DOI: https://doi.org/10.53350/pjmhs22165345

ORIGINAL ARTICLE

Bacterial Profile of Diabetic Foot Ulcer with duration and Types of Diabetes and Antibiotic Therapy

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ABSTRACT

Objective: To find out the gram negative bacteria causing the diabetic foot ulcers and most effective antibiotic therapy.
Study Design: Cross-sectional descriptive study
Place and Duration of Study: Diabetic Center Hayatabad, Kabir Medical College Peshawar from 1st September 2021 to 28th February 2022.
Methodology: Sixty nine admitted patients for the treatment of diabetes having type 1 diabetes mellitus and type 2 diabetes mellitus were enrolled. All the patients were on treatment of antibiotic such as gentacin, augmentin, amikacin and clindamycin. The specimens were analyze in microbiology laboratory and extracted by needle aspiration of material from the infected site and inoculate within 1hour after collection using gram staining smear for the detection or cytology of bacteria and its presence and absence in a specimens, for the isolation specimens were plated onto chocolate, phenyl ethyl alcohol (PEA) and MacConkey agar plate. To check the antibiotic susceptibility pattern Kirby Bauer test was performed.
Results: There were eight anaerobic gram negative bacteria included in the study. In type 1 diabetes the Escherichia coli extended-spectrum-β-lactamase (ESBL) was 4.8% while in type 2 it was 95.2% in case of Klebsiella oxytoca. There is no bacteria in type1 while in type2, 100% were detected among 13 samples out of 69. E. coli (ESBL) was found in 66.7% in diabetic patients >10 years with foot ulcers while 33.3% in <5 years and Klebsiella oxytoca was 61.5% in >10 years while in <5 years, 23.1% was found in the lesions. In the wound all the presence of E. coli (ESBL) was high as compared to other gram negative bacteria. In antibiotic therapy E. coli was 76.2% were resistant while in Klebsiella oxytoca 69.2% were show resistant while 23.1 % were sensitive while Enterobacter was 100 % and Proteus mirabillus has 50.0% sensitivity. Serratia was 75% resistant and E. colicephalosporin producer were 77.8% resistant to augmentin. The sensitivity of clindamycin was zero percent in all gram negative bacteria. Amikacin is 90.5% sensitive to E-coli and 9.5% resistant while gentacin is 66.7% sensitive and 33.3% resistant to bacteria e-coli.
Conclusions: Escherichia coli extended-spectrum-β-lactamase was found to be the most common gram negative bacteria detected in wound of diabetic foot ulcers.
Keywords: Escherichia coli, Diabetic foot ulcers, Antibiotic therapy, Sensitivity, Resistant

INTRODUCTION

Diabetes is a metabolic disease. It has two types on the basis of which it is divided into type 1 and type 2 diabetes mellitus, whereas type1 was found among 5-10 percent while type 2 was found in 90 to 95 % of individuals.[1] According to the international diabetes federation the number of individuals with diabetes will increase from 240 million in 2007 to 380 million in 2025.[2] Among all diabetic ulcers, foot ulcers are the most common in patient visiting to hospitals and their prevalence is 25%. Among diabetic individuals 567 are become infected frequently from 40% to 80%.[3] If the ulcers get infected it spread quickly to massive destruction of tissue which further leads to amputation of the lower extremities or lower limbs.[4] The key reason for amputation of lower limbs are diabetic foot ulcers but it is a most dreaded factor and influence on the diabetic patient life, early detection and identification of factors can resolve the condition which leads to amputation.[5] The bacterial pathogens isolated the mild diabetic foot ulcers caused by gram positive cocci or aerobic bacteria's like S. aureus enterococcus spp and staphylococcus epidermidis and gram negative bacilli and anaerobes like Escherichia coli, pseudomonas spp, citrobacter spp, bacteriodes spp, peptostreptococcus spp. clostridium and peptostreptococcus spp.[6,7]

According to the diabetic foot ulcers-epidemiology forecast to 2025 (diabetic foot ulcer) they includes data from seven other countries and reported 1 million of the diabetic patients in 2015 and the rate become increasing 4% per year in those seven countries while in Kuwait 22% the prevalence was found among diabetics and rank this country among 20 highest worldwide. The aetiology of diabetic foot ulcer (DFU) because of multiple factors are involve in it with neuropathy foot deformity and (pad) peripheral arterial disease which leads to amputation increased hospital admissions associated with DFU. Diabetic foot infection is present if two cardinal signs of inflammation are there in the location of infection it can be increase in pain sensation, increase temperature, discharge will be purulent and pain are the common features. The diabetic foot infection is mild to moderate and severe but consist of multiple bacteria called polymicrobial infection where various bacteria are identified at the site of infection. The most common pathogens are gram negative (gram -ive) and gram positive (gram +ive) among them due to improper use of antibiotics the incidence of methicillin resistant staphylococcus aureus (MRSA) is very high. The nature of pathogens in DFU was studies and concluded that Staphylococcus aureusis the main causative agent but some reported that gram negative aerobes this is due to ecological, socioeconomic conditions, depth of wound nature of infection, personal hygiene, geographical variation and sampling technique can trigger the results outcomes. The oral antibiotics and parenteral antibiotics guided by culture can prevent amputation.[8]

The antibiotics are very important to treat such type of wound now-a-days there is an increase prevalence of multi-drug resistance (MDR) organisms. The most common among them are methicillin-resistant S. aureus and beta lactamase producing gram negative bacteria is an alarming condition for the treatment of such infections in the community and in the patient admitted in the hospitals.[9]For diabetic ulcers bacteriological assessment play a vital role to find out the causative agents. A correct knowledge about the bacterial profile causing the diabetic foot ulcer is important to know, for the selection of antibiotics in reducing the severe conditions and for appropriate therapy. It is important to for health care professionals to treat and manage the diabetic patients and decrease the amputation.[5] The purpose of this study was to find out the gram negative bacteria causing the diabetic foot ulcers and most effective antibiotic therapy.

MATERIALS AND METHODS

It was a descriptive study held at Diabetic Center Hayatabad, Kabir Medical College Peshawar from 1st September 2021 to 28th February 2022 and 69 patients were enrolled. All the patients were on antibiotic therapy such as augmentin, gentacin, amikacin and clindamycin and vancomycin. All patients from 40-80 years with diabetic foot ulcer visiting to the tertiary care hospital and type 1 and type 2 diabetes mellitus were included. Patients with any pathology any foot deformities, undergoing any surgery were excluded. The collected sample was processed for the identification of anaerobic bacteria in diabetic ulcers. The specimens were sent to the microbiology laboratory during sample collection the infection site was first scrubbed with povidone iodine and specimens were obtained by needle aspiration of material from the depth of infected site, specimens were sent to laboratory within 20 minutes and inoculated within 1 hour after collection. Gram staining smear was used for the cytology detection of bacteria and showing absence and presence of bacteria in specimens, for the isolation specimens were plated onto chocolate agar, sheep blood 5%, phenyl ethyl alcohol (PEA) and MacConkey agar plate. The plate were under 10% CO2 incubated at 37ºC and examined at 24 and 48 hours. To check the antibiotic susceptibility pattern antibiogram test by Kirby Bauer test was performed and CLSI guide line were used. The excel sheet was entered and evaluated through spss-version 22.

RESULTS

There were eight anaerobic gram negative bacteria included in the study. In type 1 diabetes the E. coli (ESBL) was 4.8% while in type 2 it was 95.2% in case of Klebsiella oxytoca there is 0% in type1 while in type2, 100% are present and the was detected among 13 samples out of 69. E. coli (ESBL) was found in 66.7%, 33.3% in less than 5 years while Klebsiella oxytoca was 61.5% in more than 10 years while in less than 5 years 23.1% was found in the lesions. I n the wound all the bacteria were found positive but the frequency of E. coli (ESBL) was high as compared to other gram negative bacteria it was found in 21 samples out of 60 while Klebsiella oxytoca were 13 out of 69 and other remaining bacteria were decrease in number as compared to E. coli (ESBL). In antibiotic therapy 76.2% were resistant while in Klebsiella oxytoca 69.2% were show resistant while 23.1 % were sensitive while enterobacter has100% and proteus mirabillus has 50.0% sensitivity while serratia was 75% resistant necolicephalosporinase producer were 77.8% resistant to augmentin. The sensitivity of clindamycin was zero percent in all gram negative bacteria (Tables 1-5).

Table 1: Percentage of anaerobic bacteria in diabetes mellitus type1 and type 2

Anaerobes	Diabetes mellitus		Total
	Type 1	Type 2	
E-coli (ESBL)	1 (4.8%)	20 (95.2%)	21 (100%)
Enterobacter species	-	7 (100%)	7 (100%)
Proteus mirabillus	-	8 (100%)	8 (100%)
Serratia	-	4 (100%)	4 (100%)
Enterobacter	-	1 (100%)	1 (100%)
Proteus vulgaris	-	6 (100%)	6 (100%)
Klebsiella oxytoca	-	13 (100%)	13 (100%)
E. coli (Cephalosporinase producer)	-	9 (100%)	9 (100%)

Table 2: Anaerobes with the duration of diabetes mellitus

Anaerobes	Duration of diabetes mellitus			Total
	<5 years	5-10 years	>10 years	
E-coli (ESBL)	7 (33.3%)	-	14 (66.7%)	21 (100%)
Enterobacter species	-	3 (42.9%)	4 (57.1%)	7 (100%)
Proteus mirabillus	-	3 (37.5%)	5 62.5%)	8 (100%)
Serratia	1 (25%)	2 (50%)	1 (25%)	4 (100%)
Enterobacter	1 (100%)	-	-	1 (100%)
Proteus vulgaris	2 (33.3%)	-	4 (66.7%)	6 (100%)
Klebsiellaoxytoca	3 (23.1%)	2 (15.4%)	8 (61.5%)	13 (100%)
E. coli (cephalosporinase producer)	2 (22.2%)	4 (44.4%)	3 (33.3%)	9 (100%)

Table 3: Presence of anaerobes in wound culture

Anaerobes	Wound culture +ve	
	No.	%
E-coli (ESBL)	21	30.43%
Enterobacter species	7	10.14%
Proteus mirabillus	8	11.5%
Serratia	4	5.79%
Enterobacter	1	1.44%
Proteus vulgaris	6	8.69%
Klebsiellaoxytoca	13	18.84%
E. coli (Cephalosporinase producer)	9	13.04%

Table 4: Sensitivity and resistance of anaerobes with antibiotic gentacin and augmentin

Anaerobes	Gentacin		Augmentin	
	Sensitive/Resistant	Not Tested	Sensitive/Resistant	Not Tested
E. coli (ESBL)	14/7	-	0/16	5
	66.7%/33.3%	-	0%/76.2%	23.8%
E. coli (Cephalosporinase producer)	5 /3	1	0/7	2
	55.6% /33.3%	11.1%	0%/77.8%	22.2%
Enterobacter species	2 /5	-	1/5	1
	28.6% /71.4%	-	14.3%/71.4%	14.3%
Proteusmirabillus	3 /4	1	4/3	1
	37.5% /50.0%	12.5%	50.0%/37.5%	12.5%
Serratia	3 /0	1	0/ 3	1
	75.0% /0.0%	25.0%	0%/75.0%	25%
Klebsiellaoxytoca	11 /2	-	3/9	1
	84.6% /15.4%	-	23.1%/69.2%	7.7%
Proteus vulgaris	3 /3	-	2/4	-
	50.0% /50.0%	-	33.3%/66.7%	-
Enterobacter	1 /0	-	1/0	-
	100.0% /0.0%	-	100%/0%	-

Table 5: Sensitivity and resistance of anaerobes with antibiotic amikacin and clindamycin

Anaerobes	Amikacin		Clindamycin	
	Sensitive/Resistant	Not Tested	Sensitive/Resistant	Not Tested
E. coli (ESBL)	19/2	-	-	19
	90.5%/9.5%	-	-	100%
E. coli (Cephalosporinase producer)	9/0	-	0/3	5
	100%/0%	-	0%/37.5%	62.5%
Enterobacter species	5/2	-	-	5
	71.4%/28.6%	-	-	100%
Proteus mirabillus	7/1	-	-	7
	87.5%/12.5%	-	-	100%
Serratia	3/0	1	0/1	3
	75%/0%	25%	0%/25%	75%
Klebsiellaoxytoca	12/1	-	0/1	11
	92.3%/7.7%	-	0%/8.3%	91.7%
Proteus vulgaris	6/0	-	-	6
	100%/0%	-	-	100%
Enterobacter	-	1	-	1
	-	100%	-	100%

DISCUSSION

Diabetes is the risk factor for the development of infections characterized by severity and atypical localization. This infection leads to foot ulcers amputation and at last mortality.[10] For anaerobic culture 26.25% anaerobes were isolated from diabetic foot ulcer cases because it is a ploymicrobial infection with both aerobes and anaerobes are present in it. Gram negative aerobes were also found in the ulcers but majority of them were multi-resistant to antibiotics like cephalosporin, pencillin and sensitive to piperacillin-tazobactam while isolation of anaerobes required special technique for collection, transport, handling growth, inoculation of specimens and to reduce the exposure to oxygen and required very strict anaerobic conditions are for isolation and incubation. These anaerobes are sensitive to metronidazole and imipenem while less sensitive drugs for anaerobes are pencillin, clindamycin and cefoxitin. In the present study gentacin is more sensitive and less resistant to anaerobes and augmentin is less sensitive and more resistant to these bacteria. While clindamycin is less sensitive and amikacin is very sensitive and more resistant to anaerobes in the present study.[11]

Seventy (72.2%) were males and (30) 27.8% were females. In the study among 108 specimens 44.4 % were poly microbial while 44.4% were monomicrobial and 11.1% with no growth.[12] Sekharet al[12] also showed polymicrobial infection was less 35.5% than monomicrobial infection 43.5%. In the present study the wound is polymicrobial and containing various gram negative rods like E. coli (ESBL) is 30.43%,enterobacter species 10.14%,Proteus mirabillus 11.5%, Proteus vulgaris 8.69% while Klebsiella oxytoca 18.84% E. coli (cephalosporinase producer) 13.04%. In another study a microbiological assessment of diabetic ulcers showed 56% prevalence of gram negative bacteria which was more than that of the gram positive bacteria 44% it is in accordance with the present study gram negative bacteria are more prominent as compared to other microbes. However ecoli (ESBL), Klebsiella oxytoca, proteus mirabillus and enterobacter species were more prominent in wound culture as compared to other gram negative bacteria in the present study gentacine is more sensitive as compared to augmentin while amikacin is more sensitive as compared to clindamycin. Alvi et al[13] conducted a study in North India also found that gram negative bacteria were prominent in diabetic ulcers 63.8% as compared to gram positive 36.1%.The study done by AlBenwan et al[6] showed that most prevalent microbes were gram negative as compared to gram positive bacteria while in admitted patients with diabetic ulcers multi drug resistance is very common.

In another study gram negative rods are 54.8% E. coli 23.4%. Only gram negative bacteria were very prominent in the foot ulcers as compared to other bacteria.[12] In another study escherichia coli was major gram negative organism (23.8%) in the present study E. coli is also the most common bacteria in wound 30.43% another most common gram negative were Proteus mirabilis(9.5%) in the present study it is 11.5% while Pseudomonas aeruginosa and enterobacter species were also present.[13] All the gram negative isolates were resistant to amoxicillin and clindamycin which is in accordance with the present study. It is a polymicrobial infection therefore presence of multidrug resistant is commonly seen. Increasing prevalence of MDR in diabetic foot ulcer leads to limited antibiotic used for treatment which leads to poor prognosis or delay in wound healing process. It requires clinical guidelines to manage the MDR.

CONCLUSION

Eschericia coli extended-spectrum-β-lactamase was found to be the most common gram negative bacteria detected in wound 0f diabetic foot ulcers therefore definitive antibiotic therapy is needed.

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