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## Research Article

# PREVALENCE OF GRAM-NEGATIVE ORGANISMS IN BACTERIAL ISOLATES AND ITS ANTIBIOGRAM FROM PATIENTS OF DIABETIC FOOT ULCERS AT TERTIARY HEALTH CARE CENTRE, JAMNAGAR, GUJARAT

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### ABSTRACT

**Introduction:** Diabetic foot infection is one of the most common complication of diabetes mellitus. It leads to morbidity & mortality in diabetic patients. The Primary requirement for proper management of diabetic foot ulcers is the isolation of organisms & accurate selection of antibiotics. **Aim and Objective:** (1) To isolate and identify of gram-negative bacteria associated with diabetic foot ulcer patients. (2) To study antibiotic susceptibility patterns for the same. **Materials & Methods:** Total of 408 samples from wound discharge of diabetic foot ulcer patients were collected with all aseptic precautions & processed according to standard guidelines. **Result:** Out of 408 proceeded samples, 182 (44.61%) were culture positive, among which 165 (90.66%) were Gram Negative organisms & 17(9.34%) were Gram Positive. Among Gram- Negative Bacteria, *Pseudomonas aeruginosa* 48(29.09%) was the most predominant organism followed by *Escherichia coli* 46(27.87%), *Klebsiella pneumoniae* 33(20%), etc. Among all Gram Negative organism, most of them were sensitive to Imipenem 97.58%, followed by Piperacillin – tazobactam 93.94% and Gentamicin 84.24%. **Conclusion:** From this study, we can conclude that Gram-negative organisms are the most prevalent organisms which are isolated from diabetic foot infections. These organisms show a variety of sensitivity pattern to the commonly used antimicrobials, which requires prompt antibiotic sensitivity test according to which, the treatment can be streamlined to minimize morbidity & mortality among the patients.

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## INTRODUCTION

Diabetes Mellitus is a metabolic & vascular syndrome of multiple etiologies characterized by chronic hyperglycemia with disturbances in carbohydrate, fat & protein metabolism.<sup>1</sup> resulting it affects almost all the systems of the body. In India 37, 54,055 persons were diagnosed with diabetes with the prevalence rate of 8.05% recorded in the year 2020-21.<sup>2</sup>

There are mainly four types of diabetes mellitus (1) Type-1 diabetes: The destruction of  $\beta$ -cells leads to an absolute deficiency of insulin.(2) Type-2 diabetes: Insulin secretory defect with insulin resistance.(3) Gestational diabetes: High blood sugar level in pregnancy.(4) Other specific types of diabetes: e.g. MODY 1, MODY 2, Drugs or chemical induced.<sup>1</sup>

Diabetes mellitus leads to multiple complications because it causes alteration in host defence mechanisms that might increase risk of certain infections such as diabetic foot, cellulitis, abscess, necrotizing fasciitis, etc. Diabetic foot ulcers are a very serious complication of diabetes because it causes

neuropathy, angiopathy & immunopathy. Due to neuropathy, patients can't feel minor trauma & which leads to limb-threatening infections.<sup>3</sup> Diabetic foot ulcer Infections are often polymicrobial and associated with inadequate glycemic control.<sup>4</sup> These organisms are Gram Negative bacteria like *P.aeruginosa*, *E.coli*, *K.pneumoniae* and *proteus* spp. etc. & Gram positive cocci like staphylococcus aureus.<sup>5</sup>

So early diagnosis & appropriate antibiotic therapy is the most important measures to prevent further major complications like cellulitis and foot amputation due to gangrene. Proper management of these infections requires early microbial isolation, accurate selection of antibiotics & identification of complications that require surgical intervention.<sup>3</sup> Therefore, the present study was conducted to assess the role of Gram-Negative organism in Diabetic foot ulcer & their antibiotic susceptibility pattern.

#### Inclusion Criteria

All patients with a diagnosis of diabetic foot ulcers, both sexes & who were willing to participate in the study are included.

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### Exclusion Criteria

Patients with ulcers on the foot due to medical conditions other than diabetes mellitus & patients not willing to participate in this study.

## MATERIALS AND METHODS

### Sample Collection

This retrospective study was conducted at Tertiary Health Care Centre, Jamnagar, Gujarat from January 2021- December 2021. Total of 408 samples from wound discharge of diabetic foot ulcer patients were collected. All the samples were collected based on clinical history & reports of patient. Samples are collected with all aseptic precautions as per standard laboratory protocol. Wound exudate was collected by sterile swabs from the site of the ulcer of the diabetic foot. All the samples were immediately brought to the laboratory & proceeded as per standard protocols.

### Sample Processing

#### 1<sup>st</sup> Day

Direct smear examination: Smear was Prepared from collected samples & gram staining was done to identify any pathogenic organism.

Culture: All the samples were inoculated on Blood agar & MacConkey agar. Culture plates were incubated at 37°C for 24 hours under aerobic conditions.

#### 2<sup>nd</sup> Day

Inoculated culture plates were noted for any pathogen isolated or not. Seen for Morphology of colonies on blood agar & MacConkey agar. Smear was prepared from the colonies & gram staining was done to differentiate Gram Positive & Gram Negative organisms. According to that organism, further processing was done with relevant biochemical reactions as per standard guidelines.

Antibiotic sensitivity test (Kirby-Bauer disk diffusion method): 0.5 McFarland standard suspension of the isolate is made and lawn culture is done on Muller Hinton Agar plate & inoculated overnight at 37°C.

#### 3<sup>rd</sup> Day

Results of all biochemical tests & Antibiotic sensitivity tests were noted.

After incubation, zones of inhibition were noted, measured and compared with reference zones according to CLSI guidelines.

Antibiotics which were used are: Ampicillin/sulbactam (20µg), Co-Trimoxazole (25µg), Cefotaxime (30µg), Piperacillin (100µg), Chloramphenicol (30µg), Ciprofloxacin (5µg), Ceftizoxime (30µg), Tetracycline (30µg), Ofloxacin (5µg), Gentamycin (10µg), Amikacin (30µg), Gatifloxacin (10µg), Piperacillin-Tazobactam (100µg-10 µg), Imipenem (10µg). As per CLSI Guidelines Antibiotic sensitivity of the drug is calculated by zone of inhibition of bacterial growth around the disk, according to results as “sensitive”, “intermediately sensitive”, “resistant”.<sup>6</sup>

## RESULT

Out of 408 samples, 226 were culture-negative & 182 were culture-positive. (Table.1)

**Table 1** Culture result of DM Foot ulcer patients

Total Tested	Culture-Positive	Culture-Negative
408	182 (44.61%)	226 (55.39%)

Out of 182 (44.61%) culture-positive samples, 165 were Gram-Negative bacilli on gram staining. So the prevalence of Gram-Negative bacilli is 90.66%.(Table.2)

**Table.2** Organisms isolated from culture

Total Culture positive	Gram-Negative Bacilli	Gram-Positive Cocci
182	165 (90.66%)	17 (9.34%)

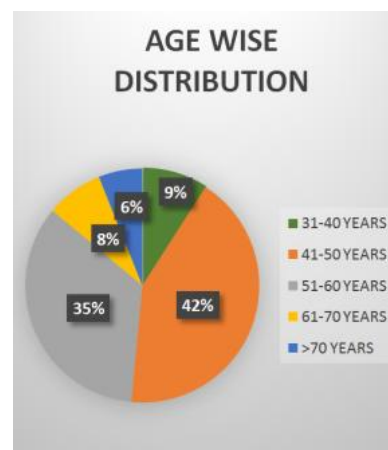
In this prevalence of males is (56.86%) more than females (43.13%).(Table.3)

**Table.3** Gender- wise distribution

	Male	Female	Total
n	232 (56.86%)	176 (43.13%)	408

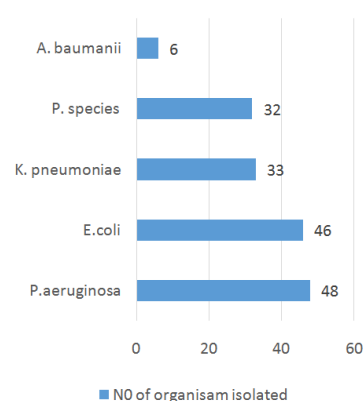
Their ages ranged from 33 years to 86 years with an average of 59 years. The maximum number of patients having diabetic foot ulcer infections belonged to 41-50 years followed by 51-60 years.(Fig.1)

**Fig.1** Age Wise distribution



**Fig.2** No. of organisms isolated

NO of organism isolated



Most common organism isolated was P.aeruginosa 48(29.09%) followed by Escherichia coli 46(27.87%), Klebsiella pneumoniae 33(20%), Proteus spp.32 (19.39%), Acinetobacter

6(3.63%) (Fig.2). Antibiotic sensitivity Results of all this organisms were shown in Table 1.

**Table 1** Antibiotic sensitivity pattern of Gram- negative organisms.

Antibiotic Tested	SENSITIVITY %				
	<i>P. aeruginosa</i>	<i>E. coli</i>	<i>K. pneumoniae</i>	<i>Proteus spp.</i>	<i>A. baumannii</i>
Ampicillin/sulbactam (AS)	IR	32.61%	39.40%	IR	IR
Co-Trimoxazole(BA)	IR	34.78%	42.42%	46.88%	33.34%
Cefotaxime(CF)	IR	69.56%	66.67%	59.38%	66.67%
Piperacillin(PC)	72.91%	71.73%	69.70%	65.63%	66.67%
Chloramphenicol (CH)	IR	15.22%	39.40%	34.38%	IR
Ciprofloxacin(RC)	18.75%	21.74%	24.24%	21.88%	16.67%
Ceftizoxime(CI)	25%	67.40%	69.70%	68.75%	50.00%
Tetracycline(TE)	IR	69.57%	66.67%	IR	66.66%
Ofloxacin(ZN)	14.58%	36.96%	39.39%	37.5%	33.34%
Gentamycin(GM)	83.33%	84.78%	87.88%	81.25%	83.33%
Amikacin(AK)	62.50%	73.91%	72.73%	71.88%	66.67%
Gatifloxacin(GF)	43.75%	56.52%	51.52%	50.00%	50.00%
Piperacillin-Tazobactam(PT)	93.75%	93.48%	93.93%	93.75%	100%
Imipenem(IPM)	95.83%	97.82%	96.97%	100%	100%

(IR: Intrinsic Resistance)

## DISCUSSION

A Diabetic foot ulcer is one of the most debilitating complications in diabetics.<sup>7</sup> Diabetic foot infections lead to suffering and disability but also prolong hospitalization hampering both the mental and economic status of patients, leading to great expenses to the patient as well as the community.<sup>3</sup> Antibiotic therapy for diabetic foot infections is started empirically in accordance with the likely causative organism. The definitive treatment is later modified according to bacterial culture and sensitivity report.<sup>7</sup> Diabetic nephropathy which occurs in approximately a third of diabetic patients and increasing incidence of multi- drug resistant infection in diabetic foot ulcer compounds the challenge faced by clinicians in treating these patients.<sup>8</sup>

In the present study, Diabetic foot infections were more common in males (56.66%) than females (43.34%) is compared with the study of Saseedharan *et al* (59.7%) and Singh *et al.* (69.6%).<sup>7,9</sup> This could be because diabetes mellitus is more common in men and are prone to trauma because of their outdoor occupation.<sup>3</sup> In our study diabetic foot infections were more common in 40-60 years age group which is around 77% of total cases. Which was similar to the study by Rani *et al.* (70%) and Jain & barman *et al.* (63%).<sup>3,10</sup>

In this study, the most prevalent organisms were Gram Negative 165 (90.66%) similar to the study of Rani *et al.*(87.03%).<sup>3</sup> Among which, the commonest isolated organism was *P.aeruginosa* (29.09%) which is similar to the study of sree Vaddadhi *et al* (23%) and Saseedharan *et al* (20.9%).<sup>11, 9</sup> *Escherichia coli* (27.87%) isolated in this study is similar to the study of Rani *et al* (25.46%).<sup>3</sup>

*Klebsiella pneumoniae* (20%) in the present study is similar to the study of Pathare *et al*(14.1%) and Anandi *et al*(16.9%).<sup>12,13</sup> In this study prevalence of *Proteus spp.* (19.39%) is similar to the study of Sannathimappa *et al.*(13%).<sup>14</sup>

Specific therapy is an important factor in the management of diabetic foot infections. In patients who appear severely ill or

having necrotising infections, broader antibiotic coverage is indicated.<sup>3</sup>

In the present study, *P. Areuginosa* was most sensitive to Imipenem (95.83%) which is similar to the study of Hena *et al.*(100%)<sup>15</sup>, sensitivity to Piperacillin-Tazobactam (93.75%) which is similar to the study of sree Vaddadhi *et al.* (96%)<sup>11</sup>, and sensitivity to amikacin in the present study (62.50%) is similar to the study of Saseedharan *et al.* (68%).<sup>9</sup>

In this study, *E.coli* was most sensitive to Imipenem (97.82%), followed by Piperacillin-Tazobactam (93.75%), Gentamycin (84.78%), Amikacin (73.91%), and third generation cephalosporin (67.40%) which compared with the study of Saseedharan *et al*, it shows the similar result with imipenem (88%).<sup>9</sup>

According to present study, in *K. pneumoniae* & *Proteus spp.* sensitivity of imipenem in concordance with the study of Hena *et al.*<sup>15</sup> *Acinetobacter baumannii* shows 100% sensitivity to Imipenem & Piperacillin-Tazobactam is similar to the study of Miyan *et al.*<sup>16</sup>

## CONCLUSION

In our study, we have found that gram- negative organisms are more predominant in diabetic foot ulcer infections. Significant resistance to a commonly used antibiotics is a very serious problem nowadays for clinicians. Delayed referral and inappropriate use of broad-spectrum antibiotics may be the main cause for Multi-drug resistance isolates. The use of antibiotic without a prescription or often over use by Patients, made situations worse. Without urgent action, we are heading for a post-antibiotic era, in which common infections and minor injuries can once again kill a human life. So, it is necessary to follow antimicrobial stewardship programmes strictly & avoid the unnecessary use of broad spectrum antibiotics to minimize morbidity & mortality among the patients.

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### Conflict Of Interest

The authors declare that there is no conflict of interest.

## FUNDING

None.

### Data Availability

All data generated in this study was collected from the Bacteriology section, Microbiology Department.

### Ethics Statements

The study was approved by the Institutional ethics committee, M. P. Shah Govt. Medical College and Guru Gobind Singh hospital, Jamnagar.

## References

1. Bloor Archith, Nayak Ramadas, Exam Preparatory manual for undergraduates Medicine.3<sup>rd</sup> edition. Jaypee brothers medical publishers, New Delhi (India), 2021.p.94-5.

2. 2021-2022 ANNUAL REPORT, Department of Health & Family Welfare, ministry of Health & Family Welfare ,Government of India.
3. Rani KL, Madhumathi, Belwadi S, BV. Bacteriological profile of diabetic foot ulcer. *IJPRBS*. 2013; 2:36-45
4. 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: 92-5.
5. Frykberg R. An evidence-based approach to diabetic foot infections. *Am J Surg*, 2003; 186(5): 44–54.
6. Kateshiya PR, Aring BJ, Gavali DM. Bacteriological profile and antibiotic sensitivity pattern of diabetic foot ulcer at tertiary care center, jamnagar, gujarat. *ejpmr*, 2021,8(7), 345-349.
7. Singh AK, Yeola M, Singh N, Damke S. A study on diabetic foot ulcers in Central rural India to formulate empiric antimicrobial therapy. *J Family Med Prim Care* 2020; 9:4216-22.
8. Keane WF, Brenner BM, de Zeeuw D, Grunfeld JP, McGill J, Mitch WE, *et al*. The risk of developing end-stage renal disease in patients with type 2 diabetes and nephropathy: The RENAAL Study. *Kidney Int*. 2003; 63:1499-507. [PubMed] [Google Scholar]
9. Saseedharan S, Sahu M, Chaddha R, Pathrose E, Bal A, Bhalekar P, Sekar P, Krishnan P. Epidemiology of diabetic foot infections in a reference tertiary hospital in India. *Braz J Microbiol*. 2018 Apr-Jun; 49(2):401-406.
10. 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 J Endocr Metab* 2017; 21:688-94.
11. Shanthi sree Vaddadhi, P. Ratna Kumari and R.Lakshmi Kumari. (2019); Study of bacterial pathogens with their antibiogram causing diabetic foot ulcers. *Int. J. of Adv. Res.* 7 (Apr). 908-914] (ISSN 2320-5407). [www.journalijar.com](http://www.journalijar.com)
12. NA Pathare, ABal.GV Talvalkar and DU Antoni All India Institute of diabetes S L Raheja hospital, Mahim, Mumbai Diabetic foot infections; A study of microorganisms associated with the different Wagner grades *Indian J Pathol Micribiol* 41(4); 437 441 1998.
13. AnandiC, Alaguraju D,NatarajanV,Ramanatham M, Subramaniam CS, Microbiology Rajah muthaiah medical Thulasiram M.Sumithra S, Department of college and hospital Annamalai University, Tamilnadu, India. Bacteriology of Diabetic foot lesions *Indian Journal of Medical Microbiology* (2004) 22(3):175-178.
14. Sannathimmappa MB, Nambiar V, Aravindakshan R, Al Khabori MS, Al-Flaiti AH, Al-Azri KN, *et al*. Diabetic foot infections: Profile and antibiotic susceptibility patterns of bacterial isolates in a tertiary care hospital of Oman. *J Edu Health Promot* 2021; 10:254.
15. Hena, J. and L. Growther, Studies on bacterial infections of diabetic foot ulcer. *African Journal of Clinical and Experimental Microbiology*, 2010. 11(3)
16. Miyan Z, Fawwad A, Sabir R, Basit A. Microbiological pattern of diabetic foot infections at a tertiary care center in a developing country. *J Pak Med Assoc.* 2017 May; 67(5):665-669. PMID: 28507348.

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