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Diabetic Foot Infections: Characterization and Antibiotic Resistance Pattern of Aerobic Bacterial Isolates in a Tertiary Care Hospital of North Kerala

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A B S T R A C T

Foot Infection is a chief complication of diabetes mellitus leading to complications like gangrene and limb amputation and constitutes common diabetes-related cause of hospitalization. This study was undertaken to characterize the aerobic bacterial isolates from diabetic foot infections and analyze their antibiotic resistance pattern in a tertiary care hospital of north Kerala. A retrospective study was carried out on samples of exudates from129 patients with diabetic foot infections over one year period from January through December 2016. Data Analysis was done using WHO NET ANTIBIOTIC RESISTANCE SURVILLENCE SOFTWARE; data was entered in Excel and analyzed using EPI INFO 2013 software. Out of 129 samples studied, 100(77.5%) showed growth of organisms.The most vulnerable age group was 51-60 years where the incidence was 39%. Of these 100 culture positive samples, 66 were monomicrobial and 34 polymicrobial**.** In all a total of 139pathogens were isolated; *Staphylococcus aureus and Pseudomonas aeruginosa* were the predominant isolates. All strains of *S. aureus* were sensitive to vancomycin and linezolid**.** *Pseudomonas aeruginosa* exhibited least resistance to imipenem and meropenem. It is mandatory to characterize the causative agents, determine antibiotic susceptibility and initiate appropriate antibiotic therapy to ensure successful outcome of diabetic foot infections.

Introduction

Diabetes mellitus (DM) is a serious public health problem and remains an important cause of morbidity and mortality worldwide. Foot infection is a major complication of diabetes, eventually leading to development of gangrene and lower extremity amputation (Raja, 2007) and constitutes the most frequent diabetes-related cause of hospitalization (Spichler *et al.*, 2015). Chronic foot infections in patients with diabetes mellitus are common and difficult problems and usually complicate the initially uninfected ulcerations that follow minor trauma in patients with peripheral neuropathy, chronic neuropathic ulcers, or arterial vascular insufficiency and take the form of cellulitis, soft tissue necrosis or osteomyelitis (Pasternack MS *et al.*, 2015). The most frequently isolated pathogens are Gram-negative bacteria including *Proteus* spp., *Pseudomonas aeruginosa*, *Klebsiella pneumoniae* and *Escherichia coli* and Gram-positive bacteria such as *Staphylococcus aureus* including Methicillin-resistant S. *aureus* (MRSA), *Group B streptococci* and

Enterococcus spp, *diphtheroids*, anaerobes and even fungi (Banashankari *et al.,* 2012), (Goldstein *et al.,* 1996). In patients with moderate to severe Diabetic foot infections (DFI) who have not recently received antibiotic therapy, these infections are generally polymicrobial, with mixed gram-positive and gram-negative species (Citron *et al.,* 2007). For infections of greater severity, empiric therapy usually includes activity against both aerobic gram-positive and gram-negative organisms (Matsuura *et al.,* 2013). Given the involvement of different microorganisms and emergence of multidrug resistant strains, clinicians are advised to consider culture before initiation of empirical therapy (Anvarinejad *et al.,* 2015). Any delay in identifying the pathogens and initiation of appropriate antibiotic therapy can result in potential life threatening situation. Hence this study was undertaken to characterize the aerobic bacterial isolates from diabetic foot infections and analyze their antibiotic resistance pattern in a tertiary care hospital of north Kerala.

Materials and Methods

After getting approval from Institutional Ethics Committee, a retrospective study was carried out, based on review of records of 129 patients from whom samples of exudates were collected and processed in the diagnostic section of Department of Microbiology, over one year period from January 2016 through December 2016, meeting inclusion and exclusion criteria. The basic socio-demographic information and data regarding age, gender, details of aerobic bacterial isolates from diabetic foot ulcers and their antibacterial resistance pattern were collected using predesigned Performa, according to standard protocol. During the study period from January 2016 to December 2016, 129 patients with diabetic foot infections were identified based on review of records and request forms sent to the the diagnostic section of Department of Microbiology along with clinical specimens. Non diabetic patients with foot infections due to any other causes and diabetic patients with lesions in regions other than foot were excluded.

All specimens of exudates including pus, tissue or swab samples -were subjected to culture and inoculated onto 5% sheep Blood and McConkey agar plates and incubated at 37°C for 48 hours and Gram stain (KOH preparation if indicative) (Winn *et al.,* 2006). The bacterial isolates were identified based on the study on colony morphology, gram stain and biochemical reactions (Joseph *et al.,* 2017). For Gram positive organisms biochemical tests like catalase, coagulase and mannitol fermentation and for Gram negative fermenters- indole, methyl red, voges-prausker, citrate, urease, TSI agar, oxidase, mannitol motility, nitrate reduction test and esculin hydrolysis were used; additional tests such as fermentation of 10% lactose, decarboxylation of arginine, ornithine and lysine, oxidative fermentation of (OF) of Hugh-Leifson- glucose, lactose, sucrose, mannitol, maltose and xylose and arabinose fermentation tests were done for non-fermenters.

Antibiotic susceptibility testing for the isolates was performed on Muller Hinton agar by the 'Kirby Bauer disc diffusion' method as recommended by the Clinical and Laboratory Standards Institute (CLSI). The antimicrobials for Gram positive organisms were penicillin (10 IU), oxacillin (1µg), ampicillin (10µg), amoxyclav (20/10 µg) cephalexin(30µg), erythromycin (15 µg), gentamycin (10µg), amikacin (30µg), clindamycin (2µg), vancomycin (30µg), linezolid (30µg) ciprofloxacin (5 µg), chloramphenicol, co-trimoxazole (1.25/23.75 µg) and tetracycline (30 µg). Against the Gram negative isolates ampicillin (10µg), cephalexin (30µg), cefuroxime (30µg), gentamycin (10µg), amikacin (30µg), cefotaxime (30µg),

ceftazidime (30µg), ceftazidime+ clavulanic acid (30µg+10µg), cefoperazone + sulbactum (75µg+10µg), cefipime (30µg), ciprofloxacin (5 µg), piperacillin (100µg), piperacillin+ tazobactam (100µg+10µg), imipenem (10 µg), meropenem (10 µg), co-trimoxazole (1.25/23.75 µg) chloramphenicol (30µg) and cefoxitin (30µg) were used.The resistance and susceptibility were interpreted according to CLSI guidelines. *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeruginosa* (ATCC 27853) were used as reference strains. *Data Analysis was done by using WHO NET ANTIBIOTIC RESISTANCE SURVILLENCE SOFTWARE*; data was entered in Excel and analyzed using EPI INFO 2013 software.

Results and Discussion

Out of the total of 129 samples taken up for the study 100(77.5%) showed growth of organisms. Of these positive cultures the incidence was 70% and 30% in male and female patients respectively. In relation to age factor, the most vulnerable age group was 51-60 years where the incidence was 39% (Table 1). Out of these 100 culture positive samples, 66 were monomicrobial and 34 polymicrobial where 29 and 5 showed 2 and 3 organisms respectively (Fig. 1). In all, a total of 139pathogens were isolated from 100 samples, Gram positive and negative organisms constituted 44 (31.6%) and 95 (68.4%) respectively. Among the Gram positive cocci, *Staphylococcus aureus* was the predominant isolate (17.9%) followed by *Enterococci* (10.4%). The leading Gram negative isolate was *Pseudomonas aeruginosa* (18.8 %) followed *by E.coli* (15.2%) and *Klebsiella pneumoniae* (12.9%). In addition, *Candida tropicalis* was isolated from one sample along with *E. coli* (Fig. 2).

All strains of *S. aureus* were sensitive to vancomycin and linezolid; however 84% were resistant to penicillin and ampicillin. *Enterococci* spp. showed 0% resistance to vancomycin and linezolid and 7.1 % resistance to penicillin ampicillin and chloramphenicol. While all isolates of *E. coli* were resistant to ampicillin, resistance to imipenem, meropenem and amikacin was 4.7%, 9.4% and 14.2% respectively. *Klebsiella pneumoniae* organisms were 83.3% resistant to ampicillin and least resistant to imipenem 16.6%. *Pseudomonas aeruginosa* isolates show a high degree of susceptibility to aminoglycosides, higher generation cephalosporins, carbapenems and piperacillin. The antimicrobial resistance pattern of Gram-positive cocci and Gram-negative bacilli is summarized in tables 2 and 3 respectively. The single strain of *Candida tropicalis* isolated was sensitive to nyststin, amphotericin B, itraconazole, ketoconazole, fluconazole and voriconazole.

Table.1 Age and gender distribution among 100 culture positive patients with Diabetic Foot Infections

Age in years	Gender		Number of patients
	M	F	
31- 40	4	3	7(7)
41-50	16	3	19(19)
51-60	23	16	39(39)
61-70	22	5	27(27)
>70	5	3	8(8)
Total	70	30	100(100)

Figures in parenthesis indicate percentage

Table.2 Percentage of antibacterial resistance of Gram positive cocci in DFI

Organisms/ Antibiotics	*Staphylococcus aureus*(25)	*Enterococci* (14)	*B-hemolytic Streptococci*(5)
Penicillin G	84	7.1	20
Ampicillin	84	7.1	20
Amoxyclav	76	-	-
Cephalexin	36	-	-
Gentamycin	20	HLG-0	-
Amikacin	4	-	-
Ciprofloxacin	36	-	0
Clindamycin	24	-	0
Erythromycin	52	21.4	0
Vancomycin	0	0	0
Linezolid	0	0	-
Tetracycline	8	28.5	20
Co-trimoxazole	16	-	-
Oxacillin	36	-	-
Chloramphenicol	-	7.1	-

Total number of isolates given in parenthesis

Table.3 Percentage of antibacterial resistance of Gram negative bacilli in DFI

Organism/ Antibiotics	*Ps. aeruginosa (26)*	*E.coli (21)*	*Kl. pneumoniae (18)*	Proteus. Spp (11)	*Citrobacter spp*. (10)	*Enterob acter* Spp. (4)	*Acineto bacter* (2)	*Kl. oxytoca* (2)
Ampicillin	-	0	83.3	27.2	100	100	100	100
cephalexin	-	33.3	55.5	0	80	75	-	0
cefuroxime	-	47.6	72.2	0	90	75	-	50
Ceftazidime	34.6	-	-	-	-	-	100	-
Cefotaxime	-	80.9	66.6	0	90	75	-	0
Cefipime	30.7	61.9	77.7	0	70	50	100	0
Cefoperazone/Sulbactum	30.7	42.8	55.5	0	60	25	100	0
Gentamicin	19.2	33.3	44.4	0	40	25	100	0
Amikacin	15.3	14.2	33.3	0	30	25	50	0
Ciplox	42.3	66.6	50	0	60	25	100	0
Ofloxacin		57.1	33.3	0	60	25	100	0
Piperacillin	34.6	-	-	-	-	-	-	-
Piperacillin Tazobactum	23	42.8	50	0	60	25	100	0
Cotrimoxazole	-	52.3	50	18.1	50	25	-	50
Chloramphenicol	-	9.4	22.2	27.2	40	25	-	50
Imipenem	7.6	4.7	16.6	0	40	0	50	0
Meropenem	7.6	9.4	27.7	0	40	0	0	0

Total number of isolates given in parenthesis

Fig.1 Number of organisms isolated per DFI from 100 culture positive samples

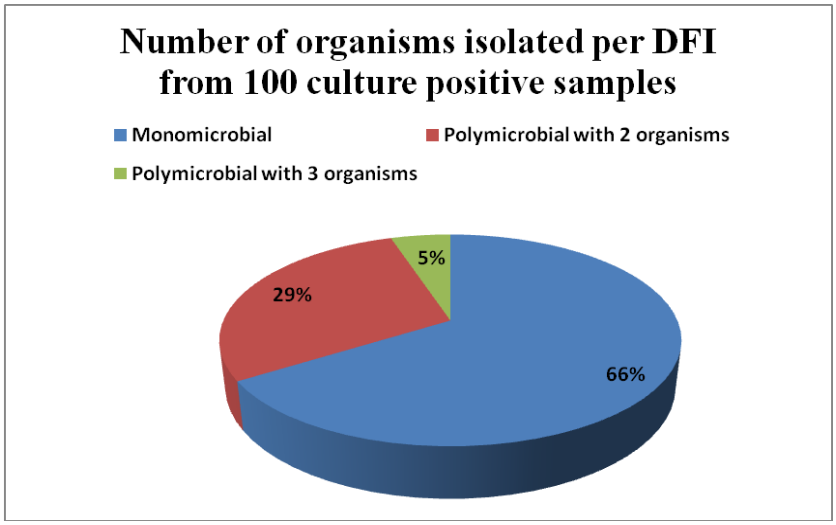
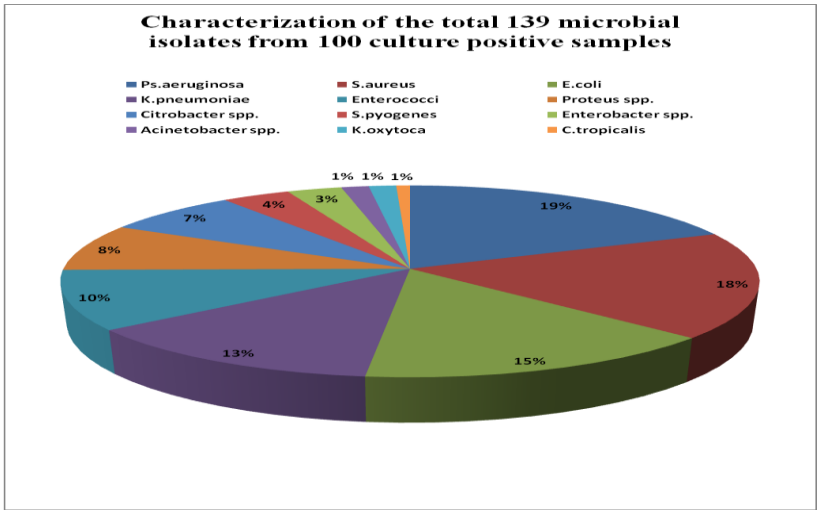


Fig.2 Characterization of the total 139 microbial isolates from 100 culture positive samples



Diabetic foot disease is a major medical, social and economic problem that is seen in every continent and constitutes a major burden to the patient and the health care system (Otu *et al.*, 2013). People with diabetes have about a 25% chance of developing a foot ulcer in their lifetime (Spichler *et al.*, 2015). Most lower extremity amputations in the United States are preceded by a foot ulcer (Boike *et al.*, 2010). In a study by Raja polymicrobial growth was found in 42.8% and 57.2% patients had pure growth whereas in our study the rate of isolation was 34% and 66% respectively. *Staphylococcus aureus* is reported to be the predominant isolate in various studies (Goldstein *et al.*, 1996), (Perim *et al.*, 2015) whereas Gram negative organisms predominated in a study by Banashankari et al (Banashankari *et al.*, 2012) and the common isolate was *Proteus* Spp. followed by E. coli. In our study *Pseudomonas aeruginosa* (18.8 %) and *Staphylococcus aureus* (17.9%) were the predominant isolates and 36 % of *S. aureus*

isolates turned out to MRSA. *S. aureus* and *P. aeruginosa* were the most common causes of diabetic foot infections as reported by Abdulrazak (Abdulrazak *et al.,* 2005).

S. aureus showed 100% susceptibility to vancomycin and linezolid and only 4% resistance to amikacin. In case of gram negative bacilli, *Ps. aeruginosa* and *Proteus* spp. were relatively susceptible to the commonly used antimicrobials whereas *K. pneumoniae* exhibited high level resistance. Imipenem and meropenem showed least resistance to the gram negative organisms. Abdul razak et al reported vancomycin as the most effective agent against gram-positive organisms and imipenem, meropenem, and cefepime against gram-negative organisms (Abdulrazak *et al.,* 2005). The majority (60–80%) of foot ulcers will heal, while 10–15% of them will remain active, and 5–24% of them will finally lead to limb amputation within a period of 6–18 months after the first evaluation (Alexiadou *et al.,* 2012).

Diabetic Foot Infections invariably start in superficial soft tissues, later involve deeper structures, including bone that lead to devastating complications and long-term morbidity. Complications may include necrotizing fasciitis, soft tissue gangrene, septic arthritis, and osteomyelitis. Therefore it is essential to evaluate the different microorganisms infecting the wound and to determine the antibiotic susceptibility patterns of the isolates from the infected wound. This knowledge is vital for planning treatment with the appropriate antibiotics, reducing resistance patterns, and minimizing healthcare costs. Management of DFI depends on the severity of infection and include the route and choice of antibiotic, the need for hospital admission, consideration of surgical intervention, and overall length of therapy. Patients with DFIs usually have several hospitalizations and are exposed to multiple courses of antibiotics. Although diabetic patients with foot infections are initially treated empirically, appropriate antibiotic therapy following microbiological results focussed on the causative organisms will improve the outcome. Therefore it is mandatory to identify the causative agents, initiate appropriate antibiotic therapy for the achievement of a successful outcome.

In conclusion, diabetic foot infection is a serious complication of diabetes which is a major medical, social and economic problem worldwide. Diabetic foot infection is either monomicribial or polymicrobial in nature involving both Gram positive cocci and Gram negative bacilli. Increasing awareness among the physicians on the microbiology of DFI will help in preventing chronic diabetic foot ulceration and reduce the risk of amputation. Therefore it is mandatory to characterize the causative agents, determine antibiotic susceptibility and initiate appropriate antibiotic therapy to ensure successful outcome of diabetic foot infections.

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