Dr. Sarita
B.J. Medical College, Civil
Hospital, Asarwa Sahibagh,
Ahmedabad, Gujarat, India

Dr. Praveg Gupta
B.J. Medical College, Civil
Hospital, Asarwa Sahibagh,
Ahmedabad, Gujarat, India

Dr. Priyadarshani
B.J. Medical College, Civil
Hospital, Asarwa Sahibagh,
Ahmedabad, Gujarat, India

Bacteriological profile in diabetic foot infection

Dr. Sarita, Dr. Praveg Gupta and Dr. Priyadarshani

Abstract
1. I have covered the morphological profile in diabetic foot infection and there antimicrobial susceptibility testing.
2. Study the prevalence of multidrug resistant pattern of isolates.
3. Suggest an effective, economical Antimicrobial policy for treatment of Diabetic foot infection.
4. Gram positive organism was predominant isolate from infected diabetic foot infection and among gram negative organism *E. coli* was most commonly isolated.
5. There were higher prevalence of both MRSA isolates and MBL producing Gram negative organism confirm the MDRO infection was higher in our patients on treatment for diabetic foot infection.

Keywords: Bacteriological profile, diabetic foot infection

Introduction

Diabetes mellitus is a health problem of first order as evidenced by the high prevalence and numerous consequences [1]. Approximately 8.3% of the world population suffers from the disease with a similar proportion of undiagnosed patients. Further the incidence increases with age reaching to 11% in above- 60 age group.

It is the fourth common cause of death all over world as a direct cause not taking into account the cardiovascular mortality due to Diabetes [2]. Diabetic foot infections are frequently occurring, complicated and costly problems in the lifetime of a diabetic [3]. It ranks first among the most common diabetes related causes of lower limb amputation making upto 25% of all hospital admissions and prolonged hospital stay.

Diabetic foot ulcers constitute the most common neurotraumatic cause of Amputation [5, 6] as about 59% of the patients require a minor or major amputation [7]. There is 20 to 45 fold increased risk of requiring amputation than non-diabetics. Diabetic foot infection increases the need for surgical management like amputation at various levels by 50% when compared to uninfected Diabetic foot ulcers [1].

The major predisposing factor for diabetic foot infections is presence of ulcer which is often a consequence of disease related neuropathy, vascular disease and compromised immunity [11, 12].

Material and Method

This study was conducted at B.J. Medical College, Ahmedabad in microbiology department laboratory, duration of January 2017 to December 2017. Total 120 samples were received for testing.

Specimen Collection and Transport

Specimens for microbiological assessment (frank pus & also swab by scrapping the wound) were obtained at the time of admission & at the time of visit to OPD, after thorough vigorous saline wash followed by wound debridement of superficial slough and exudates. Specimens were collected by scraping the ulcer base or the deeper portion of the wound edge with sterile curette into a wide- mouthed sterile container or scavenged using sterile swabs and transported to the microbiology lab without undue delay [85, 86].

Correspondence Author:
Dr. Praveg Gupta
B.J. Medical College, Civil
Hospital, Asarwa Sahibagh,
Ahmedabad, Gujarat, India

Specimen Processing
Microscopy
Direct smears were made from the specimens; gram staining was done and examined under oil immersion for the presence of pus cells, epithelial cells, bacteria and fungi and to assess quality of the sample.

Culture Media Used
MacConkey agar, Blood agar, Nutrient agar, Chocolate agar, Muller- Hinton agar

Culture Method
Inoculum was made with the specimen loaded swab or by loading sterile inoculation loop with the curetted material and Streak culture was done using flame sterilized loop. Plates were incubated overnight at 35 - 37°C in incubator. And examined the next day for growth and observations were recorded. The isolated colonies were identified by adopting the procedures of Gram staining, motility and routine biochemical reactions.

Antimicrobial Sensitivity Testing [87]
The antimicrobial sensitivity pattern for all the isolates were done in Muller Hinton Agar by modified Kirby – Bauer disc diffusion method as per CLSI guidelines using antibiotic discs (Himedia).

Mcfarland Turbidity Standard
0.5 McFarland turbidity standard was prepared for testing.

Inoculation of Mha Plates
A sterile swab is dipped in the inoculum within 15 minutes of adjusting the turbidity of the inoculum and pressed firmly against the sidewall of the test tube to drain the excess broth. Muller Hinton agar plate was inoculated by streaking the swab over the entire sterile agar surface the closed plate was left for 3-5 minutes to allow any excess surface moisture to be absorbed before applying antimicrobial discs

Application of Antimicrobial Discs
The battery of drugs to be applied was determined and the following antimicrobial discs - Gentamicin, Amikacin, Amoxycillin, Ampicillin, Oxacillin, Erythromycin, Doxycycline, Ciprofloxacin, Ofloxacin, Cephelexin, Cefixime, Ceftazidime, Ceftriaxone, Cefotaxime, Cefipime, Ceftazidime Clavulanicacid, Aztreonam, Imipenam, and were tested for Gram negative organism. Along With the above drugs Cefoxitin, Linazolid and Vancomycin were tested for gram positive cocci and Piperacillin-Tazobactum was used only for *E. coli, Klebsiella* and *Pseudomonas*. The plates were then incubated at 37° C for 16 – 18 hrs in incubator. Control strains were also inoculated following the same procedure.

Interpretation
The zones of complete inhibition from the centre of the discs was measured. The zones were measured to the nearest millimeter using zone scale (Himedia Zone of inhibition were the margin showing no obvious visible growth detected with naked eyes and interpreted by referring to the CLSI standard guidelines updated from time to time. The organism was reported as sensitive or resistant to the drugs that were tested. An intermediate zone of inhibition was also reported but the clinical application of the data were doubtful. Control plates were also read using the same procedure and reliability of the test was ensured.

Result
During the study period from January 2017 to December 2017, a total number of 120 samples were collected from patients attending SURGERY Out Patient Department and those admitted at civil hospital, Ahmedabad. And all samples were processed in the Department of Microbiology Civil Hospital.

Among the study population, 66 were males (55%) and 54 were females (45%). Out of the 120 specimens processed, 102 (85%) showed significant growth and 18 (15%) yielded no growth of organisms.

The processed 102 samples yielded a total of 148 organism, the polymicrobial isolation being the reason behind. 63(61.8%) samples yielded monomicrobial growth while 39 samples (38.2%) showed polymicrobial growth.

Among 148 organisms *Staphylococcus aureus* constitute 48(33%), *Escherichia coli* – 26 (18%), *Pseudomonas* spp. - 20(13%), *Klebsiella* spp. - 18(11%), *Acinetobacter*-13(9%) proteus sps 8 (6%), *Enterobacter*-7(5%), *Streptococcus* sps 4 (3%), CONS - 2(2%).

Distribution of isolates

Table 1: Distribution of isolates

Organism	No.	%
Staphylococcus aureus	48	33%
E. coli	26	18%
Pseudomonas sp	20	13%
Klebsiella	18	12%
Acinetobacter	13	9%
Proteus sp	9	6%
Enterobacter	8	5%
Streptococcus	4	3%
CONS	2	1%
Total	148	100%

Correlation between Glycemic Control and Amputation

Table 2: Correlation between Glycemic Control and Amputation

Glycemic Control	Total	MDRO		Amputation	
		No.	%	No.	%
FBS>110	76	18	23.7	39	52
PPBS>160	120	64	53.3	71	59

Poor glycemic control in patients is identified as an independent risk factor for MDRO infection and in turn to amputation that was in accordance with a study in AIIMS [43].

Amputation rates were higher when there was infection with multi drug resistant organism. Among the patients with MDRO infection 59% of them underwent amputation. 59 % of total amputations were performed on patients with MDRO infection.

Table 3: Clinical Outcome

	Conservative	Amputation	Total
Total patients	49	71	120
Percentage	41%	59%	100%

Table 4: Correlation between MDRO and Amputation

	Amputation	MDRO	Polymicrobial growth
No. Of patients	71	64	39
Percentage	59%	53.3%	32.5%

Discussion
As the domestic and international incidence of diabetes and its related complications continues to rise, medical fraternity needs to continue to improvise the management of the same. Early recognition of severe infections in addition to other modalities of management is a crucial component of managing diabetic foot infections.

Age and sex wise distribution
In this study the incidence of the disease was more among men than women as supported by various international and national data95. Among the total 120 patients 55% constitutes male population, the remaining 45% being the female population.

The mean age of the subjects was 60- 69 years. Older population falling in the age group of 60 – 69 years contributes the majority (32%) of the diseased undergoing treatment for DFI in our hospital.

Associated Complications
The incidence of other diabetes related complications in patients presented with DFI was as follows – 120 among 120 i.e.100% of the patients presented with Neuropathy. Various studies all over the world3, 14,98,99,100,101 including The IDSA guideline support and confirm this finding. Peripheral vascular disease was observed in 65% of the patients. Retinopathy – 25(21%), Hypertension - 36(30%). Nephropathy – 31 (26%) were the other diabetes related comorbidities observed. There was a positive correlation between the incidence of MDRO and peripheral vascular disease which was supported by other studies.

Multidrug Resistent
The MDR isolates among the total isolates constitutes 43% i.e.64 out of 148 isolates. MDR was proportional to the duration of stay in hospital and increase duration of illness. Patients who stayed in the hospital for ≥ 4weeks harboured 70.4% of the MDR isolates. Increasing prevalence of multidrug resistance among hospital flora explains this scenario. Patients with prolonged duration of illness showed increased isolation of MDR organisms amounting to 80.3% of total MDRO isolated in this study

Conclusion
This study presents a comprehensive clinical and bacteriological survey of diabetic foot infection in our locality. The non-availability of local data regarding the profile of organisms and their antimicrobial sensitivity pattern was a stimulus for this study.

Though earlier data suggest the Gram Positive aerobic bacteria as predominant isolates from infected diabetic foot ulcers, the aerobic Gram negative bacilli were the most frequently isolated. Hence the major etiological factors for DFI in our patients were different. In this study Gram negative organism were predominant, which constitute about 94 (64%) of total organism. And among gram negative organism Escherichia Coli was most common which constitute about 26 (18%).

In Gram positive organism Staph aureus was most common which constitute 48 (33%) of total isolates. Isolation of multidrug resistant Pseudomonas aeruginosa and increasing MRSA among Staph aureus raises a serious concern about the treatment modality.

Higher prevalence of both MRSA isolates and MBL producing Gram negative organisms confirms that MDRO infection was alarmingly higher in our patients on treatment for diabetic foot infection. Frequent usage of broad spectrum antibiotics and the non compliance of the patients to prolonged treatment may be the possibility behind.

The increased duration of Diabetes per se, prolonged hospital stay, poor glycemic control, associated peripheral vascular disease in addition to neuropathy are identified as significant risk factors for MDRO infections in this study that was also supported by earlier Indian and global studies as mentioned earlier. The need for surgical management is found to be more in these cases. This finding suggests the necessity to develop an effective economical empirical antimicrobial policy to reduce mortality and morbidity among these patients because of multi drug resistant.

References
1. Blanes JI *et al*. Consensus document on treatment of infections in diabetic foot. Rev Esp Quimioter. 2011; 24(4):233-262, 2.
2. American Diabetes Association. Report of the Expert Committee on the diagnosis and classification of diabetes mellitus. Diabetes Care. 2000; 23(Suppl. 1):S4-19.
3. Pecoraro RE, Reiber GE, Burgess EM. Pathways to diabetic limb amputation: basis for prevention. Diabetes Care. 1990; 13:513-21.
4. Fincke BG, Miller DR, Turpin R. A classification of diabetic foot infections using ICD-9-CM codes: application to a large computerized medical database. BMC Health Serv Res. 2010; 10:192.
5. Fincke BG, Miller DR, Turpin R. A classification of diabetic foot infections using ICD-9-CM codes: application to a large computerized medical database. BMC Health Serv Res. 2010; 10:192.
6. Boulton AJM. Peripheral neuropathy and the diabetic foot. The foot Caputo GM, Cavanagh PR, Ulbrecht JS, Gibbons GW, Karchmer AW. 1992; 2:67-72, 15.
7. Assessment and management of foot disease in patients with diabetes. N Engl J Med. 1994; 331:854-60.
8. Flynn MD, Tooke JE. Diabetic neuropathy and the microcirculation. Diabet Med. 1995; 12:298-301.
9. National diabetes fact sheet, US Department of Health and Human Services. Center for Disease Control and Prevention: Atlanta, GA, 2011.