

Anaerobic Bacteriological Profile of Infected Diabetic Foot Ulcers with their Antimicrobial Susceptibility Pattern: Need of the Hour

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ABSTRACT

Introduction: Diabetes has emerged as a major health care problem in developed as well as in developing country like India. Diabetic foot ulcers are the most common leading causes of morbidity and frequent indication for hospital admission. Limb-threatening diabetic infections are habitually polymicrobials in nature involving numerous aerobic and anaerobic organisms. *Streptococcus* species, *Staphylococcus aureus*, Enterobacteriaceae, *Bacteroides fragilis*, *Peptostreptococcus* species and *Peptococcus* species are the familiar organisms isolated from the cultures of diabetic foot ulcers. The upcoming resistance of anaerobes to the commonly used antibiotics is undiagnosed leading to treatment failure in diabetic foot ulcers.

Aim: Isolation and identification of anaerobic bacteria from diabetic foot infections with their susceptibility pattern.

Materials and Methods: A prospective study was carried out on pus samples from 100 clinically diagnosed diabetic foot

ulcers patients admitted in the surgical ward of Tertiary Care Hospital of North India. Isolation and identification of anaerobic bacteria with their susceptibility testing was done.

Results: Diabetic ulcers were graded as per the Wagener's classification (Grade 1 to Grade 5). Majority of patients belong to Wagner's Grade 2(73/100) followed by Grade 3(24/100), Grade 4(2/100) and Grade 5(1/100). In our study, out of 100 samples, 22 anaerobes were isolated. The predominant anaerobic bacteria were *Bacteroides* species (11%), followed by *Peptostreptococcus* species (5%), *Peptostreptococcus anaerobius* (2%), *Eubacterium* species (2%) followed by *Peptococcus* species and *Prevotella* species 1% each. Overall resistance was 27.27% to metronidazole, 13.63% to penicillin. There was no resistance to rest of the antimicrobials tested.

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.

Keywords: Bacteroides, Polymicrobials, Resistance, Wagner's grade

INTRODUCTION

Diabetes has been emerged as major public health problem in developing country like India. It has been labelled as Diabetic capital of the World because of the alarming fact that India has more people with diabetes than any other country [1,2]. Among the various complication of the diabetes, one of the most dreadful and difficult to treat complication is diabetic foot ulcers. The predisposing factors for diabetic foot ulcers are poorly controlled diabetes and peripheral neuropathy [3]. In such conditions these foot ulcers are prone to colonized with various organisms with tendency to invade to deeper tissues leading to gangrene [4]. So in most of the cases this may be the cause of amputation [5]. These infections are usually polymicrobials involving multiple aerobic and anaerobic infections usually considered starting the empirical treatment [6].

Among aerobic infections, *Staphylococcus*, *Streptococcus*, Enterobacteriaceae family, *Pseudomonas* species and

Acinetobacter species are common organisms. Among anaerobic infections, *Bacteroides*, *Peptostreptococcus*, *Peptococcus* are common organisms [7]. Aerobic bacteria are predominantly associated with superficial foot infections, more the deeper tissue invasion, and more chances of association of anaerobic bacteria [3,8].

The management of these infections requires isolation and identification of the microbial flora, appropriate antibiotic therapy according to the sensitivity patterns. Aerobic culture and sensitivity is routinely done in all the clinical microbiology laboratories for the identification and antibiotic sensitivities to guide the treatment of the organism to the clinicians but there is lack of anaerobic culture facilities in most of the routine laboratories [2,9]. As resistance in aerobic bacteria is the cause of major concern, but now a day, resistance is also emerging among anaerobes against the empirically used antibiotics because of their indiscriminate use, thus

failure to recognize and control the infectious process, may have devastating consequences like limb amputation, sepsis, and mortality [4,8,10]. So for treatment of limb threatening infections appropriate choice of antimicrobials is commanding in preventing amputation.

Since, the presence of anaerobic organisms has been shown to be a risk factor for severe infections of the diabetic foot ulcers already described in literature and also the emergence of resistance among anaerobic pathogens poses a problem in the choice of empirical antibiotic regimens. By keeping in mind about these facts, a study was planned with the following objectives:

- To isolate and identify anaerobic bacteria by using automated anaerobic culture techniques (Anoxomat) from diabetic foot infections.
- To determine antimicrobial susceptibility of the anaerobic isolates.

MATERIALS AND METHODS

A prospective study was carried out in Department of Microbiology on pus samples from 100 diabetic patients admitted with foot ulcers at the surgical ward of Tertiary Care Hospital of North India. The duration of the study was one year From June 2013 to May 2014. The inclusion criterion for the study was previously diagnosed or newly diagnosed diabetic of age 18 and above, presenting with lower limb infection. Exclusion criterion was limb infections in patients without any diabetic history. Patient's consent as well as ethical clearance for the study was obtained.

The extent of the lower extremity infection on admission was assessed based on Wagner's classification as follows: [11]

Grade I: Ulceration involving only the dermis

Grade II: Ulceration involving tendons and/or joint capsule

Grade III: Extending to bone, usually causing osteomyelitis

Grade IV: Localized gangrene

Grade V: Gangrene involving a major part of foot

Sample Collection: Pus sample was collected from margins and base of the ulcers. All the universal safety precautions were followed while collecting the sample. Pus was aspirated with sterile syringe after rinsing the wound with normal saline. Sample was inoculated in Robertson's Cooked Meat medium (RCM) at the bed side. The specimens were processed in the laboratory for anaerobes by standardized procedure [4,12].

Gram stain of the smear was examined under microscope and findings recorded. RCM was processed after 24 hours of incubation. Cultures were put up on Brain Heart Infusion agar supplemented with haemin and vitamin K, L-cysteine, yeast extract with preliminary disks like metronidazole (5 µg), vancomycin (5 µg) and colistin (10 µg) Sodium Polyanethol Sulphonate (SPS) discs for anaerobic incubation by automated anaerobic system (Anoxomat), Blood agar and MacConkey agar were put up for aerobic incubation, for first screening. RCM was kept reserved for backup cultures.

Further, processing was done and pure isolates were obtained by standard procedure and these pure culture isolates were further identified by standard biochemical methods [12,13]. Antimicrobial susceptibility testing was done with various commonly used antimicrobial agents that are recommended by Clinical Laboratory Standard Institute 2012 for anaerobes by the disc diffusion method [14].

RESULTS

In the present study, none of the patient belongs to Grade 1. Majority of patients belong to Wagner's Grade 2 (73/100) followed by Grade 3 (24/100), Grade 4 (2/100) and Grade 5 (1/100). None of the Grade 2 ulcers grow anaerobic bacteria. But all the ulcer belongs to Grade 4 and 5 and 19 ulcers of Grade 3 grew anaerobic bacteria [Table/Fig-1]. In our study, out of 100 pus samples, 22 samples showed anaerobic growth. So the rate of isolation of anaerobic bacteria from diabetic foot ulcers was 22%. Also, Gram stain of five samples of Grade 3 showed the characteristics of anaerobic gram positive bacilli but failed to grow on culture which can be due to some lapses in techniques or prior antimicrobial therapy.

Various risk factors associated with limb amputation in

Wagner's Grade	Number	Anaerobic Growth
1	0	0
2	73	0
3	24	19
4	2	2
5	1	1
Total	100	22

[Table/Fig-1]: Wagner's grade of diabetic foot ulcers and anaerobic growth.

diabetic foot lesions like neuropathy, duration of ulcer, uncontrolled diabetes, duration and type of diabetes, high Wagner's grade ulcers were present in all the 22 patients from where anaerobes were isolated which is itself a indicator for severity of infection. All the patients in our study belong to Type II diabetes with duration from 4 years to 30 years and duration of ulcer was 7 days to 1.5 month old.

Out of 22 samples which showed anaerobic growth, the predominant anaerobic bacteria were *Bacteroides* species. (11%), followed by *Peptostreptococcus* species (5%), *Peptostreptococcus anaerobius* (2%), *Eubacterium* species (2%) followed by *Peptococcus* species and *Prevotella* species 1% each. Anaerobic isolates are shown in [Table/Fig-2].

In the present study all the isolates of *Peptostreptococcus* species (5%), *Peptostreptococcus anaerobius* (2%), and *Peptococcus* species and *Prevotella* species (1%) showed sensitivity to all the antibiotics tested. Both the isolates of *Eubacterium* species showed resistance to metronidazole

Organism	Number	Percentage (%)
Bacteroides species	11	11%
Peptostreptococcus species	05	5%
Peptostreptococcus anaerobius	02	2%
Eubacterium species	02	2%
Peptococcus species	01	1%
Prevotella species	01	1%
Total	22	22%

[Table/Fig-2]: Distribution of anaerobes isolated from diabetic foot ulcers (n=100).

but showed sensitivity to rest of the antibiotics. Out of 11 Bacteroides species 4 showed resistance to metronidazole and 3 showed resistance to penicillin. So most common anaerobe which showed maximum resistance to antibiotics in our study was Bacteroides species Overall resistance observed in our study was 27.27% to metronidazole, 13.63% to penicillin.

DISCUSSION

Diabetic foot ulcer is the most awful complication of the uncontrolled diabetes with peripheral neuropathy and other risk factors. Both aerobic and anaerobic bacteria cause infection. So the treatment of infected diabetic foot ulcers is routinely begins based upon empirical therapy [3]. Later on therapy is modified based upon aerobic susceptibility testing but identification and susceptibility testing for anaerobes remains neglected, this approach leads to poor prognosis of the diabetic foot ulcers because of deficient treatment which can lead to development of antibiotic resistance to most of the empirically used antibiotics against anaerobes. So, the present study was conducted on 100 pus samples collected from infected diabetic foot ulcers to know their anaerobic profile along with their susceptibility testing.

In the present study, isolation rate of anaerobes was 22%. This finding was comparable to the results of the study done by Sushma NP et al., and Chopdekar KA et al., [3,15]. All the anaerobes were isolated from most of the Grade 3 ulcers and all the Grade 4 and 5 ulcers. In another study done by Raymundo M et al., the rate of anaerobic organism was found to be more with Wagner Grade IV and V [8]. This finding is also in agreement with Sushma NP et al., in her study [3]. This showed that anaerobic infections are associated with deeper tissue involvement while superficial ulcers associated with aerobic bacteria. These deeper tissue infections increase the risk of limb amputation. Out of 22 samples which showed anaerobic growth, the predominant anaerobic bacteria were Bacteroides species (11%), followed by Peptostreptococcus species (5%), Peptostreptococcus anaerobius (2%), Eubacterium species (2%) followed by Peptococcus species and Prevotella species (1%) each. A study done by Raymundo M et al., [8] showed predominance of Clostridium species while other studies done by Amalia

CS et al., Gadepalli R et al., Kannan I et al., showed Peptostreptococcus species was the predominant organism [4,16,17]. Other studies done by Chittur RY et al, Halpati A et al., Lily SY et al., and Swati VP showed predominance of Bacteroides species like our study [7,18-20]. So different anaerobic bacteria predominates in different geographical locales depending upon various environmental factors.

In the present study, antibiotic resistance was observed to be 27.27% to metronidazole and 13.63 % to penicillin. In our study antibiotic resistance is lower than the study done by Sushma NP et al., and Raymundo M et al., [3,8]. Both studies showed 40% and 63% to metronidazole and 70% and 47% to penicillin respectively. In another study done by Amalia CS et al., resistance observed was 48.2%, 13.8% and 24.1% to metronidazole, penicillin and clindamycin [4]. The variation in antibiotic resistance pattern may be due to random use of antibiotics in different settings. In the present study, no resistance was observed to clindamycin unlike the above studies quoted.

In our earlier study on anaerobes, there was resistance to only metronidazole that was too by Eubacterium species which is considered to be inherently resistant organism to metronidazole according to the literature. There was no resistance observed to rest of the antimicrobials tested to any other organism isolated [12]. So over the time resistance is increasing to the anaerobes which can be due to the routine use of broad spectrum antimicrobials against anaerobes and this increasing resistance in anaerobes is the cause of concern leading to treatment failure which complicates diabetic foot ulcers and turn out to be the reason for limb amputation.

CONCLUSION

Beside early recognition of risk factors, clinical grading of foot ulcers according to Wagner's classification, identification of causative organisms both aerobic and anaerobic along with their antimicrobial susceptibility is the need of the hour. Appropriate treatment can benefit to prevent further infection and thus prevent loss of limb.

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