RESEARCH

BACTERIOLOGICAL PROFILE IN DIABETIC FOOT INFECTIONS

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Abstract

Background: Diabetes mellitus (DM) is a major public health problem globally, affecting a large number of people worldwide and Diabetic foot infection (DFI) being one of the most serious complications. Many of these infections are either mono microbial or polymicrobial. Foot problems are largely preventable, and successful treatment therefore depends on the early diagnosis of causative agent involved in DFI. Selection of appropriate antibiotics to treat multi drug resistant pathogens is required to prevent future complications. Hence, this study was conducted in a private hospital in south Bangalore, and was aimed to determine the types of microorganisms isolated from patients with DFIs and their antibiotic susceptibility pattern. **Materials and Methods:** This was a prospective study conducted at patients attending diabetic clinic, in a private hospital in south Bangalore. A total of 68 infected diabetic wound samples (wound exudates, pus or tissue biopsy) from the ulcerated regions were collected over a period of 6 months. For aerobic culture, the samples were inoculated on pre dried plates of Blood agar and MacConkey agar along with anutrient broth. The colonies grown on the plates after overnight incubation at 37°C wereidentified. Antimicrobial susceptibility testing of aerobic isolates was done using Kirby-Bauer & #39; sdisc diffusion method on Muller-Hinton agar plates. **Result:** In this study a total of 68 specimens were received and processed in the department of Microbiology. The frequencies of males were 64% and 35% were females. The age group of patients ranged from 35-80 years. Most of the patients 51.4% belonged to the age group of 55-64 years. Single microbial infection was seen in 70.9% and 29.09% specimens yielded more than one bacteria (Mixed infections). Among them, the gram-negative organisms were more predominant and isolated from about 56.36% cultures and gram-positive strains were found in 43.63%. Klebsiella pneumonia 20% was the commonest Gram-negative organism and among the Gram-positive organism 18.18% S aureus was isolated. Majority of Gram-negative isolates were susceptible to amikacin, followed by piperaciillin/ tazobactum and Cefeperazone / Salbactum. Most of the Staphylococcus aureus were sensitive to Amikacin, Pipercillin / Tazobactum, Cefeperazone / Salbactum, Linezolid, and vancomycin. **Conclusion:** DFI are more common in India due to sociocultural practices like barefoot walking, inadequate facilities for diabetic care, low level of education, and poor socioeconomic conditions. Foot infections are one of the leading causes of morbidity and disability among people with diabetes, and it could lead to irreversible tissue damage / amputation. The microbiology of diabetic foot infections is diverse. Therefore, regular monitoring microbial spectrum and their anti-biogram helps in guiding clinician in initiating the empirical treatment of diabetic foot infection and the treatment must be started only after the culture and the sensitivity testing have been done. Therefore, the rapid propagation of the antibiotic resistance and its mechanism can be prevented.

INTRODUCTION

Diabetes mellitus (DM) is a major public health problem globally, affecting a large number of people worldwide.[1] The incidence is going to increase by 55% over the next 20 years, so this problem is going to get worse.[2] In India, the burden of diabetes has been increasing steadily. The prevalence of diabetes in India has risen from 7.1% in 2009 to 8.9% in 2019.

Diabetic foot infection (DFI) is one of the most serious complications of diabetes, and is a leading cause of morbidity and disability.[3,4,5,6] Patients with diabetes are particularly prone to foot infection primarily because of distal symmetric neuropathy, compromised blood flow and neutrophil dysfunction. DFI range in severity from superficial Paronychia to deep infections involving bone.

Bacterial spectrum of DFI varies greatly. Many of these infections are either mono microbial or polymicrobial.[3] In recent years multi-drug resistant (MDR) bacteria in DFI have been reported frequently and further complicate therapy which may lead to amputation and patient's might even loose lives.[4]

Foot problems are largely preventable, and successful treatment therefore depends on the correct evaluation of the patient, early diagnosis of causative agent involved in DFI. Selection of appropriate antibiotics to treat multi drug resistant pathogens is required to prevent future complications. Hence, this study was aimed to determine the types of microorganisms isolated from patients with DFIs and their antibiotic susceptibility pattern.

MATERIALS AND METHODS

This was a prospective study conducted at diabetic clinic. A total of 68 infected diabetic wound samples were collected over a period of 6 months. All the patients underwent detailed history including age, gender, type of diabetes, duration of diabetes & DFI's, presenting features and clinical examination including details of ulcer were recorded. Biochemical, hematological, serological and radiological profiles of the patients was noted. Various specimens (wound exudates, pus or tissue biopsy) from the ulcerated regions were collected. The surface of ulcer was thoroughly rinsed with sterile normal saline solution and specimens were collected using sterile cotton swabs from the base of the ulcer after debriding the superficial exudates. All the samples were processed in Microbiology department. For aerobic culture, the samples were inoculated on pre dried plates of Blood agar and MacConkey agar along with a nutrient broth. The colonies grown on the plates after overnight incubation at 37°C were identified. Antimicrobial susceptibility testing of aerobic isolates was done using Kirby-Bauer's disc diffusion method on Muller-Hinton agar plates.

RESULTS

In this study a total of 68 specimens were received and processed in the department of Microbiology. The frequencies of males were 44 (64%) and 24 (35%) were females. The age group of patients ranged from 35-80 years. No case of type 1 diabetes mellitus was reported. Most of the patients 35 (51.4%) belonged to the age group of 55-64 years, followed by the age group of 65–74 years (23.52%). Out of 68 specimens 55(80.8%) were Culture positive and Culture negative was observed in 13 (19.1%) specimens. Single microbial infection was seen in 39(70.9%) specimens and 16(29.09%) specimens yielded more than one bacteria (Mixed infections).

Table 1: ?

Age Group (Years)	No of patients -68	%
15-24	0	
25-34	0	
35-44	2	2.9%
45-54	12	17.64%
55-64	35	51.47%
65-74	16	23.52%
.>75	3	4.4%

All 55 bacterial isolates were aerobic. Among them, the gram-negative organisms were more predominant and isolated from about 31(56.36%) cultures. Gram-negative organisms included Klebsiella pneumoniae. 11(20%), E. coli 10 (18.18%) and Proteus mirabilis 5 (9.09%), Acinetobacter spp 3 (5.45%), and Pseudomonas aeruginosa 2(3.6 %). On the other hand, isolated gram-positive strains were found in 24 (43.63%) that included S aureus 10 (18.18%), CONS 8(14.5%), Enterococcus fecalis 6 (10.90%) shown in the [Table 3,4].

Table 2: Microorganisms isolated from DFI's

Aerobes	Number	
Gram Negative	31	56.36%
Gram Positive	24	43.63%
Mono microbial	39	(70.9%)
Poly microbial	16	(29.09%)
Klebsiella pneumoniae	11	20%
Staphylococcus aureus	10	18.18%
Escherichia coli	10	18.18%
CONS	8	14.5%

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Enterococcus fecalis	6	10.90%
Proteus mirabilis	5	9.09%
Acinetobacter spp	3	5.45%
Pseudomonas	2	3.6%

Table 3: Antibiotic susceptibility pattern of Gram-negative bacilli

Antibiotics	K.pneum	E.coli	Protues	Aceintbacter	Pseudomonas
	n=11	n=10	n=5	n=3	n=2
Ampicillin	5 (45.45%)	4 (40%)	3(60%)	0	0
Amikacin	11(100%)	8 (80%)	5 (100%)	3 (100%)	2(100%)
Gentamicin	9 (81.8%)	5 (50%)	4(80%)	0	1(50%)
Ofloxacin	8 (72.72%)	3 (30%)	2(40%)	0	0
Amoxyclav	5 (45.45%)	7 (70%)	3(60%)	0	0
Cefotaxime	10	3 (30%)	1(20%)	0	0
Pipercillin / Tazobactum	11(100%)	8 (80%)	5 (100%)	2 (66%)	2(100%)
Cefeperazone / Salbactum	11(100%)	10 (100%)	5 (100%)	2 (66%)	2(100%)
Cotrimoxazole	9 (81.8%)	4 (40%)	1(20%)	0	0
Impenem	11(100%)	10 (100%)	5 (100%)	3(100%)	2(100%)

Majority of Gram-negative isolates were susceptible to amikacin, followed by piperaciillin/ tazobactum and Cefeperazone / Salbactum. Furthermore, Klebsilella isolates showed 100% sensitivity to amikacin, piperaciillin/ tazobactum, cefeperazone / salbactum and imipenem, and high resistance rates to Ampicillin, Amoxy clav was seen.

Table 4: Antibiotic susceptibility pattern of Gram-positive cocci

Antibiotics	Staphyloccus aureus	CONS	Enterococcus f
	n=10	n=8	n=6
Ampicillin	5 (50%)	3(37.5%)	3(50%)
Amikacin	10(100%)	6(75%)	6 (100%)
Gentamicin	8 (80%)	5 (62.5%)	6 (100%)
Ofloxacin	8 (80%)	3 (37.5%)	3(50%)
Amoxyclav	6 (60%)	4 (50%)	3(50%)
Cefotaxime	7(70%)	3 (37.5%)	3(50%)
Pipercillin / Tazobactum	10(100%)	6(75%)	6 (100%)
Cefeperazone / Salbactum	10(100%)	8 (100%)	6 (100%)
Cotrimoxazole	8 (80%)	4 (50%)	2(33.3%)
Linezolid	10(100%)	8 (100%)	6 (100%)
Azithromycin	7(70%)	3 (37.5%)	2(33.3%)
Vancomycin	10(100%)	4 (50%)	4(66.66%)

Most of the Staphylococcus aureus were sensitive to Amikacin, Pipercillin / Tazobactum, Cefeperazone / Salbactum, Linezolid, and vancomycin. Whereas Cefeperazone / Salbactum sensitivity rate was 100%. Among the aminoglycosides, amikacin was the most sensitive drug. Coagulase-negative Staphylococcus was more resistant to the antibiotics than Staphylococcus. aureus. Enterococcus spp. isolated was sensitive to most of the antibiotics.

DISCUSSION

DFI are more common in India due to sociocultural practices like barefoot walking, inadequate facilities for diabetic care, low level of education, and poor socioeconomic conditions. DFI's account for one of the most common causes of hospitalizations. Foot infections are one of the leading causes of morbidity and disability among people with diabetes, and it could lead to irreversible tissue damage / amputation. Spectrum of bacteria vary widely in diabetic foot infections. Therefore, regular monitoring microbial spectrum and their anti-biogram helps in choosing appropriate antibiotics. Effective management of diabetic foot infection requires appropriate antibiotic therapy, surgical drainage, debridement and resection of dead tissue, appropriate wound care, and correction of metabolic abnormalities.[7]

Earlier studies reported preponderance of males the same was observed in our study major difference was observed between males were 44 (64%) and 24 (35%) were females.[8] Culture positive was seen in 55980.8%), culture negative was observed in 13(19.1%) specimens. The sterile culture in such cases may be due to the prior treatment with broad-spectrum antibiotics or topical application of antibiotics to the infected part.[9]

In our study, Gram negative organisms were more predominant and isolated from about 31(56.36%) cultures, while Gram positive strains were found in 24 (43.63%) which was comparable to Mohanasoundaram et al who also reported gram negative isolates as the most predominant aerobic infection in diabetic foot infections. Among Gram

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negative bacteria, Klebsiella pneumoniae.[10,11] (20%), E. coli 10 (18.18%) was commonly isolated pathogen followed by, Proteus mirabilis 5 (9.09%), Acinetobacter spp 3 (5.45%), and Pseudomonas aeruginosa 2(3.6 %). According to Mohanasundaram, S.aureus (26.1%) was the most common pathogen, followed by E.coli (18.4%). However, our results are similar to the study conducted by Ramakanth et al,[12] he studied the changing trends of bacteriological spectrum in diabetic foot infections ulcers for a period eight years and reported the isolation rate of Gram negative bacteria from 50.6%-66%.

The prevalence of S. aureus was 76 and 78% in studies according to Goldstein et al. and Kajetan et al. However, we have reported Staphylococcus aureus 10 (18.18%) a much lower prevalence when compared with earlier reports. In our study Enterococcus fecalis 6 (10.90%) was isolated. Enterococci are considered to be commensals and exhibit low virulence, excluding those who are in an immunocompromised state such as those with DM in which commensals may also become opportunistic pathogens.[13,14,15,16]

In our study, majority of specimens yielded single microbial infection 39(70.9%) specimens and 16(29.09%) specimens yielded more than one bacteria (mixed infections). This finding correlate with Pappu et al study who reported 92% mono microbial growth.[9] Anandi et al,[10] Zubair et al,[11] Rama Kant et al,[12] and Citron et al,[13] have reported 19%, 56%, 23% and 16.2% monomicrobial growth and 67%, 33%, 66%, and 83% polymicrobial growth infections respectively. Klebsiella pneumoniae. 11(20%) was common bacteria isolated from polymicrobial infections.[10] The higher incidence of single microbial infection in this study than studies done by Chincholikar and Amalia et al is probably due to the higher prevalence of mild and superficial ulcers.[17,18,19,20,21,22,23]

Piperacillin–Tazobactam, Amikacin and Imipenem were the greatest advantageous antimicrobials against aerobic Gram-negative bacteria. As for aerobic Grampositive cocci, the most effective antimicrobials where Vancomycin.[24] Furthermore, initiation of treatment with broad-spectrum antimicrobials including Carbapenems and Piperacillin-Tazobactam for more extensive chronic moderate and severe infections appears to be a safe measure. Definitive treatment can subsequently be initiated upon confirmation of offending pathogen via culture and sensitivity testing, susceptibility data and the patient's response to empirical therapy from a clinical aspect. In order to implement targeted and correct antimicrobial therapy, it is essential to have knowledge and awareness of the common offending pathogens in DFIs.

Sensitivity pattern of the microbes in diabetic foot infections is often heralded by the presence of multidrug-resistant strains. The presence of MDR organisms is the only significant independent predictor of glycemic control. This development is likely due to a lack of strict antibiotic prescription guidelines alongside the lack of adherence to infection control measures in the hospital with a higher prevalence of multidrug-resistant strains in the community. If not rectified, these practices will likely alter empirical antimicrobial therapy.

In our study, no anerobic bacterial culture was performed. Involvement of anaerobic bacteria in diabetic foot infections is not clear and few studies reported minor role of anaerobic bacteria while other studies reported preponderance of anaerobic bacteria.[14,15]

Present study has some limitations such as, no anerobic culture was performed and other multi drug resistant bacteria (MRSA, ESBL strains, Amp C beta lactamases, carbapenamases and metallo beta lactamases) were not detected. Moreover, in recent times a high proportion of E.Coli and Klebsiella spp. isolates are positive for ESBLs. Hence, routine screening towards ESBL-producing Enterobacteriaceae should be emphasised.

CONCLUSION

In conclusion, the microbiology of diabetic foot infection has been well characterised using classical microbiological techniques. In our study, diabetic foot infections were principally due to Klebsiella pneumoniae, followed by Stahylococcus aureus. Coagulase-negative Staphylococci observed in this study, was likely to reflects a combination of sample contamination and genuine pathology caused by the introduction of commensals into tissues.

High degree of antibiotic susceptibility was exhibited by all Gram-negative bacteria towards amikacin, followed by piperaciillin/ tazobactum and Cefeperazone / Salbactum whereas linezolide remained as most susceptible antibiotic towards Gram positive cocci.

Cefeperazone/ sulbactum, Amikacin and piperacillin/ tazobactum showed good susceptibility was found to be effective against both Gram positive cocci and Gram negative bacilli. It has also been stated that male diabetic patients with multidrug-resistant Gram-negative bacilli-infected foot infections have poor glycemic control and have higher mortality than their female counterparts.[19]

Clinicians are also encouraged to obtain adequate specimens after wound debridement for proper culture in addition to requesting for prompt microbiological reporting of all organisms which are obtained from the specimens. Considering the limited suitability of antibiotics, determination of the appropriate antimicrobial treatment should be guided by clinical correlation in addition to the local pattern of bacterial aetiology and its sensitivity.

The microbiology of diabetic foot infections is diverse. Regular monitoring of the antibiotic resistance pattern helps in guiding clinician in initiating the empirical treatment of diabetic foot

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infection and the treatment must be started only after the culture and the sensitivity testing have been done. Therefore, the rapid propagation of the antibiotic resistance and its mechanism can be prevented.

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