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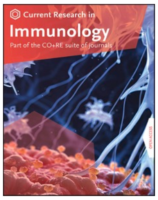

Battle royale: Immune response on biofilms – host-pathogen interactions

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ABSTRACT

The research interest of the scientific community in biofilm-forming microorganisms is growing due to the problems caused by their infections affecting humans and animals, mainly because of the difficulty of the host immune system in eradicating these microbial complex communities and the increasing antimicrobial resistance rates worldwide. This review describes the virulence factors and their interaction with the microbial communities of four well-known and highly biofilm-forming pathogens, more exactly, *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus* spp., and *Candida* spp. The innate and adaptive immune responses caused by the infection with these microorganisms and their evasion to the host immune system by biofilm formation are discussed in the present work. The relevance of the differences in the expression of certain virulence factors and the immune response in biofilm-associated infections when compared to planktonic infections is usually described as the biofilm architecture protects the pathogen and alters the host immune responses, here we extensively discussed these mechanisms.

1. Introduction

Microbial biofilms are communities of microorganisms organized in a matrix of extracellular substances with the presence of persistent cells and highly structural organization, associated with a phenotypic shift expression, and irreversibly adhered to a biotic or abiotic surface that contributes to the protection of microorganisms against extreme conditions such as environment, administration of antibiotics and antifungals, and host immune mechanisms in response to infection (Atiencia-Carrera et al., 2022; Cangui-Panchi et al., 2022). The importance of studies on biofilm-forming strains lies in the increased resistance of these organisms to antimicrobial agents, the severity of infections that they can cause in humans and animals due to their difficult eradication, and the survival of microorganisms on abiotic surfaces like medical devices (Yin et al., 2019).

The host's immune response is triggered by the detection of various virulence factors of microorganisms which are the necessary traits to establish an infectious process and interact directly with host cells. On the other hand, pathogenic microorganisms enhance immune response evasion to survive in a hostile environment and spread to different tissues (Staniszewska, 2020). Several studies carried out on biofilm-forming species have determined that certain virulence factors can positively contribute to biofilm formation of these microbial communities and trigger major problems related to existing antimicrobials resistance and pathogenic microorganisms' survival on abiotic surfaces (Atiencia-Carrera et al., 2022; Holm et al., 2015; Phillips and Schultz, 2012; Schroeder et al., 2017). For this reason, the main goal of this review is to compile the available information about the host immune response during infections with biofilm-forming strains and its relationship with the expression of other virulence factors. For this analysis, four well-known pathogens usually associated with biofilm formation and causing infections in humans and animals have been chosen (Cangui-Panchi et al., 2022), more exactly *Pseudomonas aeruginosa*, *Escherichia coli*, *Staphylococcus* spp., and *Candida* spp.

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1.1. *Pseudomonas aeruginosa*

P. aeruginosa is one of the most important pathogens involved in nosocomial infections, which can be found in different environments, and can form strong biofilms as monospecies and multispecies (Jeske et al., 2022; Reynolds and Kollef, 2021). Patients in hospitals with compromised immune systems, burns, and cystic fibrosis are affected by this bacterium, causing activation of extensive immune system pathways leading to tissue damage and a long-term infection (Gharieb et al., 2022; Rajabi et al., 2022). Indeed humans, have been directly affected by *P. aeruginosa*, but it is also present in animals, inert surfaces, and food, as domestic animals, seafood, and medical devices, increasing its ability to infect when it forms biofilms (Gharieb et al., 2022; Płókarz et al., 2022; Shahrokhi et al., 2022).

P. aeruginosa represented around 8% of healthcare infections in the urinary tract, skin, and lung abscesses among others, its various mechanisms of antibiotics resistance, including carbapenems, aminoglycosides, and cephalosporins, became a topic of international interest to researchers worldwide. The understanding of bacterial virulence factors and host immune response to infections caused by biofilms of this pathogen is of extreme interest and so we summarized the main virulence factors associated with *P. aeruginosa*-related biofilm infections in Table 1 (Gharieb et al., 2022; Weiner et al., 2016).

P. aeruginosa develops biofilm on a variety of surfaces, as living and inners, becoming resistant to different antibacterial agents, biofilm composition includes a variety of elements, such as proteins, exopolysaccharides, and DNA, among other structures (Rajabi et al., 2022) that are further discussed in the review.

Two types of mechanisms in the motility of *P. aeruginosa* have been identified involving type IV pili and flagellum. As well-known, type IV pili is associated with adhesion and motility as well as the organization of the microcolonies, which helps the formation of the biofilm (Talà et al., 2019). On the other hand, the flagellum contributes to the biofilm formation and maturation, providing a successful biofilm, where it has been demonstrated that *P. aeruginosa* mutants of type IV pili and flagellum cannot develop a precise structure, forming a weak biofilm that is susceptible to antibiotics. Employing confocal laser scanning microscopy, micrographs suggested an incomplete matrix of the biofilm and deficient chemotaxis among colonies (Barken et al., 2008). Moreover, pyocyanin and pyoverdine are special virulence factors in the *Pseudomonas* genus that are responsible to give colonies a representative color and obtain extracellular iron from the environment and host proteins in different ways (H. Li et al., 2017). In a mouse model infection, lung cells were affected by both virulence factors, preventing also phagocytosis process and suppressing cytokines activity, where lung tissue evidenced morphological changes (metaplasia and hyperplasia) and thus contributing to severe infection by the destruction of air spaces in the alveolus and cytotoxicity of the host (Farrant et al., 2020), as illustrated in Fig. 1A.

Lipopolysaccharides (LPS) are recognized as pathogen-associated molecular patterns (PAMPs) and considered one of the most potent activators of the immune responses, which lead Toll-Like Receptor 4 (TLR4) signal pathway and, in some cases, a hyperinflammatory response. In the biofilm state, LPS undergoes structural modifications affecting the lipid A and polysaccharide moieties promoting a higher production of tumor necrosis factor (TNF) and interleukin-6 (IL-6) than their planktonic counterpart and partially contributing to an increase in the inflammatory response. Therefore, the immune response is not effective because of LPS immunogenicity and its structural variations, as evaluated in mice model with a knockout of TLR5, leading to a severe lung infection and showing the hypersusceptible of the immune system host against the pathogen (Pier, 2007; Raoust et al., 2009). However, even with the presence of TLR5, the immune system is not enough to control the infection after biofilm formation.

During biofilm development, quorum sensing (QS) is one of the most important pathways of intercellular communication in *P. aeruginosa*. In fact, after the lecture on different cytokines of the host immune system, *P. aeruginosa* biofilm can change the expression of multiple genes and proteins such as protease IV and elastases LasA and LasB, preventing many mechanisms of degradation, evading the immune response, and producing tissue damage in lungs. As previously reported in studies, inactivation of the mentioned genes or proteins decreases biofilm formation and QS signals, promoting phagocytosis (Alayande et al., 2018; Holm et al., 2015), as shown in Fig. 1B. During biofilm-associated *P. aeruginosa* infection, elastases are also able to promote inflammation and tissue damage, changing the cell morphology and causing the death of macrophages and neutrophils, thus neutralizing the innate immune response and leading to the colonization of new tissues by dispersed planktonic cells (from previously formed biofilms) into new *P. aeruginosa* biofilms (Drusano et al., 2011; Yeung et al., 2014).

Table 1
Virulence factors of *P. aeruginosa* species related to biofilm formation.

Virulence factor	Virulence genes	Mechanism of action of the virulence factor	Association with biofilm formation	Immune response evasion	References
Structural					
Type IV pili	*pilH* and *pilA*	Mediates bacterial adhesion, and colonization	Facilitates the biofilm-forming and QS circuit	N/A	(Barken et al., 2008; K. Li et al., 2013)
Flagellum	*flgB, flgE, flgG, fliC*, and *fliI*	Mediates the adherence to epithelial cells	Facilitates the biofilm-forming and motility	N/A	Barken et al. (2008)
LPS	N/A	Stimulates of immune response	Facilitates the adherence and biofilm formation	Prevents the phagocytosis	(Lam et al., 2011; Raoust et al., 2009)
Quorum sensing proteins	*las* and *rhl*	Regulates host inflammatory responses	Facilitates the biofilm formation and infection	Inhibits cytokines	(Alayande et al., 2018; Luo et al., 2017)
T3SS	*exoU, exoT, exoS*, and *exoY*	Inhibits host defense after invasive infection by cell death	Facilitates the biofilm formation and infection	Leads rapid death of host cells	(Chung et al., 2013; K. Li et al., 2013)
Enzymatic					
Elastases LasA and LasB	*lasR* and *rhlR*	Mediates the increment of inflammation and tissue damage	Facilitates the biofilm infection in cystic fibrosis	Degrades IgA and IgG	Luo et al. (2017)
Protease IV	*lasR* and *rhlR*	Degrades immunoglobulins, inhibition of complement proteins, and fibrinogen	Protects the biofilm	Prevents opsonization and neutrophil phagocytosis	(Holm et al., 2016; H. Li et al., 2017)
Toxin					
Exotoxin A and B	*lasR* and *rhlR*	Inhibits eukaryotic protein synthesis leading to cell death	N/A	Leads repression of cytokines in the blood	(Jeske et al., 2022; H. Li et al., 2017)
Pyocyanin and pyoverdin	*lasR* and *rhlR*	Leads to tissue damage and necrosis of respiratory epithelium cells	Facilitate the biofilm formation and QS communication	Inhibits TNF-α and IL-1 in macrophages	(She et al., 2020; Stellari et al., 2015)

Legend – QS: quorum sensing; T3SS: type three secretion system; TNF-α: tumor necrosis factor-alpha; IL: interleukin, MIP-2: macrophage inflammatory protein; C: complement component; Ig; immunoglobulin; N/A: Not Available.

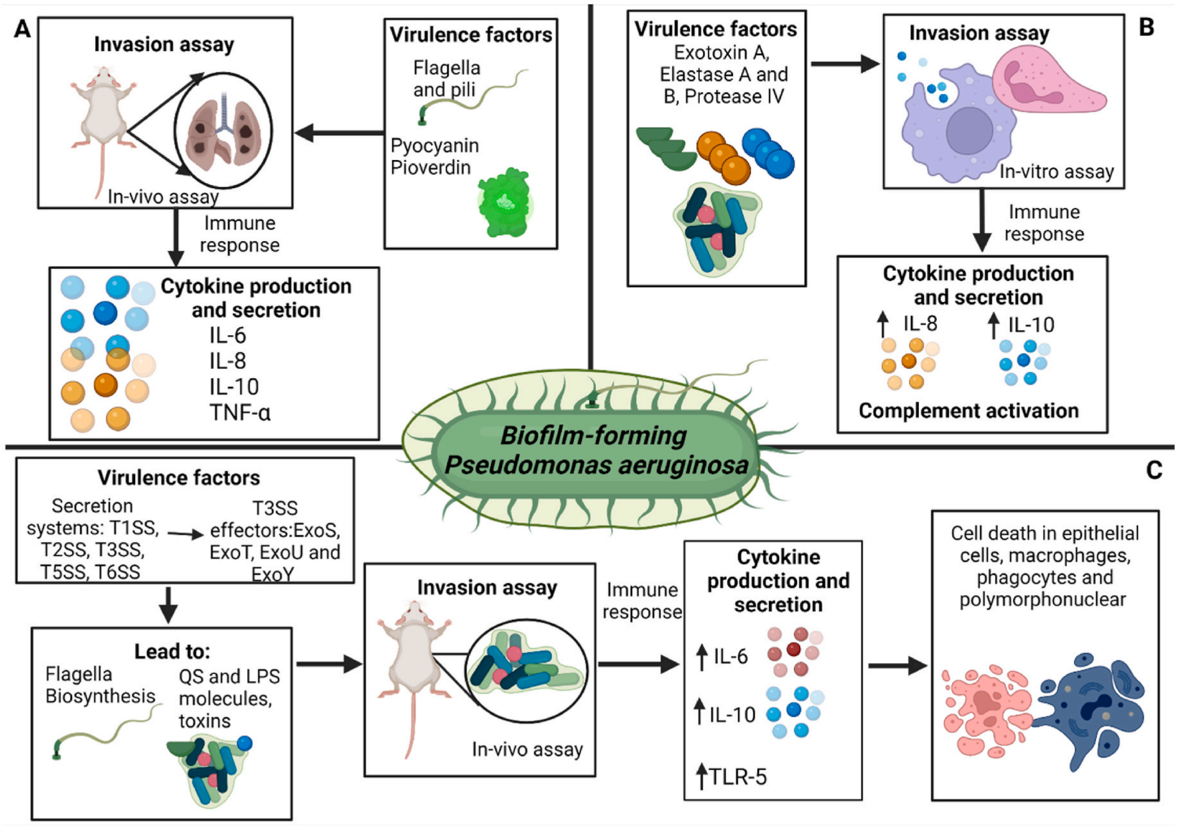

Fig. 1. Host immune response against virulence factors of biofilm-forming *P. aeruginosa* strains.

Meanwhile, during the inflammation, protease IV degrades complement components and immunoglobulins, blocking the opsonization and complement pathways. The combined virulence factors expressed in the biofilm-associated *P. aeruginosa* infection are capable of totally evading the innate immune system and partially adaptive humoral response incrementing also host tissue damage (Mauch et al., 2018).

Furthermore, the type III secretion system (T3SS) is a membrane-embedded virulence apparatus found in several Gram-negative bacteria to inject toxins and other effector proteins directly into eukaryotic cells, being one of the most important virulence factors. It translocates a specific subset of exotoxin effector proteins (ExoU, ExoT, ExoS, and ExoY), and this subset is directly deposited in the host cells, inducing pathogenesis, as a disruption of cellular signaling, causing cell death in phagocytes and epithelial cells (Chung et al., 2013). T3SS plays a role during the survival strategy under environmental stresses and several studies already demonstrated that its absence reduces motility and consequently biofilm formation (Chung et al., 2013; Zhu et al., 2016), which is represented in Fig. 1C. Other examples of injected toxins by T3SS in biofilm-associated *P. aeruginosa* infection are exotoxins A and B being potent exocellular components and leading to cellular toxicity in the host. When released by T3SS into eukaryotic cells, exotoxins A and B inhibit protein synthesis and also interfere with immune cells, facilitating the development of biofilms in the host (Shadman et al., 2021).

Host immune responses against *P. aeruginosa* infection are various, which can be summarized in terms of pro-inflammatory cytokine production and recognition pathways regarding the virulence factors of the bacteria and the recognition of PAMPs. One of the initial immune system activations is through the activation of TLR5 after the first virulence factors exposure, such as flagellin components, limiting *P. aeruginosa* motility (BenMohamed et al., 2014). In murine assays, alveolar macrophages lead the secretion of cytokines such as TNF-α, IL-1β, and other interleukins responding to the exposure by exotoxins, T3SS, and LPS structures during biofilm development (Chung et al., 2013; Mijares et al., 2011; She et al., 2020). Massive recruitment of neutrophils happens in the lungs when infected or colonized by *P. aeruginosa*, however, neutrophils are not able to eradicate the initial infection and, when the biofilm is established, the activation of complement components cannot modulate the infection, suggesting that *P. aeruginosa*-associated biofilm is not susceptible against opsonization, as well as other mechanisms of host immune system (Mauch et al., 2018; Shaikh et al., 2022). The compilation of host immune responses against *P. aeruginosa*-associated biofilm is summarized in Table 2. It is important to mention that IL-10 plays an important role in lung infections, being responsible for maintaining an inflammatory restriction of the immune response to prevent tissue damage and anaphylaxis, but also limits immune efficiency against *P. aeruginosa* infection and facilitates bacterial adhesion to the mucosal epithelium, tissue damage, and biofilm establishment (Belo et al., 2021; Stellari et al., 2015).

1.2. *Staphylococcus spp.*

The genus *Staphylococcus* includes a diverse group of Gram-positive commensal bacteria, the relevant species from this genus are *S. epidermidis* and *S. aureus*. These bacteria are usually benign and colonize the skin and mucous membranes of mammals, but under host immunocompromised conditions become pathogens (Stefani and Goglio, 2010). These results in a life-threatening problem when opportunist bacteria form biofilms into medical devices such as intravenous catheters and lead to chronic diseases. The common main characters of nosocomial infections acquiring in a hospital are *S. aureus* and *S. epidermidis* which can also form biofilms *in vivo*. *S. aureus* is the most common cause of worldwide infections highly related to various types of infections from no severe infections to pneumonia and even sepsis (Tong et al., 2015). The ability to attach to foreign material and form biofilms allows these bacteria to cause device-related infections and acquired resistance to immune response (Scherr et al., 2014). Additionally,

Table 2
Host immune responses to biofilm-forming *P. aeruginosa* strains.

Molecules or pathways	Host immune response	References
Proinflammatory cytokines		
IL-6	Stimulation of host response with serin proteases and macrophages leads to the neutrophil recruitment and secretion of chemokines MIP-2	Farrant et al. (2020)
IL-8	Recognition of bacteria by neutrophils, stimulation of host response with serin proteases, contributing to the tissue inflammation during infection	Holm et al. (2016)
IL-10	Production of the anti-inflammatory cytokines attenuates the pro-inflammatory effects after bacteria colonization and virulence factors expression	Belo et al. (2021)
TNF-α	Recognition by macrophages and PMNs, the liberation of reactive oxygen and nitric oxide, proinflammatory effects, and anti-inflammatory molecules	(Holm et al., 2015; H. Li et al., 2017)
IL-lβ	Secretion of the proinflammatory cytokines after induction of caspase-1 in pulmonary infections	(Jyot et al., 2011; Mijares et al., 2011)
Signaling pathways		
TLR4	Recognition by the host initiates the protective responses	Stellari et al. (2015)
TLR5	Recognition by the host initiates the protective responses and limits the inflammatory responses	BenMohamed et al. (2014)
C3a, C5a	Production of inflammatory effects and opsonization in lungs after bacterial infection	Mauch et al. (2018)
PAMPs	Production and recognition by macrophages, keratinocytes, and granulocytes	(Drusano et al., 2011; She et al., 2020)
Th17	Production of proinflammatory cytokines	Shaikh et al. (2022)

Legend – MIP-2: macrophage inflammatory protein-2; IL: interleukin; TNF-α; tumor necrosis factor-alpha; PMNs: polymorphonuclear neutrophils; TLR: Toll-like receptors; PAMPs: pathogen-associated molecular pattern molecules; Th: T helper cells.

S. epidermidis is an opportunistic pathogen that lives in human skin or mucosa by standard and is considered harmless and commensal bacterium, but it became a pathogen when can colonize medical devices as prosthetic devices and forms biofilm allowing so a protection against immune response and antimicrobials (Lee and Anjum, 2022).

Staphylococcus genus possesses different virulence factors depending on the species (Chessa et al., 2016). Several virulence factors allow these species to disperse and became pathogens under favorable conditions. The presence of *Staphylococcus* genus around mammals' bodies permits them to be close to whatever wound that host shows and take the opportunity to invade and form biofilm. *S. aureus* and *S. epidermidis* present different virulence factors as shown in Table 3.

Biofilm production is essential to colonize and develop an infection able to successfully evade the immune system, involving many virulence factors in this process. Biofilm formation allows proper colonization, evasion of the immune response, and antimicrobial resistance (Kiedrowski and Horswill, 2011). In *S. aureus*-associated infections, the establishment of biofilms on medical devices plays a key role in the dissemination of this pathogen. Chessa and colleagues evaluated Staphylococcus isolates from eighty breast implants and demonstrates that *S. aureus* strains achieved strong biofilm production maintaining high cell viability for a long extension of time and that certain S. epidermidis strains produced higher levels of biofilm equally as the most virulent S. aureus isolates being also classified as strong biofilm producers (Chessa et al., 2016). These findings suggest a strong common virulence background between Staphylococcus species allowing biofilm formation and avoiding bacterial recognition by the innate immune response (Chessa et al., 2016), as illustrated in Fig. 2. Biofilm is usually detected by the immune system through the presence of polysaccharide intercellular adhesin (PIA). It is well-known that most mastitis cases caused by *S. aureus* are also enhanced by biofilm production (Schönborn and Krömker, 2016). A study reveals that the production of biofilm could be regulated by autoinducer 2 (AI-2) of QS via an *icaR*-activation pathway, through inactivated *luxS* assays which unable to encode AI-2, leading to an increased PIA level and showing the association of biofilm formation with *rbf* expression as positive regulator (R. Ma et al., 2017).

Quorum sensing shows an important role in the expression of virulence factors. In Staphylococcus isolates, the *agr* QS system is extensively studied evidencing the production of certain virulence factors involved during biofilm-related infections through protease activity regulation. The *agr* QS system is known to coordinate the invasive mode of *Staphylococcus* species, increase the production of virulence factors (Table 3), and reduce surface or membrane proteins (Bezar et al., 2019). It allows *S. aureus* controls the synthesis of exoenzymes by regulating the proteolytic process (Kies et al., 2003; Tam and Torres, 2019). Several infection models demonstrated that inhibition of *agr* activation leads to virulence reduction during biofilm-associated infection (Fig. 2). The importance of *agr*-mediated expression of virulence genes has also been shown in several studies through *agr* operon, which consists of four genes: *agrB*, *agrD*, *agrC*, and *agrA* that encode the components of the QS system (Bezar et al., 2019). Moreover, the produced proteases allow *Staphylococcus* species to develop and exploit nutrition sources and play important roles in the evolution of the immune system by degrading numerous host proteins (Kies et al., 2003; Tam and Torres, 2019). Moreover, host immune responses are also able to produce proteases that help in degrading bacteria, although the main problem is the size of the biofilm which avoids phagocytosis. However, a recent study demonstrated that protease cathepsin G (CG) can degrade biofilms and allows polymorphonuclear neutrophils (PMN) to penetrate biofilm and phagocytose staphylococcal cells (Kavanaugh et al., 2021). CG is a well-known serine protease that controls the functional state of innate immunity cells and is traditionally associated as one of the effectors of inflammation and other physiological processes (digestion, smooth muscle contraction, epithelial renewal, tissue remodeling, and others) (Zamolodchikova et al., 2020). Several studies aval that staphylococcal biofilm is the biggest problem in bacterial invasion and severe infection worldwide.

As shown in Fig. 2A, Scherr and colleagues evaluated the phenotypic expression of biofilm-associated *Staphylococcus* infections against polynuclear lymphocytes (Scherr et al., 2013). The study showed how *S. aureus* biofilm can differentiate its gene expression (*sodA*, *saeS*, *saeR*, *agrB*, *rsbU*, *atl*, *recA*, and *nuc*) depending on the leukocyte subset encountered in the host, allowing us to comprehend why biofilms are harder to phagocytose by the innate immune system (macrophages and neutrophils), as the first line of immune defense against invading pathogens. In the case of macrophages, a comparison between 1 and 24 h macrophage action against *S. aureus* biofilms evidenced an upregulation of certain genes involved in metabolism (*gltS*), virulence (*spa*), cell wall (*pbp1*, *dat*, and *sdrD*), and transcription/translation/replication processes (*glnR*) while numerous genes were downregulated within the biofilm (*fabG*, *rpsA*, *hutG*, *rpmB*, *isdC*, *cap5E*, *nuc*, *epiE*, *arlR*, *asp23*, and *dmpI*). In contrast to macrophages, the exposure of *S. aureus* biofilms to neutrophils affects its gene transcription during 1–4 h, evidencing upregulation of metabolism (1h: *pyrE*, *feoB*, and *carB*; 4h: *purK*), virulence (1 h: *lytS*), and cell wall (4 h: *gltB*) but also downregulation of certain metabolism/regulation genes (*groES*, *grpE*, and *groEL*) (Scherr et al., 2013). Meanwhile, Peton and colleagues further evaluated the role of *sigS* regulator gene during biofilm-associated *Staphylococcus aureus* O11 mastitis by *in vivo* murine model measuring cytokines levels (Peton et al., 2016), as shown in Fig. 2B. When comparing to *S. aureus* O11 *ΔsigS* mutant, the *sigS* regulator gene in biofilms promoted the higher expression of IL-1α, IL-1β, and TNF-α by local innate response (Peton et al., 2016). Finally, Begun and colleagues showed that the

Table 3
Virulence factors of *Staphylococcus* species related to biofilm formation.

Virulence factor	Virulence gene	Strain isolates	Mechanism of action of the virulence factor	Association between biofilm and virulence factor	Evasion to immune response	References
Structural						
Surface Protein	*sasX*	*S. aureus* ST239-SCCmec III	Provides a successful establishment, colonization, immune evasion, and subsequent invasion.	High adherence to epithelial cells and abundant biofilms formation.	Allows intercellular aggregation and biofilm formation to enhance immune evasion mechanisms.	(Bakthavatchalam et al., 2019; De Backer et al., 2019; M. Li et al., 2012)
Surface Protein	*sesI*	*S. epidermidis* RP62A	Keeps adherence and invasion of uroepithelial cells	Abundant biofilms production and key role in the gathering phase	Permits a higher resistance to clinically antimicrobial agents and prevalence of *mecA* and *mupA* genes	Qi et al. (2018)
PIA	*icaADBC*	*S. epidermidis* 1457 and M10	Allows extracellular matrix material formation	Necessary to biofilm matrix formation	Inhibits bacterial killing and sequesters host proinflammatory products	Vuong et al. (2004)
Accumulation-associated protein	*aap*	*S. epidermidis* RP62A	Enhancer of exopolysaccharide-independent biofilm formation	Adherence and accumulation of *Staphylococcus epidermidis* strains on surfaces	Accumulation avoids phagocytoses	Alabdullatif and Ramirez-Arcos (2019)
Proteases	*sarA, sepA,* and *agr*	*S. epidermidis* 1457	Creates channels in the biofilm	Allows nutrient accessibility to different layers	Avoids human neutrophil elimination and degrades bactericidal AMPs	Lai et al. (2007)
Virulence Proteins	*nuc*	MRSA USA300	Thermonuclease precursor	Related to the reduction of sigma factor σ^B and inhibits biofilm formation	Avoids NET-mediated killing	(Kiedrowski et al., 2011; Thammavongsa et al., 2013)
Cyclophilin PpiB	*ppiB*	*S. aureus* MRSA USA300	Contributes to Nuc activity	Enhance biofilm formation	Improves virulence factors secretion	Keogh et al. (2018)
Sensor histidine kinase LytS	*lytS*	*S. aureus* MSSA SA113 and CHS101 *S. aureus* MRSA YUSA139 and YUSA145	Gene regulation	Regulates autolysis and adhesion of cells to biofilm	Adaptation to the external environment and regulate virulence gene expression	(Y. S. Liu et al., 2022; Zhang et al., 2022)
Teichoic acids	*tarS*	*S. aureus* ATCC 35556 wild types, *icaADBC* mutant, and *dltA* mutant	d-alanine-modified charged cell wall	The first step of biofilm formation	Resistance to antimicrobial host peptides	Gross et al. (2001)
Leukocidins	*pvl, lukAB, hlgAB, hlgCB,* and *lukED*	*S. aureus* USA300 LAC	Contributes to bacterial survival in wounds	Biofilm release leucocidins	Leukocytes destruction and necrosis production	(Bhattacharya et al., 2018; Golla et al., 2020)

Legend – PIA: polysaccharide intercellular adhesin; AMPs: antimicrobial peptides; MRSA: methicillin-resistant *Staphylococcus aureus*; MSSA: methicillin-sensitive *Staphylococcus aureus*; NET: neutrophil extracellular trap.

icaADBC locus, which synthesizes biofilm-associated polysaccharide intercellular adhesin (PIA) in staphylococci, is needed for the biofilm formation of a lethal *S. epidermidis* infection in *Caenorhabditis elegans* intestine nematode model (Begun et al., 2007), as shown in Fig. 2C. In this study, both *C. elegans* immunocompromised *sek-1(km4)* mutant and *C. elegans* Bristol N2 wild-type were infected by two different *S. epidermidis* strains, more exactly, *S. epidermidis* 9142 (PIA producer) and *S. epidermidis* 9142-M10 (non-PIA producer). The findings demonstrated that *S. epidermidis* 9142 were able to evade the immune responses of the immunocompetent *C. elegans* Bristol N2 through biofilm formation while *S. epidermidis* 9142-M10 was almost removed from the intestine of the *in vivo* model when compared to the *C. elegans* immunocompromised *sek-1(km4)* control that allowed a similar growth of both *S. epidermidis* strains (Begun et al., 2007).

Several immune system mechanisms (cytokines and signaling pathways) are frequently activated against pathogens' invasion and initial infection, as summarized in Table 4. Regarding initial infection and *Staphylococcus* planktonic cells, the immune system triggers a huge amount of macrophages and neutrophils, which can generally counteract the first stage of staphylococcal infection (Abdul Hamid et al., 2021). However, when an irreversible adhesion and initial biofilm are established, *Staphylococcus* biofilms can enhance the expression of numerous virulence factors reducing the ability of neutrophils and macrophages to eradicate staphylococcal cells of the host (Kavanaugh et al., 2021). It is important to mention that neutrophils and macrophages are unable to phagocyte biofilm staphylococcal cells even in the initial phase of biofilm formation (microcolonies).

Cytokines are necessary for different immune mechanisms against pathogens such as cytotoxic, humoral, cell-mediated, or allergic responses (Table 4). Microorganisms initially activate the production of IL-1 family members triggering several innate immune responses and acting also as mediators. However, IL-1 can also cause lethargy, sleep, and anorexia in patients (Jewett and Krueger, 2012). While TNF-α is related to hypotension of septic shock in patients besides its initial role as an activator and recruiter of phagocytes. During a *Staphylococcus* infection, the proinflammatory cytokines IL-1, TNF-α, and IL-6 lead to other cytokines production, phagocyte activation and recruitment, and promote the M1 macrophages phenotype. NF-κB pathway is immediately induced for the previous expression of proinflammatory cytokines, which is also related to the production of IL-2 necessary to control lymphocyte proliferation and differentiation (Ren et al., 2017). Another immune pathway induced to protect the host against *Staphylococcus*-associated infections is AIM2/ASC signaling pathway which acts as a filamentous signaling platform to prepare host defense against cytoplasmic dsDNA prevenient from pathogens and damaged organelles (Chen et al., 2021).

1.3. *Escherichia coli and pathotypes*

The importance of the different pathogenic variants of *Escherichia*

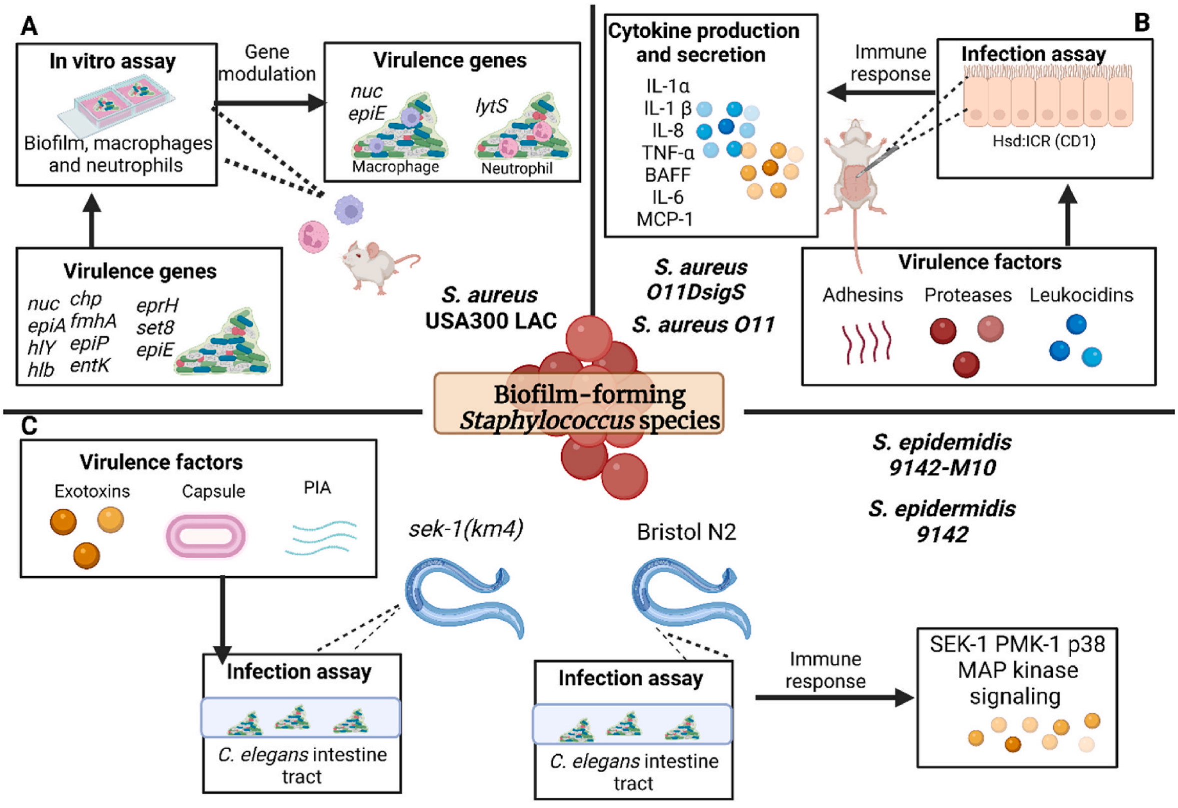

Fig. 2. Host immune response against virulence factors of biofilm-forming *Staphylococcus* species and strains.

coli resides in their ability to cause serious diseases in humans and animals that become difficult to eradicate by the formation of biofilms as metabolic and regulatory abilities of the pathotypes are increased making them more virulent and resistant to antibiotics, enhancing also their ability to evade immune response (Gunardi et al., 2021; Mittal et al., 2015; Sharma et al., 2016). This combination worsens infectious diseases caused by these enteropathogenic strains such as enteric syndromes, Crohn's disease, intestinal bleeding caused by toxin-producing intestinal pathogenic *E. coli* species (IPEC) or urinary tract infections (UTI), sepsis, meningitis prostatitis, and mastitis caused by extra-intestinal pathogenic *E. coli* species (ExPEC) (Leimbach et al., 2013). Most of the virulence factors of the different pathotypes of *Escherichia coli* contribute to the formation of biofilms, mainly in the adhesion and self-aggregation phases, thus promoting microbial growth, persistence at the site of infection, and evasion of the host immune response (Table 5). Biofilms are formed in favorable conditions with the help of several virulence factors like structural factors (type I pili or fimbriae), which provide *E. coli* with the motility necessary for interaction between planktonic cells and initial adhesion with biotic or abiotic surfaces to initiate the biofilm formation process (Conte et al., 2016; Martinez, 2000; Mittal et al., 2015; Nam, 2013). The extracellular matrix of the *E. coli*-associated biofilm is formed by different adhesin structures such as curli and cellulose fimbriae that contribute to establishing an irreversible adhesion and increase bacterial resistance enabling it to withstand adverse environmental conditions (Rochon and Römling, 2006; Tükel et al., 2010). This enhances the colonization of the epithelial mucosa and subsequent irreversible adhesion and microcolonies in various *E. coli* pathotypes (uropathogenic, enteropathogenic, and enteroaggregative *E. coli*) (Schiebel et al., 2017) or bacterial internalization into nearby host cells by forming intracellular bacterial communities (IBC) in uropathogenic *Escherichia coli* (UPEC) (Conover et al., 2016).

Several virulence factors that contribute to the formation of biofilms have been studied in their distinct phases, which vary according to the pathotypes of *E. coli*. From IPEC, the most studied is UPEC which causes urinary tract infections and can express a wide range of virulence factors like type I fimbriae, hemolysin, and transporters (sorbitol and cellulose) that cooperate in biofilm formation. Other virulence factors could also be associated with UPEC such as iron acquisition systems, protectins, and mycelia, whose genes are expressed at high levels in biofilm-forming isolates, and contribute to pathogen virulence in the processes of colonization, invasion, and protection of the bacteria against host immune response cascades and cytokines (Conte et al., 2016; Mittal et al., 2015). Likewise, adherent invasive *E. coli* (AIEC) strains are strong biofilm producers able of adhering to, invading, and surviving within epithelial cells (Martinez-Medina et al., 2009). Most virulence factors are similar to those reported in UPEC strains, but the main contribution to AIEC-associated biofilm formation is the IbeA protein (i.e., invasion of the brain endothelium protein A), which is involved in colonization and proliferation processes in infections such as prostatitis in humans (Cieza et al., 2015; Conte et al., 2016), respiratory tract infections, pericarditis, perihepatitis, peritonitis, and salpingitis in birds with the avian pathogenic *E. coli* (APEC) strain (Pilatti et al., 2016; Wang et al., 2011).

Among ExPEC strains, the enterohemorrhagic *E. coli* (EHEC) pathotype is responsible for causing hemorrhagic colitis, hemolytic uremic syndrome, and thrombotic thrombocytopenic purpura, contributing to biofilm formation through cell-cell self-aggregation by expression of the *ehaA* and *ehaB* genes (Wells et al., 2008, 2009). Although EhaA and EhaB (also called UpaC) proteins are both autotransporters and important virulence factors, EhaA is also associated with EHEC, enteroaggregative *E. coli* (EAEC), and enteroaggregative hemorrhagic *E. coli* (EAHEC) isolates while EhaB is found predominantly in ExPEC and B2 commensal strains (Clark and Maresso, 2021). Recently, Clark and Maresso suggested that *ehaA* and *ehaB* genes are important virulence factors for intestinal pathogenesis, and probably *ehaB* gene is an adherent invasive *E. coli* (AIEC)-associated virulence factor (Clark and Maresso, 2021). So, these proteins constitute an important mechanism for adhesion and biofilm formation. However, the relationship between

Table 4
Host immune response to biofilm-forming *Staphylococcus* species.

Molecules or pathways	Host immune response	References
Proinflammatory cytokines		
IL-1β	Promotes an immune response against invading microorganisms through caspase-1 activation	(J. Ma et al., 2017)
TNF-α	Triggers the transcription of nuclear factor-κB (NF-κB) and influences on proliferation and differentiation of B cells	(Aggarwal et al., 2012; J. Ma et al., 2017)
IL-6	Regulates the immune acute response, hemopoiesis, and inflammation	(Chen et al., 2021; Mihara et al., 2012)
IL-8	Major chemoattractant for multiple immune cells	(Baggiolini and Clark-Lewis, 1992; Tajima et al., 2006)
Signaling pathways		
NF-κB pathway	Expression of proinflammatory genes including chemokines, adhesion molecules, and cytokines (IL)-2, IL-12, and interferon-γ that control lymphocyte proliferation and differentiation	(Ren et al., 2017; Yamamoto and Gaynor, 2001)
AIM2/ASC signaling pathway	Triggers caspase-8-dependent apoptosis	Pierini et al. (2012)
TLR2	Activates NF-κB, increases the production of proinflammatory cytokines (e.g., TNF-α, IL-1β, and IL-6), and promotes M1 macrophages phenotype characterized by elevated expression of iNOS	(Di Lorenzo et al., 2020; Qian et al., 2012)
AMPK/Nrf2	Promotes M2 macrophage phenotype and exerts an anti-inflammatory effect	(Y.-X. Wu et al., 2021)

Legend – IL: interleukin; TNF-α; tumor necrosis factor-alpha; TLR: Toll-like receptors; iNOS: inducible nitric oxide synthase; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; AIM2/ASC: absent in melanoma 2/apoptosis-associated speck-like protein containing a CARD; AMPK/Nrf2: AMP-activated kinase/nuclear factor erythroid 2-related factor 2.

different virulence factors of other *E. coli* species and biofilm formation remains to be elucidated and further studies must be realized.

Several studies have focused on determining the host immune responses against biofilm-forming *E. coli* strains (Mittal et al., 2015; Sharma et al., 2016; Tükel et al., 2010), which in most cases are induced by the detection of different virulence factors, such as group 2 capsule antigens, iron acquisition proteins (FyuA, IutA, and Sit), adhesins (SinH, Afa, Pap, Sfa, and Iha), and numerous toxins (Usp, Sat, Vat, Cdt, Cnf1, and HlyA) (Clark and Maresso, 2021; Crémet et al., 2016). Nam studied the interaction between canine UPEC species and human bladder epithelial cells by determining the presence of virulence factors, evaluating biofilm formation, and their association with host immune responses (Nam, 2013). The findings showed a high production of proinflammatory cytokines IL-6 and IL-8 and the high zoonotic potential of these strains (Fig. 3A). A similar study was conducted by Conte and colleagues, where the ability of biofilm-forming AIEC strains to infect prostate cells as an extraintestinal target was evaluated (Conte et al., 2016), showing the increase of the same cytokines (IL-6 and IL-8) and strong inflammatory response that induced phosphorylation of mitogen-activated protein kinases and NF-κB pathways (Fig. 3B). A previous study evidenced that curli fimbriae act as an adhesive component with flagellin during biofilm formation in *E. coli*, which triggers the immune response by infecting epithelial cells and thus causes a significant release of IL-8 (Rochon and Römling, 2006), as shown in Fig. 3C. Likewise, Wu and colleagues confirmed that some *E. coli* strains causing pyelonephritis improved their adhesion and colonization in the biofilm growth mode showing a better expression of type 1 fimbriae, hemolysins, and mannose (K.-Y. Wu et al., 2022). The higher expression of the virulence factors triggered inflammatory responses that attack epithelial cells, increasing the gene expression of proinflammatory cytokines (IL-6, IL-1β, CXCL1, and CCL2) and thus activating ERK1/2 and NF-κB signaling pathways (Fig. 3D).

As demonstrated by various studies, the host immune response varies depending on the *E. coli* pathotype since the expression of virulence factor genes is also different in each *E. coli* strain. A compilation of host immune responses (cytokines, pattern recognition receptors, signaling pathways, and adaptive responses) caused by biofilm-forming *E. coli* strains is summarized in Table 6.

Host immune system against *E. coli* strains is initiated by TLR stimulation (especially, TLR4) attracting polymorphonuclear neutrophils to the infection site and eliciting cytokines, chemokines, and inflammatory regulators (reactive oxygen and nitric oxide species) from infected epithelial cells and immune innate cells (Adamus-Białek et al., 2019; Mazzulli, 2002). Biofilm formation allows bacteria to protect themselves against the immune system and express multiple virulence factors to be internalized in nearby cells, persisting inside damaged cells and surviving on different surfaces by adapting the environment according to the needs of the microbial species that make up the biofilms (Jarry et al., 2015).

1.4. *Candida spp.*

Fungi belonging to *Candida* genus usually play a commensal role, mainly colonizing the skin, vaginal, gastrointestinal, and pharyngeal cavities, composing a fundamental part of the host's normal or healthy microbiota. Certain conditions can disrupt the homeostasis of *Candida* spp. causing it to undergo a transition from commensal to an opportunistic pathogen. Reported clinical manifestations of *Candida*-related infection range from superficial and localized diseases to fatal candidiasis involving multiple organs and systems (Atiencia-Carrera et al., 2022; Salinas et al., 2020). In several studies, the latter infections are associated with a 50% mortality rate due to the presence of different virulence factors and resistance to first-line antifungals (Atiencia-Carrera et al., 2022; Atriwal et al., 2021; Cangui-Panchi et al., 2022; Pohl, 2022). Over the years, the increased mortality and morbidity of these infections have been associated with the presence of biofilm on both host and abiotic surfaces. The importance of the study of biofilm generated by *Candida* spp. and its role in disease development lies in the characterization of new pathogenic species happening around the world where each one of these exhibits differences in terms of virulence and biofilm formation (Atiencia-Carrera et al., 2022; Nett, 2016).

Candida species are well-known to colonize and invade different anatomical sites with unique physiological environments due to their ability to form biofilms, possessing diverse types of virulence factors. Structural virulence factors are directly related to biofilm formation controlling the phenotypic shift expression for yeast-to-hyphal change under specific niche growth conditions (S. Y. Liu et al., 2022). The ability to switch from yeast to hyphal is a common strategy adopted by *Candida* species to adapt to diverse environments. Although all *Candida* species have the potential for filamentous growth, it is assumed that some *Candida* species are not able to phenotypic switching during infections (Kadosh and Mundodi, 2020). As shown in Table 7, phenotypical switching has been repeatedly reported on *C. albicans, C. glabrata,* and *C. tropicalis*, however, cases of filamentation have been reported for *C. auris* (Yue et al., 2018). Villa and colleagues showed that *Candida albicans* can develop a cascade of gene expression, triggering morphological changes that cause filamentous growth (Villa et al., 2020). Several genes (*ASH1*, *ACE2*, *EFG1*, *FLO8*, and *NDT80*) are overexpressed in the presence of certain substrates like albumin and glycoprotein breakdown derivatives (N-acetylglucosamine and proline). Likewise, the typical temperature range of a fever (38–39 °C) and an acid pH like the oxidative stress induced by reactive oxygen species (ROS) promote filamentation and hyphal morphogenesis increasing *Candida* species' resistance to evade the host immune responses, such as phagocytosis by

Table 5
Virulence factors of *E. coli* species related to biofilm formation.

Virulence factor	Virulence gene	Strain isolates	Mechanism of action of virulence factor	Association with biofilm formation	Immune response evasion	References
Structural						
Type 1 pili or fimbriae	*fimH*	UPEC, AIEC	Mediates adherence to and invasion of uroepithelial cells and other host tissues	Involved in the early steps of biofilm formation	Triggers host cell signaling cascades that lead to bacterial internalization	(Conte et al., 2016; Martinez, 2000; Mittal et al., 2015; Nam, 2013)
Curli amyloid fibrils	*csgDEFG* and *csgBA* operons	*E. coli*	Directs the biosynthesis of extracellular structures composed of the CsgA protein	Major extracellular matrix component of the biofilm	Inactivates the TLR2 receptor making it unable to initiate inflammatory gene expression	(Rochon and Römling, 2006; Tükel et al., 2010)
IbeA	*ibeA*	APEC, AIEC	Associated with virulence factors involved in the invasion and adhesion process	Its function is not determined but it is known that it does not contribute to aggregation	Contributes to the spread and invasion of other cells causing systemic infections	(Cieza et al., 2015; Wang et al., 2011)
EhaA AT protein	*ehaA*	EHEC, EPEC	Contributes to adhesion to primary epithelial cells	Involved in cell-to-cell aggregation	Contributes to the spread and invasion of other tissues	(Clark and Maresso, 2021; Wells et al., 2008)
EhaB AT protein	*ehaB*	EHEC, AIEC	Mediates adherence to the ECM proteins collagen I and laminin	Contributes to biofilm growth	Contributes to the spread and invasion of other tissues	(Clark and Maresso, 2021; Wells et al., 2009)
IIC sorbitol-specific transporter	*srlA*	UPEC	Involved in sorbitol uptake	Sorbitol is assumed to serve as an energy source in biofilms	N/A	Conover et al. (2016)
Enzimatic						
Cellulose	*bcsA*	UPEC	Decreases adhesion to the uroepithelium to facilitate eradication from the urinary tract	Extracellular matrix component of the biofilm	Protects the bacteria from the action of neutrophils	Adamus-Białek et al. (2019)
Beta galactosidase	*lacZ*	UPEC	Cleaves a linkage between galactose and glucose for the utilization of these sugars	Beta-galactoside is assumed to serve as an energy source for biofilms	N/A	Conover et al. (2016)
Invasin	*ychO*	APEC	Important in pathogenicity, adhesion, and invasiveness	N/A	Contributes to the spread and invasion of other tissues	Pilatti et al. (2016)
Toxins						
Hemolysin A	*hlyA*	UPEC	Involved in the colonization of the urinary tract, lysis of host cells, and helps bacteria cross mucous membranes.	N/A	Contributes to the spread and invasion of other tissues	Engelsöy et al. (2019)

Legend – AT: autotransporter; ECM: extracellular matrix; UPEC: uropathogenic *E. coli*; AIEC: adherent invasive *E. coli*; EHEC: enterohemorrhagic *Escherichia coli*; APEC: avian pathogenic *Escherichia coli*; EPEC: enteropathogenic *E. coli*; N/A: not available.

macrophages and neutrophils (Villa et al., 2020). The ability of *C. albicans*, *C. tropicalis*, and *C. glabrata* to undergo morphological change has been well-known in the last decades (Kadosh and Mundodi, 2020). Recently, *Candida auris*, known to be a commensal microorganism of the skin, was found to also display a filamentous phenotype morphologically similar to true hyphae when exposed to mammalian cells of mucosal cavities (Yue et al., 2018). It has been hypothesized that this ability of morphological change allows *C. auris* to invade the epidermal layer, providing greater stability during the infectious process (Yue et al., 2018). In addition, all *Candida* species can produce melanin to a certain extent (García-Carnero et al., 2020). Melanin is produced by enzymatic oxidation of several aromatic precursors, being a pigment within the cell wall that alters its composition and physically avoids the PAMPs' recognition by immune receptors. It can be eventually released to the extracellular environment showing strong antioxidant properties that allow *Candida* species and biofilms to resist oxidative damage caused by the host macrophages and neutrophils (García-Carnero et al., 2020; Smith et al., 2022) and inactivate antifungal drugs, as well as, antimicrobial peptides and enzymes that the host immune system produces to eradicate fungi infections (Smith et al., 2022). Furthermore, enzymatic virulence factors comprise a set of proteins that serve a variety of functions, being their primary role to inflict damage and promote host colonization (Riceto et al., 2015). Secreted aspartyl proteinases (SAPs) are expressed to inflict damage on host cells and maintain a constant supply of nutrients for *Candida* cells' survival. In a recent study realized by Garcia-Bustos and colleagues, *C. auris* also expressed SAPs to promote biofilm formation allowing its nutrients supply maintenance and structural remodeling. The results showed that the SAPs enzymatic activity was functional even at high temperatures of 42 °C, being much higher than in *C. albicans* (Garcia-Bustos et al., 2022) These findings agree with previous reports on other *Candida* species (Yue et al., 2018), where the functionality of competent filamentous cells was based on the active SAPs secretion. Moreover, another study with *C. albicans* correlated SAP9 and SAP10 expression to maintain cell wall integrity with biofilm formation and their antifungal resistance (Kadry et al., 2018). This correlation was explained by active extrusion mechanisms allowing us to relate the phenotypic characteristics with the severity of biofilm-associated infection, as shown in Table 7. Meanwhile, hemolytic activity followed by iron acquisition facilitates tissue invasion in *Candida* species degrading hemoglobin and ferritin to acquire iron substrate (Wan et al., 2015). The hemolytic activity of different *Candida* isolates demonstrated alpha, beta, and gamma hemolytic activities (Noori et al., 2017). In fact, Noori and colleagues reported that *C. albicans* (22.7%), *C. glabrata* (63.6%), and *C. krusei* (50%) showed the highest rates of alpha, beta, and gamma hemolysin production in their study set, respectively. Likewise, iron extraction from ferritin enables biofilm formation as it plays the role of stabilizing the polysaccharide matrix. The acquisition of iron is facilitated by agglutinin-like sequence proteins being also involved in cell-to-cell cleavage and yeast adherence to host ferritin receptors (Chakraborty et al., 2020).

In 2022, Pokhrel and colleagues compared the virulence in *Candida albicans*-related infections between biofilm-forming and non-biofilm-forming isolates through an *in vivo* zebrafish larvae model (Pokhrel et al., 2022), as shown in Fig. 4A. Three *C. albicans* strains (140, 104, and 57) with phospholipase, protease, hemolytic, and biofilm-forming activity were evaluated. Hemolytic and biofilm-forming activities were demonstrated to be important virulence factors for *C. albicans*, showing a higher larvae mortality for *C. albicans* 140 and 57 (Pokhrel et al., 2022). In addition, a similar study conducted in approximately seven-month-old zebrafish with *C. albicans* infection showed the augmentation of IL-1β, TNF-α, and inducible nitric oxide synthase in the 2–15 h post-infection window evidencing a peak expression at 8 h (Chao

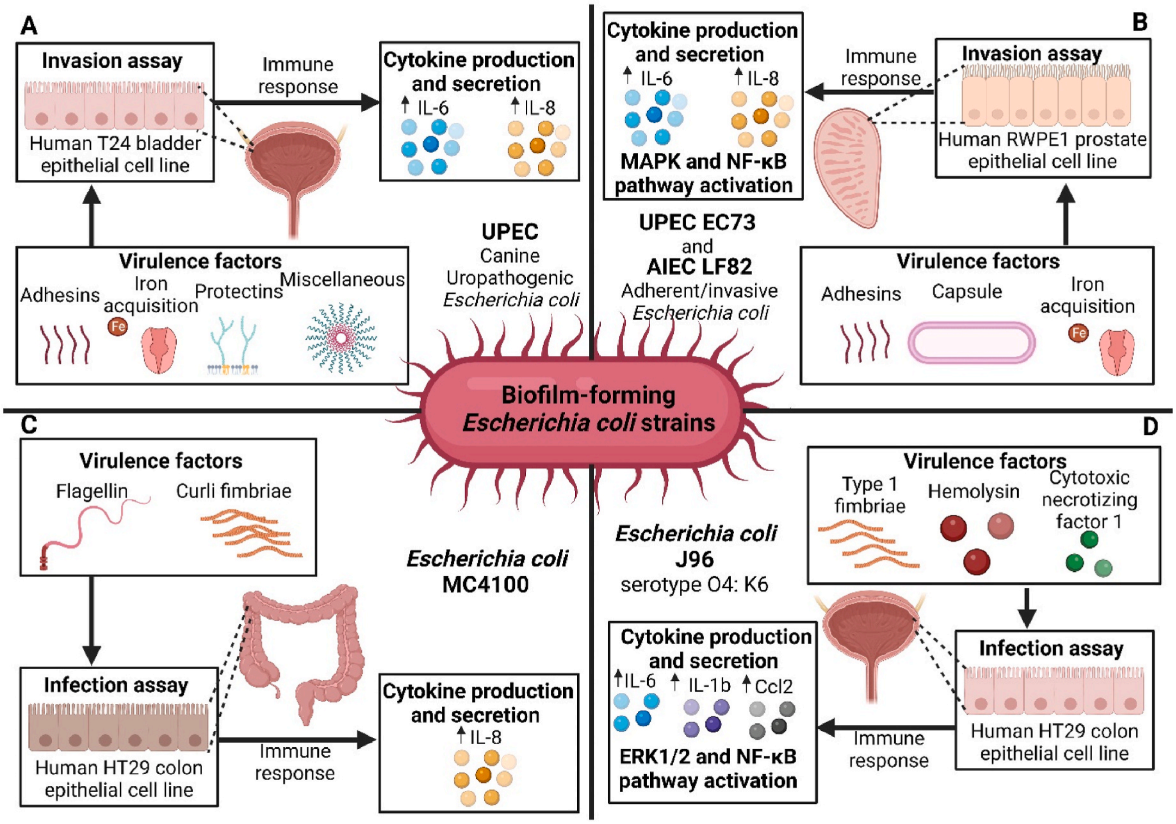

Fig. 3. Host immune response against virulence factors of biofilm-forming *E. coli* pathotypes and strains.

Table 6
Host immune response to biofilm-forming *E. coli* strains.

Molecules or pathways	Host immune response	References
Proinflammatory cytokines		
TNF-α	Is needed to start the systemic inflammatory response cascade	Didem et al. (2008)
IL-6	Correlates with disease severity and neutrophil-attractant chemokine	Mittal et al. (2015)
IL-8	Mediates the elimination of bacteria during UTI	
IFN-γ	Important for increased pathology in the ilea and ceca	Cieza et al. (2015)
Pattern recognition receptors		
TLR1 and TLR2	Recognizes amyloids in fimbriae and stimulates innate immune responses	Tükel et al. (2010)
TLR4, TLR5, TLR11	Recognizes the LPS from bacteria and induces exocytosis	(Conover et al., 2016; Engelsöy et al., 2019)
Signaling pathways		
MAPKs and NF-κB	Essential for intracellular survival of bacteria and induce secretion of proinflammatory cytokines	Conte et al. (2016)
TRPML3	Inflammasome activation, programmed urothelial exfoliation, and bacterial expulsion	Miao et al. (2015)
Adaptive immune response		
IgA	Production of antibodies against various outer membrane proteins and other secreted proteins	Wells et al. (2009)

Legend – TNF-α: tumor necrosis factor-alpha; IL: interleukin; IFN-γ: interferon-gamma; TLR: Toll-like receptors; MAPKs: mitogen-activated protein kinases; NF-κB: nuclear factor kappa-light-chain-enhancer of activated B cells; TRPML3: transient receptor potential channel 3; IgA: immunoglobulin A; UTI: urinary tract infection; LPS: lipopolysaccharide.

et al., 2010).

The interactions of *Candida auris* and the immune system were also studied by Garcia-Bustos and colleagues in an *in vivo Galleria mellonella* larvae model using two pathogenic strains, more exactly, *C. auris* CJ175 (non-aggregative) and CJ101 (aggregative) (Garcia-Bustos et al., 2022), as shown in Fig. 4B. Both *C. auris* strains induced high tissue density at the expense of plasmatocyte nodule formation regardless of their phenotype and these nodules with increased size and membrane irregularity suggested a high immune response (Garcia-Bustos et al., 2022). Another study using SAP2 protein from the *Candida parapsilosis* vaccine in wild-type BALB/c mice infected with *Candida tropicalis* was realized to evaluate the inhibition of biofilm formation (Shukla and Rohatgi, 2020), as illustrated in Fig. 4C. The colony-forming units (CFU) quantification of *C. tropicalis* infection spread was analyzed in the kidney, liver, lung, and brain and the cellular immune response was evaluated in SAP2-immunized mice at 3, 6, and 9 days post-infection. Although lower CFU count was observed in the SAP2-immunized mice group, the systemic *C. tropicalis*-associated biofilm infection significantly increased Th1, Th2, and Th17 lymphocyte populations suggesting also a vital role of B lymphocytes (IgM and IgG production) in the biofilm inhibition during early stages of infection spread (Shukla and Rohatgi, 2020). Finally, Rodrigues and colleagues studied *Candida glabrata* ATCC2001 biofilm-associated infections in the CD1 mice model, as shown in Fig. 4D. A clear tropism of *C. glabrata*-associated infection towards liver tissue was denoted by a high CFU count and a predominant presence of inflammatory myeloid cells with the surface marker F4/80 was found but the number of macrophages and neutrophils remained at control values (Rodrigues et al., 2019). These findings could be explained by the fact that *C. glabrata* infections are not associated with massive neutrophil infiltration, which makes the immune response less inflammatory when compared with other *Candida* species. When treated with two echinocandins (caspofungin and micafungin), the two-dose treatment did not show a significant impact in mice infected with *C. glabrata*

Table 7
Virulence factors of *Candida* species related to biofilm formation.

Virulence factor	Virulence gene	Strain isolates	Mechanism of action of the virulence factor	Association with biofilm formation	Evasion to immune response	References
Structural						
Transcriptional factors involved in hyphal morphogenesis	*ASH1*, *ACE2*, EFG1, FLO8, and NDT80	*C. albicans* and *C. tropicalis*	Cascade genes required for filamentous growth	Facilitates cellular intertwining in biofilm	Morphological switch to hyphae allows to penetrate and rupture immune cells	Villa et al. (2020)
Transcriptional regulator-encoding genes	*CPH1* and *FLO8*	*C. auris*	Enables the appearance of the filamentous phenotype	N/A	N/A	Yue et al. (2018)
Adhesin-like proteins	*AWP1-7*, *EPA1*, *EPA3*, *EPA6*, and *EPA7*	*C. glabrata*	Mediates adherence to fibronectin and prevents detachment	Mediates a strong interaction with the extracellular matrix	Virulence is based on endocytosis without damaging the host cell by not inducing inflammation	(Timmermans et al., 2018; Zajac et al., 2016)
Agglutinin-like sequence proteins	*ALS1* and *ALS3*	*C. albicans*	Mediates adherence to surfaces and Als3 binds to host cell ferritin	Involved in cell auto auto-adhesion in biofilm	N/A	(Deorukhkar et al., 2014; Noori et al., 2017; Pokhrel et al., 2022)
CTRG ALS-like proteins	*CTRG_02293* (*ALS1-like*) and *CTRG_03786* (*ALS2-like*)	*C. tropicalis*	Mediates adherence to host and abiotic surfaces	Enables cell-to-cell union	N/A	Galán-Ladero et al. (2019)
Melanin	N/A	*C. auris*, *C. albicans*, *C. tropicalis*, and *C. glabrata*	Mediates binding and inactivation of antimicrobial peptides and enzymes	N/A	Resistance to oxidative damage caused by immune cells and masking PAMPs	(García-Carnero et al., 2020; Smith et al., 2022)
Enzymatic						
Yapsin-related aspartyl proteinases	*YPS2*, *YPS4*, and *YPS6*	*C. glabrata*	Mediates survival in macrophages	N/A	Impediment of Syk-dependent IL-1β secretion	(Rasheed et al., 2018; Sikora et al., 2011)
Secreted aspartyl proteinases	*SAP1–3*, *SAP4*, *SAP5*, *SAP6*, *SAP9*, and *SAP10*	*C. albicans*, *C. auris*, and *C. tropicalis*	Degrades immunoglobulins and complement proteins	N/A	Reduces the susceptibility to anti-fungal activity	(Garcia-Bustos et al., 2022; Kadry et al., 2018; Sikora et al., 2011)
Phospholipase B and Phospholipase D	*PLB1* and *PLD1*	*C. albicans*, *C. auris*, and *C. tropicalis*	Disrupts the epithelial cell membrane and allows the hyphal tip to enter the cytoplasm	N/A	N/A	(Deorukhkar et al., 2014; Garcia-Bustos et al., 2022; Ramos et al., 2015)
Hemolysin-like proteins	*HLP*	*C. albicans*, *C. auris*, *C. krusei*, *C. glabrata*, and *C. tropicalis*	Demonstrates a diversity of hemolysis (alpha, beta, and gamma hemolysis) and facilitates cellular growth by iron acquisition	Explores iron from intracellular ferritin	N/A	(Deorukhkar et al., 2014; Noori et al., 2017; Pokhrel et al., 2022)

Legend – Als: agglutinin-like sequence protein; CTRG ALS-like genes: prefixed *C. tropicalis* agglutinin-like sequence proteins Syk: spleen-associated tyrosine kinase; IL: interleukin; PAMPs: pathogen-associated molecular patterns; N/A: not available.

biofilm cells evidencing the higher resistance of biofilms against both treatments and immune responses when compared to planktonic cells (Rodrigues et al., 2019).

The studies described above indicate all *Candida* species display different virulence factors, including the ability to establish biofilm, activating therefore alternative signaling pathways by the host immune system in response to the initial infection. However, the immune system possesses a series of innate and adaptive responses to deal with *Candida*-associated biofilm infections (Table 8). The innate immune system ensures a general and effective response within 0–96 h trying to stop the spread of the initial infection before biofilm establishment and/or microbial cell dissemination to the host body. Usually described as the first line of host defense, the innate immune response lacks specificity by being composed mainly of physiological barriers (epithelial and mucous membranes) and numerous non-specific immune cells such as neutrophils, macrophages, dendritic cells, natural killer (NK) cells, mast cells, basophils, and eosinophils among others. Depending on the recognition of PAMPs, this type of immune system is commonly unable to adapt against the diversity of virulence factors of the different *Candida* species and neither avoids biofilm establishment. On the other hand, the adaptive immune system can adapt its responses against different virulence factors and *Candida* species, but its response time is long with a window greater than 96 h, and unable to inhibit biofilm formation. When activated, the immune responses realized by T and B lymphocytes are not able to reach *Candida* cells within the biofilm and therefore cannot eradicate the biofilm-related infections without clinical treatments (Marshall et al., 2018).

In summary, interleukin-1β (IL-1β) is one of the main proinflammatory cytokines secreted by cells belonging to the innate immune system, in particular monocytes/macrophages. This interleukin is secreted extracellularly in response to pathogen-associated molecular patterns (PAMPs) that were recognized by mononuclear cells (Lopez-Castejon and Brough, 2011). IL-1β is a key factor in mediating the inflammatory response and facilitates synaptic activity and pain transmission which initiates the adaptive response. Similarly, tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine produced intracellularly by activated monocytes and macrophages. Its activity is mediated by the binding of TNF type I and II receptors that are present in almost all cell types and thus triggering acute inflammatory processes as well as signaling processes of cell necrosis and apoptosis (Gerriets et al., 2022). Finally, as a central part of the adaptive immune system, Th1, Th2, and Th17 lymphocytes are a type of effector lymphocytes differentiated from helper T lymphocytes in the activation phase of the adaptive immune response and they are responsible to control all the processes involved in biofilm eradication (Cohn et al., 2014). Th1 lymphocytes play a role against intracellular bacteria and dimorphic

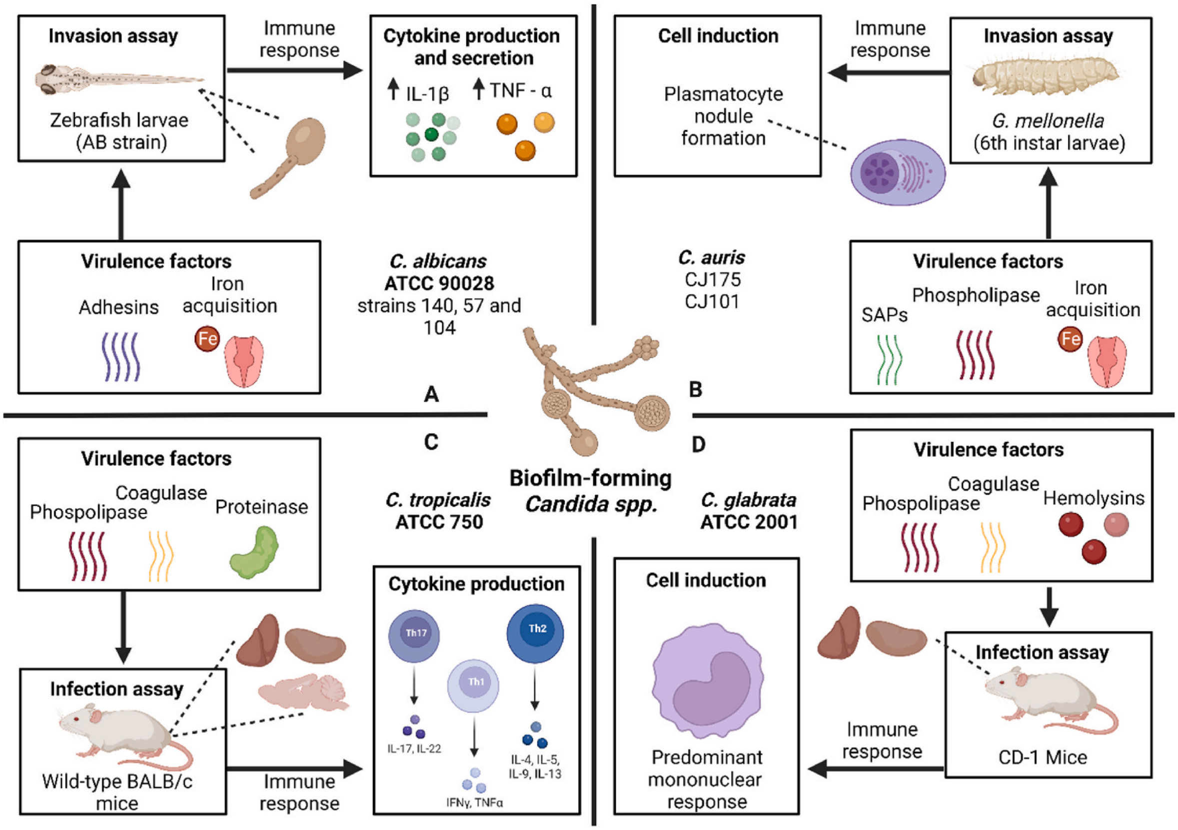

Fig. 4. Host immune response against virulence factors of biofilm-forming *Candida* species and strains.

Table 8
Host immune response to biofilm-forming *Candida* species.

Molecules or pathways	Host immune response	References
Proinflammatory cytokines		
IL-1β	Exacerbation of inflammatory damage during the tentative biofilm eradication process leads to severe tissue injury	Lopez-Castejon and Brough (2011)
TNF-α	Interferes with the production of the extracellular matrix of the biofilm	Rocha et al. (2017)
Lymphocyte population		
Th1	Stimulation of macrophages, lymphocytes, and PMNs in the destruction of pathogens and development of cytotoxic lymphocytes	Shukla and Rohatgi (2020)
Th2	Mediates the activation and maintenance of the humoral immune responses including antibody production, through the production of IL-4, IL-5, IL-6, IL-9, IL-13, and IL-17E	Shukla and Rohatgi (2020)
Th17	Mediates the production of IL-17, IL-21, and IL-22, being critical components of the antimicrobial response to biofilm eradication	Shukla and Rohatgi (2020)

Legend – IL: interleukin; TNF-α: tumor necrosis factor-alpha; PMNs: polymorphonuclear neutrophils; Th: T helper cell.

fungi infections, being differentiated by early exposure to IL-12 and IFN-γ. Meanwhile, Th2 lymphocytes realize an immunological effect against parasites and allergens by the promotion of immunoglobulin E secretion favoring the infiltration of eosinophils in the infected tissues (Cohn et al., 2014). Last, but not least, Th17 lymphocytes are responsible for protection against extracellular pathogens, facilitate neutrophil infiltration, and mediate immune protection against several pathogens (fungi or bacteria) involved in biofilm-associated infections (Cohn et al., 2014).

2. Conclusions

Several studies have explored the correlation between microorganisms' pathogenicity and biofilm formation. Numerous virulence factors have the function of facilitating biofilm formation by taking advantage of available resources in the host or fulfilling another type of function by cooperating in the evasion of the innate and adaptive immune response. It is worth mentioning that these virulence factors and their activity vary depending on the species as well as the immune response reported in the host, where the presence of cytokine-secreting mononuclear cells that subsequently activate other responses by several signaling pathways stands out. Understanding the dynamics between immune response and virulence factors of biofilm-forming pathogens is important and useful for future studies and medical applications/treatments.

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CRediT authorship contribution statement

Sandra Pamela Cangui-Panchi: Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, All authors have read and agreed to the published version of the manuscript. **Anahí Lizbeth Ñacato-Toapanta:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, All authors have read and agreed to the published version of the manuscript. **Leonardo Joshué Enríquez-Martínez:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, All authors have read and agreed to the published version of the manuscript. **Gabriela Alexandra Salinas-Delgado:** Conceptualization, Formal analysis, Investigation, Data curation, Writing – original draft, All authors have read and agreed to the published version of the manuscript. **Jorge Reyes:** Conceptualization, Writing – review & editing, All authors have read and agreed to the published version of the manuscript. **Daniel Garzon-Chavez:** Conceptualization, Resources, Writing – review & editing, Funding acquisition, All authors have read and agreed to the published version of the manuscript. **António Machado:** Conceptualization, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Supervision, Project administration, Funding acquisition, All authors have read and agreed to the published version of the manuscript.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Antonio Machado reports financial support was provided by San Francisco University of Quito. Antonio Machado reports a relationship with San Francisco University of Quito that includes: employment and funding grants.

Data availability

No data was used for the research described in the article.

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