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RESEARCH ARTICLE

The Role of Material Technologies Targeting *P. Aeruginosa* and *S. Aureus* Quorum Sensing in Biofilm Formation

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ABSTRACT
Periprosthetic joint infections are a major complication after total joint arthroplasty. *Pseudomonas aeruginosa* and *Staphylococcus aureus* are of great clinical concern, due to high antibiotic resistance, which is even increased when they form a biofilm on the implant surface. In this qualitative systematic review based on the PRISMA statement, biofilm formation of *S. aureus* and *P. aeruginosa* was studied, with a particular emphasis on the role of quorum sensing (QS). Treatment of infection by these bacteria by regulation or inhibition of the QS system via material technology was also reviewed. Pubmed, Google Scholar and Embase were searched. Articles were selected on their titles and use of the English language. All abstracts of the remaining articles were judged on the quality of the article, and fit with the scope of the review. This led to a selection of 62 articles, which consisted of 29 research articles and 33 reviews. In *P. aeruginosa* different QS systems are present in a hierarchal structure. These QS systems play an important role for formation of virulence factors and polymeric substances used in biofilm formation. In *S. aureus,* QS regulates biofilm dispersal, but not biofilm formation. Different treatments, either preventative by inhibiting biofilm formation or curative by biofilm dispersal are described in the literature. Combinations of QS inhibitors (QSI) and either antibiotics or metallic (nano)particles showed most promise in biofilm inhibition. QSI's and other biofilm inhibitors are only under preclinical investigation. Furthermore, QS has different functions in different bacteria, and treatment using QSI's are not suitable for every infection. While this makes QSI therapy unfit as an alternative to antibiotics, QSI's in combination with antibiotics can be a potent strategy to prevent biofilm formation.

Keywords: quorum sensing; biofilm; material technology; Pseudomonas aeruginosa; Staphylococcus aureus; periprosthetic joint infection

1 Introduction

Periprosthetic joint infection (PJI) is one of the major complications after joint arthroplasty, with an incidence of 1-2% for primary arthroplasties, up to and over 15% for revision arthroplasties.[1-4] PJI is associated with poor treatment outcome, high patient morbidity, and high recurrence rates.[5] The most commonly identified pathogens in PJI are bacteria of the *Staphylococcus* genus, predominantly *S. epidermidis* and *S. aureus*. Other frequently detected species are *Streptococcus* spp., *Enterococcus* spp., and Gram-negative bacteria, inter alia *Pseudomonas aeruginosa*.[6,7] Two bacteria are of particular concern in PJI; *P. aeruginosa* and *S. aureus,* with specific emphasis on methicillin resistant *S. aureus* (MRSA). *P. aeruginosa* is a Gram-negative rod bacterium, and a potential pathogen, that can lead to a multitude of infections. PJI's caused by *Pseudomonas aeruginosa*, although infrequently encountered, are characterized by their extreme treatment difficulty and associated poor treatment outcome.[7] *S. aureus* is a Gram-positive coccus and a commensal nasal and skin flora that can be found permanently on about 30% of the world's population.[8,9] Both bacteria are part of the so-called 'ESKAPE' pathogens, a group of bacteria with high resistance to antibiotics.[10] In a publication from 2017, the World Health Organization (WHO) also listed both these bacteria as among the highest priority for new antimicrobial therapy development.[11]

In addition to their high antibiotic resistance, the ability of these bacteria to form biofilms on implant surfaces, complicates their treatment.[12] A biofilm is a communal vessel containing extracellular polymeric substances (EPS), extracellular DNA (eDNA), small proteins and peptides, and one or more strains of bacteria.[13]

Biofilms protect bacteria from external forces, both mechanical and biological and the immune system of the host body. One of the major advantages for bacteria in a biofilm is their decreased susceptibility to antibiotics.[14] The minimal inhibitory concentration (MIC), the lowest concentration of an antibiotic that will inhibit the growth of a microorganism, can be up to a thousand-fold higher for bacteria in a biofilm compared to bacteria in a planktonic state.[15,16] Biofilms function as a physical barrier and consist of both cationic and anionic molecules, which hinder antibiotic penetration into the biofilm.[17] Furthermore, bacteria in a biofilm can express a physiological state of reduced metabolic activity, the so called persister state, making these bacteria less susceptible to antibiotics interacting in replicating bacteria.[18] There is also evidence that bacteria in the persister state can develop resistance to other types of antibiotics, making this subpopulation of bacteria a dangerous threat to human health.[19] Finally, bacteria in a biofilm show increased amounts of horizontal gene transfer, leading to transferring resistance genes like β-lactamases to susceptible bacteria.[20] These characteristics make treating biofilms with conventional antibiotics challenging and often lead to implant extraction and extensive debridement surgery.[21] New treatments that are effective against these bacteria and that do not lead to development of antimicrobial resistance are therefore urgently needed.[5]

One suggested new treatment strategy is to target the quorum sensing (QS) system of bacteria.[22] Quorum sensing is a complex and multi-faceted communication system used by bacteria, and is one of the underlying systems that regulates the formation and dispersion of biofilms in PJI.[23] Regulation or inhibition of QS systems via material technology might therefore be a possible alternative therapy for the treatment and prevention of PJI.[24]

In this review, three databases, Embase, Pubmed, and google Scholar, were systematically searched for information on *P. aeruginosa* and *S. aureus* with a focus on the role of QS systems and antibacterial treatments related to these systems. This review describes the general function of QS, as well as the specific functions in *P. aeruginosa* and *S. aureus.* Furthermore, a section on treatments of these bacteria, based both on disruption of QS systems as well as other techniques, has been written. In the discussion section, the findings were discussed, and future perspectives were given.

2 Materials and Methods

This review was performed in accordance with the PRISMA statement.[25] For this review all articles on the topic of quorum sensing, quorum quenching and biofilm in either *P. aeruginosa* or *S. aureus,* as well as anti-infectives for treatments of these bacteria were searched, from 2015 to the moment of the literature search, May 20th 2021, were considered eligible. No selection filters were used, other than use of English language, if possible. All publication statuses and forms were considered. Databases that were searched were PubMed, Google Scholar and Embase (Ovid search engine). The review focused on biofilm formation, in particular on the role of quorum sensing therein, of the bacteria *P. aeruginosa* and *S. aureus,* and the treatment and prevention of biofilm, either by interventions already used in the clinic or promising new

interventions. MeSH terms were found for the relevant terms, and these terms were altered to Emtree terms for the Embase search.
For the PubMed and Google scholar literature search, the following search string was used: "Quorum sensing" AND "biofilm formation" AND ((pseudomonas aeruginosa OR staphylococcus aureus) AND "anti-infective agents"). The Ovid search string can be found in **Table 1** below.
Using these search strings, 69 articles were found on PubMed, 423 on Google Scholar and 187 on Embase for a total of 679 articles. After removal of duplicates, 616 articles were left. 424 articles were omitted by screening their titles on relevancy to the review and poor/non-use of the English language. Abstracts of the 193 remaining articles were retrieved, for which 10 proved irretrievable. The remaining 183 abstracts were read and assessed on eligibility within the scope of the review. Assessment was initially was done by Max van de Voort (MvdV) and Raymond Bevers (RB) independently. In case of disagreement, a third reviewer, Chris Arts (CA), was consulted. This procedure led to a selection of 65 articles, of which 62 have been used to write this review. The 62 articles consisted of 29 research articles and 33 reviews. respectively (**Figure 1**). Data was collected from the report independently by MvdV by critical deduction of relevant information, after which the data was reviewed by RB. In addition, the review has been supplemented with 13 additional articles, based on the review of the references from the other selected papers.

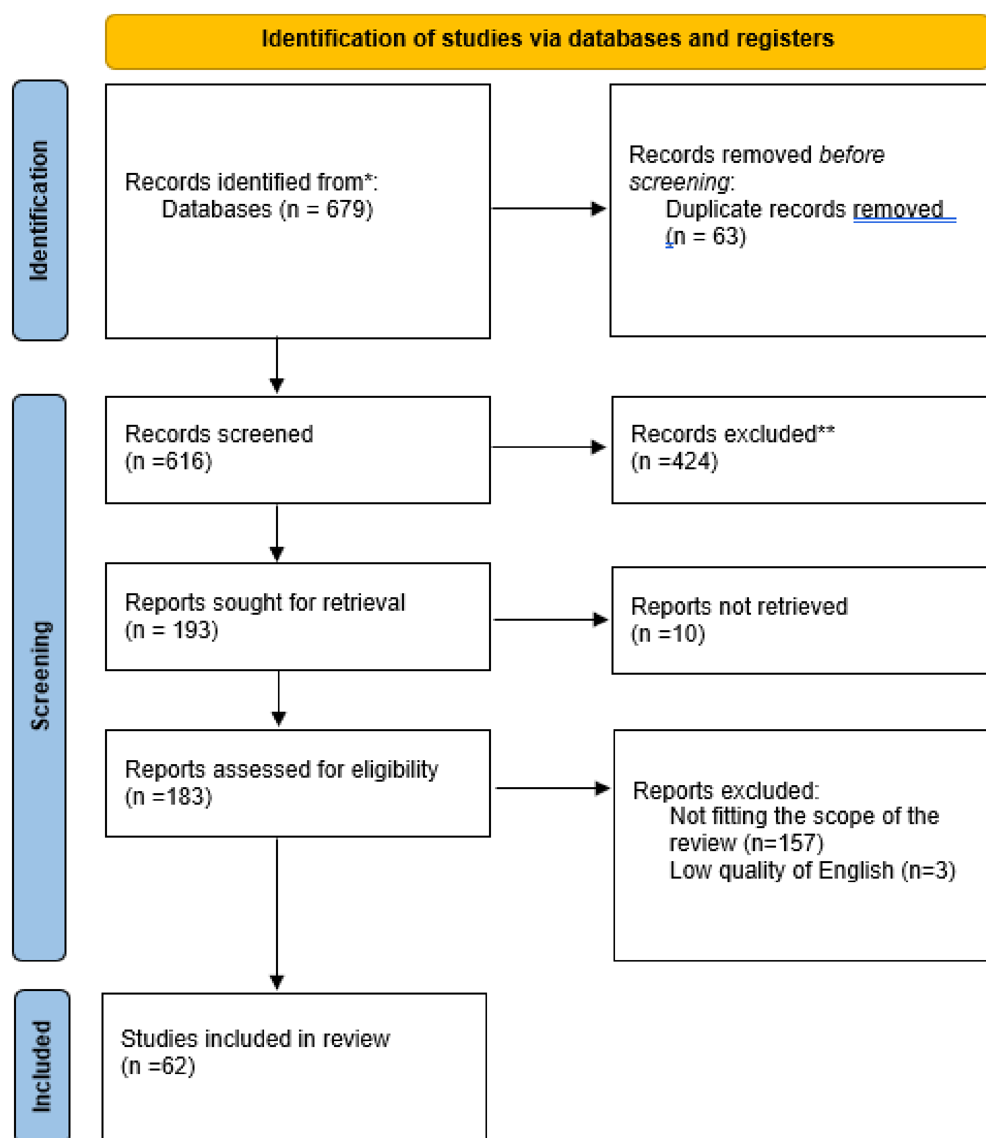

Figure 1: *PRISMA standardized flow diagram of article selection, modified from Page et al, 2021.*[25]

Table 1: the OVID Embase search string used to find articles, conform with the PRISMA statement.

Keyword number	keyword
1	exp quorum sensing/
2	('quorum sensing' or 'quorum quencing').ti,ab,kw.
3	1 or 2
4	exp biofilm/ or biofilm matrix/
5	(biofilm or 'biofilm matrix').ti,ab,kw.
6	4 or 5
7	Pseudomonas aeruginosa/
8	Staphylococcus aureus/
9	7 or 8
10	antiinfective agent/
11	3 and 6 and 9 and 10
12	limit 11 to English language

3 Biofilm formation and quorum sensing

Classical biofilm formation is divided in four steps: 1) reversible primary attachment to the carrier surface,[26] 2) irreversible adhesion to the biotic or abiotic surface, 3) maturation of the biofilm and 4) dispersion (**Figure 2**).[13,27] Preceding the first step of biofilm formation, preconditioning of the (a)biotic surface by bodily proteins is necessary for bacteria to attach, and can heavily influence the type of bacteria that attach.[28] During initial attachment of the microbe, the most important regulators are electrostatic and hydrodynamic forces. During the adhesion period, specific bacterial structures, like pili and flagella, as well as eukaryotic surface proteins like adhesins and collagens are most important.[29] The maturation of biofilm is dependent on signals received outside of the bacterial cell, stimulating processes like quorum sensing (QS), signaling molecules like cyclic-di-GMP and two component systems. In its mature form, biofilm is a complex multicellular colony, consisting of microcolonies of cells, which signal each other and different microcolonies in the biofilm to change the phenotype of cells, distribute nutrients as well as oxygen and necessary metal ions, and even to autolyze cells in the biofilm for formation of eDNA.[30,31] During dispersal, signaling systems will reach different thresholds, leading to release of planktonic cells from the biofilm to infect the body at a different location.[27,29,32]

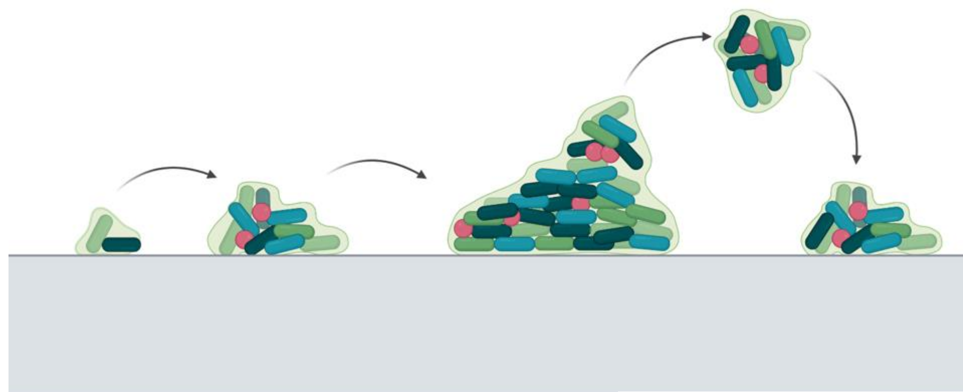

Figure 2: *The life cycle of a biofilm: following irreversible adhesion to the biotic or abiotic surface, the biofilm develops and matures, ultimately leading to dispersal and further colonization.*

One of the mechanisms behind biofilm formation is quorum sensing (QS). QS is both an inter- and intracellular communication tool for bacteria, which can activate genes regarding motility, biofilm formation, virulence factors formation and other genes important for the survival and spread of bacteria.[13,29,33] The key mechanism in QS is an autoinducer (AI) molecule, that is released by bacteria. When a certain number of bacteria in an area is attained, the AI threshold in this area is reached and gene effectors are activated. In many bacteria, these genes translate to formation of AIs, leading to a positive feedback loop .[34] There are three basic QS systems; one found in Gram positive bacteria, one found in Gram negative bacteria, and a third QS system that is based on autoinducer 2 (AI-2) and is found in both Gram positive and negative bacteria .[35] Combinations of more than one QS systems can be found in bacteria, as will be seen in *P. aeruginosa*. In Gram positive bacteria, peptide AIs are formed by the bacteria and released from the cell. These autoinducers bind to a cell surface bound kinase, which is phosphorylated and activates a response regulator. This regulator regulates downstream gene transcription, both affecting formation of new AI as well as other regulatory processes. This is called two-component system, an extensive family of regulatory mechanisms.[33,34] The Las QS system in *P. aeruginosa* pictured in **Figure 3** is part of this family. In Gram negative bacteria, the main AIs are N-acylhomoserine lactones (AHL), or similar structures.[36] The Agr system in **Figure 4** is an example of Gram negative QS systems. The most important components of these QS systems are autoinducer forming proteins, commonly indicated with the protein family name and ending with the letter I, and responder proteins, which end with letter R.[34]

3.1 Pseudomonas Aeruginosa

3.1.1 Biofilm formation

Biofilm formation in *P. aeruginosa* is initiated with the attachment phase. In this phase, lipopolysaccharide A and B are important molecules to change the hydrophilicity of the bacteria to attach to the (a)biotic surface.[37] Lipopolysaccharide A increases the hydrophobicity of the cell and therefore makes it easier to bind to hydrophobic surfaces, while lipopolysaccharide B has a hydrophilic effect. Psl, a polysaccharide also found in the later formed biofilm, forms a complex with CdrA, an external adhesin,[38] at the outer bacterial matrix to stabilize the matrix and create elasticity, leading to more favorable binding towards both biotic and abiotic surfaces [37]. After attachment, irreversible adhesion of *P. aeruginosa is* mediated by adhesins, most notably type IV pili and flagella.[26]

P. aeruginosa biofilm is formed from three types of EPS: pel, psl and alginate.[29] The structure of pel is not yet characterized but is believed to be very glucose rich.[39] The formation of pel is regulated by an inner membrane complex and is transported by a protein called pelf.[40] Because of its cationic characteristics, it plays a protective role against specific antibiotics like aminoglycosides.[41] Psl is composed of L-rhamnose, D-glucose and D-mannose repeats. Inside the cell, it enhances elasticity of the cell matrix, making it an important molecule for the primary attachment to both biotic and abiotic surfaces.[37] Without psl, viscosity of the cell matrix increases, leading to diffusion of the biofilm. Psl also is found to have effects on the growth of microcolonies, and on the differentiation of biofilm. In a mature biofilm psl is mainly found on the outside of the biofilm.[37] Pel and psl are used in different quantities by different strains of *P. aeruginosa*. In the highly virulent and major laboratory used strain PA01, research shows it uses mainly psl as an EPS, causing difficulties in attachment for *psl* PA01 mutant bacteria. However, a *psl* PA01 mutant would be able to form biofilm using pel. The PA14 strain uses pel as its main EPS, and a *pel* PA14 mutant had difficulties both attaching to a surface and was not able to form a full biofilm.[42,43] CdrA, although it binds mainly to psl in psl-heavy *P. aeruginosa* strains, can also bind to pel and other extrapolymeric substances.[38]

Alginate is a high weight D-mannuronic acid homomer, which receives O-acetyl substitution outside the bacterial cell, leading to an EPS consisting of β-1-4 glycosidic linked to α-L-guluronic acid and β-D-mannuronic acid. It is used in biofilm almost exclusively in mucoid phenotypes, for instance in cystic fibrosis patients, and therefore does not play a great role in nosocomial infections.[44] Alginate also acts as a virulence factor, recruiting host defense systems to form overabundant amounts of reactive oxygen species (ROS).[37] Furthermore, it protects *P. aeruginosa* against ROS and various antibiotics like tobramycin by creating a more well-ordered biofilm.[32,37] By attacking different host defense systems like neutrophils and macrophages, freeing eDNA for the biofilm in progress.[44] Alginate lyase can cut the long polymers into oligosaccharides, making it an

important protein in the dispersal of biofilm in the final stage of biofilm formation.[37]

eDNA is another important substance in biofilm. It is extracted both by autolysis of bacteria in the biofilm, as well as by destruction of host defense systems.[29] It can form a strong mesh with psl to further stabilize the integrity of the biofilm, as well as being an excellent protection against antibiotics because of its strong anionic charge. Furthermore, it forms a nutrient source for the bacteria in the biofilm as it is rich in amino acids.[29,37,45] In *P. aeruginosa*, eDNA can also transfer antibiotic resistant genes between bacteria, both of its own genus as well as other species. This causes all bacteria in the biofilm to become more resistant to antibiotics.[46]

3.1.2 Quorum sensing

Formation of biofilm is controlled by different communication systems found both inter and intracellularly. These communication systems are important for formation of the EPS, eDNA, and different virulence factors.[33,34] Quorum sensing in *P. aeruginosa* has a well-structured communication hierarchy consisting of four different QS systems, The Las, Rhl, *pseudomonas* quinolone signal (PQS) and integrated quinolone signal (IQS) systems.[29] The Las system is the highest QS system in the hierarchy. Its AI is 3-oxo-S12-homoserine lactone (HSL), which is formed by LasI and binds to LasR.[47] LasR then binds to regulate different parts of gene transcription, promoting formation of for instance psl and elastase, and inhibiting pel formation.[48] LasR also is the primary stimulator of siderophores, which are components in the structural integrity of biofilm, as they recruit iron for facilitating EPS cross binding by carboxyl groups.[49,50] LasR stimulates formation of both RhlI and RhlR, as well as stimulation of PQS and IQS. It is also proposed that active LasR blocks binding of RhlR with its AI, C4-HSL, leading to a negative control system (**Figure 3**).[33] The AI of the Las system, 3-oxo-S12-HSL, has its own effects on the host immune system, affecting different inflammation molecules.[33] LasI mutants have been shown to produce flat unstructured biofilms.[51]

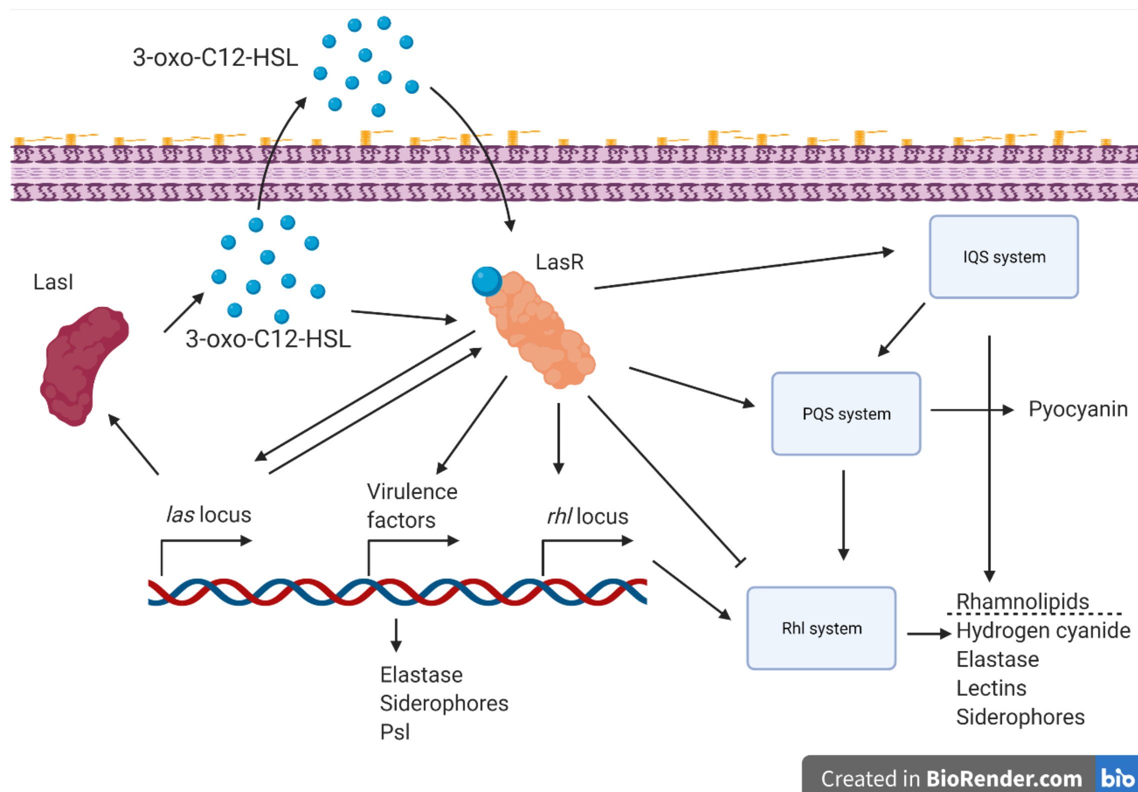

Figure 3: *the mechanics of the Las system and its effect on the other quorum sensing systems. 3-oxo-C12-HSL is formed by LasI and stabilizes LasR. LasR then can promote different genes for transcription of Las proteins, Rhl proteins and virulence factors. It further stimulates the IQS system and PQS system, while inhibiting the Rhl system. The virulence factors formed by the other QS systems are also given.*

The Rhl system is an important system for the formation of different virulence factors, mainly rhamnolipids, although it is also linked to the formation of pyocyanin, hydrogen cyanide, elastase, and lectins. Rhamnolipids are key factors in the late stages of biofilm. Rhamnolipids are formed by *P. aeruginosa* in response to detection of host defense cells and can lyse neutrophils and macrophages and scavenge their DNA.[29] Two types, mono- and dirhamnolipids, are produced by *P. aeruginosa,* and are important for the structure of the biofilm, which is described as mushroom like in late stages. Furthermore, rhamnolipids create an infrastructure to provide nutrients to the different microcolonies of the biofilm. In the dispersal phase, copious amounts of rhamnolipids are released for biofilm dispersal.[52] RhlI mutants, lacking primary formation of rhamnolipids, have shown up to 70% less biofilm formation as wild type *P. aeruginosa*.[53] both RhlR and LasR have been shown to affect the expression of AmpC, which is an important factor for formation of β-lactamases and thereby β-lactam resistance.[54]

The PQS system is found only in the *pseudomonas* class of bacteria. It is synthesized by the pqsABCDE and pqsH genes.[55] In *P. aeruginosa* it regulates over 90 genes, while also binding directly to hundreds of unidentified proteins.[55] Most importantly, PQS regulates the genes forming the virulence factor pyocyanin. The main contributors in the system are PQS itself as a signaling molecule, as well as its precursor 2-heptyl-4-quinolone (HHQ), which is 100 times less effective. HHQ is formed by PqsH, and both AI bind to PqsR.[56] The protein involved in processing this precursor into PQS is controlled by the Las system.[33] PQS is transported through the cell by outer membrane vesicles, and PQS stimulates formation of these vesicles.[32] The PQS system is a promoter of the Rhl system, and it is suggested that it is the main activator of the system, instead of the Las system.[56] A small RNA called phrS is linked to stimulation of the PQS system.[57] As phrS is only formed under oxygen rich circumstances, it is likely that the PQS system is regulated by oxygen availability.[32]

The integrated quorum sensing (IQS) system is a newly found QS, based around IQS, also known as 2-(2-hydroxylphenyl)-thiazole-4-carbaldehyde.[49] First claimed to be a product of the ambACDE genes, it is still debated what the origin of the molecule exactly is.[58] The IQS system is important in the formation of pyocyanin, a phenazine which is an important virulence factor for *P. aeruginosa*. Pyocyanin disrupts host defense by suppressing signaling by neutrophils, altering calcium ion signaling and blocking IL-2 release.[29] Furthermore, it can damage epithelial cells, and binding with eDNA will lead to formation of hydrogen peroxide, causing cell lysis of both host cells and bacterial cells for formation of more eDNA. The IQS system can also stimulate rhamnolipid production and is able to stimulate the PQS system, making it an alternative communication mechanism in bacteria with a Las mutation.[29]

3.1.3 Other regulators of biofilm formation

Another important mechanism in biofilm formation for *P. aeruginosa* is Bis-(3′-5′)-cyclic-dimeric-guanosine monophosphate (cyclic-di-GMP) formation and communication. Cyclic-di-GMP is formed by different diguanylate cyclases, which are activated through outside signals like nutrient levels, as well as inside signals like the Rhl system AI C4-HSL and psl. Cyclic-di-GMP is quickly degraded by phosphodiesterases and is therefore comparable with the cAMP in human cells.[59] Cyclic-di-GMP levels are a direct factor for the state of the bacteria, with low cyclic-di-GMP levels causing high motility and a planktonic lifestyle, for instance by stimulation of type IV pili formation,[44] and sudden drops in cyclic-di-GMP levels cause quick dispersal of the biofilm.[59] High levels of cyclic-di-GMP are key for biofilm formation by regulation of alginate, pel and psl formation. Formation of psl, which in turn stimulates cyclic-di-GMP formation, is a notable positive feedback loop which might be a possible mechanism for planktonic cells to become part of an existing biofilm.[27] Cyclic-di-GMP additionally is important during the attachment phase of *P. aeruginosa* biofilm formation, regulating formation of cup fimbriae and adhesins. Small colony variants, a phenotype of *P. aeruginosa* which are very hard to eradicate and are comparable to persister cells, are characterized by their very high levels of cyclic-di-GMP.[29,32,37]

Two component systems are a final key mechanism for the formation of biofilm in *P. aeruginosa*. The two-component system most important for biofilm formation is the GacS and GacA system.[60] GacS is a membrane bound receptor, which receives signals from outside the cell. Upon activation, it phosphorylates GacA. GacA regulates then promotes transcription of RmsZ and RmsY. These two small RNAs are inhibitors of a protein, RmsA, which normally inhibits different processes important for

the formation of biofilm. RmsA inhibits the formation of two AI, 3-oxo-s12-HSL and C4-HSL, and therefore lowers the activity of different QS systems.[29] RmsA also binds to the *pel* and *psl* operons, decreasing formation of the EPS.[37] GacS is further regulated by two hybrid sensor kinases, RetS and LadS. RetS can form a heterodimer with GacS, inhibiting autophosphorylation of GacS and therefore lowering biofilm formation. Contrarily, LadS can activate GacS and is therefore seen as a contributor to biofilm formation. This system built around GacS and GacA is proposed to be a switch for *P. aeruginosa* between an acute, more virulent infection called type III secretion system and a chronic, biofilm-based infection called type VI secretion system.[44] The trigger for activation of GacS, as well as RetS and LadS, has however not been found (**Figure 4**).[29] The Wsp two component system is used primarily by *P. aeruginosa* to limit biofilm formation, and achieve higher motility. The WspR protein regulates c-di-GMP formation, which will lead to downregulation of FleQ, which in turn downregulates the formation of EPS such as pel and psl.[60]

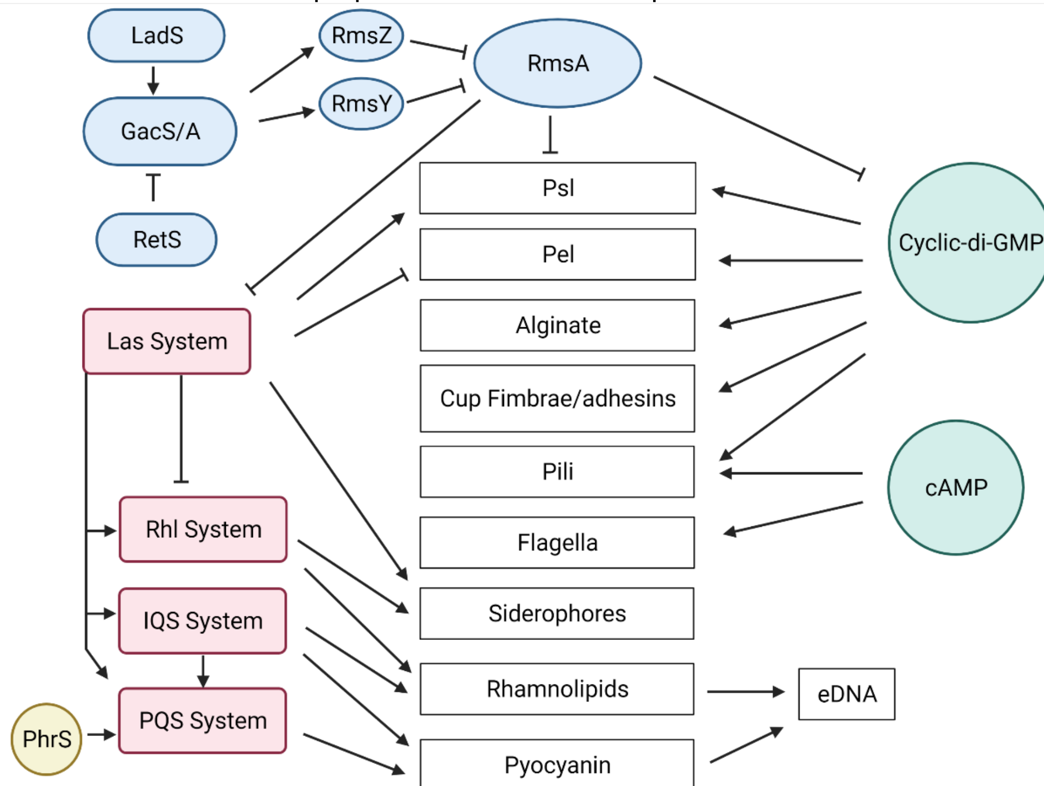

Figure 4: *The different systems playing a role in biofilm formation of* P. aeruginosa*. cAMP is important for the mobility of the bacteria, while cyclic-di-GMP stimulates formation of different biofilm structures in high concentrations. The QS systems are mostly important for formation of virulence factors. RmsA is an inhibitor of biofilm formation, but is itself inhibited by stimulation of GacS/A*

3.2 Staphylococcus aureus

3.2.1 Biofilm formation

Initial adhesion in *S. aureus* is regulated by the staphylococcal autolysin AtlA, which is vital for changing the hydrophobicity of the cell surface and acting as an adhesin.[61] AtlA deficient *S. aureus* has shown to have rough surfaces, and primary attachment on both hydrophobic and hydrophilic surfaces was decreased in methicillin sensitive *S. aureus* (MSSA). In methicillin resistant *S. aureus* (MRSA), only attachment on hydrophobic surfaces was impaired.[13] Teichoic acids, which are one of the main substances in the biofilm of *S. aureus*, also affect the initial attachment to a surface, by changing the net charge of the cell surface. After these non-specific adhesion interactions, the attachment of *S. aureus* is directed by microbial surface components recognizing adhesive matrix molecules (MSCRAMMs).[13] MSCRAMMs can bind to many matrix proteins, for instance adhesins, fibronectin and fibrinogen. These proteins are

readily available on biotic surfaces, but additionally colonize abiotic surfaces like catheters and implants in short amounts of time after entering the body. Teichoic acids furthermore play a role in the attachment by interacting with the epithelial cells.[8,13]

The biofilm of *S. aureus* is less developed than the *P. aeruginosa* biofilm and can be compared more to a slime layer consisting of bacteria, EPS, proteins and eDNA than a well-structured colony with communication and nutrient infrastructure. The most abundant EPS in the *S. aureus* biofilm is teichoic acid.[32] At least two different forms of *S. aureus* biofilms are found, both consisting of its own type of EPS. In the *ica*-dependent biofilm formation, polymeric *N*-acetyl-glucosamine (PNAG) is formed by the *ica*ADBC gene, which is regulated by the *ica*R gene.[62] The icaR gene itself is regulated by several factors. Staphylococcal accessory regulator (SarA) has been shown to affect both the regulation of *ica*R as well as upregulation of cell wall proteins and downregulation of proteases.[32] SarA mutants have been found to produce impaired biofilms, both ica-dependent and independent.[32] Alternative sigma factor B, part of alternative sigma factors which are found in many different bacteria, is linked to upregulation of *icaR* and regulation to over 250 genes, and *S. aureus* mutants lacking this factor have been shown to form a weaker biofilm and to upregulate the agr system, leading to dispersal of bacteria.[13,32,62] Rbf (regulator of biofilm formation) and *spx* are down regulators of icaR.[62]

The *ica*ADBC gene transcribes for four proteins that are important for formation of PNAG.[36] IcaA is a transmembrane protein that can form short saccharide chains of up to 20 residues in combination with IcaD. IcaC is responsible for crosslinking these short chains, as well as transporting these polymeric substances through the cell and possibly translocating them through the cell membrane. IcaB is a secreted protein, and deacetylates the PNAG to produce positively charged residues. This final product is called polysaccharide intercellular adhesion (PIA).[36] PIA is able to bind to eDNA forming a stronger mesh of biofilm.[13] PIA induction can happen both under aerobic and anaerobic conditions, the latter being induced by the SrrAB regulator.[32]

Some strains of *S. aureus,* like MRSA strains, however, do not depend on the *ica* gene to form biofilm, and the biofilm is more proteinaceous. In *ica*-independent biofilm formation, the main constituents are MSCRAMMs called fibronectin-binding proteins (FnBPs).[63] As the name suggests, these FnBPs primarily create a biofilm by binding to fibronectin, in combination with teichoic aids and eDNA. Two forms of FnBPs have been identified, named FnBPA and FnBPB.[63] Knock out versions of one of the proteins in bacteria were still able to form a similar biofilm as the wild type, suggesting that the FnBPA and FnBPB have indistinctive functions. Two membrane bound proteins, accumulation associated protein and biofilm associated protein, have been strongly linked to *ica*-independent biofilm formation as well.[13]

eDNA in *S. aureus* is predominately released by cell lysis of bacterial cells caused by holing homolog CidA and other proteins, like AtlA.[61] This cell lysis happens in all stages of biofilm formation, and eDNA is demonstrated to be important at early biofilm formation for cell attachment, as well as in late-stage biofilm formation, in which massive cell lysis takes place.[32,63] Like in *P. aeruginosa,* eDNA in *S. aureus* can transfer antibiotic resistance genes between bacteria in the biofilm.[46] Mature biofilm is furthermore shaped by so called phenol-soluble modulins, which can both be found in their solute state, in which it causes dispersal of the biofilm, as well as in a aggregated solid state, in which it stabilizes the biofilm structure.[63,64]

3.2.2 Quorum sensing

The predominant and most studied quorum sensing system in *S. aureus* is the accessory gene regulation (agr) system.[13,61] The agr system can regulate up to 150 genes, of which at least 16 are encoding for virulence factors.[13] The system consists of the autoinducer peptide (AIP) which is formed by AgrD and released from the bacteria by AgrB. AIP can bind to the transmembrane protein AgrC, which will phosphorylate and in turn phosphorylate AgrA. AgrA then can promote two different gene loci, RNAII and RNAIII.[33,34] RNAII is mostly responsible for forming different proteins found in the agr system. RNAIII produces the virulence factors, dispersal molecules like phenol-soluble modulin (PSM) surfactants, peptidases, and nucleases. RNAIII effector genes also regulate MSCRAMM formation (**figure 5**). Four allelic variants of AIP exist, which are competitive and are able to inhibit each other.[65] QS and its role in biofilm for *S. aureus* has been a discussion in the past.[51] Although the agr system has been shown to produce virulence factors and dispersal factors, it does not seem to produce any molecules in favour of biofilm formation. Biofilms in agr system knock outs were found to be thicker than

normal biofilm, and no problems in forming biofilm in those mutants was found. QS therefore is seen as a biofilm dispersal mechanism in *S. aureus.*[13,32,63]

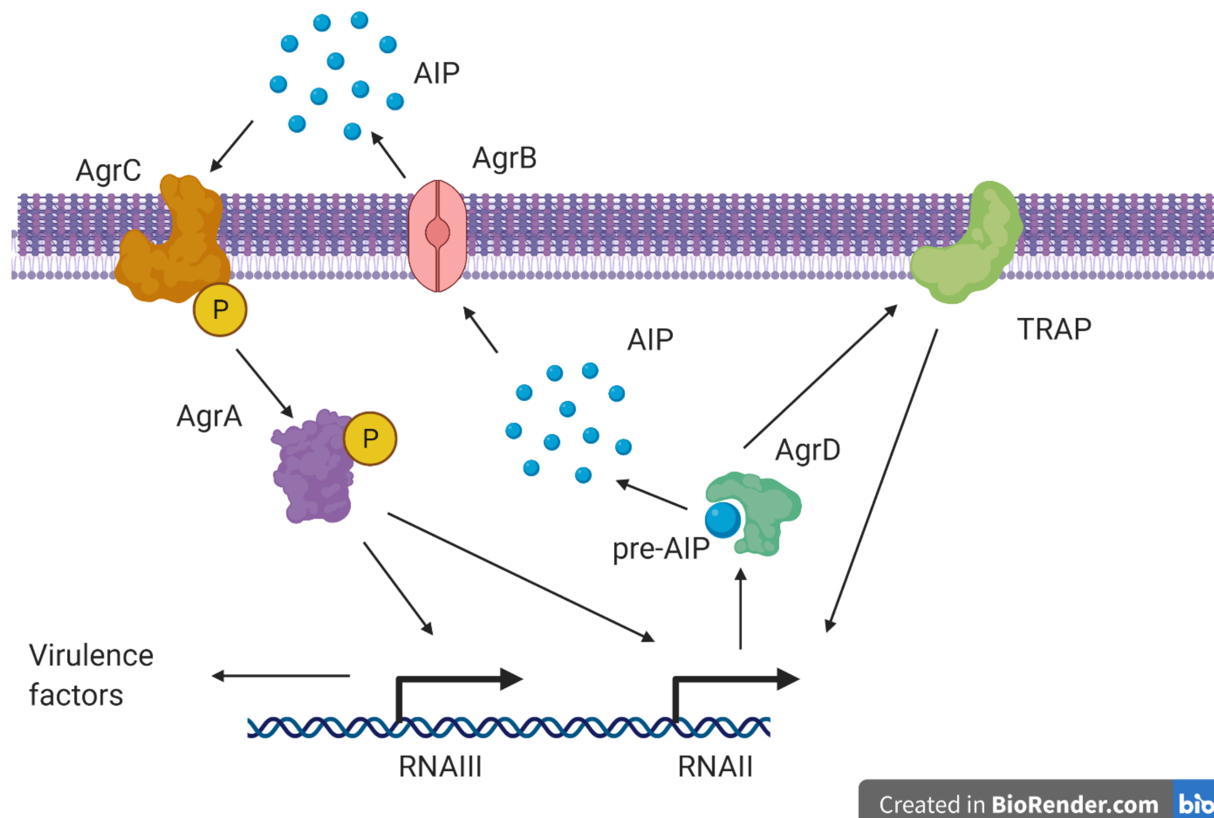

Figure 5: *The quorum sensing system of* S. aureus *based around autoinducing protein (AIP). AIP is formed by AgrD and released out of the cell by AgrB. AIP can then activate AgrC, which in turn phosphorylates AgrA which promotes different genes. TRAP receives outside signals and stimulates RNAII to form different agr proteins.*

3.2.3 Other components in biofilm formation regulation

The role of cyclic-di-GMP in biofilm formation is ambiguous. It was found in one strain of *S. aureus* that a protein called GdpS, which can affect the formation of biofilm, presented with the GGDEF domain, which is associated with cyclic-di-GMP activation. In another strain of *S. aureus,* the same effect of GdpS was observed, but cyclic-di-GMP did not seem to affect this protein, and therefore was not linked to biofilm formation.[32] Another small second messenger, cyclic-di-AMP, also seems to have some links to dispersal of biofilm formation, but no conclusive research has been performed.[63]

Two different two component systems have also been linked to biofilm formation. The Sae two component system, consisting of SaeS and SaeR, has been identified and hyperactivity of this system leads to inability of *S. aureus* to form robust biofilm.[63] The LytSR two component system, mostly essential for cell lysis and death of *S. aureus,* rationally affect the amount of eDNA released in biofilm formation, and therefore the strength of the biofilm.[63] A transcriptional repressor, CodY, likewise affects the formation of biofilm by repressing *ica* and *agr* proteins, SarA and other proteins, depending on the nutrient level in and around the biofilm. Nutrient levels are therefore predicted to be of great importance in the creation of biofilm.[63]

3.3 Treatment of biofilms

Treatment of bacterial infections involving biofilm is a great challenge, and development of new treatment plans is growing in the last years. In theory, one can divide the treatment of biofilm infections into two groups: antimicrobial and antifouling. In antimicrobial treatment, bacteria and their biofilm are combatted, either by bacteriostatic or bactericidal agents. Antibiotics and metal particles belong in this category. Antifouling

treatments are based on affecting biofilm formation itself, but not the bacteria. They can either be based on the inhibition of bacteria to adhere or attach to a surface, or to inhibit biofilm formation.[66] Quorum sensing inhibitors (QSIs) and quorum quenching enzymes (QQEs) are examples of this last category. The first disrupts QS pathways and therefore formation of biofilm, while the second are enzymes that inactivate specific signals.[47]

3.3.1 Antibiotics

The golden standard of treating bacterial biofilm infections is still by means of antibiotics. Macrolides, lincosamides, tetracyclines, rifampycins, quinolones, fusidic acid, nitroimidazole, sulfonamides and oxazolidinones are the most suitable antibiotics, and a combination of antibiotics is normally given. β-lactams [67] Broad spectrum antibiotics, like gentamycin, are a primary choice, especially because a biofilm can consist of multiple bacterial species, both Gram positive and negative.[30]

However, challenges arise when subinhibitory doses of antibiotics are reached, either admitted systematically or through local administration by a coating. Not only does this lead to bacterial resistance, but it has also been shown that subinhibitory doses have a strong pro-biofilm effect.[27] Translation inhibiting antibiotics for instance, can promote cyclic-di-GMP formation in bacteria, and antibiotics focused on inhibiting peptidoglycan synthesis can enhance polysaccharide and eDNA formation, for instance in *S. aureus*.[27] Because of the proven QSI effects of azithromycin, studies of sub-MIC levels of other antibiotics on bacterial growth have been done to find similar characteristics. A study adding sub-MIC levels of gentamycin and azithromycin showed both to have a antibiofilm and QS inhibiting effect on *P. aeruginosa,* inhibiting RhlR/I and LasR/I.[68] The antibiotic furvina, which is used as a dermal, topical antibiotic, was tested on its QS inhibiting characteristics. Although QSI characteristics were found, they were very close to MIC, and bacterial cell growth of *P. aeruginosa* was inhibited over 30% for concentration levels with significant QSI effects.[24] Sub-MIC levels of ciprofloxacin were given to 5 different strains of *P. aeruginosa* and around 30% lower biofilm formation was found. Virulence factors like rhamnolipids and protease were also lowered.[69] Sub-MIC levels of tobramycin, ceftriaxone, norfloxacin and ceftazidime lowered biofilm formation, but not significantly.[70] An *in vivo* murine model using azithromycin and ciprofloxacin was tested to find reduction of infection. Urinary catheters, either untreated or treated with both antibiotics were introduced in mice. Only mild levels of inflammation were found after seven days and onwards in azithromycin treated catheters, while untreated catheters showed high levels of inflammation. The researchers based this effect at least partly on the fact that the antibiotics had sub-MIC anti-QS effects.[71]

An intervention with tobramycin in combination with fumarate, a potentiator of tobramycin and a substance used to reawaken persister cells,[27] was used to try and kill persister cells of *P. aeruginosa*. Complete eradication of both the bacteria and biofilm was found in mucoid strains when adding 15 mM fumarate and 8 μg/mL or more tobramycin. However, planktonic persister cells were not at all more susceptible to the combination of tobramycin and fumarate compared to tobramycin alone. The researchers hypothesized that the combination was able to destroy persister cells as they formed, but not as they reawakened.[72]

3.3.2 Material technology: Metal ions and nanoparticles

Although metal ions can be toxic for bacteria and therefore detrimental for biofilm formation, safe levels of metals ions like iron, zinc, copper, and aluminum protect against erosion of biofilm. Sub-MIC levels of metal ions can therefore have a similar effect as sub-MIC levels of antibiotics, aiding bacteria and biofilm instead of destroying them.[50] Three copper complexes were tested on their QS inhibiting characteristics, based on their effects on the production of virulence factors. Alginate, pyocyanin and elastase were all significantly reduced by the copper complexes, but only at near MIC or MIC levels.[73] A cobalt complex $Co(HL)_2$, with the (E)-2-(2-(pyridin-2-ylmethylene)hydrazinyl)-4-(p-tolyl)thiazole (HL) as a coordination ligand, was found to have great effects on the Las system, blocking the system completely in under MIC levels. This led to biofilm reduction of over 50%. The docking analysis found that the complex itself did not have a strong binding towards LasR, but HL was found to be a stronger binder to LasR than natural AI 3-oxo-C12-HSL. The cobalt complex may be important for delivery to the active site however.[74] Gold nanorods, infused with a protease, were found to inhibit *S. aureus* growth for at least 48 hours, and biofilm formation was also severely inhibited. Combined with near infrared light, it decimated existing biofilm. These findings were not found with standard gold nanorods.[75] In a study where silver nanoparticles (AgNP) as well as copper

nanoparticles were tested, copper was found to effectively have zero effect on biofilm formation, while AgNPs were shown to have a strong antibiofilm effect. This effect was explained by the upregulation of RhlR, which might lead to dispersion of biofilm by rhamnolipids.[76] Two other articles proved the effect of AgNPs which were formed by using natural products to reduce cost and environmental damage, created from aqueous extracts from piper beetles and the fungus *Rhizopus arrhizus*.[77,78] AgNPs have been reported to affect various mechanisms of biofilm forming bacteria, such as inhibition of biofilm formation, destruction of biofilm and inhibition of bacterial adhesion.[79]

3.3.3 Material technology: Antimicrobial peptides

Antimicrobial peptides (AMPs) are molecules produced by animals, plants and other bacteria to gain as an innate immune response or as a competitive advantage against bacteria.[61,80] Three classes of AMPs exist, which are divided in whether they are post-translationally modified, and in size.[61] AMPs are easily modified by bioengineering and are therefore a very novel field which has gained a lot of interest as treatment for bacteria. Although AMPs have many different mechanisms of action, the most observed mechanism of action is by manipulating the formation of the bacterial cell membrane, because of their positive charge caused by lysine and arginine residues.[61,81] Although AMPs are still in pre-clinical development phases, some have demonstrated strong inhibition results against biofilm formation of biofilm, while not displaying high effectiveness against planktonic bacteria, and are therefore useful as a synergistic treatment in combination with bactericidal treatments.[82] Of note in this category, the AMP LL-37 has performed as an inhibitor of biofilm in *P. aeruginosa* and *S. aureus* as well as other bacteria.[82] Although LL-37 itself was found to be easily cleaved by endogenous enzymes, derivatives of this molecule have mitigated this problem.[81] Another AMP, nisin, is part of the antibiotic type of AMPs, which acts as a cell-wall biosynthesis inhibitor. This molecule interestingly acts on the same target molecule as vancomycin, the cell wall precursor lipid II.[61]

3.3.4 Other antimicrobials

A non-steroidal anti-inflammatory drug (NSAID), meloxicam, was tested in an *in vitro* cell culture for its QS inhibiting characteristics as NSAIDs were found earlier to have synergistic effects with antibiotics for treating infections. No effects on *P. aeruginosa* growth were found, but less biofilm formation was found at higher concentrations (7.81-31.25 µg/ml) of meloxicam. Furthermore, combination therapy of meloxicam with sub-MIC levels of tobramycin, ceftriaxone, norfloxacin, ofloxacin and ceftazidime showed synergistic effects. Especially ofloxacin with 31.25 µg/ml meloxicam led to a great reduction of biofilm formation.[70] Surfactin, a biosurfactant created by *Bacillus subtilis,* has been tested to inhibit the adhesion and biofilm formation of *S. aureus*. Both were inhibited, which was explained by the inhibition of *icaD* and *icaA* expression, as well as inhibition of alkali-soluble polysaccharide production. On a mature biofilm, surfactin did not have a significant effect.[83] Dispersin B and DNAase I, which can degrade PNAG and eDNA respectively, have been used to combat *S. aureus* biofilm formation.[84] DNAase I was also used as a coating, which almost completely inhibited adhesion of *S. aureus* and *P. aeruginosa* for up to 14 h.[66]

3.3.5 Material technology: Combination treatments

Combination of QSIs and antibiotics is a field that has been researched in depth. Curcumin has been combined with gentamycin and azithromycin to look for synergistic effects. In this study, all three treatments were given in sub-MIC levels, either separately or together. Especially the combination curcumin and azithromycin at 1/4 MIC lowered twitching, swarming and biofilm formation. The combination curcumin and gentamycin had a weaker, but still strongly significant effect. Both gentamycin and azithromycin alone had a stronger effect than curcumin alone.[68] A similar study tested curcumin in a combination with ceftazidime and ciprofloxacin. Twitching and swarming was lowered in all combinations, but only ceftazidime alone, as well as the combination of curcumin and ceftazidime had significant effects at 1/16 MIC. At 1/4 MIC, all treatments had effect, although the combination curcumin/ceftazidime had the most significant effect.[85] NPs from alginate, which has been shown to disrupt biofilm formation, were formed containing ciprofloxacin and the QSI ACNQ, which disrupts PQS signaling. The alginate NPs were shown to penetrate the biofilm better than standard polystyrene NPs, and the combination of antibiotic and QSI caused major cell death of *P. aeruginosa* in a mature biofilm.[86] *N*-(2-pyrimidyl)butanamide, a QSI nicknamed C11, was tested alone as well as in combination with different antibiotics. Alone, it was able to completely suppress biofilm formation in anaerobic conditions at a concentration of 8 µg/mL. At 1 µg/mL in combination with 4 µg/mL

Tobramycin, the same effects were found. 1 µg/mL C11 combined with 16 µg/mL colistin was able to completely suppress biofilm formation in both aerobic and anaerobic conditions. C11 was found to inhibit *lasR, lasI, rhlR* and *rhlI* expression.[53]

AgNPs have been combined with different QSIs to find a synergistic effect. In a study were a combined treatment of AgNPs and the QSI 4NPO was tested, significant effects were found with concentrations of 6.25 µg/mL of Ag and 4NPO, with complete inhibition at concentrations of either 50 µg/mL AgNPs or 200 µg/mL 4NPO.[87] In an article where curcumin NPs, AgNPs and a combination of those called AgSNPs were tested, complete inhibition of biofilm formation of both *S. aureus* and *P. aeruginosa* was found at concentrations of AgSNPs that led to non-significant inhibition with similar concentrations of the single substance NPs. Established biofilms could be reduced by up to 65% with AgSNPs containing 400 µg/mL curcumin and 50 µg/mL Ag. *S. aureus* biofilm was reduced more than *P. aeruginosa* biofilm.[88] A newly formed NP containing Ag and eugenol, an active product from the piper beetle, was able to inhibit formation of biofilm by almost 80% at 8 µg/mL without affecting bacterial growth. Virulence factors were severely inhibited as well. Docking assays showed that the NP was able to bind to LasI, LasR and PqsR, inhibiting QS in *P. aeruginosa*.[14]

3.3.6 Material technology: Antifouling treatments

Polyethylene glycol (PEG) is a hydrophilic polymer that has been extensively researched. It is theorized that repulsive electric forces, as well as compressed polymer chains create a tightly bound water layer, which is thermodynamically unfavorable to remove. Grafted on glass, PEG was able to reduce *P. aeruginosa* adhesion, but failed to do so with *S. epidermis* and *C. albicans*.[30] Polyurethane mixed PEG was able to reduce *S. aureus* adhesion, but not *P. aeruginosa* adhesion. Poly-zwitterionic materials, like PEG, form a tight water layer to block proteins and bacteria adhesion, but do so using ionic forces. They are more stable than PEG and have been found to prevent the adhesion of *P. aeruginosa* and *S. epidermis* on a surface. A third group of antifouling coatings, superhydrophobic coatings, were tested on titanium surfaces. These superhydrophobic coatings are normally created using hydrophobic compounds, organized in a particular nanostructure. The titanium surface was formed in this nanostructure using laser treatment and created a rough surface able to prevent adhesion of *P. aeruginosa,* but not of *S. aureus*. It is theorized that this is because of the spherical shape of the latter *versus* the rod shape of the former.[30] In a combination of adhesion and biofilm formation inhibition antifouling, grafting of Glycidyl methacrylate onto polyvinyl chloride (PVC) inhibited *S. aureus* from binding to the surface. Adding the QQE acylase on this surface caused *P. aeruginosa* to be unable to grow.[89]

4 Clinical Outlook and Future Perspective

In this review, a comprehensive literature overview was provided about biofilm formation and the role of quorum sensing (QS) for the bacteria *P. aeruginosa* and *S. aureus*. Furthermore, possible material technology treatments against biofilm formation and for biofilm eradication were reflected upon and their clinical viability was assessed and discussed. Biofilm formation in *P. aeruginosa* relies heavily on QS, making the QS system a very appealing target for treatment and prevention of biofilm formation. In *S. aureus* however, biofilm formation is independent of QS, but QS might be part of the mechanism of biofilm dispersal.[13,32]

Antibiotics have been used as a standard treatment for biofilms and are still seen as the golden standard.[30] This treatment poses a double threat of failure however. Exposure to sub-MIC levels of antibiotics of bacteria dwelling in a biofilm stimulates the development of antimicrobial tolerance and resistance, as well as even stronger biofilm formation.[27] It is of note that azithromycin has been shown to have QS inhibiting characteristics at sub-MIC levels, and other antibiotics, like gentamycin and ciprofloxacin seem to have similar effects on *P. aeruginosa* and *S. aureus,* based on the articles reviewed, though none of these have been tested for this purpose in clinical trials so far.[35] Only a handful of the range of QSIs found in literature have made it into human clinical trials, all of which were repurposed drugs, like azithromycin, and the anti-cancer drug 5-Fluoruracil.[35,44]

For the bacteria discussed in this review, one component found in both their respective biofilms is eDNA. eDNA plays a triple role in the biofilm; 1) as a cross-binding molecule to strengthen the biofilm mesh, 2) as direct protection against antibiotics due to its high anionic charge, and 3) as a source of Antibiotic Resistance Genes. For *P. aeruginosa,* it is also a source of nutrients for the bacteria. Deoxyribonuclease I (DNase I) has been proven to have an anti-biofilm effect, but no antibacterial effect, either on *P. aeruginosa* nor on *S. aureus*. In

the clinic, recombinant DNase I forms are used to treat cystic fibrosis patients.[82] DNase I also has been proven to work as a coating, although its activity decreased significantly after 14 to 24 hours.[20,30] DNase I or comparable substances might therefore be a viable treatment option.[84]

One confounding factor in the reviewed studies has been the confusion between anti-biofilm, anti-QS and antibacterial effects. In most studies the tested treatments were effective only at concentrations very close to MIC. This can lead to findings that can be misinterpreted as anti-biofilm or QS inhibiting, while in reality a bacteriostatic effect is observed.[24,69] This can be a problem as opposed to inhibiting biofilm formation this might lead to bacterial resistance.[44] In other studies, the results demonstrated a bactericidal effect, even though the researchers discussed an antibiofilm effect.[90]This misunderstanding of effects linked to inhibition of biofilm and QS complicates the search towards successful antibiofilm or anti-QS treatments.

Based on this review, it can be concluded that material technology, e.g. silver nanoparticles (AgNPs) can be a potent treatment by themself or in addition to traditional antibiotics. AgNPs have produced strong effects on both biofilm formation and eradication in different formulations. It should be noted however that AgNPs might be toxic to mammalian cells at concentrations as low as 2-5 µg/mL, although some claim viability of cells at 100 µg/mL still.[88] In the reviewed articles, the most potent treatments were all well above 2 µg/mL, although the combination treatment from Shah et al. did show significant effects at 2 µg/mL.[14] As these concentrations are all *in vitro*, it will be a challenge to treat patients with an effective dose while having a non-toxic concentration, and local application will be needed.

Antifouling implant surfaces are another method to combat biofilm formation, but have not been touched upon extensively in this review. An antifouling surface is a surface too hydrophobic or hydrophilic for bacteria to adhere and attach to. This could be an interesting method to prevent bacterial adhesion and subsequent infection.[30] The discussed antifouling treatments only inhibit adhesion and attachment of some particular species of bacteria. Creating an antifouling surface that is effective against all bacterial species is likely a difficult, if not impossible, task. Although such surfaces have shown to be effective *in vitro*, they have not been tested *in vivo*, and there their effectiveness, considering the complexity of host fluids, is unknown.[82] In paradox, surfaces that stop adhesion of bacteria might also prevent adhesion of host cells, which for most orthopedic implants is the exact opposite of what is required for osteointegration.

Although treatment results for combatting bacterial biofilm formation found in some articles seem formidable, it is of particular note that unless stated otherwise, all these results were found in *in vitro* experiments, most of which were under static conditions. This is in stark contrast to an *in vivo* setting, in which there is not only diffusion, but active mass transport through fluid flow. The published data of these potential treatments are very early stage and possibly not reproducible in a physiologically more relevant model, such as a dynamic *in vitro/ex vivo* bioreactor or an *in vivo* model.[35]

P. aeruginosa and *S. aureus* infection and subsequent biofilm formation pose a significant risk for joint arthroplasty patients. QSIs and similar treatments have been proposed for years as a possible alternative treatment for biofilm forming bacteria, particularly in the case of antibiotic resistant strains. However, in all these years, no great advancements have been made in this field, and most QSIs discussed in research are still at the *in vitro* stage, early proof of effect studies, and no QSI has been used clinically up to date.[47] Combined with the fact that biofilms can harbour different kinds of bacteria, which all have their own QS systems, it is unlikely that QSIs will be used in the clinics as an individual treatment, either as treatment for established biofilms or as inhibitors of biofilm formation. QSIs may still be a viable treatment, especially in combination with antibiotics or AgNPs, but research is still in an early stage, and it will take many years to bring these treatments to the clinic. Furthermore, QS has different functions in different bacteria, and treatments using QSI's may not be suitable for every infection.

Considering the upcoming epidemic of antimicrobial resistance, development of material technology to inhibit and eradicate different kinds of biofilms is essential and development of combination therapies of material technology treatments such as AgNP, treatments that eradicate eDNA, and /or antifouling surfaces will be needed in curbing antimicrobial resistance now and in the near future.

5 Conflict of Interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

6 Author Contributions

Raymond Bevers: Conceptualization, methodology, formal analysis, writing—original draft preparation, writing—review and editing, supervision, project administration, funding acquisition, Max van de Voort: Investigation, formal analysis, writing—original draft preparation, visualization, Jacobus Arts: Conceptualization, methodology, validation, writing—review and editing, supervision, funding acquisition, Jan Geurts: validation, writing—review and editing, Inge van Loo: validation, writing—review and editing. All authors have read and agreed to the published version of the manuscript.

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8 References

1. Aggarwal VK, Rasouli MR, Parvizi J. Periprosthetic joint infection: Current concept. *Indian J Orthop*. Jan 2013;47(1):10-7. doi:10.4103/0019-5413.106884
2. Izakovicova P, Borens O, Trampuz A. Periprosthetic joint infection: current concepts and outlook. *EFORT Open Rev*. Jul 2019;4(7):482-494. doi:10.1302/2058-5241.4.180092
3. Tande AJ, Patel R. Prosthetic joint infection. *Clin Microbiol Rev*. Apr 2014;27(2):302-45. doi:10.1128/cmr.00111-13
4. Goud AL, Harlianto NI, Ezzafzafi S, Veltman ES, Bekkers JEJ, van der Wal BCH. Reinfection rates after one- and two-stage revision surgery for hip and knee arthroplasty: a systematic review and meta-analysis. *Archives of Orthopaedic and Trauma Surgery*. 2021/09/30 2021;doi:10.1007/s00402-021-04190-7
5. Zardi EM, Franceschi F. Prosthetic joint infection. A relevant public health issue. *J Infect Public Health*. Dec 2020;13(12):1888-1891. doi:10.1016/j.jiph.2020.09.006
6. Signore A, Sconfienza LM, Borens O, et al. Consensus document for the diagnosis of prosthetic joint infections: a joint paper by the EANM, EBJIS, and ESR (with ESCMID endorsement). *Eur J Nucl Med Mol Imaging*. Apr 2019;46(4):971-988. doi:10.1007/s00259-019-4263-9
7. Shah NB, Osmon DR, Steckelberg JM, et al. Pseudomonas Prosthetic Joint Infections: A Review of 102 Episodes. *J Bone Jt Infect*. 2016;1:25-30. doi:10.7150/jbji.15722
8. Craft KM, Nguyen JM, Berg LJ, Townsend SD. Methicillin-resistant Staphylococcus aureus (MRSA): antibiotic-resistance and the biofilm phenotype. 10.1039/C9MD00044E. *MedChemComm*. 2019;10(8):1231-1241. doi:10.1039/C9MD00044E
9. Totté JE, van der Feltz WT, Bode LG, van Belkum A, van Zuuren EJ, Pasmans SG. A systematic review and meta-analysis on Staphylococcus aureus carriage in psoriasis, acne and rosacea. *Eur J Clin Microbiol Infect Dis*. Jul 2016;35(7):1069-77. doi:10.1007/s10096-016-2647-3
10. Pendleton JN, Gorman SP, Gilmore BF. Clinical relevance of the ESKAPE pathogens. *Expert Rev Anti Infect Ther*. Mar 2013;11(3):297-308. doi:10.1586/eri.13.12
11. Tacconelli E, Carrara E, Savoldi A, et al. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect Dis*. Mar 2018;18(3):318-327. doi:10.1016/s1473-3099(17)30753-3
12. Zimmerli W, Sendi P. Orthopaedic biofilm infections. *Apmis*. Apr 2017;125(4):353-364. doi:10.1111/apm.12687
13. Hogan S, Stevens NT, Humphreys H, O'Gara JP, O'Neill E. Current and future approaches to the prevention and treatment of staphylococcal medical device-related infections. *Current Pharmaceutical Design*. 01 Dec 2015;21(1):100-113.
14. Shah S, Gaikwad S, Nagar S, et al. Biofilm inhibition and anti-quorum sensing activity of phytosynthesized silver nanoparticles against the nosocomial pathogen Pseudomonas aeruginosa. *Biofouling*. Jan 2019;35(1):34-49. doi:10.1080/08927014.2018.1563686
15. Stewart PS. Mechanisms of antibiotic resistance in bacterial biofilms. *Int J Med Microbiol*. Jul 2002;292(2):107-13. doi:10.1078/1438-4221-00196
16. Andrews JM. Determination of minimum inhibitory concentrations. *J Antimicrob Chemother*. Jul 2001;48 Suppl 1:5-16. doi:10.1093/jac/48.suppl_1.5
17. Dincer S, Uslu F, Delik A. Antibiotic Resistance in Biofilm. 2020.
18. Kaldalu N, Hauryliuk V, Tenson T. Persisters—as elusive as ever. *Applied Microbiology and Biotechnology*. 2016/08/01

2016;100(15):6545-6553. doi:10.1007/s00253-016-7648-8
19. Barrett TC, Mok WWK, Murawski AM, Brynildsen MP. Enhanced antibiotic resistance development from fluoroquinolone persisters after a single exposure to antibiotic. *Nature Communications*. 2019/03/12 2019;10(1):1177. doi:10.1038/s41467-019-09058-4
20. Ivanova K, Ramon E, Hoyo J, Tzanov T. Innovative Approaches for Controlling Clinically Relevant Biofilms: Current Trends and Future Prospects. *Current Topics in Medicinal Chemistry*. // 2017;17(17):1889-1914.
21. Tande AJ, Gomez-Urena EO, Berbari EF, Osmon DR. Management of Prosthetic Joint Infection. *Infect Dis Clin North Am*. Jun 2017;31(2):237-252. doi:10.1016/j.idc.2017.01.009
22. Ricciardi BF, Muthukrishnan G, Masters EA, Kaplan N, Daiss JL, Schwarz EM. New developments and future challenges in prevention, diagnosis, and treatment of prosthetic joint infection. *J Orthop Res*. Jul 2020;38(7):1423-1435. doi:10.1002/jor.24595
23. Mukherjee S, Bassler BL. Bacterial quorum sensing in complex and dynamically changing environments. *Nat Rev Microbiol*. Jun 2019;17(6):371-382. doi:10.1038/s41579-019-0186-5
24. Borges A, Sousa P, Gaspar A, Vilar S, Borges F, Simões M. Furvina inhibits the 3-oxo-C12-HSL-based quorum sensing system of Pseudomonas aeruginosa and QS-dependent phenotypes. *Biofouling*. 2017;33(2):156-168. doi:10.1080/08927014.2017.1280732
25. Page MJ, McKenzie JE, Bossuyt PM, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. *Bmj*. Mar 29 2021;372:n71. doi:10.1136/bmj.n71
26. Garrett TR, Bhakoo M, Zhang Z. Bacterial adhesion and biofilms on surfaces. *Progress in Natural Science*. 2008/09/10/ 2008;18(9):1049-1056. doi:https://doi.org/10.1016/j.pnsc.2008.04.001
27. Plakunov VK, Mart'Yanov SV, Teteneva NA, Zhurina MV. Controlling of microbial biofilms formation: Anti- and probiofilm agents. *Microbiology*. 2017;86(4):423-438. doi:10.1134/s0026261717040129
28. Dutta Sinha S, Chatterjee S, Maiti PK, Tarafdar S, Moulik SP. Evaluation of the role of substrate and albumin on Pseudomonas aeruginosa biofilm morphology through FESEM and FTIR studies on polymeric biomaterials. *Prog Biomater*. 2017;6(1-2):27-38. doi:10.1007/s40204-017-0061-2
29. Jakobsen T, Tolker-Nielsen T, Givskov M. Bacterial Biofilm Control by Perturbation of Bacterial Signaling Processes. *International Journal of Molecular Sciences*. 2017;18(9):1970. doi:10.3390/ijms18091970
30. Francolini I, Vuotto C, Piozzi A, Donelli G. Antifouling and antimicrobial biomaterials: an overview. *APMIS*. 2017;125(4):392-417. doi:10.1111/apm.12675
31. Galarraga-Vinueza ME, Mesquita-Guimarães J, Magini RS, Souza JCM, Fredel MC, Boccaccini AR. Anti-biofilm properties of bioactive glasses embedding organic active compounds. *Journal of Biomedical Materials Research Part A*. 2017;105(2):672-679. doi:10.1002/jbm.a.35934
32. Wolska KI, Grudniak AM, Rudnicka Z, Markowska K. Genetic control of bacterial biofilms. *Journal of applied genetics*. 2016;57(2):225-238.
33. Haque S, Yadav DK, Bisht SC, et al. Quorum sensing pathways in Gram-positive and -negative bacteria: potential of their interruption in abating drug resistance. *Journal of Chemotherapy*. 2019/05/19 2019;31(4):161-187. doi:10.1080/1120009X.2019.1599175
34. Haque S, Ahmad F, Dar SA, et al. Developments in strategies for Quorum Sensing virulence factor inhibition to combat bacterial drug resistance. *Microbial Pathogenesis*. 2018;121:293-302. doi:10.1016/j.micpath.2018.05.046
35. Rémy B, Mion S, Plener L, Elias M, Chabrière E, Daudé D. Interference in bacterial quorum sensing: a biopharmaceutical perspective. *Frontiers in pharmacology*. 2018;9:203.
36. Venkatesan N, Perumal G, Doble M. Bacterial resistance in biofilm-associated bacteria. Review. *Future Microbiology*. November 2015;10(11):1743-1750.
37. Skariyachan S, Sridhar VS, Packirisamy S, Kumargowda ST, Challapilli SB. Recent perspectives on the molecular basis of biofilm formation by Pseudomonas aeruginosa and approaches for treatment and biofilm dispersal. *Folia Microbiologica*. 2018;63(4):413-432. doi:10.1007/s12223-018-0585-4
38. Reichhardt C, Jacobs HM, Matwichuk M, Wong C, Wozniak DJ, Parsek MR. The Versatile Pseudomonas aeruginosa Biofilm Matrix Protein CdrA Promotes Aggregation through Different Extracellular Exopolysaccharide Interactions. *J Bacteriol*. 2020;202(19):e00216-20. doi:10.1128/JB.00216-20
39. Jennings LK, Storek KM, Ledvina HE, et al. Pel is a cationic exopolysaccharide that cross-links

extracellular DNA in the Pseudomonas aeruginosa biofilm matrix. *Proceedings of the National Academy of Sciences*. 2015;112(36):11353. doi:10.1073/pnas.1503058112

40. Whitfield GB, Marmont LS, Ostaszewski A, et al. Pel Polysaccharide Biosynthesis Requires an Inner Membrane Complex Comprised of PelD, PelE, PelF, and PelG. *J Bacteriol*. Mar 26 2020;202(8)doi:10.1128/jb.00684-19

41. Khan W, Bernier SP, Kuchma SL, Hammond JH, Hasan F, O'Toole GA. Aminoglycoside resistance of Pseudomonas aeruginosa biofilms modulated by extracellular polysaccharide. *Int Microbiol*. Dec 2010;13(4):207-12. doi:10.2436/20.1501.01.127

42. Colvin KM, Gordon VD, Murakami K, et al. The Pel Polysaccharide Can Serve a Structural and Protective Role in the Biofilm Matrix of Pseudomonas aeruginosa. *PLoS Pathogens*. 2011;7(1):e1001264. doi:10.1371/journal.ppat.1001264

43. Colvin KM, Irie Y, Tart CS, et al. The Pel and Psl polysaccharides provide Pseudomonas aeruginosa structural redundancy within the biofilm matrix. 2012;14(8):1913-1928. doi:10.1111/j.1462-2920.2011.02657.x

44. Rasamiravaka T, Labtani Q, Duez P, El Jaziri M. The Formation of Biofilms byPseudomonas aeruginosa: A Review of the Natural and Synthetic Compounds Interfering with Control Mechanisms. *BioMed Research International*. 2015;2015:1-17. doi:10.1155/2015/759348

45. Miquel S, Lagrafeuille R, Souweine B, Forestier C. Anti-biofilm Activity as a Health Issue. Review. *Frontiers in Microbiology*. 2016-April-26 2016;7(592)doi:10.3389/fmicb.2016.00592

46. Bernier SP, Surette MG. Concentration-dependent activity of antibiotics in natural environments. *Frontiers in microbiology*. 2013;4:20.

47. Fong J, Zhang C, Yang R, et al. Combination Therapy Strategy of Quorum Quenching Enzyme and Quorum Sensing Inhibitor in Suppressing Multiple Quorum Sensing Pathways of P. aeruginosa. *Scientific reports*. 18 Jan 2018;8(1):1155.

48. Magalhães AP, Jorge P, Pereira MO. Pseudomonas aeruginosa and Staphylococcus aureus communication in biofilm infections: insights through network and database construction. *Critical Reviews in Microbiology*. 2019;45(5-6):712-728. doi:10.1080/1040841x.2019.1700209

49. Pérez-Pérez M, Jorge P, Pérez Rodríguez G, Pereira MO, Lourenço A. Quorum sensing inhibition in Pseudomonas aeruginosa biofilms: new insights through network mining. 2017:1-15. doi:10.1080/08927014.2016.1272104

50. Cândido EDS, De Barros E, Cardoso MH, Franco OL. Bacterial cross-resistance to anti-infective compounds. Is it a real problem? *Current Opinion in Pharmacology*. 2019;48:76-81. doi:10.1016/j.coph.2019.05.004

51. Brackman G, Coenye T. Quorum sensing inhibitors as anti-biofilm agents. *Current Pharmaceutical Design*. 01 Dec 2015;21(1):5-11.

52. Proctor CR, McCarron PA, Ternan NG. Furanone quorum-sensing inhibitors with potential as novel therapeutics against Pseudomonas aeruginosa. Review. *Journal of Medical Microbiology*. 2020;69(2):195-206.

53. Furiga A, Lajoie B, El Hage S, Baziard G, Roques C. Impairment of Pseudomonas aeruginosa Biofilm Resistance to Antibiotics by Combining the Drugs with a New Quorum-Sensing Inhibitor. *Antimicrob Agents Chemother*. Dec 28 2015;60(3):1676-86. doi:10.1128/aac.02533-15

54. Zhao J, Jiang H, Cheng W, et al. The role of quorum sensing system in antimicrobial inducedampCexpression inPseudomonas aeruginosabiofilm. *Journal of Basic Microbiology*. 2015;55(5):671-678. doi:10.1002/jobm.201300987

55. Lin J, Cheng J, Wang Y, Shen X. The Pseudomonas Quinolone Signal (PQS): Not Just for Quorum Sensing Anymore. *Front Cell Infect Microbiol*. 2018;8:230. doi:10.3389/fcimb.2018.00230

56. Soheili V, Tajani AS, Ghodsi R, Bazzaz BSF. Anti-PqsR compounds as next-generation antibacterial agents against Pseudomonas aeruginosa: A review. Review. *European Journal of Medicinal Chemistry*. 15 June 2019;172:26-35.

57. Sonnleitner E, Gonzalez N, Sorger-Domenigg T, et al. The small RNA PhrS stimulates synthesis of the Pseudomonas aeruginosa quinolone signal. *Molecular microbiology*. 03/01 2011;80:868-85. doi:10.1111/j.1365-2958.2011.07620.x

58. Cornelis P. Putting an end to the Pseudomonas aeruginosa IQS controversy. *MicrobiologyOpen*. 2020/02/01 2020;9(2):e962. doi:10.1002/mbo3.962

59. Caly DL, Bellini D, A. Walsh M, Maxwell Dow J, P. Ryan R. Targeting Cyclic di-GMP Signalling: A Strategy to Control Biofilm Formation? *Current Pharmaceutical Design*. // 2015;21(1):12-24.

60. Francis VI, Stevenson EC, Porter SL. Two-component systems required for virulence in

Pseudomonas aeruginosa. *FEMS Microbiol Lett*. Jun 15 2017;364(11)doi:10.1093/femsle/fnx104
61. Kranjec C, Morales Angeles D, Torrissen Mårli M, et al. Staphylococcal biofilms: Challenges and novel therapeutic perspectives. *Antibiotics*. 2021;10(2):131.
62. Parastan R, Kargar M, Solhjoo K, Kafilzadeh F. Staphylococcus aureus biofilms: Structures, antibiotic resistance, inhibition, and vaccines. *Gene Reports*. 2020:100739.
63. Schilcher K, Horswill AR. Staphylococcal Biofilm Development: Structure, Regulation, and Treatment Strategies. Review. *Microbiology and Molecular Biology Reviews*. 2020;84(3)e00026-19.
64. Graf AC, Leonard A, Schäuble M, et al. Virulence factors produced by Staphylococcus aureus biofilms have a moonlighting function contributing to biofilm integrity. *Molecular & Cellular Proteomics*. 2019;18(6):1036-1053.
65. Kim MK, Zhao A, Wang A, et al. Surface-attached molecules control Staphylococcus aureus quorum sensing and biofilm development. *Nature microbiology*. 2017;2(8):1-12.
66. Li X, Wu B, Chen H, et al. Recent developments in smart antibacterial surfaces to inhibit biofilm formation and bacterial infections. 10.1039/C8TB01245H. *Journal of Materials Chemistry B*. 2018;6(26):4274-4292. doi:10.1039/C8TB01245H
67. Wu H, Moser C, Wang H-Z, Høiby N, Song Z-J. Strategies for combating bacterial biofilm infections. *International journal of oral science*. 2015;7(1):1.
68. Bahari S, Zeighami H, Mirshahabi H, Roudashti S, Haghi F. Inhibition of Pseudomonas aeruginosa quorum sensing by subinhibitory concentrations of curcumin with gentamicin and azithromycin. *Journal of Global Antimicrobial Resistance*. 2017;10:21-28. doi:10.1016/j.jgar.2017.03.006
69. Gupta P, Chhibber S, Harjai K. Subinhibitory concentration of ciprofloxacin targets quorum sensing system of Pseudomonas aeruginosa causing inhibition of biofilm formation & reduction of virulence. *Indian J Med Res*. May 2016;143(5):643-51. doi:10.4103/0971-5916.187114
70. She P, Wang Y, Luo Z, et al. Meloxicam inhibits biofilm formation and enhances antimicrobial agents efficacy by Pseudomonas aeruginosa. *MicrobiologyOpen*. 2018;7(1):e00545. doi:10.1002/mbo3.545
71. Saini H, Vadekeetil A, Chhibber S, Harjai K. Azithromycin-Ciprofloxacin-Impregnated Urinary Catheters Avert Bacterial Colonization, Biofilm Formation, and Inflammation in a Murine Model of Foreign-Body-Associated Urinary Tract Infections Caused by Pseudomonas aeruginosa. *Antimicrob Agents Chemother*. Mar 2017;61(3)doi:10.1128/aac.01906-16
72. Koeva M, Gutu AD, Hebert W, et al. An Antipersister Strategy for Treatment of Chronic Pseudomonas aeruginosa Infections. *Antimicrobial Agents and Chemotherapy*. 2017;61(12):e00987-17. doi:10.1128/AAC.00987-17
73. Al-Shabib NA, Husain FM, Khan RA, et al. Interference of phosphane copper (I) complexes of β-carboline with quorum sensing regulated virulence functions and biofilm in foodborne pathogenic bacteria: A first report. *Saudi Journal of Biological Sciences*. 2019;26(2):308-316.
74. Borges A, Simões M, Todorović T, Filipović N, García-Sosa A. Cobalt Complex with Thiazole-Based Ligand as New Pseudomonas aeruginosa Quorum Quencher, Biofilm Inhibitor and Virulence Attenuator. *Molecules*. 2018;23(6):1385. doi:10.3390/molecules23061385
75. Li W, Geng X, Liu D, Li Z. Near-Infrared Light-Enhanced Protease-Conjugated Gold Nanorods As A Photothermal Antimicrobial Agent For Elimination Of Exotoxin And Biofilms. *Int J Nanomedicine*. 2019;14:8047-8058. doi:10.2147/ijn.S212750
76. Gholamrezazadeh M, Shakibaie MR, Monirzadeh F, Masoumi S, Hashemizadeh Z. Effect of nano-silver, nano-copper, deconex and benzalkonium chloride on biofilm formation and expression of transcription regulatory quorum sensing gene (rh1 R) in drug-resistance Pseudomonas aeruginosa burn isolates. *Burns*. 2018;44(3):700-708. doi:10.1016/j.burns.2017.10.021
77. Singh BR, Singh BN, Singh A, Khan W, Naqvi AH, Singh HB. Mycofabricated biosilver nanoparticles interrupt Pseudomonas aeruginosa quorum sensing systems. *Sci Rep*. Sep 8 2015;5:13719. doi:10.1038/srep13719
78. Srinivasan R, Vigneshwari L, Rajavel T, et al. Biogenic synthesis of silver nanoparticles using Piper betle aqueous extract and evaluation of its anti-quorum sensing and antibiofilm potential against uropathogens with cytotoxic effects: an in vitro and in vivo approach. *Environ Sci Pollut Res Int*. Apr 2018;25(11):10538-10554. doi:10.1007/s11356-017-1049-0
79. Zhang Y, Pan X, Liao S, et al. Quantitative Proteomics Reveals the Mechanism of Silver Nanoparticles against Multidrug-Resistant Pseudomonas aeruginosa Biofilms. *Journal of*

Proteome Research. 07 Aug 2020;19(8):3109-3122.
80. Belete TM. Novel targets to develop new antibacterial agents and novel alternatives to antibacterial agents. *Human Microbiome Journal*. 2019;11:100052. doi:10.1016/j.humic.2019.01.001
81. Galdiero E, Lombardi L, Falanga A, Libralato G, Guida M, Carotenuto R. Biofilms: Novel Strategies Based on Antimicrobial Peptides. *Pharmaceutics*. 2019;11(7):322. doi:10.3390/pharmaceutics11070322
82. Vuotto C, Donelli G. Novel Treatment Strategies for Biofilm-Based Infections. *Drugs*. 2019/10/01 2019;79(15):1635-1655. doi:10.1007/s40265-019-01184-z
83. Liu J, Li W, Zhu X, et al. Surfactin effectively inhibits Staphylococcus aureus adhesion and biofilm formation on surfaces. *Applied Microbiology and Biotechnology*. 2019;103(11):4565-4574. doi:10.1007/s00253-019-09808-w
84. Jorge P, Alves D, Pereira MO. Catalysing the way towards antimicrobial effectiveness: A systematic analysis and a new online resource for antimicrobial–enzyme combinations against Pseudomonas aeruginosa and Staphylococcus aureus. *International Journal of Antimicrobial Agents*. 2019;53(5):598-605. doi:10.1016/j.ijantimicag.2019.01.001
85. Roudashti S, Zeighami H, Mirshahabi H, Bahari S, Soltani A, Haghi F. Synergistic activity of sub-inhibitory concentrations of curcumin with ceftazidime and ciprofloxacin against Pseudomonas aeruginosa quorum sensing related genes and virulence traits. 2017;33(3)doi:10.1007/s11274-016-2195-0
86. Singh N, Romero M, Travanut A, et al. Dual bioresponsive antibiotic and quorum sensing inhibitor combination nanoparticles for treatment of Pseudomonas aeruginosa biofilms in vitro and ex vivo. *Biomater Sci*. Sep 24 2019;7(10):4099-4111. doi:10.1039/c9bm00773c
87. Liu L, Li J-H, Zi S-F, et al. AgNP combined with quorum sensing inhibitor increased the antibiofilm effect on Pseudomonas aeruginosa. *Applied Microbiology and Biotechnology*. 2019;103(15):6195-6204. doi:10.1007/s00253-019-09905-w
88. Loo CY, Rohanizadeh R, Young PM, et al. Combination of Silver Nanoparticles and Curcumin Nanoparticles for Enhanced Anti-biofilm Activities. *Journal of agricultural and food chemistry*. 30 Mar 2016;64(12):2513-2522.
89. Costoya A, Velazquez Becerra LE, Melendez-Ortiz HI, et al. Immobilization of antimicrobial and anti-quorum sensing enzymes onto GMA-grafted poly(vinyl chloride) catheters. *Int J Pharm*. Mar 10 2019;558:72-81. doi:10.1016/j.ijpharm.2018.12.075
90. Singh VK, Mishra A, Jha B. 3-benzyl-hexahydro-pyrrolo[1,2-a]pyrazine-1,4-dione extracted from exiguobacterium indicum showed anti-biofilm activity against pseudomonas aeruginosa by attenuating quorum sensing. *Frontiers in Microbiology*. 2019;10(JUN)1269.