Pseudomonas aeruginosa behaviour in polymicrobial communities: The competitive and cooperative interactions conducting to the exacerbation of infections

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Abstract

Pseudomonas aeruginosa is mostly associated with persistent infections and antibiotic resistance as a result of several factors, biofilms one of them. Microorganisms within the polymicrobial biofilm (PMB) reveal various transcriptional profiles and affect each other which might influence their pathogenicity and antibiotic tolerance and subsequent worsening of the biofilm infection. *P. aeruginosa* within PMB exhibits various behaviours toward other microorganisms, which may enhance or repress the virulence of these microbes. Microbial neighbours, in turn, may affect *P. aeruginosa*'s virulence either positively or negatively. Such interactions among microorganisms lead to emerging persistent and antibiotic-resistant infections. This review highlights the relationship between *P. aeruginosa* and its microbial neighbours within the PMB in an attempt to better understand the mechanisms of polymicrobial interaction and the correlation between increased exacerbations of infection and the *P. aeruginosa*-microbe interaction. Researching in the literature that was carried out in vitro either in co-cultures or in the models to simulate the environment at the site of infection suggested that the interplay between *P. aeruginosa* and other microorganisms is one main reason for the worsening of the infection and which in turn requires a treatment approach different from that followed with *P. aeruginosa* mono-infection.

Key words:
Pseudomonas aeruginosa, polymicrobial biofilms, co-culture, *Staphylococcus aureus*, oral streptococci, fungi

1. Introduction

Pseudomonas aeruginosa becomes more ferocious and difficult to eradicate day by day owing to its virulence factors that are either intrinsic or acquired factors. Biofilms are one of the contributing factors to the virulence and pathogenicity of bacteria. Biofilms are microbial gatherings embedded in self-produced thick extracellular polymeric substances (EPS) which serve as a matrix attaching the cells to each other and/or on other surfaces (Flemming et al., 2016). For many bacterial infections, biofilms are a prominent factor in the persistence and non-healing of the infections, owing to the protective role of biofilms to protect the bacterial cells from the immune system and antimicrobial agents (Vestby et al., 2020), contributing to increased morbidity and mortality (Pozo, 2017). *P. aeruginosa* is the most common opportunistic pathogen and has recently emerged as one of the multidrug-resistant (MDR) bacteria (Al-Wrafy et al., 2017). *P. aeruginosa* can exist alone within the biofilm (monomicrobial biofilm, MMB) or with two or more microorganisms (dual-species biofilm or polymicrobial biofilm respectively, DSB and PMB) such as *Staphylococcus aureus* (B. L. Price et al., 2020) and *Candida albicans* (Bandara et al., 2020).

Microorganisms in PMB display various interactions with each other, influencing their pathogenicity and antibiotic tolerance (Lopes et al., 2021; Mitra et al., 2022). The microorganisms respond effectively to each other through competitive or cooperative interactions, leading to a kind of stimulation or suppression effects. The interactions between two microorganisms "interspecies or intraspecies interactions" can result in alterations in the lifestyle of both or one of them that will subsequently be closely related to the progression of the infection (Bisht et al., 2020). In PMB infections, the length and persistence of illness are affected by the microbial lifestyle. The microbial cells that adopt a competitive lifestyle exhaust their potential at the site of infection faster because they will deal with the surrounding microbes, harsh environmental conditions such as lack of nutrients, immune cells, and finally antibiotics. While the microbial cells with a cooperative lifestyle benefit from the secretions of their neighbouring microbes and at the same time do not consume their energy on eliminating these microbes, therefore they focus their capabilities on the

harsh surrounding conditions and resistance to immune cells and antibiotics, hence achieving higher levels of survival at an infection site (Baishya and Wakeman, 2019). Several studies conducted on animal models showed that polymicrobial colonization caused severe damage to the models used and increased death rates among them compared to monomicrobial colonization (Wang et al., 2016; Whiley et al., 2014). Extracellular factors (or exoproducts) produced by a microbe as a response to the presence of another microbe increase epithelial cell damage due to their cytotoxic and immune-modulatory impacts (Hotterbeekx et al., 2017). Polymicrobial colonization also induces a host response that is different from that induced by monospecies infection, whereby, the enhancement of virulence factors by exoproducts leads to the induction or repression of proinflammatory mediators (Song et al., 2015; Wang et al., 2016; Whiley et al., 2014). In the co-infection of two species of bacteria, one species can alleviate the host immune response induced by another one. For instance, the *P. aeruginosa*-*S. mitis* interaction leads to the production of lower levels of cytokine proteins, such as IL-6 (Wang et al., 2016) and IL-8 (Song et al., 2015). Whereas *P. aeruginosa's* interaction with the *Streptococcus anginosus* group (SAG; also referred to as the *Streptococcus milleri* group), which is involved in pulmonary exacerbation in patients with CF, induces the IL-8 (Whiley et al., 2014). On the other hand, the use of antibiotics to treat polymicrobial infections may be somewhat risky, as the use of a particular antibiotic may weaken one microbe, allowing the activity of the other (Price et al., 2016).

To better understand the role of the PMB in exacerbating infection, several studies have highlighted the interactions between microbes in vitro, whether in co-culture (Orazi et al., 2020; Pallett et al., 2019) or in models such as DSB on cell-derived matrices (Gounani et al., 2020), human keratinocytes (Alves et al., 2018), human bronchial epithelial cells (Filkins et al., 2015) and otitis media rat-model (Yadav et al., 2017), CF-derived bronchial epithelial cells (Price et al., 2016) and biofilm slide chamber model (Beaudoin et al., 2017). Comprehending the physiology of *P. aeruginosa* interactions with other microorganisms and mechanisms by which these microorganisms are able to coexist together contributes to designing better control methods to eliminate this kind of infection. This review discusses the relationship between *P. aeruginosa* and other microorganisms as well as the mechanisms that occur in the polymicrobial community which finally lead to the emergence of persistent infections.

2. The interactions of *P. aeruginosa* in polymicrobial communities

P. aeruginosa exerts a kind of synergistic or antagonistic effects on others present within the biofilm (Briard et al., 2015; Mcdaniel et al., 2020; Murray et al., 2022). *P. aeruginosa* is mostly dominant owing to its several mechanisms adapting to changing hostile environments (Filkins et al., 2015). However, the cooperative or competitive ability of *P. aeruginosa* strains depends on their phenotypic and genetic diversity and the consequent alteration in its exoproducts (Gomes-Fernandes et al., 2022), in addition to surrounding circumstances of microbes (Pallett et al., 2019). In the DSB of *P. aeruginosa* and *Stenotrophomonas maltophilia*, an opportunistic and nosocomial pathogen implicated in lung exacerbations in CF patients, *S. maltophilia* displays an increased bacterial load and well-integrated biofilms formation compared to the mono-biofilm culture (Mcdaniel et al., 2020). However, it has been shown that adding *P. aeruginosa* to the PMB of *S. aureus*, *Staphylococcus epidermidis* and *Micrococcus luteus*, after 20 h had little effect on the viability of these bacteria. Such may be attributed to the synergy between these species of Gram-positive bacteria or the inhibitory effect of one of them upon *P. aeruginosa* (Jordana-Lluch et al., 2020). This disparity also occurs when *P. aeruginosa* grows with other microorganisms such as fungi, it has been observed that the growth of *Aspergillus fumigatus* decreased during co-culture with *P. aeruginosa* (Briard et al., 2015). In contrast, *A. fumigatus* secretions might stimulate *P. aeruginosa* growth in the co-cultures (Margalit et al., 2020).

The competitive interactions were also observed between strains of *P. aeruginosa* which explains why certain strains of *P. aeruginosa* dominate during CF infection while not others. The competitive interactions among strains may occur due to *P. aeruginosa* products such as R-pyocins, the type of tailocins produced by some *P. aeruginosa* strains, mediate the competitive and environmental interactions among strains of *P. aeruginosa* within biofilm in CF patients. The strains producing R-pyocins are more able to dominance through negatively influence on other strains of *P. aeruginosa* biofilm. (Oluyombo et al., 2019). *P. aeruginosa* may also contribute to the protection of other bacteria within biofilm from the host immune by its secretions such as the type III secretion system (T3SS) (Mcdaniel et al., 2020) and proteins that weaken the epithelial barriers and mediate the killing of phagocytes (Al-Wrafy et al., 2017). Moreover, the increasing biofilm matrix thickness is associated with *P. aeruginosa* EPS components, providing a stronger protective barrier (Lee et al., 2017). There are several mechanisms that describe the molecular interactions between microorganisms within PMB that are often mediated by factors produced by microorganisms. *P. aeruginosa*

can compete or cooperate with neighboring microbes via various mechanisms, the most prominent of which are described below.

2.1 Interactions of *P. aeruginosa* and Gram-positive bacteria

Despite *P. aeruginosa* often seeming to be dominant in the co-cultures with Gram-positive bacteria (G +ve bacteria), such dominance may not be related to an increase in its population compared to their growth in monoculture, whereas the G +ve bacteria are often accompanied by an increase in their population (Korgaonkar et al., 2013; Whiley et al., 2014). G +ve bacteria show different effects on *P. aeruginosa*, which are often stimulating or suppressive effects on its virulence and pathogenicity and are not associated with influence on growth or persistence (Korgaonkar et al., 2013; Malešević et al., 2020; Whiley et al., 2014). The G +ve bacteria have several products that can act as inducers for the upregulation of several virulence genes of *P. aeruginosa* (Korgaonkar et al., 2013; Rickard et al., 2006). In co-culture, *P. aeruginosa* responds to N-acetyl glucosamine (GlcNAc), a part of the G +ve bacteria cell wall peptidoglycan, which can act as a cue to *P. aeruginosa* to produce Pseudomonas quinolone signal (PQS). PQS is a quorum sensing (QS) molecule that regulates several virulence factors such as pyocyanin, elastase, and 2-heptyl-4-hydroxyquinoline N-oxide (HQNO) that play a critical role in the interaction of *P. aeruginosa* in the PMB (Korgaonkar et al., 2013). However, changing the local environment by G +ve bacteria might also negatively affect *P. aeruginosa* within PMB (Tan et al., 2022).

2.1.1 Competitive and cooperative interactions of *P. aeruginosa* and *S. aureus*

P. aeruginosa and *S. aureus* cause co-infection which are frequently chronic and persistent in individuals with CF, diabetic foot ulcers, and other wounds. The interactions between *P. aeruginosa* and *S. aureus* contribute to worsening the infection and antibiotic tolerance (Fischer et al., 2021; Hotterbeekx et al., 2017; Suryaletha et al., 2018) as well as enhance bacterial invasion and impair the proinflammatory response in damaged keratinocytes, leading to persistent microbial colonization and delayed wound healing (Alves et al., 2018). *P. aeruginosa* influences *S. aureus* by enhancing or diminishing its virulence and cell viability in a strain-dependent manner, whereby both mucoid and non-mucoid strains of *P. aeruginosa* decrease populations for many strains of *S. aureus* whether in a biofilm or planktonically (Filkins et al., 2015). Nevertheless, the interaction between both bacterial species varies according to clinical isolates and their exoproducts (Bernardy et al., 2020; Radlinski et al., 2017). In a recent study conducted on different strains isolated from

CF patients, *S. aureus* was divided into three main groups according to their susceptibility to *P. aeruginosa, S. aureus* strains are killed only by *P. aeruginosa* non-mucoid strains, *S. aureus* strains are killed by both non-mucoid and mucoid *P. aeruginosa* strains, and the third group not killed by either strain of *P. aeruginosa* (Bernardy et al., 2020).

P. aeruginosa affects *S. aureus* by exoproducts known as antistaphylococcal factors that are mostly the reason for *P. aeruginosa* predominance at the site of infection (**Table 1**) (Machan et al., 1992). The term "antistaphylococcal" may not always be appropriate because the exoproducts may act against the *S. aureus*, but otherwise may act as promoting or stabilizing agents (**Fig. 1**). In a study conducted on several clinical strains of *P. aeruginosa* isolated from burn wounds and CF patients, it was noted that there is a difference in the effect of each strain on the susceptibility of *S. aureus* to tobramycin, whereby the influence ranged between increased sensitivity, no effect and decreased sensitivity to tobramycin (Radlinski et al., 2017). Worth noting that *P. aeruginosa* might adopt different phenotypes that allow coexistence with *S. aureus* during infection. It has been observed that *P. aeruginosa* isolates from patients with mono-infection were more antagonistic toward *S. aureus* than those isolates from patients with co-infection (Limoli et al., 2017). The HQNO and PQS system components, the prominent factors that mediate the interspecies competitiveness of *P. aeruginosa* (Fugère et al., 2014), were isolated from the sputum of CF patients who were infected with *P. aeruginosa* in the absence of *S. aureus* (Machan et al., 1992).

Impacts of *P. aeruginosa's* exoproducts on the susceptibility of *S. aureus* to the antimicrobial agents

HQNO influences antibiotic susceptibility, growth, and biofilm formation of *S. aureus* (Gomes-Fernandes et al., 2022; Orazi et al., 2019; Orazi and O'Toole, 2017). The HQNO has been reported to increase the sensitivity of *S. aureus* biofilm to membrane-targeting antiseptics such as chloroxylenol and many antibiotics such as fluoroquinolones. HQNO interacts directly with the cell membrane to cause membrane fluidity and increase its permeability, which allows the increased entry of these compounds into the cell and promotes the *S. aureus* killing (Orazi et al., 2019). *P. aeruginosa* also enhances norfloxacin's efficacy against *S. aureus* cells through membrane fluidizing caused by other LasR-regulated factors along with HQNO. However, the accumulation of norfloxacin intracellular *S. aureus*

might also attribute to the ability of *P. aeruginosa*-secreted products to modulate the influx of this antibiotic by affecting multidrug efflux pumps in *S. aureus* (Orazi et al., 2020).

The *S. aureus* lysis by vancomycin can be promoted in an HQNO-independent manner by the *P. aeruginosa*-produced LasA endopeptidase that increases *S. aureus* sensitivity to vancomycin via cleaving pentaglycine cross-bridges in the peptidoglycan of the cell wall (Radlinski et al., 2017). In contrast, other strains of *P. aeruginosa* can protect *S. aureus* from vancomycin and other antibiotics targeting cell wall and protein synthesis in HQNO- and siderophore-dependent manner by interference with *S. aureus* electron transport chain (ETC) function and shifting *S. aureus* to fermentative growth (Orazi and O'Toole, 2017). HQNO and pyoverdine and pyochelin, the two major siderophores chelate, mediate the reduction of *S. aureus* populations as well as shift *S. aureus* from aerobic respiration to lactic acid through induction of *S. aureus ldh*, *pflB*, and *adh* expression, the genes responsible for lactate fermentation pathways in *S. aureus* (Filkins et al., 2015). Inhibition of the ETC decreases protein synthesis rates required in metabolic and biosynthesis processes which subsequently arrest (Hoffman et al., 2006; Orazi et al., 2019). Such in turn, lead to the inefficacy of the antibiotics due to most of them acting by inhibiting or influencing metabolic processes (Orazi and O'Toole, 2017). In fact, The defective strains in ETC originate as genetically mutant strains termed small colony variants (SCV) which keep the persistence of *S. aureus* with *P. aeruginosa* (Biswas et al., 2009).

As mentioned above, there is a discrepancy between *P. aeruginosa* strains in affecting the susceptibility of *S. aureus* to tobramycin due to their exoproducts. The strains of HQNO-producing *P. aeruginosa* promote *S. aureus* ability to resist the tobramycin by hindering tobramycin uptake into the cell due to the inhibiting the proton-motive force, responsible for tobramycin uptake, as a result of suppression of ETC by HQNO (Hoffman et al., 2006; Radlinski et al., 2017). Conversely, other strains of *P. aeruginosa* increase the *S. aureus* sensitivity to tobramycin by producing rhamnolipids that interact with the plasma membrane, increasing cell permeability and facilitating tobramycin uptake into *S. aureus* cells (Radlinski et al., 2017). *P. aeruginosa* exoproducts might also confer with *S. aureus* other stabilizing properties such as promoting its capability to form biofilm efficiently (Fugère et al., 2014). Furthermore, increase the tolerance of *S. aureus* to copper and silver by reducing the bioavailability of the metal in the medium by binding it or affecting its metabolism (Monych and Turner, 2020).

P. aeruginosa*'s exoproducts enhance the coexistence with *S. aureus

The competitive capability of *P. aeruginosa* might also associate with the presence or absence of certain proteins. *P. aeruginosa* produces the ClpPX, a peptidase intracellular protein degradation, which represents a negative regulator of competitiveness of *P. aeruginosa* via breaking down the proteins responsible for the production of PQS. Therefore, the strains with a deletion mutant of *clpX* or *clpP* have high competitiveness toward *S. aureus* while those with the *clpX* or *clpP* have no effect on the *S. aureus* viability due to their inability to produce PQS implicated in the lysis or the growth inhibition of *S. aureus* (Yang et al., 2020). The mucoid *P. aeruginosa* strains exhibit lesser antagonism toward *S. aureus* than non-mucoid strains and the ability of the non-mucoid strains of *P. aeruginosa* to *S. aureus* killing might attenuate in the presence of mucoid strains (C. E. Price et al., 2020). The mucoid *P. aeruginosa* strains produce alginate, an exopolysaccharide in the EPS, which can hinder the production of pyoverdine, HQNO, and rhamnolipids required for *S. aureus* killing (Limoli et al., 2017). The alginate causes transcriptional downregulation of *pvdA* expression, a gene required for the production of pyoverdine and pyochelin. It also interferes with the PQS system, leading to reduced PQS-regulated products such as HQNO and phenazines including pyocyanin (C. E. Price et al., 2020). Thus, the overproduction of alginate enhances the coexistence of *P. aeruginosa* with *S. aureus* (Limoli et al., 2017).

Table 1. *P. aeruginosa* exoproducts and their effects on *S. aureus*

Exoproduct	Influence	Mechanism	ref
HQNO	Increasing the sensitivity to fluoroquinolones and chloroxylenol	Causing membrane fluidity and change in its permeability facilitating *S. aureus* killing	(Orazi et al., 2019)
	Induces tolerance of *S. aureus* to ciprofloxacin	Respiration inhibition and reduction of intracellular ATP generation	(Radlinski et al., 2017)
	Decrease the susceptibility to vancomycin	Converting *S. aureus* to the anaerobic fermentative state	(Orazi and O'Toole, 2017)
Siderophores, pyoverdine and pyochelin,	Decreasing growth Decrease the susceptibility to vancomycin	Converting *S. aureus* to the anaerobic fermentative state leads to low growth and stopping metabolic processes that the antibiotic affects.	(Orazi and O'Toole, 2017)
Dihydroaeruginoate	Increase the tolerance of *S.*	Reducing copper bioavailability	

	aureus to copper		
Serine, threonine, PQS	Increase the tolerance of *S. aureus* to silver	Bind up the silver	(Monych and Turner, 2020)
LasA endopeptidase	Promote lysis of *S. aureus* by vancomycin	Cleaving pentaglycine cross-bridges in the peptidoglycan of the cell wall.	(Radlinski et al., 2017)
Rhamnolipids	Increase *S. aureus* sensitivity to topramycin	Facilitate proton-motive force-independent tobramycin uptake	
Multiple LasR-regulated factors	Increase *S. aureus* norfloxacin susceptibility	Causing membrane fluidity and change in its permeability Interfering with the ability of *S. aureus* to efflux the antibiotic effectively	(Orazi et al., 2020)

Impacts of *S. aureus's* exoproducts on *P. aeruginosa*

Despite *P. aeruginosa* seems more competitive against *S. aureus*, *S. aureus* might predominate in early biofilm during aggregation, attachment and growth phases and has a significant role as either an inducer or inhibitor during aggregation of *P. aeruginosa* (Armbruster et al., 2016; Beaudoin et al., 2017). *S. aureus* strains can also accelerate their growth and compromise *P. aeruginosa* growth at the onset prior to physical contact. (Niggli et al., 2021). *S. aureus* secretes several exoproducts that can act as inhibitory or stimulatory factors on certain isolates of *P. aeruginosa* (Armbruster et al., 2016; Beaudoin et al., 2017; Pallett et al., 2019) (**Table 3**). Apart from the impact sort, these interactions are dependent on the strains of both bacteria (Niggli et al., 2021).

S. aureus secretes staphylococcal protein A (SpA), an important virulence factor that enables *S. aureus* to elude host immune responses (Schneewind and Missiakas, 2019), which reveals a protective or competitive influence on *P. aeruginosa* (Armbruster et al., 2016). The SpA bind to the Fcγ domain of mammalian IgG, this, in turn, blocks the effector function of IgG via covering the cell surface of *S. aureus* with IgG molecules, oriented outward, hindering the *S. aureus* binding to the Fc receptor of the neutrophil and thus inhibiting opsonophagocytic process (Schneewind and Missiakas, 2019). In the same vein, SpA can act a similar function for *P. aeruginosa* whereby SpA bind with the *P. aeruginosa* cell surface structures such as type IV pili, an external protein component in the flagella, and Psl, an

exopolysaccharide in the EPS, then binds to the Fcγ domain of IgG, thus hindering the opsonophagocytosis and bacterial killing (Armbruster et al., 2016). Otherwise, SpA inhibits biofilm formation in non-Psl-producing *P. aeruginosa* clinical strains through binding to PilA, a type IV pili (Armbruster et al., 2016). The type IV pili mediate the attachment to surfaces and to other bacterial cells, the initial step in the formation of biofilm (Thi et al., 2020). The Psl-producing *P. aeruginosa* can avoid the inhibitory effects of SpA through Psl-hindering to the SpA binding to type IV pili (Armbruster et al., 2016).

S. aureus also mediates in the attachment of *P. aeruginosa* to human keratinocytes as well as contributes to integrating into the environment of the early biofilm (Alves et al., 2018). Furthermore, under anoxia circumstances, *S. aureus*-derived exoproducts can promote *P. aeruginosa* motility (Pallett et al., 2019). The exoproducts might also decrease the biomass of *P. aeruginosa* biofilms and increase the resistance to tobramycin in *P. aeruginosa* Psl-producing strains as a result of the binding between some *S. aureus* proteins and *P. aeruginosa* Psl (Beaudoin et al., 2017). Conversely, it has been reported that *S. aureus* increases the *P. aeruginosa* susceptibility toward ciprofloxacin and aminoglycosides when they grow together (Trizna et al., 2020).

2.1.2 Competitive and cooperative interactions of *P. aeruginosa* and oral Streptococci

The interaction between *P. aeruginosa* and *Streptococcus* spp, on either co-cultures or CF airway cell models, leads to different effects on both (Price et al., 2016; Scott et al., 2019; Stoner et al., 2022; Whiley et al., 2014). Several species of oral commensal streptococci such as SAG involving *Streptococcus anginosus*, *S. intermedius*, and *S. constellatus*, and *Streptococcus mitis* group (SMG) involving *S. mitis*, *S. oralis*, *S. sanguinis*, and *S. gordonii*, might contribute to pulmonary exacerbation in CF patients, particularly during co-infection with *P. aeruginosa* (Scoffield et al., 2017; Scott and O'Toole, 2019; Whiley et al., 2015). The *P. aeruginosa*-Streptococci interactions occur through exoproducts for both microbes (**Fig 2)**. The mucoid strains of *P. aeruginosa* enhance *Streptococcus parasanguinis* biofilm formation in a BapA1-dependent manner, a sortase A-controlled surface adhesins involved in biofilm formation by *S. parasanguinis*. In co-culture, the BapA1 mediates *P. aeruginosa* enhancement of the *S. parasanguinis* biofilm by seizing alginate produced by mucoid strains of *P. aeruginosa* (Scoffield et al., 2017). Likewise, non-mucoid strains of *P. aeruginosa* might promote biofilm formation of *Streptococcus salivarius*, the most common streptococcal species in individuals with stable CF, using *S.*

salivarius proteins. *S. salivarius* produces the maltose-binding protein which interacts with *P. aeruginosa* Psl, enhancing biofilm formation and establishing *S. salivarius* within the CF airway (Stoner et al., 2022).

The interaction of *P. aeruginosa* and *Streptococcus* spp. might also occur due to the competition for iron. Siderophore-producing *P. aeruginosa* strains hinder the *S. sanguinis* growth due to iron sequestration from the surrounding environment. Whereas the *S. sanguinis* exhibits enhanced growth when co-culturing with non-siderophore-producing *P. aeruginosa* strains (Scott et al., 2019). In the DSB of *P. aeruginosa* and *S. constellatus*, the β-hydroxyalkanoyl–β-hydroxyalkanoic acids (HAAs) and monorhamnolipids (MRL) produced by some strains of *P. aeruginosa* reduce *S. constellatus* biofilm viability. However, it has been observed that *S. constellatus* biofilm formation is enhanced upon tobramycin exposure (Price et al., 2016). Tobramycin alters gene expression in *P. aeruginosa* through the activation or repression of certain genes (Anderson et al., 2008). Therefore, tobramycin might repress the production of HAAs and MRL leading to the arresting of its repressive activity against the biofilm formation process in *S. constellatus* (Price et al., 2016). This, in turn, might indicate that the antibiotic in such cases may indirectly contribute to the aggravation of the infection.

Impacts of oral streptococci on *P. aeruginosa*

Oral streptococci also display a kind of stimulation to *P. aeruginosa* when they are together. It has been observed an increase in pyocyanin and elastase production by less virulent clinical strains of *P. aeruginosa* in co-culture biofilm with SAG and SMG compared to monoculture biofilms (Whiley et al., 2015, 2014). Streptococci produce the autoinducer-2 (AI-2), a small diffusible quorum-sensing molecule, which acts as a signal and mediates interspecies communication among bacteria. AI-2 promotes mutualistic biofilm growth for two species of bacteria in a concentration-dependent manner (Rickard et al., 2006). *S. mitis* can enhance the adhesion and biofilm formation of *P. aeruginosa* and relieve the host immune response induced by *P. aeruginosa* (Song et al., 2015). In co-inoculation of the endotracheal intubation rat models, *S. mitis* AI-2 enhanced the biofilm formation, pathogenicity, and QS gene expression of *P. aeruginosa*. Consequently increased rat mortality and worsened lung infection compared with those infected with the mono-species biofilm of *P. aeruginosa* (**Table 3**) (Wang et al., 2016).

2.2 Interactions of *P. aeruginosa* and Gram-negative bacteria

The Gram-negative bacteria (Gram -ve bacteria) exhibit competitive abilities toward *P. aeruginosa* and alter its pathogenicity through several mechanisms that lead to inhibiting or minimizing its growth or virulence. Gram -ve bacteria secrete many products that enhance their viability and virulence and might also act as anti-*P. aeruginosa*. *S. maltophilia* produces VirB/D4 T4SS, a type IVA secretion system, which performs in two different directions whereby promoting the growth of *S. maltophilia* and suppressing the growth of both clinical and environmental isolates of *P. aeruginosa* in a contact-dependent manner (Nas et al., 2019). *Burkholderia cepacia*, an opportunistic pathogen that causes life-threatening infections in patients of CF and chronic granulomatous (Sfeir, 2018), produces YtnP and Y2-aiiA lactonases that act as self-control of acyl-homoserine lactone (AHL)-mediated QS. Nevertheless, Y2-aiiA displays broader efficacy and responds to different QS signal producers in the PMB. The extracellular Y2-aiiA lactonase hinders biofilm formation by *P. aeruginosa* without affecting growth through impairing the QS system AHL, a signal molecule responsible for initial cell adhesion and biofilm development of *P. aeruginosa* (Malešević et al., 2020).

In contrast to competitive interactions, cooperative or synergistic interactions can protect both microorganisms from the immune system and antibiotics and then survive both for a longer time at the site of infection. Cooperative interactions might occur between two QS pathways of both microorganisms. *S. maltophilia* produces the diffusible signaling factors (DSF) that can be sensed by *P. aeruginosa* which produces smoR, a LuxR family regulatory protein that responds to AHL signals. Signaling through DSF is one-sided mostly, whereas both organisms can sense the AHL signals. The phenotypes resulting from *P. aeruginosa* signaling involve increased gene expression of bacterial stress tolerance and antibiotic resistance, leading to cooperative behaviour of these bacteria during CF disease (Martínez et al., 2015).

2.3 Interactions of *P. aeruginosa* and fungi

The fungi such as *A. fumigatus* represent one of the pathogens that caused pulmonary infection in CF patients. The co-infection of *P. aeruginosa* and *A. fumigatus* is associated with exacerbations in CF patients and leads to poorer health (Keown et al., 2020; Reece et al., 2017). Despite both *P. aeruginosa* and *A. fumigatus* can exhibit mutually antagonistic effects on each other (Reece et al., 2018), *P. aeruginosa* seems to be the dominant competitor in co-cultures as a result of the inhibitory effects of its secretions such as

phenazines (Briard et al., 2019, 2015), pyoverdine (Sass et al., 2018) and dirhamnolipids (diRhls) (Briard et al., 2017). Besides the ability to inhibition of *A. fumagtus* growth (Nazik et al., 2020; Sass et al., 2019, 2018; Wurster et al., 2020), *P. aeruginosa* exoproducts display extreme impacts on the A. fumagtus proteins. *P. aeruginosa* exoproducts alter the properties of proteins required for the biosynthesis of secondary metabolites (e.g. gliotoxin, fumagillin, pseurotin A) and increase the proteins involved in oxidative stress (e.g. formate dehydrogenase) and detoxification (e.g. thioredoxin reductase) (Margalit et al., 2022). *P. aeruginosa* produces four phenazines, pyocyanin, phenazine-1-carboxamide, 1-hydroxyphenazine, and phenazine-1-carboxylic acid, that promote its growth and at the same time act as inhibitors or toxins against microbial and mammalian cells surrounding it (Nadal Jimenez et al., 2012). During co-existence with *A. fumigatus*, phenazines can inhibit the *A. fumigatus* growth by inducing the generation of reactive oxygen species (ROS) and reactive nitrogen species (RNS) which targets fungal hyphae and mitochondria, as well as by chelating the iron ions, subsequence depriving the *A. fumigatus* of iron (Briard et al., 2015). In addition, the PQS can inhibit *Aspergillus* growth by interfering with fungal iron metabolism (Nazik et al., 2021). In the co-culture, *A. fumigatus* can also show a different growth phenotype characterized by multibranched hyphae and thicker cell walls rich in chitin compared with its growth alone. *P. aeruginosa* produces diRhls which are composed of two rhamnose moieties linked to the fatty acyl chain which in turn hinders β-1,3 glucan synthase activity in *A. fumigatus* leading to inhibiting its growth and subsequently altering the cell wall architecture (Briard et al., 2017). However, a thickening of the cell wall resulting from an increased chitin concentration can later attenuate the diRhls impact.

As mentioned above, the dominance of *P. aeruginosa* depends on the strain, it has been observed that the non-mucoid *P. aeruginosa* strains are more able to inhibit growth and induce poorly hyphae formation of *Lomentospora prolificans* and *Scedosporium aurantiacum*, commonly fungi associated with patients suffering from chronic pulmonary disease, compared with mucoid *P. aeruginosa* strains (Chen et al., 2017). Such impacts might be attributed to the signal molecules of the DSF and other exoproducts such as pyocyanin (Homa et al., 2019). The alginate in mucoid strains interferes with the response to PQS which regulates the production of several exoproducts, leading to lower exoproducts than non-mucosal strains (Yang et al., 2017). However, *P. aeruginosa* and *Scedosporium* spp. influence also each other also in a contact-dependent inhibitory manner (Homa et al., 2019).

C. albicans, an opportunistic fungal pathogen that can be found in burn wounds and CF patients' lungs, is frequently co-isolated with the *P. aeruginosa* from clinical sputum samples of CF patients (Grainha et al., 2020; Haiko et al., 2019; Hattab et al., 2022) and can reveal diverse responses when coexistence with *P. aeruginosa* (Fourie et al., 2021; Hattab et al., 2022; Morales et al., 2013). Phenazines influence *C. albicans* in a concentration-dependent manner, whereby fungal morphogenesis, intercellular adherence, and biofilm development are inhibited at low and nonlethal concentrations as a result of inhibiting respiration by decreasing the ability to transfer electrons to oxygen (Morales et al., 2013). N-3-oxo-dodecanoyl-L-Homoserine lactone (3-oxo-C12-HSL) is a QS signaling molecule produced by *P. aeruginosa* and plays role in its pathogenicity. The 3-oxo-C12-HSL exerts inhibitory effects on *C. albicans* hyphal growth via repression of adenylyl cyclase (Cyr1p) in the cyclic AMP (cAMP)/protein kinase A (PKA) signaling pathway, a hyphal growth regulatory pathway (Hall et al., 2011). The 3-oxo-C12-HSL is also able to induce the resistance of *C. albicans* to the fluconazole by affecting the biosynthesis pathway of ergosterol, stimulating the multidrug efflux pump activity, decreasing the oxidative stress response, and keeping membrane integrity of yeast cells (Bandara et al., 2020). However, a recent study demonstrated that *P. aeruginosa* can support the efficacy of this antifungal drug against *C. albicans* during swim bladder infection in zebrafish (Hattab et al., 2022) (**Table 2**).

Table 2. Impacts of *P. aeruginosa* on fungi within the polymicrobial community

Fungus	Influence	Exoproduct and mechanism	ref
A. fumigatus	Decrease fungal growth	Phenazines promote ROS and RNS generation and induce the iron starvation	(Briard et al., 2015)
A. fumigatus	Inhibition of forming and preformed biofilms	Depriving the fungus to iron by pyoverdine	(Sass et al., 2018)
A. fumigatus	Alteration of the fungal growth phenotype and inhibition of its growth	diRhls blocks β-1,3 glucan synthase activity in fungus and induces the production of an extracellular matrix rich in galactosaminogalactan, 1,8-dihydroxynaphthalene, and pyo-melanin, surrounding its hyphae	(Briard et al., 2017)

C. albicans	Induce the resistance to the fluconazole	Reversing the fluconazole's impact on the biosynthesis pathway of ergosterol via N-(3-Oxododecanoyl)-L-homoserine lactone	(Bandara et al., 2020)
C. albicans	Inhibition hyphal growth	Repression of the cAMP/PKA signaling pathway	(Hall et al., 2011)
Scedosporium spp.	Decrease the rate of germination and the growth	Diffusible signal factor	(Homa et al., 2019)

Despite the dominance of *P. aeruginosa* when coexistence with fungi, the fungi also might exhibit antagonism or synergism against/with *P. aeruginosa*. In the co-cultures, A. fumigatus shows anti-pseudomonal activity through gliotoxin, a toxin with inhibitory influences on the host immune response, which can act as an anti-biofilm (Reece et al., 2018). Nevertheless, increased gliotoxin production may be attended by a decrease in fungus growth (Margalit et al., 2022). Furthermore, it has been observed that the fungal metabolite farnesol reduces the production of the PQS and pyocyanin and inhibits swarming motility in *P. aeruginosa* CF strains due to affecting transcript levels of *pqsA*, a required gene in the PQS biosynthetic operon (McAlester et al., 2008). On the other hand, *C. albicans* can promote *P. aeruginosa* biofilm tolerance to meropenem through mannan and glucan production into the extracellular matrix of *P. aeruginosa*-*C. albicans* biofilm, which might sequester or inhibit the activity of the meropenem (**Table 3**) (Alam et al., 2020).

2.4 Interactions of *P. aeruginosa* and viruses

P. aeruginosa exerts a sort of interaction with the viruses when existing with viruses, whether animal viruses infect humans or bacteriophages, viruses that infect bacteria. The outcome of these interactions is reflected in the behavior of *P. aeruginosa* which might later influence the severity of the infection.

2.4.1 *P. aeruginosa* and animal viruses

During the co-infection of *P. aeruginosa* and viruses in the airway, *P. aeruginosa* can modulate the antiviral response, indicating CF patients with intermittent *P. aeruginosa* infection are at more risk of viral infections. It has been observed that the Human Rhinovirus (HRV) load was higher in samples of CF patients, which were culture-positive for *P. aeruginosa* at the time of sampling (Sörensen et al., 2020). Interactions between *P.*

aeruginosa and respiratory viruses in the airway epithelium were investigated in vitro using different epithelial cell models (Endres et al., 2022; Hendricks et al., 2016; Sörensen et al., 2020). In the co-culture model of *P. aeruginosa* and respiratory syncytial virus (RSV), the RSV infection enhanced *P. aeruginosa* secondary infection and biofilm development in airway epithelium. RSV infection and subsequent immune system disruption provide a favourable environment for secondary infection by *P. aeruginosa*. Moreover, the induction of antiviral interferons in response to RSV infection, as well as the increased apical release of the host iron-binding protein transferrin during infection, enhance biofilm formation in *P. aeruginosa* (Hendricks et al., 2016).

In contrast, *P. aeruginosa* can promote RSV infections by inhibiting the antiviral response of airway epithelial cells by producing alkaline protease A (AprA) which degrades epithelial-derived IFNλ resulting in inhibition of IFN signaling (Sörensen et al., 2020). It has also been observed that *P. aeruginosa* infection can influence the response of bronchial epithelial cells to HRV infection. Co-infection with HRV and non-mucoid *P. aeruginosa* strains increased IL-1β protein concentrations and significantly decreased levels of IL-6 protein compared to virus infection alone, which might be due to the ability of these strains to degrade IL-6 protein. Worthily, these impacts were not shown in mucoid *P. aeruginosa*-HRV co-culture (Endres et al., 2022), indicating that *P. aeruginosa*-virus interaction also depends on the bacterial phenotype.

2.4.2 *P. aeruginosa* and Bacteriophages

Bacteriophages infect *P. aeruginosa,* which significantly impacts bacteria by killing it, weakening it, or enhancing its virulence according to the type of cycle infection of phage, a lysogenic or lytic cycle. After the viral DNA injects into the bacterial host, it creates new phage particles, and self-assemble to form new phages that release from bacteria, causing bacterial lysis and death, to called lytic phages. In the lysogenic cycle, the phage genome remains in bacteria for several generations, whereby it might integrate into the bacterial genome or exists as an extrachromosomal plasmid within the bacterial cell, these prophages are called temperate phages. Integration of the phage genome in the bacterial chromosome or remaining in the cytoplasm as a plasmid might provide the bacteria with new properties (Johnson et al., 2022; Tsao et al., 2018). Several temperate phages (prophages) were isolated from clinical *P. aeruginosa* strains with genes that are capable to alter some properties of *P. aeruginosa,* such as Pf8 phage has been isolated from clinical *P. aeruginosa* strains. The

bacteriophage control infection (*bci*), a gene in Pf8 phage and is implicated in the phage infection ability, encodes many proteins that have a role in regulating the QS system, biofilm formation, motility, and pyocyanin production in the *P. aeruginosa* (Ambroa et al., 2020). Temperate phage infection can also alter the susceptibility of *P. aeruginosa* to antimicrobials at subinhibitory concentrations of antibiotics, indicating they may be introductory to antibiotic resistance (Tariq et al., 2019). On the other hand, whole phages or their products can interact with the components of EPS, cell wall, and outer membrane in *P. aeruginosa* which increase its susceptibility to antibiotics (Al-Wrafy et al., 2019; Chan et al., 2016).

Table 3. Effect of the microorganisms on *P. aeruginosa* inside polymicrobial biofilms

microorganism	Exoproduct	Effect and mechanism	ref
S. aureus	GlcNAc	Induces PQS to produce pyocyanin, elastase and HQNO	(Korgaonkar et al., 2013)
S. aureus	SpA	Inhibition of biofilm formation in non-Psl producing *P. aeruginosa* through binding to type IV pili Protection of *P. aeruginosa* from phagocytosis by neutrophils via hindering *P. aeruginosa* binding to the neutrophil Fc receptor	(Armbruster et al., 2016)
S. mitis	AI-2	Enhance the adhesion, biofilm formation pathogenicity and PQS genes expression	(Rickard et al., 2006; Song et al., 2015; Wang et al., 2016)
S. mitis, *S. gordonii* and *S. sanguinis*	H2O2	Inhibit *P. aeruginosa* growth when cultured as a primary coloniser prior to *P. aeruginosa* inoculation	(Whiley et al., 2015)
E. faecalis	Lactate dehydrogenase	Inhibit *P. aeruginosa* growth by reduced pH of the surrounding environment. Lactate dehydrogenase causes L-lactate production during fermentative growth which reduced pH	(Tan et al., 2022)
S. maltophilia	VirB/D4 T4SS	Enhances the death of *P. aeruginosa* strains	(Nas et al., 2019)
B. cepacia	Y2-aiiA lactonase	Active against N-tetradecanoyl-dl-homoserine lactone (C14-HSL), leading to biofilm formation disrupting	(Malešević et al., 2020)

C. albicans	Fungal farnesol	Decreases the PQS production and pyocyanin, and inhibits swarming motility through inhibits transcribing the responsible gene for PQS biosynthesis	(McAlester et al., 2008)
C. albicans and *C. dubliniensis*	mannan and glucan	Promotes *P. aeruginosa* biofilm tolerance to meropenem	(Alam et al., 2020)
C. albicans	phenazines (pyocyanin, phenazine methosulfate, and phenazine-1-carboxylic acid)	Inhibiting filamentation, intercellular adherence, and biofilm development	(Morales et al., 2013)

3. The surrounding environmental conditions contribute to directing the interactions in the polymicrobial communities

It should be noted that interaction mechanisms between species, whether in vitro or ex vivo, might differ when they occur at the site of infection. From this perspective, in addition to the phenotype of the microbe, several investigations of interactions between *P. aeruginosa* and other microbes in co-cultures indicate that environmental conditions may also play a role in guiding these interactions (Nazik et al., 2020; Whiley et al., 2015). In fact, the co-existence demands specific adaptations of either one or both pathogens (Fugère et al., 2014). PQS production might be reduced due to the phenotype of strain or as a result of certain circumstances. The mucoid strains of *P. aeruginosa* produce lower levels of the PQS pathway products, subsequently lower levels of siderophores and other exoproducts compared to the non-mucoid strains (Limoli et al., 2017). The ability of *P. aeruginosa* to chelate the iron might differ in vitro than in vivo. As mentioned above, *P. aeruginosa* deprives the *A. fumigatus* to iron by pyoverdine and phenazines, resulting in inhibition of the fungal growth and biofilm formation in the co-cultures. However, *A. fumigatus* persists in CF airways for the late stages of the disease, such might be attributed partly to the decrease in the hostility of *P. aeruginosa*. The environmental conditions in inflamed lungs such as oxygen levels and iron derived from blood might diminish *P. aeruginosa* antagonism, providing beneficial conditions for coexisting *A. fumigatus* (Sass et al., 2018). Iron restriction resulting from the host's innately availability at infection sites might also impact microbial interactions. *Enterococcus faecalis*, common isolates with *P. aeruginosa* in

polymicrobial biofilm-associated infections of wounds and the urinary tract, inhibits *P. aeruginosa* growth within biofilms through a reduction in local environmental pH under conditions of iron restriction and anoxia (Tan et al., 2022). Under anaerobic conditions, the production of the PQS decreases, and then the levels of siderophores (Scott et al., 2019). The anoxia might also reduce the ability of *P. aeruginosa* strains to dominate over other bacteria (Pallett et al., 2019). The response of *C. albicans* toward *P. aeruginosa* is also associated with anoxia, which may lead to differential regulation of genes responsible for the phenotype, cell membrane synthesis, and biofilm formation (Fourie et al., 2021).

It is noteworthy that the oral commensal streptococci can modulate the pathogenicity of *P. aeruginosa* strains positively and negatively according to environmental circumstances. Under aerobic conditions, *P. aeruginosa* and SMG in co-culture display cooperative interaction, whereby SMG achieves higher populations, while the pyocyanin and elastase production increases by *P. aeruginosa* compared to in monoculture. In contrast, *S. oralis* becomes more antagonistic in an atmosphere containing CO_2 through *P. aeruginosa* growth inhibition by hydrogen peroxide (H_2O_2) production (Whiley et al., 2015). In co-culture supplemented with nitrite, *S. parasanguinis*, *S. sanguinis*, and *S. gordonii* inhibit the *P. aeruginosa* growth. The nitrite combines with H_2O_2 which is produced by oral streptococci to generate the reactive nitrogenous intermediate (RNI), peroxynitrite, which may be transported by a *P. aeruginosa* ABC transporter permease. Peroxynitrite diffuses across cell membranes and induces the oxidation or nitration of DNA, lipids, and proteins or promotes membrane damage (Scoffield and Wu, 2015). However, *P. aeruginosa* produces Nitrite reductase, an enzyme in the denitrification pathway, which converts the nitrite to nitric oxide, thus preventing the accumulation of nitrite and generation of peroxynitrite in the co-culture (Scoffield and Wu, 2016). Such was also observed in the co-culture with fungi whereby the impacts of *P. aeruginosa* on the growth of *Scedosporium apiospermum*, the second most frequently fungal isolated after *A. fumigatus* from CF patients, ranged between the inhibition and enhancement relying on the culture conditions (Homa et al., 2019).

4. Concluding Remarks

The data collected in this review indicate the importance of understanding the mechanisms of polymicrobial interactions is crucial for expanding knowledge of infection and then improving the therapeutic approach and controlling the deterioration of the infections. Polymicrobial infection is more complicated than monomicrobial infection, there are various interactions that arise between host and microbe, microbe and microbe, microbes

and immune system, and finally microbes and antibiotic. The interactions between microbes may take their results towards improving or inhibiting the virulence of the microbes, and here the microbe will become more able to resist the immune system and antibiotics, or it will be the opposite. However, it is often noted that polymicrobial infection is the most persistent and resistant to antibiotics, which means that polymicrobial interactions often tend to support the infection. The PMB contributes in two different manners to the microbe's pathogenicity by enhancing or repressing its virulence. The interactions in the polymicrobial community can be led to upregulation or downregulation of gene expression of one or both microbes, and stimulation or inhibition of microbial growth, virulence factors, and susceptibility to antibiotics. Interactions can also be synergistic, resulting in microbes protecting each other from killing agents by the immune system or antimicrobials, such in turn cause exacerbate infection compared to monomicrobial infection.

Some remarks emerged during the literature review that has been summarized as follows:

1- The variable influence of *P. aeruginosa* is dependent upon strains and their ability to generate exoproducts.

2- Overproduction of some factors by *P. aeruginosa* during infection could affect microbial dynamics.

3- The phenotypic and genotypic variation of *P. aeruginosa* and other microorganisms in CF patients might explain the difference in interactions between them and the consequent worsening of infection and variation in severity.

4- The ability to antibiotic resistance depends not only upon the genotype of the pathogen being targeted or on the defensive barriers of biofilm but also on interactions between microorganisms within the biofilm.

5- The environment of the site of infection might influence the interaction between microorganisms which is reflected in the infection.

6- It should be taken into account that the treatment method may differ in the case of PMB from in the case of MMB.

7- Many exoproducts that act as inhibitors against other microbes could be exploited as promising antimicrobials such as those that have activity against QS and virulence.

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References

Al-Wrafy, F., Brzozowska, E., Górska, S., Drab, M., Strus, M., Gamian, A., 2019. Identification and characterization of phage protein and its activity against two strains of multidrug-resistant *Pseudomonas aeruginosa*. Sci. Reports 2019 91 9, 1–14. https://doi.org/10.1038/s41598-019-50030-5

Al-Wrafy, F., Brzozowska, E., Górska, S., Gamian, A., 2017. Pathogenic factors of *Pseudomonas aeruginosa* – the role of biofilm in pathogenicity and as a target for phage therapy. Postepy Hig. Med. Dosw. 71, 78–91. https://doi.org/10.5604/01.3001.0010.3792

Alam, F., Catlow, D., Di Maio, A., Blair, J.M.A., Hall, R.A., 2020. *Candida albicans*enhances meropenem tolerance of *Pseudomonas aeruginosa* in a dual-species biofilm. J. Antimicrob. Chemother. 75, 925–935. https://doi.org/https://doi.org/10.1093/jac/dkz514

Alves, P.M., Al-Badi, E., Withycombe, C., Jones, P.M., Purdy, K.J., Maddocks, S.E., 2018. Interaction between *Staphylococcus aureus* and *Pseudomonas aeruginosa* is beneficial for colonisation and pathogenicity in a mixed biofilm. Pathog. Dis. 76, 3. https://doi.org/doi: 10.1093/femspd/fty003

Ambroa, A., Blasco, L., López-Causapé, C., Trastoy, R., Fernandez-García, L., Bleriot, I., Ponce-Alonso, M., Pacios, O., López, M., Cantón, R., Kidd, T.J., Bou, G., Oliver, A., Tomás, M., 2020. Temperate Bacteriophages (Prophages) in *Pseudomonas aeruginosa* Isolates Belonging to the International Cystic Fibrosis Clone (CC274). Front. Microbiol. 11, 2299. https://doi.org/10.3389/fmicb.2020.556706

Anderson, G.G., Moreau-Marquis, S., Stanton, B.A., O'Toole, G.A., 2008. In vitro analysis of tobramycin-treated *Pseudomonas aeruginosa* biofilms on cystic fibrosis-derived airway epithelial cells. Infect. Immun. 76, 1423–1433. https://doi.org/10.1128/IAI.01373-07

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. https://doi.org/doi:10.1128/mBio.00538-16

Baishya, J., Wakeman, C.A., 2019. Selective pressures during chronic infection drive microbial competition and cooperation. npj Biofilms Microbiomes 2019 51 5, 1–9. https://doi.org/10.1038/s41522-019-0089-2

Bandara, H.M.H.N., Wood, D.L.A., Vanwonterghem, I., Hugenholtz, P., Cheung, B.P.K., Samaranayake, L.P., 2020. Fluconazole resistance in *Candida albicans*is induced by *Pseudomonas aeruginosa* quorum sensing. Sci. Reports 2020 101 10, 1–17. https://doi.org/10.1038/s41598-020-64761-3

Beaudoin, T., Yau, Y.C.W., Stapleton, P.J., Gong, Y., Wang, P.W., Guttman, D.S., Waters, V., 2017. *Staphylococcus aureus* interaction with *Pseudomonas aeruginosa* biofilm enhances tobramycin resistance. npj Biofilms Microbiomes 2017 31 3, 1–9. https://doi.org/10.1038/s41522-017-0035-0

Bernardy, E.E., Petit, R.A., Raghuram, V., Alexander, A.M., Read, T.D., Goldberg, J.B., 2020. Genotypic and phenotypic diversity of *Staphylococcus aureus* isolates from cystic fibrosis patient lung infections and their interactions with *Pseudomonas*

aeruginosa. MBio 11, 1–18. https://doi.org/10.1128/ mBio.00735-20

Bisht, K., Baishya, J., Wakeman, C.A., 2020. *Pseudomonas aeruginosa* polymicrobial interactions during lung infection. Curr. Opin. Microbiol. 53, 1–8. https://doi.org/10.1016/J.MIB.2020.01.014

Biswas, L., Biswas, R., Schlag, M., Bertram, R., Götz, F., 2009. Small-colony variant selection as a survival strategy for *Staphylococcus aureus* in the presence of *Pseudomonas aeruginosa*. Appl. Environ. Microbiol. 75, 6910–6912. https://doi.org/https://doi.org/10.1128/AEM.01211-09

Briard, B., Bomme, P., Lechner, B.E., Mislin, G.L.A., Lair, V., Prévost, M.-C., Latgé, J.-P., Haas, H., Beauvais, A., 2015. *Pseudomonas aeruginosa* manipulates redox and iron homeostasis of its microbiota partner *Aspergillus fumigatus* via phenazines. Sci. Rep. 5, 8220. https://doi.org/10.1038/srep08220

Briard, B., Mislin, G.L.A., Latgé, J.P.J.P., Beauvais, A., 2019. Interactions between *Aspergillus fumigatus* and Pulmonary Bacteria: Current State of the Field, New Data, and Future Perspective. J. Fungi 2019, Vol. 5, Page 48 5, 48. https://doi.org/10.3390/JOF5020048

Briard, B., Rasoldier, V., Bomme, P., Elaouad, N., Guerreiro, C., Chassagne, P., Muszkieta, L., Latgé, J.P., Mulard, L., Beauvais, A., 2017. Dirhamnolipids secreted from *Pseudomonas aeruginosa* modify anjpegungal susceptibility of *Aspergillus fumigatus* by inhibiting β1,3 glucan synthase activity. ISME J. 2017 117 11, 1578–1591. https://doi.org/10.1038/ismej.2017.32

Chan, B.K., Sistrom, M., Wertz, J.E., Kortright, K.E., Narayan, D., Turner, P.E., 2016. Phage selection restores antibiotic sensitivity in MDR *Pseudomonas aeruginosa*. Sci. Reports 2016 61 6, 1–8. https://doi.org/10.1038/srep26717

Chen, S.C.A., Patel, S., Meyer, W., Chapman, B., Yu, H., Byth, K., Middleton, P.G., Nevalainen, H., Sorrell, T.C., 2017. *Pseudomonas aeruginosa* inhibits the growth of *Scedosporium* and *Lomentospora* in vitro. Mycopathol. 2017 1831 183, 251–261. https://doi.org/10.1007/S11046-017-0140-X

Endres, A., Hügel, C., Boland, H., Hogardt, M., Schubert, R., Jonigk, D., Braubach, P., Rohde, G., Bellinghausen, C., 2022. *Pseudomonas aeruginosa* afects airway epithelial response and barrier function during Rhinovirus infection. Front. Cell. Infect. Microbiol. 12, 143. https://doi.org/10.3389/fcimb.2022.846828

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, 2252–2264. https://doi.org/doi:10.1128/JB.00059-15

Fischer, A.J., Singh, S.B., LaMarche, M.M., Maakestad, L.J., Kienenberger, Z.E., Peña, 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, 328–338. https://doi.org/10.1164/rccm.202004-1322OC

Flemming, H., Wingender, J., Szewzyk, U., Steinberg, P., Rice, S.A., Kjelleberg, S., 2016. Biofilms: An emergent form of bacterial life. Nat. Rev. Microbiol. 14, 563–575. https://doi.org/10.1038/nrmicro.2016.94

Fourie, R., Cason, E.D., Albertyn, J., Pohl, C.H., 2021. Transcriptional response of *Candida albicans*to *Pseudomonas aeruginosa* in a polymicrobial biofilm. G3 Genes|Genomes|Genetics 11. https://doi.org/10.1093/G3JOURNAL/JKAB042

Fugère, A., Séguin, D.L., Mitchell, G., Déziel, E., Dekimpe, V., Cantin, A.M., Frost, E., Malouin, F., 2014. Interspecific Small Molecule Interactions between Clinical Isolates of *Pseudomonas aeruginosa* and *Staphylococcus aureus* from Adult Cystic Fibrosis Patients. PLoS One 9, e86705. https://doi.org/10.1371/journal.pone.0086705

Gomes-Fernandes, M., Gomez, A.C., Bravo, M., Huedo, P., Coves, X., Prat-Aymerich, C., Gibert, I., Lacoma, A., Yero, D., 2022. Strain-specific interspecies interactions between co-isolated pairs of *Staphylococcus aureus* and *Pseudomonas aeruginosa* from patients with tracheobronchitis or bronchial colonization. Sci. Reports 2022 121 12, 1–15. https://doi.org/10.1038/s41598-022-07018-5

Gounani, Z., Şen 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. Methods 175, 105994. https://doi.org/10.1016/J.MIMET.2020.105994

Grainha, T., Jorge, P., Alves, D., Lopes, S.P., Pereira, M.O., 2020. Unraveling *Pseudomonas aeruginosa* and *Candida albicans*communication in coinfection scenarios: Insights through network analysis. Front. Cell. Infect. Microbiol. 10, 676. https://doi.org/10.3389/fcimb.2020.550505

Haiko, J., Saeedi, B., Bagger, G., Karpati, F., Özenci, V., 2019. Coexistence of Candida species and bacteria in patients with cystic fibrosis. Eur. J. Clin. Microbiol. Infect. Dis. 38, 1071–1077. https://doi.org/10.1007/S10096-019-03493-3/FIGURES/2

Hall, R.A., Turner, K.J., Chaloupka, J., Cottier, F., de Sordi, L., Sanglard, D., Levin, L.R., Buck, J., Mühlschlegel, F.A., 2011. The quorum-sensing molecules farnesol/homoserine lactone and dodecanol operate via distinct modes of action in candida albicans. Eukaryot. Cell 10, 1034–1042. https://doi.org/10.1128/EC.05060-11

Hattab, S., Dagher, A.-M., Wheeler, R.T., 2022. *Pseudomonas* Synergizes with Fluconazole against *Candida* during Treatment of Polymicrobial Infection. Infect. Immun. 90. https://doi.org/10.1128/IAI.00626-21

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, 1642–1647. https://doi.org/https://doi.org/10.1073/pnas.1516979113

Hoffman, L.R., Déziel, E., D'Argenio, D.A., Lépine, 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. 103, 19890–19895. https://doi.org/10.1073/PNAS.0606756104

Homa, M., Sándor, A., Tóth, E., Szebenyi, C., Nagy, G., Vágvölgyi, C., Papp, T., 2019. In vitro interactions of *Pseudomonas aeruginosa* with scedosporium species frequently associated with cystic fibrosis. Front. Microbiol. 10, 441. https://doi.org/10.3389/fmicb.2019.00441

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. https://doi.org/10.3389/fcimb.2017.00106

Johnson, G., Banerjee, S., Putonti, C., 2022. Diversity of *Pseudomonas aeruginosa* Temperate Phages. mSphere 7. https://doi.org/https://doi.org/10.1128/msphere.01015-21

Jordana-Lluch, E., Garcia, V., Kingdon, A.D.H., Singh, N., Alexander, C., Williams, P., Hardie, K.R., 2020. A simple polymicrobial biofilm keratinocyte colonization model for exploring interactions between commensals, pathogens and antimicrobials. Front. Microbiol. 11, 291. https://doi.org/10.3389/FMICB.2020.00291

Keown, K., Reid, A., Moore, J.E., Taggart, C.C., Downey, D.G., 2020. Coinfection with *Pseudomonas aeruginosa* and *Aspergillus fumigatus* in cystic fibrosis. Eur. Respir. Rev. 29, 1–12. https://doi.org/10.1183/16000617.0011-2020

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, 1059–1064. https://doi.org/https://doi.org/10.1073/pnas.1214550110

Lee, K., Lee, K.M., Kim, D., Yoona, S.S., 2017. Molecular determinants of the thickened matrix in a dual-species *Pseudomonas aeruginosa* and *Enterococcus faecalis* biofilm. Appl. Environ. Microbiol. 83, e01182-17. https://doi.org/. https://doi.org/10.1128/AEM.01182-17

Limoli, D.H., Whitfield, G.B., Kitao, T., Ivey, M.L., Davis, 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, e00186-17. https://doi.org/https://doi.org/10.1128/mBio.00186-17

Lopes, S.P., Jorge, P., Sousa, A.M., Pereira, M.O., 2021. Discerning the role of polymicrobial biofilms in the ascent, prevalence, and extent of heteroresistance in clinical practice. Crit. Rev. Microbiol. 47, 162–191. https://doi.org/https://doi.org/10.1080/1040841X.2020.1863329

Machan, Z.A., Taylor, G.W., Pitt, T.L., Cole, P.J., Wilson, R., 1992. 2-Heptyl-4-hydroxyquinoline N-oxide, an antistaphylococcal agent produced by *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. 30, 615–623. https://doi.org/10.1093/JAC/30.5.615

Malešević, M., Stanisavljević, N., Novović, K., Polović, N., Vasiljević, Z., Kojić, M., Jovčić, B., 2020. *Burkholderia cepacia* YtnP and Y2-aiiA lactonases inhibit virulence of *Pseudomonas aeruginosa* via quorum quenching activity. Microb. Pathog. 149, 104561. https://doi.org/10.1016/J.MICPATH.2020.104561

Margalit, A., Carolan, J.C., Sheehan, D., Kavanagh, K., 2020. The *Aspergillus fumigatus* secretome alters the proteome of *Pseudomonas aeruginosa* to stimulate bacterial growth: implications for co-infection. Mol. Cell. Proteomics 19, 1346–1359. https://doi.org/10.1074/MCP.RA120.002059

Margalit, A., Sheehan, D., Carolan, J.C., Kavanagh, K., 2022. Exposure to the *Pseudomonas*

aeruginosa secretome alters the proteome and secondary metabolite production of *Aspergillus fumigatus*. Microbiol. 168, 001164. https://doi.org/https://doi.org/10.1099/mic.0.001164

Martínez, P., Huedo, P., Martinez-Servat, S., Planell, R., Ferrer-Navarro, M., Daura, X., Yero, D., Gibert, I., 2015. *Stenotrophomonas maltophilia* responds to exogenous AHL signals through the LuxR solo SmoR (Smlt1839). Front. Cell. Infect. Microbiol. 0, 41. https://doi.org/10.3389/FCIMB.2015.00041

McAlester, G., O'Gara, F., Morrissey, J.P., 2008. Signal-mediated interactions between *Pseudomonas aeruginosa* and *Candida albicans*. J. Med. Microbiol. 57, 563–569. https://doi.org/10.1099/jmm.0.47705-0

Mcdaniel, M.S., Schoeb, T., Swords, W.E., 2020. Cooperativity between *Stenotrophomonas maltophilia* and *Pseudomonas aeruginosa* during polymicrobial airway infections. Infect. Immun. 88, e00855-19. https://doi.org/10.1128/IAI.00855-19.

Mitra, S., Mallick, A., Priyadarshini, S., 2022. Effect of polymicrobial interactions on antimicrobial resistance: an in vitro analysis in human ocular infections. Future Microbiol. 17, 491–504. https://doi.org/10.2217/fmb-2021-0114

Monych, N.K., Turner, R.J., 2020. Multiple compounds secreted by *Pseudomonas aeruginosa* increase the tolerance of *Staphylococcus aureus* to the antimicrobial metals copper and silver. mSystems 5, e00746-20. https://doi.org/10 .1128/mSystems.00746-20

Morales, D.K., Grahl, N., Okegbe, C., Dietrich, L.E.P., Jacobs, N.J., Hogan, D.A., 2013. Control of *Candida albicans*metabolism and biofilm formation by *Pseudomonas aeruginosa* phenazines. MBio 4, e00526-12. https://doi.org/10.1128/mBio.00526-12

Murray, E.J., Dubern, J.-F., Chan, W.C., Chhabra, S.R., Williams, P., 2022. A *Pseudomonas aeruginosa* PQS quorum-sensing system inhibitor with anti-staphylococcal activity sensitizes polymicrobial biofilms to tobramycin. Cell Chem. Biol. 29, 1187-1199.e6. https://doi.org/10.1016/J.CHEMBIOL.2022.02.007

Nadal Jimenez, P., Koch, G., Thompson, J.A., Xavier, K.B., Cool, R.H., Quax, W.J., 2012. The multiple signaling systems regulating virulence in *Pseudomonas aeruginosa*. Microbiol. Mol. Biol. Rev. 76, 46–65. https://doi.org/10.1128/MMBR.05007-11

Nas, M.Y., White, R.C., DuMont, A.L., Lopez, A.E., Cianciottoa, N.P., 2019. *Stenotrophomonas maltophilia* encodes a VirB/VirD4 type IV secretion system that modulates apoptosis in human cells and promotes competition against heterologous bacteria, including *Pseudomonas aeruginosa*. Infect. Immun. 87, e00457-19. https://doi.org/10.1128/IAI.00457-19

Nazik, H., Sass, G., Déziel, E., Stevens, D.A., 2020. Aspergillus is inhibited by *Pseudomonas aeruginosa* volatiles. J. Fungi 6, 118. https://doi.org/10.3390/JOF6030118

Nazik, H., Sass, G., Williams, P., Déziel, E., Stevens, D.A., 2021. Molecular modifications of the Pseudomonas quinolone signal in the intermicrobial competition with Aspergillus. J. Fungi 7, 343. https://doi.org/10.3390/JOF7050343

Niggli, S., Wechsler, T., Kümmerli, R., 2021. Single-cell imaging reveals that

Staphylococcus aureus is highly competitive against *Pseudomonas aeruginosa* on surfaces. Front. Cell. Infect. Microbiol. 11, 785. https://doi.org/10.3389/fcimb.2021.733991

Oluyombo, O., Penfold, C.N., Diggle, S.P., 2019. Competition in biofilms between cystic fibrosis isolates of *Pseudomonas aeruginosa* is shaped by R-pyocins. MBio 10, e01828-18. https://doi.org/10.1128/mBio.01828-18

Orazi, G., Jean-Pierre, F., O'Toole, G.A., 2020. *Pseudomonas aeruginosa* PA14 enhances the efficacy of norfloxacin against *Staphylococcus aureus* newman biofilms. J. Bacteriol. 202, e00159-20. https://doi.org/10 .1128/JB.00159-20

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, e00873-17. https://doi.org/10.1128/MBIO.00873-17

Orazi, G., Ruoff, K.L., O'toole, G.A., 2019. *Pseudomonas aeruginosa* increases the sensitivity of biofilm-grown *Staphylococcus aureus* to membrane-targeting antiseptics and antibiotics. MBio 10, 143–176. https://doi.org/10.1128/mBio.01501-19

Pallett, R., Leslie, L.J., Lambert, P.A., Milic, I., Devitt, A., Marshall, L.J., 2019. Anaerobiosis influences virulence properties of *Pseudomonas aeruginosa* cystic fibrosis isolates and the interaction with *Staphylococcus aureus*. Sci. Reports 2019 91 9, 1–18. https://doi.org/10.1038/s41598-019-42952-x

Pozo, J.L. Del, 2017. Biofilm-related disease. Expert Rev. Anti. Infect. Ther. 16, 51–65. https://doi.org/10.1080/14787210.2018.1417036

Price, B.L., Morley, R., Bowling, F.L., Lovering, A.M., Dobson, C.B., 2020. Susceptibility of monomicrobial or polymicrobial biofilms derived from infected diabetic foot ulcers to topical or systemic antibiotics in vitro. PLoS One 15, e0228704. https://doi.org/10.1371/JOURNAL.PONE.0228704

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, e00559-19. https://doi.org/10.1128/JB.00559-19/SUPPL_FILE/JB.00559-19-S0002.PDF

Price, K.E., Naimie, A.A., Griffin, E.F., Bay, C., O'Toole, G.A., 2016. Tobramycin-treated *Pseudomonas aeruginosa* PA14 enhances *Streptococcus constellatus* 7155 biofilm formation in a cystic fibrosis model system. J. Bacteriol. 198, 237–247. https://doi.org/10.1128/JB.00705-15/SUPPL_FILE/ZJB999093864SO1.PDF

Radlinski, L., Rowe, S.E., Kartchner, L.B., Maile, R., Cairns, B.A., Vitko, N.P., Gode, C.J., Lachiewicz, A.M., Wolfgang, M.C., Conlon, B.P., 2017. *Pseudomonas aeruginosa* exoproducts determine antibiotic efficacy against *Staphylococcus aureus*. PLOS Biol. 15, e2003981. https://doi.org/10.1371/JOURNAL.PBIO.2003981

Reece, E., Doyle, S., Greally, P., Renwick, J., McClean, S., 2018. *Aspergillus fumigatus* inhibits *Pseudomonas aeruginosa* in co-culture: Implications of a mutually antagonistic relationship on virulence and inflammation in the CF airway. Front. Microbiol. 9, 1205. https://doi.org/10.3389/FMICB.2018.01205/BIBTEX

Reece, E., Segurado, R., Jackson, A., McClean, S., Renwick, J., Greally, P., 2017. Co-

colonisation with *Aspergillus fumigatus* and *Pseudomonas aeruginosa* is associated with poorer health in cystic fibrosis patients: An Irish registry analysis. BMC Pulm. Med. 17, 1–8. https://doi.org/10.1186/S12890-017-0416-4

Rickard, A.H., Palmer, R.J., Blehert, D.S., Campagna, S.R., Semmelhack, M.F., Egland, P.G., Bassler, B.L., Kolenbrander, P.E., 2006. Autoinducer 2: a concentration-dependent signal for mutualistic bacterial biofilm growth. Mol. Microbiol. 60, 1446–1456. https://doi.org/10.1111/J.1365-2958.2006.05202.X

Sass, G., Ansari, S.R., Dietl, A.M., Déziel, E., Haas, H., Stevens, D.A., 2019. Intermicrobial interaction: *Aspergillus fumigatus* siderophores protect against competition by *Pseudomonas aeruginosa*. PLoS One 14, e0216085. https://doi.org/10.1371/JOURNAL.PONE.0216085

Sass, G., Nazik, H., Penner, J., Shah, H., Ansari, S.R., Clemons, K. V., Groleau, M.C., Dietl, A.M., Visca, P., Haas, H., Déziel, E., Stevens, D.A., 2018. Studies of *Pseudomonas aeruginosa* mutants indicate pyoverdine as the central factor in inhibition of *Aspergillus fumigatus* biofilm. J. Bacteriol. 200, 345–362. https://doi.org/10.1128/JB.00345-17

Schneewind, O., Missiakas, D.M., 2019. Staphylococcal protein secretion and envelope assembly. Microbiol. Spectr. 7, 10.1128. https://doi.org/10.1128/MICROBIOLSPEC.GPP3-0070-2019

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, e1006300. https://doi.org/10.1371/JOURNAL.PPAT.1006300

Scoffield, J.A., Wu, H., 2016. Nitrite reductase is critical for *Pseudomonas aeruginosa* survival during co-infection with the oral commensal *Streptococcus parasanguinis*. Microbiol. 162, 376–383. https://doi.org/10.1099/MIC.0.000226

Scoffield, J.A., Wu, H., 2015. Oral streptococci and nitrite-mediated interference of *Pseudomonas aeruginosa*. Infect. Immun. 83, 101–107. https://doi.org/10.1128/IAI.02396-14

Scott, J.E., Li, K., Filkins, L.M., Zhu, B., Kuchma, S.L., Schwartzman, J.D., O'Toole, G.A., 2019. *Pseudomonas aeruginosa* can inhibit growth of streptococcal species via siderophore production. J. Bacteriol. 201, e00014-19. https://doi.org/10.1128/JB.00014-19

Scott, J.E., O'Toole, G.A., 2019. The yin and yang of *Streptococcus* lung infections in cystic fibrosis: A model for studying polymicrobial interactions. J. Bacteriol. 201, e00115-19. https://doi.org/10.1128/JB.00115-19

Sfeir, M.M., 2018. *Burkholderia cepacia* complex infections: More complex than the bacterium name suggest. J. Infect. 77, 166–170. https://doi.org/10.1016/J.JINF.2018.07.006

Song, S., Du, L., Yu, J., Ai, Q., Pan, Y., Fu, Y., Wang, Z., 2015. Does *Streptococcus mitis*, a neonatal oropharyngeal bacterium, influence the pathogenicity of *Pseudomonas aeruginosa*? Microbes Infect. 17, 710–716. https://doi.org/10.1016/J.MICINF.2015.08.001

Sörensen, M., Kantorek, J., Byrnes, L., Boutin, S., Mall, M.A., Lasitschka, F., Zabeck, H.,

Nguyen, D., Dalpke, A.H., 2020. *Pseudomonas aeruginosa* modulates the antiviral response of bronchial epithelial cells. Front. Immunol. 11, 96. https://doi.org/10.3389/FIMMU.2020.00096

Stoner, S.N., Baty, J.J., Scoffield, J.A., 2022. *Pseudomonas aeruginosa* polysaccharide Psl supports airway microbial community development. ISME J. 16, 1730–1739. https://doi.org/10.1038/s41396-022-01221-y

Suryaletha, K., John, J., Radhakrishnan, M.P., George, S., Thomas, S., 2018. Metataxonomic approach to decipher the polymicrobial burden in diabetic foot ulcer and its biofilm mode of infection. Int. Wound J. 15, 473–481. https://doi.org/10.1111/IWJ.12888

Tan, C.A.Z., Lam, L.N., Biukovic, G., Soh, E.Y.-C., Toh, X.W., Lemos, J.A., Kline, K.A., 2022. *Enterococcus faecalis* antagonizes *Pseudomonas aeruginosa* growth in mixed-species interactions. J. Bacteriol. 204. https://doi.org/10.1128/JB.00615-21

Tariq, M.A., Everest, F.L.C., Cowley, L.A., Wright, R., Holt, G.S., Ingram, H., Duignan, L.A.M., Nelson, A., Lanyon, C. V., Perry, A., Perry, J.D., Bourke, S., Brockhurst, M.A., Bridge, S.H., De Soyza, A., Smith, D.L., 2019. Temperate Bacteriophages from chronic *Pseudomonas aeruginosa* lung infections show disease-specific changes in host range and modulate antimicrobial susceptibility. mSystems 4, e00191-18. https://doi.org/10.1128/MSYSTEMS.00191-18

Thi, M.T.T., Wibowo, D., Rehm, B.H.A., 2020. *Pseudomonas aeruginosa* Biofilms. Int. J. Mol. Sci. 21, 8671. https://doi.org/10.3390/IJMS21228671

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. Reports 2020 101 10, 1–18. https://doi.org/10.1038/s41598-020-71834-w

Tsao, Y.F., Taylor, V.L., Kala, S., Bondy-Denomy, J., Khan, A.N., Bona, D., Cattoir, V., Lory, S., Davidson, A.R., Maxwell, K.L., 2018. Phage morons play an important role in *Pseudomonas aeruginosa* phenotypes. J. Bacteriol. 200, e00189-18. https://doi.org/10.1128/JB.00189-18

Vestby, L.K., Grønseth, T., Simm, R., Nesse, L.L., 2020. Bacterial biofilm and its role in the pathogenesis of disease. Antibiotics 9, 59. https://doi.org/10.3390/ANTIBIOTICS9020059

Wang, Z., Xiang, Q., Yang, T., Li, L., Yang, J., Li, H., He, Y., Zhang, Y., Lu, Q., Yu, J., 2016. Autoinducer-2 of *Streptococcus mitis* as a target molecule to inhibit pathogenic multi-species biofilm formation in vitro and in an endotracheal intubation rat model. Front. Microbiol. 7, 88. https://doi.org/10.3389/FMICB.2016.00088

Whiley, R.A., Fleming, E. V., Makhija, R., Waite, R.D., 2015. Environment and colonisation sequence are key parameters driving cooperation and competition between *Pseudomonas aeruginosa* cystic fibrosis Strains and oral commensal Streptococci. PLoS One 10, e0115513. https://doi.org/10.1371/JOURNAL.PONE.0115513

Whiley, R.A., Sheikh, N.P., Mushtaq, N., Hagi-Pavli, E., Personne, Y., Javaid, D., Waite, R.D., 2014. Differential potentiation of the virulence of the *Pseudomonas aeruginosa* cystic fibrosis Liverpool epidemic strain by oral commensal streptococci. J. Infect. Dis.

209, 769–780. https://doi.org/10.1093/INFDIS/JIT568

Wurster, S., Sass, G., Albert, N.D., Nazik, H., Déziel, E., Stevens, D.A., Kontoyiannis, D.P., 2020. Live imaging and quantitative analysis of *Aspergillus fumigatus* growth and morphology during inter-microbial interaction with *Pseudomonas aeruginosa*. Virulence 11, 1329–1336. https://doi.org/10.1080/21505594.2020.1827885

Yadav, M.K., Chae, S.W., Go, Y.Y., Im, G.J., Song, J.J., 2017. In vitro multi-species biofilms of methicillin-resistant *Staphylococcus aureus* and *Pseudomonas aeruginosa* and their host interaction during in vivo colonization of an otitis media rat model. Front. Cell. Infect. Microbiol. 7, 125. https://doi.org/10.3389/FCIMB.2017.00125

Yang, J., Toyofuku, M., Sakai, R., Nomura, N., 2017. Influence of the alginate production on cell-to-cell communication in *Pseudomonas aeruginosa* PAO1. Environ. Microbiol. Rep. 9, 239–249. https://doi.org/10.1111/1758-2229.12521

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, 423–442. https://doi.org/10.1111/MMI.14519

Figure legends:

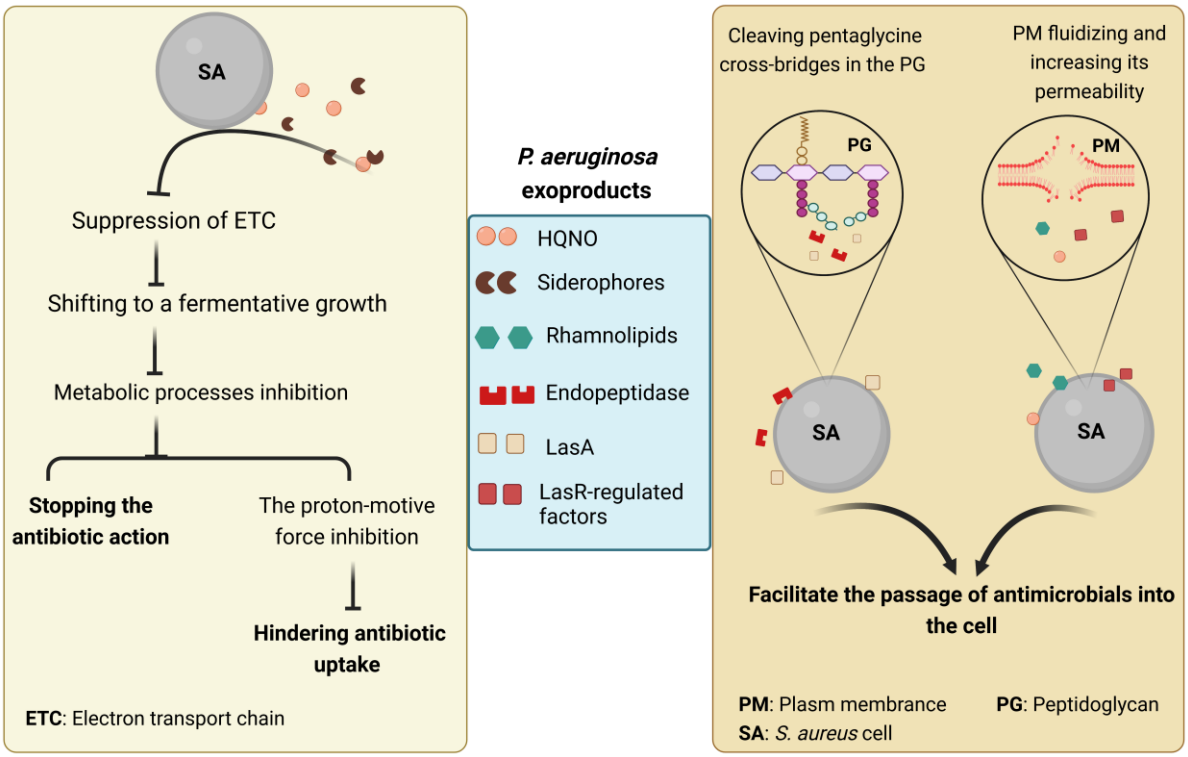

Fig. 1. Impacts of *P. aeruginosa*'s exoproducts on the susceptibility of *S. aureus* to the antimicrobial. The exoproducts can change the susceptibility of *S. aureus* via several mechanisms including causing membrane fluidity and change in its permeability, cleaving

pentaglycine cross-bridges in the peptidoglycan of the cell wall, and converting *S. aureus* to the anaerobic fermentative state.

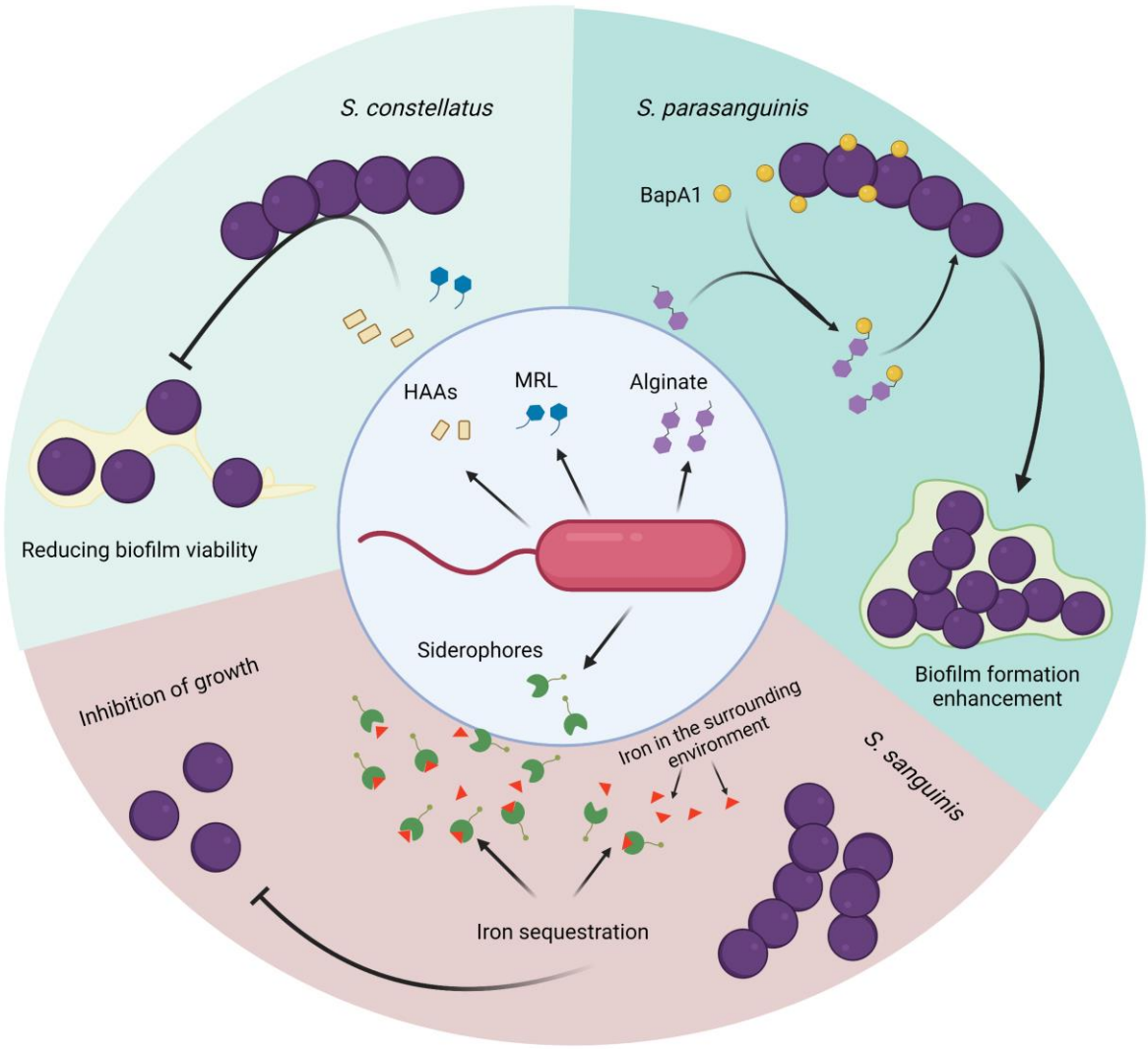

Fig. 2. Impact of *P. aeruginosa* on oral streptococci. *P. aeruginosa* strains produce alginate,β-hydroxyalkanoyl–β-hydroxyalkanoic acids (HAAs), monorhamnolipids (MRL), and siderophore, that mediate its interactions with *Streptococcus* spp. Alginate enhances *S. parasanguinis* biofilm formation by binding to BapA1. Whilst HAAs and MRL inhibit biofilm formation in *S. constellatus*. Siderophore hinders the *S. sanguinis* growth by sequestering the iron from the surrounding environment.

Graphical abstract: