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Bacteriological profile of diabetic foot infection with special reference to ESBL and MRSA in a coastal tertiary care teaching hospital

Y. Kavitha[1,*], S. Mohan[2], S. Khaja Moinuddin[3]

[1]Assistant Professor, [2]Associate Professor, [3]Tutor, Dept. of Microbiology, Vinayaka Mission Medical College, Puducherry

***Corresponding Author:**
Email: drkavitapes@gmail.com

Abstract

Introduction: One of the most important complications of diabetes mellitus is foot infection. Bacterial spectrum of diabetic foot infections vary greatly. The increasing association of multi-drug resistant (MDR) bacteria in diabetic foot infections further complicates therapy. Hence, we aimed at determining the prevalence of bacteria in diabetic foot ulcers and their anti-biogram.

Materials and Methods: This is a prospective, observational study. A total of 217 infected diabetic wound samples (pus swab/discharge) were collected from patients seen both IP department and OP departments of Vinayaka Mission's Medical College & Hospital. Antimicrobial resistance pattern was performed, as per standard microbiological procedures including methicillin resistant S. aureus and extended spectrum of beta lactamase (ESBL).

Results: Overall 207 bacteria were isolated. Among them, 122 (58.94%) were Gram negative bacilli and others were Gram positive cocci, 85(41.06). The most common isolate was Pseudomonas (23.67%) followed by Staphylococcus aureus (22.70%), Coagulase negative Staphylococci (15.94%), Klebsiella species (14.97%), Escherichia coli (9.18%). Among 122 Gram negative bacilli, 57 were identified as ESBL producing strains. A total of 47 isolates of Staphylococcus aureus were recorded, 22 were identified as MRSA strains. Majority of Gram negative isolates were susceptible to piperaciillin/tazobactum followed by amikacin. All isolates remained susceptible to cefeperazone/sulbactum and imipenam except non fermenting Gram negative bacilli.

Conclusion: Regular monitoring of bacterial susceptibility patterns helps in guiding clinician to choose apt antibiotic to treat infected diabetic foot. Treatment should be initiated only after performing culture and sensitivity testing. Therefore, the rapid propagation of multi drug resistance can be prevented.

Keywords: Diabetic Foot Infection, ESBL, MRSA, Imipenam, Linezolide.

Introduction

Diabetes is a major public health problem globally which affects a large number of people. Chief cause of morbidity and mortality among diabetic patients is diabetic foot infection.(1) At certain point, all diabetic patients develop foot infection which accounts approximately 15% and around 28% of them may require amputation.(2) Most important predisposing factor for the development of foot infection is usually peripheral neuropathy and impaired circulation.(3) Various microbes are associated with chronic wounds especially in the margin of ulcers. Both aerobic and anaerobic microbes are responsible for causing diabetic foot ulcers.(4) Pseudomonas spp, E. coli, Proteus, S.aureus, and Enterococcus spp are the common pathogens isolated from diabetic foot infections.(5) However, the etiology of wound infection differs from country to country and from hospital to hospital even within the same region.(6) Usually mild diabetic foot infections yield single microbe and severe wound infection exhibit polymicrobial growth.(7)

Association of multi drug resistant bacteria in diabetic foot infections amplify the problem faced by clinician in treating diabetic ulcers.(8) In recent years, high rates of multi drug resistant (MDR) bacteria such as methicillin resistant S.aureus (MRSA) and extended spectrum of ß-lactamase(ESBL) have been reported from different parts of the country and also globally especially among hospitalized diabetic patients. Presence of such multi drug resistant strains make the treatment more complicated which may lead to amputation and even threat to patient's lives.

Therefore, early diagnosis of causative agent involved in diabetic foot infection and selection of appropriate antibiotics to treat multi drug resistant pathogens is required to prevent future complications.

Hence, we aimed at determining the prevalence of bacteria in diabetic foot ulcers and their anti-biogram.

Materials and Methods

This is a prospective, observational study in which a total of 217 infected diabetic wound samples (pus swab/discharge) were collected from patients seen both inpatient and outpatient departments of Vinayaka Mission's Medical College and Hospital, over a period of one year. All the samples were processed in the department of microbiology by inoculating on blood agar, chocolate agar and Mac Conkey agar plates and incubated aerobically for 24 to 48 hours at 37°C. Identification of bacteria was done as per standard procedures.(9)

Exclusion/inclusion criteria: Wound infection was suspected if a wound was not healing well, getting bigger, exuding pus or fluid in diabetic patients, were included. Those who have undergone amputation and antibiotic therapy two weeks prior to the study were excluded.

Antibiotic Susceptibility testing: Susceptibility pattern was done by using Kirby Bauer disc diffusion method according to CLSI guidelines 2014.[10] A pure culture of the organism, which had been freshly.

Grown on blood agar was suspended in normal sterile saline to form a suspension equivalent to 0.5 MacFarland standard turbidity. Suspension inoculated on Mueller Hinton agar and zone size was read after incubating agar plates at 37^0 c for overnight. Following antibiotic discs were used. Ampicillin (20µg), Gentamicin (10µg), Amikacin (30µg), Cefepime (30µg), Cefoperazone/ sulbactam (75/10µg), Piperacillin/ tazobactam (100/10µg), Imipenem (10µg), Azithromycin (15µg), Cefoxitin (30µg), Vancomycin (30µg), Ciprofloxacin (5 µg), Ofloxacin (5µg), Linezolid (30µg), Cotrimaxazole (25 µg) Amoxicillin/Clavulanic acid (20/10µg).

MRSA and ESBL strains were also detected as per CLSI guidelines, 2014.[10]

MRSA detection: MRSA detection was done by using cefoxitin (30µg) disc. Inhibition zone size which was equal to or more than 22mm accounted as cefoxitin sensitive and bacteria was reported as MRSA. Inhibition zone size which was less than or equal to 21mm were reported as MSSA.

Quality control strains:

Methicillin sensitive *S. aureus* (MSSA) ATCC 25923

Methicillin resistant *S. aureus* (MRSA) ATCC 43300

ESBL detection: ESBL strains identified by using discs of Ceftazidime (30 µg) and Ceftazidime/Clavulanic acid (30/10µg) respectively. Organism to be tested was inoculated on a Muellar Hinton agar plate and the above mentioned discs were placed on the plate and incubated at 37^0C overnight. An increase in the zone diameter, which was equal to or more than 5 mm for the antimicrobial agent which was tested in combination with clavulanic acid, in comparison to the antimicrobial which was tested alone, indicated that the strain was an ESBL producer.

Quality control strains:

*K. pneumoniae*ATCC 700603 (ESBL positive control)

E. coli ATCC 25922 (ESBL negative control)

Result analysis was done by simple percentage method.

Results

A total 217 non repetitive specimens from diabetic foot ulcers were received and processed in the department of clinical microbiology. In this study, the age group of patients ranged from 35-80 years. Majority of patients suffered from diabetic foot ulcers ranged between 60-70 years. Out of 217 specimens 179(82.49%) yielded bacterial growth. No bacteria was found in 38(17.51%) specimens. Single bacteria (monomicrobial) was isolated from 154(86.03%) specimens and 25(13.97%) specimens yielded more than one bacteria (polymicrobial). Staphylococcus aureus was the most commonly isolated bacterium in lesions where more than one organism involved. A bacterium isolated from the same patient on more than one occasion was considered to be one isolate if it had the same spectrum of antibiotic resistance.

A total of 207 bacteria were isolated. Among them, 122 (58.94%) were Gram negative bacilli and others were Gram positive cocci, 85(41.06). The commonest isolate was Pseudomonas spp (23.67%) followed by Staphylococcus aureus (22.70%), Coagulase negative Staphylococci (15.94%), Klebsiella species (14.97%), Escherichia coli(9.18%), Proteus mirabilis(3.86%), Citrobacter species (3.38%), Enterococci (2.42%), Non fermenting Gram negative bacilli(1.93%), Providencia species (1.93%). The number and percentage of isolated bacteria from diabetic foot infections presented in Table 1.

Table 1: Bacterial isolates from diabetic foot infections

Organism	Percentage
Pseudomonas species	23.67
Staphylococcus aureus	22.70
CONS	15.94
Klebsiella species	14.97
Escherichia coli	9.18
Proteus mirabilis	3.86
Citrobacter species	3.38
Enterococci	2.42
NFGNB	1.93
Providencia species	1.93

Antibiotic susceptibility pattern of isolates were displayed in tables (Table 2 & 3). Among 122 Gram negative bacilli, 57 were identified as ESBL producing strains. A total of 47 isolates of Staphylococcus aureus were recorded, 22 were identified as MRSA strains. Majority of Gram negative isolates were susceptible to piperaciillin/ tazobactum followed by amikacin. All isolates remained susceptible to cefeperazone/ sulbactum and imipenam except non fermenting Gram negative bacilli. Two strains of non-fermenting Gram negative bacilli were resistant toimipenam and one strain alone was susceptible to cefeperazone/ sulbactum. Susceptibility of ESBL strains shown in Table 4.

Table 2: Susceptibility pattern of gram negative bacilli

Antibiotic	Pseudomonas n=49	Klebsiella spp n=31	E.coli n=19	Proteus spp n=8	Citrobacter n=7	NFGNB n=4	Providencia n=4
Ampicillin	9(18.36)	7(22.58)	5(26.31)	0(0)	2(28.57)	0(0)	2(50)
Amikacin	42(85.71)	25(80.64)	19(100)	8(100)	5(71.42)	2(50)	4(100)
Gentamicin	29(59.18)	15(48.38)	9(47.36)	7(87.5)	3(42.85)	0(0)	4(100)
Ciprofloxacin	25(51.02)	14(45.16)	12(63.15)	2(25)	1(14.28)	0(0)	2(50)
Ofloxacin	32(65.30)	18(58.06)	14(73.68)	4(50)	1(14.28)	0(0)	3(75)
Amoxyclav	31(63.26)	23(74.19)	17(89.47)	5(62.5)	1(14.28)	0(0)	4(100)
Cefepime	19(38.77)	13(41.93)	10(52.63)	2(25)	3(42.85)	0(0)	4(100)
Piperacillin/ Tazobactum	48(97.95)	29(93.54)	19(100)	8(100)	7(100)	1(25)	4(100)
Cefeperazone/ Sulbactum	49(100)	31(100)	19(100)	8(100)	7(100)	1(25)	4(100)
Cotrimaxazole	19(38.77)	14(45.16)	13(68.42)	2(25)	3(42.85)	0(0)	4(100)
Imipenam	49(100)	31(100)	19(100)	8(100)	7(100)	2(50)	4(100)

NFGNB: Non fermenting Gram negative bacilli

Table 3: Antibiotic susceptibility pattern of Gram positive cocci

Antibiotic	MSSA n=25	MRSA n=22	CONS n=33	Enterococci n=5
Ampicillin	14(56)	0(0)	13(39.39)	0(0)
Amikacin	25(100)	13(59.09)	31(93.93)	5(100)
Gentamicin	19(76)	4(18.18)	21(63.63)	5(100)
Ciprofloxacin	12(48)	11(50)	17(51.51)	0(0)
Ofloxacin	23(92)	15(68.18)	19(57.57)	2(40)
Amoxyclav	15(60)	3(13.63)	19(57.57)	2(40)
Cefepime	19(76)	7(31.81)	25(75.75)	1(20)
Piperacillin/ Tazobactum	25(100)	20(90.90)	29(87.87)	4(80)
Cefeperazone/ Sulbactum	25(100)	22(100)	33(100)	5(100)
Cotrimaxazole	21(84)	13(59.09)	15(45.45)	3(60)
Vancomycin	25(100)	11(50)	19(57.57)	3(60)
Linezolide	25(100)	22(100)	33(100)	5(100)
Azithromycin	19(76)	7(31.81)	14(42.42)	2(40)

MSSA: Methicillin sensttive staphylococcus aureus
MRSA: Methicillin resistant staphylococcus aureus
CONS: Coagulase negative staplycococci

Table 4: Susceptibility pattern of ESBL strains

Antibiotic	Pseudomonas n=21	Klebsiellaspp n=15	E.coli n=10	Proteus spp n=4	Citrobacter n=4	NFGNB n=4
Ampicillin	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Amikacin	17(80.95)	13(86.66)	10(100)	4(100)	2(50)	2(50)
Gentamicin	10(47.61)	5(33.33)	2(20)	3(75)	0(0)	0(0)
Ciprofloxacin	5(23.80)	3(20)	3(30)	0(0)	0(0)	0(0)
Ofloxacin	9(42.85)	5(33.33)	5(50)	1(25)	0(0)	0(0)
Amoxyclav	15(71.42)	8(53.33)	8(80)	1(25)	0(0)	0(0)
Cefepime	5(23.80)	2(13.33)	2(20)	1(25)	0(0)	0(0)
Piperacillin/ Tazobactum	20(95.23)	13(86.66)	10(100)	4(100)	4(100)	1(25)
Cefeperazone/ Sulbactum	21(100)	15(100)	10(100)	4(100)	4(100)	1(25)
Cotrimaxazole	7((33.33)	3(20)	4(40)	0(0)	0(0)	0(0)
Imipenam	21(100)	15(100)	10(100)	4(100)	4(100)	2(50)

Discussion

Diabetic foot have more tendency to develop bacterial infections that spread rapidly, leading to irreversible tissue damage.[11] Spectrum of bacteria vary widely in diabetic foot infections. Therefore, regular monitoring microbial spectrum and their anti-biogram helps in choosing appropriate antibiotics.[12] Majority of patients in our study belonged to the age group of above 55 years. This could be due to the fact that foot lesions occur commonly among patients with diabetes, particularly the elderly and those with sensory neuropathy.[13] Earlier studies reported preponderance of males.[14] However, in our study major difference was not observed between male (n=119) and female (n=98) patient numbers. No bacterial growth was found in 38(17.51%) specimens. The sterile culture in such cases may be due to topical application of antibiotics to the infected part.

In our study, majority of specimens yielded single isolate (86.03%).This finding correlate with Pappu et al study who reported 92% monomicrobial growth.[15] Anandi et al.,[1] Zubair et al.,[16] Rama Kant et al.,[17] and Citron et al.,[7] have reported 19%, 56%, 23% and 16.2% monomicrobial growth and 67%, 33%, 66%, and 83% polymicrobial growth infections respectively. bacteria in Staphylococcus aureus was common bacteria isolated from polymicrobial infections.[1]

In our study, no anerobic bacterial culture was performed. Involvement of anaerobic bacteria in diabetic foot infections is not clear and few studies reported minor role of anaerobic bacteria[18] while other studies reported preponderance of anaerobic bacteria.[19]

In our study, Gram negative bacteia isolated predominantly (58.94%), while Gram positive cocci accounted for 41.06%. Among Gram negative bacteria, Pseudomonas species (23.67%) was commonly isolated pathogen followed by Staphylococcus aureus (22.70%). In contrast, according to Mohanasundaram, S.aureus (26.1%) was the most common pathogen, followed by E.coli (18.4%).[20] However, our results are similar to the study conducted by Ramakanth et al,[17] he studied the changing trends of bacteriological spectrum in diabetic foot infections ulcers for a period eight years and reported the isolation rate of Gram negative bacteria from 50.6%-66% and predominant pathogen was Ps.aeruginosa.

Extended-spectrum β-lactamases (ESBLs) are a rapidly evolving group of enzymes which have the capability to hydrolyze third-generation cephalosporins and aztreonam but are inhibited by clavulanic acid. Very broad antibiotic resistance extending to multiple antibiotic classes is now a frequent characteristic of ESBL-producing isolates. ESBL production by clinical isolates is a therapeutic challenge in view of the expense, usage of broad-spectrum antibiotics, frequent need of intravenous therapy, and infection control considerations. Management of systemically stable patients in hospital setting may give rise to cross infection, escalated cost and increased morbidity. Therefore, the knowledge of antibiotic susceptibility pattern is mandatory for choosing appropriate therapy. In our study, 46.72% ESBL producers were recorded. ESBL production was high among Klebseilla species (15 out of 16). All ESBL producers remained susceptible to imipenam except two strains of non-fermenting Gram negative bacilli. In our study, high degree of susceptibility was observed against all isolates with cefeperazone/ sulbactum followed by piperacillin/ tazobactum. Gadepalli et al[21] also reported, ESBL production in 44.7% of bacterial isolates while Umadevi et al[22] and Akhi et al[23] demonstrated that ESBL production was found in 56% and 31.3%. of *Enterobacteriacea* members respectively Among Gram positive cocci, S. aureus remained as predominant pathogen. MRSA exhibit resistance to beta lactum antibiotics and drug of choice is limited for treating such strains. Inspite of MRSA strains susceptibility to few beta lactums in vitro, clinically they are ineffective.[24] Even though the oxacillin disc diffusion test has more reliability for detecting methicillin resistance, cefoxitin disc method was performed in our study. The accurate identification of methicillin resistance in Staphylococci by the oxacillin disc diffusion method may be affected by various factors such as medium, temperature, and the time of incubation.[24]

In this study, 46.81% MRSA were isolated. Previous studies reported 15-30% MRSA from diabetic foot ulcers. In our study, 11 strains (50%) of MRSA and 2 strains (40%) of Enterococci exhibited resistance to vancomycin. However, all Gram positive bacteria remained susceptible to linezolide. According to Michele et al, 26% strains of MRSA exhibited resistance to vancomycin by modified Kirby-Bauer disk diffusion method. This method was validated using MIC, and only 3 isolates were found to be resistant to vancomycin. In last few years, therapeutic failures have been reported with vancomycin resistant MRSA strains in the clinical setting.[25] Umadevi et al,[22] demonstrated that 65.5% of S aureus were MRSA positive while other studies on diabetic foot infections which have reported only 10–44% MRSA.[21] The most important factor responsible for the emergence of multidrug resistant bacteria is the prior usage of broad-spectrum antibiotics.[26] Usually diabetic foot ulcers are chronic in nature and patients are exposed to multiple doses of antibiotics. This could be the major predisposing factor for the development of antibiotic resistance.[27]

Present study has some limitations such as, no anerobic culture was performed and other multi drug resistant bacteria (Amp C beta lactamases, carbapenamases and metallo beta lactamases) were not detected. MRSA and ESBL strains were detected by phenotypic methods. ESBL sub type cannot be detected by phenotypic tests. Few ESBL strains can't be detected by disc diffusion technique and results in treatment failure. Nuesch & Hachler stated that genotypic

techniques are more reliable than phenotypic methods. But genotypic methods are expensive, time requiring, requirement of special apparatus and expertise limits its application in routine usage.[28] Application of advanced techniques, such as rDNA PCR, ERIC PCR, *etc.*, to evaluate the infection status and bacterial diversity of the isolates in diabetic foot wounds was suggested in the literature. Infected and non-infected foot ulcers can also be differentiated by measuring of inflammatory markers.[29,30] However, culture and sensitivity remains superior over the molecular techniques for choosing of antibiotics.

In conclusion, diabetic foot infections were principally due to Pseudomonas aeruginosa followed by Stahylococcusaureus. High degree of susceptibility was exhibited by all Gram negative bacteria towards imipenam whereas linezolide remained as most susceptible antibiotic towards Gram positive cocci. Cefeperazone/ sulbactum showed good susceptibility towards ESBL strains. Amikacin and piperacillin/ tazobactum was found to be effective against both Gram positive cocci and Gram negative bacilli. Regular monitoring of the antibiotic resistance pattern helps in guiding clinician in initiating the empirical treatment of diabetic foot infection and the treatment must be started only after the culture and the sensitivity testing have been done. Therefore, the rapid propagation of the antibiotic resistance and its mechanism can be prevented.

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