sulbactam/amoxicillin-clavulanate. *Clin Infect Dis* 2004, 38:17–24.
- [5] Lavery LA, Armstrong DG, Wunderlich RP, Tredwell J, Boulton AJ: Diabetic foot syndrome: evaluating the prevalence and incidence of foot pathology in Mexican Americans and non-Hispanic whites from a diabetes disease management cohort. *Diabetes Care* 2003, 26:1435–1438. <https://doi.org/10.2337/diacare.26.5.1435> PMID: 12716801.
- [6] Epicast: EpiCast Report: Diabetic Foot Ulcers—Epidemiology Forecast to 2025. pp. 1–59; 2017:1–59. 5.IDF: IDF Diabetes Atlas. vol. 9th edition. pp. 1–176: International Diabetes Federation; 2019:1–176.
- [7] Messenger G, Masoetsa R, Hussain I, Devarajan S, Jahromi M: Diabetic foot ulcer outcomes from a podiatry led tertiary service in Kuwait. *Diabet Foot Ankle* 2018, 9:1471927. <https://doi.org/10.1080/2000625X.2018.1471927> PMID: 29868165.
- [8] Prompers L, Huijberts M, Apelqvist J, Jude E, Piaggese A, Bakker K, et al: High prevalence of ischaemia, infection and serious comorbidity in patients with diabetic foot disease in Europe. Baseline results from the Eurodiale study. *Diabetologia* 2007, 50:18–25.