Received: February 7, 2023
Accepted: May 8, 2023

Acad Med J 2023;3(2):11-18
UDC: 616.5-022.7
https://www.doi.org/10.53582/AMJ2332011mt
Review article

MICROBIOME AND MYCOBIOME IN CHRONIC WOUNDS

Mitrova Telenta Julija, Dimova Maja, Dohceva Karajovanov Ivana

University Clinic for Dermatovenerology, Faculty of Medicine,
Ss. Cyril and Methodius University in Skopje, Republic of North Macedonia
e-mail: julija_25mkd@yahoo.com

Abstract

Skin as the biggest organ with protective function in the human body, makes an equilibrium between microbial communities and immune system. Skin microbiome is defined as the genome of microorganisms found on the skin with which microorganisms have a complex relationship. Microbiota of healthy skin consists of resident and transient microorganisms. Two most common factors for delayed healing process in chronic wounds are infection and biofilm formation. Thus, it is important to analyze microbiome and mycobiome of chronic wounds.

Keywords: microbiome, mycobiome, chronic wounds, biofilm

Introduction

Skin is the biggest organ in the human body. Its complex structure and functionality make it natural barrier, organ with protective function, which make an equilibrium between microbial communities of human body and immune system. Microbiota which is mandatory for a healthy skin is composed of many microorganisms on skin and scalp, either commensal or facultative pathogenic. Skin microbiome is defined as the genome of the microorganisms found on the skin with which microorganisms have a complex relationship[1,2].

Healthy skin microbiota consists of resident and transient microorganisms. The first one, known as fixed group, or the core microbiota are routinely found in the skin. These are commensal microorganisms, usually harmless and beneficial to a host. The other one, also known as transient microorganisms, or tourists are not permanent residents of skin, they disappear after few hours or days living on the skin[3,4]. The three most common genera in skin microbiota are: C*orynebacteria, Propionibacteria* and *Staphylococci*, and four main phyla are: *Actinobacteria, Firmicutes, Proteobacteria* and *Bacteroides*[5].

The composition of microbiota depends on genetics, diet, lifestyle, environment, anatomical area. As a result, every human being has unique microbiota. Depending on anatomical microenvironment microbiota differs in moist, sebaceous, dry areas, and areas of apocrine, eccrine and sebaceous glands and hair follicles[1,3,5].

Microbiome in chronic wounds

Chronic wounds have impaired prolonged healing process that lasts more than expected time frame of 3-6 weeks[6,7]. The first most common factor for this delayed healing is infection and associated pathological inflammation of the wound. The second one is biofilm formation[8]. Chronic wounds may not always be infected, but just colonized by a distinct microbiome that could impact the healing process and lead to an infection. The culture-independent studies from the past decade showed that wounds microbiota was diverse.

Most frequently are isolated *Staphylococcus* spp., *Pseudomonas* spp., *Corynebacterium* spp., *Anaerococcus* spp., *and Enterococcus spp.*[9-11]. Microbiome is a complex, diverse, microbial community within the human body. When skin barrier is breached, microbes from microbiome reach the wound tissue and impair the healing process[12].

Clinical practice depends on the fact which microbial community types evade or have no effect on wound healing process. The new era of sequencing technologies for microbiome examination highlights that culture-based methods in fact underestimate the diversity and complexity of human microbiome[12]. This results in increasing the number of wound microbiome studies. Thus, bacterial microbiome of the chronic wounds and skin is well-defined.

Wolcott *et al.* in their study analyzed samples of chronic wounds from venous leg ulcers, diabetic foot ulcers, nonhealing surgical wounds, and decubitus ulcers. The most frequent species found in all wound types were *Staphylococcus, Pseudomonas, Corynebacterium* and *Streptococcus*[9]. The microbial composition of the microbiome of chronic wounds was not influenced by wound type nor by demographic characteristics of patients[9].

It is very important for chronic wound microbiome to identify correlations of the microbiome to healing outcomes. Loesche *et al.* found that positive healing outcomes were associated with temporal instability of communities, particularly the transition between several distinct community types[10]. It is important to know which organisms are beneficial or detrimental for evaluating prognosis or probiotic interventions. There are still no reports on specific metabolic types which could be predictive for healing outcomes[10].

The common skin commensals *Micrococcus, Paracoccus,* and *Kocuria* are associated with normal skin, such as *Corynebacterium* and *Staphylococcus (S. hominis, S. haemolyticus,* and *S. cohnii*). On the other side, wound colonizers and pathogens *S. aureus, S. capitis, Proteus, Enterobacter, Helcococcus,* and *Pseudomonas*. *Staphylococcus spp.* are associated with both skin and wounds[13].

Verbanic *et al.* compared wound swabs before and after debridement of wounds, and there was no significant difference in the microbiome composition of wounds[13]. But that was not the case with healed *vs*. unhealed wounds, because there was the over-representation of facultative anaerobes in the microbiome of nonhealing wounds. On the other hand, anaerobes enriched healed wounds. As a result, infections in which anaerobes play a key role are easily healed and the level of oxygen increases in tissue, so anaerobic microorganisms are disfavoured[14]. On contrary, infections in which facultative anaerobes play the key role, would be more tolerant to the conditions of a healing which are changing and that is the reason for their persisting. All this has implications in treatments based on increasing oxygen tension in the wound, hyperbaric oxygen treatments[15]. Wounds with presence of pathogenic facultative anaerobes are refractory to oxygen therapies, hence these treatments should be used in wounds with low levels of facultative anaerobes. Recent studies have found that variable oxygen tension is a dominant stress in the environment of the biofilm, and having in mind the fact that facultative anaerobes may better tolerate the substantial oxygen gradients within the biofilm they cause biofilm persistence[16,17].

A biofilm is a community of cells adhered to a surface, encased in an extracellular matrix (ECM) which is self-produced (Figure 1). Microorganisms in biofilms transit from free-floating to sessile, and have increased antimicrobial tolerance and virulence compared to their planktonic counterparts[18,19]. The biofilm formation is controlled by the quorum sensing system. Quorum sensing (QS) is a cell-to-cell communication between bacteria in biofilm mediated with small molecules, chemical signals called autoinducers. When the bacterial density increases, the signalling molecules accumulate in the surrounding environment. When the minimal threshold of signalling molecules concentration is reached, they bind to receptor proteins, and the expression of genes is activated which is associated with biofilm formation. This communication can be stopped with QS inhibiting agents, including QS inhibitors and

quorum quenching enzymes, using variety of mechanisms, consequently inhibiting the formation of biofilms. QS inhibiting agents can increase bacterial sensitivity to antibiotics. The use of QS inhibiting agents can be one of the promising new treatment approaches to control bacterial infections[20].

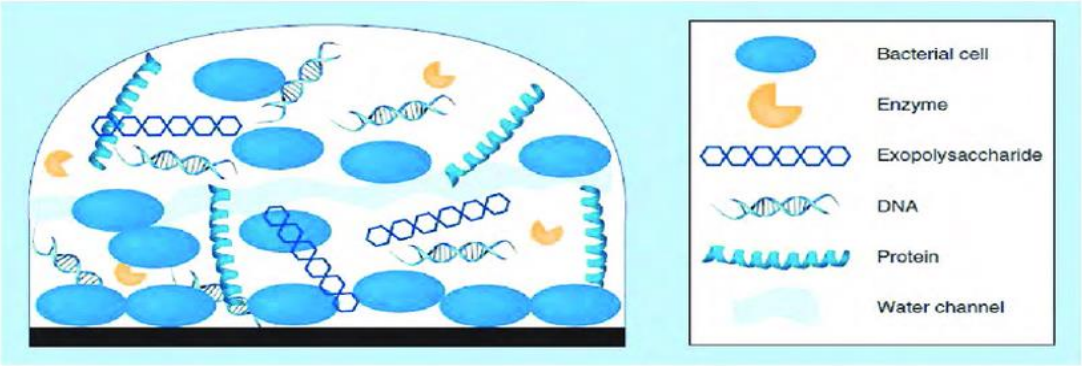

Fig. 1. Structure of biofilm
(Sangwan S, Pratibha P, Hemender T. Anti-biofilm enzymes: a strategy to remove biofilms: Agrobios Volume XVII, Issue No 12, 2019)

Studies that have focused on biofilm infections in chronic wounds showed that *Pseudomonas aeruginosa*, an opportunistic pathogen that is not found in healthy skin microbiome, formed aggregates within the host and used many of the virulence factors such as the LasR quorum sensing system[21].

It is important to note that the chronic wound microbiome is a complex entity, and biofilms are multi-species. Multi-species biofilms compared to single-species biofilms have increased recalcitrance to antimicrobials, which has been highlighted in recent studies. Inoculum consisting of *S. aureus* and *P. aeruginosa* was with higher rates of infection, which was observed in a rat model[22]. Similarly, in a murine infection model the anaerobic bacteria, *Prevotella bivia* increased pathogenicity of *S. aureus*[23]. Dalton *et al.* in their study found that complex multi-species biofilms, containing *Enterococcus faecalis, Finegoldia magna, P. aeruginosa*, and *S. aureus*, resulted in impaired healing process while remaining viable over a period of 12 days. They also reported that multi-species biofilms increased antimicrobial tolerance to treatments compared to single-species biofilm counterparts[24].

Role of fungi in chronic wounds - mycobiome

Fungi are very important; they are more than 100 times larger than bacteria and make up a considerable part of the microbiome causing infections and high levels of mortality and morbidity[25].

The skin is home to bacteria, fungi, and viruses, and that is the first line of defense against foreign microorganisms[26]. The composition of mycobiome is often determined by the body site, with *Malassezia spp*. dominating most sites. The mycobiome of the foot and moist areas comprises genera such as *Candida, Aspergillus*, and *Penicillium*[26].

Using culture-dependant techniques, and confirmed with the next generation sequencing methods, *Malassezia, Aspergillus, and Candida species* are found to be the most cultured fungi from the skin[12,27]. On the other side, the role that fungi play in chronic wounds and their effect on wound healing process is still debated in the literature. In 80% of samples culture-independent studies have identified fungi[27]. Most of the data refers to fungi isolates in diabetic foot ulcers. Chellan *et al*. in their study identified that 30% of cases with diabetic foot ulcers had fungal infections, with *Candida spp.* being the most prevalent, and the presence of *Aspergillus spp.* and *Trichosporon spp*. was also shown[28]. Kalan *et al*. found that longer healing time was associated with an increased abundance of *Ascomycota*[10]. These studies

confirm that mycobiome have impact on wound healing similarly as bacterial microbiota, where increased bacterial diversity is associated with delayed wound healing process[29].

Fungi are thought to be opportunistic pathogens, so when wound is treated with antibiotics and fungi are colonising the surrounding skin an ideal environment is created for fungal infection. Higher levels of blood glucose make *Candida* isolates to display higher activity of enzyme and that results in higher virulence, so *Candida spp.* from commensal becomes pathogen species[29].

Kalan *et al.* by using PCR-based amplicon sequencing of the fungal ITS1 region precisely defined the prevalence and structure of fungal communities in diabetic foot ulcers over a period of 6 months, in an attempt to link the clinical outcomes with polymicrobial microbiomes[12]. The most important finding was that fungi were very diverse and prevalent members of diabetic foot ulcer microbiomes. The mycobiome was with interpersonal, intrapersonal and temporal variation, and patients with systemic antibiotics had significantly higher fungal diversity in their wounds than those who were not taking antibiotics. As a result, use of antibiotics targeting bacteria may create an environment favourable to fungal colonization and expansion[12]. The most commonly isolated yeasts from diabetic foot ulcers were *Candida species* with 3 most frequently detected *(C. parapsilosis, C. tropicalis,* and *C. albicans*).

Mycobiome in diabetic foot ulcers is more frequently composed of *Cladosporium spp.* with other allergic fungi such as *Penicillium spp., Aspergillus spp., Alternaria spp., Fusarium spp.* and *Pleospora spp*. *Candida spp., Trichosporon asahii*, and *Rhodotorula spp.* as pathogenic and opportunistic fungi. They have been associated with poor outcomes in the healing process, such as amputation, or open wounds for 6 months and longer. These polymicrobial fungi communities in chronic wounds are mostly isolated from necrotic, nonviable wound tissue. It is still unclear if the polymicrobial mycobiome contributes to, or is a result of, necrosis of chronic wounds[12].

Dowd *et al*. in their retrospective study evaluated molecular diagnostic reports from 915 chronic wounds, and the results showed that 23% of clinical specimens were positive for fungal species[30]. The classification of the wounds was made in 5 categories: pressure ulcers, diabetic foot ulcers, non-healing surgical wounds, venous leg ulcers and general chronic wounds. The results showed identification of 48 different species of fungi, from 34 genera with predominance of *Candida* genus in all wound types. The most frequently isolated were *C. albicans* and *C. parapsilosis* species. In four of five wound types, *Malasezia restricta* and *Curvularia lunata* were also identified. The other fungal species isolated from chronic wounds were *Aureobasidium, Cladosporium, Ulocladium, Engodontium,* and *Trichtophyton*, which were also prevalent components of these polymicrobial infections[30]. The study found a significant negative correlation between *Staphylococcus* and *Candida* and significant relationships between both bacterial and fungal genera and patient metadata, including gender, diabetes status, and cardiovascular comorbidities. The authors concluded that fungi were more important wound pathogens and opportunistic pathogens than previously reported, and with the application of modern cost-effective and comprehensive molecular diagnostics, clinicians could identify and address this significant component of chronic wound bioburden with targeted therapies, thereby improving healing trajectories[30].

The study by Mehra *et al.* investigated the incidence of mycotic (fungal) infections in diabetic foot ulcers. The study found that 30 of 105 patients (28.6%) were positive for fungal elements on direct microscopy in 10% KOH mount, while fungal cultures on Sabouraud's dextrose agar [SDA] were positive in 21 (20%) patients. *Candida* species were the most common fungus isolated (11.43%), followed by *Aspergillus* (3.81%), *Fusarium* species (2.86%), and *Trichophyton* species (1.90%)[30].

The study found that males with diabetes were more prone to developing foot ulcers and infection than females, and that most of the patients had diabetes for more than 10 years with poor glycemic control. The study suggests that treating clinicians often focus on bacterial infections in diabetic foot ulcers, which may lead to longer hospital stays and a protracted course of illness. The authors recommend further research to better understand the microbiological profile of diabetic foot ulcers and the role of fungal infections in their development and treatment[31].

Multi-species wound biofilms

The wound microbiome consists of mixed bacterial-fungal communities, which is a result of the interactions between some clinically important bacterial and fungal species[12]. These interactions are best studied *in vitro* to better understand the antagonistic and synergistic virulence potential of interkingdom interactions. It has been reported that *Candida spp.* interacts with diverse bacteria such as *Staphylococcus aureus, Streptococcus spp, Pseudomonas aeruginosa, Esherichia coli, Enterococcus faecalis, Acinetobacter baumannii,* and *Burkholderia cenocepacia*[32]. Fungal hyphae adhere to surfaces and make a substrate to which bacteria are bound. As a result, increased resistance to antimicrobial agent occurs in biofilms.

The relationship between *S. aureus* and *C. albicans,* two pathogens often found in diabetic foot ulcers, is well studied. It is known that tolerance of *S. aureus* to antibiotics is increased because of increased production of extracellular DNA and fungal components of extracellular matrix of biofilm. Also, there is increased production of toxin, as a result of increased virulence by upregulating the agr quorum sensing pathway[33-35]. It is also found that this relationship is reciprocal, so that *S. aureus* upregulates *C. albicans* biofilm and virulence genes[36]. The presence of *C. albicans* within chronic wound biofilm was identified as one of the main reasons for antimicrobial tolerance. As a result, the importance of fungi in wound biofilm has been emphasized, and targeting the fungal scaffold within biofilms may yield better treatment outcomes[37]. There are reports that *Streptococcus agalactiae* inhibit formation of *Candida albicans* hyphae by inhibiting expression of HWP and EFG [38]. On contrary, *Candida albicans* increases colonisation of *Streptococcus agalactiae* in the murine model[39].

Polymicrobial nature of biofilm makes wound treatment more difficult, increasing antibiotic resistance and providing expansion of fungal infections. Current literature suggests use of antifungal drugs such as fluconazole, amphotericin B and antibacterial therapy, or use of a broad-spectrum topical antimicrobial that targets both[40].

The table below shows the most frequent bacteria and fungi isolated from normal skin, chronic wounds and biofilm in chronic wounds based on the current literature (Table 1).

Table 1. The most frequent bacteria and fungi in normal skin, chronic wounds and biofilms in chronic wounds

	Normal skin	Chronic wound	Biofilm in chronic wounds
Bacteria	Staphylococcus spp (St. hominis, St. haemolyticus, St. cohnii); Corynebacteria spp; Propionibacteria spp;	Staphylococcus spp (St.aureus, St.capitis) Enterobacter spp; Pseudomonas aeruginosa; Streptococcus spp	Staphylococcus aureus; Streptococcus agalactiae; Pseudomonas aeruginosa; Esherichia coli; Enterococcus faecalis; Acinetobacter baumannii
Fungi	Malasezia spp; Candida spp; Aspergillus spp; Penicillium spp;	Candida spp (C. albicans, C. parapsilosis, C. tropicalis); Aspergillus spp; Trichosporon asahii; Ascomycota spp; Cladosporium spp; Fusarium spp	Candida albicans

Conclusion

Microbes do not live in isolation. Chronic wounds have polymicrobial nature, but the fungal component requires additional studies. The mechanisms of interactions between bacteria and fungi are still unclear. All these aspects play a key role in treatment of chronic wounds which is a challenge for further investigations.

Conflict of interest statement. None declared.

References

1. Lejeune P. Contamination of abiotic surfaces: What a colonizing bacterium sees and how to blur it. *Trends Microbiol* 2003; 11(4): 179-184. doi: 10.1016/s0966-842x(03)00047-7.
2. Rendueles O, Ghigo JM. Multi-species biofilms: How to avoid unfriendly neighbors. *FEMS Microbiol. Rev* 2012; 36(5): 972-989. doi: 10.1111/j.1574-6976.2012.00328.x.
3. Kong KF, Vuong C, Otto M. Staphylococcus quorum sensing in biofilm formation and infection. *Int J Med Microbiol* 2006; 296(2-3): 133-139. doi: 10.1016/j.ijmm.2006.01.042.
4. White C, Sharman AK, Gadd GM. An integrated microbial process for the bioremediation of soil contaminated with toxic metals. *Nat Biotechnol* 1998; 16(6): 572-575. doi: 10.1038/nbt0698-572.
5. Wilking JN, Zaburdaev V, De Volder M, Losick R, Brenner MP, Weitz DA. Liquid transport facilitated by channels in Bacillus subtilis biofilms. *Proc Natl Acad Sci USA* 2013; 110(3): 848-852. https://doi.org/10.1073/pnas.1216376110.
6. Olsson M, Järbrink K, Divakar U, Bajpai R, Upton Z, Schmidtchen A, *et al*. The humanistic and economic burden of chronic wounds: A systematic review. *Wound Repair Regen* 2019; 27(1): 114-125. doi: 10.1111/wrr.12683.
7. Martinengo L, Olsson M, Bajpai R, Soljak M, Upton Z, Schmidtchen A, *et al.* Prevalence of chronic wounds in the general population: systematic review and meta-analysis of observational studies. *Ann Epidemiol* 2019; 29: 8-15. doi: 10.1016/j. annepidem.2018.10.005.
8. Leaper D, Assadian O, Edmiston CE. Approach to chronic wound infections. *Br. J. Dermatol* 2015; 173(2): 351-358. doi: 10.1111/bjd.13677.
9. Wolcott RD, Hanson JD, Rees EJ, Koenig LD, Phillips CD, Wolcott RA, *et al*. Analysis of the chronic wound microbiota of 2,963 patients by 16S rDNA pyrosequencing. *Wound Repair Regen* 2016; 24(1): 163-174. doi: 10.1111/wrr.12370.
10. Loesche M, Gardner SE, Kalan L, Horwinski J, Zheng Q, Hodkinson BP, *et al.* Temporal Stability in Chronic Wound Microbiota Is Associated With Poor Healing. *J Invest Dermatol* 2017; 137(1): 237-244. doi: 10.1016/j.jid.2016.08.009.
11. Kalan LR, Meisel JS, Loesche MA, Horwinski J, Soaita I, Chen X, *et al*. Strain- and Species-Level Variation in the Microbiome of Diabetic Wounds Is Associated with Clinical Outcomes and Therapeutic Efficacy. *Cell Host Microbe* 2019; 25(5): 641-655.e5. doi: 10.1016/j.chom.2019.03.006.
12. Kalan L, Grice EA. Fungi in the Wound Microbiome. *Adv Wound Care (New Rochelle)* 2018; 7(7): 247-255. doi: 10.1089/wound.2017.0756.
13. Verbanic S, Shen Y, Lee J, Deacon J, Chen I. Microbial predictors of healing and short-term effect of debridement on the microbiome of chronic wounds. *Biofilms Microbiomes* 2020; 6: 21. https://doi.org/10.1038/s41522-020-0130-5.
14. Rodrigues M, Kosaric N, Bonham CA, Gurtner GC. Wound healing: a cellular perspective. *Physiol. Rev* 2019; 99(1): 665-706. doi: 10.1152/physrev.00067.2017.
15. Londahl M, Katzman P, Nilsson A, Hammarlund C. Hyperbaric oxygen therapy facilitates healing of chronic foot ulcers in patients with diabetes. *Diabetes Care* 2010; 33: 998-1003. doi: 10.2337/dc09-1754.

16. James GA, Ge Zhao A, Usui M, Underwood RA, Nguyen H, Beyenal H, *et al*. Microsensor and transcriptomic signatures of oxygen depletion in biofilms associated with chronic wounds. *Wound Repair Regen* 2016; 24(2): 373-83. doi: 10.1111/wrr.12401.
17. Morgan SJ, Lippman SI, Bautista GE, Harrison JJ, Harding CL, Gallagher LA, *et al.* Bacterial fitness in chronic wounds appears to be mediated by the capacity for high-density growth, not virulence or biofilm functions. *PLoS Pathog* 2019; 15: e1007511. https://doi.org/10.1371/journal.ppat.1007511
18. Di Domenico EG, Farulla I, Prignano G, Gallo MT, Vespaziani M, Cavallo I, *et al.* Biofilm is a major virulence determinant in bacterial colonization of chronic skin ulcers independently from the multidrug resistant phenotype. *Int J Mol Sci* 2017; 18(5): 1077. doi: 10.3390/ijms18051077.
19. Høiby N, Bjarnsholt T, Givskov M, Molin S, Ciofu O. Antibiotic resistance of bacterial biofilms. *Int J Antimicrob Agents* 2010; 35(4): 322-332. doi: 10.1016/j. ijantimicag.2009.12.011.
20. Zhou L, Zhang Y, Ge Y, Zhu X, Pan J. Regulatory Mechanisms and Promising Applications of Quorum Sensing-Inhibiting Agents in Control of Bacterial Biofilm Formation. *Front Microbiol* 2020; 11: 589640. doi: 10.3389/fmicb.2020.589640.
21. Bjarnsholt T, Kirketerp-Møller K, Jensen PØ, Madsen KG, Phipps R, Krogfelt K, *et al.* Why chronic wounds will not heal: a novel hypothesis. *Wound Repair Regen* 2008; 16(1): 2-10. doi: 10.1111/j.1524-475X.2007.00283.x.
22. Hendricks KJ, Burd TA, Anglen JO, Simpson AW, Christensen GD, Gainor BJ. Synergy between Staphylococcus aureus and Pseudomonas aeruginosa in a rat model of complex orthopaedic wounds. *JBJS* 2001; 83(6): 855-661. doi: 10.2106/00004623-200106000-00006.
23. Mikamo H, Kawazoe K, Izumi K, Watanabe K, Ueno K, Tamaya T. Studies on the pathogenicity of anaerobes, especially Prevotellabivia, in a rat pyometra model. *Infect Dis Obstet Gynecol* 1998; 6(2): 61-65. doi: 10.1002/(SICI)1098-0997(1998)6:2<61:: AID-IDOG6>3.0.CO;2-A.
24. Dalton T, Dowd SE, Wolcott RD, Sun Y, Watters C, Griswold JA, *et al.* An in vivo polymicrobial biofilm wound infection model to study interspecies interactions. *PLoS ONE* 2011; 6(11): e27317. doi: 10.1371/journal.pone.0027317.
25. Short B, Bakri A, Baz A, Wiliams C, Brown J, Ramage G. There Is More to Wounds than Bacteria: Fungal Biofilms in Chronic Wounds. *Curr Clin Micro Rpt* 2023; 10: 9-16. https://doi.org/10.1007/s40588-022-00187-x
26. Findley K, Oh J, Yang J, Conlan S, Deming C, Meyer JA, *et al.* Topographic diversity of fungal and bacterial communities in human skin. *Nature* 2013; 498 (7454): 367-370. doi: 10.1038/nature12171.
27. Kalan L, Loesche M, Hodkinson BP, Heilmann K, Ruthel G, Gardner SE, *et al.* Redefining the chronic-wound microbiome: fungal communities are prevalent, dynamic, and associated with delayed healing. *mBio* 2016; 7(5): e01058-16. doi: 10.1128/ mBio.01058-16.
28. Chellan G, Shivaprakash S, Ramaiyar SK, Varma AK, Varma N, Sukumaran MT, *et al.* Spectrum and prevalence of fungi infecting deep tissues of lower-limb wounds in patients with type 2 diabetes. *J Clin Microbiol* 2010; 48(6): 2097-2102. doi: 10.1128/ JCM.02035-09.
29. Fatahinia M, Poormohamadi F, Mahmoudabadi AZ. Comparative study of esterase and hemolytic activities in clinically important Candida species, isolated from oral cavity of diabetic and non-diabetic individuals. *Jundishapur J Microbio* 2015; 8(3): e20893. doi: 10.5812/jjm.20893.

30. Dowd SE, Delton Hanson J, Rees E, et al.Survey of fungi and yeast in polymicrobial infections in chronic wounds. *J Wound Care* 2011; 20(1): 40-47. doi: 10.12968/jowc.2011.20.1.40.
31. Mehra BK, Singh AK, Gupta D, Narang R, Patil R. A clinicomicrobiological study on incidence of mycotic infections in diabetic foot ulcers.ijss-sncom. *INJSS* 2017; 4: 50-54.
32. Allison DL, Willems HME, Jayatilake JAMS, Bruno VM, Peters BM, Shirtliff ME. Candida-bacteria interactions: their impact on human disease. *MicrobiolSpectr*. 2016; 4(3): 103-136. doi: 10.1128/microbiolspec.VMBF-0030-2016.
33. Kong EF, Tsui C, Kucharíková S, Andes D, Dijck PV, Jabra-Rizk MA. Commensal protection of Staphylococcus aureus against antimicrobials by Candida albicans biofilm matrix. *mBio* 2016; 7(5): e01365-e01316. doi: 10.1128/mBio.01365-16.
34. Todd OA, Fidel PL Jr, Harro JM, Hilliard JJ, Tkaczyk C, Sellman BR, et al. Candida albicans Augments Staphylococcus aureus virulence by engaging the Staphylococcal agr Quorum Sensing System. *mBio* 2019; 10(3): e00910. doi: 10.1128/mBio.00910-19.
35. Vila T, Kong EF, Montelongo-Jauregui D, Van Dijck P, Shetty AC, McCracken C, *et al.* Therapeutic implications of C. albicans-S. aureus mixed biofilm in a murine subcutaneous catheter model of polymicrobial infection. *Virulence* 2021; 12(1): 835-851. doi: 10.1080/21505594.2021.1894834.
36. Short B, Delaney C, McKloud E, Brown JL, Kean R, Litherland GJ, *et al.* Investigating the transcriptome of Candida albicans in a dual-species Staphylococcus aureus biofilm model. *Front Cell Infect Microbiol* 2021; 11: 791523. doi: 10.3389/fcimb.2021.791523.
37. Townsend EM, Sherry L, Kean R, Hansom D, Mackay WG, Williams C, *et al.* Implications of antimicrobial combinations in complex wound biofilms containing fungi. *Antimicrob Agents Chemother* 2017; 61(9): e00672. doi: 10.1128/AAC.00672-17.
38. Yu XY, Fu F, Kong WN, Xuan QK, Wen DH, Chen XQ, *et al*. Streptococcus agalactiae inhibits Candida albicans hyphal development and diminishes host vaginal mucosal TH17 response. *Front Microbiol* 2018; 9: 198. doi: 10.3389/fmicb.2018.00198.
39. Shing SR, Ramos AR, Patras KA, Riestra AM, McCabe S, Nizet V, *et al*. The fungal pathogen Candida albicans promotes bladder colonization of group B Streptococcus. *Front Cell Infect Microbiol* 2020; 9: 437. doi: 10.3389/fcimb.2019.00437.
40. Felton T, Troke PF, Hope WW. Tissue penetration of antifungal agents. *Clin Microbiol Rev* 2014; 27(1): 68-88. doi: 10.1128/CMR.00046-13.