Original Article

Study of bacterial infection and antimicrobial sensitivity pattern in diabetic foot lesion

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Abstract

A cross sectional study was carried out on patients with diabetic foot lesions to determine the spectrum of aerobic microbial flora and to determine the microbial pathogens of the diabetic foot lesions and their antimicrobial sensitivity pattern. A total of 226 organisms were isolated from 218 patients and polymicrobial infection was found in 3.7% cases. Age of the study population ranged from 20 to 85 years of which most of the patients were from 40 to 70 yrs (81%). Male female ratio was= 3:1. In this study, *Pseudomonas* sp. (22.1%), *Proteus mirabilis* (16.4%) and *S. aureus* (14.6%) were the predominant organisms isolated. Antimicrobial susceptibility pattern of the isolates were done in which imipenem and ciprofloxacin were found to be the most effective against all organisms.

Key words: Diabetic foot, Antimicrobial susceptibility, Imipenem

Introduction

Infection is a common complication of diabetic foot lesion. Once diabetic foot becomes infected, it is very difficult to treat and ultimately amputation of limb needs to be done[1]. Diabetes is a metabolic disorder of the endocrine system which involves approximately 17 million people world wide. Each year over 700,000 new cases are diagnosed of which 12,000 to 14,000 are children, teenagers and young adults[2]. Diabetes is often accompanied by serious complications and still today there is no cure[3]. Foot infection is the most common complication of diabetic foot and play main role in the development of moist gangrene[4]. Fifteen percent of people with diabetes develops lesion at some time during their life and 85 % of major leg amputations begin with a foot lesion[5].

Early diagnosis of microbial infection and it's magnitude is essential to use the appropriate antimicrobial therapy and to avoid further complication[6,7]. However, these infections are difficult to treat because these patients have impaired microvascular circulation which limits the access of phagocytic cells and poor concentration of antibiotic in the infected tissue[8]. The organisms that occur in foot infection are generally *Staphylococcus aureus* and *Streptococcus pyogenes* arising from the patient's own skin and *Enterococcus sp.* from bowel[16]. *Pseudomonas sp., Enterococcus sp.* and *Proteus sp.* play a special role and are responsible for continuing and extensive tissue destruction with a poor blood circulation of the foot[9]. Many of these microorganisms develop resistance to commonly used antibiotics largely due to their indiscriminate use[10].

The present study was undertaken to determine the microbial pathogen of the diabetic foot lesions and their antimicrobial sensitivity pattern.

Material and methods

A cross sectional study was carried out on 218 diabetic patients with foot infection during the period of January 2010 to December 2010 in Bangladesh Institute of Health Sciences Hospital, Dhaka. Wound swab and pus were collected from the lesion particularly from sloughy or inflamed tissue. From each patient two swabs were collected. Sterile cotton swabs were moistened with sterile saline before collecting the specimens. One swab was used for isolation of bacteria and the other was used for Gram stain.

For isolation of bacteria, the media used were blood agar and the MacConkey agar, which were incubated at 37°C for 24 hours. The organisms isolated were subjected to antibiotic

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susceptibility testing on Mueller Hinton Agar (Brain heart infusion agar in case of Enterococcus sp.) using Kirby- Bauer disc diffusion method as recommended by CLSI[11].

Antibiotic used were: Amoxiclav (20/10 μg), Ceftazidime (30 μg), Cefixime (5 μg), Ceftriaxone (30 μg), Cefotaxime (30 μg), Carbenicillin (100 μg), Cephradine (30 μg), Cefuroxime (30 μg), Ciprofloxacin (5 μg), Cotrimoxazole (1.25/23.75 μg), Gentamicin (10 μg), Imipenem (10 μg), Chloramphenicol (30 μg), Piperacillin (100 μg), Azithromycin (15 μg), Cefoxitin (30 μg), Oxacillin (1 μg), Penicillin (10 units), Clindamycin (2 μg) and Vancomycin (30 μg). All discs were purchased from Becton-Dickinson, USA.

Rusults

Out of 218 diabetic patients with foot infection, 166 were male and 52 were female (M: F=3:1). Their age ranged from 20 to 85 years. The maximum number of patients having diabetic foot infection belonged to the age group of 60 to 70 years (33.9 %) followed by age group 50 to 60 yrs (31.2 %) (Table I).

Table I: Distribution of patients by age and sex

Age group	Male	Female	Total
20 - 30 yrs	03 (60.0)	02 (40.0)	05 (2.3)
30 - 40 yrs	07 (41.2)	10 (58.8)	17 (7.8)
40 - 50 yrs	24 (70.6)	10 (29.4)	34 (15.6)
50 - 60 yrs	60 (88.2)	08 (11.8)	68 (31.2)
60 - 70yrs	60 (81.0)	14 (18.9)	74 (33.9)
70 - 80 yrs	09 (69.2)	04 (30.8)	13 (6.0)
Above 80 yrs	03 (42.8)	04 (57.1)	07 (3.2)
Total	166 (76.1)	52 (23.8)	218 (100)

Note: Figures in parenthesis indicate percentage.

Table II: Distribution of organisms isolated from sample

Name of organisms isolated	No. of organisms
Pseudomonas sp.	50 (22.1)
Proteus mirabilis	37 (16.4)
Staphylococcus aureus	33 (14.6)
Escherichia coli	27 (11.9)
Enterococcus sp	24 (10.6)
Klebsiella pneumoniae	20 (8.8)
Staphylococcus epidermidis	10 (4.4)
Enterobacter sp	10 (4.4)
Citrobacter sp	09 (4.0)
Acinetobacter sp	05 (2.2)
Morganella morganii	01 (0.4)
Total	226 (100)

Note: Figures in parenthesis indicate percentage.

Out of 218 patients studied, single aerobic bacteria was isolated in 210 cases (96.33 %) and two organisms in 8 cases (3.66 %). *Pseudomonas* sp. (22.1 %) was the predominant organism followed by *P. mirabilis* (16.4 %) and *S. aureus* (14.6 %) (Table II).

Table III shows antibiogram status of the isolated organisms. It was observed that *Pseudomonas* species was found resistant to most of the antibiotics of which Imipenem was found sensitive in 62 % cases followed by Piperacillin (56 %). *Staphylococcus aureus* was isolated from 33 (14.6 %) cases of which 17 (48.5 %) strains were Methicillin resistant *Staphylococcus aureus* (MRSA). It was also observed that out of 27 *E. coli* isolated, 14 (51.8 %) were Extended Spectrum Beta Lactamase (ESBL) positive.

Table III: Antibiotic sensitivity pattern of organisms isolated from diabetic foot lesions

Antibiotics	Pseudomonas sp. N=50	P.mirabilis N=37	S. aureus N=33	E. coli N=27	Enterococcus N=23	K. pneumoniae N=20	S.epidermidis N=10	Enterobacter N=10	Citrobacter N=09	Acinetobacter N=05	M.morganii N=01
Amoxiclav	0 (0.0)	17 (45.9)	17 (51.5)	10 (37.0)	16 (69.6)	6 (30.0)	4 (40)	3 (30.0)	1 (11.1)	0 (0.0)	0 (0.0)
Ceftazidime	5 (10.0)	23 (62.2)	17 (51.5)	9 (33.3)	_	9 (45.0)	6 (60)	6 (60.0)	4 (44.4)	1 (20.0)	01 (100)
Cefixime	2 (4.0)	16 (43.2)	17 (51.5)	5 (18.5)	_	7 (35.0)	6 (60)	4 (40.0)	1 (11.1)	1 (20.0)	0 (0.0)
Ceftriaxone	2 (4.0)	22 (59.4)	17 (51.5)	7 (25.9)	_	10 (50.0)	6 (60)	5 (50.0)	4 (44.4)	1 (20.0)	01 (100)
Cefotaxime	3 (6.0)	19 (51.3)	17 (51.5)	8 (29.6)	_	10 (50.0)	6 (60)	5 (50.0)	1 (11.1)	1 (20.0)	01 (100)
Cephradine	2 (4.0)	15 (40.5)	17 (51.5)	5 (18.5)	_	6 (30.0)	7 (70)	4 (40.0)	1 (11.1)	1 (20.0)	0 (0.0)
Cefuroxime	0 (0.0)	17 (45.9)	17 (51.5)	5 (18.5)	_	6 (30.0)	7 (70)	4 (40.0)	2 (22.2)	1 (20.0)	0 (0.0)
Ciprofloxacin	20 (40.0)	21 (56.7)	22 (66.7)	7 (25.9)	12 (52.2)	11 (55.0)	3 (30)	7 (70.0)	5 (55.5)	2 (40.0)	01 (100)
Cotrimoxazole	2 (4.0)	19 (51.3)	13 (39.4)	4 (14.8)	1 (4.3)	9 (45.0)	3 (30)	6 (60.0)	6 (66.7)	2 (40.0)	01 (100)
Gentamicin	11 (22.0)	27 (73.0)	29 (87.9)	17 (63.0)	17 (73.9)	11 (55.0)	10 (100)	8 (80.0)	7 (77.8)	1 (20.0)	01 (100)
Imipenem	31 (62.0)	36 (97.3)	31 (93.9)	24 (88.9)	22 (95.6)	17 (85.0)	10 (100)	10 (100)	_	4 (80.0)	01 (100)
Chloramphenicol	_	_	_	23 (85.1)	22 (95.6)	_	_	_	_	_	_
Piperacillin	28 (56.0)	_	_	32 (97.0)	25 (92.6)	22 (95.6)	_	_	_	_	_
Carbenicillin	18 (36.0)	_	_	18 (66.7)	_	_	_	_	_	_	01 (100)
Azithromycin	_	_	2 (6.0)	_	_	_	_	_	_	_	_
Cefoxitin	_	_	16 (48.5)	_	_	_	_	_	_	_	_
Oxacillin	_	_	16 (48.5)	_	_	_	_	_	_	_	_
Penicillin	_	_	12 (36.4)	_	_	_	_	_	_	_	_
Clindamycin	_	_	11 (33.3)	_	_	_	_	_	_	_	_
Vancomycin	_	_	33 (100)	_	_	_	_	_	_	_	_

Note: Figures in parenthesis indicate percentage

Discussion

Foot infection is one of the most significant complications of diabetes and precedes lower extremity amputation. [1]Although infection is rarely implicated in the etiology of diabetic foot lesion, these lesions are susceptible to infection[12]. Infection of diabetic foot lesion occurs usually by single organism, but infection by multiple organisms (polymicrobial) may occur sometimes[13, 14].

In this study, 226 organisms were isolated from 218 patients. Single organism was isolated in 210 cases and double organisms were isolated in 8 cases (3.7%) (Table II). In the study of Hena et al [16] and Bansal et al [15] Polymicrobial infection was found in 21% (n=111) and 35% (n=143) cases respectively.

Out of 226 organisms isolated, Pseudomonas sp. (22.1%) was predominant organisms followed by *P. mirabilis (16.4%), S. aureus (14.6%), E. coli (11.9%), Enterococcus sp. (10.6%),*

K. pneumoniae (8.8%) S. epidermidis (4.4%), Enterobacter (4.4%), Citrobacter (4%), Acinetobacter sp (2.2%) and Morganella sp (0.4%) (Table II). Same organisms were isolated by other authors but their distribution correlated with some and contrasted with other authors. Bansal *et al* [15] (n=142) found *P. aeruginosa (22%), S. aureus (19%), E. coli (18%), K. pneumoniae (17%), Proteus mirabilis (11%), Enterococcus sp. (5%), Acinetobacter sp. (4%) and Citrobacter sp*. (1%) in their study. Here *Pseudomonas* was predominant organism like ours but distribution of other organisms was different. However, studies of Chincholikar *et el* [16] and Ramani *et al* [13] showed completely different distribution of organisms. Chincholikar *et al* [16] (n=105) reported highest positivity of S. aureus (31 %) followed by *P. aeruginosa (19 %), K. pneumoniae (18 %), E. coli (15 %)* and *Proteus mirabilis (9.3 %)*. Ramani *et al* [13] (n=75) reported *Proteus sp. (20.7 %)* as their predominant organisms followed by *K. pneumoniae (12.3 %)* and *Pseudomonas sp. (11.7 %)* as the common isolates.

Pseudomonas sp. was the most predominant isolate in this study which showed highest sensitivity to imipenem (62%), followed by Piperacillin (56%), Ciprofloxacin (40%), Carbenicillin (36%) and Gentamicin (22%). In the study of Bansal *et al* [15] sensitivity of *Pseudomonas aeruginosa* (n=31) to Imipenem was (100%) followed by Piperacillin (83.3%), and Ciprofloxacin (n=27) (62.5%) which are higher than ours. In the study of Hena *et al* [17] sensitivity of *Pseudomonas* (n=27) to Imipenem was 100% followed by Piperacillin (81.5%), Gatifloxacin (63%) which are also higher than our study.

P. mirabilis (16.4%) was the 2nd highest isolate in this study, 97.3% of them were sensitive to Imipenem followed by Gentamicin (73%), Ceftazidime (62.2%), Ceftriaxone (59.4%) and Ciprofloxacin (56.7%). *P. vulgaris* (6.3%) (n=7) isolated in the study of Hena *et al* [17] showed sensitivity nearer to our study. However, in the study of Bansal *et al* [15] *Proteus* sp (11%) (n=15) was 100% sensitive to Imipenem, Gentamicin and Ceftazidime which is much higher than ours. However sensitivity of other drugs was almost similar to our study.

In this study, out of 33 *S. aureus*, 17 (51.5%) were methicilin resistant *S. aureus* (MRSA). In other studies the incidence of MRSA was higher than our study; Bansal *et al* [15] found 55.6%, (n=27) Hena et al 16 76.6% (n=47) and Alavi *et al* [8] also found 91% (n=11) MRSA in their studies. Overall sensitivity pattern of *S. aureus* in our study was Vancomycin 100%, Imipenem 93.9%, Gentamicin 87.9%, Ciprofloxacin 66.7%, Amoxiclav, Ceftazidime, Cefixime, Ceftriaxone,

Cefotaxime, Cephradine and Cefuroxime each 54.5%, Cotrimoxazol 39.4%, Penicillin 36.4%, Clindamycin 33.3% and Azithromycin 6%. Some of the sensitivity pattern of our study correlates and some other contrast to finding of other authors. Bansal *et al* [15] found sensitivity of Imipenem 100%, Ceftriaxone 100%, Cefuroxime 75%, Erythromycin 71.4%, Amoxiclav 74%, Ciprofloxacin 70%, Gentamicin 68.4%, and Cotrimoxazol 50%. Here sensitivity of ciprofloxacin and imipenem correlated with ours. Hena *et al* [17] found Gentamicin 38.2%, Vancomycin 29.7%, Ciprofloxacin 25.5%, Cefepime 73.4% and Penicillin 4.2%. Here excepting cefepime all other drugs were less sensitive than ours. Alavi *et al* [8] found Ciprofloxacin 91%, Clindamycin 46%, Vancomycin 37.7%, Ceftriaxone 19% and Amoxicillin 9% sensitive. Here ciprofloxacin and Clindamycin shows higher sensitivity than ours. It was noted that Vancomycin was 100% sensitive to *S. aureus* in our study whereas it was 29.7% and 37.7% sensitive in the study of Hena *et al* [17] and Alavi *et al* [8] respectively.

In this study among the 26 (11.9%) isolates of *E. coli* sensitivity to Piperacillin was (92.6%) followed by Imipenem (88.9%) Gentamicin 63%, Amoxicalv (37%), Ceftazidime (33.3%), Ciprofloxacin (25.9%), Cephradine (18.5%), Cefuroxime (18.5%), and Cotrimoxazol (14.8%).

In the study of Hena *et al* [17] sensitivity of *E. coli* (n=17) to Imipenem (94%) and Gentamicin (58.8%) was similar to our study but sensitivity to other drugs was higher than ours. However Bansal et al [15] reported sensitivity of *E. coli* (n=26) to Imipenem 100%, Ciprofloxacin 33.3%, Amoxiclav 26.9% which are in concordance with our studies. Sensitivity of Gentamicin in their study was 30% which was lower than that of our study. However sensitivity to other drugs is much higher than our study.

In our present study, out of 27 *E. coli,* 14 (51.8%) were ESBL positive but no *Klebsiella* was found to be ESBL positive. The incidence of ESBL positive of *E. coli* correlates with the study of Varaiya *et al* [19] (43.4%) (n=54). However, they also found 51.7% of *K. pneumoniae* ESBL positive [19] which was absent in our study.

Other organisms isolated in our study were of lesser frequency and showed varied degree of sensitivity pattern which correlates well with that of Bansal *et al*.[15] When effectivity of drugs was considered Imipenem and Ciprofloxacin was found to be the most effective than other drugs against all Gram negative and Gram positive organisms. This correlates with the study of Hena *et al*[17].

Conclusion

Varieties of organisms were isolated from infected diabetic foot lesions which showed drug resistance to varying degrees. So selection of appropriate drugs should be done after getting culture and sensitivity report. However, Imipenem and Ciprofloxacin may serve as a drug of choice for empirical treatment before getting culture and sensitivity report.

References:

1. Sing N, Armstrong D G, Lipsky B A. Preventing foot ulcers in patients with diabetes. JAMA. 2005; 293: 217-28.

2. Lipsky B A, Pecoraro R E, Larson S A, Hanley M E, Ahroni J H. Out patient management of uncomplicated lower extremity infection in diabetic patients. Arch. Intern. Med. 1990; 150: 790-797.

3. Shea K W. Antimicrobial therapy for diabetic foot infections. A practical approach. Postgrad Med. 1999; 106(1): 85-86, 89-94.

4. Smith J M B, Payne J E, Berue T V. Diabetic foot lesions of skin and soft tissue infections of surgical importance. Chapter 14. In: The surgeons Guide to Antimicrobial Chemotherapy. 2002; 218-21.

5. Ramani A, Ramani R, Shivanda P G and Kundaje G N (1991). Bacteriology of diabetic foot ulcer. Indian. J Pathol. Microbiol. 1991; 34: 81-7.

6. Frykberg R G. Diabetic foot ulcer: current concepts. J. Foot Ankle Surg. 1998; 37: 440-46.

7. Baily T S, Yu H M, Rayfield E J. Pattern of foot examination in a diabetic clinic. Am J Med. 1985; 78: 371-74.

8. Alavi S M, Khosravi A D, Sarami A, Dashtebozorg A, Montazeri E A. Bacteriologic study of diabetic foot ulcer. Pak J Med Sci. 2007; 23 (5): 681-85.

9. Armstrong D G, Lavery L A. Diabetic foot ulcers: Prevention, Diagnosis and Classification. Am Farm Physician. 1998; 57:1325-32.

10. Pitte D, Wyss B, Herter- Clevel C, Kursteiner K, Vaucher J, Lew P D. Out come of diabetic foot infections treated conservatively a retrospective cohort study with long term follow up. Arch Inter Med. 1998; 159: 851-6.

11. Performance standards for antimicrobial susceptibility testing. 12th informational supplement. CLSI document M100- S 12, 22 NO.1, Pensylvania USA, 2002.

12. Frykberg R G, Armstrong D G, Giurini J, Edwards A, Kravette M, Kravitz S, et al. Diabetic foot disorders: A clinical practice guideline. American College of Foot and Ankle Surgeons. J Foot Surg. 2000; 39 (5Suppl):51-60.

13. Ramani A, Ramani R, Shivanda P G and Kundaje G N (1991). Bacteriology of diabetic foot ulcer. Indian. J Pathol. Microbiol. 1991; 34: 81-7.

14. Criado E, De Stefano A A, Keagy B A, Upchurch G R, Johonson G Jr. The course of severe foot infection in patients with diabetes. Surg Gynecol obstet. 1992; 175: 134-40.

15. Bansal E, Garg A, Bhatia S , Attri A K, Chander J. Spectrum of microbial flora in diabetic foot ulcers. Depart ment of Surgery and Microbiology, Government Medical College and Hospital Chandigarh, India. Indian Journal of Pathology and Microbiology. 2008; 51(2): 204-8.

16. Chincholikar D A, Pal R B. Study of fungal and bacteriological infections of the Diabetic foot. Indian J Pathol Microbiol. 2002; 50: 15-22.

17. Hena J V, Growther L. Studies on Bacterial infections of diabetic foot ulcer. AFR J. Cln.Microbiol. 2010; 11(3): 146-9.

18. Viswanathan V, Jasmine J J, snehalatha C, Ramachandran A. Prevlence of pathogens in diabetic foot infection in South India type 2 diabetic patients. J Assoc Physicians India. 2002; 60: 1013-6.

19. Varaiya A Y, Dogra D J, Kulkarni H M, Bhalekar N P. Extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella Pneumoniae in diabetic foot infections. Department of Microbiology, S.L Rahe Hospital, Mumbai. India. 2008; 51(3): 370- 72.