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Adherence of *Neisseria gonorrhoeae* to surface of SiHa cells (human cervical carcinoma cell line)

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INFECTIONS CONNECTED WITH ORGAN AND TISSUE TRANSPLANTATION

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Abstract: Transplantology is a branch of medicine that is developing rapidly. Transplanted whole organs or segments of organs may be recovered from either living or deceased donors. New methods of preserving transplanted solid organs, including the kidney, liver, heart, lung, and pancreas, as well as tissues, such as the cornea and skin, are being developed. Preservation fluid, which is used to perfuse and store the donated organ or tissue, should reduce biological deterioration of organs and tissue, attenuate ischemia/reperfusion-induced cell/tissue injury, and protect against damage. Lowering the storage temperature of organs significantly reduces the risk of damage. Efforts are also made to shorten the time between collecting the organ or tissue from the donor and transplanting it in the recipient. However, during transplantation, the recipient may become infected, primarily with bacteria and fungi. Infections of organ recipients occur most often due to unhygienic organ collection, improper handling and transport, and inappropriate preservation conditions, especially contamination of preservation fluid. The literature on contamination of organ preservation fluid and infections in graft recipients is very diverse, both in terms of the isolated bacterial and fungal species and the number of incidents. A large percentage of contaminating microorganisms belong to the generally non-pathogenic skin microbiota, but there are also cases of multidrug-resistant bacteria. Besides, the transplanted organs themselves may pose a danger. They may contain latent microorganisms, mainly viruses and parasites, that could be activated in a patient who has been subjected to immunosuppression to reduce the risk of organ rejection.

Keywords: contamination of organ preservation fluid, infections in organ recipients, microbial contamination of transplants (grafts), post-transplant infections, transplantology

1. Introduction

Organ transplantation (or grafting) amongst humans has developed over the past 70 years (Giwa *et al.* 2017). The first successful transplantation of the human kidney between identical twins was performed by dr. Joseph E. Murray in 1954 (Merrill *et al.* 1956). In 1990, Murray and E. Donnall Thomas were awarded with Nobel Prize in Physiology or Medicine for "their discoveries that have enabled the development of organ and cell transplantation into a method for the treatment of human disease" (The Nobel Prize, 1990). Currently, transplantation is the only effective therapy for patients with end-stage disease. So, transplants can save lives, but they can also restore function in patients with vital organ failure, thus improving their quality of life. Several factors are responsible for therapeutic success: selection of the right donor with a high-quality and efficacious organ; preservation of the organ to ensure it is in a good condition for transplantation; ensuring there is enough time to organize staff, facilities, and equipment, and to perform the tests and the actual procedure; appropriate immunosuppressive medication; and post-transplant care of the organ recipient (Jing *et al.* 2018).

European Committee (Partial Agreement) on Organ Transplantation of the Council of Europe developed special guide for specialists, intended to ensure the

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quality and safety of the donation and transplantation of organs, tissues and cells as well as technical guidance to ensure the safety and quality of human organs intended for transplantation (European Committee (Partial Agreement) on Organ Transplantation of the CE, Guide 2022). The chapter 8 of this guide draws attention to risk of transmission of microbial diseases, especially viral infections. The issues related to infections of people – potential organ donors, are also discussed. Moreover attention is paid to geographic distribution, endemic zones and risks of infectious diseases, that can be transmitted by solid organ transplantation.

Considering the data collected during the collaboration between the World Health Organization (WHO) and the Spanish Transplant Organization (Organización Nacional de Transplantes [ONT]), the most frequently transplanted solid organs (grafts) regardless of geographical region, are, in order: kidney, liver, heart, lung and pancreas (ONT-WHO Global Observatory on Donation and Transplantation, 2022). Over 150,000 these organs were transplanted in 2022 (Table I). According to Finger *et al.* (2023), the percentage of patients from the United States who survived with an implanted organ for 1 and 5 years is 90.9% and 78.6%, respectively, for heart transplants; 86.7% and 47.3%, respectively, for a single lung transplant; 87.7% and 58.6%, respectively, for a double-lung transplant; 80.9% and 50.2%, respectively, for a heart-lung transplant; 92.3% and 83.3%, respectively, for a liver transplant from a living donor; 91.2% and 75%, respectively, for a liver transplant from a deceased donor; 98.8% and 92.1%, respectively, for a kidney transplant from a living donor; 96.3% and 83.3%, respectively, for a kidney transplant from a deceased donor; and 90.9% and 79.6%, respectively, for a pancreas transplant. In addition to the above mentioned solid organs, small bowel, eyes and cornea, bones, and soft tissues/skin called vascularized composite allotransplant, are also grafted.

The aim of this review is to draw attention to the infections of organ recipients and to recognize the severity of the microbiological problem connected with the use of preservation fluid (PF) for organs before transplantation. PF contains substances that protect cells against degradation, but does not contain antimicrobial agents. Thus, it is interesting to investigate how often infections occur in organ recipients due to contaminated PF. Attention should also be paid to latent infections, mainly viruses, that develop in immunosuppressed organ recipients, as well as to parasites that may be transmitted along with the transplanted organ and that may cause infection in the organ recipient. This review provides information for microbiologists working in transplantation units, as well as medical staff directly involved in the transplantation process.

2. General aspects of transplantology and organ preservation

There are four types of transplants based on the genetic relationship between the donor and the recipient: xenotransplant, where the donor is an animal and the recipient is a human; allotransplant, where the donor and recipient are from the same species; isotransplant, where the donor and recipient are identical twins; and autotransplant, where the donor and recipient are the same person. The chance of organ rejection decreases in the order given above (Oli *et al.* 2022). Transplanted organs, either the whole organ or segments/fragments, may be recovered from either living or deceased donors (for the latter, donors who have been declared brain dead or after cardiac death). Living donor donation often takes place between related people; however, there are also anonymous and altruistic donors.

In the initial period of transplantation, organs were preserved at room temperature with the use of blood-

Table I
Organ transplants in different geographical regions in 2022 (ONT-WHO Global Observatory on Donation and Transplantation).

Organ transplantation in 2022	Geographical region							
	Poland	European	Americas	Eastern Mediterranean	Western Pacific	South-East Asia	African	Global
Kidney	874	25,361	39,196	6,364	18,219	12,696	286	102,122
Liver	362	9,840	13,387	1,817	8,325	4,067	(–)	37,436
Heart	173	2,444	4,996	184	1,090	274	(–)	8,988
Lung	93	2,073	3,313	55	1,199	144	(–)	6,784
Pancreas	18	611	1,171	56	158	30	(–)	2,026
Small bowel	(–)	40	90	14	23	3	(–)	170
Total organ transplants	1,520	40,369	62,153	8,490	29,014	17,214	286	157,526

(–) – data not available

based perfusates. Chemically defined cell culture media were developed in the 1950s (Jing *et al.* 2018). The use of blood-based perfusates versus chemically defined preservative solutions is still a concern. There are unfavorable phenomena related to blood, including hemolysis, thrombus formation, immune-mediated responses, and blood-borne infectious transmission, mainly of a viral origin (Jing *et al.* 2018). During the development of transplantology, researchers found that lowering the temperature of PF reduces biological deterioration of organs, attenuates ischemia/reperfusion-induced cell/tissue injury, and protects organs from damage. Cooling reduces cellular metabolism and the oxygen requirements. The aspects of cellular injury and microvascular dysfunction in the pathogenesis of ischemia/reperfusion during organ preservation have been discussed elsewhere (Petrenko *et al.* 2019: Datta *et al.* 2021). Renal preservation by ice cooling was first used during kidney transplantation (Calne *et al.* 1963).

Currently, there are two ways to preserve organs: static cold storage (0–8°C) and dynamic storage based on machine perfusion (Jing *et al.* 2018; Guibert *et al.* 2011). Using perfusion equipment, which provide enhanced nutrient and oxygen delivery, various procedures have been developed depending on the temperature (Jing *et al.* 2018; Petrenko *et al.* 2019): hypothermic machine perfusion (0–12°C), midthermic machine perfusion (13–24°C), subnormothermic machine perfusion (25–34°C), and normothermic machine perfusion (35–38°C). Besides, controlled oxygenated rewarming (8–20°C) has been used to preserve kidneys and livers. In this method, the perfusate temperature rises gradually to weaken ischemia/reperfusion injury (Jing *et al.* 2018). PF continuously pumped by machine perfusion systems through the organ (e.g., kidney) provides nutrients and oxygen, carries away toxic waste products, and delivers buffers that absorb metabolites produced by the organ (e.g., lactic acid and adenosine monophosphate). The use of an ultra-low temperature to protect tissues and organs has also been considered.

It should be underline, that PF in unopen containers must be sterile (European Pharmacopoeia 11, 2023, 2.6.1. Sterility). These preparations are prepared by specialized manufacturers that meet the requirements of Good Manufacturing Practice (GMP). The microbiological quality of these fluids should be similar to fluids used for cell culture.

Many chemically defined solutions that can replace blood have been evaluated and used to preserve tissues and organs before transplantation (Guibert *et al.* 2011; Latchana *et al.* 2015; Jing *et al.* 2018; Petrenko *et al.* 2019; Datta *et al.* 2021; Finger *et al.* 2023). However there is no consensus among transplantation centers as to which of PFs is the best (Salehi *et al.* 2018). The compositions of several PFs are listed in Table II. These solutions are intended to provide appropriate physiological and biochemical conditions, oxygenation, and temperature to ensure cell survival and to reduce damage associated with ischemia/reperfusion injury. The most known and frequently used PFs in solid organ transplantation are EC or Euro-EC (Collins *et al.* 1969) and UW (Belzer *et al.* 1968; Southard and Belzer 1995). Generally, PFs contain electrolytes, buffers, antioxidants, and pharmacological agents. Importantly, PFs do not contain antimicrobial agent, except for the modified UW liquid listed in Table II (Guibert *et al.* 2011), which contains penicillin G (200,000 IU/L). PFs can be differentiated based on the Na^+/K^+ ratio. High Na^+ and low K^+ concentrations correspond to extracellular solutions, whereas high K^+ and low Na^+ concentrations correspond to intracellular solutions, which are intended to prevent cellular edema by maintaining intracellular ion concentrations upon cold-induced dysfunction of Na^+/K^+ pumps (Jing *et al.* 2018; Datta *et al.* 2021).

Taking into account the risk of infection of the organ recipient through contaminated PF, it is recommended to perform a microbiological examination of a PF sample taken from the container in which the organ was stored just after removing the organ to transplantation process. The test should be performed in accordance with the procedures applicable in a given microbiological laboratory for clinical samples taken from the patient. Public Health England (PHE) in partnership with the NHS described in UK standards for microbiology investigations (UKSMIs) Document "Abdominal organ transport fluid testing" (UK Standards for Microbiology Investigations, B62, 2020). According to these recommendations, the sample of specimen from fluid surrounding the organ should be taken immediately after the organ has been lifted from the transport bag for implantation. Volume of transport fluid to submit for analysis differs depending on the size of organ being transplanted. A minimum of approximately 5% of the total volume in the organ transport bag should be used for analysis (ideally a minimum of 20 mL). PF fluid should be centrifuged at $1200 \times g$ for 5 min. and spread on appropriate media such as blood agar, Sabouraud agar and CLED agar (for *Enterobacterales*). All cultured microorganisms should be identified to species level. A separate issue is transplantation of corneas, the most commonly transplanted human tissue (Fabre *et al.* 2021). Corneal disease is the second major cause of blindness worldwide (Li *et al.* 2019). Corneal transplantation, also known as keratoplasty, involves replacing part of the recipient's corneal tissue with tissue taken from a deceased donor. The first recorded therapeutic corneal transplantation on a human, unfortunately unsuccessful, was reported in 1838; the first successful human corneal transplant was performed by Zirm in 1905 (Crawford *et al.* 2013).

Table II
Composition of preservation solutions (Jing *et al.* 2018; Guibert *et al.* 2011; Finger *et al.* 2023; Petrenko *et al.* 2019; Latchana *et al.* 2015; Datta *et al.* 2021).

Solution	Electrolytes (nmol/L)	Buffer	Antioxidant	Colloid/ Impermeant	Amino acids	Other	pH	Osmolality (mOsm/L)	Intracellular/ Extrracellular
EC (Euro-Collins)	K^+ (115), Na^+ (10), Mg^{2+} (5), Cl^- (15)	Phosphate (50; 57.5; 60), Bicarbonate (10)	–	–	–	Glucose (19.5; 180; 195)	7.3; 7.4	340; 355; 375	I
UW (University of Wisconsin, Viaspan)	K^+ (125), Na^+ (25), Mg^{2+} (5), Cl^- (20)	Phosphate (25)	Glutatione (3), Allopurinol (1)	Pentafraction – HES (50 g/L), Lactobionate (100), Raffinose (30)	–	Sulphate (5), Adenosine (5) Insulin (40 U/L), Dexamethasone 16 mg/L), Penicillin G 200,000 UI/L)	7.40 (25°C)	320; 324	I
Celsior	K^+ (15), Na^+ (100), Mg^{2+} (13), Ca^{2+} (0.25), Cl^- (28; 41.5; 71)	Histidine HCl (30)	Glutatione (3), Mannitol (60)	Lactobionate (80), Mannitol	Histidine, Glutamate (20)	Sucrose	7.30 (20°C)	242; 360; 368	E
HTK (Custodiol)	K^+ (9; 10), Na^+ (15), Mg^{2+} (4), Ca^{2+} (0.015), Cl^- (32; 50)	Histidine (180) Histidine HCl (18)	Tryptophan (2), Mannitol (30), α-Ketoglutarate (1)	Mannitol	Histidine, Tryptophan, Glutamate		7.02 –7.20 (25°C)	310	E
HTK-New (Custodiol-N)	K^+ (10), Na^+ (16), Mg^{2+} (8), Ca^{2+} (0.02), Cl^- (30)	Histidine (124), N-acetyl-L-histidine (57)	Tryptophan, α-Ketoglutarate (2)	–	Histidine, Glicyne (10) Tryptophan (2), Alanine (5), Arginine (3), Aspartate (5)	Sucrose (33), Deferoxamine (25×10^{-3}), LK-614 (7.5×10^{-3})	7.0–7.1	302	E
IGL-1 (Institut Georges Lopez-1)	K^+ (25; 30), Na^+ (120; 125), Mg^{2+} (5), Ca^{2+} (0.03–0.5), Cl^- (0; 20)	Phosphate (25)	Glutatione (3), Allopurinol (1)	Lactobionate (100), Raffinose (30), PEG-35 (0.03; 1)	–	Nitroglycerin, Adenosine (5) Sulphate (5)	7.40 (25°C)	290; 320	E
LPDG (Perfadex)	K^+ (6), Na^+ (138), Mg^{2+} (0.8), Ca^{2+} (0.3), Cl^- (142)	Phosphate (0.8), Tris or Bicarbonate (1)	–	Dextran 40 (50)	–	Glucose (5) Sulphate (0.8)	7.4	295	E
Ep4 (EP-TU)	K^+ (26), Na^+ (141), Mg^{2+} (4), Cl^- (103)	Phosphate	–	Dextran 40	–	Glucose (10)	–	–	E
ET-Kyoto (ETK)	K^+ (44), Na^+ (100)	Phosphate (25)	–	Pentafraction – HES (30), Trehalose (41; 120)	–	Sulphate, Gluconate (100), Dibutyryl cAMP	7.4	370	E
HOC – hypertonic citrate	K^+ (79), Na^+ (84), Cl^- (30)	Citrate	Mannitol (185)	–	–	–	–	–	I
PBS – phosphate buffered sucrose	Na^+ (120)	Phosphate (60)	–	–	–	Sucrose (140)	7.40	310	I
Scot	K^+ (5), Na^+ (118), Mg^{2+} (1.2), Ca^{2+} (1.75), Cl^- (128.9)	–	–	PEG-20 (30)	–	Glucose (11)	7.50	302	E
HTK-TiProtec	K^+ (93), Na^+ (16), Mg^{2+} (6; 8), Ca^{2+} (0.05), Cl^- (103)	Phosphate (1) or Histidine (198) N-acetyl-L-histidine (30)	α-Ketoglutarate (2)	–	Glicyne (5; 10) Tryptophan (2), Alanine (5), Aspartate (5; 8)	Glucose (10), Sucrose (20; 37), Deferoxamine (82×10^{-3}), LK-614 (17×10^{-3})	7.0	305	I
KH (Krebs-Henseleit)	K^+ (5.9), Na^+ (143), Mg^{2+} (1.2), Ca^{2+} (1.25), Cl^- (125.2)	Phosphate (1.2) Bicarbonate (25)	–	–	–	Sulphate (1.2), Glucose (11)	7.8	380	E

PEG polyethylene glycol, HES – hydroxyethyl starch, LK-614 – iron chelator. All units are shown as (mmol/L) unless otherwise indicated; Dextran 40, Pentafraction, PEG are in g/L

Currently, donor tissue is routinely retrieved by eye banks either through *in situ* excision of the cornea or enucleation of a complete eyeball. Of note, these tissues still carry ocular surface microbiota upon arrival at the eye bank, and these microorganisms may be a source of infection. Eye tissues are stored either in hypothermic storage at 4°C for 5–10 days (an approach mainly used in the United States) or in organ culture at 31–37°C for up to 35 days (an approach mainly used in Europe) (Li *et al.* 2019; Gibbons *et al.* 2020; Fabre *et al.* 2021). The mission, history, and tasks of the European Eye Bank Association and Eye Bank Association of America (EBAA) have been presented elsewhere (Jones *et al.* 2012; EBAA 2021).

In a systematic review, Gimenes *et al.* (2022) compared several specially developed corneal storage media with *Optisol-GS*, the most widely used medium to preserve corneas for transplantation. All of them contain antibiotics, most often gentamicin and streptomycin. However, considering the incidence of devastating fungal infection, most often caused by *Candida* sp., amphotericin and voriconazole have sometimes been added to *Optisol-GS* (Layer *et al.* 2014; Mistò *et al.* 2020; Gimenes *et al.* 2022). There are also objections to supplementation of hypothermic corneal storage media with amphotericin, mainly due to the high cost and low effectiveness of this antifungal chemotherapeutic at low concentrations (Tu 2021). On the other hand, Gibbons *et al.* (2020) concluded that the use of the fungicidal amphotericin B for endothelial keratoplasty was more cost-effective than the use of the fungistatic voriconazole or caspofungin. While the opinions are divided, cost-effectiveness also plays a role in developing treatments.

To minimize the risk of infections in organ recipients caused by microorganisms that contaminate tissues, a special monograph was introduced into the European Pharmacopoeia in 2023: "Microbiological Examination of Human Tissues" (Ph. Eur. 2023). An example of the content included in this source is a microbiological control strategy for cornea. This example was elaborated because the preservation of ocular tissue and corneal transplantation deserves special attention due to the large number of grafts performed (Chu 2000; Jones *et al.* 2012). In addition, to ensure long-term storage of corneas, Chaurasia *et al.* (2020) suggested their sterilization by gamma irradiation (17–23 kGy from a cobalt-60 source). This strategy effectively stabilizes tissue grafts and eradicates contaminating microorganisms, including viruses.

In addition to the above-mentioned preservation methods, cryopreservation has also been developed (Whaley *et al.* 2021). It requires freezing the tissue/organ to a temperature below 0°C to slow deterioration by reducing the rate of metabolism. Such preserved material, like bone marrow, blood components, and gametes, can be stored for many weeks (Datta *et al.* 2021; Ozgur *et al.* 2023). However, problems arise when large organs and other three-dimensional structures are cooled below 0°C, because ice crystals can form immediately inside cells and cause severe mechanic destruction. Cryoprotective agents (CPAs) are crucial for cryobiology (Elliott *et al.* 2017; Jang *et al.* 2017; Whaley *et al.* 2021). Solutions containing alcohols (e.g., methanol, ethanol, glycerol, ethylene glycol, and propylene glycol), sugars (e.g., trehalose, sucrose, mannitol, and raffinose), polymers (e.g., polyethylene glycol, dextrans, and hydroxyethyl starch), dimethyl sulfoxide, dimethyl acetamide, and glutamine, among other components, are commonly used for cryopreservation of microorganisms and cells. CPAs can mitigate cryoinjury caused by ice nucleation, crystal growth, and cellular dehydration during freezing, all of which influence post-thaw survival (Elliott *et al.* 2017; Ozgur *et al.* 2023). Considering the penetration of CPA into solid organs, it is necessary to use hypothermic perfusion with CPAs at a temperature of 10°C prior to cooling the material to –80°C (Elliott *et al.* 2017). Microorganisms that contaminate tissues or organs do not multiply at such a low temperature. Supercooling (to about –10°C) in the presence of CPAs largely protects cells against the negative impact of ice crystal formation (Ozgur *et al.* 2023). Experimental results concerning vitrification – rapid cooling to below –100°C to form a noncrystalline glassy phase in animal, rabbit, and rat organs (e.g., heart, liver, kidney), are promising (Ozgur *et al.* 2023; Berendsen *et al.* 2014), based on the successful cryopreservation of hepatocytes, pancreatic islets, gametes, and stern cells (Whaley *et al.* 2021). However, rewarming vitrified material and preserving functionality and viability are notable challenges. A thorough understanding of the chemical and biological processes behind freezing and thawing will be necessary for the future development of a safe and effective cryopreservation method (Weissenbacher *et al.* 2019).

There are a myriad of sources of infections in organ recipients (Oriol *et al.* 2019; European Committee (Partial Agreement) on Organ Transplantation of the CE 2022; Li *et al.* 2022; Manuel *et al.* 2023). Endogenous pathogens, mainly viruses and less often parasites, may be present in the transplanted organ and transmitted to the recipient. The donor might be unaware of their infection or be asymptomatic, especially when latent viruses become virulent in a recipient subjected to immunosuppression due to the risk of transplant rejection. Exogenous microorganisms in PF may occur during the procurement process – for example, they may come from the surface of the donor's body or from the environment. Most often, infections in organ recipients occur due to unhygienic organ collection, improper

handling and transport, and inappropriate preservation conditions, especially contamination of the PF used for perfusion and storage. Antibiotic therapy in recipients before transplantation reduces the risk of developing infection in case a contaminated organ is transplanted.

Besides, some potential organ recipients often travel abroad, mainly to Asian countries, to reduce costs and to shorten the waiting time for transplantation. In some geographic regions, there is limited medical and microbiological screening of donors. Furthermore, in countries where payment for organ donation is legal, donors typically come from lower socioeconomic areas where endemic infections such as tuberculosis and malaria may occur (Len *et al.* 2014).

Len *et al.* (2014) developed and proposed recommendations for screening donors and recipients prior to organ transplantation to minimize transmission of donor-derived infections. The authors considered latent and acute bacterial, fungal, viral, and protozoal infections. In addition, the Infectious Diseases Community of Practice of the American Society of Transplantation has published guidelines concerning screening of donors and candidate prior to solid organ transplantation (Malinis *et al.* 2019). Moreover, the online medical portal *emedicine.medscape.com* provides current information on transplantation of the kidney (Collins 2021), liver (Manzarbeitia and Arvelakis 2022), pancreas (Rao and Finger 2022), intestines (Andacoglu and Greenstein 2021), lung (Whitson 2022), and heart (Botta and Mancini 2023), as well as post-transplantation complications and infections accompanying the transplantation of these organs.

Although immunosuppressive therapies are involved at the time of transplantation, the early post-transplant period (up to 1 month) is notable for hospital-acquired infections, especially related to surgical procedures including implant placement and the use of medical devices like intravenous and urinary tract catheters. The intermediate post-transplant period (months 1–6) is the time where there is the greatest risk for opportunistic infections. In stable recipients, infections are less frequent 6 months after transplantation (Fishman 2017; Sawinski and Blumberg 2019). Transmission of infection during transplantation of solid organs grafts is uncommon but potentially life threating. Fishman (2017) provided a comprehensive overview of the epidemiology of infections during organ transplantation, diagnosis, and therapy. Regardless of whether infections are directly related to the process of organ transplantation, one should remember that these severely ill and immunosuppressed patients are also exposed to nosocomial pathogenic microorganisms in their environment. For this reason, some microorganisms isolated from infected patients may differ from microorganisms found in contaminated PF.

3. Bacterial infections in organ transplant recipients and contamination of preservation fluid

Microbiological contamination of PF is a potential source of post-transplantation infections and requires patients to be treated with antibiotics or antimicrobial chemotherapeutics. The most frequently isolated microorganisms from these fluids are staphylococci, Gram-negative rods, and *Candida* spp. strains (Sotiropoulos *et al.* 2018). An important issue that requires microbiological and clinical analysis is whether all isolated microorganisms should be treated as actual pathogens and antimicrobial therapy should be implemented in recipients (Manuel *et al.* 2023). Moreover, the protocols for screening organ or tissue donors for infectious risks are inconsistent and vary according to the type of graft, national standards, and the availability of the screening tests.

A number of publications have focused on PF contamination or infections in solid organ recipients. Mattner *et al.* (2008) investigated the extent to which bacterial and fungal donor organ contamination caused post-transplant nosocomial infections in solid organ transplant recipients. Out of 282 organ recipients (140 lung, 71 liver, 51 heart, and 16 heart-lung recipients), 150 (53.2%) received contaminated organs. The lung and heart-lung transplants were the most contaminated based on PF or organ swab microbiological examination. In the lung transplant group, 126 Gram-positive bacteria, 102 Gram-negative rods, and 57 fungi were isolated, whereas in the heart-lung transplant group, 18 Gram-positive bacteria, 5 Gram-negative rods, and 6 fungi were cultured. Of note, polymicrobial contamination was frequent.

Colvara Mattana *et al.* (2011), analyzed 136 PF samples used to store kidneys and pancreas. The contamination rate of these samples was 27.9%, mainly by coagulase-negative staphylococci, *Bacillus* spp., and *Enterococcus* spp. (representing 55% of contaminated PF samples). The authors concluded that infections of the organ recipients were not associated with contaminated PF. Sotioropoulos *et al.* (2019) reviewed 19 studies published from 2000 to 2016 regarding bacterial and fungal contamination of PF samples used to store solid organs before transplantation. Of 5647 patients, 1428 (25.3%) had positive microbial cultures. The bacteremia data showed a wide range (0%–69%), which precluded the authors for drawing conclusions or making recommendations.

Peghin *et al.* (2024) recently described skin and soft tissue infections in solid organ transplants. The authors drew attention to the possibility of infections caused by staphylococci and streptococci, as well as microorganisms that are rare in such a situation, including *Nocardia* sp., *Bartonella* sp., and Mycobacteria. Some of these

infections may be related to the patient's hospital stay and the environmental contamination there.

Isabel Oriol together with a group of Spanish researchers (Oriol *et al.* 2016; Oriol *et al.* 2018; Oriol *et al.* 2019) have conducted multicenter investigations, systematic reviews, and meta-analysis on the impact of culture-positive PF on solid organ transplantation for several years. In one study, they found that 46 out of 50 liver grafts had been stored in contaminated PF, but only in 14 cases were there pathogenic microorganisms. They mainly isolated coagulase-negative staphylococci. There was no infection among the recipients (Oriol *et al.* 2016). The authors also emphasized that the examination of PF immediately before implantation has the greatest diagnostic value. In another large multicenter cohort study, out of 622 transplanted organs (362 kidneys, 166 livers, 51 lungs, 32 hearts, and 11 multiple organs) and PF samples, as many as 389 (62.5%) were microbiologically contaminated, but only one fourth of positive samples contained "high risk" pathogens; *Staphylococcus aureus*, *Escherichia coli*, *Enterococcus faecalis*, and *Enterobacter cloacae* dominated (Oriol *et al.* 2019). PF contamination could be directly linked to the development of infection in only five patients. The authors concluded that clinical monitoring of solid organ recipients infected with microorganisms present in contaminated PF, regardless of their species, is important to make the proper diagnosis and to determine whether the situation requires the treatment of infections associated with PF (Oriol *et al.* 2018). Yahav and Manuel (2019), in a commentary on the publication of Oriol (Oriol *et al.* 2019), and Yu *et al.* (2019) also called for an increase in evidence, especially in terms of precise characterization of contaminating microorganisms, to limit the use of antibiotics in the prevention and treatment of infections.

What is important, Cervera *et al.* (2014) described recommendations for the management of multi-drug-resistant (MDR) bacteria in solid organ transplant patients, considering infections with methicillin-resistant *S. aureus* (MRSA); vancomycin resistant enterococci; and extended-spectrum beta-lactamase (ESBL)-, AmpC-, and carbapenemase-producing Gram-negative rods. Recently, Pilmis *et al.* (2023) analyzed MDR *Enterobacterales* infections in the context of abdominal solid organ transplantation, paying attention to donor screening for gastrointestinal tract colonization by these MDR Gram-negative bacteria. The authors also considered the prevalence of bacterial infections, including those caused by MDR Gram-negative bacteria, in kidney and liver recipients. The prevalence of infections was up to 65%, and the prevalence of MDR pathogens was up to 20%. While Bodro *et al.* (2013) analyzed the outcomes of bacteremia caused by drug resistant *Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa*, and *E. cloacae* (ESKAPE pathogens) in solid organ transplant recipients. In the 6-year study, there were 276 bacteremia cases among 190 recipients, of which 54 (19.6%) cases were caused by drug-resistant ESKAPE strains. These strains were found in 24 kidney recipients, 21 liver recipients, and 9 heart recipients. Carbapenem- and quinolone-resistant *P. aeruginosa* strains predominated in the above recipient groups – 13, 9, and 5, respectively – followed by ESBL-producing *K. pneumoniae* –7, 3, and 1 cases respectively.

Complications related to organ and tissue transplantation and PF contamination are primarily caused by bacteria that grow aerobically. Anaerobic bacteria generally do not pose a significant threat to transplant recipients, with the exception of *Clostridioides difficile*, a common nosocomial pathogen in hospital wards that causes infections especially in immunocompromised patients, such as organ recipients. The American Society of Transplantation Infectious Diseases Community of Practice (Mullane *et al.* 2019) developed guidelines to address the prevention and management of *C. difficile* infections in solid organ transplant recipients. The incidence of *C. difficile* infections varies by the type and number or organs transplanted. The prevalence of these infections in the solid organ transplant population ranges from a low of 3.2% in the pancreatic transplant population to 12.7% in those receiving multiple organ transplants (Mullane *et al.* 2019). It should be mentioned that Audet *et al.* (2011) examined PF contamination in the context of liver transplantation. Apart from a number of aerobic bacteria (mainly coagulase-negative staphylococci), they isolated anaerobes, four strains of *Propionibacterium* sp., and a strain of *Veillonella* sp. These bacteria did not cause infection in organ recipients.

Bacterial infections are generally connected with organ storage and surgical procedures and are not latent in nature, with the exception of *Mycobacterium* spp. infections. Transplant recipients are immunocompromised and vulnerable to developing tuberculosis. There have been a number of cases of tuberculosis reactivation in recipients of organs contaminated with *Mycobacterium tuberculosis* (Sidhu *et al.* 2014; Abad *et al.* 2019; Nguyen Van *et al.* 2024; Hyun *et al.* 2024). Screening of latent tuberculosis infection in donors is the cornerstone of the tuberculosis preventive strategy in recipients (Malinis and Koff 2021).

The use of antibiotics to decontaminate grafts has been evaluated (Paolin *et al.* 2018). The authors examined bacterial contamination profiles of 11,129 tissue samples as allografts retrieved from multi-tissue donors. The tissues were incubated twice at 4°C for 24–28 hours in a decontamination solution containing

ceftazidime (24 mg/L), lincomycin (120 mg/L), polymyxin B (100 mg/L), and vancomycin (50 mg/L). The samples were analyzed microbiologically. Immediately after tissue retrieval, 6130 (55%) of the samples were contaminated. Using subsequent decontamination, the number of bacteria decreased – to 1955 samples after the first decontamination step and to 113 samples after the second decontamination step. Coagulase-negative staphylococci were the dominant bacterial group.

Louart *et al.* (2019) drew some interesting conclusions when considering the risk of contamination of organ procurement in different locations. Their multivariate statistical analysis of 2535 grafts indicated that 285 (11%) were microbiologically contaminated, of which 20% were skin grafts, 12% were arterial grafts, 9% were heart valves, and 7% were corneal grafts. Regarding the location of organ retrieval, 47% were collected in standard operating rooms, 39% in dedicated non-operating rooms (hospital mortuaries), and 14% in intensive care units. The authors concluded that although standard operating rooms provide the best place to procure grafts, dedicated non-operating rooms led to a lower risk of tissue microbial contamination. This may be related to the fact that corneal samples, which constituted approximately 60% of the analyzed grafts, were mainly recovered from deceased patients stored in a cooled room. The authors reported that the percentage of contaminating microorganisms varied depending on the place of tissue collection. For tissues collected in standard operating rooms, 65.5% of the microbes were Gram-positive bacteria, 17.2% were Gram-negative rods, and 14.8% were fungi isolated. In dedicated non-operating rooms, 26.4% of the isolated microbes were Gram-positive bacteria, 39% were Gram-negative rods, and 20.7% were fungi. Finally, for the tissues collected in intensive care units, 64.3% of the microbes were Gram-positive bacteria, 24.3% were Gram-negative rods, and 5.7% were fungi (Louart *et al.* 2019).

In many cases, it is quite difficult to locate a proper organ donor. Sometimes, there is not enough time to find one and a patient qualified to receive a transplant dies. Kieslichova *et al.* (2019) presented a case report in which both kidneys, the liver, pancreatic islets, and the heart were transplanted to five organ recipients from a deceased patient with antibiotic-sensitive *K. pneumoniae* and *E. coli* in her sputum. Appropriate antibiotic prophylaxis and antibiotic therapy were administered to four of the five patients who developed ESBL-producing *K. pneumoniae* and ESBL-producing *E. coli* infections. It turned out that these strains were present in the transport medium in which the organs were stored during organ procurement. The two kidney recipients died, but the remaining three patients survived the operations with good graft function.

3.1. Infections associated with kidney transplantation

There have been many studies related to renal infection after kidney transplantation and contamination of PF used to store kidneys. Bertrand *et al.* (2013) pointed out that contamination of PF used to store kidney grafts has been examined using various methods. Sometimes, the methods used did not allow the detection of microorganisms because of incorrect growth conditions (e.g., media, temperature, and incubation time). Furthermore, the authors analyzed 200 kidney transplantations (with kidneys from a deceased donor) performed over a 3-year period. During the hospitalization period, 62 patients who received a kidney stored in contaminated PF, regardless of whether they received prophylactic antibiotic therapy, did not exhibit any invasive blood, urinary, peritoneal, or wounds infections related to the microorganisms isolated from the PF. Coagulase-negative staphylococci were mainly isolated. In a large study on infection in renal transplant recipients, Sawinski and Blumberg (2019) noted that bacterial infections are a major cause of morbidity and mortality after organ transplantation. The most common infections (23–75%) in kidney transplantation are those related to the urinary tract. Moreover, reactivation of tuberculosis may occur in transplant recipients. Opportunistic bacteria, including with *Nocardia* sp. and *E. coli*, remain the most common organism causing urinary tract infections (Parasuraman *et al.* 2013). Reticker *et al.* (2021) showed that out of 152 kidney transplant recipients, as many as 67% received organs stored in contaminated PF. However, 80% of these microorganisms can be considered to be part of the normal skin microbiota. Sixty-seven percent of patients who underwent transplantation with kidneys stored in contaminated PF were treated with antibiotics for 5 days. There was no difference in the incidence of infection between patients who received an organ stored in contaminated PF and patients who received an organ stored in culture-negative PF. Similarly to the aforementioned study, Yansouni *et al.* (2012) analyzed 331 PF samples, almost half of which had stored kidneys. They found that 62.2% of the PF samples were contaminated. However, high-risk organisms, mainly *Enterobacteriaceae* and *S. aureus* strains, accounted for only 17.8% of the isolated microbes.

Li *et al.* (2022) conducted a retrospective study to elaborate the association between organ PF pathogens and early infections after kidney transplantation. They analyzed clinical data from 514 kidney transplant donors and 808 recipients between 2015 and 2020. They found that 329 recipients showed early infections after transplantations connected with contaminated PF. The dominant pathogen isolated from the PF samples was *Staphylococcus epidermidis* (10.2%). In addition, 34.6%

of the PF samples contained pathogenic bacteria from the ESKAPE group, 21% of the PF samples were contaminated with *Candida* sp. Thirty-five percent of the infections were caused by microorganisms belonging to both groups. The recipients infected with ESKAPE pathogens and *Candida* sp., in comparison to recipients with other pathogens, had higher rate of bloodstream and transplant-site infections, 14.1% versus 6.9% and 16.7% versus 3.5%, respectively. Yu *et al.* (2019) made similar observations in a retrospective analysis of 1002 PF samples associated with kidney transplantation for microbiological contamination. They isolated 1036 microorganisms. As many as 275 (26.5%) of the recipients' PF samples were contaminated with ESKAPE pathogenic strains. It is worth noting that in the group of microorganisms obtained, 14.4% were *Candida* spp., including 6.3% of *Candida albicans*. The authors stated that patients whose PF is contaminated with ESKAPE pathogens have a significantly increased risk of infections during the early post-transplant period. Corbel *et al.* (2020) analyzed 4487 kidney grafts procedures and carefully examined the possibility of infection in the recipients. The percentage of contaminated PF samples that stored kidney grafts from living and deceased donors was similar, 20.5% and 24.1%, respectively. Nearly 60% of PF contaminants were polymicrobial. The most frequently isolated microorganisms were coagulase-negative staphylococci (65.8%) and *Enterobacteriaceae* strains (28%).

Saad *et al.* (2020) analyzed infections in the first year after renal transplant and concluded that the most often complications are the bacterial urinary tract infections (44.2%). Veroux *et al.* (2010) analyzed 62 PF samples used to store kidneys and found that 38.7% of samples were contaminated with at least one microorganism. There were five species of coagulase-negative staphylococci (13 strains) among the PF samples contaminated with bacteria. Bacterial contamination evolved without symptoms in most patients treated with prophylactic intravenous piperacillin-tazobactam therapy. Six patients received kidneys from PF contaminated with *C. albicans*.

PF contamination with MDR microorganisms possesses a great danger to organ recipients. Zhang *et al.* (2022) analyzed carbapenem-resistant *K. pneumoniae* infections in kidney transplant recipients. Among 206 PF samples tested, 20 were contaminated with carbapenem-resistant *K. pneumoniae* strains. An infection developed in 15 patients, and 6 of them died. All isolated strains were susceptible to ceftazidime-avibactam, and all but one strain were susceptible to tigecycline.

Ranghino *et al.* (2016) evaluated the clinical impact of microbial contamination of PF used to store kidneys. During a 3-year single-center retrospective study, the authors examined 290 PF samples and clinical data from patients who received a kidney transplant from deceased multi-organ donors. All of the patients received prophylactic broad-spectrum antibiotics intravenously during surgery and at least for 9 days after transplantation. If yeasts were present in the PF, fluconazole or caspofungin therapy was introduced. Of the 290 PF samples, 101 (34%) were contaminated with one or more microorganisms, mainly with coagulase-negative staphylococci (47 strains belonging to 9 species) and *E. coli* (17 strains). In addition, 10 *C. albicans* strains were isolated. The authors found that although PF contamination is frequent, the incidence of PF-related infections is very low. Preemptive therapy did not help to reduce the rate of PF-related infections, so a reasonable reduction in the use of antibiotic therapy could be made. The authors recommended close clinical and microbiological monitoring of the recipient when PF is contaminated to establish a diagnosis and to start the appropriate antibiotic therapy as soon as possible (Ranghino *et al.* 2016).

Transplant centers follow different approaches concerning the use of antibiotic prophylaxis before surgery and during the first post-transplant week. A nationwide survey regarding perioperative antibiotic prophylaxis in France indicated that antibiotic prophylaxis practices during the perioperative kidney transplant period are very heterogeneous, and this situation requires the development of a special guidelines (Le Berre *et al.* 2020). Of note, 107 of 139 respondents (77%) reported the existence of local practice guidelines for surgical antibiotic prophylaxis in kidney transplant recipients. Only 18 of 139 respondents (13%) reported that they used the following drugs for prophylaxis during the early post-transplant period: cephalosporins (13/18), fosfomycin (3/18), fluoroquinolones (1/18), glycopeptides (1/18), and fluconazole (1/18). The median drug prescription duration was 5 days.

3.2. Infections associated with liver transplantation

In solid organ transplant recipients, the second most commonly described complication (after infections related to kidney transplantation) is infection that result from contaminated PF used to store liver grafts. During a 4-year retrospective study (2007–2010), Sauget *et al.* (2011) showed that among 137 transplanted organs (90 kidneys and 47 livers), 54.5% of PF samples were contaminated with bacteria. Coagulase-negative staphylococci were dominant (66.4%), followed by *Enterobacteriaceae* (8.3%) and anaerobic bacteria, namely *Propionibacterium* spp. (7.5%). The following strains were also isolated from the transplanted kidneys and their PF samples: *Lactobacillus* spp. (4.9%); streptococci and enterococci (2.9%); anaerobic *Peptostreptococcus* spp. (2%); *S. aureus* (2%); and single strains of *Pseudomonas* sp.,

Bacillus sp., and *Micrococcus* sp. However, the dominant bacteria causing infections in transplant recipients were *Enterobacteriaceae* (54.3%), coagulase-negative staphylococci (17.2%), and streptococcal and enterococcal strains (15.5%). Pulsed field gel electrophoresis of the DNA of bacteria isolated from contaminated PF revealed no clonal identity, with the exception of a pair of *E. coli* strains. These findings indicates a small risk of developing infections in patients whose transplanted organs were stored in contaminated PF.

In a retrospective study, Chaim *et al.* (2011) analyzed contamination of PF samples in relation to recipient survival and acute cellular rejection in the context of liver transplantation. Fifteen of the 121 PF samples were contaminated with *K. pneumoniae* (n = 6), *S. epidermidis* (n = 5), and *A. baumannii* (n = 3). Only one patient with a PF-associated infection (caused by *K. pneumoniae*) died. In a 4-year prospective study, Reimondez *et al.* (2021) investigated the risk of infections from contaminated PF in liver transplant recipients. Of the 88 PF samples tested, 33 showed the presence of bacteria and one third had polymicrobial contamination; *S. epidermidis* predominated. Five recipients became infected and received antibiotic therapy based on the antibiogram. Antibiotic prophylaxis with 3 g of intravenous ampicillin-sulbactam was routinely administered in all recipients 30 minutes before skin incision and four times a day up to 48 hours after surgery. There was no significant difference in infections between patients whose transplanted liver was stored in contaminated PF and patients whose transplanted liver was stored in uncontaminated PF. Garcia-Zamora *et al.* (2015) reported similar observations when analyzing 178 liver transplants. They found bacteria or fungi in 79 PF samples (44%). Staphylococci (64%) and *Enterobacteriaceae* (17%) strains were isolated most frequently. There were 25 postoperative infections, but only 4 out of 79 liver graft recipients (5%) who received a liver stored in contaminated PF developed a postoperative infection related to the microorganism isolated from the PF. These findings indicate the low dependence of such infections on PF contamination.

Hygienic practices and procedures during preservation of transplanted organs have reduced the extent of PF contamination. In a 1-year study (March 2007-March 2008) involving 60 PF samples in which transplanted livers were stored, all but one of the samples were contaminated (Ruiz *et al.* 2009). Strains of low pathogenicity such as coagulase-negative staphylococci, *Streptococcus viridans*, and *Corynebacterium* sp. accounted for 75% of the isolated microbes. Microorganisms isolated from post-transplant infections did not match the strains isolated from the PF samples.

Sometimes, organ transplants are performed from deceased donors who have various infections. In a retrospective study, Tong *et al.* (2020) analyzed data from 211 liver donors, of which 82 (38.9%) were infected, to define whether, blood, bronchial aspirate, catheter, and urine samples had been subjected to microbiological examination. The most common isolates were *A. baumannii* (27 cases), *S. aureus* (22 episodes), and *P. aeruginosa* (13 cases). There were 17 cases of fungal infections, and *C. albicans* accounted for 53% of these cases. Among 82 liver donors, 51 were infected, of which there were 12 possible donor-derived infections and 39 non-possible donor-derived infections. The authors concluded that in the case of liver transplantation from an infected donor, the postoperative incidence of infection is high and the infection interval is short. When dealing with MDR bacteria, recipients may have serious complications and poor outcomes.

Berry *et al.* (2019) tested the effectiveness of intraoperative versus perioperative extended antibiotic prophylaxis in the context of liver transplant surgery. Liver transplant patients who received an extended 72-hour course of prophylactic antibiotics did not show a reduction in surgical site infections compared with patients who received a short course of antibiotics (the first dose 30 minutes prior to incision and the second dose 4 hours after initiation of the transplant procedure). There were 16 and 18 infections, respectively. Moreover, a similar number of vancomycin-resistant enterococci were isolated from each group.

3.3. Infections associated with pancreas and lung transplantation

Microbiological contamination of PF used to store pancreas transplants poses a great threat to recipients. The microbiological safety of islet preparations is particularly important. Meier *et al.* (2018) examined the microbiological purity of samples collected during islet isolation over a 10-year period. Microbial contamination of PF was found in 64.4% (291/452) of processed donor pancreas. Coagulase-negative staphylococci were isolated most frequently from pancreas PF (45%), followed by *S. aureus* (9.5%), streptococci (6.7%), and *Candida* spp. (5.3%). The procedure of preparing islets for administration to the recipient, although carried out under aseptic conditions, was also associated with the risk of contamination. The use of antibiotics and successive washing steps during pancreas digestion and islet isolation and purification helped to eliminate microorganisms inherited from procurement of the donor pancreas. After islet isolation and purification, 4.9% (22/452) of the preparations met the release criteria for transplantation. Finally, a total of 189 islet preparations were transplanted to 92 recipients (Meier *et al.* 2018).

Tran-Dinh *et al.* (2023) conducted a 6-year retrospective study on contamination of PF used to store

lung grafts before transplantation. The authors examined 271 patients and found that 83 patients (30.6%) received lung grafts stored in contaminated PF. The most common isolates were *S. aureus* (33 cases) and *E. coli* (13 cases). Additionally, five *Candida* spp. strains were recovered from contaminated PF samples. The authors concluded that there is a high prevalence of PF contamination, and this phenomenon may decrease the survival of lung graft recipients. They recommended routine microbiological testing of PF and treatment with targeted antibiotic therapy in case of infection after lung transplantation.

3.4. Infections associated with corneal and skin transplantation

Storing eye tissues in eye banks before keratoplasty for a certain period of time allows a number of tests to be performed, including an assessment of microbiological contamination (EEBA Technical Guidelines for ocular tissue, 2020). Deogaonkar and Roy (2023) analyzed 50 publications published from 2005 to 2021 regarding donor-related corneal infection. The post-keratoplasty infection rates were 0.2–0.77% for endophthalmitis and 6.5–10.5% for microbial keratitis. In analyzed articles, MDR Gram-negative rods and fungi (*Candida* spp. and *Aspergillus* spp.) were associated with contamination (Deogaonkar and Roy 2023). Thareja *et al.* (2020) reported slightly different observations. Apart from fungal infections, the most common causes of bacterial infection after keratoplasty were *Staphylococcus* and *Streptococcus* strains.

Mathes *et al.* (2019) performed a retrospective review (2006–2017) of the infection rates in a single eye bank, comparing corneas prepared and not prepared by the eye bank. The overall infection rate related to the donor tissue was low (2.3 in 10,000). The eye bank-prepared corneas were assumed to be more susceptible to infection due to exposure to elevated temperatures when removed from cold storage for processing. Additionally, handling corneas during eye bank preparation may increase the chance of tissue contamination. However, eye bank-prepared corneas were not linked to an increased risk of post-keratoplasty infections.

Ling *et al.* (2019) analyzed the current factors affecting corneal organ culture contamination and presented a flow diagram of donor tissue processing. The study included 4410 corneal samples, of which 110 (2.5%) were contaminated. Sixty-three fungal strains were isolated, including 38 *C. albicans* strains and 24 bacterial strains, of which coagulase-negative staphylococci dominated (14 strains).

Röck *et al.* (2017) retrospectively analyzed factors influencing the contamination rate of 1340 cultured corneas at the Tübingen Cornea Bank (Germany) from 2008 to 2014. The annual contamination rate ranged from 1.3% to 2.1%. Of note, half of the samples were contaminated with fungi, exclusively *Candida* sp., and half of the samples were contaminated with bacteria, predominantly *Staphylococcus* spp. The analysis showed an increased risk of contamination for septic donors compare with aseptic donors. The main source of fungal and bacterial contamination could be resistant skin microbiota. The mean monthly contamination rate was correlated with the mean monthly air temperature.

In a large retrospective analysis of organ-cultured human corneas in one French regional eye bank for the period of 2005–2018 (Fabre *et al.* 2021), among 127,979 donor corneas collected, 1240 samples (6.9%) were microbiologically contaminated. This group contained 930 (75%) bacterial strains, including 6 species of coagulase-negative staphylococci (n = 357), non-enterobacteria Gram-negative rods (n = 339), and enterobacteria (n = 140) – and 272 (21.4%) fungal strains – including 225 *Candida* sp. strains (*C. albicans*, n = 130) and 31 filamentous fungal strains. Besides, 38 (3.1%) microorganisms were not identified. There was some change in the annual average contamination rate and the microorganism groups from 2005 to 2018 (Fabre *et al.* 2021).

Li *et al.* (2019) investigated microbiological contamination in donor corneas preserved in the medium term, starting from the acquisition of eyeballs preserved and delivered in ice box to the eye bank. Eyeballs were soaked in 0.05% povidone iodine solution for 1–2 minutes, rinsed with sterile saline, and soaked in 2000 U/mL gentamicin sulphate solution for 10–15 minutes. Then corneal grafts were excised into medium-term preservation solution at 4–8°C for keratoplasty. After removal of the central corneas for transplantation, the corneoscleral rims were put back into the medium for 1 month at 20–25°C. Eighty-two donor corneas were included in the study. The contamination rate was 9.8%; seven of the eight identified strains were fungi.

Skin transplants are performed primarily in people with severe burns. These injuries are a significant global health problem, with over 11 million people requiring medical intervention each year and approximately 180,000 deaths annually (WHO Fact Sheet 2023). The management of severe burn injuries involves preventing and treating burn shock and promoting skin repair through a two-step procedure of covering and closing the wound. Currently, split-/full-thickness skin autografts are the gold standard for permanent skin substitution (Šuca *et al.* 2024). Burns provide an ideal environment for bacterial growth. There is an increased risk of infection of the skin and/or soft tissues during the early stages, particularly from Gram-positive bacteria, followed by Gram-negative bacteria and fungi (Kelly *et al.* 2022).

4. Fungal infections in organ transplant recipients

In addition to bacterial infections, organ recipients may experience fungal infections. Peghin *et al.* (2024) analyzed several skin and soft tissue fungal infections in solid organ transplant recipients. Infections in recipients were caused by strains from the following genera: *Candida* spp., *Cryptococcus* spp., *Aspergillus* spp., *Mucor* spp., *Fusarium* spp., *Histoplasma* spp., *Blastomyces* spp., and *Coccidioides* spp. It is extremely important to use appropriate diagnostic tests to detect the etiological factor of the infection. In addition, Sawinski and Blumberg (2019) reported that kidney transplant patients are at increased risk for opportunistic fungal infections, including *Candida*, *Aspergillus*, *Cryptococcus*, and *Pneumocystis*. Candidemia is most often an early post-transplant nosocomial infection of surgical drains or vascular access catheters. *Candida* infections may be observed in liver recipients with cholangitis, hematomas, or bile leaks (Fishman 2017). Invasive *Aspergillus* infections occur most often in debilitated or immunosuppressed organ recipients. Within 1-year after organ transplantation, opportunistic infections emerge, including by *Pneumocystis jirovecii*, which causes pneumonia, and by endemic fungi such as *Histoplasma capsulatum*, which causes Darling's disease (Fishman 2017).

Contamination of PF with yeasts during solid organ recovery can lead to life-threatening complications in the recipients. Fungal infections constitute a significant clinical problem in liver and kidney recipients. Botterel *et al.* (2010) analyzed 650 PF samples collected during a 5-year period in terms of fungal contamination using standardized procedures for systematic mycological culture. The yeast contamination rate was 4.1% and 3.1% for liver and kidney transplants, respectively. Strains belonging to the following species were identified: *C. albicans*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis*, *Candida valida*, *Pichia etchelsii*, and *Rhodotorula* sp. Audet *et al.* (2011) analyzed 91 PF samples used in liver transplantation and detected 4 *C. albicans* strains, 1 *Aspergillus fumigatus* strain, and 1 *Saccharomyces* sp. strain. Stern *et al.* (2022) analyzed the PF of 1248 hepatic and 1273 renal transplants. They found that fungal contamination in the PF of hepatic and renal grafts was 1.2% and 0.86%, respectively. Although the incidence of fungal contamination was low, contaminated PF was associated with high mortality of organ recipients. *C. albicans* was the most common organism (70.4%), followed by *C. krusei* and *C. glabrata*. The above observations are consistent with those reported by Levesque *et al.* (2015) in a 5-year multicenter study of PF contamination after liver transplantation. Among 2107 PF samples, 28 (1.33%) were contaminated with *Candida* strains, 64% of which were *C. albicans*. Eight recipients developed yeast-related complications and 1-year after transplantation, the mortality rate among this group of patients was 62.5%. Bachellier *et al.* (2014) mentioned *C. albicans* arteritis transmitted by PF after liver transplantation.

Ten years ago, the EBAA (Aldave *et al.* 2013) reported that from 2005 to 2010, the incidence of fungal infections after corneal transplantation showed an increasing trend. Thirty-one cases of culture-proven fungal keratitis (n = 14) and endophthalmitis (n = 17) were reported out of 221,664 corneal transplants performed using corneal tissue distributed by domestic eye banks.

Thareja *et al.* (2020) noticed that in post-keratoplasty infections, the main fungal pathogen is *C. albicans*, followed by *C. glabrata*. Besides, *Alternaria* sp. and *Cladosporium* sp. may also cause infections. Based on the findings, the authors proposed antifungal supplementation of PF to eliminate contaminated fungi. The arguments for and against supplementation of PF with voriconazole, caspofungin, amphotericin B, and betadine have been discussed. Malinis and Boucher (2019) provided recommendations regarding screening of solid organ donors for histoplasmosis, coccidioides, and cryptococcosis.

5. Viral infections in organ transplant recipients

The laboratory serological tests to detect viral infections, especially latent ones, should be performed in both organ donors and recipients (Grossi 2018; Malinis and Boucher 2019; Sawinski and Blumberg 2019). The risk of infection for recipients may be associated with the following viruses: herpes viruses (e.g. human herpes virus [HHV]6, HHV7, and HHV8); Kaposi sarcoma herpesvirus; cytomegalovirus (CMV); herpes simplex virus (HSV); varicella zoster virus (VZV); Epstein-Barr virus (EBV); retroviruses, including human immunodeficiency virus (HIV) and human T cell lymphotropic virus-1 and 2; and hepatitis viruses, including hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), and hepatitis E virus (HEV). Other rare viruses may also pose a threat: West Nile virus, Chikungunya virus, Zika virus, dengue virus, lymphocytic choriomeningitis virus, and rabies virus (Fishman 2017; Peghin *et al.* 2024). Saad *et al.* (2020) analyzed infections in the first year after renal transplant and found that the most frequent viral infections were caused by CMV (21.8%) 31–180 days after transplantation.

The U.S. Public Health Service has published recommendations on how to reduce the risk for transmission of several viruses through solid organ transplantation (Jones *et al.* 2020). In 2021, the Centers for Disease Control and Prevention realized that this recommendation might be unnecessary for pediatric organ

transplant candidates. Thus, a year later the guidelines were updated to specify that solid organ transplant candidates aged < 12 years at the time of transplantation who have received postnatal infectious disease testing are exempt from the recommendation for HIV, HBV, and HCV testing during hospital admission for transplantation (Free *et al.* 2022).

6. Parasitic infections in organ transplant recipients

In addition to bacteria, fungi, and viruses, parasites including *Toxoplasma gondii*, *Trypanosoma cruzi*, *Strongyloides stercoralis* and *Leishmania* spp., and amoebas including *Balamuthia* spp. and *Naegleria* spp. can cause infections related to transplants (Fishman 2017; Grossi 2018; Malinis and Boucher 2019; Peghin *et al.* 2024). Toxoplasmosis constitutes a significant danger in heart transplant recipients, when a *Toxoplasma* seropositive heart is transplanted into a *Toxoplasma* seronegative recipient. Toxoplasmosis has also been transmitted to liver and kidney recipients (Malinis and Boucher 2019). Transmission of *Strongyloides* spp., an intestinal nematode endemic to the tropics and subtropics, via transplantation hs been described with significant mortality and morbidity (Malinis and Boucher 2019; CDC 2012; Peghin *et al.* 2024).

7. Conclusions

Implantology is a field of medicine that has recently shown quite dynamic development. The number of people receiving solid organ and tissue transplants has increased markedly. After successful organ transplantation, many people with end-stage organ failure can live for a long period of time. The surgical process of transplantation involves the risk of infections, primarily with bacteria and fungi. This is facilitated by the contamination of PF in which an organs is stored, as well as noncompliance with hygiene procedures. The published data regarding the extent of contaminated PF and the bacterial and fungal strains are very diverse and largely depend on the level of the transplantation centers and the procedures they follow. The second area of microbiological danger for transplant recipients is related to the possibility of transmitting mainly viruses and parasites with the transplanted organ. Latent microorganisms like *Mycobacteria* and viruses may be reactivated in the body of a patient subjected to immunosuppression after transplantation. Opinions on the scope and method of antibiotic prophylaxis for organ donors and recipients are divided. Contamination of PF with essentially nonpathogenic microorganisms and their transfer to the organ recipient rarely results in severe infection. However, post-transplantation infections caused by MDR bacteria should undoubtedly be detected quickly and treated with the appropriate antibiotics.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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MICROBIAL URICASE AND ITS UNIQUE POTENTIAL APPLICATIONS

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Abstract: The uricase enzyme yields allantoin, hydrogen peroxide, and carbon dioxide by catalyzing the oxidative opening of the purine ring in the urate pathway. This enzyme is important for biochemical diagnosis and reduces toxic urate accumulation during various diseases (hyperuricemia, gout, and bedwetting). Direct urate oxidase injection is recommended in renal complications-associated gout and to prevent chemotherapy-linked hyperuricemia disorders. Thus, uricase is a promising enzyme with diverse applications in medicine. Microbial production of uricase is featured by high growth rates, cost-effective bioprocessing, and easy optimization of the medium. Microbes produce the enzyme extracellular or intracellular. Extracellular uricase is preferred for biotechnological applications as it minimizes time, effort, and purification processes. This review provides insights into uricase-producing microbes, bacterial uric acid degradation pathways, degrading enzymes, and uricase-encoding genes.

Furthermore, aspects influencing the microorganisms' production of the uricase enzyme, its activity, and its purification procedure are also emphasized. Cell disruption is mandatory for intercellular uricase production, which elevates production costs. Therefore, extracellular uricase-producing microbial strains should be investigated, and production factors should be optimized. Future techniques for obtaining extracellular enzymes should feature reduced time and effort, as well as a simple purification methodology. Furthermore, uricase gene-carrying recombinant probiotic microorganisms could become an effective tool for gout treatment.

Keywords: gout, hyperuricemia disorders, microbial uricase, urate oxidase, uricase, uricase applications, uricase purification

1. Introduction

Uricase, a peroxisomal (oxidoreductase) enzyme, catalyzes the oxidative opening of the purine ring in the urate pathway to yield allantoin, hydrogen peroxide, and carbon dioxide (Roman 2023). Uricase is important for the biochemical diagnosis of uric acid in the serum and biological fluids (urine). Biosensors can readily detect uric acid more precisely and accurately than other methods (Aafaria *et al.* 2022). It also alleviates the accumulation of toxic urate during various diseases (hyperuricemia, bedwetting, and gout). Uricase absence in some individuals could be attributed to metagenes, which prematurely terminate the translation process (Roman 2023). Direct urate oxidase injection is also suggested to treat renal complications-associated gout and prevent chemotherapy-linked hyperuricemia disorders (Cho *et al.* 2023). Microbes, animals, and plants can produce uricase. However, microbial production offers higher growth rates, cost-effective bioprocessing, and convenient optimization of the medium (Wan *et al.* 2023). The reported microbial uricase enzymes

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mainly include intracellular enzymes released via cell disruption. Therefore, extracellular enzyme production is crucial to reduce yielding time and purification. This review elaborates on the uricase-producing microorganisms, bacteria-based uric acid degradation pathway, uricase activity affecting factors, microbial production and purification, and its applications. Cell disruption is mandatory for intracellular uricase synthesis and purification, which enhances the production cost. Identifying extracellular uricase-synthesizing microbial strains and optimizing conditions is highly advisable. Moreover, uricase gene-carrying recombinant probiotic microbes could emerge as an efficient gout treatment strategy.

1.1. Uric acid: chemical structure and biosynthesis

Uric acid ($C_5H_4N_4O_3$) is a heterocyclic compound of carbon, hydrogen, nitrogen, and oxygen (Fig. 1). It forms different salts and ions such as acid urates, urates, and ammonium acid urate (El Ridi *et al.* 2017).

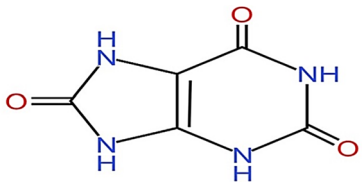

Fig. 1. Chemical structure of uric acid.

The liver and small intestine produce heterocyclic uric acid (7,9-dihydro-1H-purine-2,6,8(3H)-trione) that has a molecular weight of 168 Da. The diet contains a low urate concentration, whereas food purines are the main source of uric acid synthesis by producing new purine bases or internal breakdown of purine bases (Fauci *et al.* 2012). Hepatic uric acid generation and intestinal and renal excretion depend on multiple variables involving complicated metabolic mechanisms. Different enzymes can convert purine nucleic acids (guanine and adenine) into uric acid (Chaudhary *et al.* 2013). Two types of mechanisms initially convert Adenosine monophosphate (AMP) into inosine such as (a) deaminase-based removal of amino group to form inosine monophosphate (IMP) followed by nucleotidase-based dephosphorylation to generate inosine nucleoside, or (b) nucleotidase-based removal of phosphate group to form adenosine followed by deamination to produce inosine nucleoside. Nucleotidase also transforms guanine monophosphate (GMP) into guanosine nucleoside. Then, xanthine-oxidase (XO) oxidizes hypoxanthine to form xanthine, whereas guanine deaminase deaminates guanine to form xanthine. Xanthine oxidase further oxidizes xanthine into the final product known as uric acid. Fig. 2 demonstrates the enzymatic pathway of purine degradation. Uric acid is a weak acid at physiologic pH with a pKα value of 5.8. Uric acid mostly exists in the form of urate salt (Jin *et al.* 2012), and its crystal formation is enhanced with the high blood concentration of urate.

1.2. Benefits and hazards of uric acid

Uric acid, a urine component, is a metabolic breakdown product of purine nucleotides (guanine and adenine) and a derivative of proteins. Uric acid is a dominant natural antioxidant plasma factor that activates immunity responses in certain illnesses; however, gout and joint infections (chronic and acute) are associated with uric acid. High concentrations of blood uric acid can cause gout and other medical complications such as diabetes and kidney stone (ammonium acid urate) formation. The liver is the main uric acid-producing organ, along with the intestinal wall, endothelium of kidneys, and blood vessels (Yeum *et al.* 2004). Uric acid plays important physiological functions, and the body re-absorbs almost 90% of uric acid (Maiuolo *et al.* 2016; Roman 2023). Antioxidant activity is an essential feature of uric acid that could eliminate half of the blood plasma's free oxygen radicals (ROS) (Sautin and Johnson 2008; Roman 2023). Recent reports have highlighted uric acid-based initiation of inflammatory processes to facilitate tissue repair in addition to ROS removal (Nery *et al.* 2015). Lower blood uric acid levels might lead to mutations in renal carriers and blood cells (Sugihara *et al.* 2015). Contrarily, some studies have linked excess uric acid with kidney and cardiovascular diseases (Oberbach *et al.* 2014; Hammad *et al.* 2015; Roman 2023).

Uric acid is also known to protect the nervous system against various diseases. Autoimmune diseases such as lichen planes, Parkinson's disease, and pemphigus vulgaris are linked to lower uric acid levels (Bakhtiari *et al.* 2017; Kuwabara *et al.* 2023). Excessive protein intake or alleviated kidney-based excretion of uric acid results in increased uric acid levels in the blood (Bobulescu and Moe 2012; Xu *et al.* 2017). Higher uric acid levels damage the kidney's surface cells to cause their weak physiological activity, leading to chronic kidney diseases, particularly in Type 2 diabetes patients and individuals suffering from chemical intensification diseases (Xiao *et al.* 2015; Kim *et al.* 2015; Kuwabara *et al.* 2023). The uric acid accumulation in kidneys commonly results in kidney stone formation (Fathallah-Shaykh and Cramer 2014; Jalal 2016). Higher uric acid levels could also stimulate some autacoids and hormones to cause high blood pressure, whereas infiltration into smooth heart muscles leads to various cardiovascular illnesses (Kanbay *et al.* 2013; Kuwabara *et al.* 2023).

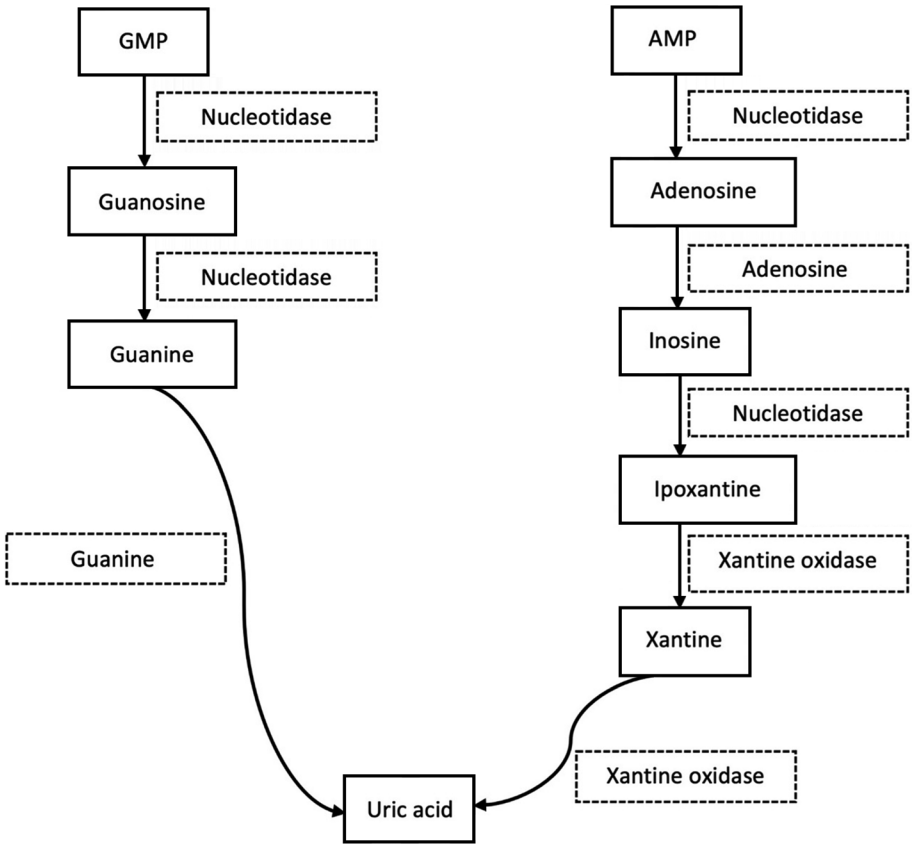

Fig. 2. Enzymatic degradation of purines to uric acid (Chaudhary *et al.* 2013).

2. Uric acid degrading microorganisms

The first microbial uricase was isolated from a fungus (*Neurospora crassa*) in 1957 (Perez-Ruiz *et al.* 2014). Later, it was discovered that various bacteria could degrade uric acid as well including *Pseudomonas aeruginosa*, *Bacillus thermocatenulatus*, *Microbacterium* sp., *Arthrobacter globiformis*, *Nocardia farcinica*, *Escherichia coli, Bacillus subtilis*, and *Bacillus fastidious* (Saeed *et al.* 2004; Suzuki *et al.* 2004; Zhou *et al.* 2005; Lotfy 2008; Xu *et al.* 2022; Chen and Li 2023). The uricase enzyme production in *Pseudomonas aeruginosa, Bacillus subtilis*, *Escherichia coli, Alcaligene*s, and *Bacillus thermocatenulatus* is extracellular whereas its production is intracellular in *Microbacterium, Proteus vulgaris, Streptomyces albidoflavus, Streptomyces graminofaciens,* and *Streptomyces exofolitus* (Zhou *et al.* 2005; Ali *et al.* 2013; Chen and Li 2023). Lactic acid bacteria (*Pediococcus* species*, Bifidobacterium*, *Leuconostoc*, *Lactobacillus*, and *Enterococcus*) can degrade uric acid as their intake in rats/mice suppressed serum uric acid level (Ogawa 2006; Li *et al.* 2023; Negm El-Dein *et al.* 2023). Different soil fungi (*Fusarium*, *Helminthosporium*, *Spondilocladium*, *Curvularia, Stemphylium*, *Aspergillus*, *Geotrichum*, *Penicillium*, *Mucor*, *Rhizopus, Alternaria*, and *Chaetomium*) are capable of producing intracellular uricase at high rates, particularly in the presence of urea or uric acid as the sole nitrogen source (Geweely and Nawar 2011; Ali *et al.* 2013; Rajagopalan *et al.* 2017; Moradpour *et al.* 2022), Table I, lists some of the significant uricase-producing microorganisms.

3. Uric acid degradation pathway in bacteria

The process of purine breakdown to uric acid is often conserved among organisms. However, uric acid degradation products could vary between species depending on the active catabolic enzymes, which can degrade or excrete uric acid in the peroxisomes (Lee *et al.* 2013). As a result of the degradation process, xanthine is the first intermediate product of all purine bases. Xanthine dehydrogenase degrades xanthine in the cytosol to generate urate. It is imported into the peroxisome and undergoes uricase-based oxidation to form 5-hydroxyisourate, which is converted to S-allantoin via 2-oxy-4-hydroxy-4-carboxy-5-ureidoi imidazoline by a functional allantoin synthase (Gabison *et al.* 2010). Urate oxidase evolution (allantoicase, uricase, and allantoinase) might be the reason behind varying degradation end-products of uric acid in different microorganisms. Most microorganisms can completely break down uric acid to ammonia through nitrogen catabolic enzymes (Marzluf *et al.* 1997). Allantoate

Table I
Uricase-producing microorganisms.

Microorganisms	Extracellular uricase	Intracellular uricase
Bacteria	*Escherichia coli*	*Proteus vulgaris*
	Bacillus pasteurii	*Streptomyces albidoflavus*
	Proteus mirabilis	*Streptomyces graminofaciens*
	Pseudomonas aeruginosa	*Saccharopolyspora* sp.
	Microbacterium spp.	
	Lactobacillus sp.	
Filamentous fungi		*Fusarium* sp.
		Geotrichum sp.
		Mucor sp.
		Alternaria sp.
		Penicillium sp.
		Aspergillus sp.
		Rhizopus sp.
Yeast	*Candida tropicalis*	*Candida utilis*

Sources: Abdel-Fattah *et al.* (2005); Ali *et al.* (2012); Azab *et al.* (2005); Chen *et al.* (2008); Chen and Li (2023); Moradpour *et al.* (2022); Negm El-Dein *et al.* (2023); Khucharoenphaisan and Sinma (2011); Rando *et al.* (1990); Rajagopalan *et al.* (2017).

amidinohydrolase (allantoicase) in certain bacteria and fungi hydrolyze allantoate to generate s-ureidoglycolate and urea (Marzluf *et al.* 1997) (Fig. 3).

4. Uric acid degrading enzymes

Uricase enzymes (protein) possess the features of cofactors, they catalyze various biochemical reactions, and their deficiency could lead to different diseases. Uricase is often used in biochemical diagnosis, blood uric acid detection, and industrial processes (El Ridi *et al.* 2017; Tandon *et al.* 2021). Therapeutic enzymes differentiate from other drugs due to their relationship with pathogens, such as activating or inhibiting a specific reaction and direct association with the disease-causing substrate via its deposition in the body (Meletis and Barker 2005). Therapeutic enzymes could be biologically extracted from fungi, plants, and bacteria or synthesized in the laboratory (Babashamsi *et al.* 2009).

Therapeutic uricase (urate oxidase) is generally not detected in humans. However, an RNA study has revealed uricase production in human liver cells (Kratzer *et al.* 2014). Uricase (urate oxidase, EC 1.7.3.3, oxidoreductase) carries out purine metabolism and activates uric acid oxidation into soluble allantoin. It is present in most vertebrates except higher apes and humans, where it became non-functional due to point mutation during evolution and formed a redundant protein (Wu *et al.* 1989). Uricase is localized in various microorganisms, including *Proteus mirabilis*, *Escherichia coli,* and *Bacillus pasteurii* (Cheristians *et al.* 1986; Rando *et al.* 1990; Nakagawa *et al.* 1996). *Microbacterium, Candida tropicalis*, *Pseudomonas aeruginosa, Streptomyces albosriseolus*, and *Bacillus thermocatenulatus* can produce extracellular uricase after the optimization of culture media (Zhou *et al.* 2005; Abdel-Fattah *et al.* 2005; Lofty 2008). Uricase enzyme, containing four subunits, attracts and converts uric acid to hydrogen peroxide and allantoin through four identical type 2 copper binding sites (Fig. 4) (Wu *et al.* 1989). Several other enzymes, including xanthine oxidase, can also degrade uric acid by inhibiting a uric acid pathway reaction (hydrolysis). Xanthine oxidase is combined with different drugs for an efficient uric acid analysis (Li *et al.* 2005).

Pseudomonas and other probiotics lactic acid bacteria produce other uric acid degrading proteins (Kanmani *et al.* 2013). Uricase catalyzes the *in-vivo* uric acid oxidation to generate CO_2 and allantoin in the presence of oxygen. The reduction of oxygen could also produce hydrogen peroxide (Fig. 5). Different types of thermostable microbial uricase enzymes are used in uric acid detection, which can sustain a wide range of pH (5, 6, 8, and 9) (Li *et al.* 2005; Ravichandran *et al.* 2015).

5. Genetics and uricase encoding genes

Fourteen functional genes have been discovered, which encode enzymes/proteins of the purine catabolic pathway. Xanthine dehydrogenase functioning requires the expression of five genes (*pucE*, *pucD*, *pucC*, *pucB*, and *pucA*), whereas two genes (*pucM* and *pucL*) encode

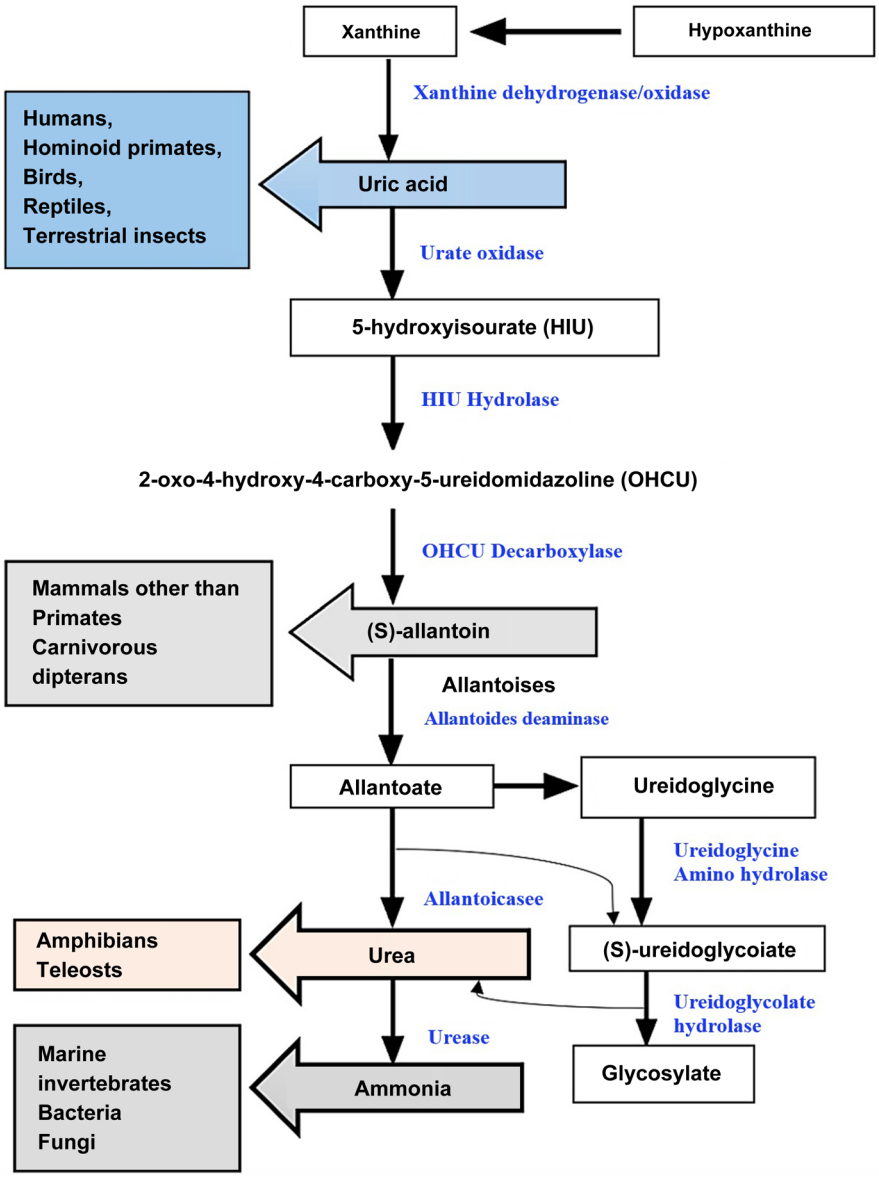

Fig. 3. The varying end products of purine metabolism in different species due to differential catabolic enzymes in the pathway (Lee *et al.* 2013).

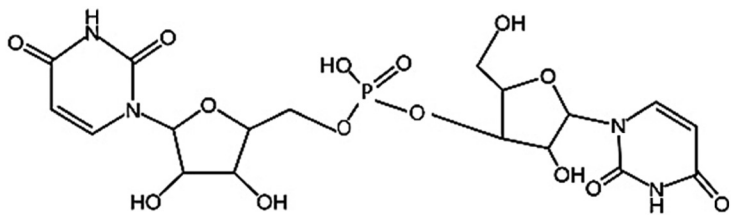

Fig. 4. Bacterial uricase (Wu *et al.* 1989).

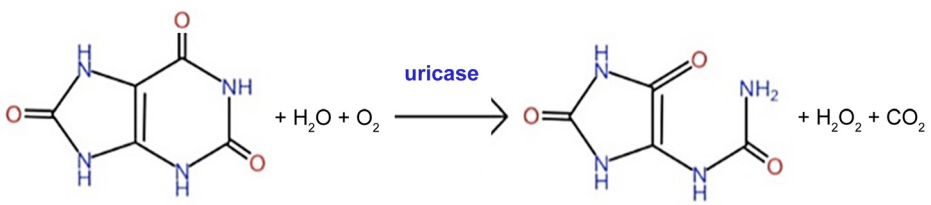

Fig. 5. Uricase catalyzes the reduction of dissolved oxygen to peroxide in the presence of uric acid (Li *et al.* 2005; Ravichandran *et al.* 2015).

uricase, and two genes (*pucK* and *pucJ*) encode uric acid transport system. The *pucI, pucH*, and *pucF* genes encode allantoin permease, allantoinase, and allantoate amidohydrolase. During a study, the *pucR*-mutant *Bacillus subtilis* expressed the lowest activity among all tested genes, indicating that PucR regulates puc gene expressions (Hafez *et al.* 2017). All 14 genes except *pucI* are located in a chromosomal gene cluster at 284–285° and participate in six transcription units. Uric acid, allantoic acid, and allantoin compounds regulate PucR for *puc* genes' expression (Argyrou *et al.* 2001). The utilization of uric acid initiates virulence factors (urease and capsule) synthesis in fatal meningitis-associated *Cryptococcus neoformans* that potentially regulate the host's immune response during the infection. Uricases (aquatic vertebrate and microbial) are mostly soluble and are found in bacterial cytoplasm or yeasts' peroxisome (Kratzer *et al.* 2014).

6. Uricase activity affecting factors

Multiple factors affect uricase activity, mainly pH and temperature. Ravichandran *et al.* (2015) reported an optimum pH (8) and temperature range (25–45°C) for uricase activity. They further noted an almost 50% decrease in uricase activity at 60°C after exposure for one hour (Chohan and Becker 2009). Contrarily, Geweely and Nawar (2011) have reported an optimum temperature of 35°C for *Aspergillus niger*-based uricase. Heavy metals serve as cofactors for some enzymes, but their large quantities could also inhibit enzyme activities (Suzuki *et al.* 2004; Witkowska *et al.* 2021). Nelson (2005) reported copper-based uricase inhibition, but Ravichandran *et al.* (2015) noted enhanced (140%) enzyme activity in response to copper stimulation. Chohan and Becker (2009) revealed enzyme stimulation by ethylenediaminetetraacettic acid (EDTA) (1 molar), whereas the same concentration inhibited the activity of ERW. Thus, unknown elements could contribute to enzyme activation and inhibition, varying in different bacteria (Ravichandran *et al.* 2015).

7. Uricase production and purification

Different carbon and nitrogen sources in the culturing media of uricase-producing microorganisms can influence uricase production. Uricase production with different carbon sources can be arranged as sucrose > glucose > cellulose > starch > maltose. Peptone is known for its higher uricase yield than other nitrogen sources (yeast extract, beef extract, and ammonium nitrate) (Pfrimer *et al.* 2010).

7.1. Extracellular uricase

Microorganisms and higher plants produce more uricase, whereas humans cannot produce this enzyme, which leads to purine breakdown-associated uric acid accumulation in the body (Hafez *et al.* 2017). Uricase alleviates hyperuricemia, whereas plant or human uricase stimulates immune responses (Roman 2023). Therefore, bacterial uricase is a therapeutic agent that removes excessive uric acid from the body (Abdel-Fattah *et al.* 2005). *Proteus mirabilis*, *Bacillus pasteurii*, and *Escherichia coli* are known to secrete uricase enzymes (Rando *et al.* 1990; Nakagawa *et al.* 1996; Hafez *et al.* 2017). *Pseudomonas aeruginosa*, *Tropical Candida*, *Thermobacilli*, *Albosriseolus*, and *Microbacterium* have been reported to yield extracellular uricase in the optimized media (Zhou *et al.* 2005; Abdel-Fattah *et al.* 2005; Anderson and Vijayakumar 2011). Extracellular uricase activity was assessed on a solidified medium by following the agar plate assay method, in which uric acid served as an inciting agent. The uric acid screening medium was comprised of sucrose (20 g/L), magnesium sulfate heptahydrate (0.5 g/L) sodium chloride (0.5 g/L), uric acid (3 g/L), di-potassium hydrogen phosphate (1 g/L), agar (15 g/L), and ferrous sulphate (0.01 g/L) (El-Naggar *et al.* 2019). The pH was adjusted to 6.8, and plates were incubated for 5–7 days at 30°C. A clear zone around the colony confirmed uricase production (Fig. 6).

7.2. Intercellular uricase

Fungal or bacterial growth is not directly associated with uric acid production. However, different fungi and bacteria can utilize uric acid as the only source of nitrogen (Baumgardner 2016). During a study, a starter culture of lactic acid bacteria (1%) was grown in uric acid (0.2%)-supplemented PGY broth to obtain intracellular uricase. The culture was incubated at 37°C for 24 hours. After fermentation, centrifugation (3000 rpm, 20 minutes) was carried out at 4°C to separate the supernatant, followed by bacterial cell-based stability testing of intracellular uricase (Carevic *et al.* 2015). Intracellular uricase production was noted in *Microbacterium* spp., *S. albidoflavus*, *P. vulgaris,* and *S. graminofaciens* (Zhou *et al.* 2005; Azab *et al.* 2005). The addition of uric acid into the growth media induced the uricase production. However, various influencing factors (heavy metals, temperature, and pH) are needed to disturb the cell to obtain intracellular uricase (Bongaerts *et al.* 1978). Generally, intracellular uricase production in the gastrointestinal system is considered more stable (O'Connel and Walsh 2007; Pugin *et al.* 2022).

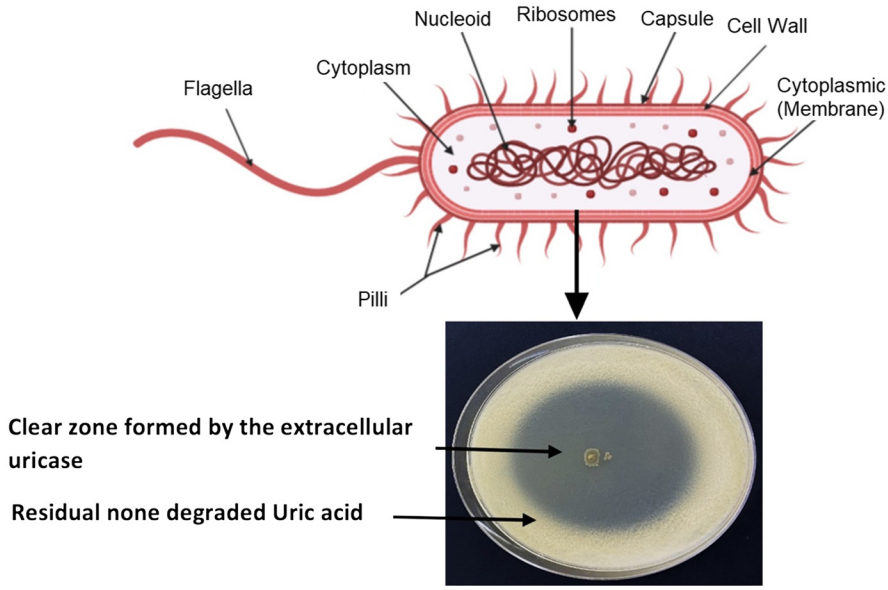

Fig. 6. A clear zone indicating *Alcaligenes faecalis*-secreted uricase on uric acid (0.3%) – supplemented BT medium.

7.3. Uricase purification from different microorganisms

Uricase purification is necessary to achieve its higher yield, which has been carried out through various approaches.

7.3.1. Precipitation by ammonium sulfate

Different concentrations of ammonium sulphate (20, 40, 60, and 70% w/v) can be used to separate uricase from secreted proteins-containing supernatant. Briefly, solid ammonium sulphate is slowly added to the culture filtrate on an ice bath with gentle stirring until the required ammonium sulphate saturation is reached. Then, the mixture is left overnight at 4°C followed by centrifugation at low temperature (8000 rpm, 30 minutes, 4°C) (Saeed *et al.* 2004; Ram *et al.* 2015).

7.3.2. Removal of ammonium sulfate salts

The Removal of ammonium sulphate is performed by dissolving the precipitate in Tris-HCl (0.01 M, 10 ml) buffer (pH 8.5). The solution is dialyzed overnight in ultra-pure distilled water/buffer (1 L) using a dialysis tube. Then, the concentrated dialyzed cell-free supernatant is subjected to the column chromatography technique (Saeed *et al.* 2004; Ram *et al.* 2015).

7.3.3. Uricase purification through ion exchange chromatography on DEAE-cellulose

A DEAE-cellulose-containing column is equilibrated with Tris-HCl (10 mM) buffer (pH 8.5). Dialyzed and concentrated cell-free supernatant is applied to it for uricase purification. The column is washed thrice with the Tris-HCl (10 mM) buffer (pH 8.5). The bound proteins are eluted in the same buffer with a linear NaCl gradient (0–0.3 M), and collected fractions are analyzed at 280 nm using a UV spectrophotometer, whereas enzyme activity and protein concentration are detected at 293 nm (Saeed *et al.* 2004; Ram *et al.* 2015).

7.3.4. Uricase purification with gel filtration column

Potassium phosphate (50 mM) buffer (pH 8.2) is used to equilibrate the Superdex 200 HR-containing gel filtration column (Amersham Pharmacia Biotech, Germany). Ammonium sulfate-based partially purified uricase is dialyzed and applied to this column. The same buffer is used to elute uricase, and all fractions (0.5 ml) with high uricase activity are concentrated using an ultrafiltration membrane (YM 10) or following the lyophilized method. The concentrated fractions are stored at –20°C (Jianguo *et al.* 1994; Saeed *et al.* 2004; Ram *et al.* 2015). According to Laemmli *et al.* (1970), the molecular weight of the purified enzyme should be determined by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) under denaturation conditions to verify the purification stages of uricase. The determination of uricase's molecular weight involves comparing its electrophoretic mobility with that of marker proteins.

8. Applications of uricase

Uricase (Urate Oxidase, EC 1.7.3.3) is a diagnostic enzyme to measure uric acid (urate) levels in the body. It also synthesizes medicines (pegloticase and rasburicase) for hyperuricemia treatment (Yang *et al.* 2012). Uricase is crucial for the human body as it disintegrates

uric acid into allantoin, carbon dioxide, and hydrogen peroxide through oxidation. Generally, uric acid is excreted through the kidneys, but its blood solubility is extremely low (6.8 mg/L) (Ravichandran *et al.* 2015; Mei *et al.* 2022). Higher blood uric acid levels are associated with uric acid nephrolithiasis, gout, cardiovascular disease, hyperuricemia, diabetes, renal failure, and tumor lysis syndrome (Ganson *et al.* 2005; Roman 2023). The uricase enzyme remains inactive in humans because of frameshift mutation during evolution. Therefore, uricase synthesis from other sources is vital to counter associated disorders (Pawar *et al.* 2018).

8.1. Diagnostic role of uricase in clinical analysis

There are diverse applications of uricase enzyme, but its most important role is treating uric acid accumulation-related illnesses (nervous system, heart) (Hafez *et al.* 2017). It is commonly applied to assess blood uric acid levels in the blood. Moreover, it is also combined with a 4-amino-antipyrine-peroxidase system to determine uric acid levels in other biological fluids (Cheung *et al.* 2020). Rasburicase is frequently used to treat organ transplants and tumor lysis-associated hyperuricemia. Uricase is also a common additive of commercial hair coloring agents (Cheung *et al.* 2020).

8.2. Biosensor for bimolecular applications

Monitoring uric acid levels in urine and blood is necessary for the disease diagnosis. There are different methods of uric acid estimation, such as mass fragmentography, enzyme electrode, colorimetry, radiochemical-HPLC, fluorescent sol-gel, commercial uric acid kits, and chemiluminescence (Domagk and Schlicke 1968; Martinez-Pérez *et al.* 2003; Zhang *et al.* 2004; Bio-Assay Systems 2007; Chu *et al.* 2012). The colorimetric method is a simple, specific, and sensitive approach that employs peroxidase and uricase (Zhou *et al.* 2005). However, these enzymes are expensive, which makes this assay more costly than other methods. Different biosensing procedures have also been devised, which involve uricase immobilization on electrode surface using ZnO nanorods, polyaniline-polypyrrole film, polyaniline, ZnO nano-flakes, and polypyrrole nanoelectrode (Uchiyama and Sakamoto 1997; Zhang *et al.* 2004; Arora *et al.* 2007; Arslan 2008; Yang *et al.* 2012)

A transducer converts the energy alterations during the interaction of biological elements (protein, antibody, and enzyme) and an analyte into a quantifiable signal (Ravichandran *et al.* 2015). Modern miniature microelectronics are featured with lower cost, better processing power, and enhanced analytical efficiency, which broaden their potential applications. Cellular interactions, enzymatic contacts, antibody-antigen interactions, nucleic acid connections, and artificial bioreceptor-based interactions are common biological recognition elements. Mass-sensitive, optical, and electrochemical transducers are frequently utilized for signal quantification (Javadi *et al.* 2018).

8.3. Agricultural applications of uricase

Biological compounds are known to enhance the quality of agricultural commodities. Soil microorganisms release vital secretions for better soil fertility, however, these microbial secretions could be hindered by different environmental factors (Javadi *et al.* 2018; Imran *et al.* 2021). Therefore, producing fungal and bacterial enzymes has been investigated to improve soil conditions and agricultural production at a reduced cost. The free uricase enzyme is utilized as calcium carbonate precipitate in the soil to promote soil mechanics by initiating urea breakdown (Hamdan *et al.* 2013).

8.4. Uricase-based detection of heavy metal water contamination

Heavy metals-containing toxic compounds are known to reduce enzyme activity, which is often used as a parameter to detect heavy metal contamination in water. The activity of uricase also decreases at varying levels in the presence of $Hg^{2+} > Ag'^{+} > Cu^{2+} > Ni^{2+} > Cd^{2+} > Zn^{2+} > Co^{2+} > Fe^{2+} > Pb^{2+} > Mn^{2+}$. Therefore, it is a toxic compound detector in water samples (Zhylyak *et al.* 1995).

8.5. Uricase application in nanomaterial manufacturing

The importance of nanomaterials has significantly increased with diverse medical, agricultural, and industrial applications. Therefore, producing environment-friendly, low-cost, and stable industrial nanomaterials is being widely investigated worldwide. In this regard, enzyme applications in nanomaterial synthesis have become quite popular during the last decade (Durán *et al.* 2014; Adelere and Lateef 2016). *Canavalia ensiformis*-isolated uricase has produced Pt, Au, and Ag nanoparticles as a stabilizing and reducing agent. Similarly, the catalytic urease has been employed to synthesize core-shell ZnO nanomaterials at an ambient temperature. Exposed enzyme residue (Cys592) facilitates the synthesis of metal alloys and metallic nanoparticles (Sharma *et al.* 2013). During the process, the Zn^{2+} binds with negatively charged surface urease at pH 9 through a weak bond reaction to form intermediate zinc hydroxide. Further, zinc hydroxide dehydration under basic conditions yields ZnO on the precipitating enzyme's surface through the 'salting out' effect (Makarov *et al.* 2002).

Conclusion and future perspectives

This review elaborates on uricase-producing microorganisms, bacterial uric acid degradation pathways, degrading enzymes, and uricase-encoding genes. Moreover, the uricase activity affecting factors, microbial uricase production, and uricase purification and applications are also discussed. Cell disruption is mandatory for intercellular uricase production, elevating production costs. Therefore, extracellular uricase-producing microbial strains should be investigated, and production factors should be optimized. Future techniques for obtaining extracellular enzymes should feature reduced time and effort and a simple purification methodology. Furthermore, uricase gene-carrying recombinant probiotic microorganisms could become an effective tool for gout treatment.

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Conflict of interest

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Data availability

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EFFECT OF REPRODUCTIVE SYSTEM DYSBIOSIS ON THE COURSE OF PREGNANCY

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Abstract: Genetic, endocrine and immunological disorders, anatomical defects in the reproductive tract, certain chronic diseases, toxic substances, or advanced age of the mother are most cited among the main causes of pregnancy loss at various stages. However, the cause of miscarriage or preterm labor in some cases remains unclear. Determination of the etiology about these clinical conditions may reduce the rate of reproductive failure. Similarly, the etiology of other obstetric disorders, such as preeclampsia or postpartum hemorrhage, has not been fully explained. One of the postulated and still under-researched causes of these disorders may be dysbiosis within the reproductive tract. A disturbed microbial balance is not always associated with the presence of an acute infection with clear clinical symptoms. Dysbiosis in conjunction with other pathophysiological factors may increase the risk of the mentioned clinical conditions. The aim of this paper is to show the information indicating the existence of a correlation between dysbiosis and an increase in the risk of obstetric disorders. Further analysis is also required to clarify the mechanism of the effect of dysbiosis on the disruption of metabolic and biochemical processes within the reproductive system during pregnancy.

Keywords: female reproductive tract microbiota, genital infections, *Lactobacillus*, pregnancy

1. Introduction

Female reproductive system microbiota is a factor affecting a woman's reproductive health. Vaginal dysbiosis may increase the risk of developing pathological conditions within the reproductive tract, for example, bacterial vaginosis, and can be a factor that hinders pregnancy (Taddei *et al.* 2018). During pregnancy significant changes occur in the body's functioning, including at the hormonal and immunological levels, leading to changes in the microbial balance of the reproductive system. At the beginning of pregnancy, a decrease in microbial diversity, including *Lactobacillus* sp. species (lactobacilli), and a decrease in pH (Xu *et al.* 2020, Escobar *et al.* 2020). During the last trimester, the vaginal microbiota normalizes again and with its composition begins to resemble the microbiota of a non-pregnant woman. Findings indicate that vaginal dysbiosis is correlated with the occurrence of such pregnancy complications as preterm labor and preeclampsia. Moreover, the use of modern molecular techniques, including PCR and DNA sequencing methods, has revealed the presence of bacteria in the uterus, placenta, and umbilical cord blood. Further studies should answer the question of whether the occurrence of certain pregnancy complications can be related to dysbiosis within these tissues (Fig. 1).

2. Microbiota during pregnancy

2.1. Vaginal microbiota

The vaginal microbiota is dominated by bacteria of the genus Lactobacillus and low bacterial diversity. These bacteria produce lactic acid which makes it unfavorable for the growth of pathogens, and the production

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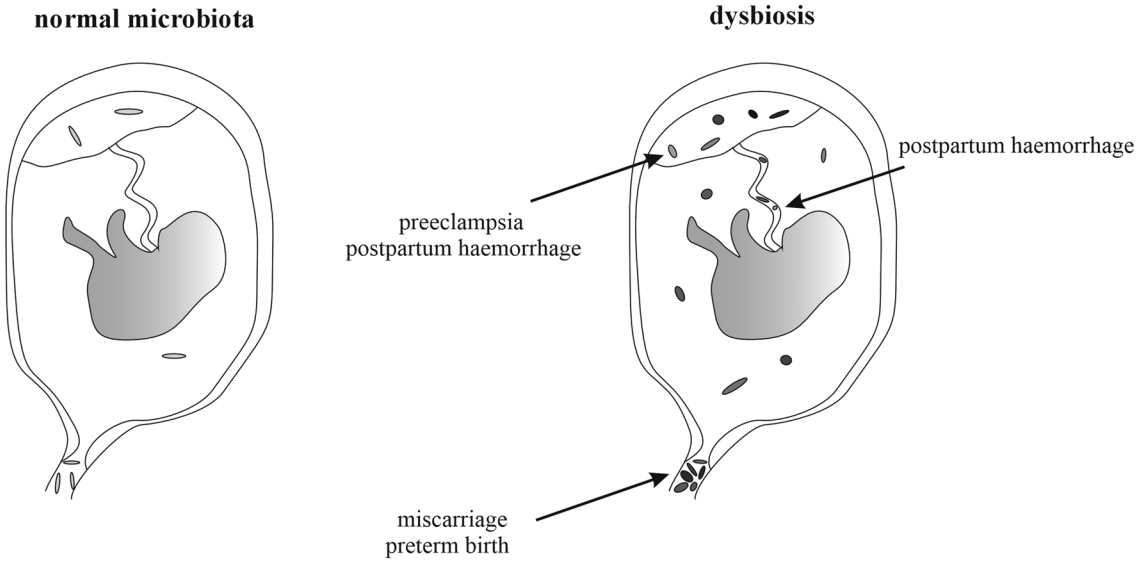

Fig. 1. Normal microbiota and dysbiosis in the vagina, uterus and placenta and possible obstetric complications.

of hydrogen peroxide and bacteriocins further enhances this effect (Greenbaum *et al*. 2018; Taddei *et al*. 2018). In addition, the vaginal microbiota is distinguished by variability and instability. As a result, it is difficult to unequivocally define the composition of normal vaginal microbiota for all women, especially given that even for a single woman the composition can change over time (Greenbaum *et al*. 2018). The composition of vaginal microbiota fluctuates under the influence of many factors (Kervinen *et al*. 2019).

During pregnancy, some of these factors change significantly for e.g. hormone value, resulting in changes in microbiota composition during this period of a woman's life. Particularly noteworthy are hormonal changes. For this reason, in the vaginal microenvironment during pregnancy, bacteria of the genus *Lactobacillus* gain an even greater advantage than before pregnancy (Taddei *et al*. 2018; Heil *et al*. 2019; Serrano *et al.* 2019). Many factors contribute to the stabilization of vaginal microbiota during pregnancy: the absence of cyclic hormonal fluctuations (Walther-António *et al.* 2014).

The multiple changes that occur in a woman's body during pregnancy affect the state of microbiota of the reproductive system. The vaginal microbiota during pregnancy is characterized by less variability and diversity than the vaginal microbiota during the non-pregnancy period. This leads to an increased degree of stability in the pregnancy microbiota, which can reduce the risk of infections and complications during pregnancy. The diversity of pregnancy microbiota is highest during the first trimester of pregnancy and decreases during the second and third trimesters of pregnancy, as estrogen levels increase in a woman's body. However, it is likely that by the final stage of pregnancy, the degree of diversity of the vaginal microbiota begins to increase again. The degree of protection against the growth of pathogenic bacteria depends on the species and strain of *Lactobacillus*. *Lactobacillus crispatus* is more characteristic of a normal vaginal microbial state than *Lactobacillus iners*, which is more prone to transition of the vaginal microbiota to an abnormal state, increasing the risk of developing bacterial vaginosis. It appears that several species of *Lactobacillus* may predominate during pregnancy (Nuriel-Ohayon *et al*. 2016; Greenbaum *et al*. 2018; Kervinen *et al*. 2019; Mei *et al*. 2019; Bagga and Arora 2020; Gupta *et al*. 2020; Pacha-Herrera *et al*. 2020; Rasmussen *et al*. 2020). During pregnancy, α-diversity and β-diversity are also reported to decrease (Nuriel-Ohayon *et al*. 2016; Schoenmakers *et al*. 2019). Interestingly, at the end of pregnancy, the gestational microbiota becomes similar again to the pre-pregnancy microbiota (Kervinen *et al*. 2019). Also, other studies confirm that there is an increase in the diversity of vaginal microbiota at the end of pregnancy (Rasmussen *et al*. 2020).

The role of *L. crispatus* and *L. iners* in pregnancy has been confirmed in many studies (Mei *et al*. 2019; Mls *et al*. 2019; Zheng *et al*. 2019). For example, the study conducted by Zheng et al. showed that the amount of *L. iners* was decreased in the second and third trimesters of pregnancy. In addition, markers of vaginal inflammation, such as the degree of vaginal purity and leukocyte esterase activity, increased as the number of *L. iners* increased (Zheng *et al*. 2019). In contrast, Serrano et al. conducted a study on women of African, Hispanic, and European descent who were and were not pregnant. During pregnancy vaginal microbiota shifted toward lactobacilli. During pregnancy, a decrease in the diversity of bacterial microbiota and a strengthening of the predominance of *Lactobacillus* in the vaginal microenvironment was observed. The decrease in the vagina

Table I
Composition of the microbiota of the reproductive organs in physiological pregnancy and pregnancy with complications.

Course of pregnancy	Organ	Microbiota
Physiological pregnancy	vagina	high abundance and low diversity of microbiota: mainly *Lactobacillus*
Physiological pregnancy	uterus	low numbers and high biodiversity of microbiota: *Lactobacillus, Cutibacterium, Escherichia, Staphylococcus, Acinetobacter, Streptococcus, Corynebacterium*
Physiological pregnancy	placenta	small numbers and high biodiversity of microbiota: *Lactobacillus, Escherichia coli, Cutibacterium acnes, Bacteroides* sp. *Neisseria lactamica, Fusobacterium* sp., *Rhodococcus erythropolis, Prevotella tannerae, Neisseria polysaccharea, Streptomyces avermitilis, Enterobacteriaceae* sp., *Cutibacterium acnes*
Physiological pregnancy	cord blood	small amount and high biodiversity of microbiota: *Enterococcus faecium, Propionibacterium acnes, Staphylococcus epidermidis i Streptococcus sanguinis*
Miscarriage	vagina	decrease in the number of *Lactobacillus*
Preterm birth	vagina	decrease in the number of *Lactobacillus* bacteria, increase in the number of bacteria: *Bacteroides (Firmicutes), Prevotella (Bacteroidetes), Klebsiella* (*Proteobacteria*) and *Mobiluncus (Actinobacteria)*
Preeclampsia	placenta	decrease in the number of *Lactobacillus*, increase in the number of *Bacteroides*
Postpartum hemorrhage	uterus and placenta	decrease in the number of *Lactobacillus*, increase in the number of *Bacteroides*

in the number of such bacteria as *Gardnerella vaginalis*, *Atopobium vaginae*, *Sneathia amnii*, and others results in a decrease in susceptibility to infection with diseases, sexually transmitted and the risk of developing bacterial vaginitis (Serrano *et al*. 2019).

Experiments by Walter-Antonio et al. showed that the dominant vaginal species is *L. crispatus*. The dominant profile in *L. crispatus* is associated with lower diversity than in *L. iners*, suggesting greater dominance of *L. crispatus* than *L. iners*. *L. iners* may suggest greater susceptibility to dysbiosis than *L. crispatus* (Walther-António *et al.* 2014). The relationship between the composition of vaginal microbiota and ethnicity has also been shown in studies by other authors (Aagaard *et al*. 2012; Freitas *et al*. 2017; Nuriel-Ohayon *et al*. 2016).

2.2 Uterine microbiota

Recent studies indicate that the uterus has its own specific microbiota. It is not easy to detect the physiological microbiota inhabiting the endometrium (uterine mucosa). Chen et al. reported that uterine microbiota is dominated by Lactobacillus (30.6%) and other microorganisms such as *Pseudomonas*, *Acinetobacter*, and *Vagococcus* (Chen *et al*. 2017). Koedooder et al. in suggested that uterine microbiota is dominant by families *Lactobacillaceae*, *Streptococaceae,* and *Bifidobacteriaceae* families (Koedooder *et al*. 2019).

The microbiological composition of the endometrium during pregnancy is of particular interest. However, the method of obtaining such material for study is problematic. As a result of such an experiment many genus were identified in the endometrium of normal pregnancy: *Cutibacterium*, *Escherichia*, *Staphylococcus*, *Acinetobacter*, *Streptococcus*, *Corynebacterium*. Bacteria of the genus *Lactobacillus* showed high variability in presence in the samples tested. The authors suggest that although a uterine microbiota with a high density of *Lactobacillus* is conducive to achieving pregnancy, the presence of these bacteria during pregnancy does not appear to be a prerequisite for a normal pregnancy (Leoni *et al*. 2019). Moreno et al. prepared a case report about women who had spontaneous miscarriages and the next physiological pregnancy. They collected data about uterine microbiota in both cases. In pregnancy that ended miscarriage, the uterine fluid included lactobacilli and appeared to have greater diversity than in physiological pregnancy (Moreno *et al*. 2020).

2.3. Placental and umbilical cord blood microbiota

The use of modern molecular techniques to identify bacteria showed the presence of bacteria in the placenta. 16S rRNA sequencing allowed the determination of the placental microbial composition (Olaniy *et al*. 2020). Using the sequencing method, the placental microbiome was characterized in more than 300 healthy, carried pregnancies and in pregnancies that terminated prematurely (Aagaard *et al*. 2012). The microbiome of isolated placental tissue included such microorganisms as *Escherichia coli*, *Cutibacterium acnes*, *Bacteroides* sp., *L. crispatus*, and *L. iners* (Aagaard *et al*. 2014). Gomez de Agüero *et al.* identified in placenta *C. acnes*, Enterobacteriaceae sp., and *Lactobacillus* (Gomez de Agüero *et al*. 2016). The movement of bacteria from the intestinal epithelium and oral mucosa through the maternal circulation allows a small number of bacteria to populate the placenta (Olanyiy *et al*. 2020). The microbiota of amniotic fluid is like that of the placenta (Schoenmakers *et al.* 2019).

As in the case of the placenta, the microbial composition of cord blood has not yet been clearly determined. Jimenez et al. identified umbilical cord blood bacteria. They detected bacteria such as *Streptococcus sanguinis*, *Enterococcus faecium*, and *Staphylococcus epidermidis*. These species occur naturally in healthy infants and are considered commensals (Jiménez *et al*. 2005). Tang et al. showed that cord blood microbiota was identified in 15 samples in women with gestational diabetes mellitus. Cord blood microbiota was dominated by *Firmicutes*, *Actinobacteria*, *Ruminococcaceae*, and *Rhodococcus* (Tang *et al*. 2020) (Fig. 1).

3. Microbiota of the female reproductive system during complicated pregnancy

3.1. Miscarriage and vaginal microbiota

Miscarriage is the most common obstetric complication and 50% of all miscarriages can be caused by chromosomal aberrations, and the causes of the remaining cases remain unclear (Larsen *et al*. 2013). Physiological pregnancy is dominated by Lactobacillus species and low bacterial biodiversity. The beginning of pregnancy is sometimes correlated with a decreased number of lactobacilli in the vagina, and this decrease may precede miscarriage (Al-Memar *et al*. 2020).

Miscarriage not caused by chromosomal aberrations can be associated with a decrease in the number of *Lactobacillus* bacteria compared to aberrant and normal pregnancies. Miscarriage may be due to the mother's inflammatory response to vaginal dysbiosis, which is often caused by a decrease in *Lactobacillus* (Grewal *et al*. 2020). Xu et al. showed that 56% of women who experienced an embryonic miscarriage showed a small amount of lactobacilli in a vaginal swab, while 88% of women in the control group showed a large amount of these bacteria. The group of women who experienced an embryonic miscarriage showed a higher level of diversity in the vaginal microbiota (Xu *et al.* 2020).

Grewal et al. reported that a reduction in the number of lactic acid bacilli in the vagina is associated with an increase in local expression of pro-inflammatory cytokines (Grewal *et al*. 2020). An increase in IL-2 levels and a decrease in IL-10 levels have been shown in a group of women who have experienced an embryonic miscarriage (Marzi *et al.* 1996; Wilczyński *et al*. 2005; Xu *et al*. 2020). In addition, a correlation has been shown between infection, the inherence of *G. vaginalis*, and increased levels of peripheral NK cells (pNK) (Kuon *et al.* 2017). The findings may indicate a relationship between vaginal microbiota, local inflammation, changes in immune parameters, and the risk of miscarriage (Kuon *et al*. 2017; Villa *et al*. 2020).

Disturbed vaginal microbiome is a risk factor for miscarriage. This factor can be modified by preventive measures (administration of prebiotics and probiotics) or therapeutic measures (antibiotic therapy) (Al-Memar *et al*. 2020).

3.2. Preterm labor and vaginal microbiota

Preterm labor (PTB) is a major obstetric problem and causes significant neonatal mortality (Parry *et al*. 1998; Bayar *et al*. 2020). Amniotic membrane rupture can lead to microorganisms from the vagina toward the uterus and fetus, and pathogenic bacteria can initiate the development of infection and inflammation. Infection can be both a cause and a consequence of the rupture of the fetal membranes (Bayar *et al.* 2020).

Before PTB occurs leukocyte activation, increases levels of pro-inflammatory cytokines and chemokines. PTB is often associated with a vaginal infection. It is suggested that matrix metalloproteinase 8 (MMP-8) changes cervical integrity and facilitates bacterial movement (Linhares *et al.* 2019). The loss of Lactobacillus species from the vaginal microbiota and the overgrowth of other bacteria contribute to the development of bacterial vaginitis (BV) and aerobic vaginitis (AV) (Donders *et al*. 2011). Elevated levels of D-lactic acid and the ratio of D-lactic acid to L-lactic acid influence the increase in extracellular matrix metalloproteinase inducer (EMMPRIN) and MMP-8 concentrations (Witkin *et al*. 2013). *L. crispatus* can inhibit EMMPRIN activation, thus preventing infection and preterm labor. *L. crispatus* has immunomodulatory functions (Witkin *et al*. 2013).

It was proven that 40% of samples taken from women who gave birth prematurely did not contain any *Lactobacillus* species, while all women with birth at term consisted of at least one or more Lactobacillus species. The most common species isolated in women who gave birth at term was *L. crispatus* (46%), followed by *L. jensenii* (25%) and *L. gasseri* (19%). Moreover, data suggest that the presence of *L. iners* was one of the causes of PTB (Aslam *et al*. 2020). Feehily et al. identified *S. amnii* and *Prevotella amnii* species as risk factors for preterm labor (Feehily *et al*. 2020). The results of other studies show that normal vaginal microbiota is associated with a 75% lower risk of PTB. The authors conclude that the absence of lactobacilli combined with a higher level of anaerobic bacteria is a stronger predictor of PTB (Kosti *et al*. 2020). These studies show that *Lactobacillus* protects against PTB (Abdelmaksoud *et al.* 2016; Stout *et al*. 2017; Di Simone *et al.* 2020).

Dysbiosis of the vaginal microbiota has also been associated with the production of other metabolites by microorganisms, which can cause PTB. Under-representation of *Lactobacillus* and consequently low lactate levels can also promote abnormal pregnancy (Fettweis

et al. 2014; 2019). For example, infection of cervical and vaginal epithelial cells by Ureaplasma urealyticum stimulates ammonia production and induces increased IL-8 production, which can lead to much higher cytotoxicity. On the contrary, *L. crispatus* appears to protect against inflammation and HeLa cell death by producing more D-lactate and less IL-8 (Cavanagh *et al.* 2020). As mentioned, the transition of microbiota composition from *Lactobacillus* spp. to *Prevotella* causes vaginal dysbiosis and production of pathogenic microbiota metabolites. For example, high levels of acetate and low levels of succinate, immunomodulatory relationships, have been associated with the occurrence of PTB. This is possible through higher pH and an increase in pro-inflammatory cytokines (Li *et al.* 2010; Mirmonsef *et al.* 2012; Amabebe *et al.* 2016; Stafford *et al.* 2017, Ansari *et al.* 2020).

Levels of vaginal inflammatory C-X-C Motif Chemokine Ligand 10 (CXCL10) and PTB were associated with the ratio of *L. crispatus*/*L. iners*, indicating possible predictive markers of PTB: cytokine levels/ *Lactobacillus* number. Many women give birth at term despite reduced bacterial counts of *Lactobacillus* species. The immune factor can modulate PTB risk regardless of *Lactobacillus* species (Fettweis *et al.* 2014; Elovitz *et al.* 2019; Di Simone *et al.* 2020).

3.3. Preeclampsia and placental microbiota

Preeclampsia (PE) is characterized by hypertension and proteinuria and is life-threatening for the pregnant woman and her baby (Goel *et al.* 2015; Rana *et al.* 2019; Olaniyi *et al.* 2020]. There are many hypotheses regarding the causes of the onset of preeclampsia, including abnormalities in the development of the placenta, disturbances in the immune mechanisms between the fetus and mother, or abnormalities in the factors responsible for vasoconstriction. These changes lead to the hypertension and multiple organ failure seen in preeclampsia syndrome (Goel *et al.* 2015; Rana *et al.* 2019; Olaniyi *et al.* 2020). The presence of bacteria in the placenta can also affect the activity of anti-angiogenic factors and pro-angiogenic factors (Olaniyi *et al.* 2020).

The placental microbiome during physiological pregnancy is dominated by *Lactobacillus* and Gram-positive and Gram-negative bacteria (Gomez *et al.* 2016). Dysbiosis of the placental microbiota, including an increase in the number of *Bacteroides* and a decrease in the number of *Lactobacillus*, alters the host immune response which can initiate the onset of various pregnancy complications, including preeclampsia and preterm labor (Gomez *et al.* 2016; Bardos *et al.* 2019; Olaniyi *et al.* 2020).

The placental microbiome has a regulatory effect on the metabolic and immune functions of the host (Olaniyi *et al.* 2020). Dysbiosis can change placental endothelial function and placental hypoxia and ischemia (Amarasekara *et al.* 2015). In addition, lipopolysaccharide can play an important role in the development of preeclampsia. Bacterial dysbiosis of the placenta can also disrupt tryptophan and fatty acid metabolism, resulting in impaired maternal and fetal energy homeostasis, which can exacerbate the course of preeclampsia (Olaniyi *et al.* 2020).

3.4. Postpartum hemorrhage versus uterine and placental microbiota

Local dysbiosis, causing activation of inflammatory cells and changes in the uterine myometrium, may be associated with the development of postpartum hemorrhage (PPH) (Farhana *et al.* 2015). After childbirth, sometimes occur a phenomenon of inability of the uterine myometrial fibers to contract. In many cases, this phenomenon may be secondary to local dysbiosis. This can promote the activation of the complement system, neutrophils, and macrophages, as well as mast cell degranulation in uterine and placental tissues, resulting in impaired uterine contractility. Indeed, an increase in the number of inflammatory cells, such as neutrophils, macrophages, and mast cells, and activation of the complement system have been observed in uterine and placental tissues in women suffering from PPH of unclear etiology (Escobar *et al.* 2020). These cells produce chemical mediators that affect the vascular myometrium and uterus. The exudate produced by the ongoing inflammatory reaction leads to edema and ultimately impairs uterine contractility (Farhana *et al.* 2015; Escobar *et al.* 2020). In summary, dysbiosis of the reproductive system can cause the development of a local inflammatory response, resulting in impaired uterine contractility and an increased risk of PPH.

4. Conclusion

The results of this study indicate a relationship between dysbiosis of the female reproductive system and obstetric complications such as miscarriage, preterm labor, preeclampsia, and postpartum hemorrhage. Acute infection is an unequivocal risk factor for obstetric disorders, while a state of dysbiosis or infection with a mild or even asymptomatic course can, along with other risk factors, influence the occurrence of pregnancy complications. Careful studies should be conducted to show the contribution of dysbiosis to the occurrence of obstetric complications, including the presence of specific bacterial species most likely to increase this risk. This will allow the introduction of new guidelines for the evaluation of pregnancy prognosis based on the microbiota status of the reproductive system.

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Conflict of interest

The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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HUMAN SKIN MICROBIOTA – ESSENTIALS FOR BEAUTY STUDIO PROFESSIONALS

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Abstract: Skin, the largest organ in the human body, presents the largest possible area for colonization by microorganisms. The aspects of the interaction between microbiota and the host cannot be omitted during procedures in a beauty studio. Professional cosmetologists should consider their work's impact on skin biodiversity and know the probable consequences. This review reviewed the current state of the art on skin microbiota with a focus on the connection between body area, microbiological biodiversity and the outcome of cooperation between the host and microorganisms. The role of the skin microbiota in maintaining the host's homeostasis was also summarized.

Keywords: biodiversity, cosmetology, dermatology, hygiene, saprophytic microorganisms

1. Introduction

In recent years, increasing attention has been paid to the best possible understanding of the human microbiome, which consists of the microbiota of individual organs and systems. The gut and human skin microbiota and their interactions with the host organism are particularly noteworthy. A growing body of scientific data indicates that the composition of commensal microorganisms living in or on the human body determines the maintenance of homeostasis and protects against the development of inflammation and the onset of many diseases, such as acne vulgaris. This article reviews the available information on the human skin microbiota, its function, composition, and variability depending on many factors and functions in a healthy body, as well as its influence on the development of acne vulgaris.

2. Habitat – skin

Human skin provides a convenient niche for microorganisms to inhabit. Therefore, the discussion of the structure and function of the microbiome must be linked to the structure and function of human skin (Fig. 1).

Human skin, the body's largest organ, is the body's outer covering. Its surface area in an adult human is 1.5–2 m^2, and its weight, including subcutaneous tissue, is about 18–20 kg (of which the epidermis alone weighs about 0.5 kg, and the dermis weighs 3 kg) (Lee *et al.*, 2019). Different areas of the skin differ in terms of the thickness of the epidermis, the distribution of appendages in it, and the humidity and temperature on its surface (Sfriso *et al.*, 2020). The total thickness of the facial skin measures 0.3–4 mm, and its surface is covered by a hydro-lipid mantle, a mixture of intrinsic and extrinsic fats, water, and exfoliated keratin (Kolarsick *et al.*, 2011; Oh *et al.*, 2016; Marks and Miller, 2019). The variable characteristics of the skin significantly affect the species composition and quantity of the microbiota (Scharschmidt and Fischbach, 2013; Gallo, 2017). It is important to remember that bacteria, viruses, fungi, and mites inhabit it. Most microorganisms inhabiting the skin are harmless, and function in symbiosis with skin cells, and interactions between microorganisms and skin cells include such phenomena as mutualism, parasitism, or commensalism (Belkaid and Segre, 2014). The skin performs many important functions, such as a protective barrier against environmental

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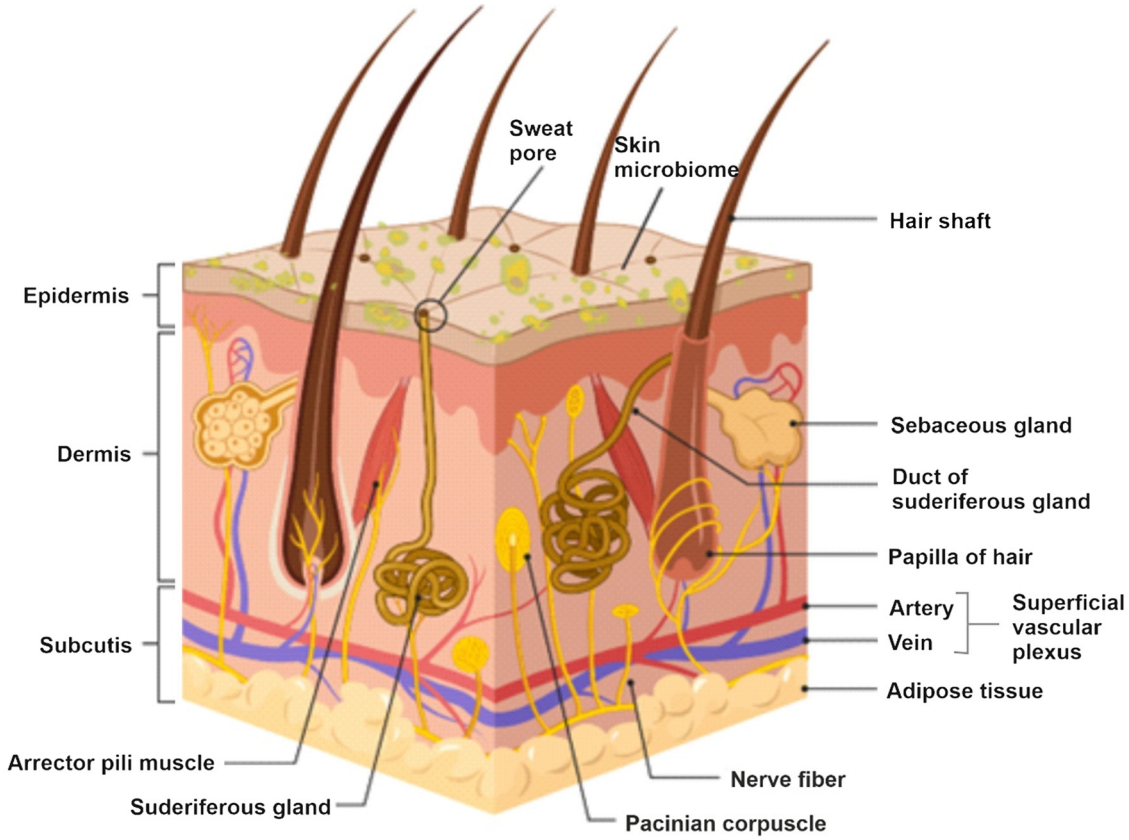

Fig. 1. General plan of human skin structure; created with Biorender.com

factors. Moreover, its proper functioning enables the body to maintain water-electrolyte balance and a constant body temperature, enabling the proper functioning of internal organs (Ladizinski *et al.*, 2014). The skin contains numerous receptors and nerve endings that enable communication with the outside world, the reception of stimuli, and the functioning of the sensory organ (Kanitakis, 2002; Wolski and Kędzia, 2019). The skin also contains Langerhans cells, or immune system cells, so one of its functions is to receive and transmit immune signals and protect the body from pathogens (Chu, 2008). In the macroscopic structure of the skin, we can observe three layers: the epidermis, dermis, and subcutaneous tissue(Kanitakis, 2002; Orłowski *et al.*, 2008). The epidermis is the outermost part of the skin, is 0.05–1.5 mm thick (the variation in thickness depends on the anatomical area), and comprises several layers of epithelial cells (Kanitakis, 2002; Wolski and Kędzia, 2019). The basal layer of the epidermis is considered the deepest layer, which includes dividing keratinocytes, melanocytes (pigment cells), and Langerhans cells (immune cells). The basal layer of the epidermis is also often called the reproductive layer. The next layer is the squamous layer, which consists of several rows of polygonal cells that, going gradually toward the surface, become progressively flattened. It is the thickest layer of the epidermis, comprising up to 12 rows of cells. Resistance fibers can also be distinguished in their structure, so the system created in this layer cushions pressure and stretching (Orłowski *et al.*, 2008). Thus, in the next layer, the granular layer, only spindle-shaped cells with an atrophic cell nucleus and numerous granules of keratohyalin rich in calcium are observed (Fairley *et al.*, 1991). In the granular layer, 1–4 rows of cells are usually observed (Orłowski *et al.*, 2008). The light layer is characteristic only of the epidermis of the palms of the hands and soles of the feet. The last, or most superficial layer, is the stratum corneum, which consists of corneocytes and flattened keratinized cells that attack cell nuclei (Sanmiguel and Grice, 2015). Corneocytes undergo gradual exfoliation, and the cycle of permanent epidermal cell renewal, or epidermal transition, lasts 28 days and increases with age to about 30–31 days (Matoltsy, 1976; Voegeli and Rawlings, 2023) The entire epidermal cycle is considered to be the proliferation and differentiation of cells in the basal layer, their further transformation and movement toward the more superficial layers, the gradual extinction of the cell nucleus and eventually reaching the stratum corneum and replacing older cells (Chu, 2008; Prescott *et al.*, 2017; Marks and Miller, 2019). The above-described arrangement of epidermal cells makes the epidermis a hostile environment for microbial growth. The surface of the epidermis is mostly dry, rough, and constantly flaking

off (Chu, 2008; Voegeli and Rawlings, 2023). Removing cells in the stratum corneum allows regular removal of microorganisms from the skin surface, preventing their unrestricted growth and biofilm formation on the skin surface (Kolarsick *et al.*, 2011). The multilayer nature of the epidermal cells, their various cell arrangement and shapes, as well as their interconnectedness (which disappears only in the superficial horny layers), as well as the presence of the hydro-lipid mantle, prevents the loss of water and skin essential products. It prevents the entry of harmful compounds and microorganisms from the environment. Moreover, the presence of the hydro-lipid mantle causes acidification of the environment to a pH value of 4 to 6.5 (Caputo and Peluchetti, 1977; Schmid-Wendtner and Korting, 2006). It also contains antibacterial compounds such as sebum, lysozyme, and dermicidin. Keratinocytes, sebocytes, sweat gland cells, and mast cells have the properties of secreting antimicrobial agents. According to modern studies, about 20 peptides with antimicrobial activity on the skin surface are called AMPs (antimicrobial peptides) (Jungersted *et al.*, 2008). AMPs include cathelicidins, defensins (HBD1, HBD2, HBD3), psoriasins, antimicrobial RNase 7 protein, and SLPI protein (Cogen *et al.*, 2010; Adamczyk *et al.*, 2018). The above allows the skin to be colonized only by specific microorganisms and strictly controls their abundance (Scharschmidt and Fischbach, 2013; Belkaid and Segre, 2014; Flowers and Grice, 2020). Notably, the epidermis is non-vascularized and draws nutrients only from the superficial vascular plexus of the dermis. The dermis, conversely, comprises connective tissue, containing cells called fibroblasts, collagen and elastic fibers, blood vessels, and skin appendages such as hair, sweat, sebaceous glands, sensory receptors, and nerve endings (Marks and Miller, 2019). A distinct boundary known as the dermal-epidermal boundary can be observed at the junction of the basal layer of the epidermis with the dermis (Kanitakis, 2002). Within the dermis, two layers can be distinguished: the papillary layer (more superficial) and the reticular layer (deeper layer). The papillary layer's papillae push into the structure of the epidermis while preventing the epidermis layers from moving against each other. Conversely, the reticular layer has numerous collagen and elastin fibers strands, with tiny fibers and nerve endings, connective tissue cells, hair, glands, and smooth muscle cells forming the adnexa muscles. Numerous blood and lymphatic vessels can also be observed in its structure (Sanmiguel and Grice, 2015). The deepest layer is the subcutaneous tissue, mainly composed of loose connective tissue and adipose tissue; its overriding function is to connect the dermis to deeper structures. This layer has glue-like and elastic fiber chambers filled with adipose tissue, making it possible to cushion damage and absorb significant water. The subcutaneous tissue also has blood vessels, lymphatic vessels, nerve fibers, and glands. Located within the dermis and subcutaneous tissue, the venous, arterial, and lymphatic vessels form the vascular system of the skin. Its characteristic features are the delicacy and small caliber of the vessels, as the larger ones are located directly in the muscles. Due to their small size, the network of dermal vessels is very dense and strongly developed, reaching under the papillary layer of the dermis, thus enabling proper nourishment of the epidermal cells (Wolski and Kędzia, 2019). Hair follicles and sweat glands have separate vascular plexuses conditioning their proper functioning (Kolarsick *et al.*, 2011).

3. Physicochemical and physiological basis of skin colonization by microorganisms

3.1. Skin microbiota

For the skin to function correctly, it must maintain its physiological pH at 4.0–6.5 (Adamczyk *et al.*, 2018). The pH value within these limits protects the body from harmful chemicals, bacteria, fungi, or viruses (Orłowski *et al.*, 2008). The pH value of the skin allows the growth of only those microorganisms that tolerate well a slightly acidic pH (Wolski and Kędzia, 2019). Therefore, it can be deduced that the guard against pathogens is the intact stratum corneum, the drying process of the skin surface (as it has been proven that the number of bacteria is reduced faster on dry skin), and the acid reaction of the lipid mantle, which is conditioned by the proper work and function of the screen sweat glands containing lactic acid and fatty acids (Schmid-Wendtner and Korting, 2006; Jungersted *et al.*, 2008; Kolarsick *et al.*, 2011; Percival *et al.*, 2012; Adamczyk *et al.*, 2018; Wolski and Kędzia, 2019).

Skin microbiota is a set of microorganisms, mainly bacteria, that form a complex ecosystem on the skin's surface in a given habitat (Sanford and Gallo, 2013) (Fig. 2). This microbiota may also include some fungi (Condrò *et al.*, 2022). Viruses and parasites, however, are always considered pathogens (Scharschmidt and Fischbach, 2013; Malinowska *et al.*, 2017; Sinha *et al.*, 2021). The qualitative and quantitative composition of the cutaneous microbiota is variable. It depends on many factors such as temperature, pH, humidity, nutrient availability, oxidoreductive potential, the climatic zone in which a person lives, race, sex, age, hormones, diet, body weight, susceptibility to stress, type of clothing worn, humidity in a particular region of the body, level of hygiene, immune status of the organism, past diseases and their treatments, antibiotic therapy used or work performed (Costello *et al.*, 2009; Belkaid and Segre, 2014; Boxberger *et al.*, 2021). How a person's skin is built, and functions determines its microbiome's

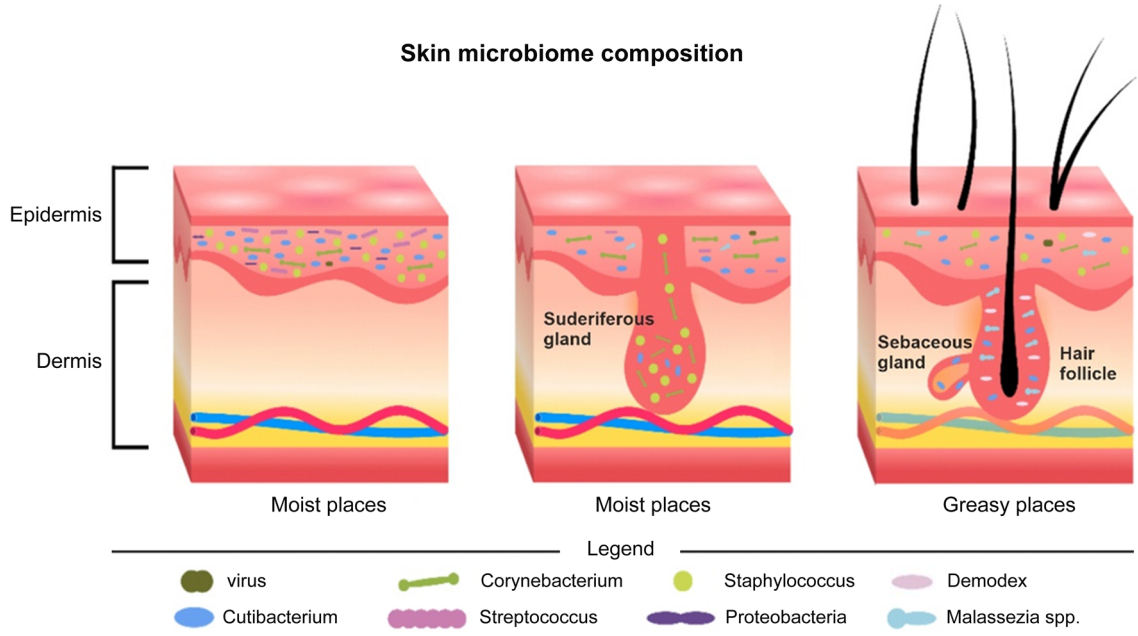

Fig. 2. Composition of the skin microbiota; own graphic inspired by Smythe and Wilkinson, 2023

stability in terms of composition, abundance, and resistance to change (Sanmiguel and Grice, 2015; Malinowska *et al.*, 2017; Lee *et al.*, 2019). On the other hand, skin microbiota has a specific role in maintaining skin homeostasis (Table I). Unfortunately, the complete species composition of the skin microbiome is not yet fully understood. Limited diagnostic capabilities are the most likely reason for incomplete knowledge on this subject (Adamczyk *et al.*, 2018; Sinha *et al.*, 2021).

The number of microorganisms in the skin of a healthy person is 10^4–10^5 cfu/cm^2. There are four main types of bacteria inhabiting human skin: *Actinobacteria* (*Corynebacterium* spp, *Cutibacterium* spp., *Microbacterium* spp., *Micrococcus* spp.), *Firmicutes* (non-hemolytic aerobic and anaerobic staphylococci *(Staphylococcus* spp.), *Clostridium* spp, α-hemolytic *streptococci* (*Streptococcus* spp.) and enterococci (*Enterococcus*), *Bacteroidetes* (*Sphingobacterium* spp., *Chryseobacterium* spp.) and *Proteobacteria* (*Janthinobacterium* spp., *Serratia* spp., *Halomonas* spp., *Delftia* spp., *Comamonas* spp.) (Cogen *et al.*, 2008; Sanford and Gallo, 2013; Scharschmidt and Fischbach, 2013; Schommer and Gallo, 2013; Wang *et al.*, 2014; Dreno *et al.*, 2017; Prescott *et al.*, 2017; Condrò *et al.*, 2022). In a healthy human, the natural skin microbiota can be divided into permanent (renewable) and transient (temporary) (Omer *et al.*, 2017). The permanent microbiota includes Gram-positive bacteria, mainly coagulase-negative staphylococci – *Staphylococcus epidermidis* (estimated to account for 50% of the bacteria residing on the skin and inhabiting the higher areas of the hair follicle mouths) and *Streptococcus* spp. and *Enterococcus* spp. and Gram-positive bacilli *Corynebacterium* spp. (mainly *C. jeikeium*), *Brevibacterium* spp., *Cutibacterium acnes* (Cogen *et al.*, 2008; Dréno *et al.*, 2018; Claudel *et al.*, 2019; Xu and Li, 2019; Brown and Horswill, 2020; Flowers and Grice, 2020).

Table I
Eight proposed roles of skin microbiota.

The role of the skin microbiota
1. Maintaining the acidic pH of the skin (pathogenic microorganisms prefer a more alkaline pH),
2. Prevent settlement and multiplication of pathogenic bacteria by limiting the available food supply (colonization resistance)
3. Keeping the commensal biota intact and eliminating disease-causing microorganisms through the production of antibacterial substances
4. Maintenance of the proper functioning of the epidermal barrier
5. Participation in metabolic processes
6. Impact on the process of tissue maturation in human individual development
7. Maintaining the homeostasis of the immune system by modulating the innate immune response and influencing the development of the acquired response
8. Regulation of pro-inflammatory cytokine expression and activation of the complement system

The skin surface also hosts *S. saprophyticus*, S. *hominis*, *S. warneri*, *S. haemolyticus,* and *S. capitis* (Dreno *et al.*, 2017). Bacteria of the genus *Micrococcus* are also isolated from the skin surface, most notably *M. luteus* and the less abundant *M. varians*, *M. lylae*, *M. sedentarius, M. roseus*, *M. kristinae* and *M. nishinomiyaensis* (Carmona-Cruz *et al.*, 2022). These bacteria belong to symbiotic species and are the most stable part of the skin microbiome (Grice *et al.*, 2008; Sanford and Gallo, 2013; Belkaid and Segre, 2014; Sanmiguel and Grice, 2015; Adamczyk *et al.*, 2018; Lee *et al.*, 2019; Condrò *et al.*, 2022). Although *Cutibacterium acnes* is one of the main commensals of the normal bacterial biota, it also contributes to the pathogenesis of acne vulgaris (Dessinioti and Katsambas, 2017; Dréno *et al.*, 2018). However, contrary to previous thinking, acne vulgaris is not associated with excessive proliferation of *C. acnes*. Present at low levels on the skin surface, *C. acnes* is the predominant bacterial species inhabiting sebaceous follicles (Omer *et al.*, 2017). In contrast, studies conducted by Byrd *et al.* on healthy volunteers also confirm the presence of *Enhydrobacter* spp. and *Veillonella* spp. (Byrd *et al.*, 2018). On the other hand, Myles *et al.* in their study focused on Gram-negative bacterial cultures and identified the following microorganisms: *Roseomonas mucosa*, *Pseudomonas* spp., *Acinetobacter* spp., *Pantoea septica* and *Moraxella asloensis* as commensally resident on human skin (Myles *et al.*, 2018). Other studies have confirmed that Gram-negative bacteria, including *Enterobacteriaceae*, non-fermenting Gram-negative bacteria, and anaerobes, are marginal. Still, commensal organisms are also part of the transient fraction of the skin microbiota. The permanent skin microbiota also includes *Micrococcus luteus*, *Staphylococcus aureus*, *Candida* spp. *Micrococcus* and *Staphylococcus* bacteria habitually colonize the surfaces of the stratum corneum, while aerobic and anaerobic lipophilic tentacles abundantly colonize the deeper parts of the hair follicles and sebaceous glands (Myles *et al.*, 2018; Boxberger *et al.*, 2021). The secretion of skin glands modifies the composition of the solid microbiota, the way of dressing, or the vicinity of mucous membranes (Adamczyk *et al.*, 2018; Prast-Nielsen *et al.*, 2019; Carmona-Cruz *et al.*, 2022). *Corynebacterium, Micrococcus, Cutibacterium* (formerly *Propionibacterium*) microorganisms are classified as *Actinobacteria*, which are Gram-positive microorganisms that produce numerous antibiotics (Scharschmidt and Fischbach, 2013; Condrò *et al.*, 2022). *Actinobacteria* account for 51.8% of all isolated microorganisms from human skin (Schommer and Gallo, 2013; Lee *et al.*, 2019). They are observed on the skin of the face, including the ears and nose, on the neck, back, lower abdomen, and feet. Fungi of the genus *Malassezia* quantitatively account for about 80% of all fungi described on human skin, but their number depends on the anatomical location of the human body (Prohic *et al.*, 2016; Adamczyk *et al.*, 2018; Claudel *et al.*, 2019; Xu and Li, 2019; Carmona-Cruz *et al.*, 2022). Fungi also include the *Candida albicans,* but also *Rhodotorula rubra, Trichosporon cutaneum, Aspergillus* spp., *Penicillium* spp., *Rhizopus* spp., *Microsporum gypseum*. These organisms are considered symbiotically mutualistic or commensals, i.e., organisms that do not harm the human organism and even benefit it (Adamczyk *et al.*, 2018). Their very presence limits the growth of other (often harmful) organisms by competing with them (Grice *et al.*, 2008; Sanmiguel and Grice, 2015). The transient microbiota is periodically variable and associated with continuous exposure and direct contact of the skin with the external environment (it can come from other people, animals, or the environment).

3.2. Interactions of microbiota with the host

The natural biota of the skin should not be destroyed, and the abundance of bacteria and fungi must remain in balance (Adamczyk *et al.*, 2018). Therefore, the overriding role of the skin microbiota will be to maintain skin homeostasis in a healthy person, fighting potential pathogens and adverse external environmental factors. It is one of the mechanisms that ensure the proper barrier function of the skin (Claudel *et al.*, 2019; Carmona-Cruz *et al.*, 2022). Moreover, skin bacteria secrete protease enzymes involved in the exfoliation and renewal of the stratum corneum. The sebum and free fatty acids produced are involved in regulating skin pH. Bacteria also produce lipase enzymes that break down superficial lipid layers (Flowers and Grice, 2020). Ureases, in turn, are responsible for the proper breakdown of urea as a secondary metabolite (Jungersted *et al.*, 2008). The cutaneous microbiota protects its host from potentially pathogenic agents by competing with them and producing antimicrobial peptides (AMPs) (Cogen *et al.*, 2010; Sanford and Gallo, 2013; Prast-Nielsen *et al.*, 2019; Flowers and Grice, 2020; Bonar *et al.*, 2021). Some bacteria produce bacteriocins that kill pathogens or produce substances that are bacteriostats, which hinder the division and multiplication of pathogens (without being harmful to the organisms producing them) (Adamczyk *et al.*, 2018). For example, the bacterium *Staphylococcus epidermidis* produces an antimicrobial peptide that destroys *Staphylococcus aureus* bacteria (Brescó *et al.*, 2017; Brown and Horswill, 2020). Another commensal bacterium, *Cutibacterium acnes,* can inhibit the growth of methicillin-resistant *Staphylococcus aureus* (MRSA) by fermenting glycerol into a series of short-chain fatty acids, which lowers the intracellular pH and inhibits the growth of *Staphylococcus aureus* (Platsidaki and Dessinioti, 2018; Spittaels *et al.*, 2020; Bonar *et al.*, 2021). Studies have shown that *S. epidermidis*

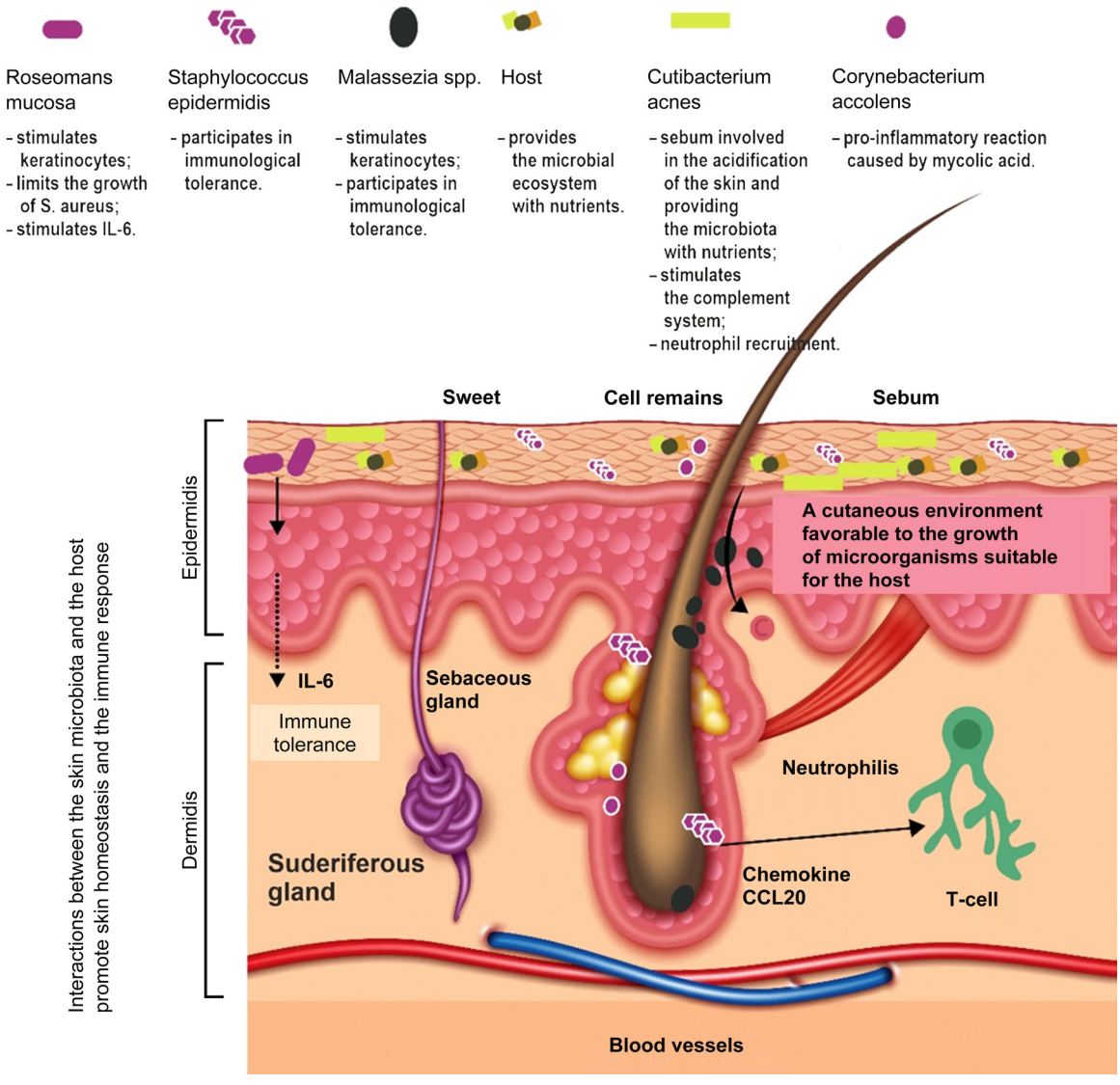

Fig. 3. Host-microbiota interactions; own graphic inspired by Liu *et al.*, 2023

is detected by keratinocytes through Toll-like receptor 2 (TLR2), thereby increasing host resistance to *S. aureus* infection through increased expression of antimicrobial peptides (defensins) (Strunk *et al.*, 2010; Scharschmidt and Fischbach, 2013; Sanmiguel and Grice, 2015; Brescó *et al.*, 2017; Dreno *et al.*, 2017). Studies by Wanke *et al.* confirm that bacteria are also involved in mast cell-mediated antiviral protection (Wanke *et al.*, 2011). TLR2 activation increases the number of mast cells activated for antiviral protection in the skin (Hooper *et al.*, 2012; Schommer and Gallo, 2013; Brown and Horswill, 2020). Fungi of the genus *Malassezia*, on the other hand, produce several indoles that inhibit the growth of unwanted yeasts and molds (Prohic *et al.*, 2016).

Relationships between constantly occurring microorganisms, those transient and pathogenic, are highly complicated and still widely studied. It turns out that it is not only the adequately composed microbiota of the skin that guards safety but also the reciprocal numerical ratio of the different types of microorganisms to each other that matters (Lee *et al.*, 2019). It is also worth remembering that microorganisms protect against infections and are responsible for a given person's smell (Flowers and Grice, 2020).

It is worth mentioning that skin commensal bacteria have a close relationship with the immune cells of their host, and T lymphocytes are taught from the beginning to respond to signals produced by skin commensals. For example, *Staphylococcus epidermidis* produces a particular type of acid that can bind to specific receptors, activating the immune system and an influx of T lymphocytes into the skin despite the absence of inflammation. T lymphocytes, in turn, promote the proliferation of keratinocytes and accelerate wound healing (Scharschmidt and Fischbach, 2013; Belkaid and Segre, 2014; Flowers and Grice, 2020). In their study, Leonel *et al.* collected current knowledge regarding the involve-

ment of commensals in wound healing. Unfortunately, the results of their observations somewhat confuse the reader (Leonel *et al.*, 2019). For example, it was shown that the absence of commensal cutaneous microorganisms positively affects the wound-healing process. On the other hand, the positive effect of *S. epidermidis*, as an agent associated with unconventional repair mechanisms in wound healing through activation of CD8 regulatory T cells, has been demonstrated (Hooper *et al.*, 2012; Leonel *et al.*, 2019; Brown and Horswill, 2020). This finding was confirmed by Lai *et al.* However, the study results are heterogeneous, indicating the need for further research on the influence of the skin microbiota on the wound healing process (Lai *et al.*, 2010). The presence of bacteria, especially *S. epidermidis,* promotes the strengthening of the skin barrier by increasing the number of tight junctions in skin cells. One study also showed that a specific strain of *S. epidermidis* can produce 6-N-hydroxyaminopurine (under the right conditions), which may be responsible for protection against skin cancer (Nakatsuji *et al.*, 2018; Severn and Horswill, 2023). The skin, a physical barrier, allows the exchange of signals between the body and the external environment. It is also an immune barrier. Keratinocytes, or epidermal cells, constantly analyze what bacteria are on their surface. It is made possible by PRR – *pattern recognition receptors* – which detect the presence of molecules produced by bacteria, immediately alerting the body to pathogens on its surface. Activation of the receptors stimulates an immune response, which triggers the production of molecules that kill bacteria, viruses, and fungi (Severn and Horswill, 2023). Hence, the conclusion is that there is a constant flow of information between the skin microbiota, keratinocytes, and the immune system, enabling an immediate response to the presence of an unfriendly microbiota (Scharschmidt and Fischbach, 2013; Belkaid and Segre, 2014; Liu *et al.*, 2023). Unfortunately, to date, the mechanisms of this communication have not been fully understood. However, it has been shown that resident bacteria interact with skin signaling molecules. Substance P, the primary skin neuropeptide modulated by pain, stress, or infection, is involved in the pathogenesis of many multifactorial skin diseases. Some of the effects of substance P are mediated through interactions with the skin microbiota. In particular, substance P can increase the virulence of staphylococci – it induces the secretion of enterotoxin C2 by *Staphylococcus aureus* and biofilm formation by *S. epidermidis,* which increases the adhesion of both bacteria to keratinocytes (Castillo *et al.*, 2019). *C. acnes* variously modulates melanocyte survival while playing a role in the post-inflammatory pigmentation of acne lesions (Wang *et al.*, 2014; Platsidaki and Dessinioti, 2018). Furthermore, the most recent and initial studies indicated that the interplay

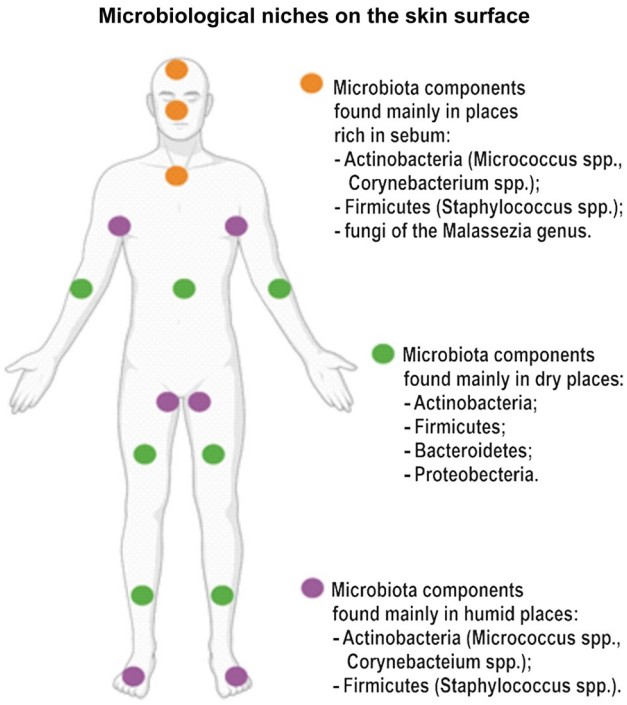

Fig. 4. Microbial niches on the surface of the skin; created with Biorender.com, inspired by Byrd *et al.*, 2018

between skin microbiota and its host is not restricted to the immune system and may be affecting brain and cognitive function (Wang *et al.*, 2024).

The qualitative composition of the human microbiome is individually specific and varies by area of skin inhabitation (Costello *et al.*, 2009; Sanford and Gallo, 2013; Sfriso *et al.*, 2020). The observed microbial niches are determined by the thickness of the skin in different areas, anatomy – pits, depressions, or folds of the skin, as well as the different distribution of skin appendages, which have their unique microbiome (Lee *et al.*, 2019; Flowers and Grice, 2020). It causes human skin to be divided into high-moisture areas, sebum-rich areas, and dry areas. More commensal microbiota are found in high-moisture areas than in dry areas. On the other hand, dry areas are more likely to have potentially invasive staphylococci, which require a less hydrated environment to grow (Sanford and Gallo, 2013). Bacterial growth is also affected by temperature (from 29.5°C on the fingers to 36.6°C in the armpit pits) and pH (from 4.2 on the cheeks to 7.9 in the armpit pits) (Costello *et al.*, 2009; Adamczyk *et al.*, 2018; Lee *et al.*, 2019).

4. Factors contributing to changes in skin microbiota

Skin microbiota composition depends on many factors (Sanford and Gallo, 2013; Sfriso *et al.*, 2020; Smythe and Wilkinson, 2023). One of them is the site on the skin. In their study, Grice *et al.* analyzed 20 different sites on the human skin of 10 healthy patients.

They proved that *Cutibacterium* and *Staphylococcus* predominated in sebaceous areas, while *Corynebacterium* species resided in the highest numbers in moist areas (Grice *et al.*, 2008). On the other hand, a strongly mixed bacterial population was observed in dry areas, with a higher frequency of β-proteobacteria and *Flavobacteria* (Prescott *et al.*, 2017). On the other hand, a study by Costello *et al.* showed much greater phylogenetic diversity at various sites on the skin compared to the microbiota of the gut, external auditory canal, or oral cavity. Ethnicity has also been shown to contribute to the diversity of the skin microbiota and is partly related to lifestyle (Costello *et al.*, 2009). For example, differences in microbiota composition have been shown between individuals of East Asian and European or African descent (Harker *et al.*, 2014). Significant differences are observed, for example, in the axillary region, where the abundance of *Staphylococcus varies* significantly concerning *Corynebacterium* (Cogen *et al.*, 2008; Dréno *et al.*, 2018; Flowers and Grice, 2020). The study by Perez *et al.* proved that the microbiota of the arm of African-American men is relatively homogeneous but significantly different from other ethnic groups. Similar conclusions were reached after studying the axillary microbiota of East Asian men; relative to the other ethnic groups, the microbial composition was quite different. What is more, East Asian individuals have a higher total amount of bacteria and proteobacteria relative to other groups (Perez *et al.*, 2016). The distribution of *Corynebacterium* species was also analyzed, and it was found that *Corynebacterium variabile* is found only in Hispanics. In contrast, *Corynebacterium kroppenstedtii* is found only in the East Asian group (Boxberger *et al.*, 2021). However, in this era of mass population migration, a complete definition of skin microbiota according to ethnicity is impossible. Physiological differences between male and female skin, such as different hormone levels, sweating rates, or skin surface pH, are also observed (Fierer *et al.*, 2008). The most remarkable differences are observed in the hand microbiota of men and women. It has also been shown that a higher amount of *Cutibacterium* and *Corynebacterium is* found in men than in women (Lee *et al.*, 2019). In contrast, bacteria from the *Enterobacteriaceae, Moraxellaceae, Lactobacillaceae,* and *Pseudomonadaceae* families are more abundant in women. *Staphylococcus* spp. occurs in significantly higher numbers in women than men, while *Corynebacterium* spp. is far more likely to colonize men's skin (Grice *et al.*, 2008; Perez *et al.*, 2016; Boxberger *et al.*, 2021; Robert *et al.*, 2022). Observing the distribution of *Malassezia* species in studies conducted by Prohic *et al.*, no significant sex effect was found (Prohic *et al.*, 2016). Leung *et al.* showed that males had higher amounts of *Cutibacterium, Staphylococcus,* and *Enhydrobacter*, while *Streptococcus* was observed in higher amounts in the female population. The *Epicoccum* and *Cryptococcus* genera were found in higher amounts in sebaceous areas in men, while *Malassezia* was mainly observed in women (Leung *et al.*, 2015). Li *et al.* observed that men have higher amounts of *Corynebacteria*, although the difference is insignificant (M. Li *et al.*, 2019). However, only males host *Corynebacterium amycolatum* and *Corynebacterium kroppenstedtii,* while females only host *Corynebacterium urealyticum* and *Corynebacterium variabile* (C. Xi Li *et al.*, 2019). It is also essential to look at the impact of the aging process on the composition of the skin microbiota. Indeed, aging is associated with many changes in skin characteristics and features, such as the appearance of spots and wrinkles and altered sebaceous gland activity, thereby affecting the composition of the skin microbiota. A study by Somboon *et al.* found a significantly higher prevalence of *Plantomycetes* and *Nitrospirae* bacteria in adolescents than in other age groups (Wilantho *et al.*, 2017). On the other hand, senile individuals show a significantly lower amount of *Cutibacterium* compared to the other age groups and an increased amount of *Corynebacterium* and *Acinetobacter* (Dessinioti and Katsambas, 2017; Dreno *et al.*, 2017). In older adults, there is also an increase in *Proteobacteria* and a decrease in *Actinobacteria* (Wilantho *et al.*, 2017). Contemporary studies also show decreased sebum production with age, reducing available nutrients for commensal bacteria and an increased possibility of spreading opportunistic bacteria (Boxberger *et al.*, 2021). Significant variation with age is also observed within *Malassezia*. For example, *Malassezia furfur* is characteristic of children's trunk skin, while *Malassezia restricta* predominates on the scalp of individuals between the ages of 21 and 35. In older people, on the other hand, *Malassezia sympodialis* predominates (Prohic *et al.*, 2016). *Demodex* spp. mites, studies show, are characteristic of older adults, and in the over-70 age group, they are found in up to 95% of (Adamczyk *et al.*, 2018; Boxberger *et al.*, 2021).

Mode of delivery, lifestyle, hygiene habits, cosmetics used, antibiotics used, geographic location, or climate can be considered external factors. In newborns, it has been indicated that the delivery type significantly impacts the skin microbiota composition. Babies born by natural childbirth have a skin microbiome shaped by bacteria present in the birth canal and the mother's vaginal area. On the other hand, babies born by cesarean section will acquire a bacterial biota similar to that of the mother's skin (Capone *et al.*, 2011; Sfriso *et al.*, 2020). The primary microbiota is transient and largely dependent on environmental factors. Later, it evolves to resemble the adult skin microbiota (Kong and Segre, 2017). During the first years of life, a highly differentiated skin microbiota develops, largely depend-

ent on the child's changing diet, increasing contact with people and animals, and exploring the environment. From 3 months, regionalization of the skin microbiota is observed in children (Dominguez-Bello *et al.*, 2010; Nagata *et al.*, 2012). Depending on the inhabited environmental zone (rural or urban), differences in the human skin microbiota related to the presence of pets are also observed. It is also worth mentioning the phenomenon of convergence of the skin microbiota in people who live together and are not related or do not have intimate relations. Studies also indicate that using facial makeup significantly increases the variability of commensal bacteria on human skin. Most cosmetics have preservatives in their formulation, which prevent the development of biofilm and the growth of *Staphylococcus aureus* or *Cutibacterium acnes* populations. Unfortunately, chemical compounds in cosmetics also inhibit the survival of commensal bacteria (Fournière *et al.*, 2020). Emulsifiers, in turn, promote the growth of potential pathogens such as *Staphylococcus aureus*. The use of topical antibiotics also affects the composition of the skin microbiota, causing a significant decrease in commensal *Staphylococcus* spp. (Reid *et al.*, 2011; Findley *et al.*, 2013; Cooper *et al.*, 2015; Baldwin *et al.*, 2017; Wallen-Russell and Wallen-Russell, 2017). Recent studies have also shown increased benefits of living in an alpine climate compared to a maritime (which significantly impacts treating atopic dermatitis in children). However, this observation needs confirmation in people with healthy skin (Nakatsuji *et al.*, 2013; van Mierlo *et al.*, 2019). Other authors indicate that after exposure to seawater, exogenous bacteria were still present on the surface for at least 24 hours after swimming and that exposure to ocean water removed physiological bacteria from human skin (Nielsen and Jiang, 2019). Altitude, associated with extreme environmental conditions, has also been proven to have a detrimental effect on skin microbiota. Add to this air pollution, which reduces the diversity of skin microbial populations (Adamczyk *et al.*, 2018).

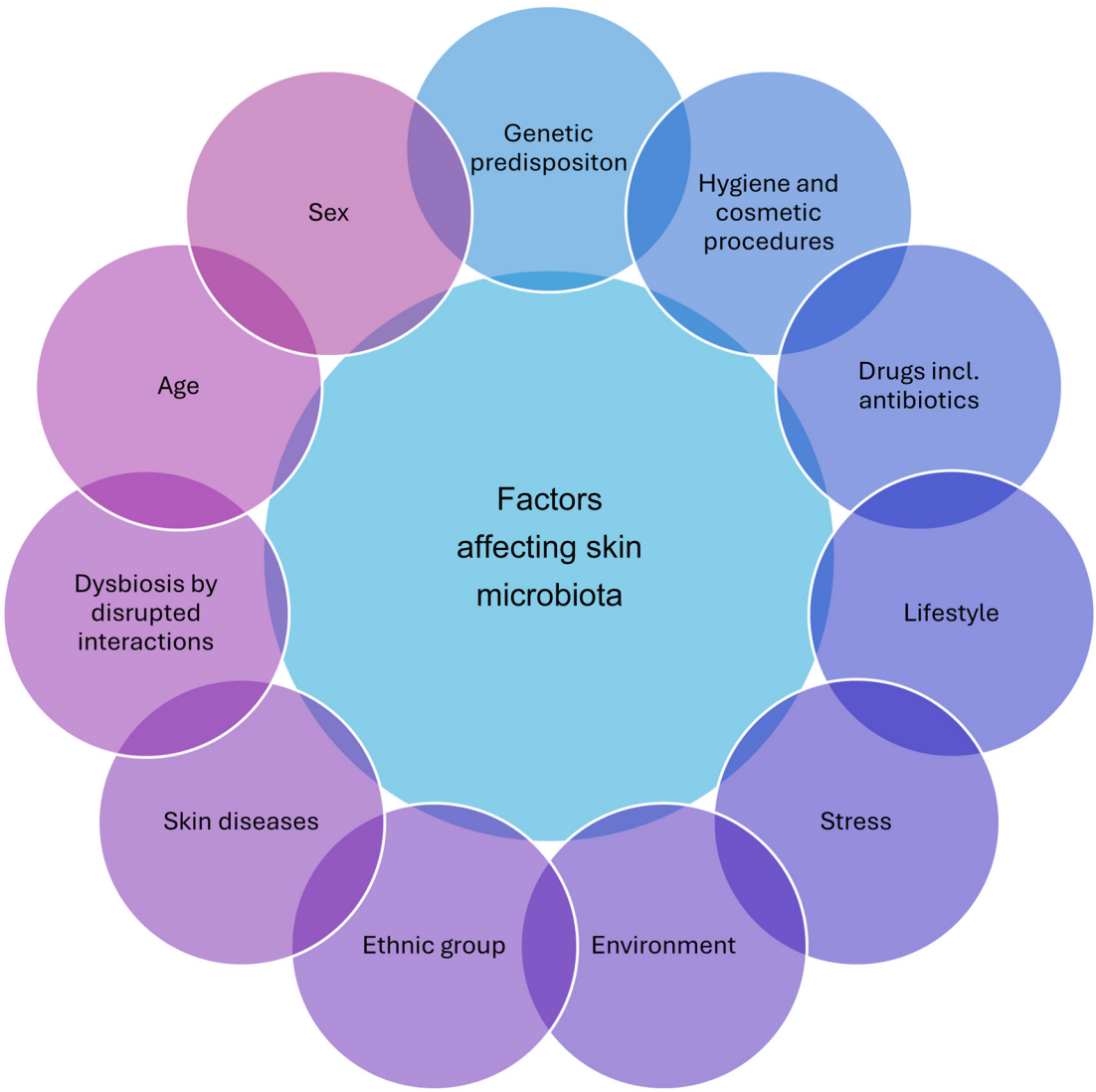

Fig. 5. Eleven factors contributing to changes in skin microbiota; own graphic

Finally, active ingredients used in cosmetics can change the composition of the skin microbiome – they can promote or inhibit the growth of certain microorganisms. According to Cundell, moisturizers can reduce the intensity of water loss from the skin and promote the skin's microflora, thereby reducing the exfoliation of dead skin cells (Cundell, 2018). Moreover, the lipid compounds of these cosmetics promote the growth of lipophilic bacteria (*Staphylococcus* and *Cutibacterium*) (Moskovicz *et al.*, 2020). Unfortunately, studies also indicate that increased levels of skin hydration can reduce the number of *Cutibacterium*, as proven by Lee *et al.* in their research (Lee *et al.*, 2018). It is also worth noting that bacteria can be used as active ingredients in cosmetics, mainly probiotic bacteria of the *Lactobacillus* genus (Butler *et al.*, 2020). They show the ability to synthesize and secrete various antimicrobial substances and block pathogens' adhesion to skin cells. However, it is essential to remember that improperly selected cosmetics for the skin type or skin problem and improper use of preparations can negatively affect the skin microbiome, reducing its diversity leading to dysbiosis (Andersen, 2019).

Based on the literature review, eleven factors affecting human skin microbiota are presented in Fig. 5.

5. Conclusion

Due to its variability and susceptibility to many factors, the human skin microbiota still requires much research and a better understanding of its functioning. At the same time, its crucial role in organizing human health and preventing the spread of inflammation should be kept in mind. Skin commensals prevent the development of many skin diseases, such as acne vulgaris, atopic dermatitis, and rosacea. However, the variability of environmental factors makes it impossible to draw uniform conclusions about the exact composition of the skin microbiota in different age groups or the context of sex differences.

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Conflict of interest

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GONOCOCCI – PATHOGENS OF GROWING IMPORTANCE. PART 2. VIRULENCE FACTORS, ANTIMICROBIAL RESISTANCE AND VACCINE DEVELOPMENT

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Abstract: *Neisseria gonorrhoeae* (gonococcus) is a human pathogen, the aetiological agent of gonorrhoea, which is the second most common bacterial sexually transmitted disease (STD) in the world. The structure of *N. gonorrhoeae* cell wall is typical of Gram-negative bacteria, poses variable antigens porin B (PorB), and opacity-associated proteins (Opa proteins), lipooligosaccharide (LOS) and type IV pili (TFP) playing an essential role in pathogenesis. In addition to adhesins, gonococcus presents other virulence factors such as reducing modifiable protein (Rmp), iron transporters, membrane pumps, and IgA peptidase. The pathogen produces outer membrane vesicles (OMVs), releases peptidoglycan (PG) fragments and is well adapted to develop infection in diverse niches of the female and male reproductive tracts. The characteristic genotypic trait of *N. gonorrhoeae* is the state of natural competence, which allows DNA uptake from the environment. The antigenic and phase variability is essential to gonococcal defence against the human immune system. Because of the increasing antimicrobial resistance (AMR) of *N. gonorrhoeae* and the high incidence rate of gonococcal infections, developing an anti-gonococcal vaccine has become an urgent need. Vaccine development difficulties are mainly due to the gonococcal ability of immune evasion, the lack of an animal model, and the limited understanding of protective immune response mechanisms.

Keywords: AMR, gonorrhoea, vaccine, virulence factor

1. Introduction

Neisseria gonorrhoeae is an absolute human pathogen, the aetiological agent of gonorrhoea. Gonococci outside the body are highly sensitive to environmental factors. They die within hours at temperatures above 40°C and are sensitive to desiccation, light and low concentrations of disinfectants. Microscopically, *N. gonorrhoeae* is a Gram-negative diplococcus arranged in characteristic pairs resembling coffee beans (Janda *et al.* 2005). It is an aerobic bacterium with high nutritional requirements. The bacterium is oxidase- and catalase-positive, oxidizes only glucose to acid, and does not metabolize other carbohydrates. It requires an energy source of glucose, pyruvate or lactate, and cysteine to grow on culture media. Due to changes in metabolic pathways, some isolates show special growth requirements for amino acids, purines, and pyrimidines (Ng *et al.* 2005; Quillin *et al.* 2018). The structure of *N. gonorrhoeae* cell wall is typical of Gram-negative bacteria. There is a thin layer of PG between the inner cytoplasmic and outer membranes. The most crucial cell wall antigens are PorB (P. I) and Opa (P. II) proteins, LOS and TFP. The increasing antimicrobial resistance (AMR) of *N. gonorrhoeae* has a multifactorial basis. It is a serious threat to public health (Unemo *et al.* 2016),

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especially in the context of a high prevalence of gonorrhoea worldwide, with over 82.4 million new cases in 2020 as estimated by WHO (WHO 2021). Gonorrhoeae is a significant health risk, especially for women, due to its often asymptomatic course and the possibility of upper genital tract complications such as pelvic inflammatory disease (PID), which can result in ectopic pregnancy and infertility. Gonorrhea infection is also connected with a higher risk of HIV acquisition.

The genus *Neisseria*, family *Neisseriaceae*, includes more than 30 different species of aerobic, Gram-negative coccus or rod species found in humans and animals. Eight commensal species are part of the human microbiome, while *N. gonorrhoeae* and *N. meningitidis* are pathogenic (Humbert *et al.* 2019). Interestingly, despite presenting different pathogenicity, these two human pathogens share a high genome identity, resulting, among others, in a sequence similarity of their outer membrane proteins. These similarities can partially explain a cross-protective effect of outer membrane vesicles (OMV), meningococcal serogroup B vaccine (MenB-4C) against gonococcal infection (Hadad *et al.* 2012; Marjuki *et al.*, 2019).

2. Genome and molecular characterization of *N. gonorrhoeae*

N. gonorrhoeae chromosome has the structure of a circular, covalently closed DNA molecule of about 2.2 million bp in length. The results of genomic analysis of 14 reference *N. gonorrhoeae* strains from the WHO collection obtained by whole genome sequencing (WGS) presented in 2016 can provide a basis for representative molecular characterization of the species (Unemo *et al.* 2016; Golparian *et al.* 2021; Sánchez-Busó *et al.* 2022). The genomes of these strains were compared and showed relatively high similarity, and the differences were mainly related to the presence of the gonococcal genomic island (GGI). It is present in about 80% of *N. gonorrhoeae* isolates and encodes the type IV secretion system (T4SS) (Dillard and Seifert 2001; Hamilton and Dillard 2006). The total number of coding sequences in the genomes of the analyzed reference strains ranged from 2295 to 2450, with an average sequence length of 0.7 kb. Core genes were estimated at 1820, and auxiliary genes at 475 to 630. The coding density in the whole genome was about 87%, with an average G+C content of 52.4%. In addition, 54 tRNAs and four copies of 16S-23S-5S rRNA operons are encoded in the gonococcal genome. The most abundant type of repeats is the so-called 10–12 bp long *DNA* uptake sequence (DUS) composed of 5'-GCCGTCTGAA-3' or 5'-ATGCCGTCTGAA-3', which typically repeats every 1.1 kb and accounts for almost 1% of the genome (Frye *et al*. 2013; Spencer-Smith *et al.* 2016). An essential feature characteristic of the species *N. gonorrhoeae* is the state of natural competence, which means the constitutive ability of bacteria to take up DNA from the environment. DNA uptake from related species of the genus *Neisseria* (Spratt *et al.* 1992), as well as from unrelated bacteria, is an essential factor in the genetic variability of gonococci (Kroll *et al.* 1998). The natural transformation process in *N. gonorrhoeae* involves TFP (Chen and Dubnau 2004). DNA uptake occurs through the interaction between pili and the DNA capture sequence (Cehovin *et al.* 2020). Human commensal species of the *Neisseriaceae* family have DUS sequences identical or differing by one or two bp (Frye *et al.* 2013; Spencer-Smith *et al.* 2016). An example of chromosomal loci transfer in *N. gonorrhoeae* is the mosaic structure of antibiotic resistance-related proteins penA. The T4SS allows to secrete fragments of single-stranded DNA (ssDNA) directly into the extracellular space, which, once recognized by the recipient pilus, can be incorporated by recombination into its genome (Dillard and Seifert 2001; Hamilton and Dillard 2006). T4SS is encoded within a GGI approximately 57 kb long (Hamilton *et al.* 2005). The GGI is a mobile genome element, probably acquired by horizontal gene transfer (HGT) (Rotman and Seifert 2014). As shown experimentally, out of 66 GGI genes, only 21 are essential for the function of the T4SS system, two-thirds of which are *tra genes* homologous to T4SS system genes of *E. coli* plasmid F (Hamilton *et al.* 2005; Pachulec *et al.* 2014; Callaghan *et al.* 2017). Gonococcal T4SS exports ssDNA directly into the extracellular space independent of contact with the host or neighbouring cell. It has been shown that this unique method of DNA secretion may have an adaptive role in the pathogenesis and acquisition of antibiotic resistance (Harrison *et al.* 2016) and in biofilm formation (Zweig *et al*. 2014).

Bacterial plasmids are autonomous, extrachromosomal, replication-capable genetic elements. Unlike the chromosome, which constitutes the core genome, plasmids are not necessary for bacterial survival and are an accessory genome. Plasmids in the form of double-stranded DNA are mostly circular. Their genes, with different functions, determine various phenotypic characteristics, such as antibiotic resistance, the ability to neutralize toxic substances, and the production of bacteriocins and toxins. Plasmids are a link of HGT that promotes variability and environmental adaptation. As autonomous, mobile genetic elements, they can move from the donor bacterial cell to the recipient cell through conjugation, transformation and transduction. In gonococci, there can be three types of circular plasmids: conjugative plasmids, β-lactamase plasmids and cryptic plasmids. Most are between 4 and 9 kb in length. Only conjugation plasmids are longer,

containing 39–42 kb. Cryptic plasmids, with a size of 4.2 kb and an undefined function for the bacterial cell, are found in most (96%) strains of *N. gonorrhoeae* (Cehovin and Lewis 2017).

3. The factors affecting the pathogenesis of gonococcal infection

3.1. Adhesins presence

N. gonorrhoeae adhesins recognize different target sites on host cells, allowing the pathogen to interact with various tissues during gonorrheal infection. Adhesion and invasion into human cells are a crucial step in developing infection. Antigenic and phase variability of adhesins protects bacteria from effective immune defense. An important virulence factor is the glycolipid outer membrane antigen LOS, composed of endotoxin-active lipid A and a core oligosaccharide. It stimulates the release of pro-inflammatory cytokines, which promotes neutrophil recruitment to the infection site (Ng *et al.* 2005; Quillin and Seiferth 2018). The structure of LOS lacks the repeated polysaccharide chains characteristic of the analogous cell wall antigen of *Enterobacterales*, lipopolysaccharide. The branched LOS molecule has three oligosaccharide chains anchored to the cell membrane by lipid A. The variable chains are attached via a 3-deoxy-D-manno-octulosonic acid molecule to two heptose residues of lipid A: Hep1 and Hep2. The oligosaccharide core shows considerable variability, even within the same *N. gonorrhoeae* strain due to phase variation in numerous glycosyl transferase genes. This enzyme, which is crucial in the synthesis of LOS, is responsible for attaching further sugars to the oligosaccharide chain. The variability of LOS is also due to the genetic diversity of alleles encoding glycosyltransferases (Apicella *et al.* 1987; Banerjee *et al.* 1998; Shafer *et al.* 2002). LOS is involved in the adherence process to epithelial cells. Variability and modifications of LOS structure, such as attachment of exogenous sialic acid molecules, promote evasion of host immune defense mechanisms. Both sialylation of gonococcal LOS and attachment of phosphoethanolamine to lipid A protect against antibodies and reduce susceptibility to bacterial killing by neutrophils, inhibit complement activation, and increase resistance to antimicrobial peptides (Lewis *et al.* 2009; Balthazar *et al.* 2011). The importance of LOS in processes leading to oviduct damage has also been described (Gregg *et al.* 1981; Cooper *et al.* 1986).

TFPs are protein filamentous surface structures anchored to the cytoplasmic membrane, passing through the outer membrane to the outside of the cell. As adhesins, they have a crucial function in the pathogenesis of gonorrheal infection. Their presence is related to the virulence of the strain. They participate in the initial phase of bacterial adhesion to human epithelial cells and interactions with neutrophils. Pili, binding to the CD46 receptor, initiates adherence to eukaryotic cells. Based on the principle of twitching motility, they provide gonococci with a degree of motility, which promotes the spread of microorganisms across the epithelial surface and facilitates their auto-aggregation. Phase variation in TFP expression enables gonococci to defend against phagocytosis and evade the host immune response. Several genes encode different subunits of pili. The main subunit of the pilE protein shows antigenic variation due to recombination with several silent copies at the *pilS* loci. Pili are also important in the natural transformation of *N. gonorrhoeae* (Chen and Dubnau 2004).

Opa proteins are a family of 24–30 kDa outer membrane proteins. As adhesins, they are involved in gonococcal auto-aggregation and receptor-mediated adherence of bacteria to eukaryotic cells and in stimulation and modification of the immune response. Opa proteins bind to host cell CEACAM receptors (carcinoembryonic antigen family adhesion molecules). They are named after a phenomenon observed macroscopically, as they cause the lack of transparency of colonies cultured on clear agar. Depending on the expression of Opa proteins, the morphology of gonococci colonies on agar varies. Opa colonies expressing Opa + are referred to as "opaque phenotype." Based on differences in binding to two types of cell receptors, two classes of Opa proteins have been distinguished, grouping 11 different types. Opa proteins class one (Opa_{50}) bind to the heparan sulfate proteoglycan receptor on epithelial cells using heparan sulfate. Opa class two (Opa_{51-60}) binds to CD66 antigen family molecules on epithelial cells, lymphocytes and neutrophils, i.e., the receptor molecules CEACAM-1, 3, 5 and 6. The ability of bacteria to interact with different types of receptors during infection activates distinct signal transduction pathways in cells and is an essential adaptive feature (Gray-Owen *et al.* 1997; Chen *et al.* 1997). Opa proteins increase bacterial resistance to complement action. Opa protein expression is subject to antigenic and phase variation. There can be up to 11 different *opa* genes in the genome of *N. gonorrhoeae* strain, with several types being expressed simultaneously. The protein sequence of all Opa proteins is 70% identical. Conservative regions of the molecule are not exposed on the surface of the outer membrane, in contrast to the highly variable outer fragments, the second and third loops. These regions are called hypervariable domains 1 and 2 (Stern *et al.* 1986; Bhat *et al.* 1992).

PorB is the most abundant outer membrane protein of *N. gonorrhoeae*, encoded by the *porB* gene. It is essential for gonococci viability as a conserved voltage-gated ion channel-like protein. It consists of three protein

subunits. Each monomer, 32–35 kDa, has a structure of β-folded barrel, 16 trans-membrane segments, and eight characteristic extracellular loops with high variability (Chen and Seifert 2013). In gonococci, there are two different Porin B isoforms – PorB1a and PorB1b-, resulting from stable expression of one of the two alleles of the *porB* gene in a given bacterial strain. Due to this diversity, two distinct phenotypes of gonococci can be distinguished depending on the type of porin they possess: P. IA and P. IB. Strains with the P. IA phenotype are characterized by increased invasiveness, which has been found based on epidemiological data describing the more frequent involvement of this particular phenotype in generalized gonococcal infections (Bash *et al.* 2005, Guglielmino *et al.* 2022). The P. IA phenotype strains are characterized by the deletion of a fragment in loop 5, which gives them resistance to trypsin digestion and higher resistance to the complement-dependent lethal effects of human serum (Blake *et al.* 1981; Ram *et al.* 2001). P. IB phenotype gonococci are more often isolated from genitourinary tract infections and usually show sensitivity to the bactericidal effect of serum. The nucleotide sequences of both types of porins show about 80% similarity. Genetic variation within PorB distinguishes strains in epidemiological studies (Fudyk *et al.* 1999; Liao *et al.* 2009). These porins are the basis for serotyping based on reactions with monoclonal antibodies (Sandström *et al.* 1980, Tam *et al.* 1982). A fragment of the third outer loop of the PorB molecule has become, along with the TbpB fragment, the basis of NG-MAST typing (Martin *et al.* 2004), and the PorB1b gene segment encoding 30 amino acids is a well-characterized determinant of antibiotic resistance, referred to as penB (Olesky *et al.* 2002; Unemo *et al.* 2011). PorB acts as a mitogen, activates B lymphocytes and stimulates cytokine production. On the other hand, this protein influences the phagocytosis process, enabling it to block complement activation through the alternative or classical pathway and protecting bacteria from opsonization. Factor H, the main regulator of the alternative complement pathway, binds directly to PorB1a. Strains with the P. IB phenotype require LOS sialylation to bind factor H. Serum resistance in gonococci is also mediated by the C4bp protein binding to different fragments – extracellular loop 1 within PorB1a of P. IA strains and loops 5 and 7 of PorB1b (P. IB). PorB can also inhibit oxidative burst and neutrophil apoptosis, facilitating pathogens to survive infection despite the inflammatory response (Chen *et al.* 2011; Chen and Seifert 2013; Palmer and Criss 2018).

Rmp is an outer membrane protein physically associated with PorB, presenting the potential of an immunology evasion (Joiner *et al.* 1985). As a highly conserved membrane protein, it stimulates the synthesis of "blocking antibodies" specific for both LOS and PorB, inhibiting serum's bactericidal effect (Gulati *et al.* 2015). The presence of antibodies to Opa proteins and the absence of "blocking antibodies" induced by Rmp was associated with the reduction of upper reproductive tract infection in high-risk women (Plummer *et al.* 1993a, 1994b).

3.2. Cytotoxic peptidoglycan fragments

PG fragments that are spontaneously released during the growth and division of bacterial cells at the site of infection are important virulence factors. Recognized by human cytosolic nucleotide-binding oligomerization domains 1 and 2, pattern recognition receptors are an element that induces the inflammatory response (Mavrogiorgos *et al.* 2014). By stimulating the inflammatory process, they are responsible for, among others, damage within the fallopian tubes and destruction of the epithelial cells. The release of pro-inflammatory peptidoglycan monomers as well as dimers and free peptides, leading to remodelling of the cell wall structure of gonococci, is possible due to the activity of bacterial enzymes, mainly lytic transglycosylases (Cloud *et al.* 2002), as well as amidases and endopeptidases (Schaub *et al.* 2019).

3.3. Outer membrane vesicles production

OMVs by *N. gonorrhoeae* (Pettit *et al.* 1992) are characteristic of many pathogenic Gam-negative bacteria (Kulp *et al.* 2010). OMVs were first described in *Vibrio cholerae* in 1967 and recognized as artefacts of *in vitro* liquid medium culture (Chatterjee SN, Das J, 1967). Vesicles are formed by the protrusion of the bacterial outer membrane, the detachment of a fragment of this membrane and the formation of a spherical, closed structure with a diameter of 20–200 nm. Inside OMVs, components of both the bacterial periplasm and cytoplasm, such as adhesins, enzymes, and DNA fragments, can be encapsulated. Examination of gonococcal OMVs proteins concentration can suggest active sorting of proteins during natural blebbing of bacteria facilitating vesicles functions (Zielke *et al.* 2014; Deo *et al.* 2018). So far, these potent single lipid bilayer spheres, carrying plenty of outer membrane lipids, proteins, LOS, periplasmatic PG fragments and proteins, cytoplasmatic proteins and nucleic acids, have shown to be the promise vaccine antigens (van der Pol *et al.*, 2015). OMVs contain many outer membrane antigens, presented in their native form, as derived straightly from the crucial pathogen adhesion and immunogenicity surface layer of Gram-negative bacterial cell wall. Polyantigenic gonococcal OMVs can potentially overcome the challenges of high phase and antigen variation of *N. gonorrhoeae*.

3.4. The ability to utilize the host's iron resources

An important phenomenon in the pathogenesis of gonorrhoea is the ability to utilize the host's iron resources with the help of gonococcal membrane transporters. Unlike most bacteria, *Neisseria* species pathogenic to humans do not produce siderophores and use iron bound to human glycoproteins, mainly transferrin (West and Sparling 1985). They can also obtain iron from lactoferrin (Mickelsen and Sparling 1981; Mickelsen *et al.* 1982), hemoglobin and heme, which are available in the female reproductive tract during menstruation. Eight iron transporters dependent on TonB proteins of the inner membrane of gonococci have been described: TbpA/TbpB, LbpA/ LbpB, HpuB/ HpuA, FetA, TdfF, TdfG, TdfH, TdfJ, which enable efficient utilization of protein-bound iron (Cornelissen and Hollander 2011). Human transferrin is found in the highest concentrations in serum, cerebrospinal fluid and joint fluid. Still, it can also be detected in semen and mucous membranes, especially in inflamed tissue. Iron acquisition requires energy and direct contact between the iron transport glycoprotein and the bacterial cell surface. Increased expression of gonococcal genes (e.g., *tbp, lbp, fbp*) responsible for iron uptake from transferrin and lactoferrin has been found during gonococcal infection of the lower genital tract (McClure *et al.* 2015).

3.5. Efflux-type membrane transporters activity

Active efflux-type membrane transporters are an essential group of surface proteins that act as pumps that remove harmful chemicals from the bacterial cell. The expression level of such efflux transporters determines the variable level of sensitivity of the microorganism to antimicrobial substances naturally present in their environment and to antibiotics. Gonococcal membrane transporters belong to different families of bacterial pumps. The FarA-FarB transporter of the Major Facilitator System (MFS) family recognizes antibacterial long-chain fatty acids (Lee and Shafer 1999). The NorM transporter is a member of the Multidrug and Toxic Compound Extrusion (MATE) family, and its overexpression can reduce the sensitivity of gonococci to ciprofloxacin and norfloxacin (Rouquette-Loughlin *et al.* 2005). The MtrC-MtrD-MtrE system belongs to the Resistance-Nodulation-Cel Division (RND) family of pumps found in Gram-negative bacteria and depends on energy drawn from ATP. The MtrCDE pump is an active transporter of substances from the cytoplasm and periplasmic space (Maness and Sparling 1973). It can remove hydrophobic compounds, naturally occurring antimicrobial peptides, bile salts, progesterone, detergent and dye-like compounds, and antibiotics (Delahay *et al.* 1997). The mtrCDE pump comprises three protein subunits, C, D, and E, passing through the outer membrane, periplasmic space, and inner membrane. It has a typical operon, and the MtrR repressor and MtrA activator regulate its expression. The *mtrR* repressor gene is an important determinant of gonococcal chromosomal antibiotic resistance. As a result of point mutations within the *mtrR* promoter or the actual gene, or due to the mosaic structure of *mtrR* resulting from HGT derived from related species of the genus *Neisseria*, there is increased expression of a pump that removes toxic substances from the bacterial cell. Phenotypically, this increases MICs for macrolide antibiotics, tetracyclines, penicillin and cephalosporins (Zarantonelli *et al.* 2001).

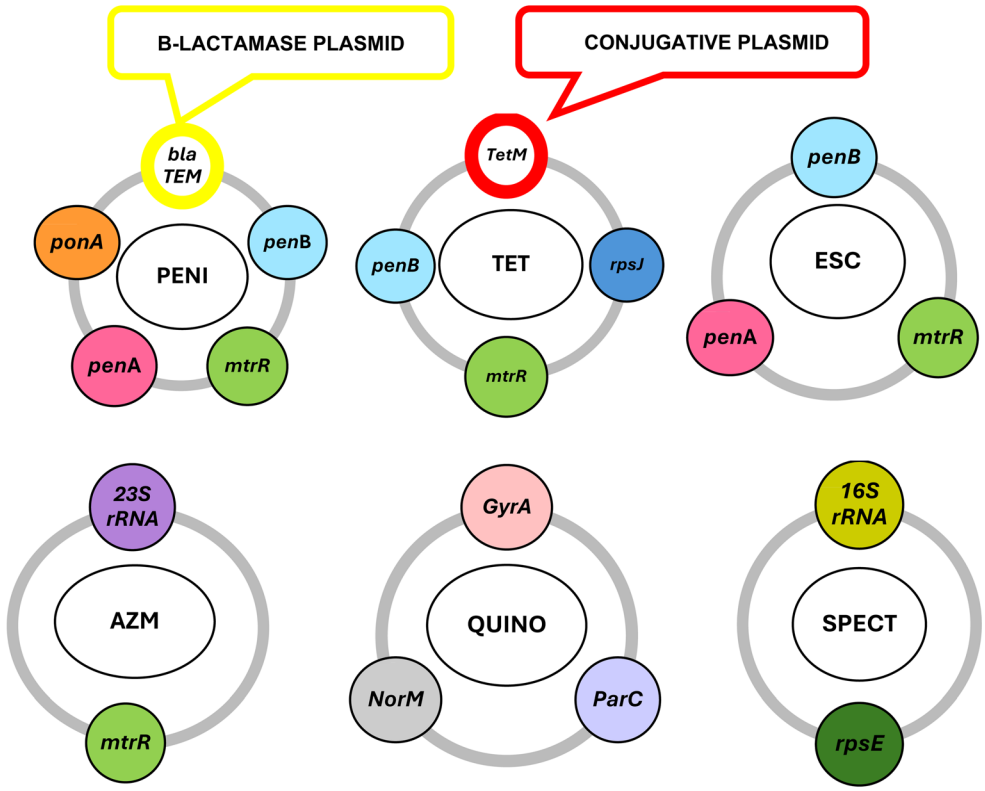
3.6. Anaerobic metabolism

Anaerobic metabolism in *N. gonorrhoeae* is enabled by two enzymes of the denitrification pathway: copper-containing nitrite reductase (AniA) that reduces nitrite to nitric oxide (NO) and nitric oxide reductase (NorB) (Barth *et al.* 2009). The NorB enzyme may be involved in removing toxic NO produced by macrophages. The expression of these enzymes is strictly regulated by oxygen availability, and elevated expression of AniA and NorB was found, e.g., in biofilms (Falsetta *et al.* 2009).

3.7. Antimicrobial resistance

The increasing antimicrobial resistance of *N. gonorrhoeae* is a global problem, and it has a complex basis, both chromosomal and plasmid-mediated (Fig. 1) (Unemo *et al.* 2016). Features of gonococci responsible for the rise of antibiotic resistance include genetic plasticity, the ability to transform naturally, high levels of antigenic and phase variation, point mutations, mosaicism of chromosomal determinants of resistance, and plasmids with resistance-determinant genes. A structurally diverse group of β-lactamase plasmids encoding blaTEM penicillinases determines penicillin resistance. In contrast, conjugative plasmids may have Tet(M) tetracycline resistance genes in their structure (Fig. 2) (Pachulec *et al.* 2014). Based on phenotypic characteristics, strains with plasmid resistance to penicillin and tetracycline, respectively, can be identified using the cephinase test for penicillinase-producing isolates (PPNG or penicillinase-producing *N. gonorrhoeae*) and a MIC value for tetracycline ≥ 16.0 mg/L, characterizing strains with high levels of tetracycline resistance – the High-Level tetracycline resistant (HLTR) phenotype (Muhammad *et al.* 2014).

Strains exhibiting low-level tetracycline resistance (LLTR), phenotypically expressed by tetracycline MIC values in the range of 1–8 mg/l, do not have the plasmid *tet*(*M*) gene, and the resistance in their case is



MECHANISM OF AMR	PENI	ESC	TET	AZM	QUINO	SPECT
SYNTHESIS OF ANTIBIOTIC INACTIVATING ENZYME	*blaTEM*					
SYNTHESIS OF NEW PROTEIN			*TetM*			
CHANGES IN MEMBRANE PERMEABILITY	***penB*** *(porB1b)*					
ACTIVE REMOVAL OF ANTIBIOTIC FROM THE CELL	*mtrR*				*NorM*	
CHANGING THE TARGET SITE OF ANTIBIOTIC ACTION	*penA*		*rpsJ*	*23SrRNA*	*GyrA*	*16SrRNA*
	ponA				*ParC*	*rpsE*

AMR – antimicrobial resistance, **PENI** – penicillin, **ESC** – extended spectrum cephalosporins, **TET** – tetracyclines, **AZM** – azithromycin, **QUINO** – quinolones, **SPECT** – spectinomycin

Fig. 1. Main chromosomal and plasmid-mediated determinants of *Neisseria gonorrhoeae* antimicrobial resistance and their mechanisms of action.

chromosomal and may be the result of changes in genes associated with drug susceptibility, among others, in the *mtrR gene* – encoding the membrane pump repressor MtrCDE or in the *porB1b (penB*) gene (Pitt *et al.* 2019). An example of chromosomal loci transfers in *N. gonorrhoeae,* including important determinants of antibiotic resistance, is the mosaic structure of PBP2 (*penA*) proteins associated with resistance to penicillin and cephalosporins (Muhammad *et al.* 2002; Nakayama *et al.* 2016) and the mosaic structure of *mtrR* and *mtrCDE* genes, encoding the pump gene repressor and membrane pump MtrCDE, respectively (Rouquette-Loughlin *et al.* 2018). Single nucleotide mutations that change the target site of antibiotic action in a bacterial cell cause resistance to penicillin, cephalosporins, macrolides, fluoroquinolones, and spectinomycin. Mutations within the genes of penicillin-binding protein PBP1 (*ponA*) cause an increase in the MIC for penicillin, while within the genes of PBP2 (*penA*) for penicillin and third-generation cephalosporins (Lindberg *et al.* 2007). Point mutations, e.g., adenine deletion (35Adel) in the promoter region and substitutions of the coding

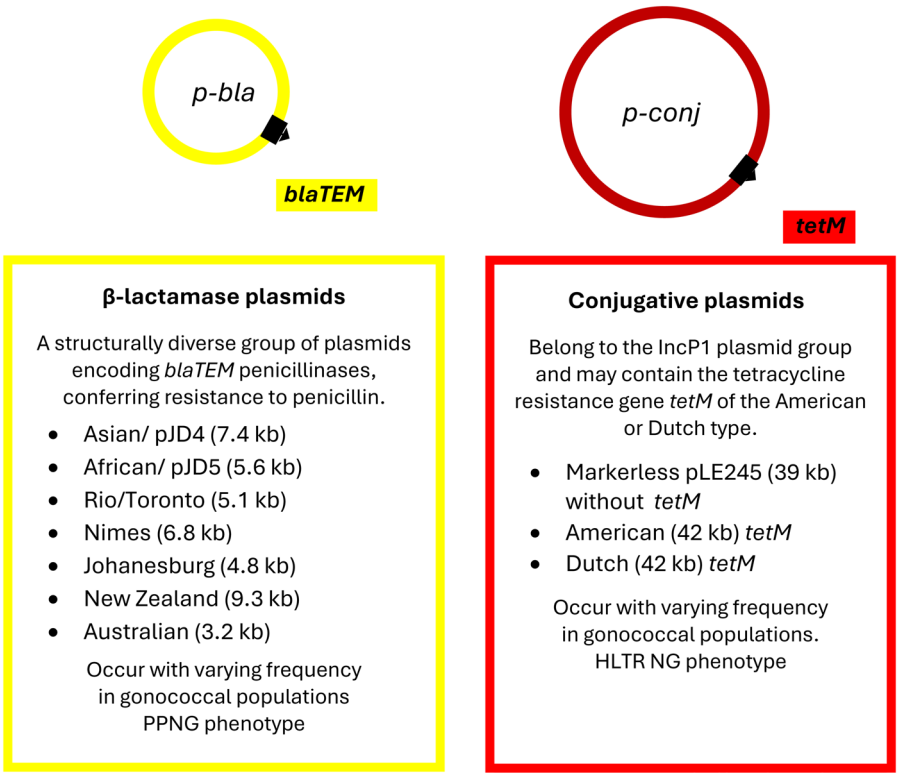


Fig. 2. Types of plasmids associated with drug resistance in *Neisseria gonorrhoeae* (according to Cehovin 2017).

region in the *mtrR* gene (A39T and G45D), can result in overexpression of the MtrCDE pump and lead to increased resistance to penicillin, tetracyclines, macrolides and cephalosporins (Zarantonelli *et al.* 2001). Mutations in the *porB1b* gene fragment, also referred to as a *penB* resistance determinant, altering the structure of the PorB1 membrane protein can result in reduced membrane permeability and impede the entry of tetracyclines, penicillin and cephalosporins into the cell. The main *PorB1b* sequence changes described so far result in amino acid substitutions of G120 and A121 (Lindberg *et al.* 2007). Resistance to azithromycin is mainly caused by mutations in the *23SrRNA* subunit allele, reducing the affinity of macrolide for the 50S ribosome unit. Mutations within the DNA gyrase (*gyrA*) and topoisomerase IV (*parC*) genes cause resistance to fluoroquinolones. Mutations in ribosomal genes encoding the 16sRNA unit and the 5S protein, *rpsE,* inhibit spectinomycin binding to the ribosome (Unemo *et al.* 2016).

3.8. Biofilm production

A bacterial biofilm is understood as a spatial structure within which bacterial cells adhere to a surface, contact each other, cooperate and are surrounded by a matrix. The extracellular polymeric mainly comprises bacterial products, glycoproteins, lipids, and nucleic acids. The matrix's biochemical nature and the biofilm's structure depend on the microorganisms' properties and environmental conditions (Stoodley *et al.* 2002; Sauer *et al.* 2003). Bacterial biofilm can form on the surface of living cells and is natural for many commensal microorganisms colonizing human mucous membranes. At the same time, the biofilm phenomenon is important in terms of the pathogenicity of microorganisms. After entering the body in planktonic form, bacterial pathogens can adhere to host cells and then gradually form a biofilm. It has been found that bacterial cells residing in biofilms are characterized by high viability and increased resistance to unfavorable environmental conditions, such as physical and chemical changes in the environment (e.g., nutrient deficiency, changes in pH or oxygen concentration), and better tolerate the presence of bactericidal substances. Growth in the form of a biofilm facilitates long-term colonization of a variety of tissues and enhances bacterial resistance to natural antimicrobial peptides, other immune system factors, and antibiotics (Donlan and Costerton 2002).

The biofilm formed by *N. gonorrhoeae* was visualized in cervical biopsy preparations (Steichen *et al.* 2008). A three-dimensional 48-hour biofilm of the reference strain *N. gonorrhoeae* 1291 formed on transformed cervical epithelial cells was imaged by confocal microscopy (Steichen *et al.* 2008). The ability of gonococci to form a biofilm on the abiotic surface under conditions of continuous flow of medium and on the surface of urethral and

cervical epithelial cells in *ex vivo* cultures was described (Greiner *et al.* 2005), according to the work of Falsetta *et al*. The transcriptomes of gonococci growing in the biofilm differ from those of planktonic cells, as demonstrated using the RNA microarray technique. Biofilms showed a significant increase in the expression of genes related to the metabolism of anaerobic respiration *AniA, NorB* and the cytochrome C peroxidase (*ccp*) gene associated with oxidative stress tolerance (Falsetta 2009).

3.9. Evasion of immune defense mechanisms

Many mechanisms of avoiding the host immune system protection have been discussed while describing gonococci's main virulence factors. *N. gonorrhoeae* is both an extra- and intracellular pathogen able to induce a strong inflammatory response through the Th-17 pathway but a weak, insufficient adaptive response. The innate immune response is a first line of defence against gonococci. Lack of protective immunity after recovery, asymptomatic infections in women and long-term complications of gonorrhoea indicate that the pathogen possesses the mechanisms of immunological evasions (Płaczkiewicz, 2019).

The ability of gonococci to evade immune defense mechanisms, including oxidative burst and killing by neutrophil granulocytes, should be underlined. Generally, neutrophils are the first phagocytic defense line in bacterial infections and represent the major component of the inflammatory response in gonococcal infection. The influx of numerous activated polymorphonuclear leukocytes (PMNs) into the urethra following gonococcal emergence on epithelium is, in fact, typical of symptomatic infection in men (Rest and Shafer 1989). The inflammatory response in symptomatic gonorrhoea is stimulated by gonococcal cell membrane adhesins, peptidoglycan fragments released naturally during cell division, A lipid of LOS, surface lipoproteins, heptoses, which are intermediates of LOS biosynthesis, and methylated DNA fragments secreted by strains possessing a type IV secretion system (Palmer and Criss 2018). Neutrophils kill microorganisms through the activity of antimicrobial proteins and synthesize ROS (Segal *et al.* 2005). However, despite the presence of numerous neutrophils in the urethra during *N. gonorrhoeae* infection, live gonococci are cultured from the purulent secretions collected from the patient, showing that PMNs are ineffective in killing *N. gonorrhoeae* (Rest and Shafer 1989). As gonococci, during infection, are exposed to different sources of reactive oxygen species (ROS), they use many factors to protect themselves from oxidative damage: catalase, cytochrome c oxidase, methionine peptide sulfoxide reductase, cytochrome c peroxidase, bacterioferritin, manganese uptake system (Criss *et al.* 2021). The IgA protease, produced and secreted by the gonococci, capable of degrading the hinge site of IgA class immunoglobulins present on human mucous membranes, is also a virulence factor helping evasion of host epithelial immunity control (Quillin and Seiferth 2018).

Host immune response to gonococci has not been fully understood. Another interesting issue is how the reproductive tract microbiota influences human susceptibility to gonococcal infections. Due to the vaginal epithelium colonization by microorganisms, the totality of which is referred to as vaginal microbiota (VM) interaction of immune cells, epithelial cells, commensals and pathogens in this niche are complex. In a physiological state, the domination of *Lactobacillus* spp. on the vaginal mucosa, with much less amounts of other microorganisms, including *Gardnerella*, *Bifidobacterium*, *Streptococcus*, *Ureaplasma*, *Corynebacterium*, *Enterococcus etc*. are observed. *Lactobacillus* spp. is responsible for maintaining stability and a healthy vaginal environment (Ravel *et al.* 2011). *Gardnerella vaginalis* is a component of the vaginal microbiota in healthy women and also the dominant microorganism in the vagina in a state of bacterial vaginosis. The reproductive tract microbiota associations with susceptibility to infection by sexually transmitted pathogens have been shown (Brotman *et al.* 2010).

4. *N. gonorrhoeae* vaccine development

The constantly increasing resistance of gonococci to antibiotics, also recommended in empirical treatment ceftriaxone, contributed to the urgency for developing both new treatment options and vaccines preventing gonorrhoea. So far, no registered vaccine against gonorrhoea has been developed, as the vaccines tested have proved ineffective. The reason may be that the correlates of immune protection in humans are not fully known, and the antigenic determinants of gonococci are highly variable. Gonococci modify their epitopes through antigenic or phase variation (Boslego *et al.* 1991) and modify and even suppress the development of a protective immune response necessary to avoid reinfection in humans. More than that, the research on vaccine construction has been challenging due to the lack of proper animal gonorrhoea infection models.

The first studies on a vaccine against *N. gonorrhoeae* tested a lysed whole-cell vaccine. This vaccine had no effect compared to the placebo group (Greenberg *et al.* 1974). A whole-cell vaccine prepared from a single strain of *N. gonorrhoeae* was tested on the Aboriginal population of Inuit in northern Canada. Before vaccination, the annual prevalence of infection was 25%, while after immunization, it was 30%, showing that the vaccine was ineffective (Greenberg *et al.* 1974). Another vaccine was the pilus vaccine tested on Americans stationed in Korea. And this vaccine was not successful. The infec-

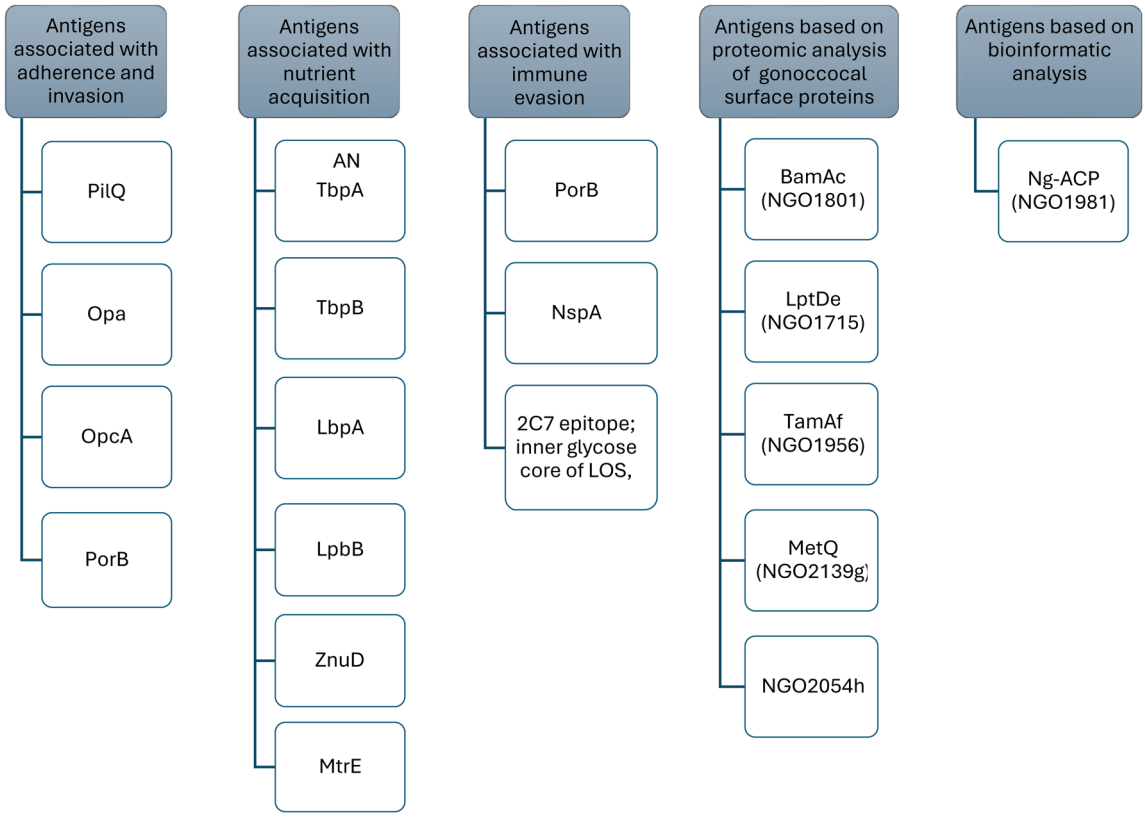


Fig. 3. Diagram of different groups of *Neisseria gonorrhoeae* vaccine candidates that induce antibodies, according to Rice et al. (2017).

tion rate was 6.9% in those receiving the vaccine and 6.5% in those receiving a placeb (Boslego *et al.* 1991).

Despite this, several promising gonococcal vaccine candidates have been identified (Fig. 3) using different research approaches. Zielke *et al.* described five potent vaccine candidates using a proteomics-driven approach, demonstrating that homologs of BamA (NGO1801), LptD (NGO1715), and TamA (NGO1956), and two uncharacterized proteins, NGO2054 and NGO2139, secreted in naturally released OMVs induce bactericidal antibodies cross-reacting with a panel of *N. gonorrhoeae* isolates (2016). In addition, the OMV vaccine against *N. meningitidis* serogroup B (MeNZB) was associated with decreased gonorrhoea rates in New Zealand. After the vaccination of over 1 million people between 2004 and 2008 with MeNZB, vaccinated individuals were significantly less likely to contract gonorrhoea compared with unvaccinated controls, with a predicted vaccine efficacy of 31% as described by Petousis-Harris *et al.* in a retrospective cohort study (Petousis-Harris *et al.* 2017). A similar prevention effect of gonorrhoea infections was observed after using the vaccine based on extracellular membrane proteins of meningococci serogroup B (Petousis-Harris *et al.* 2017). In Quebec, Canada, vaccination with 4CMenB, another anti-meningococcal vaccine, decreased the incidence of gonorrhoea (Longtin *et al.* 2017). The study conducted by Abara *et al.* again proved that MenB-4C multi-component, protein-based vaccine immunization was associated with a reduced incidence of gonorrhoea (Abara *et al.* 2022). Due to the finding that meningococcal B OMV vaccines may induce functional antibodies against gonococci (Semchenko *et al.* 2019) and the results of observational studies reporting reduced rates of gonorrhoea following the anti-meningococcal vaccination (Whelan *et al.*, 2016; Longtin *et al.*, 2017, Ochoa-Azze,) the attempts to create anti-gonococcal vaccine have been revived. Table I shows the current research on the vaccine development, approved by NIH US (National Institutes of Health USA, 2024). Further studies are needed to understand the antibody and T-cell responses to natural gonococcal infection in humans. Emerging vaccines must protect against a wide range of heterologous strains. A report suggests that the vaccine should induce a Th1 polarized response for protection (Liu *et al.* 2011; Liu *et al.* 2018; Belcher *et al.* 2023). In the future, the challenge will be the selection of specific target proteins because research shows that one of the three recombinant 4CMenB proteins is involved in the cross-protection process. However, with further discoveries regarding the pathogenesis of gonorrhoea and new vaccine antigens showing activity in preclinical studies, new studies on vaccines directly targeting *N. gonorrhoeae* will likely be designed in the future.

Table I
Research projects on gonorrhoea vaccines approved by National Institutes of Health USA
(National Institutes of Health USA, 2024.)

Project	Organization Project Leader(s)
The Gonorrhoea Vaccine Cooperative Research Center	HENRY M. JACKSON FDN FOR THE ADV MIL/MED Ann E. Jerse
Proteomics-Driven Reverse Vaccinology for Gonorrhoea	OREGON STATE UNIVERSITY Aleksandra E. Sikora
A Novel Sublingual Vaccine to Prevent *Neisseria gonorrhoeae* Infection	VIRTICI, LLC Neil A. Fanger,
STI Clinical Trials Group: Phase 2 Clinical Trial to Evaluate a Vaccine Candidate for *Neisseria gonorrhoeae*	UNIVERSITY OF ALABAMA AT BIRMINGHAM Edward Hook
Pre-clinical Vaccine Development for Emerging and Re-emerging Infectious Diseases	NIAID EXTRAMURAL ACTIVITIES Wing Pui Kong
Starve and Kill: Engineered Antigens Targeting Nutrient Acquisition Pathways Essential for Gonococcal Infection and Disease	GEORGIA STATE UNIVERSITY Cynthia N. Cornelissen
Development of nucleic acid-based vaccines against gonorrhoea	UNIVERSITY OF MASSACHUSETTS MED SCH WORCESTER Lisa Ann Lewis, Li Li, Sanjay Ram
VesiVax Vaccine Formulation Against *Neisseria gonorrhoeae*	MOLECULAR EXPRESS, INC. Gary Fujii
A novel vaccine against multidrug-resistant gonorrhoea	UNIVERSITY OF MASSACHUSETTS MED SCHOOL WORCESTER Sanjay Ram, Peter A. Rice
Production of a Gonococcal Vaccine for Countering Antimicrobial Resistance	UNIVERSITY OF CALGARY Anthony B. Schryvers, Scott D Gray-Owen, Trevor F. Moraes
Novel vaccine antigens against *N. gonorrhoeae*	TUFTS UNIVERSITY BOSTON Paola Massari

5. Conclusion

According to WHO estimations, in 2020, there were 374 million new infections in people aged 15–49 years with one of four curable STIs: chlamydia, gonorrhoea, syphilis and trichomoniasis. Unfortunately, the general knowledge of STI prevention is insufficient in many parts of the globe. As part of its mission, WHO supports countries to develop national strategic plans and guidelines, create an encouraging environment allowing them to discuss STIs, adopt safer sexual practices, and provide treatment in case of infection. Persistently increasing gonococcal antimicrobial resistance could lead to untreatable gonorrhoea in the future. For that, the WHO has awarded *N. gonorrhoeae* "superbug" status and has planned to prevent further gonococcal infections from spreading and make essential efforts to limit their incidence. Gonorrhoea prevention based on anti-gonococcal immunization is not available yet, as attempts to develop an effective vaccine have failed. Despite these, the undoubted constant progress in medicine and molecular biology, new *in vitro* and animal models, modern approaches in immunology, mathematical modelling, and especially genomics and proteomics development will hopefully succeed in combating gonorrhoea and other STDs. The step-by-step process of anti-gonococcal vaccine research should move us towards finding effective immunological protection. The global strategy of human papillomavirus vaccination proves that immunization might be both an efficient and socially approved tool for STD prevention. Currently, gonococcal infection fast diagnostic, AMR surveillance programmes, rational antibiotic therapy based on recommendations, and clinical microbiology and pharmacology knowledge are still crucial to controlling gonorrhoea. Given the presented facts, the need for further research on gonococcal biology, pathogenicity, antimicrobial resistance mechanisms, and vaccine development should be emphasized.

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The authors do not report any financial or personal connections with other persons or organizations, which might negatively affect the contents of this publication and/or claim authorship rights to this publication.

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