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Bacteriology Profile of Diabetic Foot Infection and In Vitro activity of Antimicrobial Agents

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Abstract: The study was carried out in the Department of Microbiology Index Medical College, Hospital and research centre (IMCHRC) Indore. In 100 diabetic foot patients studied, 73% were male and 27% were female. The age ranged from 31-80 yrs and majority of patients were in the age group o 51- 60 yrs. Out of 100 patients, 53 patients were diagnosed to have diabetes mellitus for> 10 yrs, 35 for 5 - 10 yrs and 12 for < 5 yrs. Out of 100 patients majority of the patients (90.29%) had ulcer for > 1 month duration. Out of 100 patients, 17 patients with ulcer belonged to the Wagner's grade 1, while 31 belonged to Grade 2, 40 belonged to grade 3 and 12 belonged to Grade 4. No growth was seen in 6 specimens out of 100 specimens. From 94 patients with culture positive specimens 170 organisms were isolated. Out of 94 culture positive cases, 22 patients had only 1 isolate, 72 patients had polymicrobial isolates Among the bacterial isolates, Gram negative comprised of 97 (57.1%) and Gram positive accounted for 73 (42.9%). Pseudomonas spp. was the most common isolates, accounting for 23.5% of all isolates followed by Staph. Aureus, Esch. Coli and Enterococci spp. comprising of 19.4%,10.5% and 10% respectively. All strains of S. aureus were sensitive to Vancomycin, 63.6% were sensitive to gentamicin and 60.60% were sensitive to cotrimoxazole. Erythromycin and penicillin showed least sensitivity 36.36% and 36.3% respectively. Enterococci spp. showed 100% sensitivity to vancomycin, 70.59% were sensitive to amikacin and gentamicin. Coagulase Negative Staphylococci showed relatively high sensitivity to all antibiotics, gentamicin 56.5% ciprofloxacin 82.6% penicillin 56.5% erythromycin 52% and cotrimoxazole 52% Among Pseudomonas spp. 90% strains showed sensitivity to Imipenem, 90% were sensitive to Amikacin, 70% were sensitive to Gentamicin. Pipercillin - Tazobactam combination showed sensitivity of 62.5 % Ceftazidime and Piperacillin had least acivity 45% and 30 % respectively. All other Gram negative bacteria were sensitive to Imipenem (100%), followed by Amikacin 71.93%, Ceftazidime 64.92%, Gentamicin 66.66 %, Cotrimoxazole 57.9 % and Amoxicillin-clavulanic acid. They were least sensitive to Ciprofloxacin, Piperacillin and Cefuroxime. 100 out of 170 isolates 58.8%were found multidrug resistant showing resistance to 3 groups of antibiotics which can be attributed due to previous antibiotic treatment and previous history of hospitalization. Penicillins, Quinolons and Cephalosporin's especially second generation showed least activity against the bacterial isolates from diabetic wound, so it should not be included in the empirical treatment regimen. A combination regimen consisting of amikacin, imipenem and vancomycin seems to be the most prudent empirical treatment of diabetic foot infection. Co-Trim oxazole can be considered in the regimen. This empirical therapy can be later modified appropriately based on the antiprogram of the isolates from the individual patients.
Keywords: Diabetic Foot Infection, Diabetes mellitus, Antimicrobial Agents.

INTRODUCTION

Diabetes mellitus is a syndrome consisting of metabolic, vascular and neuropathic components that are interrelated. It is defined as a group of metabolic diseases that are characterized by hyperglycemia resulting from defect in insulin secretion, insulin action or both [1].

Foot pathology remains the leading diabetic complication requiring hospitalization. As the incidence

of diabetes in general population is expected to raise, the prevalence of diabetic foot complications will follow [2]. It is estimated that 15% of diabetic patients will develop a foot ulcer during their lifetime. The prevalence of diabetic foot ulceration has been reported to range from 5 to 25% in diabetic patients. Foot ulcers are lesions that involve a skin break with loss of epithelium; they can extent into dermis and deeper layers, sometimes involving bone and muscles [3,4].

Diabetic foot is characterized by several pathological complications such as neuropathy, peripheral vascular disease, foot ulceration and infection with or without osteomyelitis, leading to development of gangrene and even necessitating limb amputation. Infection is a frequent (40% - 80%) and complication of these ulcers and represents a major cause of morbidity and mortality[4]. All foot ulcers are colonized with potentially pathogenic organisms. The impaired micro-vascular circulation in patients with diabetic foot limits the access of phagocytes favoring development of infection. *Pseudomonas spp., Staph aureus, Esch coli, Proteus spp*. And *Enterococcus spp*. are the most frequent aerobic pathogens contributing to progressive and widespread tissue destruction. Diabetic foot infections are often polymicrobial [5].

Pathway of foot ulceration [2, 6]

Major risk factors for the development of diabetic foot ulcers include peripheral neuropathy, peripheral vascular disease, and high foot pressure and impaired wound healing.

Sensory neuropathy, associated with pain insensitivity is the first component of the pathway. However the development of ulceration also requires the existence of trauma, usually related to the plantar tissue stress and the injury that result from the development of high foot pressure during walking. The presence of the third component, impaired wound healing due to the reduced blood flow in the ulcer area and aberrant expression of growth factors and cytokines, prevent the wound closure and leads to the development of chronic ulceration and in some cases amputation. 60 to 70% diabetic ulcers are due to neuropathy, 15 to 20% are due to ischemia and another 15 to 20% are due to combination of the above two.

Classification of diabetic foot ulcers

There has been numerous classification schemes proposed for describing diabetic foot ulcers. The most commonly used and most often referred to is Meggit Wagner system [8]. The Meggit Wagner system classifies diabetic foot ulcers into five distinct grades on the basis of anatomical location, depth of ulcer and presence of ischemia

Table-1: classification of diabetic foot ulcers - Meggit wagner's classification

Grade	Characteristics
0	Preulcer. No open lesion, skin intact, may have deformities, erythematous areas of pressure
1	Superficial ulcer. Disruption of skin without penetration of subcutaneous fat layer. Superficial infection with or without cellulitis may be present.
2	Full thickness ulcer. Penetrates through fat to tendon, or joint capsule without deep abscess osteomyelitis.
3	Deep ulcer which may or may not probe to bone, with abscess, osteomyelitis, or joint sepsis. Includes deep plantar space infections or abscess, necrotizing fasciitis, and tendon sheath infections.
4	Denotes gangrene of a geographical portion of the foot such as toes, forefoot or heel.
5	Gangrene or necrosis to the extent that the foot is beyond salvage and will require a major limb amputation.

The Wagner's classification system for diabetic foot ulcers has withstood the test of time because of simplicity, ease to use and universal ability to be communicated among various medical personnel.

Table-2: Clinical classification of diabetic Foot infection [7]

Clinical manifestations of infection	Infection severity
Wound lacking purulence or any manifestations of inflammation (i.e., erythema, pain, tenderness, warmth, or induration).	No infection
Presence of purulence and/or two or more manifestations of inflammation, but any cellulitis or erythema extends 2 cm or less around the ulcer; infection is limited to the skin orsuperficial subcutaneous tissues; no other local complications or systemic illness.	Mild
Infection (purulence and/or two or more manifestations of inflammation) in a patient who is systemically well and metabolically stable, but who has at least one of the following characteristics: cellulitis extending more than 2 cm around the ulcer; lymphangitic streaking;spread beneath the superficial fascia; deep tissue abscess; gangrene; involvement of muscle, tendon, joint, or bone.	Moderate
Infection (purulence and/or two or more manifestations of inflammation) in a patient with systemic toxicity or metabolic instability (e.g., fever, chills, tachycardia, hypotension, confusion, vomiting, leukocytosis, acidosis, severe hyperglycemia, azotemia).	Severe

Aims and objectives

- To study the bacterial agents causing diabetic foot infections.
- To study the antibiotic susceptibility pattern among the isolated organisms.
- To suggest the appropriate antibacterial therapy to avoid further complications in diabetic foot lesions.

MATERIALS AND METHODS

The study was carried out in the Department of Microbiology Index Medical College, hospital and research Centre Indore (M.P.). Specimens of diabetic foot infection were obtained from department of surgery, IMCHRC INDORE**.**

NUMBER OF CASES STUDIED: 100

Inclusion criteria

Diabetic foot infection with open lesions

Exclusinon criteria

Diabetic foot infection with only cellulitis, no open lesion. Limbs with amputation Specimens were collected, after thorough cleaning of the lesion with sterile normal saline, preferably before administration of antibiotics.

The specimens were as follows:

- Wound curettage by using a sterile scalpel.
- Aspiration from abscesses by using needle and syringe.
- Pus by using sterile swab.

Two specimens were collected from each patient. The two specimens were used for Gram stain and aerobic culture. The specimens were immediately transported to the microbiology laboratory.

Gram's staining: One of the specimen was smeared over a clean, dry microscopic slide and was stained by Gram staining technique. The film was examined for the presence bacteria and polymorphs.

Aerobic culture was carried out by directly inoculating the specimen onto blood agar and Mac Conkey agar which was incubated over night at 37^0C. All types of colony grown on these plates were read and colony description was recorded. Identification of the isolates were done by using standard conventional biochemical methods.

Antibiotic sensitivity test [9]

The antibiotic sensitivity testing was done by Kirby Bauer disk diffusion method with commercially available Hi Media disks according to clinical laboratory of standard institute (CLSI) guidelines.

The antibiotics to be tested against the isolates were determined according to the standard guidelines and also considering the local susceptibility pattern of the organism. The set of antibiotics tested for susceptibility against different organisms were as follows.

Table-3: **Antimicrobial agents tested for different isolates in present study**

Isolates	Antimicrobial agent tested
Staph. aureus	Penicillin, cefazoline, erythromycin, ciprofloxacin, and co-trimoxazole, gentamicin and vancomycin
Coagulase Negative Staphylococci Staphylococci (CoNS)	Penicillin, cefazoline, erythromycin, ciprofloxacin, co-trimoxazole, gentamicin and vancomycin
Enterococci spp.	Penicillin, gentamycin, amikacin, vancomycin.
Pseudomonas spp.	Piperacillin, Piperacillin- tazobactam, amikacin, gentamicin, ceftazidime, ciprofloxacin and imipenem.
Other Gram negative bacilli	Piperacillin, amoxicillin-clavulanicacid, gentamicin, amikacin, ciprofloxacin, ceftazidime, cefuroxime, co-trimoxazole, imipenem.

Procedure

Two to three well isolated colonies were emulsified in sterile test tube and incubated at 37 ^{0}C for 2-4 hours. The inoculum was matched with Mc Farland 0.5 standard for turbidity and a lawn culture was made in a Mueller Hinton agar plate using a sterile cotton swab after dipping into the inoculum and removing the excess amount by squeezing on to the walls of the test tube. Six antibiotic discs were placed in a 90 mm plate. The plates were incubated at 30 ^{0}C for 18-24 hours.

Interpretation

Measurement of zone diameters

After overnight incubation, zone diameters were measured using caliperor scale. The zone of the complete growth inhibition around each of the discs was measured to within the nearest millimeters. The diameter of the disc was included in the measurement. An interpretative correlation (Sensitive, intermediate or resistant) was done by using reference chart.

Table-4: Duration of diabetes mellitus and duration of diabetic Foot ulcer in the patients

Duration of diabetes mellitus	Duration of foot ulcers		
	< 1 Month	> 1 Month	Total
< 5 Years	2	10	12
5-10 Years	5	30	35
>10 Years	3	50	53
Total	10	90	100

12 Patient had diabetes mellitus for < 5 yrs. 35patients had diabetes mellitus for 5-10 yrs and 53 patients had diabetes mellitus for > 10 yrs. Majority 90 of the patients had ulcer duration > 1 month.

Table-5: Grading of diabetic foot ulcers in patients

Wagner' grading	Number of cases (%)
1	17
2	31
3	40
4	12
5	0
Total	100

Grade 0-No open lesion, only cellulitis (excluded from the present study.),Grade 1- Superficial ulcer, Grade2 - Deep ulcer without involvement of bone, Grade 3- Deep ulcer with abscess formation and involvement of bone, Grade 4- Local gangrene. Grade 5- Gangrene of entire foot. Almost three fourth of the patient with diabetic foot ulcer under this study belonged to grades 2 and 3.

Table-6: Grading of the diabetic foot ulcers and pattern of flora isolated

Wagner's grading	Total No: cases	Culture positive	Culture negative	Monomicrobial flora n (%)	Polymicrobial flora n (%)
1	17	11	6	10	1
2	31	31	-	9	22
3	40	40	-	2	38
4	12	12	-	1	11
5	0	0	-	0	0
Total	100	94	6	22	72

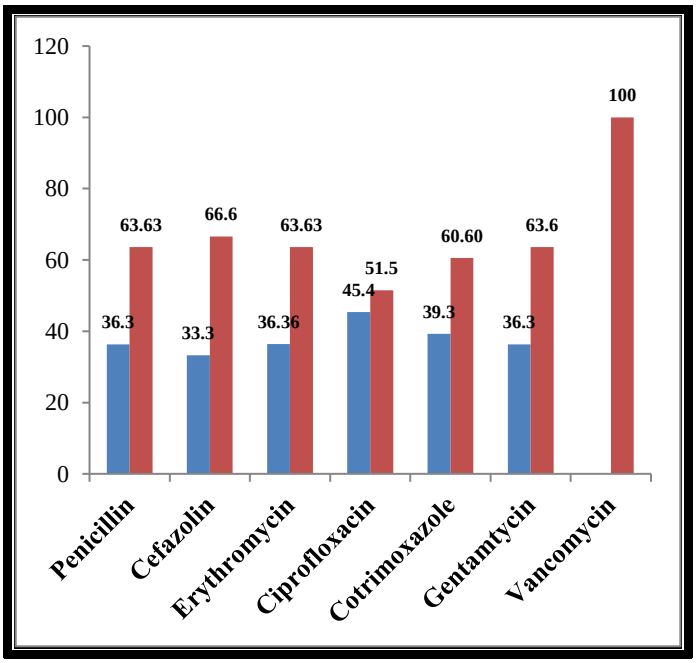
There were 6 culture negative specimens in grade 1. 22 of the patients had monomicrobial flora and 72 of the patients had polymicrobial flora. As the grade increased the percentage of the isolation of the polymicrobial flora also increased.

The most common isolates in the present study were *Pseudomonas spp.* and *Staph. aureus* (table 1).

Vancomycin was found to be the most sensitive antimicrobial agent followed by gentamycin and erythromycin Co-trimoxazole (Figure 1).

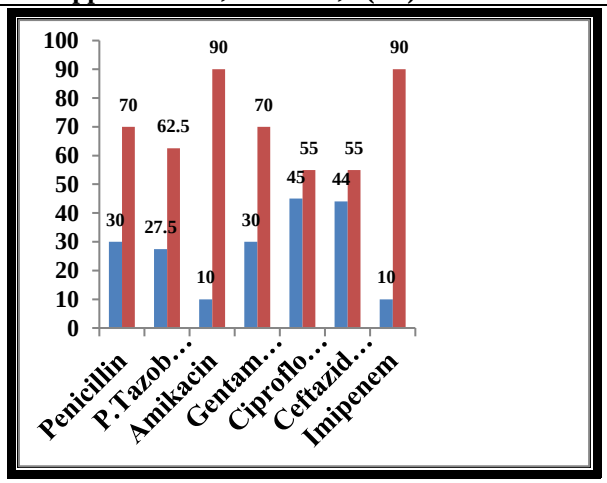
Table-7: Distribution of organisms form diabetic foot infections

Organisms	Number (%)
Gram Positive Organisms	
Staphylococcus aureus	33
Coagulase negative Staphylococci	23
Enterococci pp.	17
Gram negative organisms	
Pseudomonas spp.	40
Escherichia coli	18
Klebsiella spp.	16
Citrobacter spp.	13
Proteus spp.	10
Total	170



Blue: Sensitive Red: Resistant

Fig-1: Invitro Antimicrobial Susceptibility Pattern of *Staphylococcus aureus*

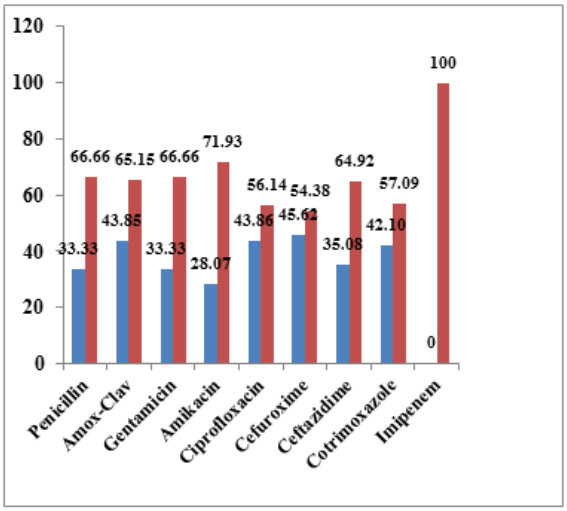


Blue: Sensitive Red: Resistant

Fig-2: Invitro Antimicrobial Susceptibility Pattern of *Pseudomonas aeruginosa*

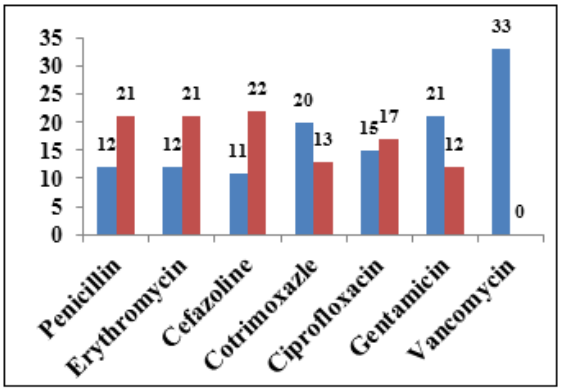
Imipenem and Amikacin found to be the most sensitive antimicrobial agent followed by gentamycin, and piperacillin. Tazobactam.

Imipenem found to be the most sensitive antimicrobial agent followed by amikacin, ceftazidime, gentamycin, Cotrimoxazole (Figure 3).



Blue: Sensitive Red: Resistant

Fig-3: Invitro Antimicrobial Susceptibility Pattern of Gram Negative Bacteria other than *Pseudomonas aeruginosa*



Blue: Sensitive Red: Resistant

Fig-4: Invitro antimicrobial susceptibility pattern of Gram positive bacteria

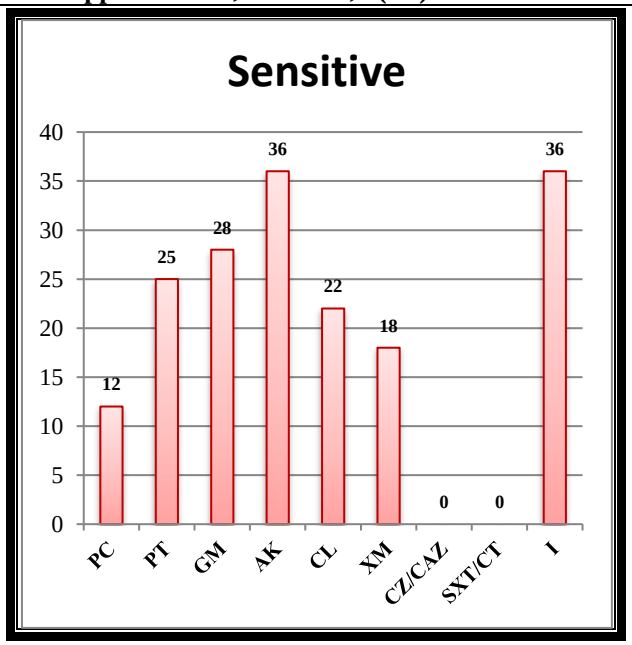


Fig-5: Invitro antimicrobial sensitivity pattern of Gram negative bacteria

CONCLUSION

- Diabetic foot infections are polymicrobial in nature.
- There is a predominance of Gram negative organisms.
- The common isolates in the present study were *Pseudomonas aeruginosa* and *Staph. aureus*.
- There is a rising prevalence of multidrug resistant bacteria isolated form diabetic foot infections.
- Gram positive bacteria are found to be most sensitive to vancomycin followed by gentamicin. Gram negative bacteria are found to be most sensitive to imipenem amikacin and gentamicin.
- A combination regimen consisting of amikacin or imipenem and vancomycin seems to be an effective combination for empirical treatment of diabetic foot infections.

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