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Research review paper

Pseudomonas aeruginosa biofilms and their partners in crime

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ABSTRACT

Pseudomonas aeruginosa biofilms and the capacity of the bacterium to coexist and interact with a broad range of microorganisms have a substantial clinical impact. This review focuses on the main traits of *P. aeruginosa* biofilms, such as the structural composition and regulatory networks involved, placing particular emphasis on the clinical challenges they represent in terms of antimicrobial susceptibility and biofilm infection clearance. Furthermore, the ability of *P. aeruginosa* to grow together with other microorganisms is a significant pathogenic attribute with clinical relevance; hence, the main microbial interactions of *Pseudomonas* are especially highlighted and detailed throughout this review. This article also explores the infections caused by single and polymicrobial biofilms of *P. aeruginosa* and the current models used to recreate them under laboratory conditions. Finally, the antimicrobial and antibiofilm strategies developed against *P. aeruginosa* mono and multispecies biofilms are detailed at the end of this review.

1. Introduction

The ability of *Pseudomonas aeruginosa* to colonize medical devices and human tissues while growing in resistant communities called biofilms is a worldwide public health concern. Biofilms are bacterial communities that grow together embedded in an extracellular matrix (ECM), which is a fundamental structural component of the bacterial community and acts as a protective shield (Ma et al., 2009). Bacteria modulate their gene expression during adaptation to biofilm growth, promoting phenotypically opposite behavior compared to their planktonic counterparts. Bacterial communication via the quorum sensing (QS) network plays a critical role during biofilm establishment, namely, in regulating the genes involved in biofilm development (Schuster and Greenberg, 2006). *P. aeruginosa* biofilms have increased antibiotic tolerance and are more resistant to host responses than their planktonic counterparts, which makes the clearance of these biofilms difficult and infections chronic (Lebeaux et al., 2014; Maurice et al., 2018).

A critical clinical trait of *P. aeruginosa* is its capacity to interact and coexist with other microorganisms in multispecies communities. From a clinical point of view, these interactions are usually detrimental to the patient, as infections caused by multiple species are often associated with worse prognosis (Peters et al., 2012). On the other hand, from a biotechnological perspective, there is a challenge to recreate the optimal conditions to grow multiple bacterial species simultaneously. *P. aeruginosa* can interact with other bacteria, fungi and viruses and together infect a wide range of human tissues (Filkins et al., 2015; Hendricks et al., 2016; Smith et al., 2015). Due to the clinical challenge of *P. aeruginosa* biofilms and the recalcitrant infections they cause, science has moved toward developing efficient and alternative antimicrobial strategies to clear *P. aeruginosa* biofilms (Barraud et al., 2009; Guillon et al., 2018; Ibaraki et al., 2020; Mwangi et al., 2019).

This review outlines the main aspects of *P. aeruginosa* biofilms and the clinical burden they represent; describes *P. aeruginosa* infections, importantly focusing on the polymicrobial interactions of this bacterium and the relative clinical outcomes; and finally, it discusses the current models used to recreate *P. aeruginosa* polymicrobial biofilms under laboratory conditions and the antimicrobial therapeutics used against *P. aeruginosa* biofilms.

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2. Extracellular matrix of *Pseudomonas aeruginosa* biofilms: the structural basis of the clinical threat

The ECM is the body of the biofilm. It maintains biofilm integrity by holding the bacterial community together and confers biofilm protection against antimicrobials and the host immune response. Hence, the ECM is a fundamental component of this multifactorial structure and is composed mainly of a mix of exopolysaccharides (EPS), extracellular DNA (eDNA) and proteins (Ma et al., 2009).

Psl and Pel are the two main polysaccharides present in the ECM of *P. aeruginosa* strains and are crucial for the integrity of the biofilm. Psl is a neutral pentasaccharide composed of D-glucose, D-mannose and L-rhamnose, while Pel is a cationic exopolysaccharide comprising 1-4 linked galactosamine and glucosamine sugars (Billings et al., 2013; Franklin et al., 2011; Jennings et al., 2015). However, during an *in vivo* infection, mutations in the anti-sigma factor encoding the *mucA* gene result in overproduction of the alginate polysaccharide and change the architecture of the biofilm's ECM (Martin et al., 1993). Alginate is an anionic polymer composed of β-D-mannuronic acid and α-L-guluronic acid. Biofilms that contain bacteria overproducing alginate occupy more space than nonmucoid biofilms, which are more densely packed (Ma et al., 2012). Each polysaccharide (Psl, Pel and alginate) provides different physiological properties to the biofilm matrix, and it is associated with different stages of biofilm development. Even though *P. aeruginosa* has the capacity to produce the three types of polysaccharide, it only does one type at any given time (Franklin et al., 2011).

The initiation and maintenance of biofilms are promoted by the interaction of Psl with the matrix adhesin CdrA. Within the matrix, bound CdrA-Psl forms robust and protease-resistant bacterial aggregates that fortify the biofilm structure (Borlee et al., 2010; Ma et al., 2009; Reichhardt et al., 2018). CdrA can also attach other yet-unknown EPS, contributing to biofilm formation and stabilizing the structure (Reichhardt et al., 2018). CdrA, Psl and Pel are bis-(3′-5′)-cyclic diguanosine monophosphate (c-di-GMP)-dependent molecules (Borlee et al., 2010). c-di-GMP is a secondary messenger that transduces the environmental signal into different cellular processes (Jenal and Malone, 2006). Under low concentrations of c-di-GMP, *P. aeruginosa* biofilms disperse and become planktonic cells. High levels of cAMP have been directly related to low c-di-GMP content, thus contributing to the inhibition of *P. aeruginosa* biofilm formation (Almblad et al., 2019). Alginate is regulated by c-di-GMP at the post-translational level (Whitney et al., 2015). Additionally, *P. aeruginosa* produces two soluble lectins: LecA and LecB. The binding between LecB and mannose present in Psl favors the positioning of Psl within the matrix, enhancing bacterial retention and aggregation (Passos da Silva et al., 2019). LecA shows specificity for binding galactose, *N*-acetyl-d-galactosamine and glucose, and it contributes to biofilm formation by cross-linking with these residues present in the biofilm matrix as well as with other glycoproteins and bacterial polysaccharides (e.g., LPS) (Diggle et al., 2006; Mitchell et al., 2005; Sabin et al., 2006).

A critical and clinically relevant event occurs *in vivo*, during the establishment of *P. aeruginosa* infection, with the organism becoming mucoid and overproducing alginate polysaccharide. From that moment on, *P. aeruginosa* induces the transition from an intermittent to chronic infection (Martin et al., 1993). Mucoid strains generally produce less Psl than nonmucoid *P. aeruginosa* strains. Nevertheless, the interactions between this EPS and the components of the matrix are also required to keep bacteria together and form robust and mature biofilms (Jones and Wozniak, 2017; Ma et al., 2012).

All three polysaccharides (Psl, Pel, alginate) play a role in biofilm adhesion, scaffolding, and stability. However, they differ in terms of biofilm protection. While Psl confers protection against the immune cells, Pel is shown to defense the biofilm against antimicrobial treatment. On the other hand, alginate production protects from both antimicrobials and the host immune response and confers additional protection to hostile environments as, for instance, the oxidative stress created during phagocytosis (Karygianni et al., 2020).

eDNA is a major component of the *P. aeruginosa* biofilm matrix that changes depending on the biofilm maturity and the surrounding environment (Whitchurch et al., 2002). In *P. aeruginosa,* eDNA is produced through a process of explosive cell lysis that subsequently forms membrane vesicles by engulfing DNA and other cytosolic content. eDNA is then released in a mechanism that is regulated by both QS-dependent and QS-independent mechanisms (Turnbull et al., 2016). Once released, eDNA interacts with extracellular Ca^{2+} and, via "cationic bridging", induces bacterial aggregation, promoting biofilm formation and subsequent maturation. Therefore, eDNA is required for the initial establishment of the biofilm (Das et al., 2014; Whitchurch et al., 2002). eDNA has been detected to interact with Psl (Wang et al., 2015) and Pel (Jennings et al., 2015) polysaccharides. During an infection, the host also impacts the presence of eDNA in the biofilm matrix through interactions with immune cells during the inflammatory response. While eDNA has been shown inside biofilms in *in vitro* studies, in *in vivo* biofilms, it has been shown to be concentrated in the external part of the biofilms (Alhede et al., 2020; Ciszek-Lenda et al., 2019; Whitchurch et al., 2002). *P. aeruginosa* strains, namely, the "rugose small colony variants" (RSCVs), isolated from patients with chronic infections are hyperbiofilm-forming strains that, unlike common laboratory strains, have fragmented eDNA within the matrix that leads to a more resistant structure (Deng et al., 2020). RSCVs are associated with high levels of c-di-GMP (Malone et al., 2010), in addition, to be able to produce alginate and Psl polysaccharides simultaneously (Franklin et al., 2011).

However, eDNA is more than just a structural biofilm component; it also influences the transcriptome of *P. aeruginosa*. This anionic polymer modulates the expression of antibiotic resistance genes such as ß-lactamases and aminoglycoside resistance genes as well as the expression of multidrug efflux pumps (e.g., EmrAB). Additionally, it alters metal homeostasis by chelating cationic ions and controlling the expression of different metal uptake and efflux systems. The acidification of the biofilm confers tolerance to acidic environments, making biofilms able to resist the infection site or phagocytose acidified vacuoles. Importantly, eDNA traps nutrients in addition to inducing genes to be able to use DNA as a nutrient source for phosphate, nitrogen or carbon (Lewenza et al., 2020; Mulcahy et al., 2008; Wilton et al., 2016). Altogether, eDNA promotes all the hallmark features of biofilms. A recently published mini-review addresses the different aspects of *P. aeruginosa* eDNA release and interactions very accurately (Sarkar, 2020).

3. *P. aeruginosa* polymicrobial coexistence

In nature, biofilms are often polymicrobial structures, meaning that different microbial species can interact and coexist within the same biofilm community. In disease, microbial interactions can affect a patient's prognosis. In this sense, the synergic interactions of organisms, whereby the combined effect is more significant than that produced by individual bacteria, can worsen the outcome of the patient (Murray et al., 2014). *P. aeruginosa* can grow and coexist with a wide range of microorganisms, including bacteria, fungi and viruses (Fig. 1).

3.1. *P. aeruginosa with Staphylococcus aureus*

One of the major partners in crime of *P. aeruginosa* is *Staphylococcus aureus.* This partnership can cause severe chronic infections promoted by biofilm aggregates in infected wounds and lungs, especially in diabetic foot ulcers and cystic fibrosis (CF). The presence of *S. aureus* and the Pel production of *P. aeruginosa* have been shown to increase the surface coverage and microcolony size of biofilms formed by both *P. aeruginosa* and *S. aureus*, and diguanylate cyclase SiaD is required for competitiveness between them (Chew et al., 2018). In CF-affected lungs, *P. aeruginosa* takes advantage of the physiology generated by the disease and the lack of iron present in the environment to produce 4-hydroxy-2-

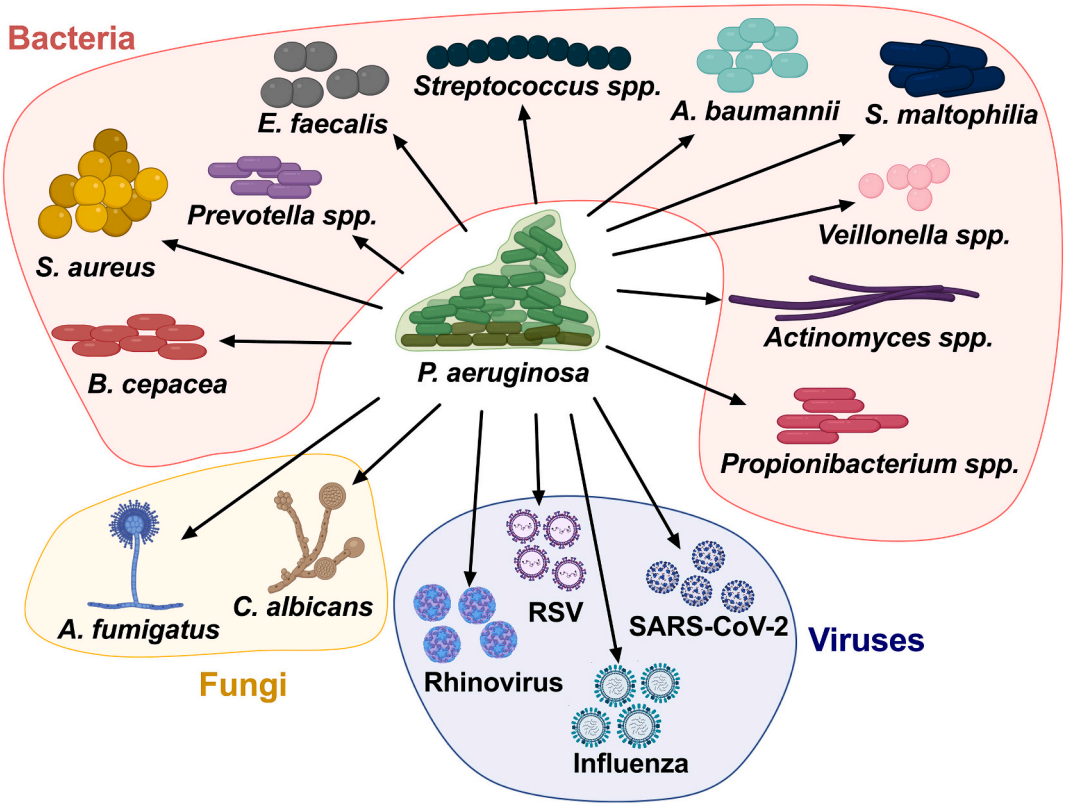

Fig. 1. Scheme of the clinically relevant microbial biofilm interactions of *P. aeruginosa*. *P. aeruginosa* can interact and coexist simultaneously with a wide range of microbes from different phyla. The figure shows the main interactions described for *P. aeruginosa* with bacteria *(Burkholderia cepacia, Staphylococcus aureus, Prevotella spp., Enterococcus faecalis, Streptococcus spp., Acinetobacter baumannii, Stenotrophomonas maltophilia, Veillonella spp., Actinomyces spp.*, and *Propionibacterium spp.*), fungi (*Aspergillus fumigatus* and *Candida albicans*) and viruses (respiratory syncytial virus (RSV), human rhinovirus, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) and influenza). Many of these interactions have been found in the disease environment. The figure was created using biorender.com.

heptylquinoline-*N*-oxide (HQNO). HQNO inhibits *S. aureus* growth by shifting its metabolism to fermentation, which eventually promotes the killing of *S. aureus*, and *P. aeruginosa* can use the iron that *Staphylococcus* stored (Filkins et al., 2015; Mashburn et al., 2005; Nguyen et al., 2015). *P. aeruginosa* produces the endopeptidase LasA, which has also been suggested to help for acquiring the iron from *S. aureus* (Mashburn et al., 2005). Transcriptional profiles performed in early cocultures of both bacteria reflect the metabolic adaptation and competition for glutamine, as nitrogen and energy sources, that the organisms face when they grow together rather than the expression of host-directed virulence factors (Tognon et al., 2019). It has also been seen that during *P. aeruginosa* coinfection with Gram-positive bacteria such as *S. aureus*, *P. aeruginosa* senses the N-acetyl glucosamine (GlcNAc) of its peptidoglycan as a cue to produce multiple virulent factors with lytic activity against prokaryotic and eukaryotic cells, therefore enhancing host killing (Korgaonkar et al., 2013). *P. aeruginosa* production of the *cis*-2-decenoic acid promotes biofilm dispersal of different bacteria, including *S. aureus* (Davies and Marques, 2009).

On the other hand, *S. aureus* can use the HQNO molecule produced by *P. aeruginosa* to increase its tolerance to certain antibiotics, such as vancomycin or tobramycin. Prolonged coculture growth of *P. aeruginosa* and *S. aureus* or increased *S. aureus* exposure to HQNO, which is abundant in CF sputum, selects for resistant *S. aureus* small-colony variants (SCV), in a mechanism dependent on the transcriptional factor sigma B (SigB) (Mitchell et al., 2010). Furthermore, *P. aeruginosa* influences *S. aureus* susceptibility to antibiotics such as vancomycin, gentamycin or ciprofloxacin (Biswas et al., 2009; Cendra et al., 2019; Hoffman et al., 2006; Orazi and O'Toole, 2017). Recent findings indicate that *S. aureus* extracellular metabolites (e.g., adhesins, enzymes, polysaccharides and peptides) can suppress *P. aeruginosa* growth, which leads to an increase in *P. aeruginosa* susceptibility to antibiotics such as ciprofloxacin and aminoglycosides (Trizna et al., 2020). The secreted staphylococcal protein A (SpA) inhibits *P. aeruginosa* biofilms through a mechanism that affects type IV pili and PsI production. This protein has also been suggested to protect *P. aeruginosa* from phagocytosis (Armbruster et al., 2016). In a clinical context, alginate overproduction of *P. aeruginosa* in the CF environment benefits *S. aureus* as it decreases the production of anti-staphylococcal molecules, enabling coexistence between these two bacteria (Limoli et al., 2017). Fig. 2 summarizes the reciprocal effect of *P. aeruginosa* and *S. aureus* interaction on their pathogenicity and the mechanism or molecule through which each beneficial or antagonistic effect is done. It is worth mentioning that a review by Hotterbeekx *et al.* thoroughly compiles the *in vivo* and *in vitro* interactions between these two organisms and the resulting phenotypic effects (Hotterbeekx et al., 2017).

On the other hand, the alpha toxin of *S. aureus* has been shown to be important in *P. aeruginosa* infection and dissemination because it mediates the disruption of host immunity and the barriers promoted by epithelial cells *(*Cohen et al., 2016*)*. Coinfection with *P. aeruginosa* and *S. aureus* leads to rapid pulmonary decline and diminished lung function, which is why these two bacteria are targets of numerous treatments to clear infections in CF lungs (Limoli et al., 2016). Due to its clinical relevance, numerous studies have been performed to culture these two bacteria *in vitro* to investigate their partnership better, but *Pseudomonas* dominance limits its *in vitro* coexistence (see Section 6 for detailed information on *in vitro* models to grow *P. aeruginosa* polymicrobial biofilms).

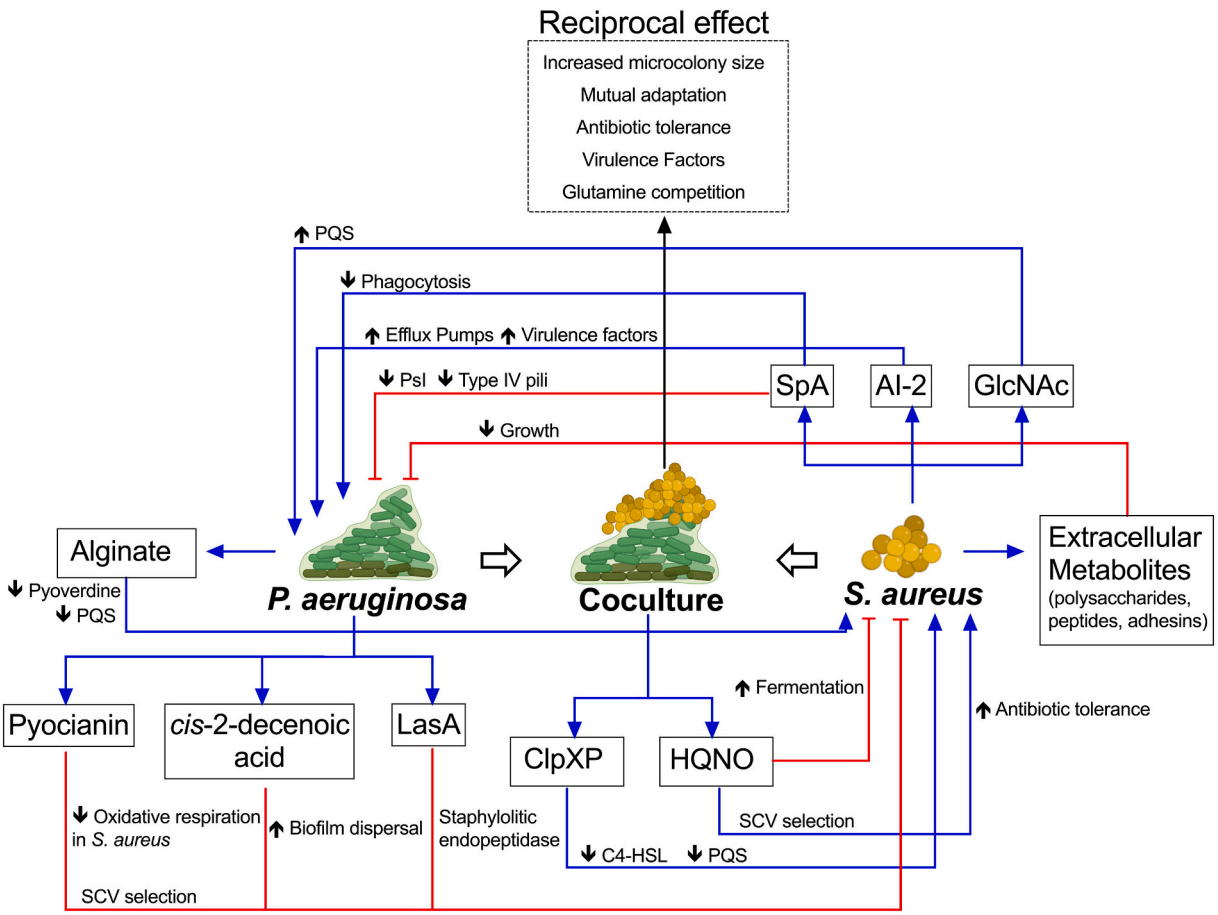

Fig. 2. Mutual effect of *P. aeruginosa* and *S. aureus* pathogenicity and interaction on each other phenotype. The scheme summarizes the effect that the products of *P. aeruginosa* and *S. aureus* cause on each other, as well as that caused by the products generated as consequence of their interaction. Blue arrows indicate a beneficial effect, while red lines indicate an inhibitory effect. In black is specified the mutual effect that the organisms receive as a consequence of their interaction. Next to each arrow is denoted the action caused by the products generated, responsible for the inhibitory or beneficial effect promoted to the receptor.

3.2. *P. aeruginosa with Streptococcus spp.*

P. aeruginosa also interacts with *Streptococcus* in a CF environment. A study revealed how a clinical CF isolate of *P. aeruginosa* (FRD1 strain) enhanced the capacity of *Streptococcus parasanguinis* to colonize and form biofilms in a mechanism wherein the latter exploited the exopolysaccharide production of *Pseudomonas* (Scoffield et al., 2017). Furthermore, in coculture growth with *Streptococcus* spp. commonly present in the flora of the host's respiratory tract, *P. aeruginosa* differentially expresses several virulence factors and drug efflux pumps, which are thought to be modulated by the QS molecule AI-2. AI-1 accumulates in CF sputum and could have a role in facilitating the intercommunication of *P. aeruginosa* with the patient flora, exacerbating CF disease. However, this relationship is complex; for instance, *Streptococcus* upregulates the production of rhamnolipids via the expression of the QS molecule AI-2, but rhamnolipids kill *Streptococcus.* The effect of these gene modulations through the AI-2 molecule has also been observed with other bacteria present in the flora, such as *Staphylococcus* spp. (Duan et al., 2003; Peters et al., 2012). It has recently been detected that both bacterial species compete for zinc, as increasing transcription of genes involved in zinc uptake was detected in *Streptococcus sanguinis* during coculture growth with *P. aeruginosa.* This study also showed that the zinc levels present in CF sputum correlate with the abundance of these two species, indicating that zinc availability may impact *S. sanguinis* and *P. aeruginosa* growth *in vivo* (Li et al., 2020). In terms of infection outcomes, coinfection with streptococci and *P. aeruginosa* shows higher damage to the CF-affected lung and microbial burden than that observed with any of the microbes alone (Duan et al., 2003; Filkins et al., 2012; Peters et al., 2012).

3.3. *P. aeruginosa with other bacteria*

P. aeruginosa has been detected coexisting with a wide range of microorganisms. Sputum analysis of CF adult patients showed the presence of many strict and facultative anaerobic bacteria including *Prevotella*, *Veillonella*, *Propionibacterium*, and *Actinomyces* together with *P. aeruginosa, S. aureus* or *Burkholderia cepacia*. Interestingly, this study revealed that *P. aeruginosa* increased the presence of anaerobic microorganisms in the sputum (Tunney et al., 2011). Specifically, coinfection with *B. cepacia* and *P. aeruginosa* has been detected to promote a higher decline in pulmonary function and worse clinical outcomes (Jacques et al., 1998). *Stenotrophomonas maltophilia* has also seen involved in polymicrobial infections with *P. aeruginosa* in CF-affected lungs. This interaction benefits *S. maltophilia* persistence in the lung (McDaniel et al., 2020).

The oral cavity is very susceptible to bacterial biofilm growth. Colonization of teeth causes dental plaque formation and consequently the formation of polymicrobial biofilms that can lead to infection like periodontal disease. Biofilm formation in the oral cavity can serve as a reservoir of respiratory pathogens (Berger et al., 2018; Vieira Colombo et al., 2016). *P. aeruginosa* with *S. aureus* and *Enterococcus faecalis* have been detected coexisting together in oral epithelial cells of people with periodontitis, and *P. aeruginosa* with *Acinetobacter* spp. have a high correlation to be found together in the oral cavity (Colombo et al., 2013;

Souto et al., 2014). Additionally, polymicrobial infections in the urinary tract have linked the presence of *E. faecalis* with more severe forms of pyelonephritis caused by *P. aeruginosa* (Tsuchimori et al., 1994).

3.4. *P. aeruginosa with fungi*

P. aeruginosa has also shown the ability to interact with organisms of other phyla such as fungi. For instance, *P. aeruginosa* has been found together with *Aspergillus fumigatus infecting lungs.* The presence of *P. aeruginosa* in lung infections caused by *A. fumigatus* has been shown to lead to higher loss of pulmonary function than in fungal infections alone. Patients with CF suffering from *P. aeruginosa* and *A. fumigatus* infections may exhibit a poorer prognosis than those patients with individual infections, as *A. fumigatus* enhances the elastase production of *P. aeruginosa* (McGuigan and Callaghan, 2015; Smith et al., 2015). Furthermore, *P. aeruginosa* secretes antifungal molecules that inhibit *A. fumigatus* biofilms; however, this inhibitory effect differs depending on the *P. aeruginosa* strain. This inhibition is higher if it is promoted by CF isolates than by non-CF isolates of *P. aeruginosa*, indicating that a selection pressure of the environment may have a role in *P. aeruginosa* and *A. fumigatus* interactions (Bisht et al., 2020).

An interaction has also been described between *P. aeruginosa* and *Candida albicans. P. aeruginosa* can kill *C. albicans* by attaching to the hyphal areas of the fungus, but it is unable to attach or to kill to the round form of the yeast (Hogan et al., 2004).

3.5. *P. aeruginosa with viruses*

P. aeruginosa interactions with respiratory viruses have also been detected in different chronic infections. In this case, there is no mutual interaction between organisms but simple coexistence. Respiratory syncytial virus (RSV) infection induces secondary infections and *P. aeruginosa* biofilm formation by dysregulating iron homeostasis in the airway epithelium (Hendricks et al., 2016). A viral infection reinforces *P. aeruginosa* adhesion to epithelial cells. In this sense, RSV has been seen to be responsible for the transition of *P. aeruginosa* to chronic colonization in CF patients (Brownlee and Turner, 2008). On the other hand, a recent study has indicated that CF-affected people suffering from intermittent infections with *P. aeruginosa* have an increased risk of being infected with human rhinovirus, in which the bacterium may be able to modulate the antiviral response *in vivo,* as was detected in a clinical setting of CF (Sorensen et al., 2020). Influenza virus (IAV) also correlates with secondary infections of *P. aeruginosa* in CF patients as well as in people with pneumonia (Hiatt et al., 1999; Scheiblauer et al., 1992). Recent findings have shown that IAV tends to exacerbate *P. aeruginosa* infection by inducing deleterious inflammatory responses (Villeret et al., 2020).

Additionally, during the COVID-19 pandemic, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), the causative agent of COVID-19, has shown capacity to be coinfecting with other microbes. A systematic review and meta-analysis that considered thirty studies, including 3834 patients, showed that 7% of hospitalized patients infected with SARS-CoV-2 suffered from bacterial coinfection, of which *P. aeruginosa* appeared in 12% of the cases (Lansbury et al., 2020). Even though it is early to have data about the nature of SARS-CoV-2 and *P. aeruginosa* coinfection, a retrospective study performed in 61 patients with COVID-19 in an Italian hospital detected a higher prevalence of *P. aeruginosa* colonization in severe COVID-19 patients than in non-COVID-19 patients (Intra et al., 2020). Further studies will be needed to elucidate the real impact of *P. aeruginosa* coinfection on COVID-19, as well as possible effects of SARS-CoV-2 on *P. aeruginosa* metabolism and biofilm growth.

4. Quorum sensing in *P. aeruginosa* biofilms

QS is an intercellular mechanism of communication that many bacterial species use to interact with each other. QS plays a critical role during the establishment of chronic infections since it modulates the expression of many different virulence factors as well as biofilm formation. In QS, autoinducers (e.g., acyl-homoserine lactones (AHLs)) serve as signaling molecules whose production is dependent on cell density (Waters and Bassler, 2005). To date, three different QS mechanisms have been described in *P. aeruginosa*: the LasI/LasR system, which produces *N*-3-oxo-dodecanoyl-homoserine lactone (3O-C12-HSL) as a signaling molecule; the RhlI/RhlR system, which produces *N*-butanoyl-homoserine lactone (C4-HSL); and the third system, which is based on 2-alkyl-4-quinolone (AQ) signals and PqsR as a transcriptional regulator. Both the LasR and RhlR systems are related to cell density, while PqsR is related to the stress response. In addition to these three primary QS circuits, the factor QscR has been detected binding to 3OC12-HSL and other long-chain AHLs without producing any AHL molecules. Furthermore, a role of 2-(2-hydroxyphenyl)-thiazole-4-carbaldehyde in the environmental stress-related *iqs* system has also been described (Gökalsın et al., 2019; Papenfort and Bassler, 2016; Schuster and Greenberg, 2006).

4.1. *Role of QS on ECM's development*

QS has a considerable impact on *P. aeruginosa* biofilm formation, specifically on the development of the biofilm matrix. As mentioned above, the eDNA of the matrix is generated via QS-dependent and QS-independent pathways. The one mediated by QS occurs via the lysis of a small population of cells, while the other liberates only basal levels of eDNA (Allesen-Holm et al., 2006). There are other ways to promote *P. aeruginosa* death and the production of eDNA, such as the intracellular increase in H_2O_2 due to pyocyanin exposure (Das and Manefield, 2012). Furthermore, the expression of the *pel* polysaccharide operon is entirely dependent on QS regulation (Sakuragi and Kolter, 2007). Biofilms formed by *P. aeruginosa* expressing deficient *las, rhl* and *pqs* systems exhibit reduced levels of eDNA in the matrix, resulting in an unstable biofilm (Allesen-Holm et al., 2006). *P. aeruginosa* with mutations affecting the production of the 3O-C12-HSL signaling molecule has been seen to form thin biofilms that lack a three-dimensional architecture (Davies et al., 1998). Recent findings have shown that treatment with tobramycin increases the presence of eDNA in the biofilm matrix through a mechanism dependent on QS. Subinhibitory concentrations of this antibiotic increase the levels of the LasR and RhlR signaling molecules 3O-C12-HSL and C4-HSL (Tahrioui et al., 2019). Additionally, rhamnolipids are amphipathic glycolipids with a critical role in the establishment and maintenance of *P. aeruginosa* biofilms. They have been shown to be required for maintaining the channel structures within the biofilm ECM that facilitate the access of nutrients and oxygen within the surrounding microcolonies (Davey et al., 2003). QS also controls rhamnolipid expression through the regulator RhlR (Medina et al., 2003). Figure 3 summarizes the role of the QS in *P. aeruginosa* ECM, including the factors involved in the composition of the ECM and the mechanisms by which the QS modulates the ECM development.

4.2. *Effect of QS on infection and P. aeruginosa polymicrobial interactions*

The QS of *P. aeruginosa* significantly impacts on the polymicrobial interactions promoted by the bacterium. Using a wound-like medium, it was demonstrated that the QS of *P. aeruginosa* is inhibited by the albumin present in the serum *in vitro*, thus making the bacteria unable to produce the virulence factors that kill *S. aureus* and allowing the survival of the cocci in the presence of *P. aeruginosa* (Smith et al., 2017). It has also been seen that alginate protects *S. aureus* from killing by *P. aeruginosa* when both organisms are growing in coculture due to a downregulation of the *pvdA* gene, which is required to produce the siderophore pyoverdine and the QS system *Pseudomonas* Quinolone System (PQS) in *P. aeruginosa* (Price et al., 2020). The PQS is responsible

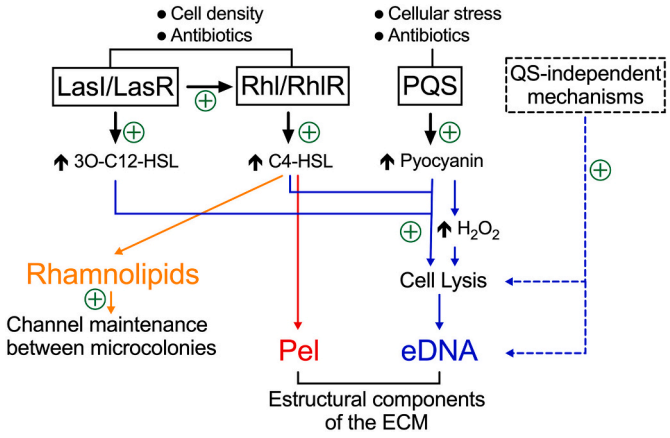

Fig. 3. Role of QS on the development of the ECM. The plot shows the effect of the main circuits of *P. aeruginosa* QS on the development of the biofilm's ECM. The principal factors described affecting QS modulation, with the respective increased autoinducers and the consequent effect on rhamnolipids (orange), Pel (red), and eDNA (blue) production, are included in the figure.

for producing the phenazine pyocyanin, which is induced by the presence of *S. aureus* (among other Gram-positive species and conditions), inhibiting the oxidative respiration of the cocci while promoting the selection of SCV (Biswas et al., 2009). Additionally, a recent study revealed that *P. aeruginosa* suppresses its antimicrobial activity against *S. aureus* through a mechanism that involves *Pseudomonas* AAA+ ClpXP protease activity on critical proteins needed to produce PQS and C4-HSL QS signal molecules (Yang et al., 2020). Otherwise, when *P. aeruginosa* interacts with *C. albicans,* pyocyanin secretion by *P. aeruginosa* is toxic in *C. albicans,* which counterattacks by producing the QS molecule farnesol. Farnesol downregulates the transcription of the pyocyanin mediator gene *pqsA (*Cugini et al., 2007*)*. During the colonization of the CF lung, farnesol may also have a protective role by reducing the levels of pyocyanin (Peters et al., 2012). In the interaction between *P. aeruginosa* and *S. maltophilia*, the latter produces a diffusible signal factor that influences the structure of *P. aeruginosa* biofilms (Ryan et al., 2008). Furthermore, *S. maltophilia* produces the fatty acid *cis*-9-octadecenoic that quenches the AHL signal of *P. aeruginosa,* inhibiting its biofilm formation (Singh et al., 2013).

Additionally, mutants of the QS regulator *LasR* have been associated with lung disease progression in CF-affected people (Hoffman et al., 2009). In these *lasR* mutant strains, QS remains active by regulating RhlR in a mechanism independent of LasR (Chen et al., 2019b). In a study that used a CF isolate, RhlR was recognized as critical for establishing chronic infection and the generation of cell toxicity in a 3D lung epithelium aggregated model (Cruz et al., 2020).

5. Clinical implications of *P. aeruginosa* biofilms – a public health issue

Microbial biofilms are involved in 65% of infectious diseases and more than 80% of chronic infections. *P. aeruginosa* is a leading nosocomial pathogen associated with this type of healthcare infection, and it is almost never found infecting alone (Bisht et al., 2020). The nature of *P. aeruginosa* biofilms *per se* is a challenge to the current known antimicrobial treatments and a nightmare for physicians.

5.1. The antimicrobial challenge of *P. aeruginosa* biofilms

The primary shield of *P. aeruginosa* biofilms is its ECM. The chemistry of the ECM hinders the penetration of positively charged antibiotics, e.g., aminoglycosides, which are sequestered by their components and impede their diffusion (Wilton et al., 2016). Biofilm growth confers intrinsic antimicrobial tolerances, sometimes requiring more than 1000 times the dose of antibiotic to be cleared than is needed to treat planktonic bacteria. Furthermore, the clinically derived mucoid phenotype, with alginate overproduction in the ECM, is even more antibiotic tolerant than the parental *P. aeruginosa.* Altogether, the high antibiotic concentrations needed to treat these biofilms are hard to achieve inside the host without causing toxicity (Goltermann and Tolker-Nielsen, 2017; Hengzhuang et al., 2013; Macia et al., 2014). Additionally, the *P. aeruginosa* biofilm community is constituted by different subpopulations of microorganisms, which have been adapted to the different microenvironments present in the multicellular system. Therefore, the bacteria residing in the inner parts of the biofilms display low metabolic activity due to the lack of oxygen and nutrients that are essentially consumed by the cells growing at the biofilm periphery, which consequently inactivates major antibiotic targets. For example, low DNA and protein synthesis affect quinolone and aminoglycoside bactericidal effects, respectively (Ciofu and Tolker-Nielsen, 2019; Stewart et al., 2016). This low-metabolic *Pseudomonas* can lead to the formation of persister cells, which are bacteria less susceptible to antibiotics and responsible for causing reinfections (Lewis, 2010). Furthermore, the hypoxic environment present in the inner parts of the biofilm is another tolerance mechanism attributed to *P. aeruginosa* biofilms since the lack of oxygen impedes the ROS necessary for the bactericidal effect of some antibiotics, such as tobramycin or ciprofloxacin (Borriello et al., 2004; Van Acker and Coenye, 2017). Antibiotic tolerance is also attributed to genetic changes occurring in these *P. aeruginosa* subpopulations, where the activation of multidrug efflux pumps due to mechanisms led by the high levels of cyclic di-GMP present in the biofilm pump antibiotics such as ciprofloxacin, gentamycin and tobramycin out of the bacterial cell at rates ten times higher than those in planktonic cells (Gupta et al., 2014; Poudyal and Sauer, 2018). Additionally, these transcriptomic changes in biofilm-forming *Pseudomonas* also occur in many other genes that are absent in free-living *Pseudomonas* that contribute to specific antimicrobial tolerance (Ciofu and Tolker-Nielsen, 2019).

Spontaneous mutations accompany the increased capacity of antimicrobial tolerance and confer resistance to certain antibiotics in *Pseudomonas* due to antimicrobial pressure. In addition to antibiotic resistance, the heterogeneity of the biofilm community and the different stresses and pressures on the subpopulations of the bacteria induce differential spontaneous mutations that benefit biofilm adaptability and persistence (Bjedov et al., 2003; Ciofu and Tolker-Nielsen, 2019; Perron et al., 2007). In this sense, RSCV in CF has increased resistance to antibiotics, and their persistence in the CF lung is thought to be due to the emergence of multidrug-resistant (MDR) variants of the mucoid phenotype (Ciofu et al., 2015; Drenkard and Ausubel, 2002).

5.2. The battle of the host immune response to clear *P. aeruginosa* biofilms

In response to a chronic infection generated by *P. aeruginosa* biofilm, the host responds by attacking the bacterial community with different types of immune cells from both the innate and adaptive systems (Maurice et al., 2018). There is an exacerbation of inflammation led by the presence of polymorphonuclear leukocytes (PMNs). Although PMNs have been detected surrounding *P. aeruginosa* aggregates, they are unable to penetrate biofilm structures and therefore eradicate them (Bjarnsholt et al., 2009). PMNs are responsible for neutrophil extracellular trap (NET) formation in a process called NETosis. NETs are DNA lattices enmeshed with PMN granule proteins secreted by PMNs. The creation of NETs has been proposed as a function of neutrophils as well as other immune cells that are still capable of phagocytosis and chemotaxis (Goldmann and Medina, 2012; Yipp et al., 2012). NETs aim to trap bacteria and kill them with antimicrobial proteins released from neutrophil azurophilic granules (Brinkmann et al., 2004).

Even though NET formation is an efficient antimicrobial mechanism,

depending on the case, *P. aeruginosa* has the potential to overcome it. Recently, *P. aeruginosa* strains that lack the LasR regulator (commonly found in CF patients) have been shown to fail to promote the generation of NETosis (Skopelja-Gardner et al., 2019). Furthermore, *P. aeruginosa* can cause tolerance to NETs by changing the negative charge of the outer surface either through the addition of aminoarabinose to lipid A in LPS or by producing surface spermidine (Halverson et al., 2015; Johnson et al., 2012).

Importantly, under healthy conditions, humans have effective immune mechanisms to clear *P. aeruginosa* infection. For instance, mucociliary clearance in the upper respiratory system and the expression of lactoferrin have been shown to effectively block the attachment and microcolony formation of *P. aeruginosa*, thus preventing the establishment of *P. aeruginosa* infection (Crabbe et al., 2014; Singh et al., 2002). However, if *P. aeruginosa* can penetrate and colonize patients affected by CF or another condition that compromises the immune system, then the situation can become dire. In CF-affected people, oxygen consumption caused by PMN activity inhibits proper production of the metabolic burst to kill phagocytosed bacteria (Bjarnsholt et al., 2009). Furthermore, the overproduction of alginate present in the aggregates of CF isolates confers additional phagocytosis protection to the biofilm (Bayer et al., 1991). Continuous exposure to the antigen indicates that IgG avidity against alginate does not significantly increase along with the progression of chronic infection, making it difficult for the immune reaction to clear the infection in CF patients (Mauch et al., 2018). Increasing the problem is the capacity of clinical isolates of *P. aeruginosa* to induce the formation of biofilms in the airways by upregulating EPS production through the assimilation of the host-derived immunometabolite itaconate (Riquelme et al., 2020).

5.3. *P. aeruginosa* chronic infections due to biofilm formation

The ability of microbial biofilms to resist exposure to a high concentration of antimicrobials and components of the host immune system makes *Pseudomonas* biofilms incredibly challenging to eradicate and a public health concern (Hoiby et al., 2015). The following section will focus on the most common chronic infections caused by *P. aeruginosa* biofilms and the organism's interactions with other microbes. Fig. 4 summarizes the characteristics, effects, and consequences of biofilm growth among the different infections. In the figure are present similarities and differences between infections.

5.3.1. *Cystic fibrosis*

People often associate *P. aeruginosa* infections with CF disease. The pathophysiology of this disease, with increased viscosity and mucus secretions which, together with the impaired mucociliary function that these patients suffer, creates a perfect environment in the airways for chronic microbial colonization, is the primary cause of morbidity and mortality of this disease (Rajan and Saiman, 2002). *P. aeruginosa* plays a leading role in CF, accounting for 40-60% of infections of this disease (CysticFibrosisFoundation, 2019). The microaerophilic environment in the CF lungs, with clear oxygen gradients promoted by abundant mucus deposition in this organ, promotes *P. aeruginosa* survival and subsequent growth. In this sense, we have recently shown a change in the metabolism of *P. aeruginosa* during the transition from aerobic to microaerophilic and, subsequent, anaerobic growth conditions (Pedraz et al.,

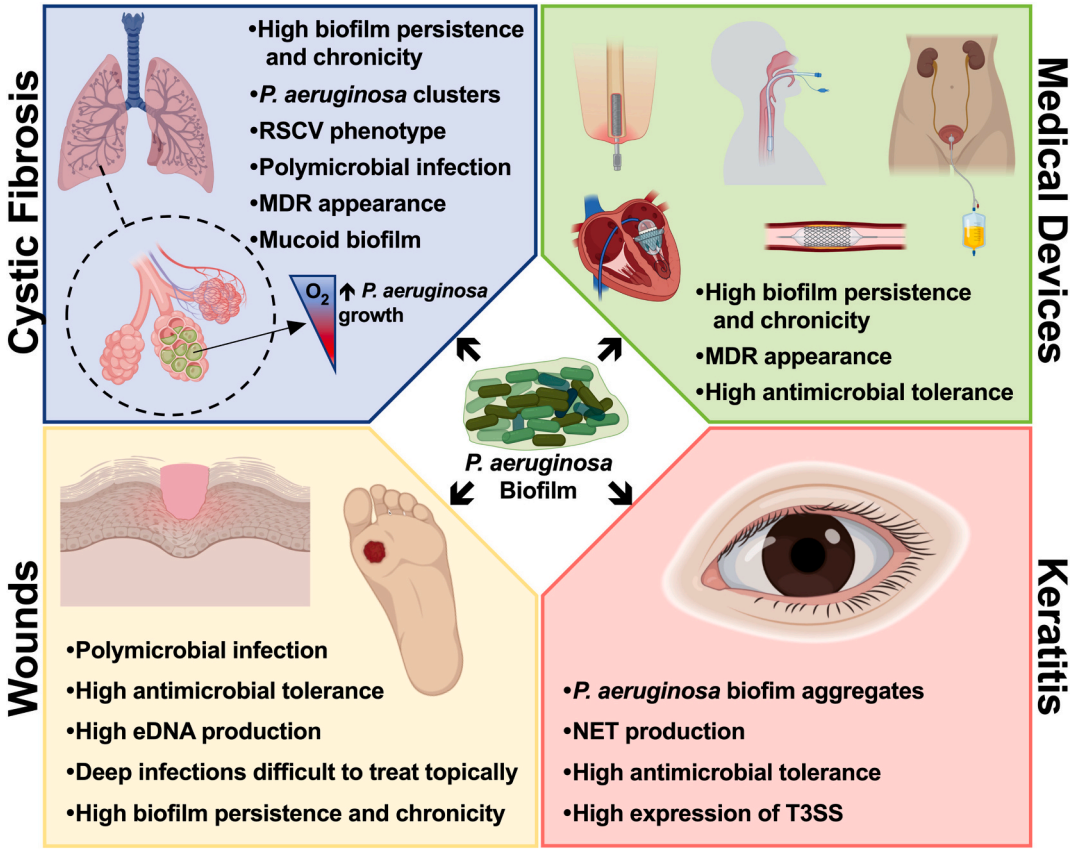

Fig. 4. Main features of *P. aeruginosa* biofilms and the respective consequences on the infection progression. The figure shows the major infections caused by *P. aeruginosa* biofilms: cystic fibrosis, wounds, infections due to biofilm growth over medical devices, and keratitis with the representative features of each one. The scheme shows the principal causes that lead to *P. aeruginosa* growth in biofilms. Hence, a lung with the alveoli completely filled with an excess of mucus is presented for CF infections, heart valves, urinary catheters, stents, and orthopedic devices are shown as examples for medical devices-related infections, a skin wound, and a foot ulcer are shown for wound infection and, finally, an eye is shown for keratitis infection. The figure summarizes the main characteristics of each infection detailed through Section 5.3. Created with biorender.com.

2020). In CF *in vivo, P. aeruginosa* aggregates in clusters rather than in compact biofilms and grows immersed within a self-produced alginate (Lam et al., 1980; Moreau-Marquis et al., 2008; Worlitzsch et al., 2002). *P. aeruginosa* can undergo mutations that increase its persistence in CF-affected lungs. As mentioned above, the mucoid phenotype of *Pseudomonas* is frequently isolated from CF exacerbations. Additionally, during *P. aeruginosa* CF infections, microorganisms can also lose the flagellum and, consequently, swimming motility. Flagellar mutants are linked to the RSCV phenotype, which has been recognized to overproduce Psl and Pel exopolysaccharides, thus enhancing the bacterial capacity to form biofilms and persist in CF lungs (Harrison et al., 2020).

However, after many investigations involving culture-dependent and culture-independent microbiological techniques as well as RNA-based studies, CF infections have been determined to be polymicrobial and different between CF patients (Acosta et al., 2020; Filkins and O'Toole, 2015). The polymicrobial community that colonizes the CF lung harbors facultative as well as obligate anaerobic bacteria (Filkins and O'Toole, 2015). A recent study using bioorthogonal noncanonical amino acid tagging revealed extensive heterogeneity of translational activity among the CF microbiota, which is unique in every CF individual. Therefore, in addition to canonical lung pathogens such as *P. aeruginosa*, there is a low abundance of other members whose activity dynamics are determinants of the acute inflammation occurring in CF, by either impacting the host or through the modulation of the other pathogen's growth and virulence (Valentini et al., 2020). Furthermore, the existence of clonal strains of *P. aeruginosa*, a consequence of patient-patient infections, which are prevalent in clusters of people who live in a defined geographical area, also impacts the microbiota of CF (Parkins et al., 2018). In this direction, we have recently demonstrated that reference strains and clinical isolates of *P. aeruginosa* behave differently during their adaptability and intracellular survival into the lung epithelium. Our work shows the importance of choosing appropriate strains when studying infectious processes with relevant translational outcomes (Cendra and Torrents, 2020).

Within the polymicrobial nature of CF infection, *P. aeruginosa* and *Staphylococcus aureus* have long been recognized as primary CF pathogens, with an increasing rate of MDR appearance (Rutter et al., 2017). Although it is commonly assumed that *S. aureus* is an early colonizer of CF lungs that is replaced by *P. aeruginosa*, a recent longitudinal retrospective, single-center cohort study that included 337 patients with CF determined that *P. aeruginosa* does not replace the *S. aureus*; rather, both species accumulate over time (Fischer et al., 2021).

5.3.2. Chronic wound infection

The skin, despite its role as a protective barrier, is susceptible to many infections, especially when it is affected by burns, wounds or ulcers (Schittek, 2011). It is estimated that approximately 1-2% of the population in developed countries will suffer from chronic wounds during their lifetimes (Gottrup, 2004). *P. aeruginosa,* together with *S. aureus,* are common bacteria usually involved in infections of skin injuries. Biofilms of *P. aeruginosa* are typically associated with deep chronic wounds, which makes treatment with topical therapies difficult (Fazli et al., 2009; Rabin et al., 2015). The chronicity of the wound is in part due to the production of elastase by *P. aeruginosa,* which deteriorates immunoglobulin G and elements of the complement system (Wilson et al., 1998).

In diabetes mellitus, the development of foot ulcers is a typical complication of the disease. These patients tend to form nonhealing ulcers on the lower extremities with higher susceptibility than nondiabetic patients (Singh et al., 2005). Insulin treatment has been shown to increase the development of *P. aeruginosa* biofilms and their antimicrobial tolerance by increasing the levels of eDNA through the lysis of neutrophils and other immune cells in wounds. Furthermore, a direct role of insulin in this prolonged inflammatory response in the wound has been demonstrated in vitro through the use of incremental levels of intracellular c-di-GMP (Watters et al., 2014; Wei et al., 2019).

Chronic wound infections are primarily affected by polymicrobial communities, in which *P. aeruginosa* and *S. aureus* play a central role (Serra et al., 2015). It has been recently seen that the genotype of the patient influences the microbiome composition of the wound, i.e., depending on the patient's genetics, certain species are more likely to colonize the wound (Tipton et al., 2020). Genomic analyses have found that genes involved in anaerobic growth, metabolic and energy pathways and membrane integrity are critical for bacterial fitness in wounds (Morgan et al., 2019).

5.3.3. Keratitis

Biofilm formation over contact lenses (CLs) is an important cause of corneal infections, as these lenses make direct contact with the corneal surface. The annual incidence rate of microbial keratitis due to CL contamination is approximately 4 per 10000 daily contact lens wearers (Morgan et al., 2005). Biofilms formed over lenses enhance bacterial resistance to antimicrobials (Zegans et al., 2002). *P. aeruginosa* is a leading cause of corneal infections promoted by CL wearers (Stapleton and Carnt, 2012). Under normal and healthy conditions, the organism can poorly colonize the cornea. Nevertheless, if there is trauma or the cornea is injured, which is often mediated by continuous CL wearing, *P. aeruginosa* can penetrate the epithelial layer and cause keratitis (Zegans et al., 2002). In the presence of phagocytic cells or corneal epithelial debris, *P. aeruginosa* can even form denser biofilms on the CL (Burnham et al., 2012; Robertson et al., 2011). While growing in biofilms, bacteria can shift their gene expression to be able to persist in the ocular environment. In this sense, *P. aeruginosa* adapts to the human corneal epithelium by modulating, mainly, the expression of virulence genes. This corneal-adapted *P. aeruginosa* forms large biofilm-like aggregates (Evans and Fleiszig, 2013). The T3SS is highly expressed in the adaptation of *P. aeruginosa* for survival on the corneal surface, and it has an essential role in preserving the biofilm against the attack of host neutrophils. This event leads to NET production, which inhibits the spread of the bacteria to the brain by forming a barrier against the pathogen (Thanabalasuriar et al., 2019). Developing new materials for contact lenses able to inhibit bacterial attachment, viability or the adaptive changes in gene expression associated with bacteria growing on surfaces could have a remarkable impact on reducing the risk of infection (Evans and Fleiszig, 2013).

5.3.4. Medical device colonization

P. aeruginosa is a major nosocomial pathogen able to colonize and form perdurable biofilms on indwelling medical devices such as endotracheal tubes (EETs), catheters, and orthopedic implants as well as on *the* inner surfaces of metal pipes in hospital water systems. *P. aeruginosa* is responsible for 10–15% of nosocomial infections worldwide (Shi et al., 2019).

Biofilm formation has been found in 95% of patients intubated with EET and mechanical ventilation for more than 24 h. ETT biofilms are perdurable and able to remain despite antibiotic treatment. This fact increases the risk of upper respiratory tract infections that eventually lead to ventilator-associated pneumonia (VAP), for which *P. aeruginosa* is the primary causative agent (Gil-Perotin et al., 2012). Furthermore, the rhamnolipids produced by *P. aeruginosa* isolates are associated with VAP development (Kohler et al., 2010). *P. aeruginosa* VAP has increased morbidity and involves intensive care unit stays with the additional cost that they represent (Safdar et al., 2005).

Urinary catheters are also susceptible to colonization by *P. aeruginosa* and lead to catheter-associated urinary tract infections (CAUTIs), the most common hospital-associated infection. *P. aeruginosa* is responsible for 12% of the CAUTIs acquired in hospitals, which generally tend to be more complicated and have a worse prognosis than those caused by other bacteria (Cole et al., 2014). Iron deficiency in urinary tract infections increases the expression of rhamnolipids in *Pseudomonas,* promoting biofilm development (Glick et al., 2010). In addition to the increased antimicrobial tolerance that bacteria have when growing in

biofilms, many clinical *P. aeruginosa* isolates from CAUTIs have been shown to encode for multiple antimicrobial resistances (Vipin et al., 2019), complicating the clearance of this infection.

6. The challenge to reproduce *P. aeruginosa* polymicrobial chronic infections

Even though we now know that biofilms are the predominant type of bacterial growth in nature, planktonic experiments have historically been used as a reference to study chronic infections *in vitro*. Therefore, it has been an urgent need to regenerate the current knowledge about these infections, with models that properly recapitulate the pathophysiology created by biofilm-related infections, as the resulting phenotype obtained from experiments done under laboratory conditions, using monoculture growth and rich media, is far different from what is happening in nature. Additionally, during polymicrobial chronic infections, microbes interact with each other and with the host, which creates an environment that is harsh to recreate *in vitro,* and that often leads to having oversimplified models. Furthermore, even though multispecies interactions can be found in nature, they usually have antagonistic relations due to nutrient and space competition, which also complicate their co-growth *in vitro*. On the other hand, if good models can be achieved, they allow high-throughput screening with the flexibility to modify conditions. For this reason, there is a biotechnological challenge to mimic the polymicrobial biofilms found in several chronic infections with low-cost and easy-to-set-up technologies (Gabrilska and Rumbaugh, 2015).

Medium optimization has been used as a strategy to recreate multispecies biofilms by including or excluding specific components that can increase *P. aeruginosa* polymicrobial coexistence. For instance, addition of albumin in the media formulation has been used to grow *P. aeruginosa* and *S. aureus* simultaneously due to the inhibitory effect of the albumin on *P. aeruginosa*'s QS (Smith et al., 2017). Similarly, the use of L-arginine or adenosine monophosphate to compromise *P. aeruginosa* pathogenesis while, consequently, increasing *S. aureus* survival, or the use of nicotinamide adenine dinucleotide phosphate (NADPH) to combat the oxidative stress created during the coculture growth, are other supplements tested to increase the coexistence of both bacteria *in vitro* (Cendra et al., 2019; Sheng et al., 2012; Zhu et al., 2007). Otherwise, modification of the physicochemical parameters in the coculture system has been another strategy to increase both species' survival *in vitro.* For instance, oxygen is an important parameter to consider, as it influences bacterial survival during the coculture biofilm growth and it has a direct impact on the spatial distribution of the microorganisms within the biofilm (Cendra et al., 2019). In this line, we have recently described an optimized medium and coculture conditions, which consider environmental parameters such as pH and oxygen and include bovine serum albumin in Dulbecco's Modified Eagle's medium, that allow the coexistence of *P. aeruginosa* and *S. aureus* in stable biofilms *in vitro* (Cendra et al., 2019). Different inoculation ratios of these two bacteria have also been used to increase both strains' viability during coculture biofilm growth (Woods et al., 2018). These optimized mediums have been tested on multispecies biofilms grown in static conditions and limited nutrients supply, using microtiter plates or Calgary biofilm devices, as well as in dynamic models such as microfluidic devices, bioreactors, or flow-system models. While static models are cost-effective and useful in high throughput screenings, dynamic models better mimic the natural environmental conditions, thus enabling the biofilm to grow closer as it does *in vivo* (Gabrilska and Rumbaugh, 2015; Stewart and Franklin, 2008).

Additionally, during *P. aeruginosa* polymicrobial biofilm growth, it has been detected that continuous flow removes toxic products that inhibit the coexistence between species. It provides fresh and oxygenated medium to the system, helping the maintenance of the pH ~ 7 (in static conditions, pH rapidly increases over pH = 8) therefore allowing *P. aeruginosa* multispecies' coexistence (Cendra et al., 2019; O'Brien and Welch, 2019). 3D printed models of *P. aeruginosa* and *S. aureus* biofilms have also been developed using gelatin-based multiphoton lithography, which demonstrated that aggregates of *S. aureus* can increase their tolerance to ß-lactams when enclosed within a shell formed of *P. aeruginosa* (Connell et al., 2013).

Even though several methods have been described *in vitro*, we are still far from mimicking the bacterial infection as it is really occurring during human or animal chronic infections. In a chronic infection promoted by biofilm growth *in vivo*, the host response is activated by immune cells that battle against the infection. Some models of lung infections use 2D monolayers or lung organoids that mimic the features of a full-sized lung (Nadkarni et al., 2016). However, these models lack vasculature and immune cells and, therefore, fail to recapitulate the complex disease environment at the organ level completely. Similarly, the lung-on-a-chip model greatly mimics the lung's mechanical properties, and it can include endothelial cells in the microfluidic system (Wu et al., 2020). Nevertheless, it has a high manufacturing cost and the limitation to do high-throughput screening. Overall, there is still a long way to go to have proper *in vitro* models able to mimic the pathophysiology generated in chronic infections.

CF and wound infections are the two infectious environments that have been more investigated to reproduce *P. aeruginosa* biofilm and polymicrobial infections.

6.1. CF models

An artificial sputum media has been developed to mimic the sputum generated in CF. However, transcriptomic profiles have revealed differences between *P. aeruginosa* grown in this medium and those isolated directly from CF sputum samples (Palmer et al., 2007). Furthermore, as it is a medium created by analyzing the metabolic pathways of *P. aeruginosa* isolates, it tends to favor *P. aeruginosa* growth, limiting polymicrobial coexistence. The use of agar and alginate-beads to grow *P. aeruginosa*, alone or with other microbes found in CF infections, has also been a strategy to simulate the CF infectious environment (Sonderholm et al., 2017). 2D models of coculture experiments growing *P. aeruginosa* and *S. aureus* on lung cells affected with CF have also been used to study this microbial interaction (Hogan et al., 2004; Orazi and O'Toole, 2017). However, the infection virulence limits the monolayer integrity and impedes proper biofilm formation as extended incubation times cannot be achieved. To overcome it, many of these experiments use bacterial supernatants instead of live-bacteria, which can be an approximation but fail to recreate the *in vivo* disease completely.

On the other hand, mice and rat models have also been used to generate the CF-disease environment to subsequently study *P. aeruginosa* infections alone and other CF pathogens such as with *S. aureus* (Cigana et al., 2018; Millette et al., 2019). However, murine secretions differ from those in humans, which hinders the direct translation of the results (Benahmed et al., 2014). *ex vivo* pig lung models are used as a replacement for live animal infection as they are clinically realistic and more ethical models (Harrington et al., 2020).

6.2. Wound models

The Lubbock chronic wound biofilm (LCWB) model was created to mimic chronic wounds infected with biofilms under laboratory conditions (Sun et al., 2008). This model uses a chopped-meat-based medium supplemented with heparinized plasma and red blood cells to grow 24 h old multispecies biofilm *in vitro*. Initially, wound-*like* biofilms grown using this model were analyzed over the abiotic surface where the bacterial strains were growing. Later, in a work that investigated *P. aeruginosa* and *S. aureus* interactions, it was detected that the LCWB coagulated after 16 h of bacterial inoculation in a mechanism triggered by a *S. aureus* coagulase-positive strain. This observation was used to employ the coagulated plasma of the LCWB directly as a scaffold to grow the mixed biofilm, thus resembling more the *in vivo* conditions (DeLeon

et al., 2014). LCWB is still the basis of a wide range of wound infection models. *P. aeruginosa* and *S. aureus* coculture biofilms grown in simulated wound fluid over collagen matrices has also been used as a wound infection model (Werthen et al., 2010). Recently, tryptic soy broth enriched with NaCl and glucose was used to grow biofilms of these two bacteria on cell-derived matrices. This model allowed us to obtain the competitive distribution of these two organisms as it occurs *in vivo* and identify the SCV morphology of *S. aureus* driven by *P. aeruginosa* (Gounani et al., 2020).

In vivo models have also been used to study wound biofilm infections, however, they are limited by their elevated cost and ethical dilemmas. Still, murine wound infection models have been used to investigate the progression of *P. aeruginosa* and *S. aureus* coinfection and wound healing, as well as to test topical treatments (Hoffmann et al., 2019). Otherwise, the porcine skin wound healing closely resembles human healing, hence, porcine burn models have also been used to study *P. aeruginosa* polymicrobial burn infection with *S. aureus* (Pastar et al., 2013).

7. Current strategies against *P. aeruginosa* biofilms

Biofilms are a terrible public health threat. However, this is not the only issue, as these bacterial communities can also cause tremendous economic losses in the industry. *P. aeruginosa* is a common biofilm producer in food, water, and textile industries (Vishwakarma, 2020). To date, numerous strategies have been developed to prevent *P. aeruginosa* biofilm formation for both medical and industrial purposes.

Due to the emergence of antimicrobial resistance, antimicrobial peptides (AMPs), biofilm-degrading agents, QS inhibitors, as well as other compounds that target specific *P. aeruginosa* molecules have been developed as an alternative to antibiotics. All these antimicrobials are used against *P. aeruginosa,* killing the bacterium directly or preventing/eradicating its biofilms. The main mechanisms of action that these molecules follow are: i) inhibition of bacterium's attachment to the surface, ii) biofilm disruption with consequent bacterial dispersion, iii) inhibition of the production of specific molecules that promotes biofilm formation, or iv) increasing the antibiotic diffusion within the biofilm. Recent reviews address these types of antimicrobial therapies and tackle the emergence of antimicrobial resistance (Makabenta et al., 2021; Pinto et al., 2020; Pircalabioru and Chifiriuc, 2020; Rumbaugh and Sauer, 2020; Verderosa et al., 2019). In Table 1, we have summarized some of the currently investigated antibiofilm treatments.

Drug delivery systems (DDS) were created to control the release of therapeutic agents at the target site. As detailed in Section 5, *P. aeruginosa* biofilms' structure and physiology challenge their treatment and, in this sense, DDS have been of great help. DDS can be used as a surface coating to prevent biofilm formation, as well as carriers to deliver the antimicrobial inside or over the biofilm efficiently. DDS enhance the antimicrobials' pharmacodynamic and pharmacokinetic effect, increasing the effective concentration in the inner parts of the biofilm for its complete clearance (Liu et al., 2021). Even though different types of DDS have been developed to improve antimicrobial treatment, this review will focus on nanoparticles (NP)-DDS. NPs are an important DDS group, which in continuous development and improvement. Nevertheless, Table 1 also includes other types of DDS (e.g., phage-delivery systems).

NPs are DDS widely used for *Pseudomonas* biofilms' treatment. NPs are classified depending on the material used in their synthesis. In this sense, they could be: i) metallic NPs, ii) non-metallic NPs, iii) polymeric NPs, iv) lipid-phased NPs, v) ceramic NPs, and vi) quantum dots (Buch et al., 2019). Recently, smart nanomaterials have been developed, which have the particularity that the delivery of the antimicrobial cargo is done in response to a stimulus as, for instance, pH or light (Chen et al., 2019a). NPs size is critical to penetrating within the biofilm properly, and it has to range between 5–500 nm, being ideally 200 nm the maximum diameter. NPs smaller than 5 nm prone to be filtrated by the

Table 1
Antibiofilm strategies against *P. aeruginosa* biofilm formation.

Antibiofilm strategy	Mechanism of action	Examples
Inhibition of QS	Use of compounds to inhibit QS signaling	• Furanones interfere with AHLs (Wu et al., 2004) • Terrein blocks QS receptors and inhibits the production of virulence factors such as elastase, rhamnolipid or pyocyanin (Kim et al., 2018) • A benzimide-benzimidazole compound inhibits the MvrF regulator, inhibiting biofilm formation (Maura et al., 2017) • Baicalin hydrate is an AHL-targeting inhibitor (Brackman et al., 2011)
Inhibition of adhesion	• Surface coating with antimicrobial NPs/molecules that prevent bacterial adhesion • Modification of the surface material to inhibit biofilm formation	• Furanones are used to coat medical devices thus preventing bacterial adhesion (Baveja et al., 2004) • Coating devices with antibiotic hydroxyapatite-based coatings (Veerachamy et al., 2014) • Hydrophilic polymers as hyaluronic acid, hydrogel coating, and heparin coating have been used for catheters coating (Chen et al., 2013) • Functionalization of surfaces with antiadhesive high-density polymers (Neoh et al., 2015) • Nanosilver coatings have been used in catheters, heart valves, and wound dressings (Khatoon et al., 2018) • Gold, titanium, and diamond nanoparticle coating treatment (Veerachamy et al., 2014) • Modification of the properties of the biomaterial, as the surface roughness, energy or hydrophilicity can alter bacterial attachment (Bazaka et al., 2012)
Inhibition of c-di-GMP	Molecules that inhibit, decrease or sequester c-di-GMP promoting biofilm dispersal	• Terrein (Kim et al., 2018) • c-di-GMP-sequestering peptide (Hee et al., 2020) • Nitric oxide (Barraud et al., 2009) • Diguanylate cyclase inhibitors (Rumbaugh and Sauer, 2020)
Antibiofilm and antimicrobial molecules and peptides	Molecules and peptides with direct antimicrobial or antibiofilm properties	• poly-L-lysine cationic polypeptides have a mucolytic activity that disrupts the biofilm (Guillon et al., 2018) • liposomes have high antimicrobial activity and antibiofilm effect (Ibaraki et al., 2020) • antimicrobial peptides (AMPs) have quick bacterial killing and a low tendency to induce resistance, for instance, AMP ZY4 kills planktonic, biofilm and

(*continued on next page*)

Table 1 (*continued*)

Antibiofilm strategy	Mechanism of action	Examples
		persister *P. aeruginosa* cells by permeabilizing the membrane (Mwangi et al., 2019). • Enzymes to disturb biofilms such as DNases, glycosidases, alginate lyases, and proteases to digest the proteins involved (Baelo et al., 2015; Blanco-Cabra et al., 2020; Rumbaugh and Sauer, 2020)
Bacteriophage therapy	*Pseudomonas*-targeted bacteriophages that infect and kill the bacterium	• ϕMR299-2 and ϕNH-4 phage therapies have been shown to kill clinical *Pseudomonas* isolates colonizing a murine lung as well as in biofilms in a pulmonary cell line (Alemayehu et al., 2012)
Bioacoustic effect	Ultrasonication increases antibiotic diffusion across biofilms	• Ultrasonication combined with gentamicin increased the killing of *P. aeruginosa,* forming a biofilm (Pinto et al., 2020)

kidney while higher than 500 nm to be recognized and cleared by the human complement system (Liu et al., 2019). The shape of the NP is another critical element, as it can influence contact killing as, for instance, it occurs with sharp NPs, which can make a hole in the bacteria and cause cytoplasmic leakage. Otherwise, including mucolytic or other degrading agents in the NPs, together with using electrostatically neutral coating, increase the efficiency of NPs penetrance into the biofilm (Tan et al., 2020). In this line, our group has demonstrated the degrading effect of DNAse coated NPs loaded with ciprofloxacin on *in vivo*-like biofilms of *P. aeruginosa*, as well as unraveled the effect of specific alginate lyases (Alg2A and A1-II') as dispersing agents of *P. aeruginosa* biofilms (Baelo et al., 2015; Blanco-Cabra et al., 2020).

On the other hand, it has also been detected that cationic nanoparticles can have a good distribution across the biofilm matrix (Baelo et al., 2015; Makabenta et al., 2021). Biocompatibility and cell toxicity are major concerns when using these DDS as depending on the material they are made of, NPs can cause important cytotoxicity, being useless for human treatment. For example, this is one drawback of metallic NPs as high metal doses are toxic for human cells, which is why these NPs are not the preferred choice to use against CF-infections due to the long-term treatment that these infections require (Makabenta et al., 2021; Vandebriel and De Jong, 2012). NPs used in medical applications are usually coated with liposomes, silica or biopolymers to enhance their biocompatibility, as well as to improve their elimination from the body. Poly-D-L-(lactic-co-glycolic acid), polylactic acid, polyethylene glycol, poly(caprolactone, dextran, chitosan, poly(urethanes), poly(ethylene imine) or poly(*N*-isopropylacrylamide) are some biopolymers frequently used for NPs coating (Tan et al., 2020). An important disadvantage of the current NPs is the amount of cargo that they can load. The vast majority only support low concentrations of the drug, making them suitable as a proof-of-concept but not feasible for clinical application. The high number of NPs needed to achieve efficient antimicrobial concentration limits the balance between antimicrobial activity and cytotoxicity.

NPs can load different antibiotics/antimicrobials to be released within the biofilm. Tobramycin, ciprofloxacin, colistin, levofloxacin, amikacin, and gentamicin are known antibiotics delivered by NPs to treat *P. aeruginosa* infections. Some of them have been used with inhaled systems, although this technology is usually inefficient to eradicate *P. aeruginosa* biofilm infection (Ho et al., 2019). Otherwise, when using NPs directly against biofilms, it has been detected that the small size of these nanocarriers allows the particle diffusion across the thick and dense mucus of CF mucoid biofilms. In this sense, delivery of amikacin antibiotic by nanoscale liposomes has been used to treat *P. aeruginosa* biofilms in lung infections (Meers et al., 2008). Delivery of nitric oxide (NO) is an additional strategy used against *P. aeruginosa* biofilms. NO, as endogenous free radical, acts as a broad-spectrum antimicrobial with low toxicity and a great capacity to eradicate biofilms. Silica NPs conjugated with NO have been tested against *P. aeruginosa* and *S. aureus* coculture biofilms (Slomberg et al., 2013). Heat generation on NPs has also been seen to improve antibiotics delivery into *P. aeruginosa* mono and polymicrobial biofilms (Teirlinck et al., 2018). Table 1 summarizes additional strategies employed against *P. aeruginosa* biofilms using NPs as DDS.

8. Conclusions

P. aeruginosa biofilms are complex structures that become even more intricate when they are formed together with other microorganisms. They represent a clinical and biotechnological burden from different perspectives; therefore, only with the continuous development and improvement of efficient antibiofilm strategies we can tackle the recurrence and chronicity caused by *P. aeruginosa* biofilm infections.

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MC and ET have designed, written and approved the final version of the review.

References

Acosta, N., Waddell, B., Heirali, A., Somayaji, R., Surette, M.G., Workentine, M.L., Rabin, H.R., Parkins, M.D., 2020. Cystic fibrosis patients infected with epidemic *Pseudomonas aeruginosa* strains have unique microbial communities. Front. Cell Infect. Microbiol. 10, 173.

Alemayehu, D., Casey, P.G., McAuliffe, O., Guinane, C.M., Martin, J.G., Shanahan, F., Coffey, A., Ross, R.P., Hill, C., 2012. Bacteriophages phiMR299-2 and phiNH-4 can eliminate *Pseudomonas aeruginosa* in the murine lung and on cystic fibrosis lung airway cells. mBio 3(2), e00029-00012.

Alhede, M., Alhede, M., Qvortrup, K., Kragh, K.N., Jensen, P.O., Stewart, P.S., Bjarnsholt, T., 2020. The origin of extracellular DNA in bacterial biofilm infections *in vivo*. Pathogens Dis. 78 (2).

Allesen-Holm, M., Barken, K.B., Yang, L., Klausen, M., Webb, J.S., Kjelleberg, S., Molin, S., Givskov, M., Tolker-Nielsen, T., 2006. A characterization of DNA release in *Pseudomonas aeruginosa* cultures and biofilms. Mol. Microbiol. 59 (4), 1114–1128.

Almblad, H., Rybtke, M., Hendiani, S., Andersen, J.B., Givskov, M., Tolker-Nielsen, T., 2019. High levels of cAMP inhibit *Pseudomonas aeruginosa* biofilm formation through reduction of the c-di-GMP content. Microbiology 165 (3), 324–333.

Armbruster, C.R., Wolter, D.J., Mishra, M., Hayden, H.S., Radey, M.C., Merrihew, G., MacCoss, M.J., Burns, J., Wozniak, D.J., Parsek, M.R., Hoffman, L.R., 2016. *Staphylococcus aureus* protein A mediates interspecies interactions at the cell surface of *Pseudomonas aeruginosa*. mBio 7 (3).

Baelo, A., Levato, R., Julian, E., Crespo, A., Astola, J., Gavalda, J., Engel, E., Mateos-Timoneda, M.A., Torrents, E., 2015. Disassembling bacterial extracellular matrix with DNase-coated nanoparticles to enhance antibiotic delivery in biofilm infections. J. Control Release 209, 150–158.

Barraud, N., Storey, M.V., Moore, Z.P., Webb, J.S., Rice, S.A., Kjelleberg, S., 2009. Nitric oxide-mediated dispersal in single- and multi-species biofilms of clinically and industrially relevant microorganisms. Microb. Biotechnol. 2 (3), 370–378.

Baveja, J.K., Willcox, M.D., Hume, E.B., Kumar, N., Odell, R., Poole-Warren, L.A., 2004. Furanones as potential anti-bacterial coatings on biomaterials. Biomaterials 25 (20), 5003–5012.

Bayer, A.S., Speert, D.P., Park, S., Tu, J., Witt, M., Nast, C.C., Norman, D.C., 1991. Functional role of mucoid exopolysaccharide (alginate) in antibiotic-induced and polymorphonuclear leukocyte-mediated killing of *Pseudomonas aeruginosa*. Infect Immun. 59 (1), 302–308.
Bazaka, K., Jacob, M.V., Crawford, R.J., Ivanova, E.P., 2012. Efficient surface modification of biomaterial to prevent biofilm formation and the attachment of microorganisms. Appl. Microbiol. Biotechnol. 95 (2), 299–311.
Benahmed, M.A., Elbayed, K., Daubeuf, F., Santelmo, N., Frossard, N., Namer, I.J., 2014. NMR HRMAS spectroscopy of lung biopsy samples: comparison study between human, pig, rat, and mouse metabolomics. Magn. Reson. Med. 71 (1), 35–43.
Berger, D., Rakhamimova, A., Pollack, A., Loewy, Z., 2018. Oral biofilms: development, control, and analysis. High Throughput 7 (3).
Billings, N., Millan, M., Caldara, M., Rusconi, R., Tarasova, Y., Stocker, R., Ribbeck, K., 2013. The extracellular matrix Component Psl provides fast-acting antibiotic defense in *Pseudomonas aeruginosa* biofilms. PLoS Pathog. 9 (8), e1003526.
Bisht, K., Baishya, J., Wakeman, C.A., 2020. *Pseudomonas aeruginosa* polymicrobial interactions during lung infection. Curr. Opin. Microbiol. 53, 1–8.
Biswas, L., Biswas, R., Schlag, M., Bertram, R., Gotz, F., 2009. Small-colony variant selection as a survival strategy for *Staphylococcus aureus* in the presence of *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. 75 (21), 6910–6912.
Bjarnsholt, T., Jensen, P.O., Fiandaca, M.J., Pedersen, J., Hansen, C.R., Andersen, C.B., Pressler, T., Givskov, M., Hoiby, N., 2009. *Pseudomonas aeruginosa* biofilms in the respiratory tract of cystic fibrosis patients. Pediatr. Pulmonol. 44 (6), 547–558.
Bjedov, I., Tenaillon, O., Gerard, B., Souza, V., Denamur, E., Radman, M., Taddei, F., Matic, I., 2003. Stress-induced mutagenesis in bacteria. Science 300 (5624), 1404–1409.
Blanco-Cabra, N., Paetzold, B., Ferrar, T., Mazzolini, R., Torrents, E., Serrano, L., Maria Lluch, S., 2020. Characterization of different alginate lyases for dissolving *Pseudomonas aeruginosa* biofilms. Sci. Rep. 10 (1), 9390.
Borlee, B.R., Goldman, A.D., Murakami, K., Samudrala, R., Wozniak, D.J., Parsek, M.R., 2010. *Pseudomonas aeruginosa* uses a cyclic-di-GMP-regulated adhesin to reinforce the biofilm extracellular matrix. Mol. Microbiol. 75 (4), 827–842.
Borriello, G., Werner, E., Roe, F., Kim, A.M., Ehrlich, G.D., Stewart, P.S., 2004. Oxygen limitation contributes to antibiotic tolerance of *Pseudomonas aeruginosa* in biofilms. Antimicrob. Agents Chemother. 48 (7), 2659–2664.
Brackman, G., Cos, P., Maes, L., Nelis, H.J., Coenye, T., 2011. Quorum sensing inhibitors increase the susceptibility of bacterial biofilms to antibiotics *in vitro* and *in vivo*. Antimicrob. Agents Chemother. 55 (6), 2655–2661.
Brinkmann, V., Reichard, U., Goosmann, C., Fauler, B., Uhlemann, Y., Weiss, D.S., Weinrauch, Y., Zychlinsky, A., 2004. Neutrophil extracellular traps kill bacteria. Science 303 (5663), 1532–1535.
Brownlee, J.W., Turner, R.B., 2008. New developments in the epidemiology and clinical spectrum of rhinovirus infections. Curr. Opin. Pediatr. 20 (1), 67–71.
Buch, P.J., Chai, Y., Goluch, E.D., 2019. Treating polymicrobial infections in chronic diabetic wounds. Clin. Microbiol. Rev. 32 (2).
Burnham, G.W., Cavanagh, H.D., Robertson, D.M., 2012. The impact of cellular debris on *Pseudomonas aeruginosa* adherence to silicone hydrogel contact lenses and contact lens storage cases. Eye Contact Lens 38 (1), 7–15.
Cendra, M., Torrents, E., 2020. Differential adaptability between reference strains and clinical isolates of *Pseudomonas aeruginosa* into the lung epithelium intracellular lifestyle. Virulence 11 (1), 862–876.
Cendra, M.D.M., Blanco-Cabra, N., Pedraz, L., Torrents, E., 2019. Optimal environmental and culture conditions allow the *in vitro* coexistence of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in stable biofilms. Sci. Rep. 9 (1), 16284.
Chen, M., Yu, Q., Sun, H., 2013. Novel strategies for the prevention and treatment of biofilm related infections. Int. J. Mol. Sci. 14 (9), 18488–18501.
Chen, M., Wei, J., Xie, S., Tao, X., Zhang, Z., Ran, P., Li, X., 2019a. Bacterial biofilm destruction by size/surface charge-adaptive micelles. Nanoscale 11 (3), 1410–1422.
Chen, R., Deziel, E., Groleau, M.C., Schaefer, A.L., Greenberg, E.P., 2019b. Social cheating in a *Pseudomonas aeruginosa* quorum-sensing variant. Proc. Natl. Acad. Sci. U. S. A. 116 (14), 7021–7026.
Chew, S.C., Yam, J.K.H., Matysik, A., Seng, Z.J., Klebensberger, J., Givskov, M., Doyle, P., Rice, S.A., Yang, L., Kjelleberg, S., 2018. Matrix polysaccharides and SiaD diguanylate cyclase alter community structure and competitiveness of *Pseudomonas aeruginosa* during dual-species biofilm development with *Staphylococcus aureus*. mBio 9 (6).
Cigana, C., Bianconi, I., Baldan, R., De Simone, M., Riva, C., Sipione, B., Rossi, G., Cirillo, D.M., Bragonzi, A., 2018. *Staphylococcus aureus* impacts *Pseudomonas aeruginosa* chronic respiratory disease in murine models. J. Infect. Dis. 217 (6), 933–942.
Ciofu, O., Tolker-Nielsen, T., 2019. Tolerance and resistance of *Pseudomonas aeruginosa* biofilms to antimicrobial agents-how *P. aeruginosa* can escape antibiotics. Front. Microbiol. 10, 913.
Ciofu, O., Tolker-Nielsen, T., Jensen, P.O., Wang, H., Hoiby, N., 2015. Antimicrobial resistance, respiratory tract infections and role of biofilms in lung infections in cystic fibrosis patients. Adv. Drug Deliv. Rev. 85, 7–23.
Ciszek-Lenda, M., Strus, M., Walczewska, M., Majka, G., Machul-Zwirbla, A., Mikolajczyk, D., Gorska, S., Gamian, A., Chain, B., Marcinkiewicz, J., 2019. *Pseudomonas aeruginosa* biofilm is a potent inducer of phagocyte hyperinflammation. Inflamm. Res. 68 (5), 397–413.
Cohen, T.S., Hilliard, J.J., Jones-Nelson, O., Keller, A.E., O'Day, T., Tkaczyk, C., DiGiandomenico, A., Hamilton, M., Pelletier, M., Wang, Q., Diep, B.A., Le, V.T., Cheng, L., Suzich, J., Stover, C.K., Sellman, B.R., 2016. *Staphylococcus aureus* alpha toxin potentiates opportunistic bacterial lung infections. Sci. Transl. Med. 8 (329), 329ra331.
Cole, S.J., Records, A.R., Orr, M.W., Linden, S.B., Lee, V.T., 2014. Catheter-associated urinary tract infection by *Pseudomonas aeruginosa* is mediated by exopolysaccharide-independent biofilms. Infect. Immun. 82 (5), 2048–2058.
Colombo, A.V., Barbosa, G.M., Higashi, D., di Micheli, G., Rodrigues, P.H., Simionato, M. R.L., 2013. Quantitative detection of *Staphylococcus aureus*, *Enterococcus faecalis* and *Pseudomonas aeruginosa* in human oral epithelial cells from subjects with periodontitis and periodontal health. J. Med. Microbiol. 62 (Pt 10), 1592–1600.
Connell, J.L., Ritschdorff, E.T., Whiteley, M., Shear, J.B., 2013. 3D printing of microscopic bacterial communities. Proc. Natl. Acad. Sci. U. S. A. 110 (46), 18380–18385.
Crabbe, A., Ledesma, M.A., Nickerson, C.A., 2014. Mimicking the host and its microenvironment *in vitro* for studying mucosal infections by *Pseudomonas aeruginosa*. Pathog. Dis. 71 (1), 1–19.
Cruz, R.L., Asfahl, K.L., Van den Bossche, S., Coenye, T., Crabbe, A., Dandekar, A.A., 2020. RhlR-regulated acyl-homoserine lactone quorum sensing in a cystic fibrosis isolate of *Pseudomonas aeruginosa*. mBio 11 (2).
Cugini, C., Calfee, M.W., Farrow 3rd, J.M., Morales, D.K., Pesci, E.C., Hogan, D.A., 2007. Farnesol, a common sesquiterpene, inhibits PQS production in *Pseudomonas aeruginosa*. Mol. Microbiol. 65 (4), 896–906.
CysticFibrosisFoundation, 2019. Cystic Fibrosis Foundation Patient Registry Highlights.
Das, T., Manefield, M., 2012. Pyocyanin promotes extracellular DNA release in *Pseudomonas aeruginosa*. PLoS One 7 (10), e46718.
Das, T., Sehar, S., Koop, L., Wong, Y.K., Ahmed, S., Siddiqui, K.S., Manefield, M., 2014. Influence of calcium in extracellular DNA mediated bacterial aggregation and biofilm formation. PloS one 9 (3), e91935.
Davey, M.E., Caiazza, N.C., O'Toole, G.A., 2003. Rhamnolipid surfactant production affects biofilm architecture in *Pseudomonas aeruginosa* PAO1. J. Bacteriol. 185 (3), 1027–1036.
Davies, D.G., Marques, C.N., 2009. A fatty acid messenger is responsible for inducing dispersion in microbial biofilms. J. Bacteriol. 191 (5), 1393–1403.
Davies, D.G., Parsek, M.R., Pearson, J.P., Iglewski, B.H., Costerton, J.W., Greenberg, E.P., 1998. The involvement of cell-to-cell signals in the development of a bacterial biofilm. Science 280 (5361), 295–298.
DeLeon, S., Clinton, A., Fowler, H., Everett, J., Horswill, A.R., Rumbaugh, K.P., 2014. Synergistic interactions of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in an *in vitro* wound model. Infect. Immun. 82 (11), 4718–4728.
Deng, B., Ghatak, S., Sarkar, S., Singh, K., Das Ghatak, P., Mathew-Steiner, S.S., Roy, S., Khanna, S., Wozniak, D.J., McComb, D.W., Sen, C.K., 2020. Novel bacterial diversity and fragmented eDNA identified in hyperbiofilm-forming *Pseudomonas aeruginosa* rugose small colony variant. iScience 23 (2), 100827.
Diggle, S.P., Stacey, R.E., Dodd, C., Camara, M., Williams, P., Winzer, K., 2006. The galactophilic lectin, LecA, contributes to biofilm development in *Pseudomonas aeruginosa*. Environ. Microbiol. 8 (6), 1095–1104.
Drenkard, E., Ausubel, F.M., 2002. *Pseudomonas* biofilm formation and antibiotic resistance are linked to phenotypic variation. Nature 416 (6882), 740–743.
Duan, K., Dammel, C., Stein, J., Rabin, H., Surette, M.G., 2003. Modulation of *Pseudomonas aeruginosa* gene expression by host microflora through interspecies communication. Mol. Microbiol. 50 (5), 1477–1491.
Evans, D.J., Fleiszig, S.M., 2013. Microbial keratitis: could contact lens material affect disease pathogenesis? Eye Contact Lens 39 (1), 73–78.
Fazli, M., Bjarnsholt, T., Kirketerp-Moller, K., Jorgensen, B., Andersen, A.S., Krogfelt, K. A., Givskov, M., Tolker-Nielsen, T., 2009. Nonrandom distribution of *Pseudomonas aeruginosa* and *Staphylococcus aureus* in chronic wounds. J. Clin. Microbiol. 47 (12), 4084–4089.
Filkins, L.M., O'Toole, G.A., 2015. Cystic fibrosis lung infections: polymicrobial, complex, and hard to treat. PLoS Pathog. 11 (12), e1005258.
Filkins, L.M., Hampton, T.H., Gifford, A.H., Gross, M.J., Hogan, D.A., Sogin, M.L., Morrison, H.G., Paster, B.J., O'Toole, G.A., 2012. Prevalence of streptococci and increased polymicrobial diversity associated with cystic fibrosis patient stability. J. Bacteriol. 194 (17), 4709–4717.
Filkins, L.M., Graber, J.A., Olson, D.G., Dolben, E.L., Lynd, L.R., Bhuju, S., O'Toole, G.A., 2015. Coculture of *Staphylococcus aureus* with *Pseudomonas aeruginosa* Drives *S. aureus* towards fermentative metabolism and reduced viability in a cystic fibrosis model. J. Bacteriol. 197 (14), 2252–2264.
Fischer, A.J., Singh, S.B., LaMarche, M.M., Maakestad, L.J., Kienenberger, Z.E., Pena, T. A., Stoltz, D.A., Limoli, D.H., 2021. Sustained coinfections with *Staphylococcus aureus* and *Pseudomonas aeruginosa* in cystic fibrosis. Am. J. Respir. Crit. Care Med. 203 (1), 328–338.
Franklin, M.J., Nivens, D.E., Weadge, J.T., Howell, P.L., 2011. Biosynthesis of the *Pseudomonas aeruginosa* extracellular polysaccharides, alginate, Pel, and Psl. Front. Microbiol. 2, 167.
Gabrilska, R.A., Rumbaugh, K.P., 2015. Biofilm models of polymicrobial infection. Fut. Microbiol. 10 (12), 1997–2015.
Gil-Perotin, S., Ramirez, P., Marti, V., Sahuquillo, J.M., Gonzalez, E., Calleja, I., Menendez, R., Bonastre, J., 2012. Implications of endotracheal tube biofilm in ventilator-associated pneumonia response: a state of concept. Crit. Care 16 (3), R93.
Glick, R., Gilmour, C., Tremblay, J., Satanower, S., Avidan, O., Deziel, E., Greenberg, E. P., Poole, K., Banin, E., 2010. Increase in rhamnolipid synthesis under iron-limiting conditions influences surface motility and biofilm formation in *Pseudomonas aeruginosa*. J. Bacteriol. 192 (12), 2973–2980.
Gökalsın, B., Berber, D., Cenk Sesal, N., 2019. Chapter 9 - *Pseudomonas aeruginosa* quorum sensing and biofilm inhibition. In: Quorum Sensing - Molecular Mechanism and Biotechnological Application, pp. 227–256.
Goldmann, O., Medina, E., 2012. The expanding world of extracellular traps: not only neutrophils but much more. Front. Immunol. 3, 420.

Goltermann, L., Tolker-Nielsen, T., 2017. Importance of the exopolysaccharide matrix in antimicrobial tolerance of *Pseudomonas aeruginosa* aggregates. Antimicrob. Agents Chemother. 61 (4).
Gottrup, F., 2004. A specialized wound-healing center concept: importance of a multidisciplinary department structure and surgical treatment facilities in the treatment of chronic wounds. Am. J. Surg. 187 (5A), 38S–43S.
Gounani, Z., Sen Karaman, D., Venu, A.P., Cheng, F., Rosenholm, J.M., 2020. Coculture of *P. aeruginosa* and *S. aureus* on cell derived matrix - an *in vitro* model of biofilms in infected wounds. J. Microbiol. Meth. 175, 105994.
Guillon, A., Fouquenet, D., Morello, E., Henry, C., Georgeault, S., Si-Tahar, M., Herve, V., 2018. Treatment of *Pseudomonas aeruginosa* biofilm present in endotracheal tubes by poly-L-lysine. Antimicrob. Agents Chemother. 62 (11).
Gupta, K., Liao, J., Petrova, O.E., Cherny, K.E., Sauer, K., 2014. Elevated levels of the second messenger c-di-GMP contribute to antimicrobial resistance of *Pseudomonas aeruginosa*. Mol. Microbiol. 92 (3), 488–506.
Halverson, T.W., Wilton, M., Poon, K.K., Petri, B., Lewenza, S., 2015. DNA is an antimicrobial component of neutrophil extracellular traps. PLoS Pathog. 11 (1), e1004593.
Harrington, N.E., Sweeney, E., Harrison, F., 2020. Building a better biofilm - formation of *in vivo*-like biofilm structures by *Pseudomonas aeruginosa* in a porcine model of cystic fibrosis lung infection. Biofilm 2, 100024.
Harrison, J.J., Almblad, H., Irie, Y., Wolter, D.J., Eggleston, H.C., Randall, T.E., Kitzman, J.O., Stackhouse, B., Emerson, J.C., McNamara, S., Larsen, T.J., Shendure, J., Hoffman, L.R., Wozniak, D.J., Parsek, M.R., 2020. Elevated exopolysaccharide levels in *Pseudomonas aeruginosa* flagellar mutants have implications for biofilm growth and chronic infections. PLoS Genet. 16 (6), e1008848.
Hee, C.S., Habazettl, J., Schmutz, C., Schirmer, T., Jenal, U., Grzesiek, S., 2020. Intercepting second-messenger signaling by rationally designed peptides sequestering c-di-GMP. Proc. Natl. Acad. Sci. U. S. A. 117 (29), 17211–17220.
Hendricks, M.R., Lashua, L.P., Fischer, D.K., Flitter, B.A., Eichinger, K.M., Durbin, J.E., Sarkar, S.N., Coyne, C.B., Empey, K.M., Bomberger, J.M., 2016. Respiratory syncytial virus infection enhances *Pseudomonas aeruginosa* biofilm growth through dysregulation of nutritional immunity. Proc. Natl. Acad. Sci. U. S. A. 113 (6), 1642–1647.
Hengzhuang, W., Ciofu, O., Yang, L., Wu, H., Song, Z., Oliver, A., Hoiby, N., 2013. High beta-lactamase levels change the pharmacodynamics of beta-lactam antibiotics in *Pseudomonas aeruginosa* biofilms. Antimicrob. Agents Chemother. 57 (1), 196–204.
Hiatt, P.W., Grace, S.C., Kozinetz, C.A., Raboudi, S.H., Treece, D.G., Taber, L.H., Piedra, P.A., 1999. Effects of viral lower respiratory tract infection on lung function in infants with cystic fibrosis. Pediatrics 103 (3), 619–626.
Ho, D.K., Nichols, B.L.B., Edgar, K.J., Murgia, X., Loretz, B., Lehr, C.M., 2019. Challenges and strategies in drug delivery systems for treatment of pulmonary infections. Eur. J. Pharm. Biopharm. 144, 110–124.
Hoffman, L.R., Deziel, E., D'Argenio, D.A., Lepine, F., Emerson, J., McNamara, S., Gibson, R.L., Ramsey, B.W., Miller, S.I., 2006. Selection for *Staphylococcus aureus* small-colony variants due to growth in the presence of *Pseudomonas aeruginosa*. Proc. Natl. Acad. Sci. U. S. A. 103 (52), 19890–19895.
Hoffman, L.R., Kulasekara, H.D., Emerson, J., Houston, L.S., Burns, J.L., Ramsey, B.W., Miller, S.I., 2009. *Pseudomonas aeruginosa lasR* mutants are associated with cystic fibrosis lung disease progression. J. Cystic Fibrosis 8 (1), 66–70.
Hoffmann, J.P., Friedman, J.K., Wang, Y., McLachlan, J.B., Sammarco, M.C., Morici, L. A., Roy, C.J., 2019. In situ treatment with novel microbiocide inhibits methicillin resistant *Staphylococcus aureus* in a murine wound infection model. Front. Microbiol. 10, 3106.
Hogan, D.A., Vik, A., Kolter, R., 2004. A *Pseudomonas aeruginosa* quorum-sensing molecule influences *Candida albicans* morphology. Mol. Microbiol. 54 (5), 1212–1223.
Hoiby, N., Bjarnsholt, T., Moser, C., Bassi, G.L., Coenye, T., Donelli, G., Hall-Stoodley, L., Hola, V., Imbert, C., Kirketerp-Moller, K., Lebeaux, D., Oliver, A., Ullmann, A.J., Williams, C., Biofilms, E.S.G.f, Consulting External Expert Werner, Z, 2015. ESCMID guideline for the diagnosis and treatment of biofilm infections 2014. Clin. Microbiol. Infect. 21 (Suppl. 1), S1–25.
Hotterbeekx, A., Kumar-Singh, S., Goossens, H., Malhotra-Kumar, S., 2017. *In vivo* and *in vitro* interactions between *Pseudomonas aeruginosa* and *Staphylococcus* spp. Front. Cell Infect. Microbiol. 7, 106.
Ibaraki, H., Kanazawa, T., Chien, Y.W., Nakaminami, H., Aoki, M., Ozawa, K., Kaneko, H., Takashima, Y., Noguchi, N., Seta, Y., 2020. The effects of surface properties of liposomes on their activity against *Pseudomonas aeruginosa* PAO-1 biofilm. J. Drug Deliv.Sci. Technol. 57 (101754).
Intra, J., Sarto, C., Beck, E., Tiberti, N., Leoni, V., Brambilla, P., 2020. Bacterial and fungal colonization of the respiratory tract in COVID-19 patients should not be neglected. Am. J. Infect. Control 48 (9), 1130–1131.
Jacques, I., Derelle, J., Weber, M., Vidailhet, M., 1998. Pulmonary evolution of cystic fibrosis patients colonized by *Pseudomonas aeruginosa* and/or *Burkholderia cepacia*. Eur. J. Pediatr. 157 (5), 427–431.
Jenal, U., Malone, J., 2006. Mechanisms of cyclic-di-GMP signaling in bacteria. Annu. Rev. Genet. 40, 385–407.
Jennings, L.K., Storek, K.M., Ledvina, H.E., Coulon, C., Marmont, L.S., Sadovskaya, I., Secor, P.R., Tseng, B.S., Scian, M., Filloux, A., Wozniak, D.J., Howell, P.L., Parsek, M. R., 2015. Pel is a cationic exopolysaccharide that cross-links extracellular DNA in the *Pseudomonas aeruginosa* biofilm matrix. Proc. Natl. Acad. Sci. U. S. A. 112 (36), 11353–11358.
Johnson, L., Mulcahy, H., Kanevets, U., Shi, Y., Lewenza, S., 2012. Surface-localized spermidine protects the *Pseudomonas aeruginosa* outer membrane from antibiotic treatment and oxidative stress. J. Bacteriol. 194 (4), 813–826.
Jones, C.J., Wozniak, D.J., 2017. Psl produced by mucoid *Pseudomonas aeruginosa* contributes to the establishment of biofilms and immune evasion. mBio 8 (3).
Karygianni, L., Ren, Z., Koo, H., Thurnheer, T., 2020. Biofilm matrixome: extracellular components in structured microbial communities. Trends Microbiol. 28 (8), 668–681.
Khatoon, Z., McTiernan, C.D., Suuronen, E.J., Mah, T.F., Alarcon, E.I., 2018. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon 4 (12), e01067.
Kim, B., Park, J.S., Choi, H.Y., Yoon, S.S., Kim, W.G., 2018. Terrein is an inhibitor of quorum sensing and c-di-GMP in *Pseudomonas aeruginosa*: a connection between quorum sensing and c-di-GMP. Sci. Rep. 8 (1), 8617.
Kohler, T., Guanella, R., Carlet, J., van Delden, C., 2010. Quorum sensing-dependent virulence during *Pseudomonas aeruginosa* colonisation and pneumonia in mechanically ventilated patients. Thorax 65 (8), 703–710.
Korgaonkar, A., Trivedi, U., Rumbaugh, K.P., Whiteley, M., 2013. Community surveillance enhances *Pseudomonas aeruginosa* virulence during polymicrobial infection. Proc. Natl. Acad. Sci. U. S. A. 110 (3), 1059–1064.
Lam, J., Chan, R., Lam, K., Costerton, J.W., 1980. Production of mucoid microcolonies by *Pseudomonas aeruginosa* within infected lungs in cystic fibrosis. Infect. Immun. 28 (2), 546–556.
Lansbury, L., Lim, B., Baskaran, V., Lim, W.S., 2020. Co-infections in people with COVID-19: a systematic review and meta-analysis. J. Infect. 81 (2), 266–275.
Lebeaux, D., Ghigo, J.M., Beloin, C., 2014. Biofilm-related infections: bridging the gap between clinical management and fundamental aspects of recalcitrance toward antibiotics. Microbiol. Mol. Biol. Rev. 78 (3), 510–543.
Lewenza, S., Johnson, L., Charron-Mazenod, L., Hong, M., Mulcahy-O'Grady, H., 2020. Extracellular DNA controls expression of *Pseudomonas aeruginosa* genes involved in nutrient utilization, metal homeostasis, acid pH tolerance and virulence. J. Med. Microbiol. 69 (6), 895–905.
Lewis, K., 2010. Persister cells. Ann. Rev. Microbiol. 64, 357–372.
Li, K., Gifford, A.H., Hampton, T.H., O'Toole, G.A., 2020. Availability of zinc impacts interactions between *Streptococcus sanguinis* and *Pseudomonas aeruginosa* in coculture. J. Bacteriol. 202 (2).
Limoli, D.H., Yang, J., Khansaheb, M.K., Helfman, B., Peng, L., Stecenko, A.A., Goldberg, J.B., 2016. *Staphylococcus aureus* and *Pseudomonas aeruginosa* co-infection is associated with cystic fibrosis-related diabetes and poor clinical outcomes. Eur. J. Clin. Microbiol. Infect. Dis. 35 (6), 947–953.
Limoli, D.H., Whitfield, G.B., Kitao, T., Ivey, M.L., Davis Jr., M.R., Grahl, N., Hogan, D.A., Rahme, L.G., Howell, P.L., O'Toole, G.A., Goldberg, J.B., 2017. *Pseudomonas aeruginosa* alginate overproduction promotes coexistence with *Staphylococcus aureus* in a model of cystic fibrosis respiratory infection. mBio 8 (2).
Liu, Y., Li, Y., Shi, L., 2021. Controlled drug delivery systems in eradicating bacterial biofilm-associated infections. J. Control Release 329, 1102–1116.
Liu, Y., Shi, L., Su, L., van der Mei, H.C., Jutte, P.C., Ren, Y., Busscher, H.J., 2019. Nanotechnology-based antimicrobials and delivery systems for biofilm-infection control. Chem. Soc. Rev. 48 (2), 428–446.
Ma, L., Conover, M., Lu, H., Parsek, M.R., Bayles, K., Wozniak, D.J., 2009. Assembly and development of the *Pseudomonas aeruginosa* biofilm matrix. PLoS Pathog. 5 (3), e1000354.
Ma, L., Wang, S., Wang, D., Parsek, M.R., Wozniak, D.J., 2012. The roles of biofilm matrix polysaccharide Psl in mucoid *Pseudomonas aeruginosa* biofilms. FEMS Immunol. Med. Microbiol. 65 (2), 377–380.
Macia, M.D., Rojo-Molinero, E., Oliver, A., 2014. Antimicrobial susceptibility testing in biofilm-growing bacteria. Clin. Microbiol. Infect. 20 (10), 981–990.
Makabenta, J.M.V., Nabawy, A., Li, C.H., Schmidt-Malan, S., Patel, R., Rotello, V.M., 2021. Nanomaterial-based therapeutics for antibiotic-resistant bacterial infections. Nat. Rev. Microbiol. 19 (1), 23–36.
Malone, J.G., Jaeger, T., Spangler, C., Ritz, D., Spang, A., Arrieumerlou, C., Kaever, V., Landmann, R., Jenal, U., 2010. YfiBNR mediates cyclic di-GMP dependent small colony variant formation and persistence in *Pseudomonas aeruginosa*. PLoS Pathog. 6 (3), e1000804.
Martin, D.W., Schurr, M.J., Mudd, M.H., Govan, J.R., Holloway, B.W., Deretic, V., 1993. Mechanism of conversion to mucoidy in *Pseudomonas aeruginosa* infecting cystic fibrosis patients. Proc. Natl. Acad. Sci. U. S. A. 90 (18), 8377–8381.
Mashburn, L.M., Jett, A.M., Akins, D.R., Whiteley, M., 2005. *Staphylococcus aureus* serves as an iron source for *Pseudomonas aeruginosa* during *in vivo* coculture. J. Bacteriol. 187 (2), 554–566.
Mauch, R.M., Norregaard, L.L., Ciofu, O., Levy, C.E., Hoiby, N., 2018. IgG avidity to *Pseudomonas aeruginosa* over the course of chronic lung biofilm infection in cystic fibrosis. J. Cyst. Fibros 17 (3), 356–359.
Maura, D., Drees, S.L., Bandyopadhaya, A., Kitao, T., Negri, M., Starkey, M., Lesic, B., Milot, S., Deziel, E., Zahler, R., Pucci, M., Felici, A., Fetzner, S., Lepine, F., Rahme, L. G., 2017. Polypharmacology approaches against the *Pseudomonas aeruginosa* MvfR regulon and their application in blocking virulence and antibiotic tolerance. ACS Chem. Biol. 12 (5), 1435–1443.
Maurice, N.M., Bedi, B., Sadikot, R.T., 2018. *Pseudomonas aeruginosa* Biofilms: host response and clinical implications in lung infections. Am. J. Respir. Cell Mol. Biol. 58 (4), 428–439.
McDaniel, M.S., Schoeb, T., Swords, W.E., 2020. Cooperativity between *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* during polymicrobial airway infections. Infect. Immun. 88 (4).
McGuigan, L., Callaghan, M., 2015. The evolving dynamics of the microbial community in the cystic fibrosis lung. Environ. Microbiol. 17 (1), 16–28.
Medina, G., Juarez, K., Valderrama, B., Soberon-Chavez, G., 2003. Mechanism of *Pseudomonas aeruginosa* RhlR transcriptional regulation of the rhlAB promoter. J. Bacteriol. 185 (20), 5976–5983.

Meers, P., Neville, M., Malinin, V., Scotto, A.W., Sardaryan, G., Kurumunda, R., Mackinson, C., James, G., Fisher, S., Perkins, W.R., 2008. Biofilm penetration, triggered release and in vivo activity of inhaled liposomal amikacin in chronic *Pseudomonas aeruginosa* lung infections. J. Antimicrob. Chemother. 61 (4), 859–868.
Millette, G., Langlois, J.P., Brouillette, E., Frost, E.H., Cantin, A.M., Malouin, F., 2019. Despite antagonism *in vitro*, *Pseudomonas aeruginosa* enhances *Staphylococcus aureus* colonization in a murine lung infection model. Front. Microbiol. 10, 2880.
Mitchell, E.P., Sabin, C., Snajdrova, L., Pokorna, M., Perret, S., Gautier, C., Hofr, C., Gilboa-Garber, N., Koca, J., Wimmerova, M., Imberty, A., 2005. High affinity fucose binding of *Pseudomonas aeruginosa* lectin PA-IIL: 1.0 A resolution crystal structure of the complex combined with thermodynamics and computational chemistry approaches. Proteins 58 (3), 735–746.
Mitchell, G., Seguin, D.L., Asselin, A.E., Deziel, E., Cantin, A.M., Frost, E.H., Michaud, S., Malouin, F., 2010. *Staphylococcus aureus* sigma B-dependent emergence of small-colony variants and biofilm production following exposure to *Pseudomonas aeruginosa* 4-hydroxy-2-heptylquinoline-N-oxide. BMC Microbiol. 10, 33.
Moreau-Marquis, S., Stanton, B.A., O'Toole, G.A., 2008. *Pseudomonas aeruginosa* biofilm formation in the cystic fibrosis airway. Pulm. Pharmacol. Ther. 21 (4), 595–599.
Morgan, P.B., Efron, N., Hill, E.A., Raynor, M.K., Whiting, M.A., Tullo, A.B., 2005. Incidence of keratitis of varying severity among contact lens wearers. Br. J. Ophthalmol. 89 (4), 430–436.
Morgan, S.J., Lippman, S.I., Bautista, G.E., Harrison, J.J., Harding, C.L., Gallagher, L.A., Cheng, A.C., Siehnel, R., Ravishankar, S., Usui, M.L., Olerud, J.E., Fleckman, P., Wolcott, R.D., Manoil, C., Singh, P.K., 2019. Bacterial fitness in chronic wounds appears to be mediated by the capacity for high-density growth, not virulence or biofilm functions. PLoS Pathog. 15 (3), e1007511.
Mulcahy, H., Charron-Mazenod, L., Lewenza, S., 2008. Extracellular DNA chelates cations and induces antibiotic resistance in *Pseudomonas aeruginosa* biofilms. PLoS Pathog. 4 (11), e1000213.
Murray, J.L., Connell, J.L., Stacy, A., Turner, K.H., Whiteley, M., 2014. Mechanisms of synergy in polymicrobial infections. J. Microbiol. 52 (3), 188–199.
Mwangi, J., Yin, Y., Wang, G., Yang, M., Li, Y., Zhang, Z., Lai, R., 2019. The antimicrobial peptide ZY4 combats multidrug-resistant *Pseudomonas aeruginosa* and *Acinetobacter baumannii* infection. Proc. Natl. Acad. Sci. U. S. A. 116 (52), 26516–26522.
Nadkarni, R.R., Abed, S., Draper, J.S., 2016. Organoids as a model system for studying human lung development and disease. Biochem. Biophys. Res. Commun. 473 (3), 675–682.
Neoh, K.G., Wang, R., Kang, E.T., 2015. Surface nanoengineering for combating biomaterials infections. Biomater. Med. Dev. Assoc.Infect. 133–161.
Nguyen, A.T., Jones, J.W., Ruge, M.A., Kane, M.A., Oglesby-Sherrouse, A.G., 2015. Iron depletion enhances production of antimicrobials by *Pseudomonas aeruginosa*. J. Bacteriol. 197 (14), 2265–2275.
O'Brien, T.J., Welch, M., 2019. A continuous-flow model for *in vitro* cultivation of mixed microbial populations associated with cystic fibrosis airway infections. Front. Microbiol. 10, 2713.
Orazi, G., O'Toole, G.A., 2017. *Pseudomonas aeruginosa* alters *Staphylococcus aureus* sensitivity to vancomycin in a biofilm model of cystic fibrosis infection. mBio 8 (4).
Palmer, K.L., Aye, L.M., Whiteley, M., 2007. Nutritional cues control *Pseudomonas aeruginosa* multicellular behavior in cystic fibrosis sputum. J. Bacteriol. 189 (22), 8079–8087.
Papenfort, K., Bassler, B.L., 2016. Quorum sensing signal-response systems in gram-negative bacteria. Nat. Rev. Microbiol. 14 (9), 576–588.
Parkins, M.D., Somayaji, R., Waters, V.J., 2018. Epidemiology, biology, and impact of clonal *Pseudomonas aeruginosa* infections in cystic fibrosis. Clin. Microbiol. Rev. 31 (4).
Passos da Silva, D., Matwichuk, M.L., Townsend, D.O., Reichhardt, C., Lamba, D., Wozniak, D.J., Parsek, M.R., 2019. The *Pseudomonas aeruginosa* lectin LecB binds to the exopolysaccharide Psl and stabilizes the biofilm matrix. Nat. Commun. 10 (1), 2183.
Pastar, I., Nusbaum, A.G., Gil, J., Patel, S.B., Chen, J., Valdes, J., Stojadinovic, O., Plano, L.R., Tomic-Canic, M., Davis, S.C., 2013. Interactions of methicillin resistant *Staphylococcus aureus* USA300 and *Pseudomonas aeruginosa* in polymicrobial wound infection. PLoS One 8 (2), e56846.
Pedraz, L., Blanco-Cabra, N., Torrents, E., 2020. Gradual adaptation of facultative anaerobic pathogens to microaerobic and anaerobic conditions. FASEB J. 34, 2912–2928.
Perron, G.G., Gonzalez, A., Buckling, A., 2007. Source-sink dynamics shape the evolution of antibiotic resistance and its pleiotropic fitness cost. Proc. Biol. Sci. 274 (1623), 2351–2356.
Peters, B.M., Jabra-Rizk, M.A., O'May, G.A., Costerton, J.W., Shirtliff, M.E., 2012. Polymicrobial interactions: impact on pathogenesis and human disease. Clin. Microbiol. Rev. 25 (1), 193–213.
Pinto, R.M., Soares, F.A., Reis, S., Nunes, C., Van Dijck, P., 2020. Innovative strategies toward the disassembly of the EPS matrix in bacterial biofilms. Front. Microbiol. 11, 952.
Pircalabioru, G.G., Chifiriuc, M.C., 2020. Nanoparticulate drug-delivery systems for fighting microbial biofilms: from bench to bedside. Fut. Microbiol. 15, 679–698.
Poudyal, B., Sauer, K., 2018. The ABC of biofilm drug tolerance: the MerR-like regulator BrlR is an activator of ABC transport systems, with PA1874-77 contributing to the tolerance of *Pseudomonas aeruginosa* biofilms to tobramycin. Antimicrob. Agents Chemother. 62 (2).
Price, C.E., Brown, D.G., Limoli, D.H., Phelan, V.V., O'Toole, G.A., 2020. Exogenous alginate protects *Staphylococcus aureus* from killing by *Pseudomonas aeruginosa*. J. Bacteriol. 202 (8).
Rabin, N., Zheng, Y., Opoku-Temeng, C., Du, Y., Bonsu, E., Sintim, H.O., 2015. Biofilm formation mechanisms and targets for developing antibiofilm agents. Fut. Med. Chem. 7 (4), 493–512.
Rajan, S., Saiman, L., 2002. Pulmonary infections in patients with cystic fibrosis. Sem. Respir. Infect. 17 (1), 47–56.
Reichhardt, C., Wong, C., Passos da Silva, D., Wozniak, D.J., Parsek, M.R., 2018. CdrA interactions within the *Pseudomonas aeruginosa* biofilm matrix safeguard it from proteolysis and promote cellular packing. mBio 9 (5).
Riquelme, S.A., Liimatta, K., Wong Fok Lung, T., Fields, B., Ahn, D., Chen, D., Lozano, C., Saenz, Y., Uhlemann, A.C., Kahl, B.C., Britto, C.J., DiMango, E., Prince, A., 2020. *Pseudomonas aeruginosa* utilizes host-derived itaconate to redirect its metabolism to promote biofilm formation. Cell Metab. 31 (6), 1091–1106 e1096.
Robertson, D.M., Parks, Q.M., Young, R.L., Kret, J., Poch, K.R., Malcolm, K.C., Nichols, D. P., Nichols, M., Zhu, M., Cavanagh, H.D., Nick, J.A., 2011. Disruption of contact lens-associated *Pseudomonas aeruginosa* biofilms formed in the presence of neutrophils. Investig. Ophthalmol. Visual Sci. 52 (5), 2844–2850.
Rumbaugh, K.P., Sauer, K., 2020. Biofilm dispersion. Nat. Rev. Microbiol. 18 (10), 571–586.
Rutter, W.C., Burgess, D.R., Burgess, D.S., 2017. Increasing incidence of multidrug resistance among cystic fibrosis respiratory bacterial isolates. Microb. Drug Resist. 23 (1), 51–55.
Ryan, R.P., Fouhy, Y., Garcia, B.F., Watt, S.A., Niehaus, K., Yang, L., Tolker-Nielsen, T., Dow, J.M., 2008. Interspecies signalling via the *Stenotrophomonas maltophilia* diffusible signal factor influences biofilm formation and polymyxin tolerance in *Pseudomonas aeruginosa*. Mol. Microbiol. 68 (1), 75–86.
Sabin, C., Mitchell, E.P., Pokorna, M., Gautier, C., Utille, J.P., Wimmerova, M., Imberty, A., 2006. Binding of different monosaccharides by lectin PA-IIL from *Pseudomonas aeruginosa*: thermodynamics data correlated with X-ray structures. FEBS Lett. 580 (3), 982–987.
Safdar, N., Dezfulian, C., Collard, H.R., Saint, S., 2005. Clinical and economic consequences of ventilator-associated pneumonia: a systematic review. Crit. Care Med. 33 (10), 2184–2193.
Sakuragi, Y., Kolter, R., 2007. Quorum-sensing regulation of the biofilm matrix genes (pel) of *Pseudomonas aeruginosa*. J. Bacteriol. 189 (14), 5383–5386.
Sarkar, S., 2020. Release mechanisms and molecular interactions of *Pseudomonas aeruginosa* extracellular DNA. Appl. Microbiol. Biotechnol. 104 (15), 6549–6564.
Scheiblauer, H., Reinacher, M., Tashiro, M., Rott, R., 1992. Interactions between bacteria and influenza A virus in the development of influenza pneumonia. J. Infect. Dis. 166 (4), 783–791.
Schittek, B., 2011. The antimicrobial skin barrier in patients with atopic dermatitis. Curr. Probl. Dermatol. 41, 54–67.
Schuster, M., Greenberg, E.P., 2006. A network of networks: quorum-sensing gene regulation in *Pseudomonas aeruginosa*. Int. J. Med. Microbiol. 296 (2-3), 73–81.
Scoffield, J.A., Duan, D., Zhu, F., Wu, H., 2017. A commensal streptococcus hijacks a *Pseudomonas aeruginosa* exopolysaccharide to promote biofilm formation. PLoS Pathog. 13 (4), e1006300.
Serra, R., Grande, R., Butrico, L., Rossi, A., Settimio, U.F., Caroleo, B., Amato, B., Gallelli, L., de Franciscis, S., 2015. Chronic wound infections: the role of *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Exp. Rev. Anti Infect. Ther. 13 (5), 605–613.
Sheng, L., Pu, M., Hegde, M., Zhang, Y., Jayaraman, A., Wood, T.K., 2012. Interkingdom adenosine signal reduces *Pseudomonas aeruginosa* pathogenicity. Microb. Biotechnol. 5 (4), 560–572.
Shi, Q., Huang, C., Xiao, T., Wu, Z., Xiao, Y., 2019. A retrospective analysis of *Pseudomonas aeruginosa* bloodstream infections: prevalence, risk factors, and outcome in carbapenem-susceptible and -non-susceptible infections. Antimicrob. Resist. Infect. Control 8, 68.
Singh, N., Armstrong, D.G., Lipsky, B.A., 2005. Preventing foot ulcers in patients with diabetes. JAMA 293 (2), 217–228.
Singh, P.K., Parsek, M.R., Greenberg, E.P., Welsh, M.J., 2002. A component of innate immunity prevents bacterial biofilm development. Nature 417 (6888), 552–555.
Singh, V.K., Kavita, K., Prabhakaran, R., Jha, B., 2013. Cis-9-octadecenoic acid from the rhizospheric bacterium *Stenotrophomonas maltophilia* BJ01 shows quorum quenching and anti-biofilm activities. Biofouling 29 (7), 855–867.
Skopelja-Gardner, S., Theprungsirikul, J., Lewis, K.A., Hammond, J.H., Carlson, K.M., Hazlett, H.F., Nymon, A., Nguyen, D., Berwin, B.L., Hogan, D.A., Rigby, W.F.C., 2019. Regulation of *Pseudomonas aeruginosa*-mediated neutrophil extracellular traps. Front. Immunol. 10, 1670.
Slomberg, D.L., Lu, Y., Broadnax, A.D., Hunter, R.A., Carpenter, A.W., Schoenfisch, M.H., 2013. Role of size and shape on biofilm eradication for nitric oxide-releasing silica nanoparticles. ACS Appl. Mater. Interfaces 5 (19), 9322–9329.
Smith, A.C., Rice, A., Sutton, B., Gabrilska, R., Wessel, A.K., Whiteley, M., Rumbaugh, K. P., 2017. Albumin inhibits *Pseudomonas aeruginosa* quorum sensing and alters polymicrobial interactions. Infect. Immun. 85 (9).
Smith, K., Rajendran, R., Kerr, S., Lappin, D.F., Mackay, W.G., Williams, C., Ramage, G., 2015. *Aspergillus fumigatus* enhances elastase production in *Pseudomonas aeruginosa* co-cultures. Med. Mycol. 53 (7), 645–655.
Sonderholm, M., Kragh, K.N., Koren, K., Jakobsen, T.H., Darch, S.E., Alhede, M., Jensen, P.O., Whiteley, M., Kuhl, M., Bjarnsholt, T., 2017. *Pseudomonas aeruginosa* aggregate formation in an alginate bead model system exhibits *in vivo*-like characteristics. Appl. Environ. Microbiol. 83 (9).
Sorensen, M., Kantorek, J., Byrnes, L., Boutin, S., Mall, M.A., Lasitschka, F., Zabeck, H., Nguyen, D., Dalpke, A.H., 2020. Corrigendum: *Pseudomonas aeruginosa* modulates the antiviral response of bronchial epithelial cells. Front. Immunol. 11, 1453.

Souto, R., Silva-Boghossian, C.M., Colombo, A.P., 2014. Prevalence of *Pseudomonas aeruginosa* and *Acinetobacter* spp. in subgingival biofilm and saliva of subjects with chronic periodontal infection. Braz. J. Microbiol. 45 (2), 495–501.
Stapleton, F., Carnt, N., 2012. Contact lens-related microbial keratitis: how have epidemiology and genetics helped us with pathogenesis and prophylaxis. Eye 26 (2), 185–193.
Stewart, P.S., Franklin, M.J., 2008. Physiological heterogeneity in biofilms. Nat. Rev. Microbiol. 6 (3), 199–210.
Stewart, P.S., Zhang, T., Xu, R., Pitts, B., Walters, M.C., Roe, F., Kikhney, J., Moter, A., 2016. Reaction-diffusion theory explains hypoxia and heterogeneous growth within microbial biofilms associated with chronic infections. NPJ Biofilms Microb. 2, 16012.
Sun, Y., Dowd, S.E., Smith, E., Rhoads, D.D., Wolcott, R.D., 2008. *In vitro* multispecies Lubbock chronic wound biofilm model. Wound Repair Regen 16 (6), 805–813.
Tahrioui, A., Duchesne, R., Bouffartigues, E., Rodrigues, S., Maillot, O., Tortuel, D., Hardouin, J., Taupin, L., Groleau, M.C., Dufour, A., Deziel, E., Brenner-Weiss, G., Feuilloley, M., Orange, N., Lesouhaitier, O., Cornelis, P., Chevalier, S., 2019. Extracellular DNA release, quorum sensing, and PrrF1/F2 small RNAs are key players in *Pseudomonas aeruginosa* tobramycin-enhanced biofilm formation. NPJ Biofilms Microb. 5, 15.
Tan, M., Reyes-Ortega, F., Schneider-Futschik, E.K., 2020. Magnetic nanoparticle-based drug delivery approaches for preventing and treating biofilms in cystic fibrosis. Magnetochemistry 6 (4), 72.
Teirlinck, E., Xiong, R., Brans, T., Forier, K., Fraire, J., Van Acker, H., Matthijs, N., De Rycke, R., De Smedt, S.C., Coenye, T., Braeckmans, K., 2018. Laser-induced vapour nanobubbles improve drug diffusion and efficiency in bacterial biofilms. Nat. Commun. 9 (1), 4518.
Thanabalasuriar, A., Scott, B.N.V., Peiseler, M., Willson, M.E., Zeng, Z., Warrener, P., Keller, A.E., Surewaard, B.G.J., Dozier, E.A., Korhonen, J.T., Cheng, L.I., Gadjeva, M., Stover, C.K., DiGiandomenico, A., Kubes, P., 2019. Neutrophil extracellular traps confine *Pseudomonas aeruginosa* ocular biofilms and restrict brain invasion. Cell Host Microbe 25 (4), 526–536 e524.
Tipton, C.D., Wolcott, R.D., Sanford, N.E., Miller, C., Pathak, G., Silzer, T.K., Sun, J., Fleming, D., Rumbaugh, K.P., Little, T.D., Phillips, N., Phillips, C.D., 2020. Patient genetics is linked to chronic wound microbiome composition and healing. PLoS Pathog. 16 (6), e1008511.
Tognon, M., Kohler, T., Luscher, A., van Delden, C., 2019. Transcriptional profiling of *Pseudomonas aeruginosa* and *Staphylococcus aureus* during *in vitro* co-culture. BMC Genomics 20 (1), 30.
Trizna, E.Y., Yarullina, M.N., Baidamshina, D.R., Mironova, A.V., Akhatova, F.S., Rozhina, E.V., Fakhrullin, R.F., Khabibrakhmanova, A.M., Kurbangalieva, A.R., Bogachev, M.I., Kayumov, A.R., 2020. Bidirectional alterations in antibiotics susceptibility in *Staphylococcus aureus-Pseudomonas aeruginosa* dual-species biofilm. Sci. Rep. 10 (1), 14849.
Tsuchimori, N., Hayashi, R., Shino, A., Yamazaki, T., Okonogi, K., 1994. *Enterococcus faecalis* aggravates pyelonephritis caused by *Pseudomonas aeruginosa* in experimental ascending mixed urinary tract infection in mice. Infect. Immun. 62 (10), 4534–4541.
Tunney, M.M., Klem, E.R., Fodor, A.A., Gilpin, D.F., Moriarty, T.F., McGrath, S.J., Muhlebach, M.S., Boucher, R.C., Cardwell, C., Doering, G., Elborn, J.S., Wolfgang, M.C., 2011. Use of culture and molecular analysis to determine the effect of antibiotic treatment on microbial community diversity and abundance during exacerbation in patients with cystic fibrosis. Thorax 66 (7), 579–584.
Turnbull, L., Toyofuku, M., Hynen, A.L., Kurosawa, M., Pessi, G., Petty, N.K., Osvath, S. R., Carcamo-Oyarce, G., Gloag, E.S., Shimoni, R., Omasits, U., Ito, S., Yap, X., Monahan, L.G., Cavaliere, R., Ahrens, C.H., Charles, I.G., Nomura, N., Eberl, L., Whitchurch, C.B., 2016. Explosive cell lysis as a mechanism for the biogenesis of bacterial membrane vesicles and biofilms. Nat. Commun. 7, 11220.
Valentini, T.D., Lucas, S.K., Binder, K.A., Cameron, L.C., Motl, J.A., Dunitz, J.M., Hunter, R.C., 2020. Bioorthogonal non-canonical amino acid tagging reveals translationally active subpopulations of the cystic fibrosis lung microbiota. Nat. Commun. 11 (1), 2287.
Van Acker, H., Coenye, T., 2017. The role of reactive oxygen species in antibiotic-mediated killing of bacteria. Trends Microbiol. 25 (6), 456–466.
Vandebriel, R.J., De Jong, W.H., 2012. A review of mammalian toxicity of ZnO nanoparticles. Nanotechnol. Sci. Appl. 5, 61–71.
Veerachamy, S., Yarlagadda, T., Manivasagam, G., Yarlagadda, P.K., 2014. Bacterial adherence and biofilm formation on medical implants: a review. Proc. Inst. Mech. Eng. H 228 (10), 1083–1099.
Verderosa, A.D., Totsika, M., Fairfull-Smith, K.E., 2019. Bacterial biofilm eradication agents: a current review. Front. Chem. 7, 824.
Vieira Colombo, A.P., Magalhaes, C.B., Hartenbach, F.A., Martins do Souto, R., Maciel da Silva-Boghossian, C., 2016. Periodontal-disease-associated biofilm: a reservoir for pathogens of medical importance. Microb. Pathog. 94, 27–34.
Villeret, B., Solhonne, B., Straube, M., Lemaire, F., Cazes, A., Garcia-Verdugo, I., Sallenave, J.M., 2020. Influenza A virus pre-infection exacerbates *Pseudomonas aeruginosa*-mediated lung damage through increased MMP-9 expression, decreased elafin production and tissue resilience. Front. Immunol. 11, 117.
Vipin, C., Mujeeburahiman, M., Arun, A.B., Ashwini, P., Mangesh, S.V., Rekha, P.D., 2019. Adaptation and diversification in virulence factors among urinary catheter-associated *Pseudomonas aeruginosa* isolates. J. Appl. Microbiol. 126 (2), 641–650.
Vishwakarma, V., 2020. Impact of environmental biofilms: industrial components and its remediation. J. Basic Microbiol. 60 (3), 198–206.
Wang, S., Liu, X., Liu, H., Zhang, L., Guo, Y., Yu, S., Wozniak, D.J., Ma, L.Z., 2015. The exopolysaccharide Psl-eDNA interaction enables the formation of a biofilm skeleton in *Pseudomonas aeruginosa*. Environ. Microbiol. Rep. 7 (2), 330–340.
Waters, C.M., Bassler, B.L., 2005. Quorum sensing: cell-to-cell communication in bacteria. Annu. Rev. Cell. Dev. Biol. 21, 319–346.
Watters, C., Everett, J.A., Haley, C., Clinton, A., Rumbaugh, K.P., 2014. Insulin treatment modulates the host immune system to enhance *Pseudomonas aeruginosa* wound biofilms. Infect. Immun. 82 (1), 92–100.
Wei, Q., Zhang, Z., Luo, J., Kong, J., Ding, Y., Chen, Y., Wang, K., 2019. Insulin treatment enhances *Pseudomonas aeruginosa* biofilm formation by increasing intracellular cyclic di-GMP levels, leading to chronic wound infection and delayed wound healing. Am. J. Transl. Res. 11 (6), 3261–3279.
Werthen, M., Henriksson, L., Jensen, P.O., Sternberg, C., Givskov, M., Bjarnsholt, T., 2010. An *in vitro* model of bacterial infections in wounds and other soft tissues. APMIS 118 (2), 156–164.
Whitchurch, C.B., Tolker-Nielsen, T., Ragas, P.C., Mattick, J.S., 2002. Extracellular DNA required for bacterial biofilm formation. Science 295 (5559), 1487.
Whitney, J.C., Whitfield, G.B., Marmont, L.S., Yip, P., Neculai, A.M., Lobsanov, Y.D., Robinson, H., Ohman, D.E., Howell, P.L., 2015. Dimeric c-di-GMP is required for post-translational regulation of alginate production in *Pseudomonas aeruginosa*. J. Biol. Chem. 290 (20), 12451–12462.
Wilson, M., Seymour, R., Henderson, B., 1998. Bacterial perturbation of cytokine networks. Infect. Immun. 66 (6), 2401–2409.
Wilton, M., Charron-Mazenod, L., Moore, R., Lewenza, S., 2016. Extracellular DNA acidifies biofilms and induces aminoglycoside resistance in *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. 60 (1), 544–553.
Woods, P.W., Haynes, Z.M., Mina, E.G., Marques, C.N.H., 2018. Maintenance of *S. aureus* in co-culture with *P. aeruginosa* while growing as biofilms. Front. Microbiol. 9, 3291.
Worlitzsch, D., Tarran, R., Ulrich, M., Schwab, U., Cekici, A., Meyer, K.C., Birrer, P., Bellon, G., Berger, J., Weiss, T., Botzenhart, K., Yankaskas, J.R., Randell, S., Boucher, R.C., Doring, G., 2002. Effects of reduced mucus oxygen concentration in airway *Pseudomonas* infections of cystic fibrosis patients. J. Clin. Investig. 109 (3), 317–325.
Wu, H., Song, Z., Hentzer, M., Andersen, J.B., Molin, S., Givskov, M., Hoiby, N., 2004. Synthetic furanones inhibit quorum-sensing and enhance bacterial clearance in *Pseudomonas aeruginosa* lung infection in mice. J. Antimicrob. Chemother. 53 (6), 1054–1061.
Wu, Q., Liu, J., Wang, X., Feng, L., Wu, J., Zhu, X., Wen, W., Gong, X., 2020. Organ-on-a-chip: recent breakthroughs and future prospects. Biomed. Eng. Online 19 (1), 9.
Yang, N., Cao, Q., Hu, S., Xu, C., Fan, K., Chen, F., Yang, C.G., Liang, H., Wu, M., Bae, T., Lan, L., 2020. Alteration of protein homeostasis mediates the interaction of *Pseudomonas aeruginosa* with *Staphylococcus aureus*. Mol. Microbiol. 114 (3), 423–442.
Yipp, B.G., Petri, B., Salina, D., Jenne, C.N., Scott, B.N., Zbytnuik, L.D., Pittman, K., Asaduzzaman, M., Wu, K., Meijndert, H.C., Malawista, S.E., de Boisfleury Chevance, A., Zhang, K., Conly, J., Kubes, P., 2012. Infection-induced NETosis is a dynamic process involving neutrophil multitasking *in vivo*. Nat. Med. 18 (9), 1386–1393.
Zegans, M.E., Becker, H.I., Budzik, J., O'Toole, G., 2002. The role of bacterial biofilms in ocular infections. DNA Cell Biol. 21 (5-6), 415–420.
Zhu, Y., Weiss, E.C., Otto, M., Fey, P.D., Smeltzer, M.S., Somerville, G.A., 2007. *Staphylococcus aureus* biofilm metabolism and the influence of arginine on polysaccharide intercellular adhesin synthesis, biofilm formation, and pathogenesis. Infect. Immun. 75 (9), 4219–4226.