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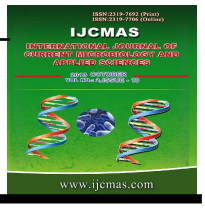

Original Research Article

Prevalence of Bacteria Isolated from Type 2 Diabetic Foot Ulcers and the Antibiotic Susceptibility Pattern

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A B S T R A C T

Keywords

Type 2 diabetes mellitus; diabetic foot infections; antibiotic susceptibility.

Diabetes mellitus is a metabolic disorder which in particular affects the metabolism of carbohydrates. Foot ulcers are a very common complication of Type 1 and Type 2 diabetes. The amputation and gangrene rates of diabetic foot infections are also increasing rapidly. Patients having diabetic foot infections face serious problems including amputations. The aim of this study was to determine the microbiology of diabetic foot infections and to assess the antibiotic susceptibility. In this study 530 patients' foot ulcer samples (who have Type 2 diabetes) were analyzed microbiologically. Total of 16 different genera of aerobic bacteria (520 isolates) were obtained. The antibiotic susceptibility using 26 antibiotics was performed and the results were tabulated.

Introduction

Foot ulcers are much feared complications of diabetes, with recent studies suggesting that lifetime risk of developing a foot ulcer in diabetic patient may be as high as 25% (Singh *et al*., 2005). Fifteen per cent of people with diabetes will develop a foot ulcer at some time during their life, and 85% of major leg amputations begin with a foot ulcer (Ramani *et al*., 1991). Infection is most often a consequence of foot ulceration, which typically follows trauma to a neuropathic foot (Rao and Lipsky, 2007). Severe infections in the foot may lead to leg amputations. In addition foot complications now account for the most frequent reason for hospitalization in diabetic patients (Alavi *et al*., 2007). Gram-negative bacteria, Gram-positive bacteria and few fungal species are reported as the common microbes present in diabetic foot infections.

Multidrug resistant Gram-negative bacteria (MDRGNB) are a major therapeutic challenge both in hospital and

community settings (Shakil *et al*., 2008). The increasing association of multi-drug resistant (MDR) pathogens with diabetic foot ulcers further compounds the challenge faced by the physician or the surgeon in treating diabetic ulcers without resorting to amputation (Yoga *et al*., 2006). Hence, usage of appropriate antibiotics is needed to avoid the risk of severity in foot infections of diabetic patients. But the presence of drug-resistant bacteria makes the antibiotic therapy more difficult.

Diabetic foot infections

Diabetic foot infections are sores on the feet that occur in 15% of diabetic patients some time during their lifetime. The risk of lower-extremity amputation is increased 8-fold in these patients once an ulcer develops. Foot disorders such as ulceration, infection, and gangrene are the leading causes of hospitalization in patients with diabetes mellitus (Boulton, 2002). Foot ulcers are a significant complication of diabetes mellitus and often precede lower-extremity amputation. The most frequent underlying etiologies are neuropathy, trauma, deformity, high plantar pressures, and peripheral arterial disease. Thorough and systematic evaluation and categorization of foot ulcers help to guide appropriate treatment (Frykberg *et al*., 2002).

Prevalence

Foot ulceration is common, affecting up to 25% of patients with diabetes during their lifetime (Singh *et al*., 2005). Over 85% of lower limb amputations are preceded by foot ulcers and diabetes remains a major cause of non-traumatic amputation across the world with rates being as much as 15 times higher than in the non-diabetic population. An ankle is lost to diabetes somewhere in the world every 30 s, a more important fact is that up to 85% of all amputations in diabetes should be preventable (Boulton *et al*., 2005). In India prevalence of foot ulcers in diabetic patients in clinic population is 3%, which is much lower than reported in the western world. A possible reasoning for the low prevalence in Indians is younger age and shorter duration of diabetes (Pendsey, 1994; International consensus on the Diabetic Foot, 1999).

Causes

Foot problems are common in people with diabetes because of their increased risk of peripheral neuropathy, peripheral vascular disease abnormal pressure on the foot, and impaired resistance to infection. These factors frequently combine and result in ulceration and infection, progression to gangrene, and subsequent lower limb amputation. In diabetic patients, multiple factors may exist that increase the risk of ulceration. A diabetic foot infection is most simply defined as any inframalleolar infection in a person with diabetes mellitus. These include paronychia, cellulitis, myositis, abscesses, necrotizing fasciitis, septic arthritis, tendonitis, and osteomyelitis. Once the protective layer of skin is breached, underlying tissues are exposed to bacterial colonization (Lipsky *et al*., 2004).

Infections of diabetic foot ulcers

Infection is a common and serious complication of diabetic foot wounds. Infection leads to formation of microthrombi, causing further ischemia, necrosis, and progressive gangrene. Massive infection is the most common factor leading to amputation. Local trauma

and/or pressure (often in association with lack of sensation because of neuropathy), in addition to microvascular disease, may result in various diabetic foot infections that run the spectrum from simple, superficial cellulitis to acute and chronic osteomyelitis and deep-skin and soft-tissue infections.

Microbiology

Anaerobic bacteria are almost always isolated with aerobes from diabetic foot infections (Goldstein *et al*., 1996; Ramani *et al*; 1991). Aerobic gram-positive cocci are the predominant microorganisms that colonize and acutely infect breaks in the skin. *S. aureus* and the beta-hemolytic streptococci (groups A, C, and G, but especially group B): (El-Tahawy, 2000 and Urbancic-Rovan and Gubina, 2009). Enterococci, Enterobacteriaceae, obligate anaerobes, *Pseudomonas aeruginosa*, nonfermentative gram-negative rods: (Pathare *et al.,* 1998). Antibiotic-resistant organisms: (e.g., MRSA or vancomycin-resistant enterococci) (Hartemann-Heurtier, 2004). Coagulase negative staphylococci and *Corynebacterium* species (diphtheroids) (Hunt, 1992). Sometimes, initial management comprises: multidrug resistant Gram-negative bacteria (MDRGNB) are a major therapeutic challenge both in hospital and community settings (Shakil *et al.,* 2008). The pathogenic role of each isolate in a polymicrobial infection is often unclear. The high prevalence of anaerobic bacteria in the foot ulcers of diabetic patients was first documented by Louie and colleagues in 1976 (Louie *et al.,* 1976).

Prevention

The detection of neuropathy before it gets severe is the best method to prevent diabetic foot infections. These are some of the ways to prevent diabetic foot infections:

General care: avoid smoking, alcohol and high glucose levels. Wearing diabetic socks to help in preventing moisture, pressure and blistering. Good diabetes control. If any ulcer is present, special care should be taken. Yearly once visit of physician. Keeping the ulcer cleaned and bandaged. Cleaning the wound using a wound dressing or bandage. Avoiding barefoot walking. Avoiding foot problems like hammer toes, corns, calluses, sores, blisters, cuts, redness, bunions, warts, dry-cracked skin, athlete's foot etc. General cleanliness of feet. A single cut or ulcer should be observed and treated in the earlier stages.

Treatment

Not all ulcers are infected; however, if an infection is found, a treatment program of antibiotics, wound care, and possibly hospitalization will be necessary. Some of the ways are:

Prevention of infection

Taking the pressure off the area, called "off-loading". Removing dead skin and tissue, called "debridement". Applying medication or dressings to the ulcer. Managing blood glucose and other health problems

Materials and Methods

Isolation of bacteria from diabetic foot ulcers

The Foot ulcer samples were collected from patients who had Type 2 diabetes and subjected to microbiological analyses. Sample collection (pus, wound exudates) had been undertaken in medical wards, after the wounds are washed vigorously with normal saline solution. Discharge

from margins and edges of ulcer was collected with help of two sterile swabs, one for gram stain and one for culture before antiseptic dressing was applied. Then swabs were immediately transported to the laboratory for culture. A total of 530 samples were collected from 530 patients. Out of 530, 410 (77.4%) were males and 120 (22.6%) were females. The female: male ratio in this study was 1: 3.41. The age range was 40–90. Then using various differential and selective media, the samples were cultured aerobically and the aerobic bacteria were isolated.

Antibiotic susceptibility test using disc diffusion method

The materials used are: Muller Hinton agar medium, Petri dishes, conical flasks, inoculation loops, antibiotic discs, forceps, sterile swabs, overnight microbial cultures etc. Muller Hinton agar was prepared and sterilized. 25 ml of MHA was aseptically poured into Petri dishes and allowed to solidify. Sterilized swabs were dipped in overnight cultures and spread evenly over the medium. The antibiotic discs were placed over the medium in each of the plates containing the swabbed cultures and incubated overnight at 37°C. After 24 hours of incubation the diameter of the zones were measured and recorded. Then the screened isolates were subjected for microbial and biochemical characterization.

In this study 26 antibiotics were used. Each belongs to different groups. Some of the standard antibiotics are: Amikacin: 10 µg, Ampicillin: 10 µg, Cefotoxime: 30µg, Ceftazidime: 30µg, Cefazolin: 30µg, Ceftriaxone: 30 µg, Ciprofloxacin: 30 µg, Gentamycin: 10 µg, Imipenem: 10 µg, Linezolid: 10 µg, Netillin: 10 µg, Ofloxacin: 2 µg, Oxacillin: 5 µg, Penicillin G: 2 µg, Vancomycin: 30 µg etc.

Results and Discussion

A total of 530 patients' foot ulcer samples (who have Type 2 diabetes) were analyzed. Out of 530, 410 (77.4%) were males and 120 (22.6%) were females, ratio was 1: 3.41. The age range was 40–90. From these 530 patients 385 (72.6) isolates were isolated. 145 (27.4%) patients had no bacterial infection. Out of 385 isolates, 65 (16.9%) isolates showed two organisms, others (320, 83.1%) one. Total isolated bacteria were 450. The results of the bacteria isolated are tabulated in Table 1. The statistical study of the isolation of bacterial pathogens according to the age of diabetic patients in the present study is tabulated in Table 2. Gram-positive bacteria comprised of 40.4% and Gram-negative bacteria 59.6%. The antibiotic susceptibility tests describe the sensitivity patterns of all the 16 bacteria (Tables 3–6). All the Gram-positive bacteria showed good sensitivity to most of the antibiotics. *Enterococcus faecalis* showed lesser sensitivity for the antibiotics.

MRSA also shows lesser sensitivity. It showed good sensitivity for amikacin, amoxicillin, chloromycetin, chloramphenicol, levofloxacin and Penicillin. The Gram negative bacteria showed good activity against amikacin, cephalexin, amoxicillin, gentamycin, ofloxacin, piperacillin-tazobactum, ticarcillin-clavulanic acid combinations. *Enterobacter* Spp., *E. coli* and *M. morganii* showed 100% resistance to Amoxicillin. *P. mirabilis*, *P. flourescens*, *M. morganii* and *Enterobacter* Spp. Showed 100% resistance towards augmentin.

Table.1 Isolation of bacteria from diabetic foot infections

S. No	Bacteria	*n* (%)
1	*Staphylococcus aureus*	97 (21.6)
2	*Pseudomonas aeruginosa*	63 (15.1)
3	*Escherichia coli*	68 (14)
4	MRSA	42 (9.3)
5	*Klebsiella pneumoniae*	34 (7.6)
6	*Proteus mirabilis*	31 (7)
7	*Enterococcus faecalis*	29 (6.4)
8	*Enterobacter* Spp.	20 (4.4)
9	*Proteus vulgaris*	17 (3.8)
10	NFGNB	15 (3.3)
11	*Beta-haemolytic-Streptococci*	9 (2)
12	*Pseudomonas flourescens*	7 (1.5)
13	*Morganella morganii*	5 (1.1)
14	*Streptococcus pyogenes*	5 (1.1)
15	*Citrobacter freundii*	4 (0.9)
16	*Acinetobacter baumannii*	4 (0.9)

Table.2 Statistical study of the isolation of bacterial pathogens according to the age of diabetic patients in the present study

S. No.	Bacteria (*n*)	AGE (%)				
		40–50	50–60	60–70	70–80	80–90
1.	*Staphylococcus aureus* (97)	22 (22.7)	29 (29.9)	17 (17.6)	18 (18.5)	11 (11.3)
2.	*Pseudomonas aeruginosa* (63)	4 (6.4)	17 (27)	16 (25.4)	20 (31.7)	6 (9.5)
3.	*Escherichia coli* (68)	15 (22.1)	19 (27.9)	20 (29.4)	10 (14.7)	4 (5.9)
4.	MRSA (42)	7 (16.6)	11 (26.2)	13 (30.9)	5 (12)	6 (14.3)
5.	*Klebsiella pneumoniae* (34)	4 (11.8)	13 (38.2)	10 (29.4)	4 (11.8)	3 (8.8)
6.	*Proteus mirabilis* (31)	4 (13)	9 (29)	9 (29)	5 (16)	4 (13)
7.	*Enterococcus faecalis* (29)	7 (24.1)	6 (20.8)	12 (41.4)	3 (10.3)	1 (3.4)
8.	*Enterobacter* Spp. (20)	3 (15)	7 (35)	3 (15)	4 (20)	3 (15)
9.	*P. vulgaris* (17)	1 (5.9)	2 (11.7)	6 (35.3)	8 (47.1)	0
10.	NFGNB (15)	2 (13.3)	4 (26.7)	0	5 (33.3)	4 (26.7)
11.	*Beta-h-Streptococci* (9)	4 (44.4)	0	3 (33.3)	2 (22.2)	0
12.	*Pseudomonas flourescens* (7)	0	4 (57.1)	3 (42.9)	0	0
13.	*Morganella morganii* (5)	1 (20)	2 (40)	2 (40)	0	0
14.	*Streptococcus faecalis* (5)	5 (100)	0	0	0	0
15.	*Citrobacter freundii* (4)	2 (50)	2 (50)	0	0	0
16.	*Acenetobacter baumannii* (4)	0	1 (25)	2 (50)	1 (25)	0

Table.3 Antibiotic susceptibility of Gram-positive bacteria

No.	Gram positive bacteria	Antibiotic sensitivity (%)																			
		AK		AM		AU		CE		CH		CD		CX		CH		CI		CT	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
1	*Staphylococcus aureus*	100	0	100	0	100	0	79	21	100	0	45	55	90	10	100	0	78	12	88	10
2	MRSA	88	12	93	7	87	13	80	20	94	6	†	†	50	50	96	4	68	32	80	20
3	*Enterococcus faecalis*	46	54	91	9	88	12	22	78	70	30	100	0	70	30	96	4	76	24	95	5
4	*Beta*-h-*treptococci*	20	80	100	0	80	20	85	15	80	20	60	40	100	0	80	20	100	0	20	80
5	*Streptococcus pyogenes*	100	0	100	0	100	0	90	10	85	15	77	23	95	5	100	0	100	0	100	0

Table.4 Antibiotic susceptibility of Gram-positive bacteria

No.	Gram positive bacteria	Antibiotic sensitivity (%)																			
		E		G		I		LE		ME		OF		OX		P		TC		TP		V	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
1	*Staphylococcus aureus*	80	20	80	20	100	0	100	0	100	0	89	11	95	5	10	90	100	0	100	0	100	0
2	MRSA	64	36	70	30	†	†	100	0	†	†	66	34	41	59	96	4	91	9	90	10	100	0
3	*Enterococcus faecalis*	58	42	61	39	70	30	100	0	70	30	55	45	90	10	90	10	20	80	95	5	100	0
4	*Beta*-h-*treptococci*	50	50	60	40	80	20	85	15	80	20	60	40	100	0	100	0	100	0	85	15	60	40
5	*Streptococcus*	65	35	70	30	83	17	90	10	85	15	70	30	100	0	100	0	100	0	90	10	70	30

Table.5 Antibiotic susceptibility of Gram-negative bacteria.

No	Bacteria	Antibiotic sensitivity (%)																			
		AK		AM		AU		CH		CD		CE		CO		CT		CI		I	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
1.	*P. aeruginosa*	76	24	81	19	76	24	38	72	81	19	88	12	40	60	20	80	78	22	88	12
2.	*E. coli*	100	0	0	100	0	100	80	20	76	24	88	12	86	14	70	30	79	21	90	10
3.	*Klebsiella* pp.	82	18	91	9	100	0	73	27	46	54	60	40	70	30	†	†	70	30	80	20
4.	*Proteus mirabilis*	100	0	52	48	0	100	61	39	70	30	76	24	77	23	68	32	64	36	100	0
5.	*Enterobacter Spp.*	85	15	0	100	0	100	100	0	85	15	†	†	85	15	10	90	60	40	†	†
6.	*Proteus vulgaris*	100	0	22	78	76	24	88	12	76	24	88	12	88	12	76	24	82	18	100	0
7.	NFGNB	58	42	72	28	72	28	93	7	60	40	†	†	58	42	30	70	51	49	†	†
8.	*Pseudomonas flourescens*	60	40	40	60	0	100	0	100	80	20	70	30	60	40	40	60	60	40	100	0
9.	*M. Morganii*	100	0	0	100	0	100	20	80	40	60	100	0	†	†	85	15	90	10	100	0
10.	*Citrobacter freundii*	100	0	100	0	40	60	60	40	†	†	70	30	†	†	100	0	100	0	100	0
11.	*Acinetobacter baumannii*	100	0	100	0	100	0	45	55	100	0	100	0	40	60	100	0	70	30	100	0

Table.6 Antibiotic susceptibility of Gram-negative bacteria

S.No	Bacteria	Antibiotic sensitivity (%)															
		G		ME		N		OF		P/T		S/C		TC		T/C	
		S	R	S	R	S	R	S	R	S	R	S	R	S	R	S	R
1.	*P. aeruginosa*	26	74	88	12	50	50	36	64	88	12	91	9	72	18	72	18
2.	*E. coli*	58	42	90	10	100	0	45	55	20	80	88	12	60	40	72	18
3.	*Klebsiella* pp.	61	39	80	20	70	30	61	39	20	80	73	27	70	30	84	16
4.	*P. mirabilis*	67	33	100	0	40	60	65	35	100	0	100	0	55	45	89	11
5.	*Enterobacter Spp.*	80	20	†	†	38	72	85	15	70	30	90	10	80	20	†	†
6.	*P. vulgaris*	82	18	100	0	44	56	76	24	100	0	100	0	46	54	87	13
7.	NFGNB	86	14	100	0	†	†	58	42	72	28	37	63	72	28	†	†
8.	*Ps.flourescens*	80	20	60	40	45	55	60	40	80	20	60	40	†	†	0	100
9.	*M. Morganii*	85	15	100	0	†	†	100	0	100	0	100	0	†	†	100	0
10.	*Citrobacter freundii*	100	0	100	0	30	70	100	0	100	0	100	0	100	0	100	0
11.	*Acinetobacter baumannii*	70	30	100	0	45	55	75	25	100	0	100	0	45	55	100	0

Many organisms showed multidrug resistance. This increasing incidence of multidrug resistant organisms is a potential risk factor in management of diabetic foot infections which may lead to devastating complications like systemic toxicity, gangrene formation and amputation of lower extremity (Jain *et al*., 2012). Nowadays combination of drugs (e.g., piperazillin+tazobactum, ticarcillin-clavulanate) shows successful remedies for the treatment of diabetic foot infections.

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