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Research Article

Isolation and antibiotic susceptibility of bacteria from foot infections in the patients with diabetes mellitus type I and type II in the district of Kancheepuram, Tamil Nadu, India

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ABSTRACT

Background: Diabetic foot infections are important cause of morbidity and mortality among persons with diabetes mellitus. The reported prevalence rates in India range from 0.9–8.3%. Diabetes foot lesions are the leading cause of non-traumatic amputations worldwide. A study has been conducted to isolate and find the antibiotic susceptibility pattern of the bacteria from diabetic foot infections from the patients of Kancheepuram district, Tamil Nadu, India.
Methods: Sixty patients previously diagnosed or newly diagnosed as diabetic, presented with lower extremity infection attending Tagore medical college and hospital and its peripheral centres were selected for the study. Various specimens (pus, wound exudates, or tissues biopsy) for microbiological studies were obtained from the infected region. The specimens were cultured on blood agar and MacConkey agar for aerobic / facultative anaerobic organisms and on Neomycin Blood Agar for anaerobic organism. The plates were then incubated at 37°C. For anaerobic culture the plates were incubated in the McIntosh anaerobic jar. Isolates obtained are identified by standard laboratory techniques.
Results: The result showed that Pseudomonas aeruginosa (48.3%) is the predominant bacterium followed by Staphylococcus aureus (38%) and other bacteria. The anaerobic bacteria are also isolated from the diabetic foot ulcers. The Peptostreptococcus species (26.7%) are the predominant bacteria followed by other bacteria. Further the results showed that 22 patients (37%) showed the multi-bacterial infection and remaining 38 patients (63%) showed mono bacterial infection. The drugs like amikacin, cefepine, ciprofloxacin, cotrimoxazole and roxythromycin are sensitive to many gram positive bacterial isolates.
Conclusion: The present study has given the data of various bacteria encountered in the diabetic foot ulcer in the district of Kancheepuram, Tamil Nadu, India and its antibiotic sensitivity pattern. The results clearly reveal that there is no definite aetiology in diabetic foot infections. Many patients presented the infection with the involvement of many bacteria. Further it is evident that many bacteria are multi drug resistant and thus complicating the management of diabetic foot infections.

Keywords: Diabetic foot infection, Bacterial isolates, Antibiotic sensitivity pattern, Kancheepuram district

INTRODUCTION

Diabetes mellitus is a worldwide disorder and affects many people. Type 2 diabetes is the most common form of diabetes in developing countries like India, Africa and China. At present 31.7 million people are diabetic in India. Hence, it has been labelled as "The diabetic capital of the world".[1] Diabetes is considered to be a serious disorder not just for its predominance but for its complications. One such important complication is the

foot infections. Foot infections are common in diabetic patients with prevalence as high as 25%.[2] It is also estimated that it is the most common cause of admission of diabetic patients in the hospital.[3]

Diabetic foot infections are important cause of morbidity and mortality among persons with diabetes mellitus.[4] The reported prevalence rates in India range from 0.9-8.3%. Diabetes foot lesions are the leading cause of non-traumatic amputations worldwide.[4] The risk of lower extremity amputation is 15 to 46 times higher in diabetics than in persons who do not have diabetes mellitus.[5]

The vast majority of diabetic foot complications resulting in amputation begin with the formation of skin ulcers. Early detection and appropriate treatment of these ulcers may prevent up to 85 percent of amputations.[6] Infected Diabetic foot ulcers may be complicated by septicaemia and may even result in the death of the patient.

Owing to severity and risk it becomes imperative to initiate antibiotic treatment immediately after reliable culture specimens are obtained from the infected ulcers without waiting for the results of culture and sensitivity.

Bacteriology of diabetic foot infections is highly complicated. It involves both aerobes and anaerobes. Many researches have presented a picture of mixed infection with both aerobic and anaerobic bacteria. The antibiotic susceptibility pattern also shows lot of variation among different geographical places and also with various periods of time. The multidrug resistant bacteria have been reported in many diabetic foot infections.[7,8]

As there is no single etiological agent for the causation of the diabetic foot infections, it becomes very important to find out the different bacteria involved in diabetic foot ulcer and also to know about its antibiotic sensitivity pattern in a particular geographical area. The physician cannot wait until culture report and an understanding of the bacteria and its sensitivity pattern involved in that particular geographical area will help to initiate the treatment without any time delay.

Hence a study has been conducted to isolate and find the antibiotic susceptibility pattern of the bacteria from diabetic foot infections.

METHODS

The ethical clearance was obtained from the institutional ethical committee before the start of the work.

Patients

Sixty patients previously diagnosed or newly diagnosed as diabetic, presented with lower extremity infection attending Tagore medical college and hospital and its peripheral centres were selected for the study. An informed consent was obtained from the patients in a prescribed form at the time of sample collection.

Case study form

A questionnaire was developed to record the medical history, Family History, examination details and investigation reports. Details regarding type of diabetes, its duration, treatment, compliance by the patient, awareness about complications, personal habits like smoking and alcohol consumption, history of hypertension, neuropathy and trauma were recorded.

On local examination, foot ulcers in diabetic patients were categorised into six grades (grade 0 - grade 5) based on Wagner classification system.[9]

Specimen collection

Various specimens (pus, wound exudates, or tissues biopsy) for microbiological studies were obtained from the infected region. For ulcer, the wound was first rinsed with sterile normal saline and then two swabs were collected from the depth of the wound to check for the presence of aerobes and anaerobes. The swab for anaerobic culture was transferred to a tube of sterile Robertson's cooked-meat broth (RCM). The other swab for aerobic culture was transferred into a sterile tube with brain heart infusion broth.

Any fluid discharged in sufficient quantity from the wound was aspirated with sterile needle and syringe aseptically. Part of the specimen collected from wound was transferred to a sterile test tube for gram staining and aerobic culture studies and the remaining part was inoculated in RCM medium for anaerobic culture. The tubes were immediately transported to the microbiology laboratory, Tagore Medical College and Hospital for further processing.

Culture

The specimens were cultured on Blood agar and MacConkey agar for aerobic / facultative anaerobic organisms, and on Neomycin Blood Agar for anaerobic organism. The plates were then incubated at 37^{O}C. For anaerobic culture the plates were incubated in the McIntosh anaerobic jar. Isolates obtained are identified by standard laboratory techniques.

Antibiotic susceptibility test

The anti-microbial susceptibility testing of aerobic isolates is performed by the Kirby Bauer disc diffusion method as recommended by the clinical and laboratory standards institute.[10]

The anaerobes were grouped based on the following antibiotic identification discs namely kanamycin (1 mg), colistin (10 μg), and vancomycin (5 μg).

Sodium polyanethol sulphonate (SPS) disc is placed near the colistin disc for rapid presumptive identification of Peptostreptococcus anaerobius.

Speciation of anaerobic isolates

Pure isolates are further processed for grouping of anaerobes and speciation of the same.

Following tests are carried out:

- 20% bile tube test
- Indole production
- Catalase production
- Nitrate reduction test
- Production of lipase and lecithinase
- Urea hydrolysis

RESULTS

A total of sixty patients who were diagnosed to have diabetes mellitus with foot ulcers were selected for this study. Out of 60 patients 36 (60%) patients were male and 24 (40%) patients were female (Figure 1).

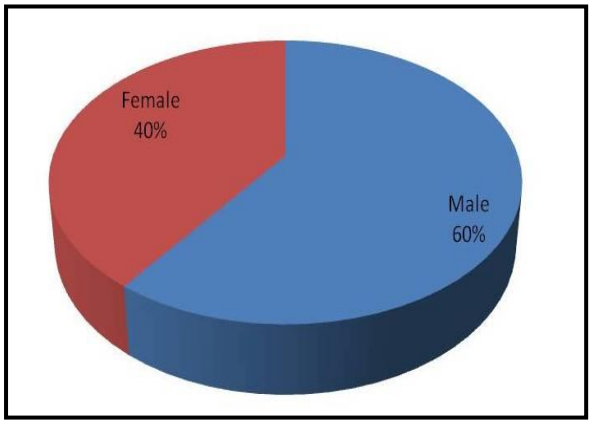

Figure 1: Showing the percentage of male and female patients.

Bacteria isolated from the diabetic foot ulcer

The Table 1 depicts the aerobic bacteria that are isolated from the diabetic foot ulcers. From the table it is evident that Pseudomonas aeruginosa (48.3%) is the predominant bacterium that is isolated from the diabetic foot ulcer followed by Staphylococcus aureus (38%) and other bacteria.

The Table 2 shows the anaerobic bacteria that are isolated from the diabetic foot ulcers. From the table it is clear that Peptostreptococcus species (26.7%) are the predominant bacteria followed by other bacteria.

Further the results showed that 22 patients (37%) showed the multi-bacterial infection and remaining 38 patients (63%) showed mono bacterial infection (Figure 2).

Table 1: Aerobic bacteria isolated from diabetic foot infection.

Name of the bacteria	Number of patients	%
Staphylococcus aureus	23	38.3
Staphylococcus epidermidis	14	23.3
Staphylococcus saprophyticus	7	11.7
Pseudomonas aeruginosa	26	43.3
Streptococcus pyogenes	4	6.7
Streptococcus mutans	2	3.3
Bacillus subtilis	3	5.0
Proteus spp.	4	6.7
Escherichia coli	6	10.0
Klebsiella pneumoniae	1	1.7

Table 2: Anaerobic bacteria isolated from diabetic foot infection.

Name of the bacteria	Number of patients	%
Peptostreptococcus species	16	26.7
Anaerobic Streptococci	6	10.0
Bacteroides fragilis group	3	5.0
Clostridium spp	1	1.7

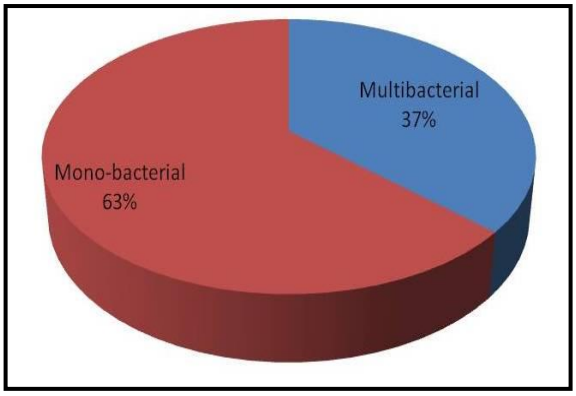

Figure 2: Showing the percentage patients with multi-bacterial and mono-bacterial foot infection.

Antibiotic sensitivity test

The Table 3 shows the antibiotic sensitivity pattern of different gram positive bacteria isolated from diabetic foot ulcer. From the table it is evident that drugs like amikacin, cefepine, ciprofloxacin, cotrimoxazole, roxythromycin are sensitive to many bacterial isolates. Other antibiotics are mostly resistant to many bacterial isolates.

The Table 4 depicts the antibiotic sensitivity pattern of different gram negative bacterial isolates. From the table it is clear that all the antibiotics tested are mostly sensitive to the bacterial isolates.

Table 3: Antibiotic sensitivity of various gram positive bacteria isolated from diabetic foot infection.

Antibiotic	Gram positive bacteria					
	S. aureus N=23	S. epidermidis N=14	S. saprophyticus N=7	S. pyogenes N=4	S. mutans N=2	B. subtilis N=3
Amikacin	23 (100%)	14 (100%)	7 (100%)	4 (100%)	2 (100%)	3 (100%)
Ampicillin	8 (34.8%)	5 (35.7%)	5 (71.4%)	0 (0%)	1 (50%)	1 (33.3%)
Cloxacillin	14 (60.9%)	8 (57.1%)	6 (85.7%)	3 (75%)	2 (100%)	3 (100%)
Cefepine	20 (87%)	13 (92.9%)	6 (85/7%)	4 (100%)	2 (100%)	3 (100%)
Chloramphenicol	12 (52.2%)	5 (35.7%)	3 (42.9%)	2 (50%)	2 (100%)	2 (66.7%)
Ciprofloxacin	19 (83.6%)	10 (71.4%)	6 (85/7%)	4 (100%)	2 (100%)	3 (100%)
Cotrimoxazole	22 (95.7%)	13 (92/9%)	7 (100%)	4 (100%)	2 (100%)	3 (100%)
Gentamycin	9 (39.1%)	3 (21/4%)	1 (14.3%)	0 (0%)	0 (0%)	2 (66.7%)
Penicillin	16 (69.6%)	11 (78.6%)	6 (85.7%)	1 (25%)	0 (0%)	2 (66.7%)
Roxythromycin	22 (95.7%)	14 (100%)	7 (100%)	4 (100%)	2 (100%)	3 (100%)
Tetracyclin	17 (73.9%)	8 (57.1%)	4 (57.1%)	3 (75%)	1 (50%)	2 (66.7%)
Vancomycin	18 (78.3%)	12 (85.7%)	4 (57.1%)	2 (50%)	1 (50%)	1 (33.3%)

Table 4: Antibiotic sensitivity of various gram negative bacteria isolated from diabetic foot infection.

Antibiotic	Gram negative bacteria			
	P. aeruginosa N= 26	Proteus spp. N=4	E. coli N=6	K. pneumoniae N=1
Ciprofloxacin	25 (96.5%)	4 (100%)	6 (100%)	1 (100%)
Ceftazidine	26 (100%)	4 (100%)	6 (100%)	1 (100%)
Piperacillin	26 (100%)	4 (100%)	6 (100%)	1 (100%)
Ceftoxine	24 (92.3%)	4 (100%)	6 (100%)	1 (100%)
Amikacin	19 (73.1%)	4 (100%)	6 (100%)	1 (100%)
Imipenam	26 (100%)	4 (100%)	6 (100%)	1 (100%)
Chloramphenicol	17 (65.4%)	3 (75%)	4 (66.7%)	0 (0%)
Gentamycin	20 (76.9%)	4 (100%)	6 (100%)	1 (100%)
Cotrimoxazole	26 (100%)	4 (100%)	6 (100%)	1 (100%)
Tetracyclin	16 (61.5%)	4 (100%)	6 (100%)	1 (100%)

DISCUSSION

In the present study a total of 14 bacterial species are isolated. This includes both aerobes and anaerobes. Among the aerobes both gram positive and gram bacteria are isolated. Thus it confirms the fact that the diabetes foot infection does not have a clear aetiology.[11] Polymicrobial nature of diabetic foot infections has been observed in various studies.[12,13] The same has been recorded in the present study as well.

The present study also had given the picture of antibiotic sensitivity of various bacterial isolates. The data differs from the various other studies.[14,15] Thus it is clear that the antibiotic sensitivity pattern differs with different geographical area.

The present study has given the data of various bacteria encountered in the diabetic foot ulcer in the district of Kancheepuram, Tamil Nadu, India and its antibiotic sensitivity pattern. The results clearly reveal that there is no definite aetiology in diabetic foot infections. Many patients presented the infection with the involvement of many bacteria. Further it is evident that many bacteria are multi drug resistant and thus complicating the management of diabetic foot infections. The present study can help the clinicians to develop antibiotic policy for the early treatment of diabetic foot infections in the district of Kancheepuram, Tamil Nadu, India.

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Ethical approval: The study was approved by the Institutional Ethical Committee

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