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Microbiological profile of diabetic foot infections in a tertiary care hospital in South India - A prospective study

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Abstract

Background: Diabetic foot infection [DFI] is one of the most common complications in diabetic patient population in which require hospitalization. This leads to long hospital stay and more morbidity.

Objectives: To study the relative frequency of bacterial pathogens from diabetic foot infections and to find their in vitro susceptibility to antimicrobial drugs in use. To find the drug resistance pattern with reference to ESBL (Extended-Spectrum beta Lactamase) production among isolates.

Methods: In our prospective study all possible specimens from DFI which were graded as per Wagner's scoring were collected and processed as per standard guidelines. The isolates were identified by standard biochemical tests and antibiogram performed by Kirby-Bauer disc diffusion method and interpreted as per The Clinical and Laboratory Standards Institute (CLSI) guidelines. Screening for MRSA and ESBL production was done as per standard procedures.

Results: A total of 378 cases with 281 males and 97 females were included.345 specimens yielded positive growth with total 425 isolates.81.16% showed single isolate and rest 2 or 3 isolates. Gram negative organisms were more common than gram positive organisms. *Staphylococcus aureus* was the commonest followed by *Pseudomonas aeruginosa* in ours study. Others were *S.epidermidis, E.coli, K.pneumoniae, Acinetobacter, Proteus sp, Enterococci sp*. Vancomcin, Linezolid and Teicoplanin demonstrated 100% sensitivity to Gram positive pathogens and MRSA. Gram negative pathogens exhibited maximum sensitivity to Colistin, Carbapenems and Ps.aeruginosa to Polymixin-B.

Conclusion: To conclude *Staphylococcus aureus*, *Pseudomonas aeruginosa* are the common pathogens in DFI. Multi drug resistance among the pathogens is an alarming condition. Vancomycin, Imipenems and Colistin are the first line drugs of choice to start empirical therapy in DFI.

Keywords: DFI (diabetic foot infections), Multi drug resistance, Antimicrobial susceptibility, Staphylococcus aureus, Pseudomonas aeruginosa, Vancomycin, Imipenem

Introduction

Globally, Diabetic foot lesions are a major medical, social and economic problem and leading cause of hospitalization in diabetic patients. Initial management of these infections is done with empirical antibiotic therapy based on susceptibility data performed from clinical data. Accurate management of these lesions depends on culture and antibiotic susceptibility of the pathogens [1]. Up to 30% of diabetic patients with foot ulcer will eventually require an amputation, of which 60% are preceded by an infected ulcer [2]. Diabetic foot infection (DFI refers to constellation of signs and symptoms, which includes the presence of purulent discharge (pus), or 2 or more signs and symptoms of inflammation (redness, swelling, pain, tenderness, and warmth) [3]. Diabetic foot infections are associated with long duration hospital stay, high financial costs and long term morbidity and mortality [4].The major predisposing factors for development of foot ulceration and infection are peripheral sensory neuropathy, peripheral vascular disease and deformity. Supporting factors include trauma, edema and hyperglycemia. In a diabetic ischemic limb ,with loss of skin integrity the foot tissues, planes are more prone to development of infection with further extension to deep fascial planes and bones complicated by necrosis and gangrene of the foot [5,6,7]. Management of DFI is dependent on microbial etiology. Diabetic foot ulcers are colonized by a variety of microorganisms. However all cases of DFI do not require antimicrobial therapy unless the bacterial etiology is proved. Several variable and contradictory studies are available regarding the microbial etiology of DFI [8]. Earlier studies have mentioned that many DFI are polymicrobial and gram positive organisms like *Staphylococcus aureus, Streptococci* are predominant etiological agents of DFI. Recent studies indicate that they are mono microbial in nature and gram negative organisms particularly members of family Enterobacteriaceae, Pseudomonas are in increasing trend [9,10]. More of concern than the agents is development of drug resistance among them and their role in DFI. These MDRO (Multi drug resistant organisms) like MRSA, ESBL producers, MBL, Amp-c producers are further complicating the situation making amputation of the foot more common. The present study was done to find the possible aerobic bacterial pathogens in DFI and to find the drug sensitivity pattern of the isolates. The study would help to design a prophylactic protocol to start an empirical therapy before results of culture and sensitivity are available.

Methods

A prospective study was conducted at Narayana medical college and Hospital during the period from March 2012 to February 2013. The study was approved by the ethical committee of the hospital. All patients attending the OPD and IPD of department of surgery with diagnosed Diabetic Foot Infections (DFI) were enrolled in the study. The patients were clinically assessed and all the data regarding age, Duration of diabetes, Type of treatment received and presence of other systemic illness was recorded. The foot infections were clinically examined and graded as per Wagner's grading system [11]. *Grade 1*: Superficial Diabetic Ulcer, *Grade 2*: Ulcer extension with Involvement of ligament, tendon, joint capsule or fascia, No abscess or Osteomyelitis, *Grade 3*: Deep ulcer with abscess or Osteomyelitis, *Grade 4*: Gangrene to portion of forefoot and *Grade 5*: Extensive gangrene of foot.

Specimen collection and processing

Specimens were collected after obtaining informed consent from the patient. Type of specimen depends upon the grade of the lesion. Two Surface swabs were collected from Grade-1 & 2 after thorough washing of the wound with sterile Distilled water and

70% alcohol from the base of the ulcer. Sterile swabs dipped in Glucose broth was used for collecting the specimen with thorough rotating the swab from the base of lesion. The other specimens collected were tissue biopsy, wound curettage, aspiration and necrotic material from the lesion depending upon the grade of the lesion. All the specimens were processed for isolation of aerobic bacteria. Anaerobic isolation was not performed due to technical difficulties. The surface swabs were inoculated on 5% sheep blood agar, Macconkey agar and chocolate agar and incubated at 37^0C in presence of 5% CO_2.The other swab was used for preparation of the smear and performed gram staining and observed under microscope. All the other specimens were processed as per the same standard guidelines [12]. The culture media was examined after incubation for 24 -48 hrs and the colonies of the growth were performed gram staining and observed under the microscope. After staining the isolates were performed standard biochemical tests for identification.[12] All the identified isolates were tested for antibiogram. The antibiogram was performed by Kirby-Bauer disc diffusion method with antibiotic discs obtained from Himedia labs Mumbai and *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25912, *Klebsiella pneumonia* ATCC 700603, *Pseudomonas aeruginosa* ATCC 27853 were used as quality control strains. [13]

The following antibiotic discs were placed on Muller Hinton agar and incubated at 37^0c for 24hrs and zone of inhibition was measured as per manufacturer's instructions. Penicillin, Ampicillin, Erythromycin, Azithromycin, Oxacillin, Cefoxitin, Cefotaxime Chloramphenicol, Clindamycin, Ofloxacin, Vancomycin, Linezolid, Teicoplanin and additional Amoxy-clav, Amikacin, Imipenem, Meropenem, Piperacillin –tazobactam, Polymixin-B and colistin for gram negative bacilli.

Results

A total of 378 patients of which 281 males (74.34%) and females 97(25.66%) were enrolled in the study. Males are predominant in the study. The age of the patients was between 17-65 years with mean age being of 41 years. By grading of the lesions 83(21.96%) were grade 1, 162(42.86%) grade 2, 103(27.25%) grade 3 and 30(7.93%) were in grade 4 & 5. In the study 345(91.27%) were positive for growth with 280(81.16%) showing single isolate, 50(14.5%) two isolates and 15(4.34%) with three isolates. A total of 425 isolates were isolated in the study **[Table-1].**

Table 1: Characteristics of Diabetic foot specimens & isolates		
Total no of cases	**:**	**378**
Males	:	281(74.34%)
Females	:	97(25.66%)
Grading of cases		
Grade 1	:	83 (21.96%)
Grade 2	:	162 (42.86%)
Grade 3	:	103 (27.25%)
Grade 4 and 5	:	30 (7.93%)
No of positive cultures	**:**	**345 (91.27%)**
No of Isolates	**:**	**425**
Single isolate	:	280 (81.16%)
Two isolates	:	50 (14.5%)
Three isolates	:	15 (4.34%)

Data of the study shows Gram negative organisms were more predominant 267(62.82%) than gram positive organisms 158(37.18%). In our study among gram positive *Staphylococcus aureus* was predominant (98/158, 23.06%) followed by *Staphylococcus epidermidis* (55/158, 12.94%) and *Enterococcus faecalis*(5/158, 1.18%) . Among gram negative pathogens *Pseudomonas aeruginosa* was predominant (80/267, 18.82%) followed by *Escherichia coli* (59/267,13.88%) and rest *Klebsiella pneumoniae* (11.29%), *Enterobacter aerogenes*(6.59%), *Citrobacter freundii*(5.18%), *Acinetobacter baumanii*

(4.24%), *Proteus vulgaris*(2.17%) and *Proteus mirabilis*(0.71%).**[Table-2].**

In our study the antibiotic sensitivity of gram positive pathogens the pathogens (%) **[Table-3].** Among *Staphylococcus aureus*, 38% were resistant to oxacillin indicating MRSA. MRSA pathogens were further confirmed by Cefoxitin (30µg) disc test. A zone of inhibition which was < than or equal to 21mm was considered as MRSA. MRSA were tested for Vancomycin, Linezolid and Teicoplanin by Kirby-Bauer disc diffusion test. All of MRSA were 100% sensitive to all the three antibiotics. Gram negative pathogens exhibited maximum sensitivity to Colistin, Imipenem and Meropenem and maximum resistance to Ampicillin and Cefuroxime. ESBL screening for the gram negative pathogens was done by using Ceftazidime and Ceftazidime/Clavulanic acid disc. An increase in zone diameter or equal to 5mm when used in combination than used alone was taken as ESBL producer. In our study of all the gram negative pathogens *Escherichia coli* was maximum ESBL producer with 22 in number, followed by *Pseudomonas aeruginosa* 20, *Klebsiella pneumoniae* 16, *Enterobacter aerogenes* 6, *Citrobacter freundii* 8, *Acinetobacter baumanii* 8 and *Proteus sp* 4 in number. Antibiogram of these isolates demonstrated maximum sensitivity to Colistin followed by Carbapenems (Imipenem & Meropenem). **[Table-4]**

Table 2: Isolation & distribution of pathogens	
Gram positive organisms (No& %)	**:158 (37.18)**
Staphylococcus aureus	98(23.06%)
Staphylococcus epidermidis	55(12.94%)
Enterococcus faecalis	5(1.18%)
Gram negative organisms (No&%)	**:267(62.82)**
Escherichia coli	59 (13.88%)
Pseudomonas aeruginosa	80 (18.82%)
Klebsiella pneumoniae	48 (11.29%)
Enterobacter aerogenes	28 (6.59%)
Citrobacter freundii	22(5.18%)
Acinetobacter baumanii	18 (4.24%)
Proteus mirabilis	9 (2.12%)
Proteus vulgaris.	3 (0.71%)

Table 3: PERCENTAGE OFANTIBIOTIC SENSITIVITY OF GRAM POSITIVE PATHOGENS (%)

	Pen	Azn	Cfxn	Ctxm	Cpml	Cdmn	Vmn	Oxn	Ermn	Ofxn	Lzd	Tpn
Staphylococcus aureus	54	78	70	81	79	90	100	72	74	91	100	100
Staphylococcus epidermidis	64	77	78	88	71	92	100	78	78	92	100	100
Enterococcus faecalis	73	82	71	86	77	92	100	82	79	90	100	100

Pen: Pencillin, Azn: Azithromycin,Cfxn: cefoxitin, Ctxm: Cefotaxime, Cpml: Chloramphenicol, Cdmn: clindamycin, Vmn: vancomycin, Oxn: Oxacillin, Ermn: Erythromycin, Ofxn :Ofloxacin, Lzd:Linezolid, Tpn: teicoplanin

Table 4: PERCENTAGE OFANTIBIOTIC SENSITIVITY OF GRAM NEGATIVE PATHOGENS(%)

	E. coli	*P. aeruginosa*	*K. pneumoniae*	*E. aerogenes*	*C. freundii*	*A. baumanii*	*P. mirabilis*	*P. vulgaris.*
Amp	36	NT	24	28	43	NT	29	32
Amxv	82	81	83	88	82	80	85	84
Akn	82	80	83	82	85	82	90	90
Ofxn	89	88	83	84	90	80	82	83
Ctxm	78	74	75	71	79	70	71	74
Cfxm	78	77	72	70	74	73	73	72
Ctzm	84	88	80	81	82	79	72	77
Cpm	88	82	89	89	81	80	82	82
cpz+slm	92	90	91	91	92	93	93	96
Piptaz	95	96	93	99	91	93	93	94
Ipm	93	92	91	95	95	95	95	96
Mpm	93	94	93	98	99	92	94	94
PxnB	NT	94	NT	NT	NT	NT	NT	NT
Cstn	100	100	100	100	100	100	100	100

Amp:Ampicillin, Amxv:Amoxyclav, Akn:Amikacin, Ofxn:ofloxacin, Ctxm:Cefotaxime, Cfxm:Cefuroxime, Ctzm:Ceftazidime, Cpm:Cefepime, Cpz+slm: Cefoperazone+sulbactam, Piptaz: piperacillin+tazobactam,Ipm:Imipenem,Mpm:Meropenem,Pmx-B:Polymyxin-B,Ctsn:Colisistin

Discussion

DFI are a serious complication in diabetic patients requiring hospitalization and amputation. DFI are associated with increase in hospital stay, financial burden and more mortality and morbidity. Our present study reveals that males are more common than females and the most common age group being 17-60yrs. The percentage of growth positivity was 91.27% which is very high when compared with the other studies of Mohd Zubair et al [14] , Anandi et al [15] Rama Kant et al [16] and Citron et al [17]. Mono microbial growth was 81.16% in our study when compared to studies of Mohd Zubair et al [14] , Anandi et al [15] Rama Kant et al [16] and Citron et al [17] who reported 56.6%, 19%, 23 % of mono microbial growth in their studies. The study of Pappu K et al [18] reported 92% of mono microbial growth in his study which parallels the findings of our study. The commonest two isolate combinations were *Pseudomonas aeruginosa* with *Escherichia coli* followed by *Escherichia coli* and *Staphylococcus aureus*. Our study analysis reveals that Grade-2 as the most common type of DFI. Gram negative organisms are more common (62.82%) than gram positive organisms (37.18%) in our study which confers with the findings of Pappu K et al [18], Pathare NA et al [19] and others. However studies of Mantey I et al[20];Dang CN et al;[21] reported Gram positive organism as predominant in their study. The ratio of gram positive to gram negative organisms in our study was 0.6:1 .This can be clearly explained by the differences in age, sex distribution, grading of ulcers, study settings may be the reason for the difference. Analysis of our study identifies *Staphylococcus aureus* as the most common pathogen followed by *Pseudomonas aeruginosa*, *Escherichia coli*. This is in agreement with many other reported studies [22]. However these findings are in contrast to findings by studies of Pappu K et al [18] and Zubair et al [14] who reported *Escherichia coli & Pseudomonas aeruginosa* as predominant pathogens. In our study 38% of *Staphylococcus aureus* are MRSA which is in accordance with studies of Raja NS [23] but against the findings of M.B. Girish et al [24] who reported only

15%. All the MRSA were sensitive to Vancomycin, Linezolid and Teicoplanin in our study. *Pseudomonas aeruginosa* was resistant to most of the antibiotics except Colistin, Polymyxin-B and Carbapenems (Imipenem & carbapenem) which coincides with the studies of Kajetan M et al; [25] and Jones EW et al[26]. ESBL production was noticed in all the isolates with maximum among *Escherichia coli* followed by *Pseudomonas aeruginosa*. But other studies reports *Pseudomonas aeruginosa* as the most common ESBL producer. Gram negative organisms exhibited resistance to commonly used Cephalosporins, but sensitivity to Ofloxacin, Piperaciilin-tazobactam, Imipenem,Meropenem and Colistin.

Conclusion

Our present study identifies the changing bacterial profile in diabetic foot infections. Gram negative pathogens and particularly Multi drug resistant organisms are increasing and dangering the situation with more concern. Awareness of the causative organisms and their antibiotic susceptibility is essential for institution of appropriate antibiotic therapy. To conclude *Staphylococcus aureus*, *Escherichia coli*, *Pseudomonas aeruginosa* are the common organisms in our study. Vancomycin, Linezolid and Imipenems are the drugs of choice to combat any polymicrobial infection in DFI.

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