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Clinico-bacteriology and risk factors for the diabetic foot infection with multidrug resistant microorganisms in north India

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Abstract
This study was carried out in diabetic patients with foot ulcer, to determine the bacterial profile of infected ulcer, antibiotic resistance of the isolates and to find out the potential risk factors for infection with multidrug resistance. Gram-negative bacilli were screened for extended spectrum β lactamase (ESBL) production and *Staphylococcus aureus* were screened for methicillin resistance. In the 60 diabetic foot patients, 37(61.6%) were males and 23(38%) were females. 49(81.6%) had T2DM, whereas only 11(18.3%) patients had T1DM. The presence of sensory neuropathy was observed in 66.6% patients. Bacterial infection was found in 86.6% DFU cases, 40% cases had mixed bacterial infection while 48.5% cases had monomicrobial infections. 23.3% DFU patients had infection by multidrug resistant (MDR) organisms. ESBL producer was found in 45.3% gram-negative isolates. 33 % gram-negative strains were positive for bla_{CTX-M} gene followed by bla_{SHV} (20%) and bla_{TEM} (6.6%) Poor glycemic control in 63.3% patients, duration of infection > 1month (43.3%) and ulcer size > 4cm2 (78.1%) was independently associated with risk of MDR organism infection.

Keywords: Diabetic foot ulcers; multidrug-resistant organisms; risk factors; outcome.

Introduction

Diabetic foot ulceration and infections are a major medical, social, economic problem and a leading cause of morbidity and mortality, especially in the developing countries like India (Ako et al., 2006; Shankar et al., 2005; Gadepalle et al., 2006). Fifteen percent of all diabetics develop a foot ulcer at some point in their lives which is highly susceptible to infections and that spreads rapidly, leading to overwhelming tissue destruction and subsequent amputation (Lipsky et al., 2004). The major predisposing factor to foot ulceration leading to infection is usually related to peripheral neuropathy (Joshi et al., 1999). Mostly the diabetic foot infections are mixed bacterial infections (Viswanathan et al., 2002; Chincholikar and Pal, 2002) and the proper management of these infections requires appropriate antibiotic selection based on culture and antimicrobial susceptibility testing. Sometimes, initial management comprises empirical antimicrobial treatment based on susceptibility data (Goldstein et al., 1996). Knowledge of the agent(s) that cause infected DFU is helpful in selecting definitive antibiotic therapy. In recent years, there has been an increase in the incidence and prevalence of ESBLs. Currently, there was paucity of data on ESBL-producing organisms from diabetic foot infections especially in this part of world. Infection with multidrug resistant organisms (MDR organisms) may increase the duration of hospital stay, cost of management and may cause additional morbidity and mortality (Hartemann-Heurtier et al., 2004). Early diagnosis of microbial infections is aimed to institute the appropriate antibacterial therapy to avoid further complications. Therefore, this study is planned with the objective to determine the bacterial profile and antibiotic resistance to find out the potential risk factors for infection with multidrug resistance.

Materials and Methods

A total of 230 diabetic patients were admitted in the Centre for Diabetes and Endocrinology, J.N.M.C, A.M.U., Aligarh, India, 60 of them who developed ulcer in their foot during Dec 2008 to Nov 2009 were included in this study.

Clinical examination

A detailed clinical history and physical examination was carried out for every subject. Age, Sex, anthropometric measurements, duration of ulcer, duration of diabetes, glycemic control, lipid profile, presence of retinopathy, serum creatinine level or presence of micro/macro-albuminuria, hypertension, history of smoking, history of previous amputation, duration of hospital stay and clinical outcome were noted for every patient. Foot ulcers were categorized into six grades (grade 0 - grade 5) based on Meggit Wagner Classification System (Wagner, 1981). Neuropathy was quantified in each patient, assessing vibration sensation using a

128 HTZ tuning fork and a 10g monofilament (absence of perception of the Semmes Weinstein monofilament at 2 of 10 standardized plantar sites on either foot).

Ulcers were assessed for signs of infection (swelling, exudates, surrounding, cellulitis, odor, tissue necrosis and crepitation) and size was determined by multiplying the longest and widest diameters expressed in centimeters squared (cm^2), and the diagnosis of extension to the bone was made by probing with a sterile probe by the resident posted in the ward. Plain radiograph was performed to all the subjects and MRI was done in Osteomyelitis suspects. The lesions were then categorized into 3 main clinical groups: (I) skin ulcer (Wagner 1 and 2); (II) deep tissue ulcer with suspected osteomyelitis (Wagner 3); and (III) gangrenous lesion (Wagner 4 and 5). All cases were monitored until discharged from the hospital. All the subjects gave informed consent and clearance was obtained from the hospital ethics committee.

Microbiological methods

Pus sample were obtained by scrapping the base of ulcer or the deep portion of the wound edge with a sterile curette (Gadepalle et al., 2006; Motta et al., 2003), which was transported to the Microbiology Department and processed for aerobic bacteria. Total transfer time to the laboratory was not more that 30 mins. Direct microscopic examination of ulcer sample was performed. Standard methods for isolation and identification of aerobic bacteria were used (Collee et al., 1996; Collee and Marr, 1996).

Susceptibility testing

Antimicrobial susceptibility testing was performed using the disk diffusion method as described by the CLSI (Clinical and Laboratory Standards Institute, 2007), Antimicrobial disk used were Imepenem (10µg), Aztreonam (30µg), Amoxyclav (30µg), Cefpodoxime (10µg), Cefepime (30µg), Cefoperazone (75µg), Cefoperazone/sulbactam (75/10µg), Cefixime (5µg), Piperacillin (100µg), Piperacillin/tazobactam(100/10µg), Ceftazidime (30µg), Ceftazidime/clavulanic acid (30/10µg), Amoxycillin (20µg), Cephotaxime (30µg), Cephotaxime/clavulanic acid (30/10µg), Ceftriaxone (30µg), Cefoxitin (30µg), Amikacin (30µg), Chloramphenicol (30µg), Gentamicin (10µg), Gatifloxacin (5µg), Ofloxacin (5µg), Levofloxacin (5µg), Sparfloxacin (5µg), Streptomycin (10µg), Erythromycin (15µg), Tobramycin (10µg), Clindamycin (2µg), Azithromycin (15µg), Oxacillin (1µg), Vancomycin (30µg) and Bacitracin (µg). All discs were obtained from Hi-Media labs, Mumbai, India. Inter-pretative criteria for each antimicrobial tested were those recommended by manufacturer's guideline (Hi-Media labs, Mumbai, India).

Phenotypic methods for MRSA and ESBL detection

Staphylococcus species were tested for methicillin resistance by using 1-µg oxacillin disc (National Committee for Clinical Laboratory Standards, 2004) and 30 µg cefoxitin disk (Anand et al., 2009). Gram-negative bacilli were first screened for the production of ESBL by disc diffusion method using Cephotaxime, Ceftriaxone, Aztreonam, Cefepime, Cefoxitin and Ceftazidime and later on confirmed by Cephalosporin/Clavulanate combination disk test (disk potential test) using Ceftazidime, ceftazidime+clavulanic acid, cephotaxime, cephotaxime+clavulanic acid, piperacillin, piperacillin+tazobactam, cefoparazone and cefoparazone+sulbactam (David and Robert, 2005). *E. coli ATCC* 25922 (non ESBL-producer), *K. pneumoniae 700603* (ESBL-producer) and *Staphylococcus aureus* ATCC 25923 were used as control strains respectively. A microorganism was classified as MDRO if it was found to be resistant to two or more classes of antimicrobials and included MRSA, ESBL producing organisms (Hartemann-Heurtier et al., 2004).

Molecular methods for ESBL detection

Preparation of DNA template: Template DNA was prepared from freshly cultured bacterial isolates by suspending 3-5 colonies in 50 µl of molecular grade water, and then heating at 95°C for 5 minutes and immediately chilling at 4°C. Positive controls harboring bla_{CTX-M}, bla_{TEM} and bla_{SHV} and negative control (*E. coli* ATCC 25922) were processed in the same way for DNA extraction.

Detection of bla genes by PCR

Molecular detection of bla_{CTX-M}, bla_{TEM} and bla_{SHV} was performed in gram-negative isolates by using polymerase chain reaction (PCR) according to the methods described previously with minor modifications (Ensor et al., 2006; Shahid et al., 2009). The primers and cycling conditions for detection of *bla genes* were same as described by Shahid et al (Shahid et al., 2009).

Antibiotic treatment

Antibiotics were selected according to published recommendation (Hartemann-Heurtier et al., 2004). In mild infections amoxicillin clavulanic acid was given

empirically by the oral route. However in moderate infections intravenous route was preferred taking into consideration the likelihood of osteomyelitis. Considering that the causative agent was polymicrobial, we initiated ampicillin-sulbactam plus an aminoglycoside/quinolone or piperacillin-tazobactam or ceftriaxone plus metronidazole/clindamycin. In the presence of severe infections, surgical debridement and amputation were performed immediately after admission. Metronidazole (500mg I.V. every 8 hours) was added to the drug regimen if cellulitis or gangrene was also present. Combinations of extended spectrum antibiotics were initiated and the treatment was later modified in accordance with the culture results. The duration of the treatment was at least 4-6 weeks and prolonged in cases of osteomyelitis. All patients also received an intensive insulin treatment.

Statistical analysis

The data was analyzed using SPSS version 13.0 for descriptive statistics. Quantitative variables were expressed as means ±SD while qualitative variables were expressed as percentage (%).

Results

In the 60 diabetic foot patients studied (TABLE 1a & 1b), the male to female ratio was 1.6:1. The average age of patients was 48.7±11.4 years (average ± s.d.). Majority of patients 38 (63.3%) were in the age group 41-60 years which include 21 males and 17 females, followed by 13 (21.6%) in age group 21-40 years (10 Males and 3 females).

Table 1a: Demographic details of DFU patients (data expressed as mean±sd or n% unless otherwise indicated).

	Overall Mean±sd or n(%)	**Male Mean±sd or n(%)**	**Female Mean±sd or n(%)**
Age Distribution (years)	**48.70±11.41**	**46.97±12.36**	**49.41±9.6**
0-20	1 (1.6)	1(1.6)	**-**
21-40	13(21.60)	10(16)	3(5)
41-60	38(63.33)	21(35)	17(28.33)
61-80	8(13.33)	5(8.3)	3(5.00)
Type of Diabetes			
Type 1	11 (18.33)	8 (13.3)	3 (5.0)
Type 2	**49 (81.66)**	29 (48.3)	20 (33.33)
Duration of Ulcer (days)	**41.72 ± 5.61**	**44.65 ± 6.07**	**35.33 ± 3.86**
< month	30(51)	18(30)	12(20)
> month	**23(38.3)**	12(20)	11(18.3)
Hospital Stay (days)	**22.13 ± 16.50**	**21.73 ± 11.41**	**21.83 ± 22.74**
Ulcer Grade (Wagner)		**37(61.6)**	**23(38.3)**
grade 0	3(5)	2(3.3)	1(1.6)
grade 1	20(33.3)	10(16.6)	10(16.6)
grade 2	21(35)	14(5.4)	7(11.6)
grade 3	12(20)	9(15)	3(5)
grade 4	1(1.6)	1(1.6)	-
grade 5	3(5)	1(1.6)	2(3.3)
Discharge Status			
alive	57(95)	34(56.66)	23(38.3)
dead	3(5.0)	3(5.0)	-
Habit			
Non-smoker	16(26.6)	7(11.6)	9(15)
Smoker	21(35.0)	20(33.33)	1(1.6)
Alcoholic	5(8.3)	5(8.3)	-
Fundus Examination	**55**	**34(61.18)**	**21(38.18)**
Normal	25(45.45)	17(30.90)	8(14.54)
Diabetic Retinopathy	29(52.72)	17(30.90)	12(21.81)
Cataract	1(1.81)	**-**	1(1.81)
History of Amputation			

Present	12(20)	8(13.3)	4(6.6)
Absent	48(80)	33(55)	15(25)
Duration of Diabetes (years)	**7.93 ± 5.95**	**8.78 ± 5.67**	**6.13 ± 5.07**
0-10	29(69)	19(45.2)	10(23.8)
11-20	12(28.5)	8(19)	4(9.5)
21-30	1(2.3)	1(2.3)	-
Size of Ulcer	**23.58±56.26**	**29.48±69.89**	**13.81±17.94**
≤4 cm^2	12(21.81)	6(10.90)	6(10.90)
>4 cm^2	43(78.1)	28(50.9)	15(27.2)
Site of Ulcer			
Planter	11(18.3)	7(11.6)	4(6.6)
Margin	10(16.6)	6(10.0)	4(6.6)
Heel	13(21.6)	9(15.0)	4(6.6)
Interdigital	20(33.3)	12(20.0)	8(13.3)
Malleoli	5(8.3)	2(3.3)	3(5.0)
Leg	3(5.0)	1(1.6)	2(3.3)
Multiple areas	7(11.6)	4(6.6)	3(5.0)

In presenting complaints, 65% patients had polyuria, polydipsia (32%), polyphagia (24%), weight loss (38%), weakness (39%), swelling in feet (42%), burning during micturition (27%), and pain in leg (35%). Among the DFU patients, 49 (81.6%) had type 2 diabetes mellitus, whereas only 11 (18.3%) patients had type 1 diabetes mellitus. The duration of diabetes for more than 10 years was observed in 28.5 % (11-20 yrs duration) and 2.3 % (>21 yrs) patients whereas 69% had diabetes for less than 10 years.

Table 1b: Clinical characteristics of DFU patients (data expressed as mean±sd or n% unless otherwise indicated).

Routine Investigations (at the time of admission)			
Blood Picture	58	35(60)	28(48.2)
WBC	10.57 ± 4.48	10.80 ± 5.32	9.80 ± 2.80
Hb	11.24±2.16	11.03±2.26	11.09±2.04
RBC	4.9±1.750	5.0±2.20	4.6±0.60
Renal Function Test	58	35(60.3)	23(39.6)
Blood sugar	202.0 ± 105	187.33 ± 114.7	215.58 ± 86.8
Blood urea	39.49 ± 15.2	39.40 ± 14.28	37.97 ± 16.97
Serum Creatinine	2.08 ± 0.4	2.06 ± 0.3	2.03 ± 0.5
Liver Function Test	55	33(60)	22(40)
SGOT/AST	19.75 ± 11.1	20.91 ± 11.5	17.17 ± 10.3
SGPT/AST	18.33 ± 12.2	19.17 ± 13.0	16.04 ± 10.8
alkaline phosphate	13.98 ± 8.7	13.23 ± 9.7	14.47 ± 7.3
bilirubin	1.73 ± 0.1	1.71 ± 0.1	1.69 ± 0.0
Serum Protein	56	33(58.9)	23(41)
Total serum protein	7.56 ± 0.8	7.54 ± 0.8	7.28 ± 0.8
Serum albumin	4.35±0.5	4.32±0.6	4.21±0.4
Serum globulin	4.28±1.1	4.22±0.7	4.20±1.5
Plasma Glucose	58	35	23
Fasting	164.50±82.96	163.86±89.23	158.62±74.31
Normal ≤125	22(37.9)	14(24.1)	8(13.7)
Postprandial	215.30±94.23	213.86±102.35	208.86±82.53
Normal ≤195	28(48.2)	16(27.5)	12(20.6)
HbA1c	11.01±2.52	10.99±2.54	10.51±2.53
6-7 %(good control)	3(6)	2(4)	1(2)
7-8 % (fair control)	2(40	1(2)	1(2)
>8 % (poor control)	41(82)	25(50)	16(32)

The mean HbA1c 11±2.52 was observed in a total of 50 patients with only 14% patients achieving an HbA1c of < 7.0 %. The mean duration of foot infection was 41.7±5.6 days of which 56.6% patients had foot infection of less than 1 month and 43.3% patients reported history of infection for more than 1 month. The mean duration of hospital stay was 22.1±16.5 days. Ulcer was found on plantar surface in 18.3% patients, on interdigits (33.3%), on margins (16.6%), on heel (21.6%), on malleoli (8.3%), and on multiple areas (≥2) was 11.6%. Size of ulcer <4 cm^2 was observed in 21.8% patients, between 4-8 cm^2 in 30.9%, 8 – 12 cm^2 in 21.8% and > 12 cm^2 in 25.4% patients. Patients were graded according to *Meggit Wagner Classification*. Grade I ulcer was found in 33.3%, Grade II in 35%, Grade III in 20%, Grade IV in 1.6%, and Grade V in 5% of patients. The number of DFU patients and their type of isolates within the Wagner grade was summarized in Table 2.

Table 2: Type of flora isolated from different grades of DFU patients.

Wagner Grade and Type of Isolate				
Wagner's Grade	**Patients**	**Sterile**	**Mono**	**Poly**
Grade-0	3	3	-	-
Grade-1	20	5	11	4
Grade-2	21	-	14	7
Grade-3	12	-	7	5
Grade-4	4	-	1	-
Grade-5	3	-	1	2
Total	**60**	**8**	**34**	**18**

In Grade 1**,** monomicrobial infection and polymicrobial infection was seen in 55% and 20% patients respectively whereas 25% showed no growth in their culture report. In Grade 2, monomicrobial infection (66.6%) and polymicrobial infection (33.3%), In Grade 3, monomicrobial infection (58.3%) and polymicrobial infection (41.7%), In Grade 5, monomicrobial infection (33.3%) and polymicrobial infection (66.7%) whereas all the patients showing monomicrobial infection were from Grade 4. 80% of patients were managed conservatively with medical therapy and/or debridement and in 20% of patient's amputation was done. 52.7% reported diabetic retinopathy in which 17 were males and 12 were females. The presence of sensory neuropathy was observed in 66.6% patients whereas 15% had no sensory neuropathy. Nephropathy and hypertension was present in 39% and 55.8% of patients respectively.

Microbiological observations

A total of 75 bacterial isolates were isolated, averaging 1.2 species per patient. 56.6% patients had monomicrobial infection and polymicrobial etiology was observed in 33% while 13.3% showed no growth in their culture report. In the direct microscopic examination of ulcer samples, 93% showed corresponding result to the culture growth on next day, 2% direct results differ in their culture growth and in 5% cases, direct examination could not been done. Among the bacterial isolates, gram-positive cocci comprised of 44% and gram-negative bacilli accounted for 56%. Gram-positive to gram-negative ratio was 1:1.3. *Staphylococcus aureus* was the most common isolate, accounting for 28%; followed by *Escherichia coli* 26.6%, *Pseudomonas aeruginosa* 10.6%, beta hemolytic *Streptococcus spp* 6.6%, *Klebsiella oxytoca 5.3%, Enterococcus faecalis 4%, Acinetobacterspp 4%, Coryneformspp 2%, CONS 2%* and *Proteus vulgaris 2%.* Prevalence of various bacterial isolates in different Wagner grades of foot ulcer was shown in Table 3.

Table 3: Frequency of isolates within Wagner grades.

Frequency of Isolates Within Wagner's Grades							
S. No.	**Name of Isolate**	**I**	**II**	**III**	**IV**	**V**	**Total**
		n	**n**	**n**	**n**	**n**	**n (%)**
1	*Staphylococcus aureus*	7	8	4	1	1	21(28)
2	*Escherichia coli*	4	8	4	2	2	20(26.6)
3	*Pseudomonas aerugenosa*	3	1	2	1	1	8(10.6)
4	*Klebsiella pneumoniae*	1	2	1	1	-	5(6.6)
5	*β hemo_streptococcus*	2	1	2	-	-	5(6.6)
6	*Klebsiella oxytoca*	-	-	2	1	1	4(5.3)
7	*Enterococcus faecalis*	-	3	-	-	-	3(4)
8	*Acinetobacter*	-	1	2	-	-	3(4)
9	*CONS*	-	2	-	-	-	2(2.6)
10	*Coryneformspp*	1	-	1	-	-	2(2.6)
11	*Proteus vulgaris*	-	1	-	-	1	2(2.6)
	Total	**18**	**27**	**18**	**6**	**6**	

In grade 1, prevalence of *S. aureus* was predominant compared to others whereas in grade 2 and 3, *E coli* and *S. aureus* showed equal number of prevalence. In grade 5, the prevalence of *E. coli* was doubled when compared with *S. aureus.* The maximum number of isolates (28) was from grade 2 infected patients followed by 21 in grade 1, 9 in grade 3, 6 in grade 5 and only 1 in grade 4.

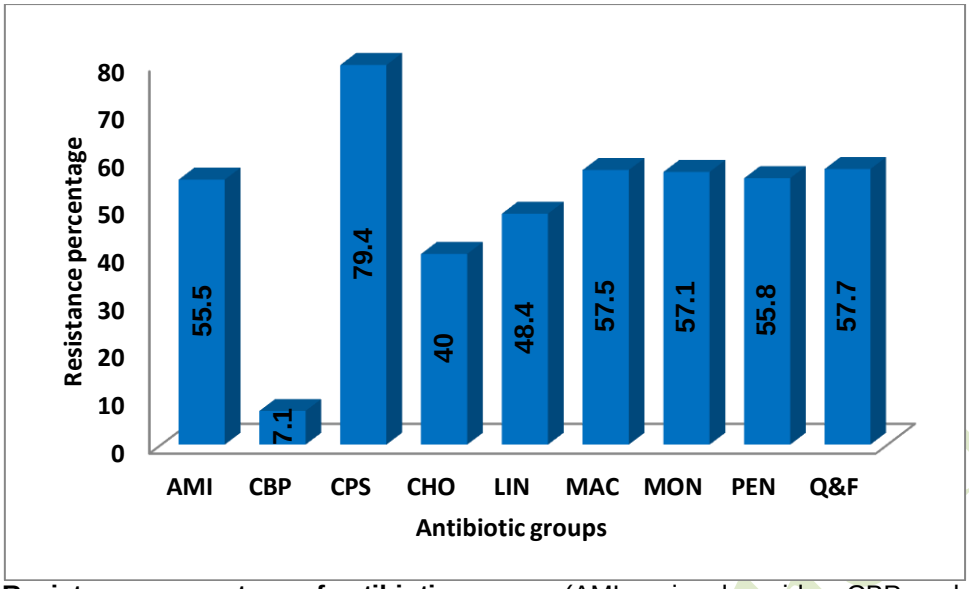
The result of resistance studies are summarized in Table 4. High degree of antibiotic resistance was observed in gram-negative bacilli (55.9%) compared to 48.3% by gram-positive cocci. In gram-positive bacteria, CONS exhibited a higher frequency (73.8%) of resistance to the antibiotics tested, followed by beta hemolytic *streptococcus* (52.7%), *E. faecalis* (52.7%), *S. aureus* (43%) and 42.8% by *Coryneform spp.* All the gram-positive isolates were uniformly susceptible to vancomycin. Methicillin resistance was found in 57.1% *S. aureus* isolates by using 1 µg oxacillin disk and 71.4% by 30 µg Cefoxitin disk. Among gram-negative bacilli, *Acinetobacter spp* showed 75.3% of resistance to the antibiotics tested, followed by *K. oxytoca* (59.7%), *P. aeruginosa* (55.9%), *E. coli* (53%), *K. pneumonia* (48.6%), and *P. vulgaris* (47%). On an average, 23.3% patients having infections in there foot by MDR Organisms.

The resistance percentage of total bacterial isolates in different groups of antibiotics tested is shown in Graph 1. Higher percentage of resistance (79.4%) was shows among the cephalosporin group followed by quinalones and fluoroquinalones (57.7%), macrolides (57.5%), monobactam (57.1%), penicillin (55.8%), aminoglycosides (55.5%), lincosamides (48.4%), chloremphenecol (40%) and carbapenems (7.1%).

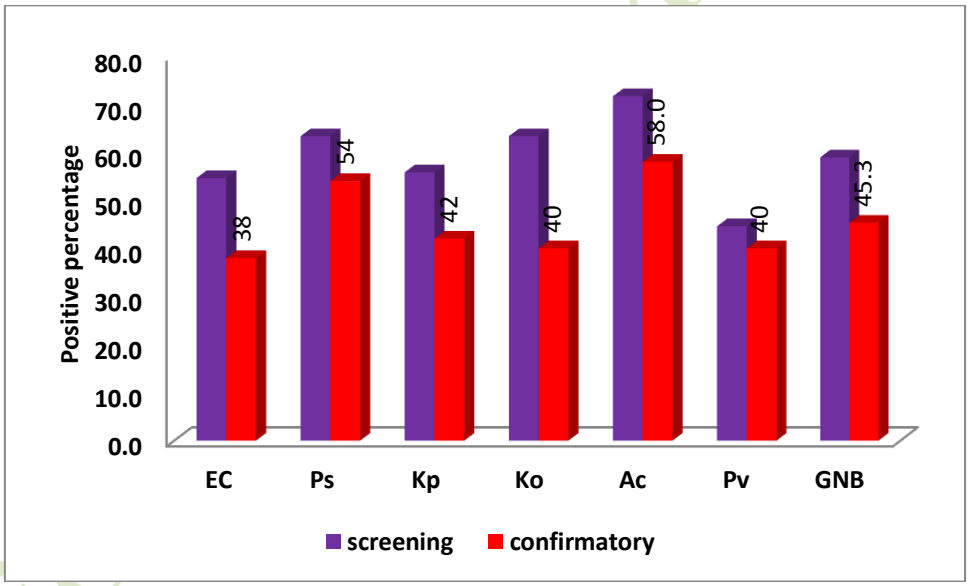
Phenotypic ESBL detection in DFU isolates

The result of phenotypic test was shown in Graph 2. Based on Kirby-Bauer disc diffusion method average production of ESBL was inferred in 71.6% of *Acinetobacter spp* followed by *P. aeroginosa* (63.3%), *K. oxytoca* (63.3%), *K. pneumoniae* (55.8%), *E. coli* (54.6%) and *P. vulgaris* (44.6%). About 57.9% gram-negative DFU isolates were ESBL positive by disc diffusion method.

In Disk potentiation method, average production of ESBL was 58% in *Acinetobacterspp* followed by *P. aeruginosa* (54%), *K. pneumoniae* (42%), *K. oxytoca* (40%), *P. vulgaris* (40%) and *E. coli* (38%). 45.3% of gram-negative DFU isolates were ESBL positive by disk potentiation test.



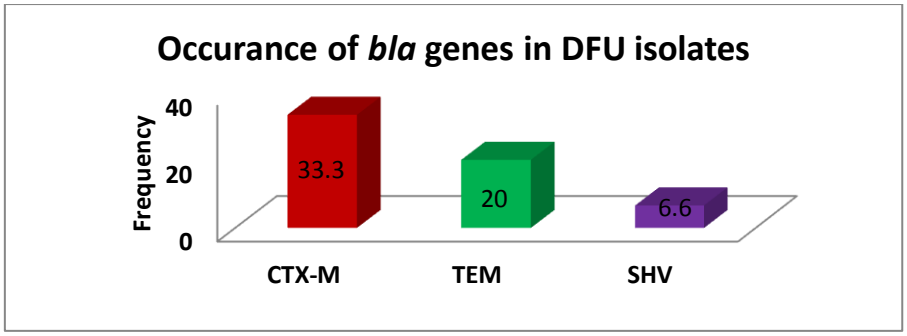
Graph 1: Resistance percentage of antibiotic groups. (AMI: aminoglycosides, CBP: carbapenems, CPS: cephalosporins, CHO: choramphenecol, LIN: lincosamides, Mac: macrolides, MON: monobactam, PEN: penicillin and Q&F: quinalones & fluoroquinalones)



Graph 2: Screening and confirmatory test result for ESBL detection in DFU isolates. (EC:*Escherichia coli*, Ps: *Pseudomonas aerugenosa*, Kp: *Klebsiella pneumoniae*, Ko: *Klebsiella oxytoca*, Ac: *Acinetobacter*, Pv: *Proteus vulgaris*, GNB: total *gram-negative*)

Occurrence of bla genes

The frequency of the occurrence of various *bla* genes in DFU isolates is shown in Graph 3, CTX-M was found to be the most prevalent ESBL noticed in 33.3%, followed by TEM in 20% isolates and SHV beta-lactamases were noticed in 6.6% isolates, respectively.



Graph 3: PCR assay results for bla_{SHV}, bla_{TEM} and bla_{CTX-M} genes.

Table 5 shows the result of factors to be associated with the presence of MDR infections. The duration of ulcer > 1 month was observed in 78.5% patients having MDR infection. The size of ulcer more than 4cm^2 was found in 85.7% patients with MDR infection and in 14.2 % patients having ulcer size less than 4 cm^2 in MDR infections. Fasting blood sugar 176±85.3 mg, HbA1c >8 (85.7%), presence of sensory neuropathy in 78.5% MDR patients and previous antibiotic use in 71.4% were significantly associated with MDR organisms infection in DFU patients.

Table 5: Association of clinical data of DFU patients with or without MDR organism infections (data expressed as mean±sd or n% unless otherwise indicated).

N 60	Non-MDR n 47	MDR n 14
Gender Distribution		
Male	29(61.7)	8(61.5)
Female	18(38.2)	6(42.8)
Age Distribution (years)		
0-20	1(2.1)	-
21-40	11(23.4)	3(23)
41-60	28(59.5)	10(76.9)
61-80	7(14.8)	1(7.6)
Type of Diabetes		
Type 1	10(21.2)	1(7.6)
Type 2	37(78.7)	13(92.8)
Duration of Diabetes (years)		
0-10	33(70.2)	8(61.5)
11-20	9(19.1)	2(15.3)
21-30	4(8.5)	3(23)
Duration of Ulcer		
< month	31(65.9)	3(23)
>month	16(34)	11(78.5)
Hospital Stay (days)	**21.7±17.5**	**20.6±15.2**
Size of Ulcer		
≤4 cm^2	11(23.4)	2(14.2)
>4 cm^2	31(65.9)	12(85.7)
Ulcer Grade (Wagner)		
grade 0	3(6.3)	0(0)
grade 1	17(36.1)	3(23)
grade 2	16(34)	5(38.4)
grade 3	9(19.1)	4(28.5)
grade 4	0(0)	1(7.6)
grade 5	2(4.2)	1(7.6)
Fundus Examination		

Diabetic Retinopathy	22(46.8)	9(69.2)
Discharge Status		
Alive	46(97.8)	12(85.7)
Dead	1(2.1)	2(15.3)
Management		
Amputation	3(6.3)	10(71.4)
Conservative	44(93.6)	4(30.7)
Blood Picture		
WBC	9.5±4.6	10.6±3.6
Hb	9.5±14.1	6.2±6.2
RBC	10.5±2.1	10.1±2.2
Previous Antibiotic Use		
Present	16(34)	9(64.2)
Absent	31(65.9)	4(28.5)
RFT		
Blood Sugar	218±110	150.8±48.7
Blood Urea	38.4±14.8	40.8±16.5
Serum Creatinine	1.0±0.3	1.3±0.7
LFT		
SGOT/AST	18.9±10.9	19.5±11.6
SGPT/AST	17.7±12.1	17.1±12.4
Alkaline Phosphate	12.3±8.6	16±8.6
Bilirubin	0.7±0.1	0.8±0.1
Serum Protein		
TSP	6.7±0.8	6.7±0.9
SA	3.4±0.6	3.2±0.5
SG	3.1±0.7	4.0±1.8
Plasma Glucose		
Fasting	176.6±85.3	127±55.4
Postprandial	231.3±97.6	167.1±50.3
HbA1c		
6-7 %(good control)	2(4.2)	1(7.1)
7-8 % (fair control)	5(10.6)	1(7.1)
>8 % (poor control)	30(63.8)	12(85.7)
Lipid Profile (mg/dl)		
Total lipid	555.3±187.9	543±165.3
Cholesterol	170.6±38.6	155.3±47.2
Triglycerides	176.6±124.8	160.3±100.4
HDL	42±7.3	39.3±10.5
LDL	90.2±28.8	84±14
VLDL	35.2±24.7	32±19.9
Phospholipids	215.4±27	202.8±34.5

Discussion

This study presents a comprehensive clinical and microbiological profile of infected diabetic foot ulcers in hospitalized patients. With the rise in the prevalence of diabetes mellitus there is increasing problem of infections among diabetic patients especially the diabetic foot infection which according to some studies accounts for 20% of hospital admissions (Shankar et al., 2005). India is the home of the largest number of diabetic individuals and their socio-economic conditions are poor. As multidrug resistance is a growing problem, effort was made to study the association of different characteristics with the presence of MDR Organisms. The prevalence of diabetic foot ulcers among male subjects was found to be 56.6% against 30% in female i.e. a ratio of 3.5:1, which may be due to higher level of outdoor activity among males compared to females. Diabetic retinopathy was observed in 52.7% patients studied. There may be one possible of the 13.3% DFU patients which shows no growth in their culture report, that,

in India, most of the patients went to local practitioner, who have little knowledge on DFU treatment. With increasing duration of diabetes, there is increased risk of diabetes related complications especially chronic complications like sensory neuropathy. This study also reports a high prevalence of neuropathy (66.6%). The prevalence of sensory neuropathy in earlier studies shows marked variation. It was 77.8% in a Nigerian study (Ako et al., 2006) and 56.8% in a south Indian study (Shankar et al., 2005). This marked variation in the prevalence may be due to difference in the methods used for the diagnosis of these conditions (10g monofilament or biothesiometer).

In the statistical analysis, duration of infection >1month, prior antibiotic use and ulcer size >4cm^2 were independent predictors of infection with MDR Organism. Thus patients with a large ulcer, with a history of prior antibiotic use and duration of infection >1month were more likely to harbor MDRO's. In the present study, mean duration of ulcer was found to be 41.7±5.6 days with 38.3% having ulcer for more than 1 month. About 78.1% presented with a large ulcer of approximate size of >4cm^2 thereby accounting for approximately 61.6% of the patients presenting with Wagner's grade II and above. 41.6% of patients had used antibiotics prior to reporting to the hospital. The reasons for presentation with advanced grade and stage of ulceration could be because of lack of structured health care delivery in the country, attempted self-medication and trust in traditional healers (Boulton and Vileikte, 2001). Moreover inadequate antibiotic treatment and the use of non sterile instruments for dressing results in the growth of multi resistant organisms necessitating hospital admission and surgical intervention (Armstrong and Lipsky, 2004). Prolonged or broad-spectrum antibiotic therapy predisposes patients to infections with antibiotic-resistant organisms like MRSA (Hartemann-Heurtier et al., 2004). This could explain the high level of MDRO infection in our study.

A bacteriological evaluation of diabetic foot ulcer infections showed that the prevalence of gram-negative organisms were found to be more than gram-positive organisms which is in accordance with the previous findings (Gadepalli et al., 2006). The gram-positive to gram-negative ratio was 1:1.3 which is similar to the findings reported earlier (Tentolouris et al., 1999).

Diabetic foot infections are usually polymicrobial in nature and this has been well documented in the literature. In our study also, we found polymicrobial etiology in 13.3% and monomicrobial in 30% patients with the rate of isolation of about 1.25 bacteria per patient which is lower than the previous studies (Gadepalli et al., 2006; Gerding, 1995) which shows rate of isolation between 2.3 -5.8. Gram-positive organisms which include MRSA were found in 23.3% of patients in reversal to the older studies which show predominance of gram-positive ones (Lipsky et al., 2004a; Lipsky et al., 1990; Lipsky et al., 2004b). The present study confirms that MDR organisms are extremely common in hospitalized patients with diabetic foot ulcers. This is in accordance with the reports of Heartemann-Heurtier (Hartemann-Heurtier et al., 2004).

There is high degree of antibiotic resistance found in our isolates, may be due to the fact that ours is a tertiary care hospital with widespread usage of broad spectrum antibiotics leading to selective survival advantage of pathogen. The antimicrobial resistance pattern was similar to the recent studies done in India and outside (Shankar et al., 2005; Raja, 2007). Gram-negative bacteria that are regarded as normal flora of the skin, like *P. aeruginosa*, may cause severe tissue damage in diabetics and should never be automatically disregarded as insignificant in diabetic foot ulcers (Mike and Ali, 2004). In our study, 57.1% of isolated *S aureus* were methicillin resistant by using 1µg oxacillin disk and 71.4 % of isolated *S. aureus* was resistant to 30µg cefoxitin disk. None of the gram-positive isolates were resistant to vancomycin (VRSA). Clinical isolates of vancomycin resistant *Enterococcus* (VRE) and MRSA resistant have also been reported from treated patients (Herrero et al., 2002; Tsiodras et al., 2001).

Mathur et al. have reported 68% prevalence of ESBL producers from India (Mathur et al., 2002). Babypadmini et al. have shown 40% of *K. pneumoniae* isolates and 41% of *E. coli* isolates to be ESBL producers in their study cohort (Babypadmini and Appalaraju, 2004). Currently there is paucity of data on the prevalence of ESBLs in diabetic foot infection. In a study conducted in Brazil, Motta et al. (2003) say that the prevalence was only 6% among *E. coli* isolates. Gadepalli et al. have reported 54.5% *E. coli* isolates to be ESBL producers (Gadepalli et al., 2006) , which have caused diabetic foot infections. In a recent study, Shobha et al. have reported 27.3% *K. pneumonia, 25.2% E. coli*, 21.42% *Pseudomonas* spp, 25% *Enterobacter* spp and 17% *Acinetobacter* spp to be ESBL producer (Shobha et al., 2009). In this study, 71.6% of *Acinetobacterspp* were positive for ESBL

screening, followed by *P. aeroginosa* (63.3%), *K. oxytoca* (63.3%), *K. pneumoniae* (55.8%), *Escherichia coli* (54.6%) and *P. vulgaris* (44.6%). The high percentage of ESBL production by disk potentiation test was observed in *Acinetobacter* spp (58%) followed by *P. aeruginosa* (54%), *K. pneumoniae* (42%), *K. oxytoca* (40%), *P. vulgaris* (40%) and *E. coli* (38%). The bla_{CTX-M} is among the most prevalent and widely disseminated genes in the clinical bacterial population in India (Ensor et al., 2006; Walsh et al., 2007). In the present study, we found 33.3% bla_{CTX-M} as the most prevalent ESBL gene followed by 20% bla_{TEM} and 6.6% bla_{SHV} in the DFU isolates tested.

It is known that MDR infections are resistant to several antibiotics, and therefore, they can be treated with extended spectrum antibiotics for longer durations. As a result, duration of hospital stay for infections with MDRMs can be longer and their treatment can be more costly. Furthermore, mortality from infections with MDRMs is twice as high as mortality from infections with microorganisms sensitive to antibiotics (Eckman et al., 1995).

In our study, the prevalence of multi-resistant bacterial strains also portends the possibility of longer period of hospitalization for patients as healing may be compromised when bacterial are highly resistant to antimicrobials. The prevalence of both MRSA isolates and ESBL producing gram-negative isolates was in accordance with the reports of Hartemann-Heurtier et al. (Hartemann-Heurtier et al., 2004). Manual minimum inhibitory concentration (MIC) was not carried out as it was time consuming and tedious for all the ESBL-producing clinical isolates obtained in the present study.

Table 4: Antimicrobial resistance pattern of bacteria isolated from diabetic foot ulcers in diabetic patients (N=75).

Antibiotic groups	Antimicrobial agents	EC	Ps	Kp	Ko	Ac	Pv	Sa	Ef	bHS	CONS	TOTAL
		20	8	5	4	3	2	21	3	5	2	
		n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	n(%)	N(%)
	Amoxycillin	17(85)	6(75)	4(80)	3(75)	3(100)	2(100)	¤	‡	‡	‡	35(83.3)
	Amoxyclav	13(65)	5(62.5)	4(80)	3(75)	3(100)	0(0)	6(28.6)	1(33.3)	5(100)	1(50)	42(56)
	Piperacillin	17(85)	6(75)	3(60)	4(100)	3(100)	1(50)	¤	‡	‡	‡	34(80.9)
Penicillin	Oxacillin	‡	‡	‡	‡	‡	‡	12(57.1)	2(66.7)	3(60)	2(100)	20(60)
	Cefoxitin	10(50)	6(75)	2(40)	1(25)	2(66.6)	1(50)	15(71.4)	0(0)	3(60)	1(50)	41(54.6)
	Ceftriaxone	11(55)	8(100)	3(60)	2(50)	3(100)	0(0)	12(57.1)	2(66.7)	1(20)	2(100)	44(58.6)
	Cefpodoxime	14(70)	7(87.5)	3(60)	3(75)	3(100)	1(50)	¤	‡	‡	‡	31(73.8)
	Ceftazidime	10(50)	6(75)	4(80)	4(100)	3(100)	0(0)	¤	‡	‡	‡	27(64.2)
Cephalosporins	Cefotaxime	15(75)	5(62.5)	3(60)	4(100)	3(100)	2(100)	4(19.0)	2(66.7)	5(100)	2(100)	46(61.3)
	Cefoparazone	20(100)	6(75)	5(100)	4(100)	3(100)	2(100)	¤	‡	‡	‡	40(95.2)
	Cefixime	16(80)	4(50)	4(80)	4(100)	1(33.3)	1(50)	11(52.4)	2(66.7)	3(60)	1(50)	48(64)
	Cefepime	13(65)	4(50)	2(40)	4(100)	1(33.3)	1(50)	¤	‡	‡	‡	25(59.1)
Monobactum	Aztreonam	10(50)	5(62.5)	4(80)	2(50)	2(66.66)	1(50)	¤	‡	‡	‡	24(57.1)
Carbapenems	Imepenem	1(5)	2(25)	0(0)	0(0)	0(0)	0(0)	¤	£	‡	‡	3(7.1)
	Amikacin	11(55)	5(62.5)	1(20)	4(100)	1(33.3)	1(50)	3(14.3)	3(100)	1(20)	2(100)	34(45.3)
Aminoglycosides	Gentamycin	8(40)	4(50)	3(60)	4(100)	3(100)	1(50)	4(19.0)	2(66.7)	3(60)	2(100)	35(46.6)
	Streptomycin	‡	‡	‡	‡	‡	‡	16(76.2)	3(100)	3(60)	2(100)	25(75.7)
	Tobramycin	‡	‡	‡	‡	‡	‡	11(52.4)	2(66.7)	3(60)	2(100)	18(54.5)
Chloremphenecol	Chloramphenicol	10(50)	3(37.5)	2(40)	1(25)	1(33.3)	1(50)	3(14.3)	2(66.7)	4(80)	1(50)	30(40)
	Ofloxacin	14(70)	3(37.5)	3(60)	4(100)	3(100)	2(100)	10(47.6)	0(0)	3(60)	2(100)	44((58.6)
Quinalones &	Sparfloxacin	‡	‡	‡	‡	‡	‡	16(76.2)	3(100)	3(60)	2(100)	25(75.7)
fluroquinalones	Gatifloxacin	11(55)	6(75)	1(20)	2(50)	2(66.6)	2(100)	6(28.6)	1(33.3)	3(60)	1(50)	36(48)
	Levofloxacin	9(45)	5(62.5)	1(20)	2(50)	2(66.6)	1(50)	11(52.4)	2(66.7)	3(60)	1(50)	37(49.3)
	Ceftazidime+Clavulanic acid	2(20)	1(12.5)	1(20)	0(0)	3(100)	0(0)	¤	‡	‡	‡	7(16.6)
β lactum inhibitors	Cephotaxime+Clavulanic acid	6(30)	(0)	1(20)	0(0)	1(33.3)	0(0)	14(66.7)	2(66.7)	0(0)	1(50)	25(33.3)
	Piperacillin+Tazobactum	3(15)	4(50)	1(20)	4(0)	3(100)	1(50)	¤	‡	‡	‡	16(38.0)
	Cefoparazone+Sulbactum	4(20)	2(25)	1(20)	0(0)	3(100)	1(50)	¤	‡	‡	‡	11(26.1)
Macrolids	Erythromycin	‡	‡	‡	‡	‡	‡	7(33.3)	2(66.7)	2(40)	2(100)	14(24.4)
	Azithromycin	‡	‡	‡	‡	‡	‡	15(71.4)	3(100)	3(60)	2(100)	24(72.7)
Lincsamides	Clindamycin	‡	‡	‡	‡	‡	‡	9(42.9)	2(66.7)	3(60)	2(100)	16(48.4)
Glycopeptides	Vancomycin	‡	‡	‡	‡	‡	‡	0(0)	0(0)	0(0)	0(0)	0(0)
Bacitracin		‡	‡	‡	‡	‡	‡	‡	‡	3(60)	‡	3(60)

‡ Not tested, ¤ All Staphylococcus resistant to oxacillin have been considered resistant to all β-lactum
Ps: *Pseudomonas aeruginosa*, Ec: *Escherichia coli*, Pv: *Proteus vulgaris*, Ko: *Klebsiella oxytoca*, Kp: *Klebsiella pneumoniae*, Ac: *Acinetobacter sp*, Sa: *Staphylococcus aureus*, BHS: Beta hemolytic *streptococcus*, CONS: Coagulase negative *staphylococcus sp*, Ef: *Enterococcus faecalis*

In conclusion, a detailed knowledge of the susceptibility to antimicrobial agents is necessary to facilitate the development of effective strategies to combat the growing problem of resistance especially the MRSA and ESBL strains. The prevalence of MDR organisms was alarmingly high in the diabetic foot patients in India because of indiscriminate use of antibiotics. The findings of the present study suggest that prospective multicentre studies are required to assess the appropriate empirical antibiotic regimen in diabetic foot ulcer infections. The study also directs us that proper management of diabetic foot ulcers with appropriate antibiotics such as carbapenems and chloramphenicol along with good glycemic control must be implemented to decrease the incidence of MDR organisms for better clinical outcome.

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