

**BACTERIOLOGICAL PROFILE AND ANTIBIOTIC SENSITIVITY PATTERN OF
DIABETIC FOOT ULCER AT TERTIARY CARE CENTER, JAMNAGAR, GUJARAT.**

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ABSTRACT

Introduction: World's largest number of Diabetes Mellitus is present in India. Most common complication due to diabetic foot ulcer is lower limb amputation. Diabetic foot ulcer is polymicrobial with multidrug resistant. **AIMS & Objectives:** This study was to isolate the bacterial pathogens and their antibiotic susceptibility pattern from discharge samples from patients with diabetic foot ulcer in microbiology department at tertiary care hospital, Jamnagar. **Materials & Methods:** 171(57%) isolates were recovered from wound discharge samples 300. Antibiotic susceptibility of microorganism was determined by using disk diffusion method as per CLSI guidelines. **Result:** Amongst 300 samples 171(57%) were positive for bacteriological culture, in which major isolates was Pseudomonas 44 (25%), followed by Proteus spp. 38 (22%), E.coli 29 (17%), Klebsiella spp. 27 (15%), S.aureus 27 (15%) and Acinetobacter 11 (6%). Among all gram negative bacilli were sensitive to Imipenem 100%, followed by Piperacillin-tazobactam 67%, followed by Gentamicin 49%. Among all gram positive cocci Vancomycin and Erythromycin were sensitive 96%, followed by Linezolid 93%. **Conclusion:** This study shows that in Diabetic foot ulcer most common organism was Pseudomonas followed by Proteus spp., E.coli, etc. Early identification of organism can help to treat with right antibiotic & help early recovery.

KEYWORDS: Diabetes Mellitus, Foot ulcer, Bacteria, Antibiotic susceptibility test.**INTRODUCTION**

Diabetes is common metabolic disorder of endocrine system which affect all the system of body, with prevalence rate of 12-17% in India.^[1] It is increasing worldwide, around 366 million people affected by 2030 due to longer life expectancy and changes in diet habit.^[2]

Three main types of diabetes mellitus 1) IDDM - Insulin dependent diabetes mellitus where enough insulin not produced by pancreas. 2) NIDDM - Non insulin dependent diabetes mellitus where enough insulin but not work means insulin resistance. 3) Gestational diabetes - where high sugar level in pregnant women. In history diabetes there has been much progress in medicine to try helping those who suffer from it but in future diabetes time-line will be one with a cure.^[3,4]

Complications arising from diabetes mellitus are macrovascular and microvascular which include neuropathy, retinopathy, nephropathy, diabetic foot ulcer with or without gangrene.^[5] Diabetic foot ulcer is most common and major cause of morbidity, longer hospital stay and amputation which feels discomfort or disability to patient.^[6] Diabetic foot ulcer is polymicrobial infection associated with inadequate glycemic control.^[7] these organisms are mainly gram positive cocci staphylococcus aureus, and gram negative bacteria-

proteus spp., E.coli, pseudomonas aeruginosa, klebsiella spp., etc.^[8] These organisms are multidrug resistance.

Prompt antibiotic therapy should be useful for reducing hospitalization and amputation.^[9] Proper identification of bacteria may help for the better outcome and also prevents complications following the infection. In view of these facts the present study has been planned in order to find out bacterial infection in diabetes foot ulcer.

AIMS AND OBJECTIVE

To isolate and identification of bacteria associated with diabetic foot ulcer and to study antibiotic susceptibility pattern of that bacterial pathogens.

INCLUSION CRITERIA

Diabetic foot ulcer with traumatic and accidental injury.

EXCLUSION CRITERIA

Foot ulcer other than diabetes mellitus.

MATERIALS AND METHODS

In this retrospective study, total 300 samples from wound discharge of diabetic foot ulcer are collected from Guru Gobind Singh Government Hospital, Jamnagar. On the basis of clinical history and finding samples are

collected. Then sample were immediately transported to laboratory for processing, and then smeared for direct microscopy, cultured & antibiotic susceptibility testing and biochemical tests.

Collection of the specimen

On the basis of clinical history and finding samples are collected as per laboratory protocol. The ulcer site from diabetic foot exudate was collected by sterile thin cotton wool swabs, aseptically. Then sample were immediately transported to laboratory for processing.

Diagnostic methods: All the samples were processed in the following manner:

1st DAY FOLLOW UP

(i) Primary smear gram stain: - Gram positive bacteria resist to decolourisation and retain violet colour while Gram negative bacteria are decolourised, appear pink colour.

(ii) Culture: -All swabs are inoculated on Nutrient agar & differential media like MacConkey agar. The culture plate is incubated at 37^o C for 24 hours. For non-fastidious organism Blood agar and Chocolate agar plates are incubated at 3-5% CO₂ in candle jar for 24 hours.

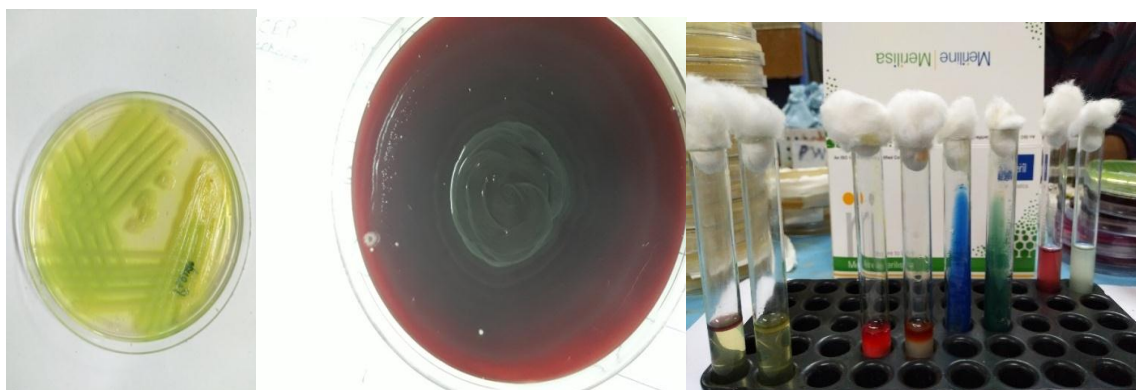


Fig. 1: *P.aeruginosa*, swarming of *Proteus*, IMViC reactions.

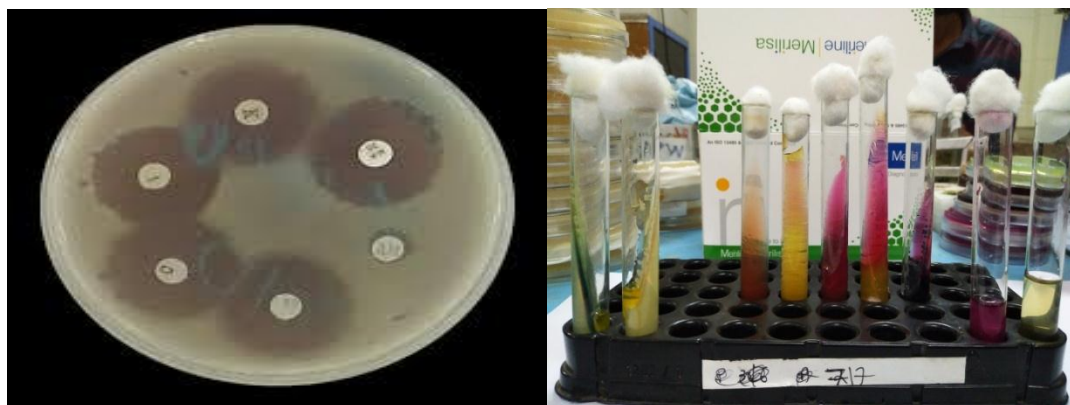


Fig. 2: TSI, PPA, Urease and Antibiotic Sensitivity Testing.

2nd DAY FOLLOW UP

Inoculated culture plates were examined for growth on media. The colonial characteristic of each different type of colonies were noted and Gram stained smear was observed.

For identification of isolates following biochemical media and reagents were used.

- Peptone water: To detect Indole production.
- Glucose phosphate broth (GPB): For Methyl Red (MR) and Voges-Proskauer (VP) test.
- Christensen's urea broth: To test urease activity.
- Triple Sugar Iron Agar (TSI): To test the fermentation of glucose, sucrose, lactose and production of hydrogen sulphide (H₂S).

- Simmon's Citrate Agar: To test utilisation of citrate as sole source of carbon.
- Phenylalanine Agar (PPA): To determine whether the microbe produces the enzyme phenylalanine.
- Sugar peptone water: To determine carbohydrate fermentation activity.
- Oxidase test: To test presence of cytochrome oxidase.
- Catalase test: To differentiate *S.aureus* from other staphylococci spp.
- Coagulase test: For the identification of *S.aureus*—formation of clot does not flow on tilting the test tube.
- Mannitol Salt Agar: *Staphylococcus aureus* produce yellow coloured colonies due to mannitol fermentation.

Antibiotic Sensitivity Testing (Kirby-bauer diskdiffussion method)

Pure broth culture suspension by adding 2-3 pure isolated colonies of the organism prepared into peptone water and incubated at 37°C for 4-6 hours & Match turbidity with 0.5 McFerlands standards. (1.5X10⁸ cfu/μl) Broth is inoculated on Muller Hinton medium, incubated overnight.

3rd Day Follow Up

All the biochemical reactions were observed and results were noted.

Antibiotic sensitivity testing

Sensitivity of drug is determined by zone of inhibition of bacterial growth around the disk, results as “sensitive”, “intermediate sensitive”, “resistant” as per CLSI guidelines.

RESULT

Out of 300 samples, 129 samples were Negative on Gram staining examination, 171 samples were positive on gram staining on direct smear examination, so prevalence for bacteria is 57%, male is more(58.74%) affected than female. 51-60 year (34.50%) age group is most affected followed by 41-50 year, 61-70 year, etc.

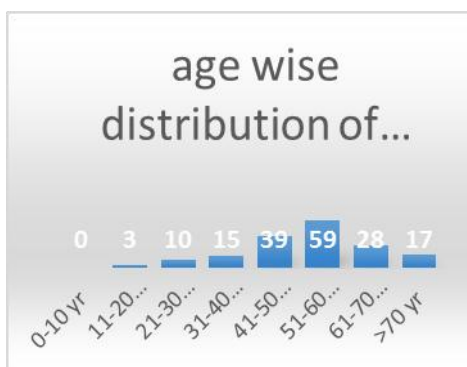


Fig. 3: Age wise distribution.

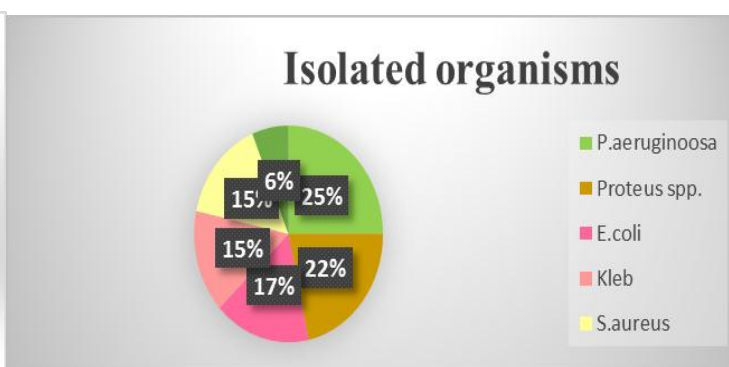


Fig. 4: Isolated organism among positive cases.

Most common isolated organism was P.aeruginosa (25%) followed by, Proteus speices (22%), E.coli (17%), Klebsiella (15%), S.aureus (15%), Acinetobacter speices (6%). Among these organism antibiotic sensitivity was

more common in Imipenem (100%), followed by Piperacillin-Tazobactam. And in Gram positive organism most sensitive antibiotic were Vancomycin (96.3%), Erythromycin (96.3%).

Antibiotics	P.aeruginosa	Proteus spp.	E.coli	Kleb	Acineto
Gentamicin (GM)	43.2%	50%	51.3%	48.1%	72.7%
Cotri-moxazole (BA)	22.3%	47.4%	72.4%	51.9%	36.7%
Amikacin (AK)	31.8%	32.6%	37.9%	29.6%	27.3%
Ofloxacin (ZN)	29.5%	39.5%	27.6%	37%	18.2%
Piperacillin-Tazobactam (PT)	70.5%	78.9%	65.5%	51.9%	54.5%
Imipenem (IPM)	100%	100%	100%	100%	100%

Fig. 5: Antibiotic sensitivity pattern of different organism.

Antibiotics	S.aureus
Cefotaxime (CF)	88.9%
Ampi-salbactam (AS)	81.5%
Linezolid (LZ)	92.6%
Levofloxacin (QB)	85.2%
Vancomycin (VA)	96.3%
Erythromycin (E)	96.3%

Fig. 6: Antibiotic sensitivity of S.aureus.

DISCUSSION

Diabetes foot ulcer may be associated with some predisposing risk factors like smoking, alcoholic, trauma, previous ulcer, prior amputation, previous ulcer leading to amputation, neuropathy, etc.

Ulcer may be due to diabetes any of its complication, which may include bacterial pathogens which may show

different antibiotic sensitivity pattern which may affect the treatment of the patient and their recovery rate.

Various organisms causing infection in foot, out of them the organism showing resistance to common antibiotics are of main concern. It is one of the leading cause of complications. The random use of antibiotics by clinician to treat the foot ulcer, may further lead to chronicity of the infection, complications, any other infections.

So, the study was conducted for isolate bacterial pathogens and resistance pattern of the bacterial isolated from patients with diabetes foot ulcer.

Total	Kandati J et al (2015) ^[10]	Khare J et al(2017) ^[11]	Seung T S et al(2017) ^[12]	Present study
Positive cases	91.27%	62%	82.2%	57%
Male	74.34%	72.58%	72.5%	58.47%
Female	25.66%	27.42%	27.5%	41.53%

Fig. 7: Comparison of positive cases and sex wise distribution in different studies.

Organism	Mehta V J et al(2014) ^[7]	Kandati J et al (2015) ^[10]	Seung T S et al(2017) ^[12]	Present study
P.aeruginosa	27%	18.82%	9.4%	25%
Proteus spp.	7%	2.83%	-	21.5%
E.coli	19%	13.88%	7.2%	16.5%
Kleb	22%	11.29%	3.2%	15.3%
S.aureus	7%	23.06%	13.7%	15.3%
Acineto	2%	4.24%	1.6%	6.4%

Fig. 8: Comparison of various isolates in different studies.

In present study, among the positive samples the highest number of isolation is Pseudomonas aeruginosa (25%), which was similar to Mehta V J et al(2014)^[7] (27%).

It has been noted that different studies have found different figures for antibiotic susceptibility and resistance for a single organism at different times at according to the susceptibility of a particular strain to various antibiotics prevailing at that time.

Name of antibiotic	Mehta V J et al(2014) ^[7]	Kandati J et al (2015) ^[10]	Yerat R C et al (2015) ^[13]	Omaina A et al (2016) ^[14]	Present Study
Gentamicin	25%	-	26.3%	-	49%
Cotri-moxazole	40%	-	9.5%	-	45%
Amikacin	38%	84.5%	53.5%	74.9%	32.2%
Ofloxacin	-	84.1%	52.3%	49.1%	32.2%
Piperacillin-Tazobactam	38%	94%	48.1%	53.1%	67.1%
Imipenem	100%	93.66%	100%	-	100%
Cefotaxime	-	81%	76.19%	36.4%	88.9%
Ampi-salbactam	70%	-	76.19%	-	81.5%
Linezolid	100%	100%	-	-	92.6%
Levofloxacin	-	-	-	54.5%	85.2%
Vancomycin	100%	100%	100%	68.2%	96.3%
Erythromycin	-	74%	95.2%	63.6%	96.3%

Fig. 9: Comparison of antibiotic Sensitivity in different studies.

Among comparison studies, various organisms are observed to be sensitive to Imipenem. 100% by Mehta V J et al, 93.66% by Kandati J et al, 100% by Yeart R C et al and in present study 100%. Organisms show more resistance to amikacin and ofloxacin both in present study and comparison studies.

Among comparison studies, various organisms are observed to be sensitive to Linezolid. 100% by Mehta V J et al and in Kandati J et al which is 92.6% sensitive by present study.

CONCLUSION

P. aeruginosa (25%) and E. coli (16.5%) have high prevalence in present study. Among antibiotic susceptibility maximum sensitivity in Gram negative bacilli was Imipenem (100%) and Piperacillin-

Tazobactam (67.1%), while in Gram Positive cocci was Linezolid (92.6%) and Vancomycin (96.3%).

Early diagnosis of the patients on clinical ground as well as diagnosis of the causative organism and to know effective antibiotic for the same is of very much importance for the positive outcome.

Study of antibiotic susceptibility pattern of various species isolated shows the alarming level of resistance to a number of antibiotics tested. It strongly suggests the need for careful planning of antibiotic usage policy in the future.

Proper personal hygiene along with awareness of cleanliness may help the situation. Strict hospital ward and operation theatre cleanliness is also required.

Frequent changing of antiseptic solution bottles and judicious use of antibiotics are of importance.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

AUTHOR'S CONTRIBUTION

All authors (Dr. Binita Aring, Dr. Pushpa Kateshiya, Dr. Dipali Gavali) listed have made a substantial, direct and

intellectual contribution to the work, and approved it for publication.

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DATA AVAILABILITY

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

ETHICS STATEMENTS

The study was approved by Institute ethics committee, M. P. Shah Govt. Medical college and Guru Gobind Singh hospital, Jamnagar with Ref. no. IEC/Certi/113/2017.

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