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Original Research Article

Bacteriological profile of diabetic foot ulcer

Shwetha DC[1]*, Venkatesha D[2]

[1]Dept. of Microbiology, BGS Global Institute of Medical Sciences, Bangalore, Karnataka, India
[2]Dept. Professor & Head, Adichunchanagiri Institute of Medical Sciences Adichunchanagiri University, BG Nagara, Karnataka, India

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ABSTRACT

Introduction: Infected Diabetic foot ulcer is the common cause of hospitalization among patients with diabetes mellitus. Amputation of the lower limbs is the most common complication of Diabetic foot ulcer & infection is the cause in majority of the cases. Hence, Characterizing the bacteriological profile & antibiotic susceptibility testing is very important for the effective management of Diabetic foot ulcer.
Materials and Methods: Swabs from infected Diabetic foot ulcer were inoculated on to Blood agar & MacConkey agar. The bacterial identification was done according to standard protocol. The Antibiotic susceptibility testing for the isolated bacteria was done on Mueller Hinton agar by Kirby Bauer's Disc Diffusion method.
Results: 134 aerobic bacteria were isolated from 110 diabetic patients. Among Gram positive cocci, *Staphylococci aureus* (11.9%) was the predominant isolate followed by Coagulase negative Staphylococci (4.5%) & *Enterococci* spp (7.5%). *E. coli* (18.6%) was the predominantly isolated Gram-negative bacilli followed by Klebsiella spp (17.1%), *Citrobacter* spp (11.2%), Pseudomonas spp (7.5%), *Proteus* spp (7.5%), *Enterobacter* spp (7.5%), Non-fermenting Gram negative bacilli (5.2%) & *Acinetobacter* spp (1.5%). We found variations in the antibiotic sensitivity of isolated pathogens. 13.7% of Gram-negative bacilli were Extended Spectrum Beta Lactamase (ESBL) producers & 31.2% of Staphylococci were Methicillin Resistant Staphylococcus aureus (MRSA).
Conclusion: Our study shows that the repeated bacteriological evaluation & their antibiotic susceptibility is paramount in the management of infected Diabetic foot ulcer & the abuse of antibiotics should be restrained to prevent the emergence of antibiotic resistance.

1. Introduction

Diabetes Mellitus is a chronic disorder affecting a large population & acts as a major public health problem in India.[1] Infected foot ulcer is a common cause of morbidity in diabetic patients, leading to dreaded complications like gangrene & amputations.[2] Three main factors responsible for this are neuropathy, angiopathy & immunopathy. Neuropathy is the most important factor: minor irritations & trauma can lead to life threatening infections without the patient feeling the changes.[3]

Most diabetic foot diseases are valid crises: Anti-toxin treatment ought to be begun quickly to work on the possibilities rescuing the appendage.[4] Contaminations are frequently polymicrobial, Multi drug safe & related with deficient glycemic control. Subsequently, there is a requirement for consistent observation of safe microscopic organisms to give the premise to exact treatment & lessen the gamble of intricacies.[5]

Many examinations have given an account of the bacteriology of Diabetic Foot Contaminations throughout recent years, however the outcomes have been shifted &

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* *Corresponding author*.
E-mail address: shwetha.dasarahalli@gmail.com (Shwetha DC).

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frequently incongruous. These errors could be because of the distinctions in the causative life forms which has happened over the long haul, topographical varieties or the sort & the seriousness of the contamination, as announced in the examinations.[6] The current review was embraced to decide the bacteriological profile of Diabetic foot ulcer in our space.

2. Materials and Methods

This is a cross-sectional, observational study conducted over a period of 6 months (November 2021 to April 2022) in the Department of Microbiology. The study includes 110 samples from patients with Diabetic foot ulcer attending the outpatient department of Surgery. The ulcer grading was done according to Wagner Meggitt classification. Patients with ulcer grade one or more were included in the study & patients with grade zero or limb amputations were excluded from the study.

Specimen like pus, exudates or tissue biopsy were obtained from the ulcer. Pus & exudates were collected from the margin & the base of the ulcer using two sterile swabs & transported in a sterile test tube. Tissue biopsy was taken using a sterile blade in wedge shape including the base & margin of the ulcer & transported in sterile normal saline. The specimens were sent immediately to the microbiology laboratory for further processing.

One swab was used for Gram's staining & the second swab was used to inoculate on Blood agar & MacConkey agar for the isolation of aerobic bacteria. The Gram stained smear was examined under microscope for the presence of pus cells & bacteria. The inoculated plates were incubated overnight at 37°C. The bacterial growth obtained was identified based on morphology, cultural characteristics & biochemical reactions according to standard protocol.[7] The antibiotic susceptibility testing of the isolated bacteria was done by Kirby Bauer's Disc Diffusion method on Mueller Hinton agar using the antibiotics & interpretation was done based on CLSI (Clinical Laboratory Standard Institute) Guidelines. Due to lack of facilities, anaerobic culture was not done. Results were analyzed for aerobic bacteria only.

3. Results

Among 110 clinically diagnosed cases of diabetic foot ulcer that were included in the study 73 (66.4%) showed monomicrobial growth, 29 (26.4%) showed polymicrobial growth & 8 (7.2%) cases were sterile on culture (Table 1).

Table 1: Number of bacteria isolated from Diabetic foot ulcer

Monomicrobial growth	73	66.4%
Polymicrobial growth	29	26.4%
No Growth	08	7.2%
Total	110	100%

In our study, 134 aerobic bacteria were isolated from 110 diabetic patients. 102 (76.1%) were Gram negative bacilli & 32 (23.9%) were Gram positive cocci. Further distribution of pathogens is depicted in Table 2. Among Gram negative bacilli, *E. coli* (18.6%) was the most common isolate followed by *Klebsiella* spp (17.1%), *Citrobacter* spp (11.2%), *Pseudomonas* spp (7.5%), *Proteus* spp (7.5%), *Enterobacter* spp (7.5%), Non-fermenting Gram negative bacilli (5.2%) & *Acinetobacter* spp (1.5%). Among Gram positive cocci, *Staphylococci aureus* (11.9%) was frequently isolated followed by Coagulase negative Staphylococci (4.5%) & *Enterococci* spp (7.5%) respectively.

Table 2: Various Bacteria identified from diabetic foot ulcer

Gram negative bacilli (n=102)		**Percentage**
E. coli	25	18.6%
Klebsiella spp	23	17.1%
Citrobacter spp	15	11.2%
Pseudomonas spp	10	7.5%
Proteus spp	10	7.5%
Enterobacter spp	10	7.5%
NFGNB	07	5.2%
Acinetobacter spp	02	1.5%
Gram positive cocci (n=32)		
Staphylococcus aureus	16	11.9%
CONS	06	4.5%
Enterococci spp	10	7.5%
Total	**134**	**100%**

In the present study, most of the Gram-negative bacilli were sensitive to Amikacin (61.8%) followed by Gentamicin (56.9%), Meropenem (50.9%), Imepenem (49.0%), Piperacillin-tazobactum (43.1%), Ciprofloxacin (30.4%), Cefipime (23.5%), Amoxyclav (16.6%), Ceftrixone (12.7%) & less sensitivity to Cephotaxime (10.8%) shown in Table 3.

Table 3: Antibiotic susceptibilitypattern of Gram negative bacilli

Antibiotic	**Number (n=102)**	**Percentage (%)**
Amikacin	63	61.8%
Gentamicin	58	56.9%
Meropenem	53	50.9%
Imepenem	50	49.0%
Piperacillin tazobactum	44	43.1%
Ciprofloxacin	31	30.4%
Cefipime	29	23.5%
Amoxyclav	17	16.6%
Ceftriaxone	13	12.7%
Cephotaxime	11	10.8%

The sensitivity pattern of Staphylococci is shown in Table 4. All the Staphylococcal isolates showed 100% sensitivity to Vancomycin & Linezolid followed by Clindamycin (77.2%), Gentamicin (63.6%), Ciprofloxacin (63.6%), Tetracycline (63.6%), Erythromycin (40.9%),

Table 4: Antibiotic susceptibility pattern of Staphylococci

Antibiotic	**Number (n=22)**	**Percentage (%)**
Vancomycin	22	100%
Linezolid	22	100%
Clindamycin	17	77.2%
Gentamicin	14	63.6%
Ciprofloxacin	14	63.6%
Tetracycline	14	63.6%
Erythromycin	09	40.9%
Amoxyclav	07	31.8%
Cephotaxime	04	18.2%
Penicillin	00	00

Table 5: Antibiotic susceptibility pattern of *Enterococci*

Antibiotic	**Number (n=10)**	**Percentage (%)**
Vancomycin	10	100%
Linezolid	10	100%
Amoxyclav	08	80%
Tetracycline	06	60%
Ciprofloxacin	06	60%
High Level Gentamicin	05	50%
Penicillin	04	40%
Cefotaxime	03	30%

Table 6: Antibiotic susceptibility pattern of isolated Gram negative bacilli

Organism (n=102)	**Amc**	**Ak**	**Gen**	**Cip**	**Ctx**	**Ctr**	**Cpm**	**Pit**	**Ipm**	**Mrp**
E. coli (n=25)	12%	64%	56%	20%	00	4%	16%	40%	48%	60%
Klebsiella (n=23)	13%	52.2%	47.8%	21.7%	8.6%	8.6%	13%	30.4%	52.1%	47.8%
*Citrobacte*r (n=15)	13.3%	80%	66.6%	46.6%	20%	20%	33.3%	66.6%	60%	53.3%
Pseudomonas(n=10)	10%	90%	90%	80%	10%	30%	40%	60%	40%	50%
Proteus (n=10)	40%	50%	40%	10%	20%	10%	40%	80%	90%	70%
Enterobacter (n=10)	10%	60%	50%	30%	30%	30%	30%	20%	30%	40%
NFGNB (n=7)	42.8%	42.8%	71.4%	28.6%	00	00	14.3%	14.3%	14.3%	28.6%
Acinetobacter (n=2)	00	00	00	00	00	00	00	00	00	00

Note: Amc- Amoxyclav, Ak- Amikacin, Gen- Gentamicin, Cip- Ciprofloxacin, Ctx- cefotaxime, Ctr- Ceftriaxone, Cpm- Cefepime, Pit- Piperacillin tazobactum, Ipm- Imepenem, Mrp- Meropenem

Table 7: Antibiotic susceptibility pattern of isolated Gram positive cocci

Organism (n=32)	**P**	**Amc**	**Gen**	**Cip**	**Ctx**	**E**	**Cd**	**Te**	**Va**	**Lz**
S. aureus (n=16)	00	37.5%	62.5%	68.7%	25%	43.7%	75%	68.7%	100%	100%
Enterococci (n=10)	40%	80%	-	60%	30%	-	-	70%	100%	100%
MRCONS (n=6)	00	16.7%	66.7%	50%	00	33.3%	83.3%	50%	100%	100%

Note: P- Penicillin, Amc- Amoxyclav, Gen- Gentamicin, Cip- Ciprofloxacin, Ctx- cefotaxime, E- Erythromycin, Cd- Clindamycin, Te- Tetracycline, Va- Vancomycin, Lz- Linezolid

Amoxyclav (31.8%), Cephotaxime (18.2%). None of the isolates were sensitive to Penicillin.

All the *Enterococci* isolates showed sensitivity to Vancomycin & Linezolid. The sensitivity to Amoxyclav was 80% followed by Tetracycline (60%), Ciprofloxacin (60%), High Level Gentamicin (50%), Penicillin (40%) & Cephotaxime (30%) shown in Table 5.

Sensitivity pattern of isolated Gram negative bacilli & Gram positive cocci is presented in Tables 6 and 7 respectively. Amikacin was the most susceptible antibiotic to all bacterial isolates & Cephalosporins were the most resistant antibiotics. Non-fermenters (*Pseudomonas* spp, *Acinetobacter* spp & others) showed decreased sensitivity to carbapenems compared to *Enterobacteriaceae* (*E. coli*, Klebsiella spp, *Citrobacter* spp, *Proteus* spp, *Enterobacter* spp). Both the *Acinetobacter* isolates showed resistance to all antibiotics that were tested. In our study 13.7% of Gram negative bacilli were Extended Spectrum Beta Lactamase (ESBL) producers & 31.2% of Staphylococci were MRSA.

4. Discussion

Diabetic foot ulcer is a significant entanglement of Diabetes mellitus. Untreated diabetic foot ulcers will become tainted prompting different outcomes like gangrene or removal of the appendage.[8] The diabetic foot contaminations are for the most part blended bacterial diseases. In our review, 26.4% of cases showed polymicrobial development which is as per numerous different examinations.[9–11]

In the current review, bacterial profile of diabetic foot ulcer showed lion's share of Gram negative microorganisms (76.1%) more than Gram positive microbes (23.9%) & overwhelming confines were individuals from *Enterobacteriaceae* which is likewise seen in different examinations.[12,13] It is consistently important to assess various microorganisms contaminating the injury on a normal premise notwithstanding standard glycemic control, wound care, careful debridement, pressure-offloading & keeping up with satisfactory blood supply.[14] to execute focused on & right antimicrobial treatment, having information & consciousness of the normal culpable microbes in diabetic foot infections is fundamental.[15]

In the current review, the most often detached microorganisms is *E. coli* (18.6%) trailed by *Klebsiella* spp (17.1%), S. aureus (11.9%) & *Citrobacter* spp (11.2%) which is like review done by Ako-Nai et al.[16] conversely, different examinations show S. aureus & *Pseudomonas* spp as dominating microorganisms.[17–20]

Our review shows that the detached microorganisms had different responsiveness designs against normally utilized anti-microbials. A large portion of Gram-negative bacilli were impervious to routine anti-toxins like cephalosporins & Non fermenters showed diminished aversion to carbapenems. Greater part of gram-positive cocci showed protection from Penicillin, Cefotaxime & Erythromycin which corresponds with the aftereffects of different investigations.

Diabetic foot ulcers are exceptionally inclined to colonization with antimicrobial-safe life forms, including methicillin-safe Staphylococcus aureus & expanded range beta-lactamase delivering gram negative creatures.[21] In our review 13.7% of Gram-negative bacilli were ESBL makers & 31.2% of Staphylococci were MRSA. In a concentrate by Saraswathy KM et al, 68.8% of the Gram-negative bacilli were ESBL makers & 21% of Staphylococci were impervious to Methicillin.

Factors liable for MDR might be regular hospitalization, utilization of wide range anti-infection agents, lacking careful source decrease, persistent injuries, silly utilization of anti-microbials, & the exchange of obstruction qualities. To lighten what is happening & furthermore to decrease the pace of removal, clinicians ought to recommend anti-infection agents normally, convenient & adequately & there ought to be occasional oversights on the medication utilization by the individual association. Clinicians ought to change to culture report based utilization of smaller range treatment. A satisfactory & ideal careful mediation is fundamental to accomplish contamination source decrease.[22] In this way, early identification of causative microbes & determination of suitable anti-toxins in view of the antimicrobial testing is need of great importance for the legitimate administration of diabetic foot ulcer.

5. Conclusion

Diabetic foot ulcer infections are one of the major problems in diabetic patients & requires a team approach for its effective management. *E. coli*, *Klebsiella* spp, S. aureus & *Pseudomonas* spp were found to be the prevalent bacteria & unfortunately these bacteria have shown increased resistance to most effective antibiotics. Hence, there is need for periodic bacteriological evaluation which improves treatment outcome, reduces complications as well as emergence of multidrug resistance.

6. Source of Funding

None.

7. Conflict of Interest

None.

References

1. Chavan SK, Karande GS, Mohite RV. Clinico-bacteriological profile of diabetic foot ulcer among the patients attending rural tertiary health care centre. *Int J Med Res Health Sci*. 2014;3(4):861–5.
2. Bansal E, Garg A, Bhatia S, Attri AK, Chander J. Spectrum of microbial flora in diabetic foot ulcers. *Indian J Pathol Microbiol*. 2008;51(2):204–8.
3. Rani L, Madhumathi K, Belwadi S, Ramana BV. Bacteriological profile of diabetic foot ulcer. *Int J Pharm Res Bio-Sci*. 2013;2(2):36–45.
4. Perim MC, Borges JC, Celeste SC, Orsolin EF, Mendes RR, Mendes GO, et al. Aerobic bacterial profile & antibiotic resistance in patients with diabetic foot infections. *Rev Soc Bras Med Trop*. 2015;48(5):546–54.
5. Mehta VJ, Kikani KM, Mehta SJ. Microbiological profile of diabetic foot ulcers and its antibiotic susceptibility pattern in a teaching hospital, Gujarat. *Int J Basic Clin Pharmacol*. 2014;3(1):92–5.
6. Shanmugam P, Jeya M, and SLS. The Bacteriology of Diabetic Foot Ulcers with a Special Reference to Multidrug Resistant Strains. *J Clin Diagn Res*. 2013;7(3):441–5.
7. Collee JG, Duguid JP, Fraser AG, Marmion BP, Simmons A. Laboratory strategy in the diagnosis of infective syndromes. Mackie & McCartney practical medical microbiology. 14th ed. Singapore: Churchill Livingstone; 1996. p. 53–94.
8. Shareef J, Sunny S, Bhagavan KR. Study on bacteriological profile & antibiotic susceptibility pattern in patients with diabetic foot ulcers in a tertiary care teaching hospital. *J Soc Health Diabetes*. 2018;6(1):40–7.
9. Otta S, Debata NK, Swain B. Bacteriological profile of diabetic foot ulcers. *CHRISMED J Health Res*. 2019;6(1):7–11.
10. Mourya K, Sharma R, Vyas A, Khoja HO, Vyas N. Clinico Microbiological Spectrum of Diabetic Foot Ulcer with Multidrug Resistant Organisms at Tertiary Care Hospital. *Jaipur Journal of Medical Science & Clinical Research*. 2020;8(7):83–91.

11. Shah P, Eswarawaka M, Reddy AD, Shah S, Srivastava P, N. Bacteriological profile of diabetic foot. *Int Surg J*. 2021;8(2):704–713.
12. Darwis I, Hidayat H, Wisnu G, Mentari S. Bacteriological profile & antibiotic susceptibility pattern of diabetic foot infections in a tertiary care hospital in Lampung, Indonesia. *Malays J Med Sci*. 2021;28(5):42–53.
13. Shs AM. Spectral & Antibiotic Susceptibility of Pathogens Isolated from Saudi Patients with Diabetic Foot Infections. *Microbiol Res*. 2021;12:16–20.
14. Pankaj D, Muni S, Jha IN, Kumari A. Clinical presentation & bacteriological profile of diabetic foot in Eastern Bihar, India. *Int J Res Med Sci*. 2015;3:3058–64.
15. Goh TC, Bajuri MY, Nadarajah SC, Rashid AHA, Baharuddin S, Zamri KS, et al. Clinical & bacteriological profile of diabetic foot infections in a tertiary care. *J Foot Ankle Res*. 2020;13(1):36. doi:10.1186/s13047-020-00406-y.
16. Ako-Nai AK, Ikem I, Akinloye OO, Aboderin AO, Ikem R, Kassim OO, et al. Characterization of bacterial isolates from diabetic foot infections in Ile-Ife, Southwestern Nigeria. *Foot (Edinb)*. 2006;16(3):158–64.
17. Aleem S, Multani H, Bashir H. Bacteriological profile & antimicrobial sensitivity pattern of isolates from diabetic foot patients attending a teaching hospital in northern India. *Asian J Med Sci*. 2021;12(5):83–7.
18. Murshed M. Bacteriological Profile of Diabetic Foot Infection & Its Effect on Limb Salvation. *J Surg Sci*. 2020;24(1):21–5.
19. Manikandan J, Jaikumar S. Bacteriological Profile & Spectrum of Susceptibility Pattern in Diabetic Foot Ulcer Patients at Tertiary Care Hospital, Puducherry. *Ann RSCB*. 2021;25(2):3532–41.
20. Hamid MH, Arbab AH, Yousef BA. Bacteriological profile & antibiotic susceptibility of diabetic foot infections at Ribat University hospital; a retrospective study from Sudan. *J Diabetes Metab Disord*. 2020;19(2):1397–406.
21. Yadhav M, Chetana GS. Bacteriological profile of diabetic foot ulcer using hicrome UTI Agar. *Trop J Pathol Microbiol*. 2017;3(2):195–200.
22. Jain SK, Barman R. Bacteriological profile of diabetic foot ulcer with special reference to drug-resistant strains in a tertiary care center in North-East India. *Indian Journal of Endocrinology & Metabolism*. 2017;21:688–94.

Author biography

Shwetha DC, Assistant Professor

Venkatesha D, Professor & Head

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