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Original Research Article

Bacterial Profile of Diabetic Foot Ulcer - Study From Western India

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ABSTRACT

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. Fifteen percent of all diabetics develop a foot ulcer at some point in their lives.
Aim and objectives: To Study the bacterial profile of diabetic foot ulcer. To isolate and identify aerobic bacteria from diabetic foot ulcers and to determine their antibiotic resistance pattern
Material and methods: This was a prospective study, conducted over a period of 1 year. 75 diabetic patients with foot ulcer attending the Surgery OPD were included in the study. Two swabs were taken from each patient into Amie's transport medium and processed by standard techniques to isolate and identify the bacteria. Antibiotic susceptibility testing was done by Kirby-Bauer's disc diffusion method for all the isolated bacteria.
Results: A total of 110 aerobic organisms were isolated from 75 cases. Organisms isolated were *Staphylococcus aureus* (21.8%), *Enterococcus faecalis* (4.6%), *Pseudomonas aeruginosa* (18.2%), *Escherichia coli* (13.6%), *Proteus spp* (9.1%), *and Acinetobacter baumannii* (7.3%), *Klebsiella pneumoniae* (6.4%),
The prevalence rate of ESBL producing *E. coli* and *Klebsiella pneumonia* and MRSA was 60%, 57.1% and58 % respectively.
Conclusion: The prevalence of Multi Drug Resistant organisms was alarmingly high in the diabetic foot infection patients in India because of indiscriminate use of antibiotics. Mostly the diabetic foot infections are mixed bacterial infections.

Keywords: Diabetic foot ulcer, bacteria, ESBL, MRSA.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder in which there is increased level of blood glucose because of insulin deficiency leading to significant morbidity and mortality. [1] The prevalence of diabetes is rapidly increasing worldwide and a real epidemic of the disease expected in this century. Global prevalence of diabetes is 6.3% in the general population and 8.7% among persons aged 20 years and older, which correspond to total number of 171 million of patients, this number will double and would reach 366 million in 2030. [2]

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. [3,4] 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. [5] The major predisposing factor to foot ulceration leading to infection is usually related to peripheral neuropathy. [6] Mostly the diabetic foot infections are mixed bacterial infections and the proper management of these infections requires appropriate antibiotic selection based on culture and antimicrobial susceptibility testing. [7]

Many studies have been reported on the bacteriology of diabetic foot infections (DFIs) over the past 25 years, but the results have varied and have often been contradictory. A number of studies have found that *Staphylococcus aureus* is the main causative pathogen, [7,8] The role of anaerobes is particularly unclear, because in many studies specimens were not collected or cultured properly to recover these organisms. Among those that did use appropriate methods, some reported that anaerobes play a minimal role. [9,10]

The infection leads to the early development of complication even after a trivial trauma, the disease progresses and becomes refractory to antimicrobial therapy. It is essential to assess the magnitude of bacterial infection of the lesions to avoid further complications and save the diabetic foot. [11] Early diagnosis of microbial infections is aimed to institute the appropriate antibacterial therapy and to avoid further complications.

Aim and objectives

This work was done to study the bacterial profile of diabetic foot ulcer. Following were objectives - To isolate and identify aerobic bacteria from diabetic foot ulcers and to determine their antibiotic resistance pattern.

MATERIALS AND METHODS

This was a prospective study, conducted over a period of one year. 75 diabetic patients with foot ulcer attending the Surgery OPD were included in the study. Two swabs were taken from each patient into Amie's transport medium and processed by standard techniques to isolate and identify the bacteria. Antibiotic susceptibility testing was done by Kirby-Bauer's disc diffusion method for all the isolated bacteria. CLSI guidelines were used for antibiotic sensitivity testing. Special efforts were made to identify drug resistant bacteria like methicillin resistant *staphylococcus aureus* (MRSA), ESBL *E.coli* and ESBL *Klebsiella Spp.*

RESULTS

Seventy five patients with diabetic foot ulcer admitted in the surgical wards, were included in this study.

Maximum number of patients were in the age group of 51 to 60 years (36%), followed by age group 61 to 70 years (29.3%). There were only 1.3 % patients in age group 81 to 90.

Out of 75 patients studied, 55 (73.3%) were males, while females constituted only 20 (26.7%)

Out of 75 cases, 10 (13.3%) cases were of Insulin dependent diabetes mellitus and 65 (86.6%) cases were of non-Insulin dependent diabetes mellitus.

Table 1: Fasting Blood sugar levels and number of isolates in 75 cases

Fasting BSL	No. of patients	No. Of isolates
≤ 200mg %	30	40
> 200mg%	45	70

In the present study, 30 patients had blood sugar levels < 200mg% and 45 patients had BSL > 200mg%.

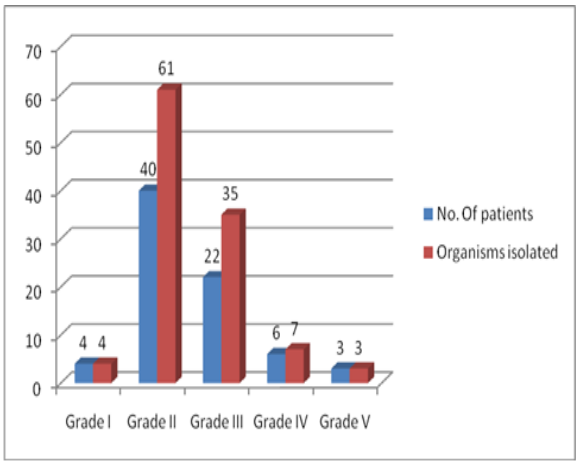

Figure 1: Correlation between grades of ulcers and bacterial isolates (*Wagner's classification*)

More patients were having Grade II and grade III ulcers. Sixty one and thirty five bacteria were isolated from these ulcer samples.

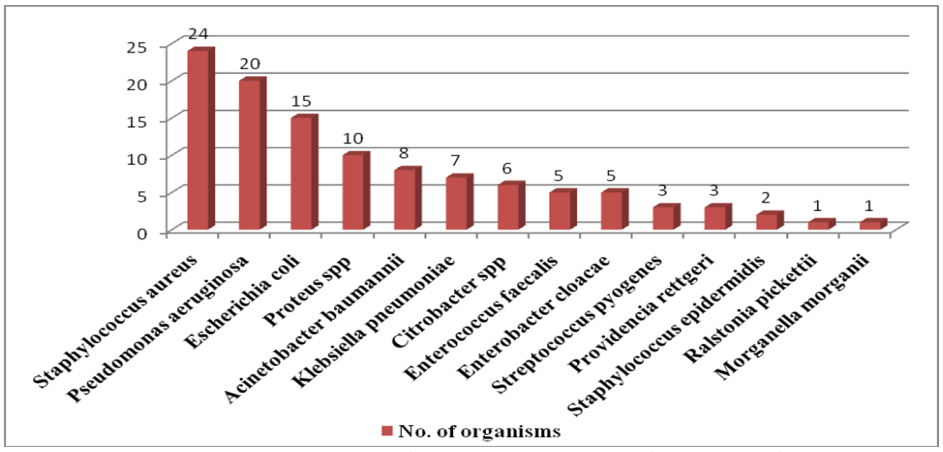

Figure 2: Number and percentage of aerobic bacteria isolated from diabetic foot ulcer

This shows out of 110 aerobic organisms isolated, Gram negative organisms (n=76) were more common than Gram positive organisms (n=34). The percentages of most frequently isolated microorganisms in Diabetic foot ulcer patients were as follows: *Staphylococcus aureus*24 (21.8%), *Pseudomonas aeruginosa* 20(18.2%), *Escherichia coli* 15(13.6%), *Proteus spp*10 (9.1%) *Acinetobacter baumannii* 8(7.3%), *Klebsiella pneumonia* 7(6.4%), *Citrobacterspp* 6(5.4%), *Enterobacter cloacae* 5(4.6%) and *Providenciarettgeri* 3(2.7%).

Out of 75 cases studied, monomicrobial infection was present in 40 (53.3%) cases and polymicrobial infection was observed in 35 (46.7%) cases.

Table 2: Antibiotic resistance pattern of Gram negative bacilli isolated from diabetic foot ulcer

Organism		No	PI %	PIT %	AMC %	CAZ %	CTR %	CPM %	AT %	IMP %	GEN %	TOB %	CIP %	CL %	PB %
E.coli	Non-ESBL	6	66.7	16.7	83.3	66.7	50	66.7	66.7	00	16.7	16.7	50	00	00
	ESBL	9	88.9	33.3	88.9	100	100	77.7	100	00	33.3	33.3	88.9	00	00
K.pneumoniae	Non-ESBL	3	66.7	33.3	66.7	66.7	66.7	33.3	33.3	00	66.7	33.3	00	00	00
	ESBL	4	75	75	100	100	100	50	100	25	75	50	25	00	00
Pseudomonas aeruginosa		20	30	20	80.	75	80	80	75	00	55	40	40	00	00
Acinetobacter baumannii		8	87.5	75	87.5	87.5	87.7	75	75	12.6	87.5	75	100	00	00

PI=Piperacillin; PIT=Piperacillin/Tazobactam; AMC=Amoxicillin/clavulanic acid; CAZ=Ceftazidime; CTR=Ceftriaxone; CPM=Cefepime; AT=Aztreonam; IMP=Imipenem; GEN=Gentamicin; TOB=Tobramycin; CIP=Ciprofloxacin; CL=Colistin; PB=Polymyxin-B.

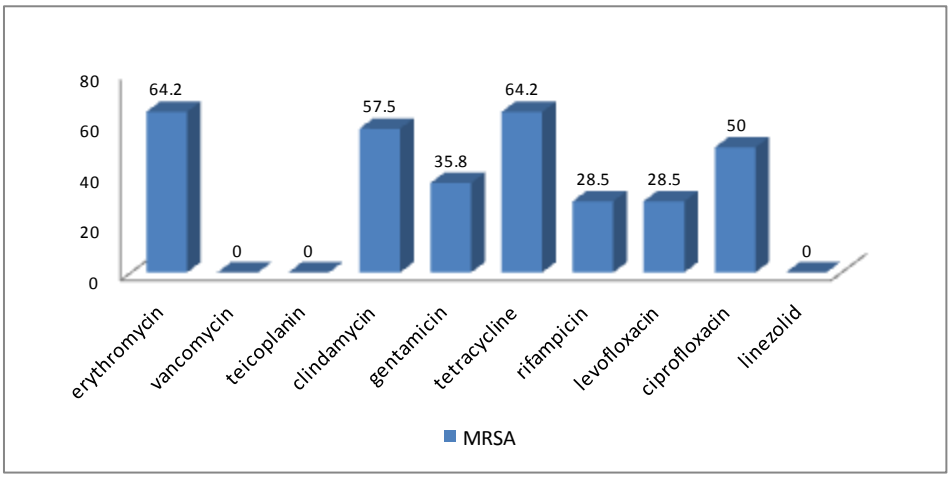

Figure 3: Percentage of Antibiotic resistance pattern of MRSA

Prevalence of MRSA in diabetic foot ulcer patients was found to be 58.3%.MRSA isolates showed maximum resistance to erythromycin (64.2%) and Tetracycline (64.2%), they showed less resistance to Rifampicin (28.5%) and Levofloxacin (28.5%). All MRSA isolates were sensitive to Linezolid, Vancomycin and Teicoplanin.

DISCUSSION

Diabetes mellitus recognized to be common in Indians of the Asian subcontinent. Currently, 50.8 million Indians have diabetes. The projections indicate that India will have the largest number of diabetic patients by the year 2030AD. [12] Diabetic foot infection is a common cause for the hospital admissions of the diabetic patients and caused by a number of socio-cultural practices in India. [13] Such practices include bare foot walking, inadequate facilities for diabetic care, low levels of education, and poor socioeconomic conditions. [12]

This study presents a microbiological profile of infected diabetic foot ulcers. 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. [3] India is the home of the largest number of diabetic individuals. As multidrug resistance is a growing problem, effort was made to study the presence of MDR Organisms.

The prevalence of diabetic foot ulcers among male subjects was found to be 73.3% against 26.7% in female. This is similar to the observations by Dipali AC *et al* (2002) [14] reported 67.6% in males and 32.4% in females,

Among the diabetic foot ulcer patients, 65 (86.6%) had type II diabetes mellitus, whereas only 10 (13.4%) patients had Type I diabetes mellitus, which is almost equal concordance with a studies by Dipali AC *et al.* (2002) [14] reported Type 2 diabetes mellitus in 76.1% and 23.8% type 1 diabetes mellitus and study by Mohammad Zubair *et al* (2010). [15] Whereas Sapico *et al* (1980) [16] has reported that Type I diabetes 69.2% was predominant.

In the present study, we observed that isolates per case was less in patients with blood sugar levels ≤ 200mg% as compared to isolates per case from patients with blood sugar levels > 200mg%. The good control of blood sugar in diabetic patient is a desirable goal in the prevention of certain infections and to ensure maintenance of normal host defense mechanisms determining resistance and response to infection. There is a significant diminution in intracellular bactericidal activity of leukocytes with *Staphylococcus aureus* and *Escherichia coli* in patients with poorly controlled diabetes.

In our study maximum patients were having Wagner grade II and III type of ulcer and maximum number of organisms was isolated from these cases. Similar observation was reported by Gonzatez *et al.,* (2003) [17] from Spain, Sharma *et al.,* (2006) [18] from Nepal and Arumugam *et al.,* (2011) [19] from India, they had also observed predominant isolation in Wagner grading II and III of Diabetic Foot Ulcer. 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 *et al* .,(2001) [20] and Mohammad Zubair *et al* (2010). [15]

In the present study, 110 organisms were isolated from 75 patients. Among 110 isolates 34 were gram positive isolates and 76 were gram negative isolates. Studies from western countries showed that Gram-positive aerobes are the predominant organisms isolated from diabetic foot ulcer patients. In contrast, two recent Indian studies have shown a preponderance of gram-negative aerobes. Gadepalli *et al.,* (2006) [21] in their study on 80 ulcer specimens, recovered 183 isolates, of which 56% were Gram negative and 44% were gram-positive. Studies by Viswanathan *et al.,* (2002), [6] from South India, reported 35% gram-positive pathogens isolates and

65% gram-negative ones, these finding emphasizing the high prevalence of gram-negative pathogens in Southern India. Three large diabetes research centers (India, Germany, and Tanzania) have obtained very similar results. [19]

Similarly in two recent studies, gram-positive bacteria were the commonest agents Arumugam *et al.*, (2011) [19] and Mohammad Zubair *et al* (2010). [15] But other studies by Asha *et al*., (2011) [22] and Ekta Bansal *et al*., (2008) [23] have documented gram-negative bacteria as the predominant organisms associated with diabetic foot infections. Therefore, there seems to be a changing trend, in the organisms causing diabetic foot infections, with gram-positive bacteria replacing gram-negative bacteria as commonest agents.

The relatively high prevalence of *Pseudomonas aeruginosa* in this study may be due to previous antimicrobial use, long hospital stay, chronic wounds and surgical procedures, factors which were contributing to infection. We also noted a relatively high proportion of the K. *pneumoniae* and *E. coli* isolates that were positive for ESBLs, compared with other study conducted in Kuwait (2012). [24] Thus, this study emphasizes the importance of routine screening for ESBL-producing Enterobacteriaceae in clinical laboratories.

Our findings demonstrate the importance of careful selection of antimicrobial therapy, based on culture findings and the antimicrobial sensitivity patterns of the isolates. From our results, it is obvious that Amoxicillin/clavulanic acid and Ceftazidime cannot be recommended for use as an empirical therapy in diabetic foot infections because these drugs were inactive against most strains of pathogens found in these infections. For severe infections and more-extensive chronic moderate infections it is safest to initiate therapy with broad- spectrum agents such as imipenem or Piperacillin/Tazobactam. Over all, none of the Gram negative isolates showed resistance to colistin and polymyxin-B. These two drugs seem to be the most effective drugs in our study with very good sensitivity. Because there is little information on the pharmacokinetic and pharmacodynamics properties of colistin and polymyxin-B, it should be used judiciously. [25] From our antimicrobial susceptibility data, we suggest that Imipenem, Piperacillin / Tazobactam, Linezolid, Teicoplanin and vancomycin to be the most effective agents against most of bacteria isolated in diabetic foot infections. This is equal concordance with a study by Gadepalli *et al.*, (2006). [21]

The high rates of antibiotic resistance observed in the present study 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 pathogens. While many factors must be considered, including previous antibiotic therapy, knowledge of the usual causative organisms in these infections and their antibiotic susceptibilities will allow clinicians to make informed choices. Certainly, empirical antibiotic therapy should include coverage for ESBL producing and for MRSA in a patient with risk factors for infection with these pathogens. As many patients with diabetic foot ulcers have polymicrobial infections, relatively broad spectrum antibiotics should be used for empiric therapy, especially for patients with severe infections and those who are immunocompromised.

The main limitation of this study is the failure to detect the anaerobic bacteria. Moreover, the risk factors for the occurrence of MDR pathogens and the production of metallo-ß-lactamases have not been studied.

CONCLUSION

The prevalence of Multi Drug Resistant organisms was alarmingly high in the diabetic foot infection patients in India because of indiscriminate use of antibiotics. Mostly the diabetic foot infections are mixed bacterial infections. Empirical antibiotic therapy should include coverage

for ESBL producing and for MRSA in a patient with risk factors for infection with these pathogens.

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