Original article

Drug resistant anaerobic infections: Are they complicating diabetic foot ulcer?

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Abstract:

Introduction: Poorly controlled diabetes and associated peripheral neuropathy often results in foot ulcers which get infected frequently resulting in gas gangrene and sometimes require amputation. Polymicrobial nature of infection is usually considered to start the empirical treatment. The aerobic culture and sensitivity is commonly practiced to guide the treatment but no efforts are taken to look for the anaerobic infection. The upcoming resistance of anaerobes to the commonly used antibiotics is under diagnosed leading to treatment failure in diabetic foot ulcers. The present study was undertaken to investigate the role of anaerobic infections in 50 clinically diagnosed diabetic foot ulcer patients admitted in a tertiary care hospital.

Aims & Objectives: To isolate & identify the aerobic & anaerobic bacteria from diabetic foot ulcers as per the standard microbiological technique.

To study the antimicrobial susceptibility pattern of both aerobic & anaerobic bacteria isolated from these ulcers.

Material & Methods: A prospective study was carried out in 50 diabetic patients admitted with foot ulcers. The pus samples collected from foot ulcers were processed for both aerobic & anaerobic culture followed by antimicrobial susceptibility testing as per standard protocol.

Results: Anaerobic organisms were isolated from 10 patients (22%), three of which grew only anaerobic bacteria (6%).The most commonly isolated anaerobe was found to be *Clostridium* species (50%) followed by *Peptostreptococcus* species (40%). Metronidazole is the drug of choice to treat anaerobic infection; in the present study anaerobes showed 40% resistance to Metronidazole. The polymicrobial infection was seen in 22% of patients which were purely aerobic infection .The most common aerobic bacteria isolated was *E.coli*. Mixed aerobic & anaerobic infection was seen in14% of patients.

Conclusion: This study demands further analysis of diabetic foot ulcers to formulate the empirical treatment considering the susceptibility pattern of both aerobic & anaerobic organisms.

Keywords: Diabetic foot ulcer, Anaerobes, Antimicrobials

Introduction

Diabetes has emerged as a major health care problem not only for developed country but also developing country like India. One of the dreadful complications of Diabetes is a foot ulcer. Poorly controlled diabetes & peripheral neuropathy are the predisposing factors for the diabetic foot ulcers. These ulcers are colonized with bacteria that may invade into deeper structures, including bone, ultimately leading to gangrene and all-too-frequently amputation.

The infection is usually polymicrobial in nature and this must be taken into account before starting empirical therapy[1]. Aerobic culture and sensitivity is commonly done to guide the treatment but no efforts are taken to identify the anaerobic infection. Most laboratories do not attempt to isolate or do the

susceptibility testing of anaerobes as it is very tedious, costly and time consuming. Recently, many authors has reported antibiotic resistance among the anaerobes, with clear evidence of treatment failure when an ineffective antibiotic is used[1,2]. One of the commonly used antimicrobial for anaerobic infection is Metronidazole, however anaerobes are developing resistance to this also[1,2]. The present study was undertaken to identify the aerobes & anaerobes causing diabetic foot infection and study their antimicrobial susceptibility pattern.

Aims & Objectives:

To isolate & identify the aerobic & anaerobic bacteria from diabetic foot ulcers as per the standard microbiological technique.

To study the antimicrobial susceptibility pattern of both aerobic & anaerobic bacteria isolated from these ulcers.

Materials and methods:

A prospective study was carried out in 50 diabetic patients admitted with foot ulcers. The inclusion criteria for the study was; previously diagnosed or newly diagnosed diabetic of age 18 & above, presenting with lower limb infection of Grade 2 to grade 5 (as per Wagner's classification) with or without empirical antibiotic treatment.

Pus or exudates were collected from margins and base of infected ulcers by taking universal safety precaution. The wound was first rinsed with normal saline and then the tissue was aspirated with sterile syringe. For abscess formation the surface was cleansed with an antiseptic ; specimens were then obtained by inserting a sterile needle and syringe. For anaerobic culture sample was inoculated in Robertson's Cooked meat medium (RCM) at the bed side. The specimens were processed in the laboratory as follows.

1) Gram Stain of the smear was done to study the morphology of infecting organisms.

2) Anaerobic culture: The inoculated RCM was incubated overnight at 37^0C.The subculture from RCM was done on Kanamycin BA (Blood Agar), Neomycin BA and Willis and Hobbs medium for anaerobic isolation. These plates were incubated under anaerobic condition in an anaerobic jar at 37^0C for 48 hrs. Colonies obtained were confirmed as obligate or facultative anaerobe by doing aerotolerance test[3]. Further identification of anaerobes was done as per the VPI manual[4].The antimicrobial susceptibility of anaerobes for Metronidazole was done by using E test (Biomerieux) method as per CLSI guidelines[4]. The antimicrobial susceptibility test for Clindamycin & Penicillin was done by disc diffusion method as recommended by VPI manual[5].

3) Aerobic culture: The sample was inoculated on Blood agar(BA), MacConkeys agar(MA) and Chocolate agar(CA) for aerobic bacterial isolation. Identification of isolated colonies was done as per standard microbiological technique[3]. Antimicrobial susceptibility testing for aerobic bacteria was done by using Kirby–Bauer's disk diffusion method as per CLSI guidelines[5].

Results:

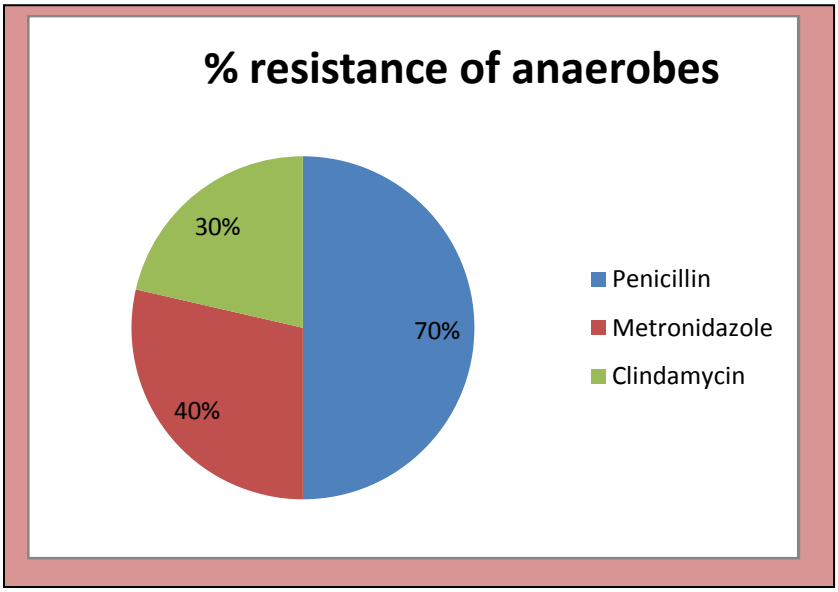
The present study was conducted to know the role of anaerobes in fifty clinically diagnosed cases of infected diabetic foot ulcer. These ulcers were graded as per the Wagener's classification. Majority of patients belong to grade 2(60%) followed by grade 3(28%). In our study, out of 50 samples followed anaerobes were isolated from 10 samples i.e. the rate of isolation was found to be 20%. Gram stain of four samples showed the characteristics of anaerobic gram positive bacilli but failed to grow on culture. Three

out of fifty samples showed pure growth of anaerobes on culture. The most commonly isolated anaerobe was found to be *Clostridium* species (50%) followed by *Peptostreptococcus* Spp.(40%)(Table 1) .On antimicrobial susceptibility testing of anaerobes they showed 70%, 40% & 30% resistance to Penicillin, Metronidazole & Clindamycin respectively (fig1).

Table : Distribution of anaerobes isolated from diabetic foot ulcers.

Anaerobes isolated	No. of isolates(n=10)	%
Peptostreptococcus Spp.	4	40
Clostridium welchii	1	10
Clostridium bifermentans	1	10
Clostridium butyricum	2	20
Clostridium limosum	1	10
Prevotella Spp.	1	10

Fig 1: Percentage antimicrobial resistance of total anaerobes isolated



Polymicrobial etiology was observed in 22% of patients while mixed infection was seen in 14% of patients. The anaerobes were more frequently isolated with grade 4 diabetic foot ulcer than grade 2 & 3. On aerobic culture, total 47 bacteria were isolated from 50 samples. Gram negative bacteria (78.8%) were predominantly isolated than gram positive bacteria (21.2%). In gram positive bacteria, *S. aureus* (12.76%) was found to be the most common isolate followed by *Enterococcus spp*.(8.5%)(Table 2). In gram negative bacteria, *E.coli* was the commonly isolated bacteria (19.14%) followed by *Klebsiella species* (17.02%).

Table 2: Aerobic bacteria isolated from infected foot ulcers

Bacteria isolated	No. of isolates	Percentage (%)
➢ Gram positive	10	21.27
S.aureus	06	12.76
Enterococcus species	04	8.51
➢ Gram negative	37	78.72
E.coli	09	19.14
Klebsiella species	08	17.02
P.aeruginosa	07	14.89
Proteus vulgaris	05	10.63
Enterobactor species	03	6.38
Citrobactor species	03	6.38
Proteus mirabilis	02	4.26

On antimicrobial susceptibility testing Gram negative isolates showed resistance to Cotrimoxazole (83.6%) followed Cefotaxim (62.3%) and Gentamycin(60.9%).All gram negative isolates were found to be 100% sensitive to Piperacillin+Tazobactam, Cefoperazone+sulbactam, Imepenem (Table 3) while Gram positive isolate showed 100% sensitivity to Linezolid(Table 4).

Table 3:Antimicrobial resistance pattern of Gram negative bacteria

Antibiotics	Gram negative R (%)
Piperacillin+Tazobactam	00
Cefoperazone+sulbactam	00
Ciprofloxacin	56.8
Cotrimoxazole	83.6

Amikacin	35.5
Gentamicin	60.9
Tetracyclin	44.6
Cefotaxim	62.3
Imepenem	00

Table 4 : Antimicrobial resistance pattern of Gram positive bacteria

Antibiotics	*S.aureus* R (%)	*Enterococcus Species* R (%)
Ampicillin	83.3	60.5%
Amoxicillin	83.3	60.5%
Amoxclav	83.3	60.5%
Ciprofloxacin	33.3	100
Cotrimoxazole	50	100
Gentamycin	50	75
Erythromycin	50	50
Linezolid	00	00

[Gram positive cocci showed 83%,60.5% &60.5% resistance to Ampicillin, Amoxicillin and Amoxclav respectively while 100% sensitive to Linezolid.]

Discussion:

Infected diabetic foot ulcer is a challenge to clinicians as often they are polymicrobial in nature[1]. The present approach for treating infected diabetic foot ulcer is largely based on empirical therapy. The treatment is modified according to the antimicrobial susceptibility pattern of aerobic bacteria. Anaerobic bacteria are often neglected leading to poor prognosis. So the present study was conducted to know the bacteriological profile of 50 clinically diagnosed diabetic foot ulcer patients with special reference to anaerobes.

Anaerobes from diabetic foot ulcer have been reported from 5% to as high as 95%[1]. In our study it was found to be 20%. Gram stain of four samples showed the characteristics of anaerobic gram positive bacilli but failed to grow on culture. This could be attributed to previous antibiotic treatment or some lack in culture method used in this study. Anaerobes were isolated more frequently with Wagner grade IV. Same observation was made by Maria Raymundo *et al* [2] where the rate of anaerobic infection was found to be more with Wagner grade IV & V. In this study, the most commonly isolated anaerobe was found to be *Clostridium* species(50%) followed by *Peptostreptococcus* Spp.(40%). Similar study conducted by Maria Raymundo *et al* have found same results with *Clostridium* as the predominant

organism with 43% rate of isolation[2]. However, C. Amalia *et al* & R. Gadepalli *et al* have found *Peptostreptococcus Spp.* as the most common isolate from diabetic foot infections [1,6].

Infection in the diabetic foot is usually of polymicrobial etiology. In more superficial infection (Wagner's Grade 1-2) aerobic bacteria are the predominant organisms. While in deeper wounds (Wagner's Grade 3-5), gram negative aerobes and anaerobes are more frequently found [1]. Polymicrobial nature of infection raises a question of providing antimicrobial coverage for every organism isolated in culture. In our study, 22% of cases were of polymicrobial in nature. Polymicrobial infection ranges from 19.2 to 80% as per the literatures[7,8] . Ozer *et al* in their study have found low rate of polymicrobial infection (19.2 %) as compare to monomicrobial infection (52.3%)[2,3]. As the anaerobic infection was not investigated in their study they have documented low rate of polymicrobial infection. In our study the low rate of polymicrobial infection could be because few samples were collected after starting antibiotic therapy.

A number of studies have reported *Staphylococcus aureus* as the main causative pathogen, but two recent investigations reported a predominance of gram negative aerobes. Gram negative bacteria were found to be predominant in the present study also .The same result was found by E. Bansal *et al* [9] and Gadepalli *et al*[6]. In the present study among gram negative organism *E.coli* (19.64%) followed by *Klebsiella pneunoniae* (17.02%) and *P.aeruginosa* (14.69%) were found to be the most common pathogens. Results were slightly different as that of E.Bansal *et al*[9] and Sharma V.K. *et al*[10], as they have described *P. aeruginosa* as the most commonly found bacteria. This variation may be due to variation in environmental conditions, geographic distribution and seasonal variation. Gadepalli *et al* described Proteus as commonly found gram negative bacteria[6]. Variation was seen in various studies based on environmental factors. Among gram positive bacteria *S.aureus* was commonly found bacteria with 12% prevalence in present study.

Methicillin resistance was seen in 66.64% of *S.aureus* which is slightly higher than E.Bansal *et al* (55.56%)[9] while Zubair *et al* [11] described 23.3% prevalence of MRSA. In the present study Gram positive organism showed 100% sensitivity to Linezolid which is in concordance with results shown by Gadepalli *et al*[6]. Among Gram negative bacteria isolated, *E.coli* was commonly found bacteria showing high resistance to Ciprofloxacin(100%), Tetracycline(77.8%), Cefotaxime(60%) , and Gentamicin(55.6%) . Similar results were observed by E.Bansal *et al*[9].In the present study, Piperacillin+Tazobactam and Cefoperazone+ sulbactam showed 100% sensitivity against the gram negative bacilli which is also shown by Gadepalli *et al*[6]as 45%-77% for Piperacillin+Tazobactam and 86%-100% for Cefoperazone+ sulbactam Cefoperazone+Sulbactam for gram negative bacteria.

The rate of anaerobic infection in diabetic foot ulcers varies widely from 5% to as high as 95% [1] . Antibiotic susceptibility pattern of anaerobes was reported by very few literatures. In the present study resistance pattern of anaerobes were found to be 40%, 70%, & 30% to Metronidazole, Penicillin & Clindamycin respectively. In the present study the antibiotic resistance was found to be slightly higher for Penicillin than M. Raymundo *et al* [2]. In his study, he showed it as 63%, 47%, and 47% for

Metronidazole, Penicillin, Clindamycin respectively. Similar study by Catherine *et al* reported resistance of 48.2%, 13.8% and 24.1% for same antibiotic[1]. This variation may be due to environmental factors & haphazard use of antibiotic resulting into high rate of antibiotic resistance.

Conclusion:

With the emerging resistance of anaerobes to the commonly used drugs it becomes mandatory to look for antimicrobial susceptibility pattern for anaerobes along with the aerobes in diabetic foot ulcers. This study demands the further evaluation of diabetic foot ulcers for large population so as to form therapeutic guidelines for the betterment of the treatment outcome.

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2012InfectiousDiseaseSocietyofAmericaClinicalPracticeGuidelinesfortheDiagnosisandTreatment of Diabetic Foot Infections. CID 2012:54(12):132-173. 3. Lipsky BA, Armstrong DG, Citron DM et al. Ertapenem versus piperacillin/tazobactam for diabetic foot infections (SIDESTEP): prospective, randomized, controlled, double-blinded, multicentre trial.Â daily living REQUIRE careful clinical assessment. DO NOT assume they are secondary to UTI.â€ VCH/ASPIRES 2014. â€¢ Urine culture and susceptibility and urinalysis testing is NOT necessary or beneficial in healthy, non-pregnant, premenopausal, non-diabetic women with acute cystitis (at least 2 of 3 cardinal symptoms â€“ dysuria, urgency or frequency) and NO vaginal discharge without functional or anatomical abnormalities of the urinary tract. Diabetic foot ulcers have a considerable negative impact on pÂ atientsâ€™ lives, and are highly susceptible to infection that all too often leads to amputation. It is essential that diabetic foot ulcers receive the best possible wound management. Successfully treating a diabetic foot ulcer requires a comprehensive uÂ nderstanding of the wound: its cause, progression, risk, and treatment. But more than this, it takes a cross functional Âapproach, where the patient also has an active role in the Âtreatment process. Diabetic foot infections (DFIs) are a common and often severe problem for people with diabetes. Though not all diabetic foot ulcers are infected, many become infected given the patientâ€™s compromised immune system and lack of peripheral sensation. These DFIs usually begin as small ulcerations or calluses, and frequently grow into larger ulcerations with rampant polymicrobial infections. Those wounds that are not infected do not require the initiation of antibiotic therapy. Diabetic foot ulcers (DFUs), a micro-vascular complication, are associated with a substantial increase in morbidity and mortality. DFUs are a complicated mixture of neuropathy, peripheral arterial diseases, foot deformities, and infections. Foot infections are frequent and potentially devastating complications. Infection prospers in more than half of all foot ulcers and is the factor that most often leads to lower extremity amputation. The complications of microbial flora span the spectrum from superficial cellulitis to chronic osteomyelitis and gangrenous extremity lower limb amputations. Wou