CHARACTERIZATION & ANTIMICROBIAL SUSCEPTIBILITY TESTING OF AEROBES FROM FOOT ULCERS OF ADULTS-COVERING LETTER

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ABSTRACT
BACKGROUND
Ulcer foot is a major problem that can impair the quality of life, require prolonged hospitalization & entail high cost to the patient, his/her family members, health care system Apart from diabetic ulcer, the non-diabetic causes of ulcer foot like burns ulcer is now a major problem in many parts of the world.
Appropriate antibiotic therapy is an important part of an ulcer foot management.
METHODS
2 Swabs were sampled and subjected to culture by standard methods. The organism obtained was identified upto genus level and antibiotic susceptibility testing was performed as per CLSI guidelines. Drug resistance was simultaneously detected using disc approximation method and the results were noted.
RESULTS
A total of 200 samples were processed, 85% yielded growth & 15% yielded, No growth. The ulcers sampled were 72 Diabetic,38 ulcer due to necrotizing fascitits,30 ulcers due to cellulitis,23 Venous ulcer,15 Non healing ulcer,10 hypertensive ulcer,9 Traumatic ulcer,1 osteomyelitis ulcer,1 Snake bite ulcer & 1 venous ulcer.
Gram negative isolates constituted 67.39% while Gram positive isolates were 32.60%. Pseudomonas aeruginosa,51 (22.1%) was the most common Gram Negative Isolate S. aureus, 33 (14.34%) was the most common Gram positive isolate.
CONCLUSION
Diabetic foot ulcer is one of the most commonly encountered cause of foot ulcers in adults. The next most common causes are Necrotizing fasciitis & ulcer due to cellulitis.

There is a potential risk factor towards the development of systemic toxicity, gangrene formation & amputation of the lower limb if there is no early medical intervention

The increasing resistance pattern of various organisms to the commonly used antibiotics is reducing the treatment options for the physician to treat the infections

KEYWORDS: Ulcer foot, Types of Ulcers, Diabetic foot, Gram Positive Bacteria, Gram Negative Bacteria, Pus

INTRODUCTION

Ulcer foot is a major problem that can impair the quality of life, require prolonged hospitalization & therefore entail high cost to the patient's family members & free health care system offered by governmental & Non-Governmental organizations [1,9]

The magnitude of ulcer foot associated with diabetes mellitus is increasing globally at an alarming rate. About 150 – 170 million populations are suffering from this disease worldwide& the prevalence is said to be double by 2025.In India25 million people suffer from diabetes mellitus, it is estimated that 15% of the diabetics develop ulcer foot with 85%of them progressing to amputations. [1]

Apart from diabetic ulcer, the non-diabetic causes of ulcer foot like burns ulcer is now a major problem in many parts of the world. Following burns, colonization of microorganisms can lead to penetration into viable tissue depending on the local wound factors & patient's immunosuppression. This would increase the overall infection related morbidity & mortality. [2]

Appropriate antibiotic therapy is an important part of an ulcer foot management. Common aerobic organisms isolated should be treated with empirical antibiotic therapy comprising of antibiotics sensitive to Gram negative & Gram positive microorganisms. [3]

Infected foot is one of the most common septic problem leading to hospitalization. Ischemia & peripheral neuropathy are the major factors leading to ulcer formation, an initial lesion that serves as the portal of entry for soft tissue, bony & even systemic infection [5]

In the recent years there has been an increase in the incidence of multidrug resistant organisms among the foot ulcers. The initial management comprises of empirical antimicrobial therapy based on antimicrobial susceptibility data. [6]

Poorly controlled diabetes is prone to skin infections because elevated blood sugar which reduces the immunity. Carbuncles, boils, and other skin infections may be hazardous & even a small cut may progress to a deep, open sore, leading to ulcer. In most cases ulceration is a consequence of the loss of protective sensation leading to ulcer foot. [7]

Diabetic foot ulcer is a frequent complication requiring hospitalization. It has emerged as one of the most common cause for non-traumatic lower extremity amputation & management of this condition requires an extensive knowledge about the common pathogens & the risk factors associated with the disease. [9]

Among the factors affecting the effectiveness of wound healing therapy are, the specific microorganisms that colonize the Chronic Venous Leg Ulcers(CVLU). For example, the presence of Pseudomonas aeruginosa can retard the healing of wounds due to their ability to form biofilms. [11]

The foot infections in patients are initially treated empirically. If the therapy is directed at the known causative organisms causing the infection, the outcome will be improved [12].

The International Diabetes Federation (IDF) has estimated that about 366million persons in the world have DM & this will increase to 552 million by 2030.The lifetime risk of a person with diabetes developing diabetes mellitus foot ulcers (DMFU) is reported to be as high as 25%. [13]

The individuals with diabetes have at least a 10-fold greater risk of being hospitalized for soft tissue and bone infections of the foot than individuals without diabetes. The Indian diabetic population is expected to increase to 57 million by the year 2025 [14]

Therefore, it is very important to have an in depth knowledge of the organisms that are predominant in different ulcer foot conditions & their sensitivity pattern to start the appropriate antimicrobial therapy for timely management of ulcer foot. [2], [4], [6]

This study will demonstrate the isolation of the causative organism & antimicrobial susceptibility of the organism which will in turn help in the timely management of foot ulcers thereby reducing the morbidity, mortality & shorten the hospital stay.

MATERIALS &METHODS

The study was conducted in the Dept. Microbiology from Dec 2021-June 2023 in a tertiary care hospital. A total of 200 patients with foot ulcers were sampled after obtaining Institutional ethical committee clearance.

Criteria for inclusion of Patients: Adults presenting with foot ulcers due to
- ✓ Non specific causes : Diabetic Ulcer, Venous ulcer, Arterial ulcer, ulcer due to blood dyscrasias etc.
- ✓ Malignant Ulcer

Criteria for Exclusion of Patients: Ulcers with a specific cause, for ex: Tubercular, Syphilitic ulcers.

Collection of Specimen

2 Swabs were collected as per the standard guidelines for collection of samples from various ulcers after giving a thorough saline wash and prior to administration of antibiotics. The swabs were transported to the laboratory immediately for culture.

Laboratory Procedures

The swabs were processed as per standard operative procedure(SOP) for Ulcer foot. The samples were inoculated to sterility check passed Blood and MacConkey agar in the same order & incubated at 37^0C for 24-48 hrs. Using standard methods, the organism(s) were identified upto species level. After the confirmation of the organism, Antibiotic susceptibility testing(AST) was performed by Kirby-Bauer disc diffusion method as per the Clinical & laboratory Standard Institute (CLSI) guidelines

Following drugs were used for the AST of the aerobic Gram negative isolates belonging to

1) Enterobacteriaceae

Piperacillin/Tazobactam-PIT(100/10μg), Ceftriaxone-CTR(30μg), Ceftazidime-CAZ(30μg), Ciprofloxacin-CIP(5μg), Co-Trimoxazole-COT(25 μg), Amikacin-AK(30μg), Gentamicin-GEN(10μg), Imipenem-IPM(10μg),

2) Staphylococcal isolates

Clindamycin-CD(2μg), Erythromycin-E(15μg), Pristinomycin-RP(15μg), Penicillin-P(15μg), Linezolid-LZ(30μg), Cefoxitin-CX(30μg), Ciprofloxacin-CIP(5μg), Co-Trimoxazole-COT(25μg) Gentamicin-Gen (10μg)

3) Streptococcal isolates

Clindamycin-CD(2μg), Erythromycin-E(15μg), Pristinomycin-RP(15μg), Penicillin-P(15μg), Linezolid-LZ(30μg), Ciprofloxacin-CIP(5μg), Gentamicin-Gen (10μg), High Level Gentamicin-HLG(120 μg), Bacitracin-B(0.04U)

4)Non Fermenting isolates

Piperacillin/Tazobactam-PIT(100/10μg), Ceftazidime-CAZ(30μg), Ciprofloxacin-CIP(5μg), Co-Trimoxazole-COT(25μg), Amikacin-AK(30μg), Ampicillin-AMP(10μg), Imipenem-IPM(10μg), Tobramycin-TOB(10μg

Antibiotic resistance was looked for simultaneously by approximation of discs while putting up AST. Following disc approximation Tests were put up: CTR,PIT,CAZ - ESβL.
CD,E,RP- Inducible resistance
CX resistance- βlactamase production in Gram positive isolates.

RESULTS
Total samples processed- 200
Total Samples with Growth-170(85%)
Samples without Growth-30(15%)

Table.1.Types of ulcers processed

Sl no	Type of Ulcer processed	Number
1.	Diabetic Ulcer	72
2.	Ulcer due to Necrotizing Fasciitis	38
3.	Ulcer due to cellulitis	30
4.	Venous ulcer	23
5.	Non Healing ulcer	15
6.	Hypertensive ulcer	10
7.	Traumatic ulcer	9
8.	Osteomyelitis ulcer	1
9.	Snake bite ulcer	1
11.	Burns ulcer	1
TOTAL		**200**

TYPES OF ULCERS

- Diabetic Ulcer
- Ulcer due to Necrotizing Fasciitis
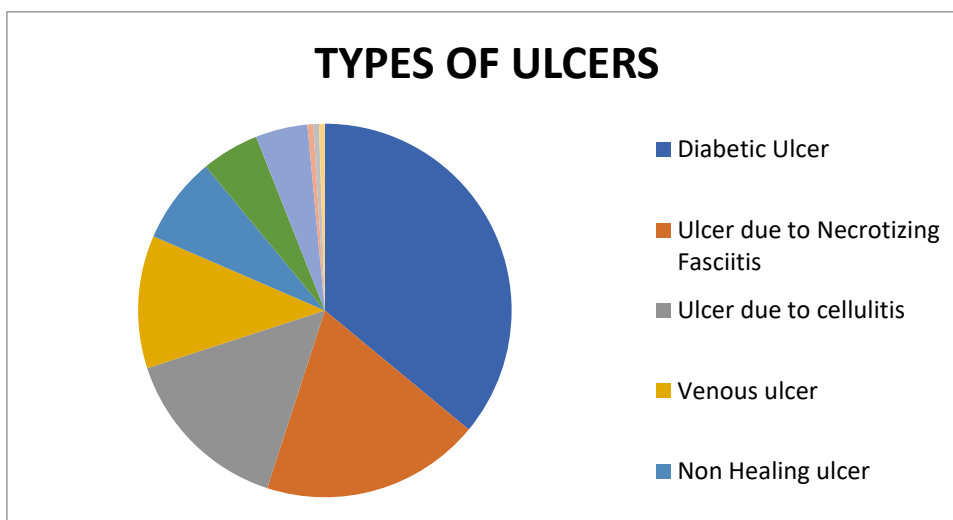- Ulcer due to cellulitis
- Venous ulcer
- Non Healing ulcer

Table 1.Shows, A total of 200 patients with ulcer foot were sampled. Out of the total samples processed, Diabetic ulcer was the most common ulcer(72) followed by Necrotizing fasciitis(38),Ulcer due to cellulitis(30),Venous ulcer(23),Non healing ulcer, Hypertensive ulcer(10), Traumatic ulcer(9), Osteomyelitic ulcer(1), Snake bite ulcer(1) & Burns ulcer(1).

Table 2.Ulcers yielding Growth & No Growth

Name of the ulcer	With Growth	No Growth	TOTAL
Diabetic Ulcer	66	6	72
Ulcer due to necrotizing Fasciitis	20	18	38
Ulcer due to cellulitis	30	0	30
Venous ulcer	19	4	23
Non Healing ulcer	15	0	15
Hypertensive ulcer	8	2	10
Traumatic ulcer	9	0	9
Osteomyelitic ulcer	1	0	1
Snake bite ulcer	1	0	1
Burns Ulcer	1	0	1
TOTAL	170	30	200

Table2. Shows, Among the total ulcers (200) sampled, 170(85%) yielded growth & 30(15%) did not yield any growth. Out of the ulcers that did not yield growth , 6 were diabetic ulcers, 18 Ulcers due to Necrotizing Fasciitis,4 venous ulcers & 2 Hypertensive ulcers.

Table 3. Total number of organisms Isolated

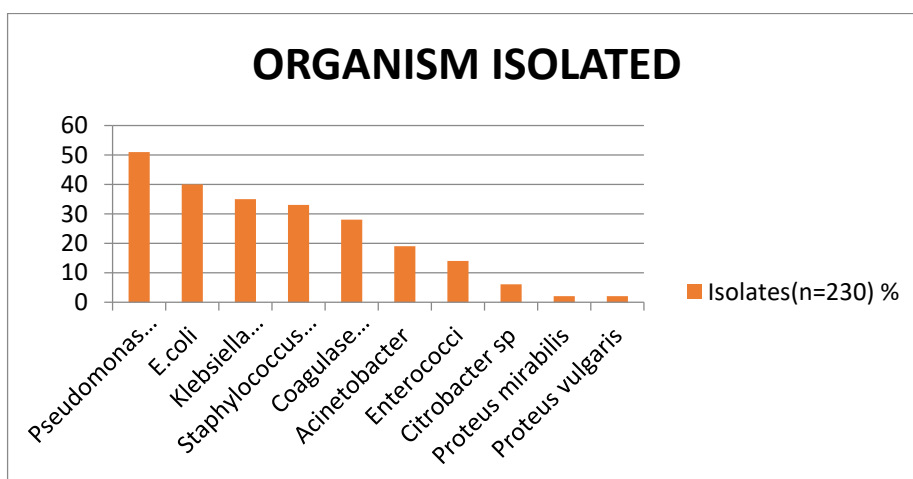| Organism	Isolates(n=230) %
Pseudomonas aeruginosa	51(22.1%)
E.coli	40(17.39%)
Klebsiella pneumonia	35(15.21%)
Staphylococcus aureus	33(14.34%)
Coagulase Negative S.aureus(CoNS)	28(12.17%)
Acinetobacter	19(8.26%)
Enterococci	14(6.08%)
Citrobacter sp	6 (2.60%)
Proteus mirabilis	2(0.86%)
Proteus vulgaris	2(0.86%)

Table 3 shows
Total organisms isolated : 230
Total Number of Gram Negative isolates: 155 (67.39%)
Total Number of Gram positive Isolates: 75(32.60%)

A total of 230 organisms were isolated. Among them the most common isolate was Pseudomonas aeruginosa(22.1%)followed by E.coli(17.39%),Klebsiella pneumonia(15.21%), S.aureus(14.34%), CoNs(12.17%), Acinetobacter(8.26%), Enterococci(6.08%), Citrobacter(2. 60%),P.mirabilis(0.86%) & P.vulgaris(0.86%).

Table.4. Antimicrobial Susceptibility pattern of Pseudomonas & Acinetobacter

Anti -microbial Agent	Pseudomonas aeruginosa (n=51)		Acinetobacter Sp. (n=19)	
	S	R	S	R
IPM	48(94.11%)	3(5.88%)	16(84.21%)	3(15.78%)
PIT	40(78.43%)	11(21.56%)	15(78.94%)	4(21.05%)
CIP	31(60.78%)	20(39.2%)	13(68.42%)	6(31.57%)
CAZ	46(90.19%)	5(9.80%)	14(73.68)	5(26.31%)
AK	34(66.66%)	17(33.33%)	11(72.70%)	8(42.10%)
TOB	38(74.50%)	13(25.49%)	12(63.15%)	7(36.84%)
COT	---------		15(78.94%)	4(21.05%)

Table 4 shows antimicrobial susceptibility pattern of pseudomonas aeruginosa & Acinetobacter. Pseudomonas showed a sensitivity of 94.11% & 78.43% to IPM & PIT respectively. A resistance of 39.2% & 33.33% was observed to CIP & AK respectively.
Acinetobacter showed a sensitivity of 84.21% & 78.94% to IPM & PIT. A resistance of 31.57% & 26.31% to CIP & CAZ was observed

Table 5. Antimicrobial susceptibility pattern of Enterobacteriaceae

Anti-micr-obial Agent	E.coli (n=40)		Klebsiella Pneumoniae (n=35)		Citrobacter Sp. (n=6)		P.mirabilis (n=2)		P.vulgaris (n=2)	
	S	R	S	R	S	R	S	R	S	R
PIT	32(80%)	8(20%)	30(85.7%)	5(14.28%)	6(100%)	-----	2(100%)	-------	2(100%)	-------
CTR	30(75%)	10(25%)	31(88.5%)	4(11.42%)	6(100%)	------	2(100%)	-------	2(100%)	--------
CAZ	29(72.5%)	11(27.5%)	29(82.8%)	6(17.14%)	5(83.3%)	1(16.66%)	2(100%)	--------	2(100%)	---------
IPM	39(97.5%)	1(2.5%)	33(94.2%)	2(5.71%)	6(100%)	--------	2(100%)	-------	2(100%)	--------
CIP	22(55%)	18(45%)	23(65.7%)	12(34.28%)	5(83.3%)	1(16.66%)	2(100%)	--------	2(100%)	---------
COT	20(50%)	20(50%)	21(60%)	14(40%)	6(100%)	--------	2(100%)	-------	2(100%)	---------
AK	25(62.5%)	15(37.5%)	19(54.28%)	16(45.71%)	5(83.3%)	1(16.66%)	1(50%)	1(50%)	1(50%)	1(50%)
GEN	27(67.5%)	13(32.5%)	21(60%)	14(40%)	6(100%)	--------	1(50%)	1(50%)	2(100%)	---------

Table 5 shows the susceptibility of Enterobacteriaceae. E. coli showed 97.5% & 80% sensitivity IPM & PIT while klebsiella pneumoniae showed a sensitivity of 94.2% & 88.5% to IPM & PIT respectively. Among the other species, P. vulgaris & P.mirabilis were 100% sensitive to PIT,CTR,IMP & CAZ.
A resistance of 37.5% & 32.5% was noted to AK & GEN among the isolates of E.coli. While a resistance of 40% & 34.28% was noted among the isolates of Klebsiella pneumoniae to COT & CIP respectively.

Table.6.Antimicrobial susceptibility pattern of Gram Positive Cocci

*AMP & HLG used for Enterococci

Antimicrobial agent	S.aureus (n=33)		CoNS (n=28)		Enterococci (n=14)	
	S	R	S	R	S	R
CD	28(84.84%)	5(15.15%)	23(82.14%)	5(17.85%)	-----	---------
E	27(81.81%)	6(18.18%)	22(78.57%)	6(21.42%)	------	----------
RP	26(78%)	7(21.21%)	18(64.28%)	10(35.71%)	-----	----------
CIP	20(60.60%)	13(39.39%)	17(60.71%)	11(39.28%)	------	-----------
CX/AMP*	22(66.66%)	11(33.33%)	25(89.28%)	3(10.71%)	0(71.42%)	4(28.57%)
LZ	33(100%)	------	28(100%)	-------	14(100%)	------
P	22(66.66%)	11(33.33%)	24(85.71%)	4(14.28%)	0(71.42%)	4(28.57%)
GEN/HLG*	23(69.69%)	10(43.47%)	22(78.57%)	6(21.42%)	14(100%)	------

Table 6 shows A sensitivity of 100% to Linezolid was observed among the isolates of S.aureus, CoNS & Enterococci. A resistence of 21.47% & 39.28% to GEN & CIP was seen among the isolates of CoNS respectively.

Table 7.Antimicrobial resistance pattern of GNB

Name of the Organism	ESβL isolates
Pseudomonas aeruginosa (n=51)	11(21.56%)
E.coli (n=40)	8(20%)
Klebsiella pneumoniae (n=35)	4(11.42%)
Acinetobacter	Nil
Citrobacter	Nil
P.mirabilis	Nil
P.vulgaris	Nil

Table 7 shows, A total of 11 isolates were ESβL producers among Pseudomonas aeruginosa while E.coli & Klebsiella pneumoniae had 8 & 4 isolates respectively

Table 8 Antimicrobial resistance pattern of GPC

Name of the Organism	Methicillin resistance	Inducible resistance to CD
S.aureus(n=33)	**11(33.33%)**	**5(15%)**
CoNS(n-28)	**3(10.71%)**	**5(17.85%)**

Table 8 shows , Among the strains of S.aureus, 33.33% of the isolates were Methicillin resistant strains & 15% showed inducible resistance to CD. Among the CoNs strains, 10.71 % were Methicillin resistant & 17.85% showed Inducible resistance to Clindamycin(CD.)

Discussion

Foot ulcers are the most common medical complications encountered in the present day Medical practice. Among them Diabetic Foot Ulcers are the most common cause. Diabetic foot ulcers are responsible for more hospitalizations than any other complication of diabetes. Ulcerations can have potential devastating complications as they cause up to 90% of lower extremity amputations in patients with diabetes.

Factors responsible for ulceration among Diabetics are
-Level of uncontrolled hyperglycemia
-Reduced circulation and arterial blood flow
-Nutrition status
-Inability to offload the affected region of the foot
-Presence of infection[16]

In the present Study out of the 200 samples processed, 72 were Diabetic ulcers. Among them 66 ulcers yielded growth while 6 yielded No growth.50 ulcers(75%) had monomicrobial etiology while 14(21.21%) had polymicrobial etiology. An analysis by citron et al found 83.8% of poly microbial & 16.2% monomicrobial etiology which was different from the present study.[17]

ESβL production was noted among 34% GNB with 3 E.coli, 4 Pseudomonas & 1 K. pneumoniae isolate. E.coli isolates were found to have a sensitivity of 80% to PIT , Ceftazidime-72%, Imipenem-90%.K.M Mohanasoundaram in their study reported 31.5% ESβL production with PIT, Cefaperazone sulbactam & Imipenem being the most sensitive antibiotics.[6]

Present study isolated 6 MRSA. Clindamycin (84%), Erythromycin(81%), Ciprofloxacin(60%) were the common drugs to which most GPC were sensitive.T.prabhakaran & Mathangi in their study isolated 42 MRSA & reported Erythromycin(80%),Gentamycin(80%) & ofloxacin(89%) to be the common antibiotics to which most GPC were sensitive.[19]

The probability of diabetic ulcers with No growth could be attributed to antibiotic therapy prior to the collection of specimen, better Glycaemic control & wound care on admission to hospital

Chronic venous leg ulcers (CVLU) are a debilitating and often painful disease that affects approximately 1% of the world's population. The primary cause is insufficient valvular function of the veins in the legs causing increased hydrostatic pressure leading to edema of the subcutaneous tissue, which predispose to ulceration [15]

In the present study, 23 venous ulcers were sampled. Among them 19 ulcers yielded growth and 4 did not yield growth. The most common organism isolated was CoNS 7(33.3%) followed by Pseudomonas aeruginosa (28.51%) & S. aureus 4(19.01%), with 1 isolate being MRSA & 2 MRCoNS.

Necrotizing fasciitis is a rapidly progressive infectious disease that primarily involves the fascia and subcutaneous tissue. It is an uncommon, life threatening infection. It can affect all parts of body and the lower extremities are the most common sites of infection. The predisposing conditions are diabetes mellitus, liver cirrhosis, alcoholism, hypertension, chronic renal insufficiency, and malignancy. Prompt diagnosis and early treatment with adequate antibiotic with or without surgical intervention are vital. [21]

Proper management of Ulcer foot requires appropriate antibiotic selection based on culture and antimicrobial susceptibility results; Although initial management comprises empirical antimicrobial therapy, it is often based on susceptibility data obtained from studies performed on various clinical samples previously. [4]

Table 9.COMPARISON OF STUDIES IN ISOLATES OF FOOT ULCERS

Isolates	Abbas et al[26]	Bhalodia et al[25]	Du et al[27]	Present study
Avg.organism/patient	1.52	1.25	1.3	1.15
Predominant isolate	GNB	GNB	GPC	GNB
Pseudomonas	22%	30.57%	19%	22.1%
S.aureus	19%	12.74%	31%	33%
Methicillin resistance	55%	55.50%	55.56%	33.3%

The increasing incidence of multi drug resistant organisms among the foot ulcers is a potential risk factor in the management of foot infections. This may lead to devastating complications like systemic toxicity, gangrene formation & amputation of lower extremity.[25]

These multi drug resistant organisms are frequently resistant to many classes of antibiotics, so it is necessary for the clinician to be aware of the prevalence of multi drug resistant organisms & their management.

CONCLUSION
The present study "Characterization & antimicrobial susceptibility testing of aerobes from foot ulcers of adults" has shown that Diabetic foot ulcer is one of the most commonly encountered cause of foot ulcers in adults. The next most common causes are Necrotizing fasciitis & ulcer due to cellulitis.

The increasing resistance pattern of various organisms to the commonly used antibiotics is reducing the treatment options for the physician to treat the infections.

This is a potential risk factor towards the development of systemic toxicity, Gangrene formation & amputation of the lower limb if there is no early medical intervention

The study will help the clinician in choosing appropriate antibiotic(s) or it combination for timely management in the treatment of foot ulcer

Management of foot infections require a combination therapy of Antibiotics & surgical drainage. The choice of antibiotic used in the treatment should depend on the culture report. Empirical antimicrobials should include antibiotics covering Gram positive & Gram Negative isolates.

Timely management of foot ulcers reduces the hospital stay & morbidity.

More studies are needed to give an in depth knowledge about the increasing trends of resistance among the commonly encountered organisms & study on anaerobes would help to know the various organisms in foot ulcers of adults.

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