Scholars Journal of Applied Medical Sciences (SJAMS) **ISSN 2320-6691 (Online)**
Sch. J. App. Med. Sci., 2017; 5(5C):1883-1887 **ISSN 2347-954X (Print)**

(An International Publisher for Academic and Scientific Resources)
www.saspublishers.com DOI: 10.36347/sjams.2017.v05i05.034

Original Research Article

Bacteriological profile and their Antibiotic Sensitivity Pattern in Diabetic Foot Infections in a Tertiary Care Hospital

Pradeep M.S.S[1], Vishnuvardhanarao K[2], Setty C.R[3]
[1]Assistant professor, [2]Professor, [3]Professor & HOD
Department of Microbiology, Dr. PSIMS & RF/Dr. NTR University of Health Sciences, India

***Corresponding author**
Pradeep M.S.S
Email: pradeepmss7@gmail.com

Abstract: Diabetes mellitus (DM) is a metabolic disorder of endocrine system which is a major health problem with rising prevalence worldwide and is associated with several complications. In India it is estimated to have 61.3% million cases. Seven lakhs new cases are diagnosed every year in which Diabetic foot Infection (DFI) is one of the leading cause of hospitalization which may also lead to amputation of infected foot, if not treated promptly. The present study was conducted to isolate and identify the bacterial pathogens associated with DFI and to find out the antibiotic susceptibility pattern of these isolates. A total of 110 samples were collected from patients with Diabetic foot Infection during the period of Jan 2016- Dec 2016, after taking informed consent. The specimens were subjected to bacteriological study by Gram's staining and culture according to standard guidelines. Isolates were identified and antibiotic susceptibility testing was done. Results: Out of 110 samples (total of 119 isolates), single organism was isolated from 73 (66.4%) samples and mixed bacterial growth was seen in 23 (20.9%) samples. Fourteen samples (12.7%) did not show any bacterial growth. In the present study gram negative organisms were isolated frequently (72.3%) than gram positive organisms (27.7%). Klebsiella pneumonia (37.2%) and Pseudomonas aeruginosa (25.6%) were the predominant gram negative bacilli whereas Methicillin Resistant Staphylococcus aureus (MRSA) (36.4%) was the most predominant in gram positive organisms. Among gram negative isolates 81.4% were susceptible to polymyxin-B followed by imipenem (76.7%), meropenem (67.4%), piperacillin/tazobactam (63.9%), cephalothin (20.9%) and ceftazidime (12.8%). Gram positive isolates were 100% susceptible to vancomycin and linezolid, followed by tetracycline (78.9%), gentamicin (75.8%), cephalothin, erythromycin and co-amoxyclav (51.5%). Conclusion: Both gram positive cocci and gram negative bacilli caused DFI and in this study there is predominance of gram negative organisms mainly Klebsiella pneumonia and Pseudomonas aeruginosa and among gram positive organisms MRSA was frequently isolated.
Keywords: Antibiotic susceptibility, Bacterial isolates, Diabetic foot infections, Gram negative isolates, MRSA

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder of endocrine system which is a major public health problem and has a rising prevalence worldwide. India is estimated to have 61.3 million people suffering with Diabetes mellitus and each year seven lakhs new cases are diagnosed which also include children, teenagers and young adults [1]. It is estimated that these cases may increase to 87 million by 2030 [2]. DM is a multifaceted disease having several complications like neuropathy, nephropathy, retinopathy, peripheral vascular disease and also Diabetic foot infections (DFI). DFI are the leading cause of hospitalization among diabetic patients [3].

It is likely that 15% of people with diabetes develop foot ulcer during their life which cause severe disability and hospitalization and also economic burden to families and health system [4, 5]. Uncontrolled or poorly controlled diabetes will reduce the effectiveness of immune cells fighting against bacteria where even a small cut may lead to an open sore called ulcer. In patients with diabetes there may be impaired micro-vascular circulation to the region of diabetic foot which limits the access of phagocytic cells resulting in bacterial infection [6].

Staphylococcus aureus and βeta hemolytic *streptococci* are widely recognized as pathogens in DFI. The role of other frequently isolated organisms is less

clear. Some studies have shown that with optimal specimen collection, transport and culture techniques used, multiple organisms were recovered from DFI [7]. The present study was undertaken to determine the microbiological profile and antibiotic sensitivity pattern of the isolates from diabetic foot infections (DFI). According to some studies the DFI with multiple organisms will lead to interactions between these organisms leading to production of virulence factors such as hemolysins, proteases, collagenases and also short chain fatty acids which cause inflammation and impede wound healing resulting in chronicity of infection [8].

MATERIALS & METHODS

A total of 110 patients with DFI were included in this study Over a period of one year (from Jan 2016-Dec 2016) and samples were collected from deeper portion of the ulcers by using two sterile swabs by making firm and rotatory movement of the swabs.. Informed consent was taken from the patients.

One swab was used for Gram's staining and the other for culture. Direct culture was done on blood agar and MacConkey agar. The inoculated plates were incubated at 37^{o}c for 18 to 24 hrs. Then they were examined for growth and further processing was done according to the nature of isolate which was by Gram's staining, colony morphology and biochemical properties.

Antibiotic susceptibility testing was done by using Kirby-Bauer disc diffusion method as per CLSI guidelines 2014-15 using Himedia discs [9] and the antimicrobial discs used were polymyxin-B (300µg) imipenem (10µg), meropenem (10µg), piperacillin/ tazobactam (100/10µg), cefaperazone/ sulbactam (50/50µg), ciprofloxacin (5µg), amikacin (30µg), gentamicin (10µg), Co-Amoxyclav (30µg), Cephalothin (30µg), cotrimoxazole (25µg), neomycin (30µg), ceftazidime (30µg), vancomycin 30(µg), linezolid (30µg), tetracycline (30µg), erythromycin (15µg) , oxacillin (1µg) and penicillin (10µg).

RESULTS

In the present study, the age of patients ranged from 20-80 yrs. The maximum no. of samples (34.5%) collected was in the age group of 50-60 yrs and least (0.9%) was in the age group of 30-40 yrs (Table-1). Out of 110 samples, single pathogen was isolated from 73 (66.4%) samples and more than one organism was isolated from 23 (20.9%) samples, whereas 14 (12.7%) samples did not show any bacterial growth even after 48hrs of incubation.

In the present study, gram negative organisms (78.1%) were more frequently isolated than gram positive organisms (30%). Among gram negative organisms, the most common isolate was *Klebsiella pneumonia* (37.2%) followed by *Pseudomonas aeruginosa* (25.6%) whereas methicillin resistant *Staphylococcus aureus* (MRSA) (36.4%) was the commonest isolate among gram positive organisms. The other Gram positive organisms isolated were methicillin sensitive *Staphylococcus aureus* (33.3%), *Coagulase negative Staphylococcus* (15.2%), *Streptococcus* Species (15.2%) and among gram negative were *Escherichia coli* (15%), *Proteus mirabilis* (17.4%), *Acinetobacter baumanii* (3.5%) and *Citrobacter* Species (1.2%) (Table-2, 3).

Out of 86 gram negative isolates,70 (81.4%) were susceptible to Polymyxin B followed by imipenem (76.7%), meropenem (67.4%), piperacillin/ tazobactam (63.9%) , cefaperazone/sulbactam (58.1%), ciprofloxacin (32.5%), amikacin (61.6%), gentamicin (48.8%), co-amoxyclav (25.6%), cephalothin (20.9%), cotrimoxazole (30.2%), neomycin (46.5%), ceftazidime (12.8%) (Table 4). Out of 33 gram positive isolates, all were susceptible to vancomycin and linezolid followed by tetracycline (78.9%), gentamicin (75.8%), cephalothin, erythromycin and co-amoxyclav (51.5%), neomycin (45.5%), cotrimoxazole (48.5%), ciprofloxacin (21.2%), oxacillin (33.3%) and penicillin (9.1%). (Table-5)

Table 1: Shows the different age groups from whom specimens were collected

Age Group	No. of Samples
20-30 yrs	11 (10%)
30-40 yrs	1 (0.9%)
40-50 yrs	26 (23.6%)
50-60 yrs	38 (34.5%)
60-70 yrs	27 (24.5%)
>70 yrs	7 (6.3%)

Table-2: Shows the different types of gram positive organisms

S.no	Name of the Organism	No. of isolates
1	*S.aureus (MRSA)*	12 (36.4%)
2	*S.aureus (MSSA)*	11(33.3%)
3	*Coagulase negative Staphylococcus*	5(15.2%)
4	*Streptococcus species*	5 (15.2%)
	Total	33(100%)

Table-3: Shows the different types of gram negative organisms

S.no	Name of the organism	No. of isolates
1	*Klebsiella spp*	32 (37.2%)
2	*Pseudomonas aeruginosa*	22 (25.6%)
3	*Proteus mirabilis*	15 (17.4%)
4	*Escherichia coli*	13 (15.15%)
5	*Acinetobacter baumanii*	3 (3.5%)
6	*Citrobacter spp*	1 (1.2 %)
	Total	86 (100%)

Table-4: Showing the Antibiotic Susceptibility of gram positive organisms to various antibiotics

S.no	Name of the Antibiotic	No. of isolates		
		Sensitive	Resistant	Intermediate
1	Co-Amoxyclav	17 (51.5%)	15 (45.5%)	1 (3.03%)
2	Cotrimoxazole	16 (48.5%)	17 (51.5%)	-
3	Cephalothin	17 (51.5%)	16 (48.5%)	-
4	Ciprofloxacin	7 (21.2%)	25 (75.8%)	1 (3.03%)
5	Erythromycin	17 (51.5%)	16 (48.5%)	-
6	Gentamicin	25 (75.8%)	8 (24.2%)	-
7	Linezolid	33(100%)	-	-
8	Oxacillin	11 (33.3%)	12 (36.4%)	-
9	Neomycin	15 (45.5%)	18 (54.5%)	-
10	Pencillin	3 (9.1%)	30 (90.9%)	-
11	Tetracycline	26 (78.9%)	7 (21.2%)	-
12	Vancomycin	33(100%)	-	-

Table-5: Showing the Antibiotic Susceptibility of gram negative organisms to various antibiotics

S.no	Name of the Antibiotic	No. of isolates		
		Sensitive	Resistant	Intermediate
1	Amikacin	53 (61.6%)	33 (38.4%)	-
2	Cefaperazone/ Sulbactam	50 (58.1%)	36 (41.9%)	-
3	Ciprofloxacin	28 (32.5%)	51 (59.3%)	7 (8.1%)
4	Co-Amoxyclav	22 (25.6%)	61 (70.9%)	3 (3.5%)
5	Cephalothin	18 (20.9%)	68 (79.1%)	-
6	Cotrimoxazole	26 (30.2%)	58 (67.4%)	2 (2.3%)
7	Ceftazidime	11 (12.8%)	10 (11.6%)	1 (1.2%)
8	Gentamicin	42 (48.8%)	44 (51.2%)	-
9	Imipenem	66 (76.7%)	20 (23.3%)	-
10	Meropenem	58 (67.4%)	28 (32.5%)	-
11	Neomycin	40 (46.5%)	40 (46.5%)	6 (7%)
12	Polymyxin-B	70 (81.4%)	16 (18.6%)	-
13	Piperacillin/Tazobactam	55 (63.9%)	31 (36.04%)	-

DISCUSSION

DFI are the major public health problem with rising prevalence worldwide and is estimated that 15% of people with DM develop foot ulcer leading to disability and hospitalization. These diabetic foot ulcers are more prone to bacterial infection which may lead to irreversible tissue damage. Complications begin with a small ulcer which is neglected or unrecognized. This gets infected and leads to severe morbidity and also some times to the extent of lower extremity amputations [10]. The progression of infection in DFI may be as a result of compromised immune status, reduction in effectiveness of immune cells due to uncontrolled or poorly controlled diabetes or delayed diagnosis or inappropriate evaluation of extent of infection or suboptimal antimicrobial therapy [11].

In the present study 66.4% of the samples yielded single bacterial isolate whereas 20.9% grew mixed bacterial isolates. According to Priyadarshini *et al.;* single bacterial and mixed bacterial isolates were seen in 50% of the samples [12]. According to Mohd Zubair *et al.;* single bacterial isolates were seen in 56.6% and mixed bacterial isolates were seen in 33% [13]. In the present study, gram negative organisms (72.3%) were isolated more than the gram positive organisms (27.7%). According to Benwan *et al.;* gram negative organisms (51.2%) were frequently isolated than gram positive organisms (32.3%) [14]. According to Vimalin Hena *et al.;* gram negative organisms were isolated in 57.6% whereas gram positive organisms were isolated in 42.3% of samples [15].

In the present study, gram negative isolates were found to be susceptible to polymyxin-B (81.4%) followed by imipenem (76.7%), piperacillin/ tazobactam (63.9%), amikacin (61.6%), cefaperazone/ sulbactam (58.1%), gentamicin (48.8%). Gram positive isolates were found 100% susceptible to vancomycin and linezolid followed by tetracycline (78.9%), co-amoxyclav (51.5%), cotrimoxazole (48.5%), and neomycin (45.5%). According to Mehta VJ *et al.;* gram negative isolates showed 100% susceptibility to imipenem followed by polymyxin-B (88%), piperacillin/tazobactam (38%), amikacin (38%), gentamicin (25%) whereas gram positive isolates showed 100% susceptibility to vancomycin and linezolid followed by tetracycline (90%), neomycin (70%), co-amoxyclav and cotrimoxazole (40%) respectively [16].

According to Manikandan *et al.;* gram negative isolates showed 93% susceptibility to imipenem followed by amikacin (89.6%), gentamicin (83.4%), ceftazidime (74.4%), ciprofloxacin (48%), co-amoxyclav (23.7%) and ampicillin (17.5%) whereas gram positive isolates showed 100% susceptibility to amikacin and gentamicin followed by vancomycin (90.1%), ciprofloxacin (79.3%), erythromycin (77.3%), co-amoxyclav (74.6%), clindamycin (59.7%), oxacillin (54.1%) respectively [17]. The bacterial isolates and their antibiotic sensitivity patterns in the present study were similar to the other studies. Early detection of the pathogens and their treatment with appropriate antibiotics will reduce the possibility of severe complications of DFI like amputation of limbs.

CONCLUSION

Both gram positive cocci and gram negative bacilli caused DFI and in this study there is predominance of gram negative bacilli. DFI are common with increasing prevalence worldwide and in past 3 decades there is marked increase in research in understanding the pathophysiology, diagnosis and treatment of DFI. Patients should be educated towards regular blood glucose monitoring, compliance to diet, life style and also foot care. There are also advanced techniques like r DNA PCR, ERIC PCR etc for evaluation of infection status and diversity of bacterial isolates in DFI. According to literature measurements of inflammatory markers can also be used to distinguish between infected and non-infected foot ulcer i.e colonization. But however, positive results of culture have always priority. Knowledge on antibiotic susceptibility pattern of isolates from DFI is crucial for planning the appropriate treatment of these cases.

It is important to implement the validated guidelines and to audit the processes for specimen collection, processing, identification and antibiotic susceptibility testing to have a better outcome. Health education and creating awareness among the patients regarding the complications of DFI and the necessary preventive measures to be taken play an important role in reducing the morbidity and hospitalization.

REFERENCES

1. Lipsky BA, Pecoraro RE, Larson SA, Hanley ME, Ahroni JH. Outpatient management of uncomplicated lower-extremity infections in diabetic patients. Archives of Internal Medicine. 1990 Apr 1; 150(4):790-7.
2. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes research and clinical practice. 2010 Jan 31; 87(1):4-14.
3. Pappu AK, Sinha A, Johnson A. Microbiological profile of diabetic foot ulcer. Calicut Med Journal 2011; 9:1-4.

4. World Health Organization Fact Sheet N0312, World Health Organization, Geneva, Switzerland 2009.
5. Crawford F, Inkster M, Kleijnen J, Fahey T. Predicting foot ulcers in patients with diabetes: a systematic review and meta-analysis. Qjm. 2007 Feb 1; 100(2):65-86.
6. Boulton AJ. The diabetic foot: a global view. Diabetes/Metabolism Research and Reviews. 2000 Sep 1; 16(S1):S2-5.
7. Bowler PG, Duerden BI, Armstrong DG. Wound microbiology and associated approaches to wound management. Clinical microbiology reviews. 2001 Apr 1; 14(2):244-69.
8. Bowler PG, Davies BJ. The microbiology of infected and noninfected leg ulcers. International journal of dermatology. 1999 Aug 1; 38(8):573-8.
9. Federation ID. IDF diabetes atlas: International Diabetes Federation. Executive Office. 2011.
10. Pecoraro RE, Ahroni JH, Boyko EJ, Stensel VL. Chronology and determinants of tissue repair in diabetic lower-extremity ulcers. Diabetes. 1991 Oct 1; 40(10):1305-13.
11. Lipsky BA, Itani K, Norden C. Treating foot infections in diabetic patients: a randomized, multicenter, open-label trial of linezolid versus ampicillin-sulbactam/amoxicillin-clavulanate. Clinical Infectious Diseases. 2004 Jan 1; 38(1):17-24.
12. Shanmugam P, Jeya M. The bacteriology of diabetic foot ulcers, with a special reference to multidrug resistant strains. Journal of clinical and diagnostic research: JCDR. 2013 Mar; 7(3):441.
13. Zubair M, Malik A, Ahmad J. Clinico-bacteriology and risk factors for the diabetic foot infection with multidrug resistant microorganisms in North India. Biol Med. 2010; 2(4):22-34.
14. Al Benwan K, Al Mulla A, Rotimi VO. A study of the microbiology of diabetic foot infections in a teaching hospital in Kuwait. Journal of infection and public health. 2012 Feb 29; 5(1):1-8.
15. Hena J, Growther L. Studies on bacterial infections of diabetic foot ulcer. African Journal of Clinical and Experimental Microbiology. 2010; 11(3).
16. Mehta VJ, Kikani KM, Mehta SJ. Microbiological profile of diabetic foot ulcers and its antibiotic susceptibility pattern in a teaching hospital, Gujarat.
17. Manikandan C, Prabhakaran P. Clinical and bacteriological profile of diabetic foot infections in Pattukkottai area hospitals, Tamilnadu, India. Int J Curr Res Aca Rev. 2015; 3(4):166-73.