Journal of Current Medical Research and Opinion
Received 18-01-2024| Revised 20-01-2024| Accepted 10-02-2024| Published Online 12-02-2024

DOI: https://doi.org/10.52845/CMRO/2024/7-2-7 ISSN (O) 2589-8779 | (P) 2589-8760
CMRO 07 (02), 2107-2114 (2024)
Original Articles

# Isolation of Fungi from Diabetic Foot Ulcer

**Rawan Ziad Tariq[1], Aliaa majeed adakhaynah[2], Fatima Rafid latif[3], Mina Jawad Shaker[4], Zahraa Ali Yasser[5], Zainab Hamed Alwan[6], Asraa Hassan Mutashar[7], Ghufran Rahim Shanaawah Malih[8], Raghda Muhammad Kazem[9], Nagham Abd Al Ameer Kadem[10]**

**Abstract:**

Among all of diabetes complications, foot ulcers are at higher risk to occur, and it is estimated that 20% of hospital admissions among DM patients result from diabetic foot ulcers (DFUs). DFUs can lead to infection, gangrene, amputation, and if proper treatment is not provided, can even cause death. In fact, once a DFU is developed there is a greater risk of amputation, and it is estimated that 50–70% of all lower limb amputations (LLAs) are due to DFUs. It is predicted that in the general population (≥45 years), the incidence of vascular LLA in the diabetic is eight times higher than in non-diabetic individuals, and when it comes to the age group ≥ 85 years, the incidence in men increases to 15 times higher and 12 times higher in women than the mean incidence rates of all population groups. Most fungus like to live in a warm, moist area, and particularly on the foot they like to live in the interdigital web spaces, which lead to maceration and fungal infection between the toes. If this is left untreated it inevitably leads to breakdown in the skin leading to ulceration, allowing other pathogens a portal to set up infection. There is very little risk of pathogens infecting the superficial skin and can easily be dealt with topical antifungals, however when the fungal infection invades the deeper structures through repeated trauma it takes a whole new angle and can lead to some devastating results. Diabetic patients are a 10-fold greater risk of being admitted as a result of soft tissue or bone infection.

**Keywords:** Diabetic, Fungi, Foot Ulcer

[1]Dhi qar university college pathological analysis
[2]Dhi qar university college pathological analysis
[3]Dhi qar university college pathological analysis
[4]Dhi qar university college pathological analysis
[5]Dhi Qar University, College of Science/Department of Chemistry
[6]Dhi Qar University, College of Science/Department of Biology
[7]Dhi Qar University, College of Science/Department of Biology
[8]Dhi qar university college pathological analysis
[9]Dhi Qar University, College of Science/Department of Chemistry
[10]Dhi qar university college pathological analysis



## Introduction:

Diabetes *mellitus* (DM) is one of the most prevalent endocrine diseases worldwide, characterised by an increase in blood glucose levels caused by defective insulin secretion, action or both [1]. Officially, diabetes is classified into three major groups, namely, Type 1, Type 2 and gestational [2]. Type 1 DM or insulin-dependent DM (IDDM) is the result of a failure of the body to produce insulin, due to destruction of pancreatic β-cells [3]. This type represents only 5–10% of all diabetes cases and is mostly associated to genetic factors [2]. Type 2 DM, also known as non-insulin dependent DM (NIDDM),

longer diabetic duration, hypertension, diabetic retinopathy and a history of smoking [9.22]. DFUs, especially chronic ulcers, can lead to amputations which can cause a significant decrease in life quality and an increase in early mortality [8]. The five-year relative mortality after a DFU is 48%, which is a higher value than that of many types of cancers [23]. However, the reported frequency of ulceration varies considerably. For example, the prevalence of active DFUs ranges from 3% in Oceania to 13% in North America, with a global mean prevalence of 6.3% [22]. This difference is even greater when comparing DFU prevalence among countries [22].

For example, Belgium has the highest prevalence of DFUs with 16.6%, whereas Australia presents the lowest value: 1.5% [22]. These disparities are justified by some differences in the healthcare systems. For instance, in Belgium, the state supports the treatment costs, but not the expenses related to preventive treatments, whereas in Australia, fewer than 50% of diabetic patients have regular foot examinations [24,25].

**Aims of study:**

1. Diagnosis of the main cause of diabetic foot ulcers.
2. Identify fungi and some types of bacteria that cause ulcers.
3. Obtaining pure cultures from the causative fungi.
4. Knowledge of isolation and cultivation methods.
5. Identify the possible causes of the injury.
6. How to protect and treat ulcers.

**Literature review**

**Fungi**

Fungi (singular: fungus) are a kingdom of usually multicellular eukaryotic organisms that are heterotrophs (cannot make their own food) and have important roles in nutrient cycling in an ecosystem. Fungi reproduce both sexually and asexually, and they also have symbiotic associations with plants and bacteria. However, they are also responsible for some diseases in plants and animals. The study of fungi is known as mycology.

represents the most common type comprising 85% of all cases [2]. This Type results from insulin resistance or insufficient insulin production, essentially associated with multihormonal disorders [4]. Gestational DM (GDM) is mainly caused by a blockage of insulin action by the pregnancy hormones, causing insulin resistance and hyperglycaemia [5]. Women with a history of GDM are more likely to develop Type 2 diabetes later in their lives [5]. The global prevalence of diabetes increases every year. In fact, the number of people with diabetes between 1980 and 2014 quadrupled, from 108 million to 422 million, and the numbers continue to escalate [6]. This increase is so fast that diabetes is now considered by the World Health Organization (WHO) as a global fast-growing epidemic [6]. According to epidemiological studies, if the current trend continues, by 2045, 700 million adults will suffer from the disease, which represents a 51% increase [7,8]. In addition to this global public health threat, there is a huge economic burden associated. The related annual global health expenditure will rise 11% from USD 760 billion in 2019 to USD 845 billion by 2045 [8].

In addition to the economic impact associated with this disease, there are other multifaceted complications that account for more than 50% of these direct costs, such as nephropathy, neuropathy, retinopathy, atherosclerosis and foot ulcers [9,10,11,12,13]. These secondary pathophysiological outcomes are a result of a deficiency in the vascular system, causing inefficient circulation [14-16]. Unfortunately, the COVID-19 outbreak has had a negative impact on healthcare delivery to patients with DFUs [17,18]. In fact, a study in Naples reported that patients with diabetes admitted to a Tertiary Care Center for DFU management had a threefold risk of amputation compared to 2019 numbers [19]. LLA has a greater negative impact on the patient's quality of life than any other complication of diabetes, such as renal failure or blindness, with depression and anxiety highly associated [12,20]. The diabetic foot is a major medical, social and economic problem, affecting 40 to 60 million people globally [21]. The main risk factors for diabetic foot ulceration are older age, male sex, Type 2 diabetes, lower body mass index,

pathogens a portal to set up infection. There is very little risk of pathogens infecting the superficial skin and can easily be dealt with topical antifungals, however when the fungal infection invades the deeper structures through repeated trauma it takes a whole new angle and can lead to some devastating results.Diabetic patients are a 10-fold greater risk [26] of being admitted as a result of soft tissue or bone infection

**Bacteria**

Bacteria microbes with a cell structure simpler than that of many other organisms. Their control centre, containing the genetic information, is contained in a single loop of DNA. Some bacteria have an extra circle of genetic material called a plasmid rather than a nucleus. The plasmid often contains genes that give the bacterium some advantage over other bacteria. For example it may contain a gene that makes the bacterium resistant to a certain antibiotic. Bacteria reproduce by binary fission. In this process the bacterium, which is a single cell, divides into two identical daughter cells.

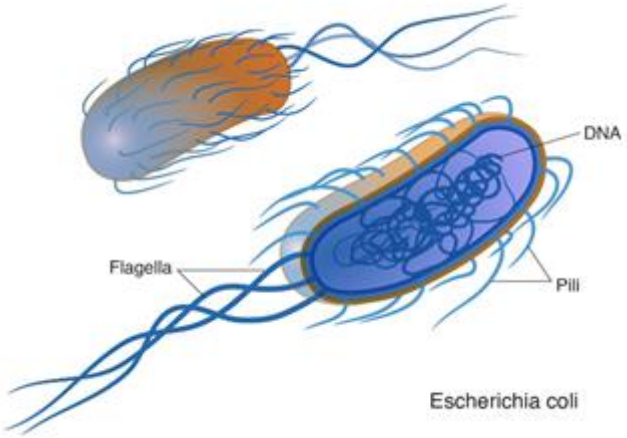


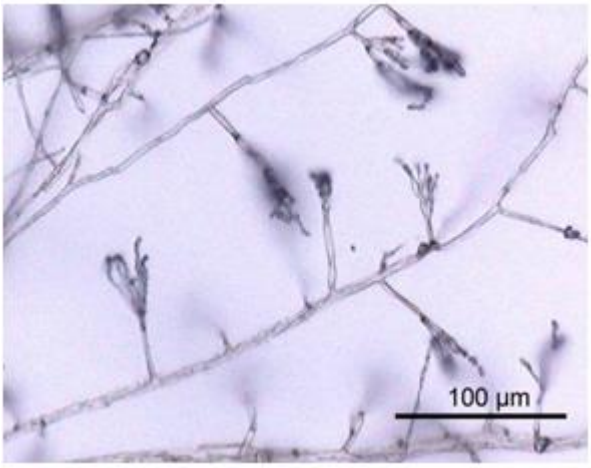

**Figure (2): Escherichia coli**

**Importance Bacteria**

Bacteria play important roles in many natural processes, including nutrient cycling and decomposition. They are also used in biotechnology, such as in the production of antibiotics, vaccines, and other medicines. However, some bacteria are harmful and can cause diseases, such as pneumonia, strep throat, and food poisoning.

**Bacteria Cases diabetic foot ulcer**

**Figure(1): These are hyphae of a Penicillium fungus.**




**Importance of fungi**

Humans have been indirectly aware of fungi since the first loaf of leavened bread was baked and the first tub of grape must was turned into wine. Fungi are everywhere in very large numbers in the soil and the air, in lakes, rivers, and seas, on and within plants and animals, in food and clothing, and in the human body. Together with bacteria, fungi are responsible for breaking down organic matter and releasing carbon, oxygen, nitrogen, and phosphorus into the soil and the atmosphere. Fungi are essential to many household and industrial processes, notably the making of bread, wine, beer, and certain cheeses. The medical relevance of fungi was discovered in 1928, when Scottish bacteriologist Alexander Fleming noticed the green mold Penicillium notatum growing in a culture dish of Staphylococcus bacteria. Around the spot of mold was a clear ring in which no bacteria grew. Fleming successfully isolated the substance from the mold that inhibited the growth of bacteria. In 1929 he published a scientific report announcing the discovery of penicillin, the first of a series of antibiotics many of them derived from fungi that have revolutionized medical practice.

**Fungi cause diabetic foot ulcers**

Most fungus like to live in a warm, moist area, and particularly on the foot they like to live in the interdigital web spaces, which lead to maceration and fungal infection between the toes. If this is left untreated it inevitably leads to breakdown in the skin leading to ulceration, allowing other

data, in 85% of diabetic patients foot ulcers preceded amputations of the same extremity; they also increase by as much as 20-fold the risk of minor or major amputation of the same extremity *[37,38]*. Bacterial infections of diabetic foot ulcers are polymicrobial and mixed aerobic-anaerobi *[35,36]*. While *Staphylococcus spp*., *Streptococcus spp*., *Enterococcus spp.*, species of *Enterobacteriaceae* and *Pseudomonas spp*. Are the most common aerobic isolates, *Peptostreptococcus spp*. and *Bacteroides spp*. are the most common anaerobic isolates *[35,36]*. Literature data on the frequency of fungal isolation from the diabetic foot ulcer differ significantly. *Candida spp*. is the most commonly isolated yeast from these ulcers (less than 5%-21%). Given the conditions prevailing in diabetic foot, even low pathogenic yeasts may cause an infection of foot ulcers. These types of yeasts often belong to the normal mycobiota of the skin around ulcers, or may colonize diabetic foot ulcers secondarily, hindering the assessment of the real role of fungal isolates from the ulcer. Because of their typical form and size, fungal elements can be visualized in histopathologic preparations of ulcer biopsy using different staining procedures [39,40]. Microscopic demonstration of tissue invasion and host reaction confirms the existence of fungal infection. There have been some reports of an increased incidence of fungal infections (dermatophytosis and candidiasis) of interdigital spaces and nails in the toes of diabetic patients, as well as of the association of these infections with the development of severe and deep inflammatory processes in feet [39,40] .

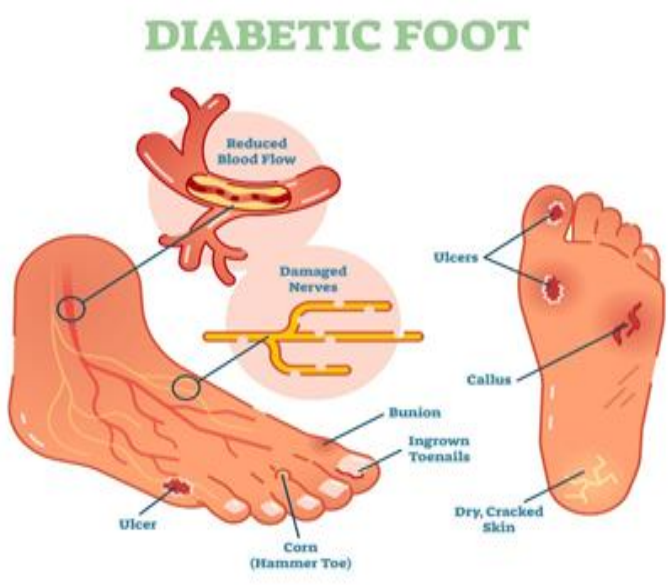



**Figure (3): Diabetic foot ulcer**

DFUs are mainly exposed to skin commensal bacteria that can colonize the wound as multi-layered microbial communities surrounded by a self-produced protective extracellular biofilm *[27]*. Biofilm makes wound healing and infections very difficult to resolve, as local access for antimicrobial agents and the host's immune system is hampered *[28]*. Colonization is defined as the presence of multiplying bacteria with no overt host immunological reaction *[29]*. In contrast, infection results when invading organisms overwhelm the host's defenses. Several factors are thought to be involved, including the *bacterial load* within the *wwound [30]*. *It has been suggested that bacterial concentration greater than* $10^5$ *colony-forming units (CFUs) per gram of tissue [31] indicates* the presence of a "critical" degree of colonization at which the host defenses are no longer able to contain it *[29]*. However, many individuals with diabetes mellitus have an impaired inflammatory response and may not show the classical signs of infection in a wound with a high bacterial load *[32]*. Another important factor that correlates with the likelihood of infection is the virulence of the colonizing microorganism, as demonstrated by the toxin secreting β-haemolytic streptococci that were able to induce tissue damage at $10^2$ CFUs per gram of tissue *[31]*. Recent works have reported that toxigenic *Staphylococcus aureus* strains (harboring exfoliatin-, EDIN-, PVL- or TSST-encoding genes) are rarely isolated from DFUs but are often present in infections with a more severe grade and systemic impact, whereas non-toxigenic strains seem to remain localized in deep structures and bone with diabetic foot *osteomyelitis [33]*.

**Diabetic foot ulcers**

Approximately 15% of persons with diabetes will have foot ulcer in their lifetime *[34]*. The pathogenesis of diabetic foot is highly complex, including polyneuropathy, peripheral vascular disease, and compromised immunity, slower wound healing, trauma and infection [*35,36]*. Complications associated with the development of infection and diabetic foot syndrome are the main cause of morbidity, nontraumatic lower extremity amputations, and diabetic patient mortality [*35,36]*. As shown by epidemiologic

with 10% povidone-iodine followed by sterile normal saline. Culture swabs were taken from the depth of the ulcers by a cotton-tipped applicator measuring around 0.5 cm x 0.5 cm and kept in routinely used normal saliva-containing sterile containers. Punch biopsy forcep was used to take tissue from the depth of a particular ulcer under the aseptic technique. A tissue sample was collected, sealed, labeled, and sent to the microbiology lab within 1 h to detect the strains of fungal pathogens.

**Laboratory procedures:**

The tissue sample was subjected to the following microscopic examination before the inoculation in culture media.

1) Microscopic examination of the KOH prepared tissue sample: tissue was kept on a slide with the addition of 10% KOH at 37°C for 2 h and then examined under the microscope.

2) Microscopic examination of Gram-stained tissue smear: a smear was obtained on a clean and dry slide from the crushed tissue sample and was dried, fixed by heating, and stained with Gram stain. After proper staining, it was examined under oil immersion for identification of Gram-positive budding cells and pseudohyphae,

specific for Candida infection.

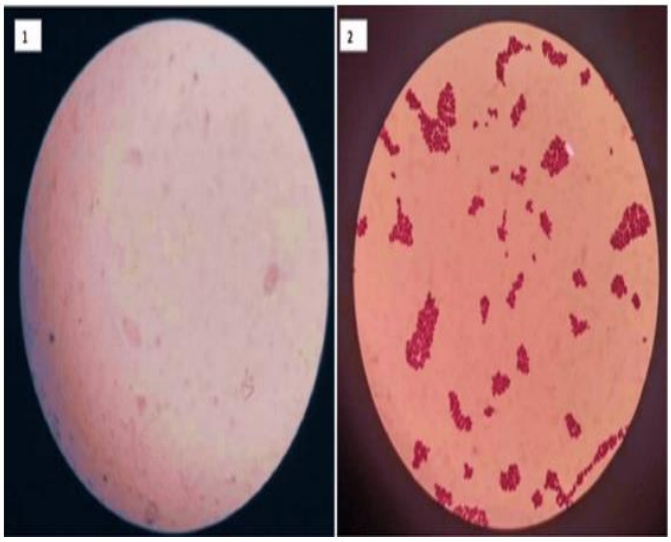



**Figure (4): 1. KOH mount for fungal identification. 2. Gram staining of fungal organism**

**Method of the culture of tissue sample**

A culture media of Sabouraud's dextrose agar (SDA) with chloramphenicol antibiotic was used

**Previous studies on DFU**

was over one hundred years ago that **Pryce**, a surgeon working in Nottingham, England, recognised the connection between diabetes and foot ulceration: "Diabetes itself may play an active part in the causation of perforating ulcers" he wrote in the Lancet, and further "it is abundantly evident that the actual cause of the perforating ulcers was a peripheral nerve degeneration" [*41,42]*. However, it was **Paul Brand (1914–2003)** who added science to the art of foot care *[43,44,45]*. When he spoke at a US Department of Health conference and was asked to make a recommendation on reducing amputation in diabetes, most listeners expected an answer promoting vascular surgery or modern medications. They were surprised to hear that his key recommendation was a national campaign to encourage physicians to remove patients' shoes and socks and to examine the feet *[43]*. Although always emphasising the art of clinical medicine in his work, **Brand also performed pioneering research** looking at abnormal foot pressures during walking, and he described the use of thermography in assessing areas at risk of imminent breakdown *[44.45]*. Progress in our understanding of the pathogenesis and management of the diabetic foot has been made in the last 20 years. This has been matched by an increasing number of publications in peer-reviewed journals. Taken as a percentage of all Pub-Med listed papers on diabetes, papers on the diabetic foot have increased from 0.7% in the 1980–88 period to more than 2.7% in the last 6 years. During the same time period, foot councils and study groups have been formed in the EASD and the ADA, and the International Working Group has published an international consensus booklet on the diabetic foot *[46]*.

**Diagnosis**

Method of isolating fungi from diabetic foot ulcer. A review of previous studies conducted from April 2018 to March 2020.

**Sample collection**

Before taking a culture from the depth of the ulcer the wound surrounding was cleaned thoroughly

Mellitus: A Chronic Endocrine Disorder. Eur. J. Biol. 2019, 4, 1–14.

3. Katsarou, A.; Gudbjörnsdottir, S.; Rawshani, A.; Dabelea, D.; Bonifacio, E.; Anderson, B.J.; Jacobsen, L.; Schatz, D.A.; Lernmark, Å. Type 1 diabetes mellitus. Nat. Rev. Dis. Prim. 2017, 3, nrdp201716.
4. Chatterjee, S.; Khunti, K.; Davies, M.J. Type 2 diabetes. Lancet 2017, 389, 2239–2251.
5. Coustan, D.R. Gestational Diabetes Mellitus. Clin. Chem. 2013, 59, 1310–1321
6. WHO..2020.Availableonline: https://www.who.int/health-topics/diabetes#tab=tab_1 (accessed on 23 March 2021).
7. Wild, S.; Roglic, G.; Green, A.; Sicree, R.; King, H. Estimates for the year 2000 and projections for 2030. Diabetes Care 2004, 27, 1047–1053.
8. IDF. IDF Diabetes Atlas, 9th ed.; International Diabetes Federation: Brussels, Belgium, 2019.
9. Forlee, M. What is the diabetic foot? The rising prevalence of diabetes worldwide will mean an increasing prevalence of complications such as those of the extremities. CME 2011, 29, 4–8.
10. Icks, A.; Scheer, M.; Morbach, S.; Genz, J.; Haastert, B.; Giani, G.; Glaeske, G.; Hoffmann, F. Time-Dependent Impact of Diabetes on Mortality in Patients After Major Lower Extremity Amputation: Survival in a population-based 5-year cohort in Germany. Diabetes Care 2011, 34, 1350–1354.
11. Kerr, M. Foot Care for People with Diabetes: The Economic Case for Change. NHS Diabetes 2012-03. 2012. Available online: http://www.diabetes.org.uk/Documents/%0Anhs-diabetes/footcare/footcare-

for culturing the tissue sample. Four such culture tubes were prepared and tissue samples were inoculated. Cycloheximide (actidione) was added to two tubes out of four tubes. Culture tubes containing both chloramphenicol and cycloheximide were incubated at 25°C-30°C. The addition of chloramphenicol inhibits the growth of bacteria whereas cycloheximide inhibits the growth of contaminant saprophytic fungi. Cultures were examined twice a week for 4-6 weeks.

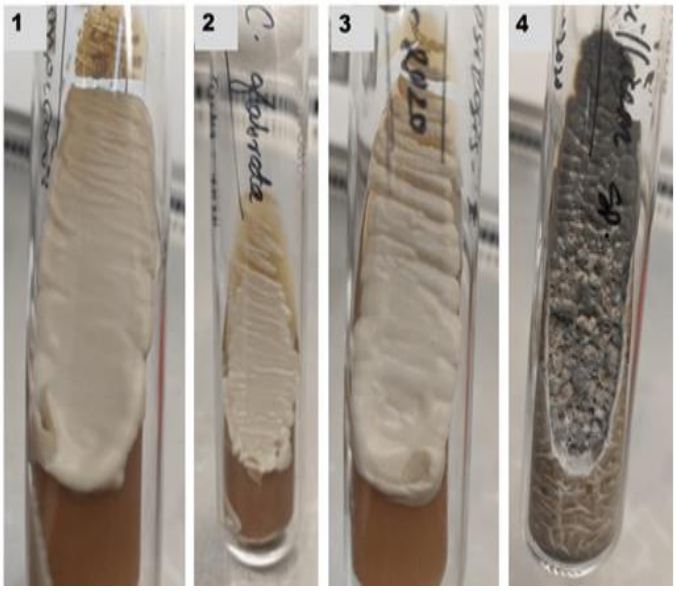



**Figure (5): Candida and Penicillium species growth in SDA medium.**

**1 – Candida tropicalis; 2 – Candida glabrata; 3 – Candida parapsilosis; 4 – Penicillium species.**

### 2.5.4 Mounting of fungal growth:

Once the growth was identified on the culture medium it was mounted using lactophenol cotton blue (LPCB) for staining filaments and spores of the fungus. Sometimes LPCB mounting disturbs fungal morphology during the process, so slide culture was added in most of the cases to protect the intact morphology of the isolated fungi.

## References

1. American Diabetes Association. Diagnosis and Classification of Diabetes Mellitus. Diabetes Care 2013, 37, S81–S90.
2. Mukhtar, Y.; Galalain, A.M.; Yunusa, U.M. A Modern Overview on Diabetes

Emergencies. Diabetes Care 2020, 43, e123–e124.

20. Martin, J.M.; Zenilman, J.M.; Lazarus, G.S. Molecular Microbiology: New Dimensions for Cutaneous Biology and Wound Healing. J. Investig. Dermatol. 2010, 130, 38–48.
21. IDF. IDF Diabetes Atlas, 8th ed.; International Diabetes Federation: Brussels, Belgium, 2017.
22. Zhang, P.; Lu, J.; Jing, Y.; Tang, S.; Zhu, D.; Bi, Y. Global epidemiology of diabetic foot ulceration: A systematic review and meta-analysis. Ann. Med. 2017, 49, 106–116.
23. Armstrong, D.G.; Wrobel, J.; Robbins, J.M. Guest Editorial: Are diabetes-related wounds and amputations worse than cancer? Int. Wound J. 2007, 4, 286–287.
24. Van Acker, K.; Oleen-Burkey, M.; De Decker, L.; Vanmaele, R.; Van Schil, P.; Matricali, G.; Dys, H.; De Leeuw, I. Cost and resource utilization for prevention and treatment of foot lesions in a diabetic foot clinic in Belgium. Diabetes Res. Clin. Pr. 2000, 50, 87–95.
25. Tapp, R.; Zimmet, P.Z.; Harper, C.A.; De Courten, M.P.; Balkau, B.; Mccarty, D.J.; Taylor, H.; Welborn, T.A.; Shaw, J.E. Diabetes Care in an Australian Population: Frequency of screening examinations for eye and foot complications of diabetes. Diabetes Care 2004, 27, 688–693.
26. Lavery LA, Armstrong DG, Wunderlich RP, Mohler MJ, Wendel CS, Lipsky BA.2006.
27. G.A. James, E. Swogger, R. Wolcott, Pulci ni,E. deLancey, P. Secor, J. Sestrich, *et al.*Biofilms in chronic wounds Wound Repair Regen, 16 (2008), pp. 37-44.
28. T.F. Mah, G.A. O'Toole Mechanisms of biofilm resistance to antimicrobial agents for-people-with-diabetes.pdf (accessed on 23 March 2021).
12. Barshes, N.R.; Sigireddi, M.; Wrobel, J.S.; Mahankali, A.; Robbins, J.M.; Kougias, P.; Armstrong, D.G. The system of care for the diabetic foot: Objectives, outcomes, and opportunities. Diabet. Foot Ankle 2013, 4, 21847.
13. WHO. Global Report on Diabetes; Geneva World Health Organization Press: Geneva, Switzerland, 2016.
14. Noor, S.; Zubair, M.; Ahmad, J. Diabetic foot ulcer—A review on pathophysiology, classification and microbial etiology. Diabetes Metab. Syndr. Clin. Res. Rev. 2015, 9, 192–199.
15. Yazdanpanah, L. Literature review on the management of diabetic foot ulcer. World J. Diabetes 2015, 6, 37–53.
16. Johannesson, A.; Larsson, G.-U.; Ramstrand, N.; Turkiewicz, A.; Wiréhn, A.-B.; Atroshi, I. Incidence of Lower-Limb Amputation in the Diabetic and Nondiabetic General Population: A 10-year population-based cohort study of initial unilateral and contralateral amputations and reamputations. Diabetes Care 2009, 32, 275–280.
17. Liu, C.; You, J.; Zhu, W.; Chen, Y.; Li, S.; Zhu, Y.; Ji, S.; Wang, Y.; Li, H.; Li, L.; et al. The COVID-19 Outbreak Negatively Affects the Delivery of Care for Patients with Diabetic Foot Ulcers. Diabetes Care 2020, 43, e125–e126.
18. Boulton, A. Diabetic Foot Disease during the COVID-19 Pandemic. Medicina 2021, 57, 97.
19. Caruso, P.; Longo, M.; Signoriello, S.; Gicchino, M.; Maiorino, M.I.; Bellastella, G.; Chiodini, P.; Giugliano, D.; Esposito, K. Diabetic Foot Problems During the COVID-19 Pandemic in a Tertiary Care Center: The Emergency Among the

diabetic. Int J Dermatol1999;38(Suppl. 2):S17-19.

38. Gupta AK, Humke S. The prevalence and management of onychomycosis in diabetic patients. Eur J Dermatol 2000;10:379-384.
39. Chandler FW, Kaplan W, Ajello L. Color atlas and textbook of the histopathology of mycotic diseases. Chicago: Year Book Medical Publisher, 1980.
40. Ajello L, Hay RJ. Medical mycology. In: Topley & Wilson's microbiology and microbial infections, 9th ed. London: Arnold, 1998.
41. Pryce TD (1887) A case of perforating ulcers of both feet associated with diabetes and ataxic symptoms. Lancet 2:11–12 .
42. Boulton AJM (1990) Lawrence Lecture: the diabetic foot: neuropathic in aetiology? Diabetic Med 7:852–858 .
43. Brand PW (1983) The Diabetic Foot. In: Ellenberg M, Rifkin H (eds) Diabetes mellitus: theory and practice, 3rd edn. Med Exam Publishing, New York, pp 829–849.
44. Bauman JH, Brand PW (1963) Measurement of pressure between foot and shoe. Lancet 1:629–632 .
45. Bergtholdt HT, Brand PW (1976) Temperature assessment and plantar inflammation. Lepr Rev 47:211–219 CAS .
46. Bergtholdt HT, International Working Group on the Diabetic Foot (1999) International consensus on the diabetic foot. Amsterdam, The Netherlands.

47.

Trends Microbiol, 9 (2001), pp. 34-39 View PDF.

29. D.T. Williams, J.R. Hilton, K.G. Harding Diagnosing foot infection in diabetes Clin Infect Dis, 39 (2004), pp. S83-S96
30. Y.N. Pierpont, M.G. Uberti, F. Ko, M.C. R obson, C.A. Smith, T.E. Wright, *et al.*Individualized, targeted wound treatment based on the tissue bacterial level as a biological marker Am J Surg, 202 (2011), pp. 220-224 .
31. G. Dow, A. Browne, R.G. Sibbald Infection in chronic wounds: controversies in diagnosis and treatment Ostomy Wound Manage, 45 (1999) 23–7, 29–40; quiz 41-42 .
32. T. Bjarnsholt, K. Kirketerp.Møller, P.Ø. Je nsen, K.G. Madsen, R. Phipps, K. Krogfelt , *et al.*Why chronic wounds will not heal: a novel hypothesis Wound Repair Regen, 16 (2008), pp. 2-10.
33. C. Dunyach-Remy, C. Ngba Essebe, A. Sotto, J.-P. Lavigne *Staphylococcus aureus* toxins and diabetic foot ulcers: role in pathogenesis and interest in diagnosisToxins, 8 (2016) .
34. Mayfield JA, Reiber GE, Sanders LJ, Janisse D,Pogach LM. Preventive foot care in people withdiabetes. Diabetes Care 1998;21:2161-2177.
35. Kahn CR, Weir GC, eds. Joslin's diabetes mellitus,13th ed. Media: Williams &Wilkins, 1994.
36. Hanèeviæ J, Coce F, Bo‘ikov V, eds. Diabetic foot.Zagreb: Medicinska naklada, 2002.
37. Rich P, Hare A. Onychomycosis in a special patientpopulation - focus on the