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Aerobic Bacterial Isolates from Diabetic Foot Ulcers and Their Antibiotic Susceptibility Pattern in A Tertiary Care Hospital

Sangeetha C. Patil[1], Sumathi S[2], Surekha Y Asangi[3], Krishna S.[4]

Author Affiliation: [1]Assistant Professor [2]Associate Professor, Department of Microbiology, Koppal Institute of Medical Science (KIMS), Koppal, Karnataka 583231, India. [3]Professor [4]Professor & Head, Department of Microbiology, Vijayanagara Institute of Medical Sciences, Ballari, Karnataka 583104, India.

Corresponding Author: **Sumathi S,** Associate Professor, Department of Microbiology, Koppal Institute of Medical Science (KIMS), Koppal, Karnataka 583231, India.

E-mail: drsumathis@yahoo.co.in

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Abstract

Background: Diabetes mellitus is a metabolic disorder in which there is increase in the levels of blood glucose because of insulin deficiency. Diabetic foot ulceration and infections are major medical, social, economical problem and it is the leading cause of morbidity and mortality in developing countries like India. The present study is an attempt made to know the aerobic bacteriological profile of diabetic foot ulcers.

Material and methods: A total of 100 patients with diabetic ulcers admitted in surgical wards were studied. Pus was collected using two swabs from each patient one for staining and the other for aerobic culture. The organisms isolated were identified using standard techniques. Antimicrobial susceptibility of the bacterial isolates was done by Kirby-Bauer disc diffusion method.

Result: Polymicrobial etiology was observed in 59% and monomicrobial etiology in 41%. A total of 165 organisms were isolated. Most common isolates were *staphylococcus aureus* 38 (23.03%), followed by *Klebsiella spp* 34 (20.6%), *Pseudomonas aeruginos*a 28 (16.96%), *Escherichia.coli* 26 (15.75%), *Proteus spp* 23 (13.93%), *Enterococcus faecalis* 8 (4.84%), *Citrobacter spp* 4 (2.42%) and *Staphylococcus epidermidis* 4(2.42%). Most sensitive antibiotics were Imipenem, amikacin, ciprofloxacin and Gentamicin.

Conclusion: Culture and sensitivity from the wound plays an important role in prescribing the appropriate antibiotic at the time of admission itself rather than starting empirical treatment. Thus proper antibiotics policy and measures to restrict the indiscriminate use of antibiotics should be taken to minimize the emergence of drug resistant pathogens.

Keywords: Diabetic Foot Ulcers; *Staphylococcus aureus; Klebsiellaspp; Citrobacter Spp*; Polymicrobial etiology.

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Introduction

Diabetes mellitus (DM), a very common endocrine disorder with major public health consequences arising from severe damage to numerous end organs. DM affects all populations worldwide and the prevalence of this disease is increasing at a very alarming rate. The International Diabetes Federation (IDF) currently estimates that about 366 million persons in the world have DM, with projections that this will increase to 552 million by 2030 [1]. The Indian diabetic population is expected to increase to 57 million by the year 2025 [2]. At present 31.7 million people are diabetic

in India. Hence, it has been labelled as "The diabetic capital of the world". Diabetes warrants a lot of attention because of its various complications like retinopathy, nephropathy, peripheral neuropathy, cardiovascular disease, peripheral vascular disease (PVD), cerebrovascular accident, hypertension and diabetic foot [3].

The diabetic foot may be defined as a group of syndromes in which neuropathy, ischemia , and infection lead to tissue break down resulting in morbidity and possible amputation [4]. About 15-25% will develop a diabetic foot ulceration (DFU) during their lifetime, Over 50% of these ulcerations will become infected. Re admission rates for DFI patients are approximately 40% and nearly one in six patients die within 1 year of their infection. The presence of infection in a patient with DFU increases the risk of a minor amputation by 50% compared to patients with ulcers which are not infected [5]. The diabetic wounds are mostly infected by pus forming microorganisms like *Enterococcius spp, Staphylococcus aureus, Pseudomonas aeruginosa, E.coli, Klebsiella spp, Proteus spp* [6].

Aims and Objectives

1. To isolate the pathogenic organisms from diabetic foot ulcers.
2. To determine the antibiotic susceptibility pattern of isolated organisms.

Materials and Methods

The present study was conducted in the Department of Microbiology, Vijayanagar Institute of Medical Sciences, Bellary for duration of one year. A total of one hundred patients with diabetic ulcers admitted in surgical wards were studied.

A proforma was filled for each patient documenting such as age, sex, address and clinical information including chief complaints, duration of symptoms, predisposing factors and any previous history of treatment.

Collection of Sample

Samples were collected in the surgical wards where the dressing was being done. The ulcer was cleaned with sterile normal saline and the surrounding area was cleaned with 70% alcohol. Debris, dead and devitalized tissue overlying the ulcer was removed using a sterile forceps and scissors. Swabs were collected from the depth of the ulcers on the feet of the diabetic patients. From each patient, two swabs were collected. One swab was used for the isolation of aerobic bacteria and the other for preparation of smear for Gram stain. [7]. Debrided necrotic material was also collected [8]. After sample collection, the specimens were processed immediately in the laboratory.

Processing of Sample

Direct microscopic examination: Smear was prepared on clean glass slide, air dried. Gram stain was done for the smear and examined under oil immersion objective for the presence of pus cells, bacteria and fungi, low power and the high power objectives for fungi [9].

Culture

Aerobic culture: The swabs were inoculated on nutrient agar, blood agar and MacConkey agar. All plates were incubated aerobically at 37^0C and evaluated at 24 hours, 48 hours and 72 hours. The organisms isolated were identified using standard techniques, based on the colony morphology, Gram staining of smear from colony and biochemical properties.

Antimicrobial susceptibility of the bacterial isolates to the commonly used antibiotics was done by Kirby-Bauer disc diffusion method [9,10].

The strength of the antibiotics discs used were [11].

Ampicillin 10 µg

Amoxicillin/Clavulanic acid

Augmentin 20 µg/10 µg

Amikacin 30 µg

Gentamicin 10 µg

Ciprofloxacin 5 µg

Ceftriaxone 30 µg

Cefotaxime 30 µg

Imipenem 10 µg

Microbial agents of diabetic foot ulcers

Most of the diabetic foot infections are polymicrobial in nature and mixed organisms are frequently encountered. Spectrum of microorganisms depends mainly on microbial flora of the lower limb, metabolic factors, food hygiene, and the use of antibiotics[2].

Results

Table 1: Age and sex distribution

Age group	Male		female		Total	
	No	%	No	%	No	%
21-30	2	3.08	0	0	2	2
31-40	1	1.54	2	5.72	3	3
41-50	14	21.53	13	37.14	27	27
51-60	27	41.54	10	28.57	37	37
>60	21	32.31	10	28.57	31	31
Total	65	100	35	100	100	100

Out of 100 cases, 65 were males and 35 were females. Among 100 cases, 37 (37%) were of age group 51-60 years, out of 37, 27 (41.54%) were males and 10 (28.57%) were females. 31 (31%) cases were of age group 61 and above. Out of 31, 21 (32.3%) were males and 10 (28.57%) were females. 27 (27%) cases were of age group 41-50 years, 3 (3%) cases were of between 31-40 years, 2 (2%) cases were of age group of 21- 30 years (Table 1).

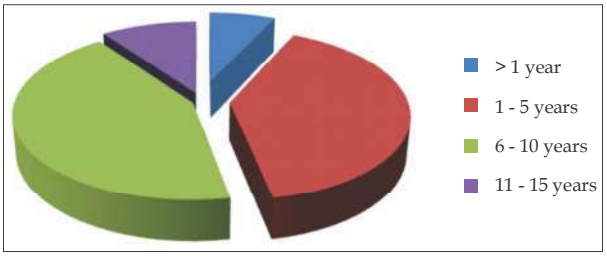

Fig 1: Showing the duration of diabetes mellitus

Above Fig. 1 shows that out of 100 cases. 43 (43%) had diabetes for 6-10 years. 40 (40%) had diabetes for 1-5 years. 10 (10%) had diabetes mellitus for 11-15 years and 7 (7%) were detected diabetic at the time of admission for the treatment of ulcer.

It is observed that most of the patients were suffering from diabetes for more than five years.

Type of Diabetes Mellitus

Out of 100 cases, 2 (2%) cases were insulin dependent diabetes mellitus and 98 (98%) cases were non insulin dependent diabetes mellitus.

Table 2: Duration of diabetic ulcer

Duration in weeks	Number of cases	Percentage
<1 week	5	5
2-4	40	40
5-7	15	15
8-10	30	30
>11 weeks	12	12
Total	100	100

Majority of the patients 40 (40%) were presented with ulcer of 2-4 weeks duration followed by 30 (30%) patients presented with ulcer of 8 -10 weeks duration, 15 (15%) patients presented with ulcer of 5- 7 weeks duration. 12 (12%) patients presented with ulcer of more than 11 weeks and 5(5%) patients had ulcer of less than 1 week (Table 2).

It is observed that, most of the patients presented with ulcer of more than two weeks duration

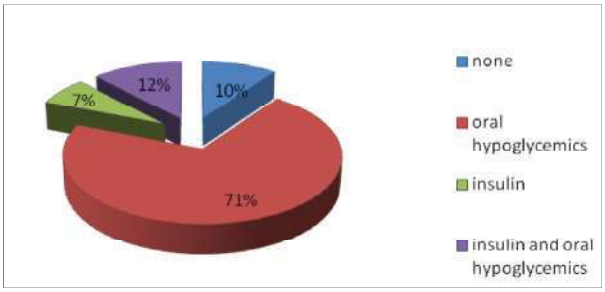

Fig. 2: Showing treatment taken for diabetes mellitus

The above Fig. 2 shows that , prior to admission, 71 (71%) patients were maintained on oral hypoglycaemic agents , 12 (12%) patients were both on insulin and oral hypoglycaemics, 7 (7%) were on insulin therapy and 10 patients were not previously diagnosed as diabetics.

Table 3: showing the different aerobic organisms isolated

Type of organism	Number of organisms	Percentage
Gram positive organisms	50	30.3
Staphylococcus aureus (38)	38	23.03
Staphylococcus epidermidis (4)	4	2.42
Enterococcus faecalis (8)	8	4.84
Gram negative organisms	115	69.6
Pseudomonas aeruginosa (28)	28	16.96
Klebsiella pneumoniae (32)		
Klebsiella oxytoca (2)	34	20.6
E.coli(26)	26	15.75
Proteus mirabilis (21)		
Proteus vulgaris (2)	23	13.93
Citrobacter freundii (3)		
Citrobacter koseri (1)	4	2.42
Total	165	100

Out of 165 organisms isolated, most common isolates were staphylococcus aureus 38 (23.03%), followed by Klebsiella spp 34 (20.6%), Pseudomonas aeruginosa 28 (16.96%), Escherichia. coli 26 (15.75%), Proteus spp 23 (13.93%), Enterococcus fecalis 8 (4.84%), Citrobacter spp 4 (2.42%) and Stapylococcus epidermidis 4 (2.42%) (Table 3).

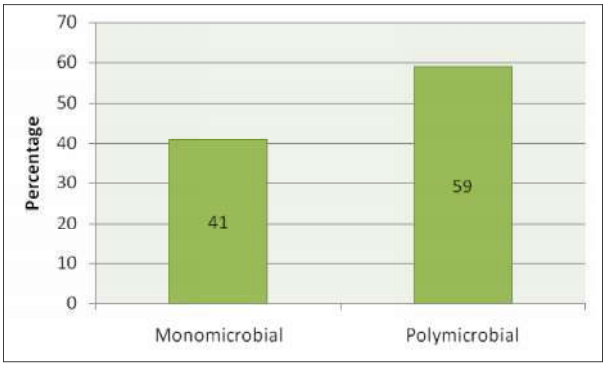

Fig. 3: Showing the distribution of organisms

The above Fig. 3 shows that monomicrobial flora isolated in 41% of cases and polymicrobial flora isolated in 59% of cases.

Table 4: Distribution of organisms in polymicrobial flora

Type of organism	Number of cases	Percentage
Gram positive organisms	2	3.38
Gram negative organisms	24	40.67
Gram positive and Gram negative organisms	26	44.06
≥ 3 organisms	7	11.86
Total	59	100

Majority of the organisms isolated Gram positive and negative oranisms from (44.06%) cases followed by Gram negative organisms in 40.67%, three organisms isolated in 11.86% of cases and Gram positive organisms in 3.38% of cases (Table 4).

to ciprofloxacin, 56 (33.9%) were sensitive to Gentamicin, 55 (33.3%) were sensitive to ceftriaxone, 48 (29%) were sensitive to cephalexin, 43 (26%) were sensitive to amoxycillin and clavulanate, 40 (24.2%) were sensitive to cefotaxime.

From the above antibiogram most sensitive antibiotics were Imipenem, amikacin, ciprofloxacin and Gentamicin.

Discussion

Patients with DM frequently require minor or major amputations of the lower limbs (15–27%), and in more than 50% of cases, infection is the preponderant factor. These more severe diabetic foot infections usually require hospitalization, parenteral antibiotic therapy and surgical procedures [12].

Most of the cases if identified early and treated appropriately initially in the community can be treated effectively with antibiotics at an early stage and in an out-patient setting. But unfortunately because of the late referrals primarily and also alternate medicines, herbal medicines, and poor medical facilities in the far flung and tribal areas, less knowledge regarding diabetes in general and foot ulcers in particular leads to loss of limbs and loss of life in some cases even when they reach a tertiary care hospital [13].

Table 5: Antibiotic susceptibility pattern of isolates

Antibiotics	*Staphylococcus aureus* (n=38)	*Staphylococcus epidermidis* (n=4)	*Enterococcus spp* (n=8)	*Pseudomonas spp* (n=28)	*Klebsiella spp*(n=34)	*E. coli* (n=26)	*Proteus spp* (n =23)	*Citrobacter spp* (n=4)	Total n=165
Amikacin	17 (44.7)	1 (25)	3 (37.4)	14 (50)	19 (55.8)	14 (53.8)	15 (65)	3 (42.9)	86 (52.1)
Amoxycillin and clavulanate	15 (39.4)	3 (75)	5 (62.5)	0 (0)	6 (17.6)	7 (26.9)	7(30.4)	0(0)	43 (26)
Gentamicin	10 (26.3)	1 (25)	2 (25)	9 (32.1)	13 (38.2)	11 (42.3)	8 (38)	1 (33.3)	56 (33.9)
Ciprofloxacin	13 (34.2)	3 (75)	5 (62.5)	11 (39.2)	21 (61.7)	18 (69.2)	11 (52.4)	3 (42.9)	85 (51.5)
Ceftriaxone	12 (31.5)	2 (50)	4 (50)	3 (10.7)	12 (35.2)	12 (46.1)	9 (39.1)	1 (25.3)	55 (33.3)
Cefotaxime	6 (15.7)	2 (50)	1(12.5)	1 (3.5)	9 (26.4)	12 (46.1)	8 (38)	1 (25.3)	40 (24.2)
Imipenem	34 (89.4)	3 (75)	6 (75)	25 (89.2)	30 (88.23)	24 (92)	21 (87)	4 (100)	147 (89)
Cephalexin	11 (28.9)	2 (50)	2 (25)	1 (3.57)	21 (61.7)	4 (15.3)	7 (33.3)	1 (25.3)	48 (29)

The above table 5 shows the antibiotic susceptibility pattern of aerobic organisms isolated in the study. Out of 165 organisms isolated 147 (89%) were sensitive to imipenem, 86 (52.1%) were sensitive to amikacin, 85 (51.5%) were sensitive

Duration of diabetes mellitus

In the present study 53 (53%) of cases were suffering from diabetes for more than 5 years. This finding is in concordance with study done by Sapico et al. [14] 8 (61.54%) were suffering from

diabetes for more than 5 years, and Leela Rani K et al. [7] reported 53.6% of cases were suffering from diabetes mellitus for more than 6 years.

IDDM/NIDDM

In the present study out of 100 cases, 98% were non- insulin dependent diabetes mellitus. This correlated with study done by Ramani et al. (81.34%) [15], Chincholikar et al. (76.19%) [16], Ravishekhar Gadepalli et al. (88.8%) [17], Azizul Hasan et al. (92%) [13], with the predominance of type 2 diabetes mellitus.

Comparison of monomicrobial and polymicrobial flora

In the present study monomicrobial etiology found in 41% of cases and polymicrobial etiology in 59%. Similar findings were observed in study done by Chincholikar et al. [16] for monomincrobial (30.5%) and polymicrobial (69.5%), Kavitha A et al. [3] for monomincrobial (25%) and polymicrobial (85%), Leela Rani et al. [7] for monomincrobial (36%) and polymicrobial (56%), Dushyanth singh et al. [18] for monomincrobial (14.75%) and polymicrobial (85.24%).

Comparison of aerobic organisms isolated

In the present study 165 aerobic organisms isolated. The most predominant organisms isolated were *staphylococcu aureus* 38 (23.03%), followed by *Klebsiella* spp 34 (20.6%), *Pseudomonas aeruginosa* 28 (16.96%), *E.coli* 26 (15.75%), *Proteus* spp 23 (13.93%), *Enterococcus fecalis* 8 (4.84%), *Citrobacter* spp 4 (2.42%) and *Staph epidermidis* 4 (2.42%).

Sapico et al. [14] reported, most predominant organisms isolated were *Proteus spp* (13.3%) followed by *Staphylococcus aureus* (10%), *E.coli* (10%), *Enterobacter spp* (9.9%), *Enterococcus spp* (5%) and each 3.3% by *Staphylococcus epidermidis*, *Streptococcus spp*, *Pseudomonas spp*, *Providendia spp*, *Citrobacter spp*.

Ramani et al. [15] reported, most predominant organisms isolated were *Staphylococcus aureus* (60%) followed by *Klebsiella spp* (20%), *Proteus spp* (19%), *Pseudomonas spp* (19%), *Enterococcus spp* (11%), *Citrobacter spp* (9.2%), *Staphylococcus epidermidis* (4.9%), *E.coli* (2.4%), and each 3.3% by *Streptococcus spp (1.8%) and Enterobacter spp (1.8%).*

Rovan urbancic et al. [18] reported, most predominant organisms isolated as *Staphylococcus aureus* (26.7%), *Staph epidermidis* (9.9%), *Enterococcus spp* (9.5%), *Klebsiella spp* (5.4%), *Enterobacter spp* (3.6%), and *E.Coli* (3.2%).

Ahamed T [19] reported, most predominant organisms isolated were *Staphylococcus aureus* (28%) followed by *Pseudomonas spp* (22%), *Proteus spp* (18%), *Streptococcus spp* (13%), *Enterococcus spp* (11%), *Staphylococcus epidermidis* (7%), *Klebsiella spp* (6%), *E.coli* (6%), *Enterobacter spp* (5.5%), *Morganella spp* (5.5%)

Ravishekhar et al. [17] reported, most predominant organisms isolated were *Pseudomonas spp* (18%), *Staphylococcus aureus* (13.7%), *Proteus spp* (12.6%), *Staphylococcus epidermidis* (12%), *E.coli* (12%), and *Enterococcus spp* (11.5%)

Dushyant singh et al. [18] reported, most predominant organisms isolated were *Enterococcus spp* (57.6%) followed by *Staphylococcus aureus* (28.8%), *Streptococcus spp* (21.1%) and *Pseudomonas spp* (15.3%).

Ozer b et al. [19] reported, most predominant organisms isolated were *E.coli* (36.5%) followed by *Pseudomonas spp* (18.9%), *Enterococcus spp* (14.9%), *Staph aureus* (10.8%), *Streptococcus spp* (6.8%), and *Staph epidermidis* (5.4%).

JJ mendes et al. [20] reported, most predominant organisms isolated as *Staph aureus* (51%) followed by *Pseudomonas spp* (12.2 %) and *Acinetobacter spp* (8.2%).

Banashankari et al. [21] reported, most predominant organisms isolated were *Pseudomonas spp* (32%) followed by *Staphylococcus aureus* (19%), *Proteus spp* (18%), *E.coli* (16%), *Staphylococcus epidermidis* (13%) and *Enterococcus spp* (9%)

Antibiotic Sensitivity

Ramani et al. [15]. reported most of the isolates were sensitive to Gentamicin (52.05%), Chloramphenicol (48.43%), Kanamycin (43.49%), Erythromycin (35.8%) and Cephalexin (34.53%) and all the anaerobes were sensitive to Metronidazole.

Grayson ML et al. [22] .compared the efficacy of Imipenem/Cilastatin and Ampicillin/Sulbactum in the treatment of limb threatening foot infection in diabetic patients, found 81% vs. 85% efficacy respectively. Rovan Urbancic V et al. [18]. reported most of the organisms were susceptible to Amoxicillin/Clavulanate (87.9%) and Ciprofloxacin (89.0%).

Chincholikar Dipali A et al. [16] reported that most of the strains were sensitive to cephalosporins and ciprofloxacin. Banashankari et al. [21] reported Enterobacteriaceae group and *P.aeruginosa* strains

were largely susceptible to imipenem (100%), piperacillin-tazobactam, ceftazidime, aminoglycosides, and ciprofloxacin. More than 70% of staphylococcus aureus sensitive to methicillin. Cefoperazone + sulbactum showed about 67% sensitivity, while ciprofloxacin and amikacin were only 23% and 44% sensitive.

In the present study most of the strains were sensitive to imipenem 89% followed by amikacin, ciprofloxacin and Gentamicin.

Conclusion

Diabetic foot ulcers not only cause hospitalization, but also affect the patient economically and may lead to increased mortality. Culture and sensitivity from the wound plays an important role in prescribing the appropriate antibiotic at the time of admission itself rather than starting empirical treatment. The lack of multi-disciplinary approach in the treatment of diabetic foot is quite obvious and there is a lot of scope of improvement in the form of holistic approach to a patient with diabetic foot rather than just treating the foot. Infection control programme and policies should be vigorously pursued in our health care facilities as well as antibiotic prescription regulation to cope with the upsurge of *resistance* to various antibiotics. Thus proper antibiotics policy and measures to restrict the indiscriminate use of antibiotics should be taken to minimize the emergence of drug resistant pathogen, whose spread would leave no option to treat gram negative infections.

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Permissions: Nil

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