ejpmr, 2018,5(6), 631-635

SJIF Impact Factor 4.897

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

BACTERIOLOGICAL PROFILE OF DIABETIC FOOT INFECTIONS

Priyanka Patil, Ravindra Khadse*, Sonal Chavan and Sharmila Raut

Department of Microbiology, Indira Gandhi Govt. Medical College, Nagpur.

***Corresponding Author: Dr. Ravindra Khadse**

Department of Microbiology, Indira Gandhi Govt. Medical College, Nagpur.

Article Received on 10/04/2018 **Article Revised on 30/04/2018** **Article Accepted on 20/05/2018**

ABSTRACT

Diabetes Mellitus (DM), with its increasing prevalence and incidence, is regarded as a serious public health problem worldwide. The 15% of all diabetic patients develop a foot ulcer at some point in their lifetime leading to hospital admissions and cause of non traumatic lower extremity amputations. A total of 150 pus samples from patients having diabetic foot ulcer were processed for 162 bacteria isolation, antimicrobial susceptibility and drug resistance. The mono-microbial infection was 65.3% and poly-microbial 21.3%. Maximum organisms were isolated from Grade 3 and 4 foot wounds. Monomicrobial flora was predominant in Grade 3, while polymicrobial flora in Grade 4. Gram negative bacilli were more prevalent (79.6%) than gram positive cocci (20.4%). In enterobacteriaceae group, Imipenem (74.3%) was the most effective drug. The 42.8% were ESBL producers, 18.6% AmpC producers, 21.4% carbapenemase producer and 15.7% were MBL producer. In *Pseudomonas aeruginosa* and *Acinetobacter* species were sensitive to Polymyxin B, Colistin and Imipenem., 22% were ESBL producers, 18.6% AmpC producers and 32.2% MBL producers. All *S. aureus* isolates were sensitive to Vancomycin and Linezolid. MRSA were 40.9% and MSSA were 59.1%. Introduction of ESBL, MBL or carbapenemase production in Gram negative bacilli and MRSA is a matter of great concern. Since there is an increasing rate of multidrug resistant organisms, there is a need for continuous surveillance to provide the basis of the empirical therapy and to reduce the risk of the complications.

KEYWORDS: Diabetic foot, Diabetic ulcer, multidrug resistance.

INTRODUCTION

Diabetes Mellitus (DM), with its increasing prevalence and incidence, is regarded as a serious public health problem worldwide. India has a diabetic population of about 50.8 million, which is expected to increase to 87 million by 2030.[1] Studies report that 15% of all diabetic patients develop a foot ulcer at some point in their lifetime. This complication accounts for approximately 20% of hospital admissions in diabetic patients. This can be easily attributed to several practices prevalent in India, such as barefoot walking, inadequate facilities for diabetes care, low socioeconomic status and illiteracy.[2]

Diabetic foot infections (DFI) include cellulitis, abscess, necrotizing fasciitis, septic arthritis, tendonitis and osteomyelitis. The most common and classical lesion is the infected diabetic "mal-perforans" foot ulcer.[3] The major factor which predisposed to the foot ulceration which led to the infection are usually related to peripheral neuropathy and an impaired circulation which limited the access of the phagocytes.[4] Diabetes is the leading cause of non traumatic lower extremity amputations and accounts for more than 50% of amputations.[3,5]

E.coli, *Proteus* spp, *Pseudomonas* spp, *S. aureus*, and *Enterococcus* spp are the most frequent pathogens which are cultured from diabetic foot ulcers. The infections in the diabetic foot are usually polymicrobial due to aerobic bacteria, anaerobes and *Candida* spp. The severe infections usually yield polymicrobial isolates, whereas the milder infections are generally monomicrobial.[6,7]

Management of these infections require isolation and identification of the microbial flora, appropriate antibiotic therapy according to the sensitivity patterns, appropriate wound care, identification of the chronic complications and proper surgical intervention for these complications.[8] Emergence of resistance among organisms against the commonly used antibiotics has been clearly outlined as being largely due to their indiscriminate use.[9]

In view of the above facts, a prospective study was carried out to determine the relative frequency of aerobic bacterial isolates cultured from diabetic foot ulcer and to assess their comparative in vitro susceptibility to the commonly used antibiotics.

MATERIALS AND METHOD

The prospective study was carried out in Indira Gandhi Government Medical College, Nagpur a tertiary care institute. The institutional ethical clearance was obtained. Patients of type1 and type2 diabetes mellitus with recent as well as recurrent infected diabetic foot ulcer were included in the study. The clinical history of the patients such as age, sex, types of diabetes, duration of diabetes, size of ulcer and duration of ulcer were recorded. The ulcers were graded according to the Meggit Wagner's classification.[9]

The ulcer was cleaned with sterile normal saline and the surrounding area with 70% alcohol. Debris, dead and devitalized tissue overlying the ulcer was removed and from each patient, two swabs were collected from the depth of the ulcers. Out of the two swabs collected, one was used for microscopic examination like Gram stain and other for culture. The isolates were identified by standard microbiological techniques by studying their colony characteristics, morphology and biochemical reactions.[10]

Antimicrobial susceptibility of all bacterial isolates was done. Each isolate was subjected to antimicrobial susceptibility test as per CLSI guidelines using Kirby-Bauer disk diffusion technique.[11,12] Commercially available (Himedia Laboratories Pvt. Ltd., Mumbai, India) disks of 6 mm diameter with recommended potency as per CLSI were used. Extended spectrum beta lactamase (ESBL) production, AmpC producers, detected by Disc diffusion test and double disc synergy test (DDST), carbapenemase production by phenotypic confirmatory test. MBL production was detected double disk synergy test, MBL E-strip test and combined disk test.

RESULTS

A total of 150 pus samples from patients having diabetic foot ulcer were processed. Diabetic foot ulcer was more common in males (71.3%) as compared to females (28.7%) with ratio 2.5:1. The mean age of the subjects was 52.07 ± 9.57 years.

Out of 150 pus samples, 130 (86.7%) yielded growth of organisms making total of 162 isolates. 65.3% had mono-microbial infection and 21.3% had poly-microbial infection. Patients were graded according to Meggit Wagner Classification. Maximum number of organisms were isolated from Grade 3 foot wounds (n= 63), followed by Grade 4 (n=47), Grade 2 (n=32) and Grade 1 (n=20). Monomicrobial flora was predominant in Grade 3, while polymicrobial flora in Grade 4.

Gram negative bacilli were more prevalent (79.6%) than gram positive cocci (20.4%). The commonest gram negative isolate was *Pseudomonas aeruginosa* (19.75%), followed by *Klebsiella pneumoniae* (17.9%), *Acinetobacter* species(16.7%), *E. coli* (14.8%), *Proteus* species 8 (4.9%), *Citrobacter* species 7 (4.3%) and *Enterobacter* species 2 (1.2%). Amongst gram positive, *Staphylococcus aureus* (13.6%) was the commonest followed by Coagulase negative *Staphylococci*(CONS) 6 (3.7%) and *Enterococcus* species 5 (3.1%).

Table 1: Antibiotic resistance pattern of Enterobacteriaceae isolates (n= 70).

Drugs	*Klebsiella pneumonia* n= 29 (%)	*E.coli* n=24 (%)	*Citrobacter koseri* n= 7 (%)	*Proteus mirabilis* n= 6 (%)	*Proteus vulgaris* n= 2 (%)	*Enterobacter* spp n= 2 (%)	Total n=70 (%)
Ampicillin	29 (100)	24 (100)	7 (100)	6 (100)	2 (100)	2 (100)	70 (100)
Amoxyclave	23 (79.3)	20(83.3)	5 (71.4)	6 (100)	2 (100)	2 (100)	58 (82.8)
Piperacillin- Tazobactum	17 (58.6)	7 (29.2)	3 (42.8)	1 (16.7)	1 (50)	0 (0)	29 (41.4)
Cefazothin	28 (96.5)	24 (100)	7 (100)	6 (100)	2 (100)	2 (100)	69 (98.6)
Cefixime	25 (86.2)	18 (75)	5 (71.4)	6 (100)	2 (100)	2 (100)	58 (82.8)
Cefotaxime	22 (75.9)	14(58.3)	5 (71.4)	5 (83.3)	2 (100)	1 (50)	49 (70)
Ceftazidime	21 (72.4)	14(58.3)	5 (71.4)	5 (83.3)	2 (100)	1 (50)	48 (68.6)
Cefipime	14 (48.3)	12 (50)	3 (42.8)	5 (83.3)	1 (50)	1 (50)	36 (51.4)
Aztreonam	17 (58.6)	16(66.7)	5 (71.4)	5 (83.3)	2 (100)	1 (50)	46 (65.7)
Imipenum	8 (27.6)	6 (25)	2 (28.6)	1 (16.7)	1 (50)	0 (0)	18 (25.7)
Gentamicin	14 (48.3)	10(41.7)	3 (42.8)	4 (66.7)	2 (100)	0 (0)	33 (47.1)
Amikacin	10 (34.5)	7 (29.2)	2 (28.6)	4 (66.7)	1 (50)	0 (0)	24 (34.3)
Tetracycline	17 (58.6)	13(54.2)	3 (42.8)	5 (83.3)	1 (50)	1 (50)	40 (57.1)
Ciprofloxacin	15 (51.7)	12 (50)	3 (42.8)	5 (83.3)	2 (100)	1 (50)	38 (54.3)
Levofloxacin	10 (34.5)	7 (29.2)	4 (57.1)	4 (66.7)	1 (50)	1 (50)	27 (38.6)

Enterobacteriaceae group showed that Imipenem (74.3%) was the most effective drug followed by Amikacin (65.7%). Ampicillin and Cefazolin were found to be totally ineffective drugs against Enterobacteriaceae. (Table 1).

Out of the 70 isolates of Enterobacteriaceae, 30 (42.8%) isolates were identified as Extended spectrum beta lactamase (ESBL) producers and AmpC producers were 13 (18.6%). Out of these 13 strains, 12 were inducible AmpC producers and a single was non-inducible AmpC producer. Fifteen (21.4%) isolates showed

carbapenemase production. MBL production was detected in the 11 (15.7%) isolates.

Table 2: Antibiotic resistance pattern of Non fermentative gram negative bacilli (*P. aeruginosa* and *Acinetobacter* species) (n=59).

Drugs	Resistant (%)		
	***P. aeruginosa* (n=32)**	***A. baumannii* (n=22)**	***A. lwoffii* (n=5)**
Piperacillin	29 (90.6)	22 (100)	5 (100)
Piperacillin- Tazobactum	10 (31.2)	15 (68.2)	3 (60)
Ceftazidime	26 (81.2)	19 (86.4)	5 (100)
Cepotaxime	--	18 (81.8)	5 (100)
Cefipime	21 (65.6)	15 (68.2)	3 (60)
Aztreonam	22 (68.7)	--	--
Imipenem	9 (28.1)	9 (40.9)	1 (20)
Gentamicin	14 (43.7)	15 (68.2)	2 (40)
Amikacin	10 (31.2)	12 (54.5)	2 (40)
Tetracyclin	--	18 (81.8)	3 (60)
Ciprofloxacin	13 (40.6)	19 (86.4)	3 (60)
Levofloxacin	13 (40.6)	18 (81.8)	2 (40)
Colistin	2 (06.25)	1 (04.5)*	0 (0)*
Polymixin B (300)	0 (0)	--	--

Antibiotic resistance pattern of Non fermentative gram negative bacilli (Table 2) shows that Polymyxin B (100%) and Colistin (93.7%) were the most sensitive drugs for *Pseudomonas aeruginosa*, followed by Imipenem (71.9%), Amikacin (68.7%) and Piperacillin-Tazobactum (68.7%). *A. baumannii* isolates were sensitive to Colistin (95.5%), Imipenem (59.1%).

Amongst the 59 *Pseudomonas aeruginosa* and *Acinetobacter* spp. isolates, 22% were ESBL producers, 18.6% were AmpC producers and 32.2% were MBL producers.

Table 3: Antibiotic resistance pattern of Gram positive organisms (n= 33).

Sr.No.	Drugs	***S. aureus* (n=22)**	**CONS (n=6)**	***Enterococcus* spp. (n=5)**
1.	Penicillin	22 (100)	6 (100)	2 (40)
2.	Ampicillin	--	--	2 (40)
3.	Cefoxitin	9 (40.9)	3 (50)	--
4.	Gentamicin	5 (22.7)	3 (50)	--
5.	High Level Gentamicin (HLG)	--	--	0 (0)
6.	Erythromycin	11 (50)	1 (16.7)	4 (80)
7.	Tetracycline	3 (13.6)	3 (50)	--
8.	Ciprofloxacin	14 (63.6)	4 (66.7)	--
9.	Levofloxacin	13 (59.1)	2 (33.3)	--
10.	Clinadmycin	7 (31.8)	3 (50)	--
11.	Chloramphenicol	8 (36.4)	0 (0)	--
12.	Linezolid	0 (0)	0 (0)	0 (0)
13.	Vancomycin	0 (0) *	0 (0) *	0 (0) *

All *S. aureus* isolates were 100% sensitive to Vancomycin and Linezolid. (Table 3).

Out of 22 *S. aureus* isolates studied, Inducible Clindamycin resistance ($iMLS_B$) was seen in 5 (22.7%) isolates and Constitutive Clindamycin resistance ($cMLS_B$) in 02 *S. aureus* isolates. 09 (40.9%) were Methicillin Resistant *S. aureus* (MRSA) and 13 (59.1%) were Methicillin Sensitive *S. aureus* (MSSA). Of the 6 CONS isolates, 3 (50%) were Methicillin resistant CONS and 3 (50%) were Methicillin sensitive CONS.

DISCUSSION

A diabetic foot infection is any inframalleolar infection in diabetes. Diabetic foot ulcer is one of the most common complications requiring hospitalization among diabetic patients in their fifth decade of life. Diabetic foot ulcers have several factors that may be associated with multidrug resistant microorganisms carriage, such as inappropriate antibiotic treatment, chronic course of the wound and frequent hospital admissions. This study noticed that maximum number of diabetic foot cases (45.3%) was found in sixth decade. The mean age of the subjects was 52.07 ± 9.57 years with male preponderance. Peripheral neuropathy is a major associated factor for diabetic foot ulcers. Similar

observations have been made in earlier studies.[13,15]

The microbial pattern varied according to the grade of ulcer with Gram positive cocci being predominant in Wagner I diabetic foot and Gram-negative organisms as the grade advanced to gangrene. The maximum number of organisms was isolated from Grade 3 foot wounds. The Grade 3 ulcers showed monomicrobial flora while polymicrobial flora is seen in Grade 4. Polymicrobial etiology in diabetic foot ulcers may often be due to previous treatment history. Most of the previous studies observed maximum microbes isolation in grade-IV foot infections.[4,14] In the superficial grades (Wagner 1 and 2), aerobic bacteria (*Staphylococcus* species, *Streptococcus* species and Enterobacteriaceae) are predominant pathogens while drug resistant bacteria and anaerobic bacteria add up in Wagner grade 3 to 5 ulcers.[9]

The some studies reported as Gram-positive aerobes as predominant agents in diabetic foot infections while some found the predominant involvement of Gram-negative isolates.[4,16] These discrepancies could be partly due to the differences in the causative organisms which occurred over time and the geographical variation or the types and the severity of the infections.[13]

Antibiotic sensitivity and resistance pattern in case of enterobacteriaceae group showed that Imipenem (74.3%), Amikacin(65.7%) were effective drugs. This study observed 42.8% ESBL and 18.6% AmpC producer. The Prevalence of 44.7% ESBL producers and 33.33% AmpC β-lactamase producers had been reported previously.[17] ESBL, AmpC, and MBL producers indicates multidrug resistance. Introduction of MBL or carbapenemase production in Gram negative bacilli is a matter of great concern.

Pseudomonas aeruginosa found to be sensitive to Polymyxin B (100%) and Colistin (93.7%), followed by Imipenem (71.9%), Amikacin (68.7%) and Piperacillin-Tazobactum (68.7%). Bengalorkar GM et al[3] reported *Pseudomonas* species were sensitive to Imipenem (68%), Amikacin (53%), Ciprofloxacin (15%). *A. baumannii* isolates were sensitive to Colistin (95.5%), Imipenem (59.1%) while *A. Lwoffii* were sensitive to Colistin (100%), 80% isolates showed susceptibility to Imipenem and Tobramycin. The 50% strains of *Acinetobacte*r were resistant to Cephalosporins, Quinolones, Penicillins, Tetracycline and sensitive to Imipenem and Meropenem.[15] Turhan et al observed 25% of the *Acinetobacter* species had Imipenem resistance.[18] ESBL production was observed in 21.9% *P.aeruginosa* isolates and 22.2% *Acinetobacter* spp. isolates. The carbapenemase production was detected in the 32.2% isolates. Priyadarshini Shanmugam et al[15] reported 30.77% carbapenemase producing *Pseudomonas* and *Acinetobacter* species with 33.3% ESBL, 16.6% carbapenemase producer.

All isolates of *S. aureus* were sensitive to Vancomycin and Linezolid and resistant to Penicillin G. MRSA were 40.9% and 59.1% were MSSA. The 55.50% Methicillin resistant *S. aureus* (MRSA) was observed previously.[14] Inducible Clindamycin resistance ($iMLS_B$) was seen in 05 isolates and Constitutive Clindamycin resistance ($cMLS_B$) in 02 *S.aureus* isolates.

The *Enterococcus* spp. isolates were 100% sensitive to Vancomycin, Linezolid and HLG while high levels of resistance found to Erythromycin, Tetracycline, and Ciprofloxacin. However, no High-level Aminoglycoside resistance was observed in the Enterococcal isolates.[16]

CONCLUSION

Since there is an increasing rate of multidrug resistant organisms, there is a need for continuous surveillance to provide the basis of the empirical therapy and to reduce the risk of the complications. The inadvertent use of broad spectrum antibiotics should be discouraged. The selection of the antibiotic treatment in diabetic foot infections should be based on the predominant organisms which are isolated and their antimicrobial susceptibility patterns. This will improve the overall antibiotic utilization and reduce the emergence of multidrug resistant organisms. It is a high time for microbiology laboratories to introduce β-lactamase testing routinely for the knowledge of their prevalence.

REFERENCES

1. Shaw JE, Sicree RA, Zimmet PZ. Global estimates of the prevalence of diabetes for 2010 and 2030. Diabetes Res Clin Pract., 2010 Jan; 87(1): 4–14.
2. Vijay V, Snehalatha C, Ramchandran A. Socio-cultural practices that may affect the development of the diabetic foot. IDF Bull, 1997; 42: 10-2.
3. Bengalorkar GM, Kumar T.N. Diabetic foot infections: A review. Int J Biol Med Res. 2011; 2(1): 453-460.
4. Ozer B, Kalaci a, Semerci E, Duran N, Davul S, Yanat a N. Infections and aerobic bacterial pathogens in diabetic foot. African J Microbiol Res., 2010; 4(20): 2153–60.
5. Smith SR, Reed JF 3rd. Prevalence of mixed infections in the diabetic pedal wound: a perspective based on a national audit. Int J Low Extrem Wounds., 2002 Jun; 1(2): 125–8.
6. Pecoraro RE, Reiber GE, Burgess EM. Pathways to diabetic limb amputation. Basis for prevention. Diabetes Care., 1990 May; 13(5): 513–21.
7. Viswanathan V, Jasmine JJ, Snehalatha C, Ramachandran A. Prevalence of pathogens in diabetic foot infection in South Indian type 2 diabetic patients. J Assoc Physicians India, 2002 Aug; 50: 1013–6.
8. Citron DM, Goldstein EJC, Merriam CV, Lipsky BA, Abramson MA. Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. J Clin Microbiol, 2007 Sep.; 45(9): 2819–28.

9. Pathare NA, Bal A, Talvalkar G V, Antani DU. Diabetic foot infections: a study of microorganisms associated with the different Wagner grades. Indian J Pathol Microbiol, 1998 Oct.; 41(4): 437–41.
10. Collee JG, Dugaid JP, Fraser AG, Marmion BP, Simmons A. Laboratory strategy in the diagnosis of infective syndromes. In: Collee JG, Fraser AG, Marmion BP, Simmons A eds. Mackie and McCartney practical medical microbiology. 14th ed, Churchill Livingstone, 53-94.
11. Performance standards for antimicrobial susceptibility testing; twenty-fourth informational supplement. Clinical Laboratory Standards Institute (CLSI), 2014.
12. Bauer AW, Kirby WMM, Sherris JC, Turk M: Antibiotic susceptibility testing by a standardized single disk method. Am J Clin Pathol, 1966; 45: 493-6.
13. Mohanasoundaram KM. The microbiological profile of diabetic foot infections. J Clin Diagnostic Res., 2012; 6(3): 409–11.
14. Bansal E, Garg A, Bhatia S, Attri AK, Chander J. Spectrum of microbial flora in diabetic foot ulcers. Indian J Pathol Microbiol, 2008; 51(2): 204–8.
15. Shanmugam P. The Bacteriology of Diabetic Foot Ulcers, with a Special Reference to Multidrug Resistant Strains. J Clin DIAGNOSTIC Res., 2013; 7(3): 441–5.
16. Gadepalli R, Dhawan B, Sreenivas V, Kapil A, Ammini AC, Chaudhry R. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care., 2006 Aug.; 29(8): 1727–32.
17. Varaiya AY, Dogra JD, Kulkarni MH, Bhalekar PN. Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae in diabetic foot infections. Indian J Pathol Microbiol, 2008; 51: 370-2.
18. Turhan V, Mutluoglu M, Acar A, Hatipoglu M, Onem Y, Uzun G, et al. Increasing incidence of Gram-negative organisms in bacterial agents isolated from diabetic foot ulcers. J Infect Dev Ctries., 2013; 7(10): 707–12.