



“COMPARATIVE STUDY OF AEROBIC BACTERIAL ISOLATES IN SUPERFICIAL WOUND SWAB AND DEEP TISSUE BIOPSY CULTURES AMONG TYPE 2 DIABETES MELLITUS PATIENTS WITH FOOT ULCERS”

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ABSTRACT**INTRODUCTION:** Diabetes is a metabolic disorder of the endocrine system and a worldwide problem.

Diabetic foot lesions are major medical, social and economic problem and are the leading cause of hospitalization for patients with diabetes. 85% major leg amputation begins with a foot ulcer. Targeted treatment of infective organism requires accurate identification of pathogens to enable refinement of antibiotic protocols to improve the outcome and to reduce antibiotic resistance

AIMS AND OBJECTIVES OF THE STUDY: 1. To isolate, identify and compare the aerobic bacteria in superficial wound swabs and deep tissue biopsies.

2. To study the antibiotic susceptibility pattern of the isolated bacteria.

MATERIALS AND METHODS: Two hundred patients with diabetic ulcers were included, ulcer swabs and tissue specimens collected, immediately transported and organisms were identified by culture and biochemical reactions. Antibiotic susceptibility test was done by using Kirby Bauer's disc diffusion method on Mueller Hinton agar under CLSI guidelines.

RESULTS AND CONCLUSION: Out of 200 samples, aerobic bacteria isolated were 77% from swab and 76% from tissue biopsy. A total of 220 isolates were obtained from 154 positive swabs and 207 isolates were obtained from 152 positive tissue biopsy specimens. 61% from swab and 65.8% from tissue biopsy yielded monomicrobial growth. The organisms isolated predominantly were *Staphylococcus aureus* (including *MRSA*), *Klebsiella spp*, *Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter spp*, *Proteus spp* and *Enterococcus spp*.

Swab culture yielded more organisms than tissue biopsy culture. Calculated P value was insignificant (0.115, P value<0.05 significant). Gram positive organisms were more sensitive to linezolid, vancomycin, amikacin and cotrimoxazole. Gram negative organisms were more sensitive to imipenem, piperacillin–tazobactam, amikacin and aztreonam. Swab is preferable because it is more economical, technique is easy and more compliant for the patient.

KEYWORDS : Diabetic ulcer, Aerobic bacteria, Swab method, Tissue biopsy

INTRODUCTION

Diabetes is a metabolic disorder of the endocrine system and a worldwide problem.¹ India has become the diabetic capital of the world; within next few years with its attendant complications it is going to burden the resources of the country.

Global prevalence of diabetes mellitus was 3%.² Global prevalence of diabetes in 2003 was 194 million. By 2030, predicted to rise to 366 million due to longer life expectancy and changing dietary habits. Prevalence in southern India for diabetic ulcer ranges from 13 to 18%.³ Diabetic foot lesions are leading cause of hospitalization.⁴ 85% major leg amputation begins with a foot ulcer.⁵

The most common pathogenic organisms in diabetic ulcers are colonizers which include *Coagulase Negative Staphylococcus*, *Enterococcus*, *Alpha hemolytic Streptococcus*, *Diphtheroids*, *Beta hemolytic Streptococcus*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Prevotella*, *Peptostreptococcus*.⁶ Targeted treatment of infective organisms require accurate identification of pathogens in order to enable refinement of antibiotic protocols to improve the outcome and reduce antibiotic resistance.⁷

Swab culture method is universal, quick and easy but susceptible to collecting contaminants including high number of colonizers and often lack the true pathogens.⁸⁻¹²

Hence the present study has been undertaken to evaluate the isolation of aerobic bacteria and their antibiotic susceptibility pattern and also for comparison of isolates between superficial swab and deep tissue biopsy.

AIMS AND OBJECTIVES OF THE STUDY

1. To isolate, identify and compare the aerobic bacteria in superficial wound swabs and deep tissue biopsies.
2. To study the antibiotic susceptibility pattern of the isolated bacteria.

MATERIALS AND METHODS

The present cross sectional comparative study was conducted at a tertiary care centre from November 2015 to April 2017 after obtaining informed consent. Institutional ethical clearance was obtained.

A total of 200 Type 2 diabetic patients with diabetic ulcer from the surgery department (inpatient and out patients) were included. Ulcer

swab and tissue biopsy samples were collected, transported to the microbiology laboratory and processed.

INCLUSION CRITERIA:

- Type 2 diabetes mellitus patients with Wagner's grade 1, 2 foot ulcers.

EXCLUSION CRITERIA:

- Type 1 diabetes patients presenting with foot ulcers.
- Patient already underwent surgical debridement.
- Patient with Wagner's grade 0,3,4,5 diabetic ulcer

WAGNER ULCER CLASSIFICATION SYSTEM¹³

Grade	Description
0	Skin intact
1	Superficial ulcer
2	Deeper, full-thickness extension of ulcer
3	Deep abscess or osteomyelitis associated with ulcer
4	Partial forefoot gangrene with ulcer
5	Extensive foot gangrene with ulcer

METHODS OF COLLECTION OF SPECIMENS**Swab collection**

Two swabs were collected under aseptic precautions, one for Gram's staining and another for culture. After thoroughly cleaning the wound with normal saline, cotton tipped swab was rubbed over the wound surface 1cm² or collected directly from the base of the ulcer with sterile swab which is in contact with wound for at least 5sec.^{6,14-16}

Tissue biopsy collection

Deep tissue sample was surgically excised, approximately 1 to 5 grams by using a new set of sterile instruments: curette, forceps and scalpel, after surrounding area of the wound was cleaned with povidone iodine solution at depth of 5mm from ulcer base.

Specimen transport - Both swab and tissue specimen were transported and processed within 15 minutes.¹¹

The samples were processed using standard protocols. Identification of aerobic bacteria was based on morphology, staining characteristics, cultural characteristics and biochemical reactions. Antimicrobial susceptibility testing of the obtained isolates was done on Mueller Hinton agar using Kirby Bauer's disc diffusion method under CLSI guidelines (2015).

RESULTS

A total of 200 patients were enrolled for the study. A total of 220 isolates were obtained from 154 positive swabs and 207 isolates were obtained from 152 positive tissue biopsy specimens.

The study group comprised predominantly males 123(61.5%) and 77(38.5%) were females.

Table 1.age Distribution

AGE IN YEARS	FREQUENCY	PERCENTAGE (%)
30 - 40	13	6.5
40 - 50	38	19.0
50 - 60	69	34.5
60 - 70	49	24.5
>70	31	15.5
Total	200	100.0

Table-2 Culture Outcome

	Swab	Tissue biopsy
Growth	154(77%)	152(76%)
No Growth	46(23%)	48(24%)
Total	200	200

Table -3 Microbial Growths Obtained In Swab And Tissue Biopsy

	SWAB	%	TISSUE	%
Monomicrobial	94	61	100	65.8
Dimicrobial	54	35.1	47	31
Polymicrobial	6	3.9	5	3.2
Total	154	100	152	100

Table- 4 Organisms Obtained From Swab And Tissue Biopsy**A.GRAM POSITIVE ORGANISMS**

	SWAB	%	TISSUE BIOPSY	%
1. <i>Staphylococcus aureus</i>	56	36.36	48	31.57
2. <i>Enterococcus spp</i>	9	5.84	5	3.28
3. <i>Staphylococcus epidermidis</i>	7	4.54	2	1.31
4. <i>Streptococcus pneumoniae</i>	1	0.64	1	0.65
5. <i>Alpha haemolytic Streptococci</i>	1	0.64	-	-
6. <i>Beta haemolytic Streptococci</i>	1	0.64	-	-
Total	75		56	

B.GRAM NEGATIVE ORGANISMS

	SWAB	%	TISSUE BIOPSY	%
1. <i>Klebsiella spp</i>	38	24.67	46	30.26
2. <i>Escherichia coli</i>	35	22.72	37	24.34
3. <i>Pseudomonas aeruginosa</i>	32	20.77	29	19.07
4. <i>Acinetobacterspp</i>	25	16.23	23	15.13
5. <i>Proteus spp</i>	11	7.14	11	7.2
6. <i>Citrobacter freundii</i>	3	1.94	4	2.63
7. <i>Enterobacterspp</i>	1	0.64	1	0.65
Total	145		151	

8 isolates of *Staphylococcus aureus*, 3 *Pseudomonas aeruginosa* and 1 *Beta haemolytic Streptococci* were grown in swab but missed from tissue biopsy specimen. However 8 isolates of *Klebsiella spp*, 2 *Escherichia coli* and 1 *Citrobacter freundii* were grown in tissue biopsy but missed from swab.

Table- 5 Concordances Between Swabs And Deep Tissue Cultures

Swabs that yielded all isolates cultured from Deep tissue	121/154 (78.571%)
Swabs that yielded all isolates found in Deep tissue plus additional growth	130/154 (84.415%)
Tissue biopsy yielded all isolates found in Swab plus additional growth	121/152(79.6%)
Isolates not grown in swabs but found in deep tissue	3/154 (1.9480%)
Only growth in Swab	24/154 (15.584%)
Only growth in Tissue biopsy	17/152 (11.038%)

Table- 6 A. Antibiotic Susceptibility Pattern Of Gram Positive Organisms

ANTIBIOTICS TESTED	SWAB		TISSUE BIOPSY	
	S	%	S	%
1. linezolid(LZ)	73	100	52	92.8
2. vancomycin(VA)	62	82.6	47	83.9
3. amikacin(AK)	46	62.1	38	67.8
4. cotrimoxazole(COT)	46	61.3	35	62.5
5. erythromycin(E)	38	58.4	33	60
6. ampicillin(AMP)	38	50.6	32	58.1
7. penicillin(P)	9	45.3	13	27
8. ciprofloxacin(CIP)	30	44.1	25	44.6
9. gentamicin(GEN)/ HLG	27	42.8	19	34.5
10. ceftaxime	13	23.2	12	25

HLG- high level gentamicin**B. ANTIBIOTIC SUSCEPTIBILITY PATTERN OF GRAM NEGATIVE ORGANISMS**

ANTIBIOTIC	SWAB		TISSUE BIOPSY	
	S	%	S	%
1. piperacillin-tazobactam(PTZ)	36	90	36	80
2. imipenem(IMP)	122	84.7	130	88.4
3. amikacin(AK)	80	55.17	86	56.9
4. aztreonam (AT)	18	54.5	26	56.5
5. ceftazidime clavulanic acid(CAC)	63	43.7	68	45.6
6. amoxicillin clavulanic acid(AMC)	60	41.3	61	40.6
7. ceftriaxone(CTR)	51	35.9	64	42.3
8. ciprofloxacin(CIP)	47	32	58	38.4
9. cotrimoxazole(COT)	43	29.6	58	38.6
10. ampicillin(AMP)	41	28.4	58	38.4
11. ceftazidime(CAZ)	38	26	50	33.1
12. gentamicin(GEN)	26	25.7	51	34

Table -7 Mrsa And Esbl Strains With Percentage

	SWAB (%)	TISSUE BIOPSY (%)	Total (%)
MRSA	43(76.8%)	36(75%)	79(75.96%)
ESBL	104(71.7%)	97(64.2%)	201(67.9%)

MRSA - methicillin resistant *Staphylococcus aureus*,
ESBL - extended spectrum β -lactamase

DISCUSSION

In the present study, a total 200 patients were enrolled over a period of 18 months. Most common age group with diabetic foot was between 50–60 years, probably due to poor glycemic control. In a study by Hena *et al*¹⁷ the common age group affected was 56–65 years with average of 58 yrs. In another study by Hafni AA *et al*¹⁸ the common age group affected was 51–60 years.

In the present study 61.5% were males and 38.5% were females. In a study conducted by Siham Sh *et al*¹⁹ in 2013, 68% were males and 32% were females. Bengalorkar G *et al*²⁰ showed 70% males and 30% females which are all in concordance with our study. Male preponderance was due to more exposure to injuries during their occupational and recreational activities.

SWAB V/S TISSUE BIOPSY**Table-8 Comparison Of Culture Outcome**

	Swab	Tissue biopsy
Present study	77%	76%
Bozkurt ²¹	68%	52%
Nelson ⁷	70%	86%
Haalboom ²²	96%	77%

Determination of the microbiological cause of diabetic foot ulcer relies on the sampling method used. Study conducted by Bozkurt *et al* and Haalboom *et al* have shown that swab technique yielded better result and to be a better method. However Nelson *et al* had shown tissue biopsy to be a better method.

In our study out of 200 patients, pathogens reported was 77% from swabs and 76% from tissue biopsies. 78.5% swabs yielded all organisms that were cultured from deep tissue biopsy. Organisms that

were not grown in swab but grown in tissue biopsies were 1 isolate of *Citrobacter species*, 2 *Escherichia coli* and 8 *Klebsiella species*. Organisms that were not grown in tissue but grown in swab were 8 *Staphylococcus aureus*, 3 *Pseudomonas aeruginosa* and 1 *Beta haemolytic Streptococci*.

Swab culture yields more organisms than tissue biopsy culture. Calculated P value is not significant. Swab is preferable because it is more specific, economical, easy technique and more compliant from patients.

Table 9 Causative Organisms

Study	<i>Staphylococcus aureus</i> and <i>MRSA</i> (%)	<i>Klebsiella spp</i> (%)	<i>Escherichia coli</i> (%)	<i>Pseudomonas spp</i> (%)
Present study	36	24.6	22	20
Hena JV <i>et al</i> ⁷	42.3	9	15.3	24.3
Gadepalli R <i>et al</i> ¹	13.7	6.6	12	9.8
Jain M <i>et al</i> ²	12.7	22.29	16.56	30.57
Anandi C <i>et al</i> ²³	13.6	13.6	27.7	11.3

Staphylococcus aureus was the most common organism isolated in our study which is similar to the studies conducted by Hena JV *et al* and Gadepalli R *et al* whereas *Pseudomonas spp* and *E.coli* were the common organisms which were isolated in the studies Jain M *et al* and Anandi C *et al* respectively.

ANTIBIOTIC SUSCEPTIBILITY

In our study Gram positive bacteria were more sensitive to linezolid, vancomycin, amikacin and cotrimoxazole Gram negative bacteria were more sensitive to piperacillin-tazobactam, imipenem, amikacin and aztreonam by swab and tissue culture.

DRUG RESISTANCE

Multidrug resistance is a worrying global health issue as infections caused by them are associated with higher morbidity and mortality. Our study shows 75.96% MRSA and 67.9% ESBL. They have limited therapeutic options to treat them which may result in poor clinical outcome. ESBL-producing organisms frequently exhibit resistance to other antimicrobial agents may be due to plasmid encoded/ chromosomal mediated. Therefore early detection of these bacteria is important to control and prevent nosocomial outbreaks in hospital settings.

CONCLUSION

This study gives us knowledge about the prevalence of diabetic foot ulcers in our hospital and an insight of the causative organisms of diabetic ulcer and their sensitivity pattern. Higher prevalence of multidrug resistance was observed in our study warranting prompt need of surveillance for the effective management of such MDR strains. It also discourages the indiscriminate use of antibiotics. Hence there is a need to select an appropriate technique to ensure continued surveillance in order to combat bacterial drug resistance.

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