Electronic Journal of General Medicine
2021, 18(6), em323
e-ISSN: 2516-3507
https://www.ejgm.co.uk/

MODESTUM

Review Article **OPEN ACCESS**

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

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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

ARTICLE INFO

Received: 7 Jul. 2021

Accepted: 26 Sep. 2021

ABSTRACT

Diabetic foot ulcer (DFU) is becoming one of the major complications around the world with associated consequences such as lower-extremity amputation, high morbidity, mortality and hospitalization. It has the possibility to become the next global epidemic. Major part of the concern comes from the diverse bacterial and fungal population that is found at the infection site and their growing antimicrobial resistance. If the threat of antimicrobial resistance is not dealt with than it will rise to become the main cause of mortality and below knee amputation in case DFU. Also, most of the time main focus is given on detecting bacterial population which causes the fungal population to go unnoticed and act as the silent enemy. Bacterial and fungal prevalence scenario from different countries have been discussed in this study along with the alarming antibiotic resistance scenario around the globe. Furthermore, choosing the correct technique to identify them also plays a vital role. With proven lacking's of the culture-based methods maybe it is time to move on to the faster and more specific molecular methods. As, many of the molecular techniques have already proven to be more efficient. This review discussed the bacterial and fungal prevalence along with their growing antimicrobial resistance and evaluated different biochemical and molecular techniques in identification process.

Keywords: DFU, causative agents, antibiotic resistance, wound infection, molecular methods, biochemical tests

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]. However, 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*, *Peptococcus 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 organism. 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 tuberculostearicum, Corynebacterium amycolatum, 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 vulgaris* (0.5%), *Bacteroides ovatus* (0.5%). Anaerobic gram positive bacteria (8.2%) comprised off, *Peptostreptococcus assachrolyticus* (4.4%), Peptrostreptococcus species (1.6%), *Peptrostreptococcus 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 *Peptrostreptococcus 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. Glabrata* (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. galbrata* (0.83%), *C. krusei* (0.83%), *Candida spp*. (3.3%), *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. List of the bacteria responsible for DFI

Organism	Prevalence (%)	Reference
Enterococcus spp.	33.9%	[20]
	10.20%	[26]
	9%	[25]
	3%	[23]
Klebsiella spp.	18.64%	[26]
	8%	[20,33]
	6.6%	[25]
Klebsiella pneumoniae	15%	[22]
	7.6%	[24]
Bacillus cereus	17%	[20]
Enterobacter hormaechei	22%	[20]
Enterobacter cloacae	13.9%	[22]
Enterobacteriaceae group	32.4%	[23]
Staphylococcus species	13%	[20]
Staphylococcus aureus	76.6%	[33]
	44%	[27]
	21.3%	[21]
	20.3%	[22]
	20.3%	[24]
	20%	[26]
	16.47%	[25]
	13.7%	[23]
Oxacillin resistant *Staphylococcus aureus*	11.7%	[23]
Oxacillin sensitive *Staphylococcus aureus*	36.1%	[23]
Staphylococcus epidermidis	15.9%	[23]
Oxacillin sensitive *Staphylococcus epidermidis*	3.3%	[23]
Staphylococcus haemolyticus	4.8%	[23]
Staphylococcus lugdunensis	4.8%	[23]
Staphylococcus spp.	85.5%	[23]
Coagulase negative Staphylococci	79%	[27]
	7.9%	[25]
	6.6%	[23]
Acinetobacter baumannii	10%	[20]
Acinetobacter spp	4.5%	[33]
Acinetobacter species	9.3%	[25]
Pseudomonas aeruginosa	25%	[27]
	19%	[22]
	9.8%	[24]
	7.06%	[25]
Pseudomonas spp	48%	[33]
	27.8%	[21]
	9.35	[26]
	3.38%	[23]
Proteus spp.	33%	[21]
	28%	[22]
	11%	[33]
Proteus mirabilis	10.59%	[24]
Proteus species	12.6%	[25]
E. coli	15.29%	[33]
	14.9%	[27]
	12%	[22]
	12%	[24]
	3.8%	[26]
	3.38%	[25]
Streptococcus agalactiae	25%	[22]
	0.8%	[33]
Streptococcus spp	41.9%	[23]
Non-fermenting gram-negative rods	7.7%	[23]
Corynebacterium spp.	25.6%	[23]
Miscellaneous gram-positive rods	11.7%	[23]
Fusobacterium spp.	2.4%	[23]
Porphyromonas spp.	11.7%	[23]
Prevotella spp	14.1%	[23]

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]
Veilonella species	1.6%	[25]
Veilonella 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]
	1.6%	[25]
Bacteroides fragilis	1.6%	[25]
Bacteroides eggerthii	1.1%	[25]
Bacteroides vulgaris	0.5%	[25]
Bacteroides vulgaris	0.5%	[25]
Diptheroids	8.16%	[26]
Eubacterium lentum	0.5%	[25]

Table 2. List of the bacteria responsible for DFI

Organism	Prevalence (%)	Reference
Aspergillus species	5%	[28]
	2%	[29]
Aspergillus niger	10%	[30]
Aspergillus fumigatus	0.83%	[19]
Penicillium	1%	[28]
Penicillium marneffei	15%	[30]
Candida tropicalis	22.7%	[28]
	22.7%	[30]
	10.5%	[19]
	4.1%	[29]
Candida parapsilosis	25.5%	[19]
	0.83%	[29]
Candida glabrata	7.5%	[30]
	2.8%	[19]
	0.83%	[29]
Candida spp.	3.3%	[19]
Candida guilliermondii	2.8%	[29]
Candida sake	2.8%	[29]
Candida globose	1.4%	[29]
Candida famata	0.7%	[29]
Candida melibiosica	0.7%	[29]
Candida lusitaniae	0.7%	[29]
Candida albicans	40%	[28]
	10.6%	[30]
	9.15	[19]
	2.9%	[29]
Candida krusei	10%	[28]
	2%	[30]
	0.83%	[19]
	0.7%	[29]
Kodmaea ohmeri	2.1%	[29]
Rhodotorula spp.	0.83%	[19]
Scopulariopsis spp	0.83%	[19]
Acremonium spp.	0.83%	[19]
T. mentagrophytes	2.5%	[19]
Fusarium	7.5%	[30]
Cladosporium	10%	[30]
Trichosporon asahii	12.8%	[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 andurease 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, anerobic 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 rRNA 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, *Corrnebacterium striatum, Propionibacterium spp., Pophyromonas somerae, 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 tests 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

[24]. 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 ertapenem. 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 maltophillia* 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, imioenem, cefuroxime and clindamycin [22].

In an investigation conducted on patients admitted to a hospital in Kenya, *Staphylococcus aureus* was detected to be resistant towards benzylpenicillin and trimethoprim. Furthermore, *E. coli* was highly resistant to ampicillin, aztrenam, cefuroxime and TMPSMX. While, *P. mirabilis* was resistant to ampicillin and *S. fonticola* species were resistant to ampicillin, amoxicillin, cefazolin, cefepime, ceftazidime, piperacillin-tazobactam and TMPSMX. 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 ß-lactam. 90% *Acinetobacter spp*. strains were also resistant to ß-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 cefoxitin. 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, augmantin (75%), ceftazidime (66.7%), ceftriaxone (75%), cotrimoxazole (97.2%), tetracycline (80.6%) etc. *Proteus sp*. showed high resistance to, ceftazidime (84%), cotrimoxazole (88%), ciprofloxacin (88%), tetracycline (84%). *Klebsiella sp*. was highly resistant to, cefotaxime (85.7%), cefuroxime (90.8%). *E. coli* exhibited high resistance to, cefuroxime (81.8%), ceftazidime (72.7%), ceftriaxone (72.7%), 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, fugal species were found to be 100% resistant to clindamycin + amikacin and cloxacillin + pipracillin + tazobactum 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

sample [41]. So, based on this and from the data discussed earlier in the article it can be said that Molecular tests are the better of the two.

Detection of DFI has some limitations. The wounds sites are full of various colonies of organisms. Thus, because of specific culture-based methods can return no definitive result [52,53]. Besides, in the biochemical methods we only grow known organisms. There is a chance that these organisms are actually laboratory weeds, which means we are not being able to detect the real organisms responsible. Also, it takes 2-3 days to cultivate and determine the sensitivity pattern of the organisms. During this 2-3 day period patients have to be given empiric antibiotic treatment which is not appropriate in 1/4th of cases [54]. Moreover, in cases of polymicrobial infection, pathogenic and harmless colonizers cannot be differentiated. Furthermore, patients who are already under antibiotic treatment sometimes gives false negative results in biochemical tests [55]. Also, molecular methods were found to be less specific than culture-based methods [24].

It is recommended to use molecular techniques (RT-PCR) for the detection process. This process is time saving and it also has the ability to identify smaller concentrations of organism than the biochemical method (standard cultures). Culture based methods often gives false negative results if the patient had a history of previous antibiotic use, this problem is not present in case of RT-PCR detection. Besides, through PCR multiple types of organism can be detected together whereas only one organism could be detected per culture [41]. Overall, RT-PCR is globally available technique. So, it would be financially affordable for mid/low-income countries as well.

All the organisms mentioned above showed resistance against different ranges of antibiotics. However, the pattern of resistance varied greatly depending on geography, microorganism prevalence and antibiotic usage. It is recommended that we try to tackle antibiotic resistance by ensuring proper usage of antibiotics.

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.

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