Bacterial Diversity with Emerging Antimicrobial Resistance of Diabetic Foot Ulceration and Current Detection Techniques: A Review

Akash Ahmed 1*, Sayeed Akhtar Alvi 1, Ishrat Binte Aftab 2, Fahmina Akhtar 1

1 BRAC University, BANGLADESH
2 Technical University of Munich, GERMANY

Corresponding Author: akash.ahmed@bracu.ac.bd

Citation: Ahmed A, Alvi SA, Aftab IB, Akhtar F. Bacterial Diversity with Emerging Antimicrobial Resistance of Diabetic Foot Ulceration and Current Detection Techniques: A Review. Electron J Gen Med. 2021;18(6):em323. https://doi.org/10.29333/ejgm/11303

INTRODUCTION

Diabetic foot is a serious diabetic complication that consists ulceration of the soft tissue or bone below the malleoli irrespective of duration due to diabetes mellitus [1,2]. Due to the high prevalence of diabetes it is gradually rising as a serious and devastating non-communicable disease [3]. According to the estimation of the International Diabetes Federation (IDF), people are losing at least one limb per 30 seconds worldwide [2]. It is also the most common cause of hospital admission and lower extremity amputation in diabetic patients [4]. The rate of developing foot infection in individuals with diabetes is approximately 25% [5]. In diabetic patients, 85% of amputations are associated by a prior foot ulceration that progresses to extreme gangrene or infection [6]. Additionally, diabetic patients with ulceration have two-fold chances to increase the mortality than nonulcerated diabetic patients [7]. Estimation of five-year mortality is around 40% [8].

Among diabetic patients, globally the prevalence of diabetic foot ulceration (DFU) is between 3% to 13% [6]. North America tops the chart with the highest ratio of DFU. An estimated 13% people suffer from DFI in North America. Followed by Africa (7.2%), Asia (5.5%), Europe (5.1%) and Oceania (3%) [6]. Currently, the world is experiencing an epidemic of diabetes which is affecting the quality of life along with significant mortality and morbidity [2]. The IDF states 425 million people in the world have diabetes mellitus, which by 2045 is expected to increase up to 628 million [9]. With the numbers skyrocketing every day, it is presumable the scenario of DFU will be worse as well. Besides that, the disease itself is a huge long term socioeconomic consequence.

DFU is a preventable disease. It is possible also to decrease the frequency of lower limb amputations to 49-87% [10]. With early detection and treatment DFU complications can redeem the ulceration by 44-85%, literature suggests [10]. For early detection and treatment it is important to understand the types of microorganism responsible for ulceration and, the optimal detection techniques. Furthermore, there is an urgent need to understand the role of antimicrobial resistance in DFU otherwise effective treatment and amputation is not easy to prevent. There are some guidelines on antibiotic use yet DFU remains hard to treat because of antibiotic resistance [11]. Study of bacterial profile and patterns of antimicrobial resistance is highly essential for now. Few studies have focused on the bacterial profile, prevalence of responsible microorganisms and antimicrobial resistance of DFU [11-14]. However, most of these studies are country specific and thus, does not provide information in broader term. Therefore, there is a need for reviewing the literature to find out what are the
most prevalent microorganisms, antibiotic resistance pattern and detection technique globally. This narrative review aims to explore the causative agents of DFU, their role in antimicrobial resistance. It also provides a brief understanding of current detection techniques available.

**CAUSATIVE AGENT AND THEIR PREVALENCE**

Study revealed that 85% of lower limb amputation is caused by polymicrobial infection which is one of the most extreme outcomes of DFU [15-17]. Polymicrobial infections involve a variety of aerobic and anaerobic infections such as, Staphylococcus aureus, Streptococcus spp., Enterobacteriaceae spp., Bacteroides fragilis, Peptostreptococcus spp. and Peptostreptococcus spp [18]. The aerobes and anaerobes are divided into gram negative and gram positive bacteria as well, which will be discussed later in the article. Apart from bacteria mycotic agents such as fungi play a major role as well. filamentous fungi and yeasts have been detected to cause DFI by various studies. Candida spp., is determined to be the main agent involved in DFI [19].

According to an investigation conducted on Diabetic Foot Care Hospital and Dhaka Medical College Hospital, Bangladesh, Enterococcus spp. (9%), Klebsiella spp. (7%), Bacillus cereus (17%), were found to be the most dominant. According to their findings, Ent. hormaechei (22%) was the organism that was found in highest number among patients. Although Citrobacter spp. was only found in 2% patients, it was responsible for 5% infections in immune compromised patients. Furthermore, staphylococcus species amounted for 13% of all isolates. But, the most important finding was the presence of a nosocomial pathogen called A. baumannii (10%)[20]. In accordance with a study conducted by BIRDEM General Hospital, Bangladesh, Polymicrobial infection was found in 75.3% cases [21]. In this study gram negative organism was found in high numbers (80%), such as Pseudomonas (48%), Proteus sp. (33%). While Staphylococcus aureus (21.3%) was the most frequent among the 19.3% gram positive pathogen found [21].

A study conducted by Hospital University Sains Malaysia reported that gram negative organisms were the principal agent in causing DFU, as 62.4% such organism was found by them. Pseudomonas spp. (27.8%), Proteus spp. (10.5%), Klebsiella spp. (8.3%) are the most dominant gram-negative organisms detected. Gram positive (38%) organisms included, Staphylococcus aureus (20.3%) and Streptococcus agalactiae (9.8%). Another study from Malaysia also indicates that gram negative bacteria (52%) is the most frequently detected organism in DFI. Some of the most frequently detected organisms were, Proteus spp. (28%), P. aeruginosa (25%), Klebsiella pneumoniae (15%), E. coli (14.9%) and Enterobacter cloacae (13.9%). On the other hand, S. aureus (44%), Group B Streptococci (25%) and Enterococcus spp (9%) were the most frequently detected gram-positive organisms. Five anaerobic bacteria were detected in this study. They were, Peptostreptococcus spp., 3 Bacteroides spp and Clostridium spp.. They also reported 43% polymicrobial infection [22].

United States-based multicenter clinical trial conducted a study from 2001-2004. According to their research, 83.8% patients were suffering from polymicrobial infection. But, more importantly 43.7% patients were infected by four or more organisms. In this study gram positive (57.2%) bacteria were more prevalent. 48% patients were infected by only aerobes and 43.7% were infected by both aerobic and anaerobic bacteria. Frequently detected aerobic bacteria included, Nonfermenting gram-negative rods (7.7%), Pseudomonas spp. (9.3%), Enterobacteriaceae group (32.4%), Corynebacterium spp. (25.6%), Miscellaneous gram-positive rods (11.7%), Enterococcus spp. (33.9%), Staphylococcus spp. (85.5%), oxacillin resistant S. aureus (11.7%), oxacillin sensitive S. aureus (36.1%), S. epidermidis (15.9%), oxacillin sensitive S. epidermidis (3.3%), S. haemolyticus (4.8%), S. lugdunensis (4.8%). Coagulase-negative staphylococci (7.9%) and Streptococcus spp. (41.9%) were also reported to be found. Anaerobic bacteria included, Bacteroides fragilis group (12.1%), Fusobacterium spp. (2.4%), Porphyromonas spp. (11.7%), Prevotella spp. (14.1%), Anaerobic cocci (48.2%), Clostridium spp. (4.4%), Non spore forming gram-positive rods (9.5%). Gram positive bacteria consisted of 80% aerobic organism. Among them S. aureus (76.6%) was the most prominent. Other organisms included, Coagulase negative staphylococci, S. epidermidis, Staphylococcus lugdunensis, Staphylococcus haemolyticus, Staphylococcus simulans, Staphylococcus hominis, Streptococcus agalactiae, Streptococcus mitis, Streptococcus milleri, Enterococci, Helcococcus, Aerococcus, Gemella, Corynebacterium tuberculosis, CORYNEBACTERIUM, Corynebacterium xerosis and Corynebacterium urealyticum. Gram negative organisms included, Pseudomonas aeruginosa, Proteus mirabilis, Klebsiella species, while Enterobacteriaceae (63.3%) was identified as the largest group of gram negative rod [23].

A research from Kenyatta National Hospital, Nairobi detected 64.71% gram negative and 29.41% gram positive bacteria in DFU patients. Frequently detected organisms were, S. aureus (16.47%), E. coli (15.29%), Proteus mirabilis (10.59%), Klebsiella pneumoniae (7.06%) and P. aeruginosa (7.06%) [24].

A study conducted on patients admitted to endocrinology ward at All India Institute of Medical Sciences reports that, most of the patients were infected from aerobic bacteria only (65%). On the other hand, 1.2% patients were infected with anaerobic bacteria only and the rest of the 33.8% were infected by both. A staggering 70% patients were suffering from a polymicrobial infection while 12.5% patients were infected by more than three species. Frequently detected aerobic gram-negative bacteria (51.4%) included, Proteus species (12.6%), E. coli (12.0%), Pseudomonas aeruginosa (9.8%), Acinetobacter species (9.3%), Klebsiella species (6.6%) and 0.5% Citrobacter and Enterobacter species each. Aerobic gram positive (33.3%) organisms included, S. aureus (13.7%), Enterococcus species (11.5%), Coagulase negative Staphylococci (6.6%), Micrococcus species (1.6%). Anaerobic gram negative (7.1%) included, Veillonella species (1.6%), Bacteroides species (1.6%), Bacteroides fragilis (1.6%), Bacteroides eggerthii (1.1%), Bacteroides vulgari (0.5%), Bacteroides ovatus (0.5%). Anaerobic gram positive bacteria (8.2%) comprised off, Peptostreptococcus asaachroticus (4.4%), Peptostreptococcus species (1.6%), Peptostreptococcus anaerobius (0.5%), Clostridium perfringens (0.5%), Clostridium septicum (0.5%), Eubacterium lentum (0.5%) [25]. Another study detected 73.75% aerobic and 26.25% anaerobic organisms among the study population. Frequently detected anaerobes were Peptostreptococcus spp (42.85%), Bacteroides spp. (28.57%), Veillonella spp. (14.28%), Porphyromonas spp (9.52%) and Clostridium perfringens. In this study 49.32% gram
positive and 27.27% gram negative organism were also found. The most frequent isolates were, Proteus spp. (32.20%), Staphylococcus aureus (20.33%), Klebsiella spp. (18.64%), Enterobacter spp. (5.08%), Pseudomonas spp. (3.38%), Escherichia coli (3.38%), Enterococcus spp. (10.20%), Diptheroids (8.16%) and Citrobacter [26].

A study from Turkish Society of Clinical Microbiology and Infectious Diseases reports detecting 52% monomicrobial infection. However, the fatality rate was higher among individuals with polymicrobial infection (13% vs 2.3%). The prevalence of gram-negative bacteria (56.1%) was higher than gram positive bacteria according to this study. Some of the most frequent isolates were, S. aureus (20%), P aeruginosa (19%), E. Coli (12%). They also detected 79% coagulase negative Staphylococcus and 21% multidrug resistant P. aeruginosa [27].

Apart from bacteria, fungus also play a very important role in causing DFU. Patients who had to be amputated within 15 days of admission had higher amount of fungal infection [28]. According to an investigation, researchers detected fungal infection in 16.2% patients. The frequent fungal isolates were, Candida albicans (2.9%), Candida krusei (2%), Aspergillus (2%), Penicillium (1%) while Candida tropicalis (10.5%) was the most frequent one [28].

A study focused on isolating fungi from deep tissue of diabetic foot wounds detected 27.2% fungal species which included, Candida parapsilosis (25.5%), C. tropicalis (22.7%), T. asahii (12.8%), C. albicans (10.6%), Aspergillus sp. (5%), C. guilliermondii (2.8%), Non-albicans Candida sp. (2.8%), C. glabrata (2.8%), Fusarium sp. (2.8%), Candida sake (2.8%), Zygosaccharomyces sp. (2.1%), Kodamaea ohmeri (2.1%), Candida globose (1.4%), C. krusei (0.7%), Penicillium sp. (0.7%), C. lusitaniae (0.7%), Candida famata (0.7%), Candida melibiosica (0.7%) [29]. In this study they found that 5.8% individuals were infected by fungus only and 21.4% individuals had both fungal and bacterial infection [29].

Investigation conducted by S.L.Raheja Hospital and Diabetic Research Centre, Mumbai found that, among 41 patients undergone below limb amputation, 70.73 had fungal infection. In this research they found that, 40% of the study population were infected by fungal species. The most frequent isolates were, Candida albicans (40%), Candida krusei (10%), Cladosporium (10%), Aspergillus Niger (10%), Penicillium Marneffei (15%), C. Globata (7.5%) and Fusarium (7.5%) [30].

Investigation on patients admitted to JSS Hospital, Mysore, India was able to detect fungal species along with bacterial species. The fungal species found included, Candida, Aspergillus Niger, Aspergillus fumigates, Aspergillus flavus, Fusarium, Trichophytons (Dermatophyte), Penicillium, Acremonium [31].

Fungal species detected from a study on patients admitted to Emam Reza Hospital, Iran was comprised of, C. albicans (9.1%), C. tropicalis (4.1%), C. parapsilosis (0.83%), C. glabrata (0.83%), C. krusei (0.83%), Candida spp. (3.8%), T. mentagrophytes (2.5%), Rhodotorula spp. (0.83%), Acremonium spp. (0.83%), Scopulariopsis spp. (0.83%), A. fumigatus (0.83%) [19].

All of the bacterial and fungal prevalence in DFI can be summarized in Tables 1 and 2.
Table 1 (continued). List of the bacteria responsible for DFI

| Organism                        | Prevalence (%) | Reference |
|---------------------------------|----------------|-----------|
| Anaerobic cocci                 | 48.2%          | [23]      |
| Clostridium spp                 | 4.4%           | [23]      |
| Clostridium perfringens         | 0.5%           | [25]      |
| Clostridium septicum            | 0.5%           | [25]      |
| Non spore forming gram-positive rods | 9.5%   | [23]      |
| Citrobacter species             | 0.5%           | [25]      |
| Micrococcus species             | 1.6%           | [25]      |
| Veillonella species             | 1.6%           | [25]      |
| Veillonella spp                 | 14.28%         | [26]      |
| Peptostreptococcus spp          | 42.85%         | [26]      |
| Peptostreptococcus asaccharolyticus | 4.4%   | [25]      |
| Peptostreptococcus anaerobius   | 0.5%           | [25]      |
| Bacteroides fragilis group      | 12.1%          | [23]      |
| Bacteroides spp                 | 28.57%         | [26]      |
| Bacteroides fragilis            | 1.6%           | [25]      |
| Bacteroides eggerthii           | 1.1%           | [25]      |
| Bacteroides vulgaris            | 0.5%           | [25]      |
| Bacteroides vulgarius           | 0.5%           | [25]      |
| Diphtheroids                    | 8.16%          | [26]      |
| Eubacterium lentum              | 0.5%           | [25]      |

Table 2. List of the bacteria responsible for DFI

| Organism                        | Prevalence (%) | Reference |
|---------------------------------|----------------|-----------|
| Aspergillus species             | 5%             | [28]      |
| Aspergillus niger               | 2%             | [29]      |
| Aspergillus fumigatus           | 10%            | [30]      |
| Penicillium                     | 0.83%          | [19]      |
| Penicillium marneffei           | 1%             | [28]      |
| Candida tropicalis              | 15%            | [30]      |
| Candida parapsilosis            | 22.7%          | [28]      |
| Candida glabrata                | 22.7%          | [30]      |
| Candida albicans                | 10.5%          | [19]      |
| Candida lusitaniae              | 10.5%          | [19]      |
| Candida albicans                | 4.1%           | [29]      |
| Candida krusei                  | 25.5%          | [19]      |
| Candida guillermondii           | 2.8%           | [29]      |
| Candida sake                    | 2.8%           | [29]      |
| Candida glabrata                | 2.8%           | [29]      |
| Candida albicans                | 2.8%           | [29]      |
| Candida melibiosica             | 0.7%           | [29]      |
| Candida lusitaniae              | 0.7%           | [29]      |
| Candida albicans                | 0.7%           | [29]      |

DETECTION TECHNIQUES

Determination of various microorganism that play a major role in the foot infection of diabetic patients is very important. Because it is the usual reason behind high morbidity of diabetic patients, which results in serious complications such as gangrene and amputations [34]. Different types of detection tests are done worldwide. They are either biochemical methods or molecular techniques.

In a study by Birdem General Hospital, Bangladesh, they used fermentation, indole, nitrate disk reduction, special-potency disk test, catalase and urease test, sodium polyanethol sulphonate disk test, bile esculin hydrolysis test, lipase and lecithinase test, colony observation of fluorescence study and pigment production test [35]. However, anaerobic bacteria is very difficult to detect. To detect anaerobes a specialized detection test called the simple two step combustion technique in candle jar was used [35]. The same techniques were used by a study in India as well [32]. This modified candle jar technique was cheaper and simpler option than the traditional gas pak system which is used to detect anaerobes [26,35].

PCR, DGGE, 16S rRNA gene sequencing analysis, metagenomics and metatranscriptomics have emerged as an option for researchers and scientists to get a deep understanding of the bacterial population [36]. Delftia acidovorans, Serratia nematodiphila, Streptococcus salivarius, Fusobacterium nucleatum, Flavobacterium succinicans, Staphylococcus pettenkoferi are among the species that have been detected by 16 RNA method. However, these organisms could not be detected by other detection techniques [36].

Shotgun metagenomic sequencing detects bacterial population at the infected site. Many uncommon organisms including, Cornebacterium striatum, Propioniobacterium spp., Pseudomonas aeruginosa, Brevibacterium massiliense, Klebsiella oxytoca and Coagulase-negative species such as, Staphylococcus pettenkoferi, Staphylococcus simulans and Staphylococcus lugdunensis were detected using this technique [37].

A study on patients from Riyadh Medical Complex used API 20E, API 20Strep to determine the pathogens involved. In that study 98.5% aerobic and only 1.5% anaerobic bacteria was detected [38]. Vitek 2 and API 20A was used for the identification of species [39]. In another study where API system was used for identification, 97% aerobic bacteria was detected [40].

A study compared the effectiveness of conventional culture methods and 16s rDNA PCR to detect anaerobic organisms. 52% patients was determined to have anaerobic infection by PCR, whereas only 8% patients could be determined by conventional culture methods [41]. Similarly, another study also reports that, they were able to detect 65 pathogens by bacteria specific PCR [42]. Another study used PCR to specifically detect S. aureus. They were able to detect 44% S. aureus from the study population. They also targeted the mecA gene to detect MRSA and found 25% of the samples to be positive for MRSA [43].

In a research among patients admitted to Kenyatta National Hospital, Nairobi, comparison was done between biochemical tests and molecular techniques. RT-PCR showed 58.8% sensitivity to detect S. aureus, [24] showing that molecular tests were more sensitive than biochemical tests.
Molecular tests helped detecting organisms that were not detected by biochemical tests, although it was less specific than biochemical tests [24].

An in-depth study was carried out to determine the benefit and drawbacks of molecular and biochemical tests to detect pathogens in DFI. The comparison of results shows that, 88% of total sample was positive for S. aureus by RT-PCR while culture-based method was only positive for 57%. In case of S. pyogenes 15% were positive for RT-PCR and only 1% for culture-based method. Among S. agalactiae, 30% were positive for PCR and 22% for culture-based method. In case of S. dysgalactiae, 22% and 13% samples were found to be positive by PCR and culture-based method, respectively. This result indicates molecular detection is more efficient than biochemical method [44].

**ANTIBIOTIC RESISTANCE**

The main concern while dealing with an infection is to slow down the rate of infection or to eradicate it, however, due to the growing rate of antibiotic resistance in recent years it is getting difficult to treat DFU with antibiotics.

An investigation conducted on patients admitted to two Bangladeshi hospitals detected that, Staphylococcus spp. was 100% resistant towards monobactam and 67% resistant to penicillin-G group. *Acinetobacter spp.* was 86% resistant to penicillin and cephalosporin antibiotic group. *Bacillus spp.* was 88% resistant to monobactam, cephalosporin and penicillin group. Citrobacter was 100% resistant to cephalosporin group. Also, 82% of the study population were resistant to carbapenem antibiotic group [20].

United States multicenter clinical trial conducted an investigation from 2001-2004. They found that, Enterococci and MRSA strains were resistant to etrapenem. They also detected that, cephalexin, clindamycin and ciprofloxacin were not that much effective with ciprofloxacin being the most ineffective one. A gram-negative organism called *Stenotrophomonas maltophilia* was resistant towards almost all antibiotics.

According to a study, aerobic gram-negative organisms showed higher resistance to Amoxycillin (92%), amoxycillin-clavulanic acid (60%) and cephalosporins (72%). In case of anaerobes, high resistance was observed towards clindamycin (38.09%), penicillin (23.81%), cefoxitin (19.05%), imipenem (4.76%) and metronidazole [26].

Research conducted by Department of Medical Microbiology, UMMC, Malaysia discovered that, *S. aureus* was resistant towards methicillin (16%), vancomycin (100%), rifampin (100%), fusidic acid (7%), erythromycin (16%) and clindamycin (7%). Enterococci was resistant against, imipenem (8%), ampicillin (17%) and co-trimoxazole (25%). All isolates of group B streptococci were effective against penicillin, ampicillin, vancomycin, imoenem, cefuroxime and clindamycin [22].

In an investigation conducted on patients admitted to a hospital in Kenya, *Staphylococcus aureus* was detected to be resistant towards benzypenicillin and trimethoprim. Furthermore, *E. coli* was highly resistant to ampicillin, aztreanam, cefuroxime and TMP-SMX. While, *P. mirabilis* was resistant to ampicillin and *S. fonticola* species were resistant to ampicillin, amoxicillin, cefazolin, cefepime, ceftazidime, piperacillin-tazobactam and TMP-SMX. 30.77% *S. aureus* and 40.38% gram-negative bacilli were multi drug resistant organism in this study [24].

Enterobacteriaceae family exhibited high resistant to β-lactam. 90% *Acinetobacter spp.* strains were also resistant to β-lactam in another study. Most importantly they detected that almost all of the strains of *Acinetobacter spp.* had developed resistance against the mainstream antibiotics. *Pseudomonas aeruginosa* exhibited highest resistance to cefepime. 67% staphylococci strains exhibited resistance to cefotixin. Enterobacteriaceae and staphylococci exhibited almost 90% resistance to ampicillin. 87% strains of enterococci was resistant to tetracycline and erythromycin [45].

In a research conducted by BIRDEM General Hospital, Bangladesh, 43.8% *S. aureus* were methicillin resistant. It was also resistance to cotrimoxazole (62.5%), ciprofloxacin (75%) and tetracycline (56.3%). *Pseudomonas sp.* showed high resistant to, augmentin (75%), ceftazidime (66.7%), ceftriaxone (75%), cotrimoxazole (97.2%), tetracycline (80.6%) etc. *Proteus sp.* showed high resistance to, cefazidime (84%), cotrimoxazole (88%), ciprofloxacin (88%), tetracycline (84%). Klebsiella sp. was highly resistant to, cefotaxime (85%), cefuroxime (90.8%). *E. coli* exhibited high resistance to, cefuroxime (81.8%), ceftazidime (72.7%), ceftaxone (72%), tetracycline (72.7%) [21].

MRSA is a major threat in DFI patients. According to a study, they found 36% MRSA from the study population and they were highly resistant towards ciprofloxacin and erythromycin [46]. Another study reported that, MRSA was 100% resistant towards penicillin, 94.22% towards co-amoxiclav and 81.22% towards gentamicin [47].

Fungal species are developing resistance towards antibiotics as well. For instance, they were found to be resistant towards, flucytosine (1.5%), fluconazole (3.9%), amphotericin B (6.9%), voriconazole (6.9%) and itraconazole (17.7%) [29].

In another study, fungal species were found to be 100% resistant to clindamycin + amikacin and cloxacillin + pipacillin + tazobactem and cephalosporins [30].

**DISCUSSION**

Among various studies, *Staphylococcus aureus*, *E. coli*, *Pseudomonas aeruginosa*, *Proteus spp.*, *Klebsiella spp.*, *Enterobacter spp.* and *Enterococcus spp.* were found to be most frequent organisms detected. On the other hand, *Candida albicans*, *Candida krusei*, *Candida tropicalis*, *Candida glabrata* and *Candida parapsilosis* were the most commonly detected fungi across various studies. *Staphylococcus aureus* causes soft tissue and bone infections and a major part remains present at the lower part of the feet [48]. It can even invade and enter into osteoblasts [49], fibroblasts and endothelial cell [50]. It is also highly resistant to antibiotic and antibiotic therapy [50,51].

Among biochemical tests vs molecular method, molecular method was found to be more efficient and reliable. Biochemical tests are more time consuming. It also needs viable pathogens and suitable culture conditions for growth. Furthermore, they have lower detection sensitivity and might underestimate the bacterial prevalence [44]. However, molecular method is more fast and sensitive to detecting pathogens [44]. Molecular methods were able to detect that 52% patients were infected with anaerobic infection while biochemical method could only detect 8% from the same
organisms. During this 2-3 day period patients have to be given the real organisms responsible. Also, it takes 2-3 days to empiric antibiotic treatment which is not appropriate in 1/4th of organisms. There is a chance that these organisms are actually cultivate and determine the sensitivity pattern of the.

based methods often gives false negative results if the patient than the biochemical method (standard cultures). Culture had a history of previous antibiotic use, this problem is not present in case of RT-PCR detection. Besides, through PCR techniques. This review will help to understand the diversity of microorganism prevalence and antibiotic usage. It is recommended that we try to tackle antibiotic resistance by focusing on early detection and applying easy and quick detection evolving as multi-drug resistant. To prevent this, we need to focus on early detection and applying easy and quick detection techniques. This review will help to understand the diversity of microorganism and fungus responsible for DFU along with the pattern of antibiotic resistance and optimal detection technique.

CONCLUSION

Diabetic foot ulceration is becoming a big global threat day by day. The pathogens are not only diverse but also, they are evolving as multi-drug resistant. To prevent this, we need to focus on early detection and applying easy and quick detection techniques. This review will help to understand the diversity of microorganism and fungus responsible for DFU along with the pattern of antibiotic resistance and optimal detection technique.

Author contributions: All authors have sufficiently contributed to the study, and agreed with the results and conclusions.

Funding: No funding source is reported for this study.

Declaration of interest: No conflict of interest is declared by authors.

REFERENCES

1. Gemechu FW, Curley CA. Diabetic Foot Infections. Am Fam Physician. 2013. Available at: www.aafp.org/afpAmericanFamilyPhysician (Accessed: 3 July 2021).

2. Abdissa D, Adugna T, Gerema U, et al. Prevalence of diabetic foot ulcer and associated factors among adult diabetic patients on follow-up clinic at Jimma Medical Center, Southwest Ethiopia, 2019: An institutional-based cross-sectional study. J Diabetes Res. 2020;2020. https://doi.org/10.1155/2020/4106383 PMid:32258165 PMCid:PMC7102459

3. Murphy-Lavoie HM, Ramsey A, Nguyen M, et al. Diabetic foot infections. StatPearls. StatPearls Publishing; 2021. Available at: http://www.ncbi.nlm.nih.gov/pubmed/28722943 (Accessed: 3 July 2021).

4. Khan Y, M. Khan M, Raza Farooqui M. Diabetic foot ulcers: a review of current management. Int J Res Med Sci. 2017;5:4683. https://doi.org/10.18203/2320-6012.ijrms.20174916

5. Wu SC, Driver VR, Wrobel JS, et al. Foot ulcers in the diabetic patient, prevention and treatment. Vasc. Health Risk Manag. Dove Press; 2007:65-76. PMid:PMC1994045

6. Zhang P, Lu J, Jing Y, et al. Global epidemiology of diabetic foot ulceration: a systematic review and meta-analysis. Ann. Med. 2017;106-16. https://doi.org/10.1080/07853890.2016.1231932 PMid:27585063

7. Chammas NK, Hill RLR, Edmonds ME. Increased mortality in diabetic foot ulcer patients: The significance of ulcer type. J Diabetes Res. 2016;2016. https://doi.org/10.1155/2016/2879809 PMid:27213157 PMcid:PMC4860228

8. Jupiter DC, Thorud JC, Buckley CJ, et al. The impact of foot ulceration and amputation on mortality in diabetic patients. I: From ulceration to death, a systematic review. Int Wound J. 2015;13:892-903. https://doi.org/10.1111/iwj.12404 PMid:25601358 PMcid:PMC7950078

9. Cho NH, Shaw JE, Karuranga S, et al. IDF Diabetes Atlas: Global estimates of diabetes prevalence for 2017 and projections for 2045. Diabetes Res Clin Pract. 2018;138:271-81. https://doi.org/10.1016/j.diabres.2018.02.023 PMid:29496507

10. Holstein P, Ellitsgaard N, Olsen BB, et al. Decreasing incidence of major amputations in people with diabetes. Diabetologia. 2000;43:844-7. https://doi.org/10.1007/s001250051459 PMid:10952455

11. Demetriou M, Papanas N, Panagopoulos P, et al. Antibiotic resistance in diabetic foot soft tissue infections: A series from Greece. Int J Low Extrem Wounds. 2017;16:255-9. https://doi.org/10.1177/1534734617737640 PMid:29110552

12. Sánchez-Sánchez M, Cruz-Pulido WL, Bladínieres-Cámara E, et al. Bacterial prevalence and antibiotic resistance in clinical isolates of diabetic foot ulcers in the Northeast of Tamaulipas, Mexico. Int J Low Extrem Wounds. 2017;16:129-34. https://doi.org/10.1177/1534734617705254 PMid:28682727

13. Amini M, Davati A, Piri M. Determination of the resistance pattern of prevalent aerobic bacterial infections of diabetic foot ulcer. Iran. J. Pathol. Farname Inc in collaboration with Iranian Society of Pathology, 2013. Available at: http://ijp.iranpath.org/article_8328.html (Accessed: 28 June 2021).

14. Xie X, Bao Y, Ni L, et al. Bacterial profile and antibiotic resistance in patients with diabetic foot ulcer in Guangzhou, Southern China: Focus on the differences among different Wagner’s grades, IDSA/IWGDF Grades, and Ulcer Types. Int J Endocrinol. 2017;2017. https://doi.org/10.1155/2017/8694903 PMid:29075293 PMCid:PMC5623783
References:

15. Menon VU, Kumar KV,Gilchrist A, et al. Prevalence of known and undetected diabetes and associated risk factors in central Kerala - ADEPS. Diabetes Res Clin Pract. 2006;74:289-94. https://doi.org/10.1016/j.diabres.2006.03.025 PMid:16730847

16. Pecoraro RE, Ahroni JH, Boyko EJ, et al. Chronology and determinants of tissue repair in diabetic lower-extremity ulcers. Diabetes. 1991;40:1305-13. https://doi.org/10.2337/diabetes.40.10.1305 PMid:1936593

17. Viswanathan V. The diabetic foot: Perspectives from Chennai, South India. Int. J. Low. Extrem. Wounds. 2007;3-6. https://doi.org/10.1177/1534734606297987 PMid:17344200

18. Lipsky BA, Berendt AR, Cornia PB, et al. 2012 infectious diseases society of America clinical practice guideline for the diagnosis and treatment of diabetic foot infections. Clin. Infect. Dis. Oxford Academic; 2012:e132-e173. https://doi.org/10.1093/cid/cis346 PMid:22619242

19. Saba Fata M, Saeed Modagheh MH, Faizi R, et al. Mycotic infections in diabetic foot ulcers in Emam Reza hospital. Jundishapur J Microbiol. 2011.

20. Karmaker M, Sanyal SK, Sultana M, et al. Association of bacteria in diabetic and non-diabetic foot infection - An investigation in patients from Bangladesh. J Infect Public Health. 2016;9:267-77. https://doi.org/10.1016/j.jiph.2015.10.011 PMid:26617249

21. Paul S, Barai L, Jahan A, et al. A Bacteriological Study of Diabetic Foot Infection in an Urban Tertiary Care Hospital in Dhaka City. Ibrahim Med Coll J. 1970;3:50-https://doi.org/10.3329/imjc.v3i2.4216

22. NS Raja. Microbiology of diabetic foot infections in a teaching hospital in Malaysia: a retrospective study of 194 cases. J Microbiol Immunol Infect. 2007;40:39. PMid:17332905

23. Citron DM, Goldstein EJC, Merriam CV, et al. Bacteriology of moderate-to-severe diabetic foot infections and in vitro activity of antimicrobial agents. J Clin Microbiol. 2007;45:2819-28. https://doi.org/10.1128/JCM.00551-07 PMid:17609322 PMCid:PMC2045270

24. Mutonga DM, Mureithi MW, Ngugi NN, et al. Bacterial isolation and antibiotic susceptibility from diabetic foot ulcers in Kenya using microbiological tests and comparison with RT-PCR in detection of S. aureus and MRSA. BMC Res Notes. 2019;12:1-6. https://doi.org/10.1186/s13104-019-4278-0 PMid:31036061 PMCid:PMC6489269

25. Gadepalli R, Dhawan B, Sreenivas V, et al. A clinico-microbiological study of diabetic foot ulcers in an Indian tertiary care hospital. Diabetes Care. 2006;29:1727-32. https://doi.org/10.2337/dc06-0116 PMid:16873771

26. Haldar J, Mukherjee P, Mukhopadhyay S, et al. Isolation of bacteria from diabetic foot ulcers with special reference to anaerobe isolation by simple two-step combustion technique in candle jar. Indian J Med Res. 2017;145:97-101. https://doi.org/10.4103/ijmr.IJMR_1436_14 PMid:28574021 PMCid:PMC5460581

27. Saltoglu N, Ergonul O, Tulek N, et al. Influence of multidrug resistant organisms on the outcome of diabetic foot infection. Int J Infect Dis. 2018;70:10-4. https://doi.org/10.1016/j.ijid.2018.02.013 PMid:29476898

28. Sanjayasi S, Balu J, Narayanan CD. Fungal Infection: A Hidden Enemy in Diabetic Foot Ulcers. J Foot Ankle Surg (Asia Pacific). 2015;2:74-6. https://doi.org/10.5005/jp-journals-10040-1033

29. Chellan G, Shivaprakash S, Ramaiyar SK, et al. Spectrum and prevalence of fungal infecting deep tissues of lower-limb wounds in patients with type 2 diabetes. J Clin Microbiol. 2010;48:2097-102. https://doi.org/10.1128/JCM.02035-09 PMid:20410345 PMCid:PMC2884499

30. Kareliya H, Bichile L, Bal A, et al. Fungal Infection in Diabetic Foot A Clinico-microbiological Study. Acta Sci Microbiology. 2019;2:49-55.

31. Raza M, Anurshetrus BS. Clinical study of coexistence of fungal infections in diabetic foot ulcers and its management. Int Surg J. 2017;4:3943. https://doi.org/10.18203/2349-2902.isj20175157

32. Haldar J, Mukherjee P, Mukhopadhyay S, et al. Isolation of bacteria from diabetic foot ulcers with special reference to anaerobe isolation by simple two-step combustion technique in candle jar. Indian J Med Res. 2017;145:97-101. https://doi.org/10.4103/ijmr.IJMR_1436_14 PMid:28574021 PMCid:PMC5460581

33. Sharifah Aisyah SH, Siti Asma’ H, Nurahan M. The significant association between polymicrobial diabetic foot infection and its severity and outcomes. Malaysian J Med Sci. 2019;26:107-14. https://doi.org/10.21315/mjms2019.26.1.10 PMid:30914988 PMCid:PMC6419864

34. Grayson ML. Diabetic foot infections - Antimicrobial therapy. Infect. Dis. Clin. North Am.; 1995:143-61. https://doi.org/10.1016/S0891-5520(20)30645-0

35. Mohammuddunnoobi - Jahan T, Amin A AL. Microbiological Study of Diabetic Foot Ulcer. BIRDEM Med J. 2018;8:251-6. https://doi.org/10.3329/birdem.v8i3.38133

36. Reiber GE, Smith DG, Vileikyte L, et al. Causal pathways for incident lower-extremity ulcers in patients with diabetes from two settings. Diabetes Care. 1999;22:157-62. https://doi.org/10.2337/diacare.22.1.157 PMid:10333919

37. Kalan L, Meisel J, Loesche M, et al. The microbial basis of impaired wound healing: differential roles for pathogens, “bystanders”, and strain-level diversification in clinical outcomes. bioRxiv. 2018;427567. https://doi.org/10.1101/427567

38. Alodaini HA, Shoeb AA. An overview of epidemiology and etiology of bacteria associated with diabetic injuries and their dominant infection at Central Region, Riyadh, Saudi Arabia. African Journal of Bacteriology Research, 2018;10(2):6-14. https://doi.org/10.5897/JBRL2017.0250

39. Demetriou M, Papanas N, Panopoulos M, et al. Tissue and swab culture in diabetic foot infections: Neuropathic versus neuroischemic ulcers. Int J Low Extrem Wounds. 2013;12:87-93. https://doi.org/10.1016/j.ijsu.2013.03.001 PMid:23667099

40. Kadir A, Satyavani M, Pande K. Bacteriological study of diabetic foot infections. Brunei Int Med J. 2012;19-26.

41. Aherrao N, Shahi SK, Dwivedi A, Kumar A, Gupta S, Singh SK. Detection of anaerobic infection in diabetic foot ulcer using PCR technique and the status of metronidazole therapy on treatment outcome. Wounds. 2012 Oct;24(10):283-8. PMid:235776052

42. Noor S, Raghav A, Parwez I, et al. Molecular and culture based assessment of bacterial pathogens in subjects with diabetic foot ulcer. Diabetes Metab Syndr Clin Res Rev. 2018;12:417-21. https://doi.org/10.1016/j.dsx.2018.03.001 PMid:29580872
43. Sandhu S, Rathnayake I, Huygens F. Prevalence of methicillin resistance and virulence determinants of \textit{Staphylococcus aureus} in diabetic foot ulcers. Int J Basic Clin Pharmacol. 2014;3:978. https://doi.org/10.5455/2319-2003.ijbcp20141201

44. Stappers MHT, Hagen F, Reimnitz P, et al. Direct molecular versus culture-based assessment of Gram-positive cocci in biopsies of patients with major abscesses and diabetic foot infections. Eur J Clin Microbiol Infect Dis. 2015;34:1885-92. https://doi.org/10.1007/s10096-015-2428-4 PMid:26143347 PMCid:PMC4545178

45. Murali TS, Kavitha S, Spoorthi J, et al. Characteristics of microbial drug resistance and its correlates in chronic diabetic foot ulcer infections. J Med Microbiol. 2014;63:1377-85. https://doi.org/10.1099/jmm.0.076034-0 PMid:25038136

46. Mottola C, Semedo-Lemsaddek T, Mendes JJ, et al. Molecular typing, virulence traits and antimicrobial resistance of diabetic foot staphylococci. J Biomed Sci. 2016;23:1-10. https://doi.org/10.1186/s12929-016-0250-7 PMid:26952716 PMCid:PMC4782296

47. Jafari-Sales A, Farhadi F, Ezeidyadi M, et al. Study of antibiotic resistance pattern in methicillin-resistant \textit{Staphylococcus aureus} isolated from clinical samples of hospitals in Tabriz – Iran. Int J Biomed Public Heal. 2018;1:71-5. Available at: http://www.ijbmph.com (Accessed: 28 June 2021).

48. Ambrosch A, Haefner S, Jude E, et al. Diabetic foot infections: Microbiological aspects, current and future antibiotic therapy focusing on methicillin-resistant \textit{Staphylococcus aureus}. Int. Wound J. 2011;567-77. https://doi.org/10.1111/j.1742-481X.2011.00849.x PMid:21883937 PMCid:PMC7950829

49. Shi S, Xianlong Z. Interaction of \textit{Staphylococcus aureus} with osteoblasts (Review). Exp. Ther. Med. 2012;367-70. https://doi.org/10.3892/etm.2011.423 PMid:22969897 PMCid:PMC3438663

50. Garcia LG, Lemaire S, Kahl BC, et al. Antibiotic activity against small-colony variants of \textit{Staphylococcus aureus}: Review of in vitro, animal and clinical data. J Antimicrob Chemother. 2013;68:1455-64. https://doi.org/10.1093/jac/dkt072 PMid:23485724

51. Baumert N, Von Eiff C, Schaff F, et al. Physiology and antibiotic susceptibility of \textit{Staphylococcus aureus} small colony variants. Microb Drug Resist. 2002;8:253-60. https://doi.org/10.1089/10766290260469507 PMid:12523621

52. Lavery LA, Armstrong DG, Wunderlich RP, et al. Risk factors for foot infections in individuals with diabetes. Diabetes Care. 2006;29:1288-93. https://doi.org/10.2337/dc05-2425 PMid:16732010

53. Prompers L, Huijberts M, Apelqvist J, et al. High prevalence of ischaemia, infection and serious comorbidity in patients with diabetic foot disease in Europe. Baseline results from the Eurodiale study. Diabetologia. 2007;50:18-25. https://doi.org/10.1007/s00125-006-0491-1 PMid:17093942

54. Lipsky BA, Napolitano LM, Moran GJ, et al. Inappropriate initial antibiotic treatment for complicated skin and soft tissue infections in hospitalized patients: Incidence and associated factors. Diagn Microbiol Infect Dis. 2014;79:273-9. https://doi.org/10.1016/j.diagmicrobio.2014.02.011 PMid:24661685

55. Percival SL, McCarty SM, Lipsky B. Biofilms and Wounds: An Overview of the Evidence. Adv Wound Care. 2015;4:373-81. https://doi.org/10.1089/wound.2014.0557 PMid:26155379 PMCid:PMC4486148