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The Effect of Quorum-Sensing and Efflux Pumps Interactions in *Pseudomonas aeruginosa* Against Photooxidative Stress

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Abstract
Resistant infections essentially cause mortality in a burn unit. Several bacteria contribute to burn infections; among these, *Pseudomonas aeruginosa* majorly contributes to these infections revealing significant drug resistance. Similar to other bacteria, *P. aeruginosa* reveals various mechanisms to attain highest pathogenicity and resistance; among these, efflux pumps and quorum sensing are crucial. Quorum sensing enables effective communication between bacteria and synchronizes their gene expression resulting in optimum effect of the secreted proteins; alternatively, efflux pumps increase the bacterial resistance by pumping out the antimicrobial factors as well as the QS signals and precursors. Of recent, increasing episodes of drug resistance led to new findings and approaches for killing pathogenic bacteria without inducing the drug-resistant species. Photodynamic therapy (PDT), considered as an adjuvant and innovative method for conventional antibiotic therapy, is a photochemical reaction that includes visible light, oxygen, and a photosensitizer (PS). In this therapy, after exposure to visible light, the PS generates reactive oxygen species (ROS) that are bacteriostatic or bactericidal. Furthermore, this oxidative stress can disrupt the coordination of gene expression and alter the bacterial behavior. Considering the fact that the adaption and several gene expression patterns of microorganisms within the biofilm make them notably resistant to the recent antimicrobial treatments, this study aimed to emphasize the relationship between the efflux pump and QS under oxidative stress and their role in *P. aeruginosa's* reaction to PDT.
Keywords: Antimicrobial photodynamic therapy; *Pseudomonas aeruginosa*; Quorum sensing.

Introduction

Treating burn wounds represents one of the most recent crucial issues. Considering the human body mechanism in efficient tissue repair and in preventing infections, during early hours after an injury, it is observed that about 70% of the mortality rate occurs due to resistant infection during the subsequent days after the injury.[1]

Pseudomonas aeruginosa is an opportunistic gram-negative bacterium belonging to the gamma-proteobacteria order, which is one of the main causes of infection and biofilm formation in immunocompromized and cystic fibrosis patients, in patients with bed wounds, and also in infections due to medical instruments such as catheters.[2] Known to be crucial in burn wounds, this bacterium possesses high instinct resistance toward several antibiotics and can achieve acquired resistance shortly after the antibiotic therapy. Besides, it is essential in the formation of drug-resistant biofilm. According to Centers for Disease Control and Prevention (CDC) reports, *P. aeruginosa* is placed among the top ten resistant bacteria worldwide. Its high resistance and pathogenicity are essentially due to the function of quorum sensing (QS) and efflux pumps.[2]

Quorum Sensing

Communication between similar or different species occurs via various chemical signals produced and secreted by the bacteria. These signals can be population dependent (QS) or can be secreted during several proliferation stages (e.g., indole, which is secreted in the stationary phase by *Escherichia coli*). The main difference between releasing signals in different proliferation stages and QS is that the QS signal is controlled by the increase in the population density.[3] Initially, in the 1970s, it was found that in a Gram-negative and marine bacterium *Vibrio fischeri*, light production was controlled by population density. In 1994 it was proven that a threshold density is necessary for intracellular recognition of the autoinducer (AI) signals.

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The name *quorum* was coined for threshold density and *quorum sensing* for this respective phenomenon. After *V. fischeri,* QS was studied in several gram-positive and gram-negative bacteria, and it was discovered that population density-dependent phenotype changes help bacterial growth during continuous environmental changes.[4-7]

The QS system of *V. fischeri* is considered as a model system for several Gram-negative bacteria. This system comprises 2 regulatory proteins, LuxR and LuxI. LuxI produces autoinducer signal and LuxR recognizes the signal.

LuxR type proteins have 2 separate domains; the one present in the N-terminal binds to the ligands (LBD), whereas the other present in the C-terminal binds to DNA (DBD). In the absence of AI, the majority of the LuxR protein domains fold inappropriately and degrade instantly (if they do not bind to their specific ligand); however, after binding to the ligand, AI and LuxR complex stabilizes. Ligand binding results in a conformational change, which allows DBD to bind to the promoter and initiates gene transcription. In all Lux homologs, AI reveals similar homoserine lactone structure with different length alkyl chains.[8-11] Two types of Lux protein families have been identified in *P. aeruginosa,* LasI/LasR and RhlI/RhlR, which are able to recognize (OdDHL) 3oxoC12HSL and C4HSL as AI. These systems control the expression of several genes involved in the pathogenicity and biofilm formation, such as exotoxin A, rhamnolipid, pyocyanin, alkaline phosphatase, and elastase, and are crucial in regulating more than 300 genes. In addition, PQS and IQS systems were identified as acyl homoserine lactone (AHL) independent QS systems in *P. aeruginosa*. Each of the 4 systems is responsible for inter-hierarchy regulation. LasR is on the top, which regulates several genes including *lasI*/*R*, *rhl I*/*R*, *pqs* R, and *pqs*ABCDH (genes in PQS system) through AHL. RhlI reveals similar effect as LasR and regulates the expression of *las* and *pqs* and along with self-regulation, after binding to C_4HSL. PQS system regulates its own genes and also feedbacks the active Rhl which connects the Rhl, Las, and PQS signaling systems.

Despite RhlR being the key component of QS in *P. aeruginosa* after binding of C4HSL to RhlR, another AI should also be involved in pathogenicity. In wild species, the other system is Las; however, in clinical species, Las mutant has been found. Phosphate starvation protein Pho B compensates the Las activities by activating IQS. IQS leads to *pqs* expression, thus, preparing AI for *rhl* expression. These system and interactions make these bacteria immune to the Las mutation.[12-16]

Efflux Pumps

Efflux pumps are transporter proteins which are located in cytoplasmic membrane and exert compounds. They use ATP (active transport) or can act as secondary active transporters or antiports and use proton motif force and/or sodium motif force as an energy source.[17]

All the bacterial efflux pumps presently known belong to these 6 major families, the last group being newly described:

1- ATP-binding cassette (ABC)
2- Major facilitator superfamily (MFS)
3- Division resistance nodulation cell division (RND)
4- Small multidrug resistance (SMR)
5- Multidrug and toxic compound extrusion (MATE)
6- Proteobacterial antimicrobial compound efflux (PACE)[17,18]

The Role of Efflux Pumps in Antibiotic Resistance

Efflux pumps can reduce the intracellular drug concentration and block the antibiotics in reaching their targets in the microbial cell; hence, bacteria only experience subinhibitory levels of antibiotics, which could trigger other resistance mechanisms such as change in the structure of antibiotics targets due to mutation or enzymatic deactivation of antibiotics.[19] Efflux pumps work in 3 levels of resistance. First, they make the bacteria intrinsically resistant by expression in the basal level. Second, overexpression of pumps causes acquired resistance of mutant strains. Third, in strains that grow under stress, temporary overexpression of pumps would cause resistance.[20,21]

RND efflux pumps are crucial in intrinsic and acquired resistance in *P. aeruginosa*. Twelve pump-encoding operons have been discovered in the genome of this bacterium.[22,23] These pumps are genetically and structurally similar; however, they are different in their substrate specificity and regulation.[24] Efflux pumps in *P. aeruginosa* contribute to reduced susceptibility toward most antibiotics and antibacterial agents.[25] The majorly studied pump of this bacterium is MexAB-OprM, which constitutes the widest spectrum of substrates among all bacterial effluxes like quinolones, tetracycline, chloramphenicol, trimethoprim, beta-lactam, beta-lactamase inhibitors, macrolides, azithromycin, colistin, detergents, and dyes. Other pumps involved in drug resistance are OprJ: quinolones and erythromycin, MexEF-OprN: chloramphenicol and quinolones, and Mex XY: quinolones and erythromycin.[26]

Studies on the efflux pumps of *P. aeruginosa* have not proven the exact role of these pumps in biofilm resistance; however, the expression of these pumps in biofilms is heterogeneous and cells located in the substratum reveal maximum levels of expression. Biofilm populations demonstrate several expressions due to their specific physiochemical conditions.[27] Additionally, researchers indicate that MexAB-OprM and MexCD-OprJ are essential for biofilm formation in the presence of antibiotics such as azithromycin, ciprofloxacin, colistin, and aminoglycosides.[19] MexCD-OprJ is crucial in the resistance of azithromycin and MexAB-OprM

is responsible for colistin resistance. Furthermore, planktonic cells of *P. aeruginosa* reveal overexpression of MexEF-OprN in hypoxic conditions.[27] Recently, PA1874–PA1877 is reported as a new efflux pump system involved in biofilm resistance. The expression level of PA1874 was 10-fold higher in biofilm formation than in planktonic cells, and deletion of this operon leads to changes in the biofilm susceptibility pattern to tobramycin, whereas its overexpression leads to decreased susceptibility of aminoglycosides and fluoroquinolones in the planktonic cells.[28]

Stewart et al, in 2 studies, demonstrated that efflux pumps have no effect on the biofilm resistance in *P. aeruginosa*; these studies, unlike others, reported that efflux pumps are responsible for antibiotic resistance only in the planktonic cells and their protection role in biofilm presumably depends on the physiological condition of the biofilm or the presence of specific subpopulations that take advantage of efflux expression. Moreover, regulatory effects of the efflux pumps on biofilm could be strain and condition dependent.[29,30]

In general, little data is available on the regulation of efflux pump expression in biofilms; however, Liao et al reported that presumably there exists a relation between BrlR (Mer type transcriptional factor) and efflux pump expression in biofilms. BrlR responds to the concentration of the second messenger c-diGMP and changes the expression of several genes. In addition, Liao et al demonstrated that BrlR is required for the maximum expression of MexAB-OprM and MexEF-OprN in biofilms of *P. aeruginosa*. BrlR binds to the operons of MexAB-OprM and MexEF-OprN and directly regulates their expression.[31]

Studies regarding the resistance process of antibiotic therapy against biofilm formation in burn wound infections report that inactive cells in the deeper layers of biofilms (persister cells) are resistant to a wide range of antibiotics, which target several biological processes like protein synthesis, cell division, and cell wall formation. Studies indicate that efflux pumps are responsible for the "active defense" of persisters.[32] Colistin from polymixins family, which is an effective antibiotic against a majority of the Gram-negative bacteria and acts as the last line defense in resistant infections like *P. aeruginosa, Acinetobacter baumanii, Klebsiella pneumonia,* and Enterobacteriaceae, reveals its efficiency on the persister cells in the deep layers of biofilms. The gene of resistance for colistin was initially reported in 2016,[33] and a study reported that MexAB-oprM pumps possess an active role in causing resistance in the deep layers of biofilms.[34] Moreover, this pump in cooperation with MexCD-OprJ is responsible for macrolides and azithromycin resistance against biofilms.

In general, utilizing the antiefflux pumps such as PABN, carbonyl cyanide, and chloropropharin, decrease the biofilm formation; however, the molecular mechanism is elusive.[35]

The Role of Efflux Pumps in Pathogenicity

Efflux pumps were initially presented as a resistance mechanism in *E. coli* in 1980.[36] It is assumed that considering the conserved genes of these systems among different strains and their variety of substrates,[17] other than their antibiotic resistance, these may also be physiologically essential.[18] A study reveals that detoxification of intercellular metabolites, pathogenicity in plant and animal hosts, homeostasis, and trafficking of intracellular signals are certain physiological roles of efflux pumps.[37]

Minimum pathogenicity by efflux pumps is observed in MexA mutant strains. In addition, the strains with overexpressed pumps are not pathogenic; hence, appropriate efflux pump expression is necessary for complete pathogenicity.

As aforementioned, efflux pumps are used in intracellular signaling. A group of AI can freely pass the bacterial membrane; however, several others including PQS and OdDHL, due to their hydrophobic nature, must be secreted through pumps or membrane vesicules.[34] Expression of MexAB-OprM is not controlled by QS; however, studies report that these efflux pumps specify the LasR-signal binding by exerting 3oxoCnHSL and do not allow the signals of other species in mixed cultures to transfer the inappropriate message. Additionally, this mechanism may act as a bacterial defense system during the utilization of QS inhibitors.[38,39]

OdDHL is a MexAB-OprM substrate[40] and mutants of this pump are unable to transport this AI; hence, it results in decreased expression of Las regulated virulence factors.[41] MexEF-OprN and MexGHI play an indirect role in QS by secreting anthranilate, which is a toxic metabolite and a PQS precursor.[42] C_4-homoserine lactone (C4HSL) overexpresses MexAB-OprM. Due to the interaction between QS systems, mutation in any of these systems would result in less pathogenicity and QS.[17]

Alternatively, several strains isolated from clinics and environment are QS mutants. Hence, the inability of the bacteria in QS might be advantageous. These strains are generally signal blind, which indicates that they can produce AI but cannot respond to it. This might be a result of the energy consumption. Signal production would utilize 0.01% of the cell energy, whereas signal response uses 5% of the cell energy.[43] This ability of the cell to produce and respond to the signal and to decide whether to respond in a particular situation is due to efflux. Efflux pump blocks the QS response by increasing the secretion of AI or its precursors and enables the bacteria to quickly adapt.

The General Relationship Between Efflux Pump and QS

1- Overexpression of MexAB-OprM results in increased secretion of antibiotics and OdDHL, which increases the resistance and decreases the expression of the

virulence factors controlled by QS.[38,44]

2- Expression of MexAB-OprM is induced by C4HSL, which results in more antibiotic resistance and OdDHL-LasR binding specificity and additional control of the gene expression regulated by QS.[45,39]
3- Lack of MexAB-OprM pump results in the accumulation of OdDHL in the cell and limits cell-cell communication.[38]
4- The overexpression of MexEF-OprN and production of virulence factors controlled by Las or Rhl are interlinked.[24]

Antimicrobial Photodynamic Therapy

Due to the increasing level of drug resistance, finding new therapeutic approaches and alternatives or supplementary drugs besides antibiotics remains the core priority of scientists worldwide. Photodynamic therapy (PDT is widely used for cancer treatment. It is also used for benign conditions like age-related macular degeneration or other dermatological applications.[46,47] Additionally, PDT is increasingly used as a therapeutic approach for infectious diseases.[46] When pathogens are targeted in PDT, it is referred to as photodynamic inactivation (PDI) or antimicrobial PDT (aPDT). Initially, during the commencement of the 20th century, the potential of PDT as an antimicrobial therapy was revealed when Moan and Peng studied the efficiency of acridine orange in killing paramecia in the presence of light.[48] During aPDT, the cellular structures and biomolecules are destroyed due to unspecific reactions. A nontoxic dye is used in aPDT as photosensitizer (PS) that acts as photoactive drugs, collectively with visible light on the appropriate wavelength to excite the PS. The excited PS can produce reactive oxygen species (ROS) such as hydroxyl radical and singlet oxygen that are responsible for killing the cell by energy/electrons transfer to the ground state in the presence of molecular oxygen.[49] These excited oxygen molecules are unstable; thus, they lose their energy by transpiration of light (fluorescence) or heat production to form a structure known as triplet state that lasts for microseconds, which in comparison to nanoseconds for the excited singlet, is more stable. In type II photochemical reaction, the energy transfer reaction eventually forms a singlet oxygen ($^1O_2$).[50] This ROS is produced inside or outside the bacterial cell and can cause bacterial cell death either by damaging the cell membrane or deoxyribonucleic acid (DNA). The light wavelength could differ with due attention to the PS structure. As different families of PSs are used for different bacteria, several infections are studied to find the appropriate PS for each pathogen. The perfect PS structure for aPDT would differ from the anticancer PSs; in antimicrobial aPDT, we prefer PSs with cationic charges, in particular; to target the Gram-negative bacteria the more charge of PS is desirable.[50] In 1990, studies reported cationic PS like porphyrins,[51] phenothiazinium,[52] and phthalocyanines,[53] which stimulated quick and immense light killing of gram-negative bacteria such as *E. coli* and *P. aeruginosa* on top of PDT of gram-positive bacteria and fungi (For a detailed review of the photosensitizer families, refer to reference 49).

Although cancer therapy was the chief target for PDT from 1970 to 2010, recently, with the alarming rate of drug-resistant pathogens, aPDT is considered as the antibacterial approach.[50] Few advantages of aPDT are: (1) it is board-spectrum and is effective for a wide range of targets such as both Gram-positive and Gram-negative bacteria, fungi, protozoa and even inactive viruses. (2) There is a poor possibility of developing photoresistant species even after multiple PDTs. (3) We could target aPDT with selectivity for pathogens to avoid side effect on host tissues. (4) Less chance of inducing mutagenic effect exists. (5) aPDT is faster than antibiotic therapy, requiring only a few minutes to kill the pathogens, which is in contrast to antibiotics that may require days or even weeks to achieve maximum effects. (6) Studies reported that aPDT can be effective in biofilm infections where antibiotics are generally ineffective. (7) aPDT is affordable and inexpensive.[54]

Pseudomonas aeruginosa uses enzymes like catalase and superoxide dismutase or its green-blue pigment (pyocyanin), which are partly controlled by QS, to evade the harmful effect of aPDT. Furthermore, efflux pumps can decline the effect of aPDT by extruding PS. In 2005 and 2008, 2 different studies initially reported that PS from phenothiazinium family like toluidine blue, which is widely used in aPDT, is a substrate for multidrug-resistant pumps in *P. aeruginosa, Staphylococcus aureus,* and *E. coli*.[55,56] Another study on biofilms of *Enterococcus faecalis* indicated that the presence of efflux pumps inhibitors can increase the efficiency of aPDT with methylene blue.[57]

5-aminolevulinic acid (5-ALA) is a precursor of porphyrin that is produced in the bacterial cell during heme synthesis and its intracellular accumulation can result in light sensitivity and ROS production; recently, ALA and protoporphyrin are used as PSs in aPDT for superficial infections. In 2014, a survey on *E. coli* reported that use of efflux pumps inhibitors, collectively with an iron-chelating agent, could significantly increase the efficacy of protoporphyrin-IX-mediated aPDT.[58] Another study on the virulence factor expression in *S. aureus* [59] demonstrated that oxidative damage caused by aPDT can alter the expression level of certain proteins such as functional proteins involved in cell division, metabolic activities, oxidative stress response, and sugar uptakes. A study on the effect of sub-lethal PDT (sPDT) with methylene-blue on virulence factors of *A. baumanii* indicates that sPDT increases the accumulation of efflux pumps and also decreases the expression of specific genes such as *epsA* (membrane protein responsible for polysaccharides extrusion), *csuE* (adhesion involved in biofilm formation), *abaI* (AHL-producing signal, essential for QS and biofilm development).[60] Alternatively, a recent

study on *V. harvey* reported that low dose of laser not only is bactericidal but also has a positive effect on growth and increases QS.[61]

Conclusion and Future Directions

In conclusion, *P. aeruginosa* is the chief bacteria involved in a life-threatening infection in burn patient wound; its ability to form biofilms that can adhere to both biotic and abiotic surfaces make this bacterium resistant to almost all the antibiotics and therapeutic approaches. Therefore, it is essential to find novel efficient methods to tackle drug resistance in *P. aeruginosa*'s biofilm-related infections.[62-64] QS and efflux pumps are the major mechanisms for pathogenicity and resistance of *P. aeruginosa*. These systems, in addition to their response to the extracellular environment, interact together and affect each other's functions. aPDT as an adjuvant method, besides antibiotics, received undivided attention during the last decade.

aPDT, in addition to killing the major pathogenic population, can alter the expression pattern in a limited population. This phenomenon could either upset the balance between systems and decrease the bacterial pathogenicity or increase the bacterial pathogenicity and make treatment even more difficult. Based on the review of literature referred in this study, we studied the importance of *P. aeruginosa* in infections and intercellular connections of QS and efflux pumps in *P. aeruginosa* and their effect on resistance and their reaction to aPDT. Improvement of this therapeutic approach and its applications in clinical cases of burn wounds requires specific cellular research on interactions of bacterial systems during aPDT treatment.

Ethical Considerations

Not applicable.

Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this paper.

References

1. Naqvi A, Zulfiqar S, Abbas Naqvi SM, Usman M, Naqvi S, Baqar S. Burn wound infection; significance of rule of nine in microbial surveillance. *Professional Medical Journal*. 2014;21(5):869-73
2. Agnihotri N, Gupta V, Joshi RM. Aerobic bacterial isolates from burn wound infections and their antibiograms--a five-year study. *Burns*. 2004;30(3):241-243. doi:10.1016/j.burns.2003.11.010
3. Jayaraman A, Wood TK. Bacterial quorum sensing: signals, circuits, and implications for biofilms and disease. *Annu Rev Biomed Eng*. 2008;10:145-167. doi:10.1146/annurev.bioeng.10.061807.160536
4. Fuqua WC, Winans SC, Greenberg EP. Quorum sensing in bacteria: the LuxR-LuxI family of cell density-responsive transcriptional regulators. *J Bacteriol*. 1994;176(2):269-275.
5. Waters CM, Bassler BL. Quorum sensing: cell-to-cell communication in bacteria. *Annu Rev Cell Dev Biol*. 2005;21:319-346. doi:10.1146/annurev.cellbio.21.012704.131001
6. Hawver LA, Jung SA, Ng WL. Specificity and complexity in bacterial quorum-sensing systems. FEMS Microbiol Rev. 2016;40(5):738-752. doi:10.1093/femsre/fuw014
7. Antunes LC, Ferreira RB, Buckner MM, Finlay BB. Quorum sensing in bacterial virulence. *Microbiology*. 2010;156(Pt 8):2271-2282. doi:10.1099/mic.0.038794-0
8. Papenfort K, Bassler BL. Quorum sensing signal-response systems in Gram-negative bacteria. Nat Rev Microbiol. 2016;14(9):576-588. doi:10.1038/nrmicro.2016.89
9. Bassler BL. How bacteria talk to each other: regulation of gene expression by quorum sensing. *Curr Opin Microbiol*. 1999;2(6):582-587.
10. Ng WL, Bassler BL. Bacterial quorum-sensing network architectures. *Annu Rev Genet*. 2009;43:197-222. doi:10.1146/annurev-genet-102108-134304
11. Li YH, Tian X. Quorum sensing and bacterial social interactions in biofilms. *Sensors (Basel)*. 2012;12(3):2519-2538. doi:10.3390/s120302519
12. Lee J, Zhang L. The hierarchy quorum sensing network in Pseudomonas aeruginosa. *Protein Cell*. 2015;6(1):26-41. doi:10.1007/s13238-014-0100-x
13. Schuster M, Sexton DJ, Diggle SP, Greenberg EP. Acyl-homoserine lactone quorum sensing: from evolution to application. *Annu Rev Microbiol*. 2013;67:43-63. doi:10.1146/annurev-micro-092412-155635
14. Parsek MR, Greenberg EP. Acyl-homoserine lactone quorum sensing in gram-negative bacteria: a signaling mechanism involved in associations with higher organisms. *Proc Natl Acad Sci U S A*. 2000;97(16):8789-8793.
15. Smith RS, Iglewski BH. P. aeruginosa quorum-sensing systems and virulence. *Curr Opin Microbiol*. 2003;6(1):56-60. doi:10.1016/S1369-5274(03)00008-0
16. Rasamiravaka T, El Jaziri M. Quorum-sensing mechanisms and bacterial response to antibiotics in *P. aeruginosa*. *Curr Microbiol*. 2016;73(5):747-753. doi:10.1007/s00284-016-1101-1
17. Spengler G, Kincses A, Gajdacs M, Amaral L. New roads leading to old destinations: efflux pumps as targets to reverse multidrug resistance in bacteria. *Molecules*. 2017;22(3). doi:10.3390/molecules22030468
18. Hassan KA, Liu Q, Henderson PJ, Paulsen IT. Homologs of the *Acinetobacter baumannii* AceI transporter represent a new family of bacterial multidrug efflux systems. *MBio*. 2015;6(1). doi:10.1128/mBio.01982-14
19. Alibert S, N'Gompaza Diarra J, Hernandez J, et al. Multidrug efflux pumps and their role in antibiotic and antiseptic resistance: a pharmacodynamic perspective. *Expert Opin Drug Metab Toxicol*. 2017;13(3):301-309. doi :10.1080/17425255.2017.1251581
20. Piddock LJ. Multidrug-resistance efflux pumps - not just for resistance. *Nat Rev Microbiol*. 2006;4(8):629-636. doi:10.1038/nrmicro1464
21. Alcalde-Rico M, Hernando-Amado S, Blanco P, Martinez JL. Multidrug efflux pumps at the crossroad between antibiotic resistance and bacterial virulence. *Front Microbiol*. 2016;7:1483. doi:10.3389/fmicb.2016.01483
22. Van Acker H, Coenye T. The role of efflux and physiological adaptation in biofilm tolerance and resistance. *J Biol Chem*. 2016;291(24):12565-12572. doi:10.1074/jbc.R115.707257
23. Tian ZX, Yi XX, Cho A, O'Gara F, Wang YP. CpxR activates MexAB-OprM efflux pump expression and enhances

antibiotic resistance in both laboratory and clinical nalB-Type Isolates of *Pseudomonas aeruginosa*. *PLoS Pathog*. 2016;12(10):e1005932. doi:10.1371/journal.ppat.1005932
24. Kohler T, van Delden C, Curty LK, Hamzehpour MM, Pechere JC. Overexpression of the MexEF-OprN multidrug efflux system affects cell-to-cell signaling in *Pseudomonas aeruginosa*. *J Bacteriol.* 2001;183(18):5213-5222. doi:10.1128/JB.183.18.5213–5222.2001
25. Nikaido H, Pages JM. Broad-specificity efflux pumps and their role in multidrug resistance of gram-negative bacteria. *FEMS Microbiol Rev*. 2012;36(2):340-363. doi:10.1111/j.1574-6976.2011.00290.x
26. Schweizer HP. Efflux as a mechanism of resistance to antimicrobials in *Pseudomonas aeruginosa* and related bacteria: unanswered questions. *Genet Mol Res*. 2003;2(1):48-62.
27. Blanco P, Hernando-Amado S, Reales-Calderon JA, et al. Bacterial multidrug efflux pumps: much more than antibiotic resistance determinants. *Microorganisms*. 2016;4(1). doi:10.3390/microorganisms4010014
28. Zhang L, Mah TF. Involvement of a novel efflux system in biofilm-specific resistance to antibiotics. *J Bacteriol.* 2008;190(13):4447-4452. doi:10.1128/jb.01655-07
29. Folsom JP, Richards L, Pitts B, et al. Physiology of *Pseudomonas aeruginosa* in biofilms as revealed by transcriptome analysis. *BMC Microbiol*. 2010;10:294. doi:10.1186/1471-2180-10-294
30. Stewart PS, Franklin MJ, Williamson KS, Folsom JP, Boegli L, James GA. Contribution of stress responses to antibiotic tolerance in *Pseudomonas aeruginosa* biofilms. *Antimicrob Agents Chemother*. 2015;59(7):3838-3847. doi:10.1128/aac.00433-15
31. Liao J, Schurr MJ, Sauer K. The MerR-like regulator BrlR confers biofilm tolerance by activating multidrug efflux pumps in *Pseudomonas aeruginosa* biofilms. *J Bacteriol*. 2013;195(15):3352-3363. doi:10.1128/jb.00318-13
32. Pu Y, Ke Y, Bai F. Active efflux in dormant bacterial cells - New insights into antibiotic persistence. *Drug Resist Updat*. 2017;30:7-14. doi:10.1016/j.drup.2016.11.002
33. Rapoport M, Faccone D, Pasteran F, et al. First Description of mcr-1-Mediated Colistin Resistance in Human Infections Caused by Escherichia coli in Latin America. *Antimicrob Agents Chemother*. 2016;60(7):4412-4413. doi:10.1128/aac.00573-16
34. Soto SM. Role of efflux pumps in the antibiotic resistance of bacteria embedded in a biofilm. *Virulence*. 2013;4(3):223-229. doi:10.4161/viru.23724
35. Fahmy A, Srinivasan A, Webber MA. The Relationship Between Bacterial Multidrug Efflux Pumps and Biofilm Formation. In: Li XZ, Elkins CA, Zgurskaya HI. Efflux-Mediated Antimicrobial Resistance in Bacteria: Mechanisms, Regulation and Clinical Implications. Cham: Springer International Publishing; 2016:651-663.
36. McMurry L, Petrucci RE, Jr., Levy SB. Active efflux of tetracycline encoded by four genetically different tetracycline resistance determinants in *Escherichia coli*. *Proc Natl Acad Sci U S A*. 1980;77(7):3974-3977.
37. Martinez JL, Sanchez MB, Martinez-Solano L, et al. Functional role of bacterial multidrug efflux pumps in microbial natural ecosystems. *FEMS Microbiol Rev.* 2009;33(2):430-449. doi:10.1111/j.1574-6976.2008.00157.x
38. Evans K, Passador L, Srikumar R, Tsang E, Nezezon J, Poole K. Influence of the MexAB-OprM multidrug efflux system on quorum sensing in *Pseudomonas aeruginosa*. *J Bacteriol*. 1998;180(20):5443-5447.
39. Pearson JP, Van Delden C, Iglewski BH. Active efflux and diffusion are involved in transport of Pseudomonas aeruginosa cell-to-cell signals. *J Bacteriol.* 1999;181(4):1203-1210.
40. Olivares J, Alvarez-Ortega C, Linares JF, Rojo F, Kohler T, Martinez JL. Overproduction of the multidrug efflux pump MexEF-OprN does not impair *Pseudomonas aeruginosa* fitness in competition tests, but produces specific changes in bacterial regulatory networks. *Environ Microbiol*. 2012;14(8):1968-1981. doi:10.1111/j.1462-2920.2012.02727.x
41. Rampioni G, Pillai CR, Longo F, et al. Effect of efflux pump inhibition on Pseudomonas aeruginosa transcriptome and virulence. *Sci Rep.* 2017;7(1):11392-11399. doi:10.1038/s41598-017-11892-9.
42. Moore JD, Gerdt JP, Eibergen NR, Blackwell HE. Active efflux influences the potency of quorum sensing inhibitors in *Pseudomonas aeruginosa*. *Chembiochem*. 2014;15(3):435-442. doi:10.1002/cbic.201300701
43. Minagawa S, Inami H, Kato T, et al. RND type efflux pump system MexAB-OprM of Pseudomonas aeruginosa selects bacterial languages, 3-oxo-acyl-homoserine lactones, for cell-to-cell communication. *BMC Microbiol*. 2012;12:70. doi:10.1186/1471-2180-12-70
44. Lopez CA, Travers T, Pos KM, Zgurskaya HI, Gnanakaran S. Dynamics of intact MexAB-OprM efflux pump: focusing on the MexA-OprM interface. *Sci Rep*. 2017;7(1):16521. doi:10.1038/s41598-017-16497-w
45. Maseda H, Sawada I, Saito K, Uchiyama H, Nakae T, Nomura N. Enhancement of the mexAB-oprM efflux pump expression by a quorum-sensing autoinducer and its cancellation by a regulator, MexT, of the mexEF-oprN efflux pump operon in Pseudomonas aeruginosa. *Antimicrob Agents Chemother*. 2004;48(4):1320-1328. doi:10.1128/AAC.48.4.1320–1328.2004
46. Levy JG, Obochi M. New applications in photodynamic therapy. Introduction. *Photochem Photobiol.* 1996;64(5):737-739.
47. Dougherty TJ, Gomer CJ, Henderson BW, et al. Photodynamic Therapy. *J Natl Cancer Inst*. 1998;90(12):889-905. doi:10.1093/jnci/90.12.889
48. Moan J, Peng Q. An outline of the hundred-year history of PDT. *Anticancer Res*. 2003;23(5a):3591-3600.
49. Ochsner M. Photophysical and photobiological processes in the photodynamic therapy of tumours. *J Photochem Photobiol B.* 1997;39(1):1-18.
50. Abrahamse H, Hamblin MR. New photosensitizers for photodynamic therapy. *Biochem J.* 2016;473(4):347-364. doi:10.1042/bj20150942
51. Hamblin MR, Newman EL. On the mechanism of the tumour-localising effect in photodynamic therapy. *J Photochem Photobiol B*. 1994;23(1):3-8. doi:10.1016/S1011-1344(94)80018-9
52. Iyer AK, Greish K, Seki T, et al. Polymeric micelles of zinc protoporphyrin for tumor targeted delivery based on EPR effect and singlet oxygen generation. *J Drug Target*. 2007;15(7-8):496-506. doi:10.1080/10611860701498252
53. De Rosa FS, Bentley MV. Photodynamic therapy of skin cancers: sensitizers, clinical studies and future directives. *Pharm Res*. 2000;17(12):1447-1455. doi:10.1023/a:1007612905378

54. Sharma SK, Dai T, Kharkwal GB, et al. Drug discovery of antimicrobial photosensitizers using animal models. *Curr Pharm Des*. 2011;17(13):1303-1319.
55. Tegos GP, Hamblin MR. Phenothiazinium antimicrobial photosensitizers are substrates of bacterial multidrug resistance pumps. *Antimicrob Agents Chemother*. 2006;50(1):196-203. doi:10.1128/aac.50.1.196-203.2006
56. Tegos GP, Masago K, Aziz F, Higginbotham A, Stermitz FR, Hamblin MR. Inhibitors of bacterial multidrug efflux pumps potentiate antimicrobial photoinactivation. *Antimicrob Agents Chemother*. 2008;52(9):3202-3209. doi:10.1128/aac.00006-08
57. Kishen A, Upadya M, Tegos GP, Hamblin MR. Efflux pump inhibitor potentiates antimicrobial photodynamic inactivation of *Enterococcus faecalis* biofilm. *Photochem Photobiol*. 2010;86(6):1343-1349. doi:10.1111/j.1751-1097.2010.00792.x
58. Turlin E, Heuck G, Simoes Brandao MI, et al. Protoporphyrin (PPIX) efflux by the MacAB-TolC pump in Escherichia coli. *Microbiologyopen*. 2014;3(6):849-859. doi:10.1002/mbo3.203
59. Bartolomeu M, Rocha S, Cunha A, Neves MG, Faustino MA, Almeida A. Effect of photodynamic therapy on the virulence factors of *Staphylococcus aureus*. *Front Microbiol*. 2016;7:267. doi:10.3389/fmicb.2016.00267
60. Pourhajibagher M, Boluki E, Chiniforush N, et al. Modulation of virulence in *Acinetobacter baumannii* cells surviving photodynamic treatment with toluidine blue. *Photodiagnosis Photodyn Ther*. 2016;15:202-212. doi:10.1016/j.pdpdt.2016.07.007
61. Redenski I, Sahar-Helft S, Stabholz A, Steinberg D. Er:YAG Laser irradiation induces behavioral changes in *V. harveyi*. *Photomed Laser Surg*. 2017;35(3):164-170. doi:10.1089/pho.2016.4155
62. Sharma G, Rao S, Bansal A, Dang S, Gupta S, Gabrani R. *Pseudomonas aeruginosa* biofilm: potential therapeutic targets. *Biologicals*. 2014;42(1):1-7. doi:10.1016/j.biologicals.2013.11.001
63. Hancock RE, Speert DP. Antibiotic resistance in *Pseudomonas aeruginosa*: mechanisms and impact on treatment. *Drug Resist Updat*. 2000;3(4):247-255. doi:10.1054/drup.2000.0152
64. Percival SL, Suleman L, Vuotto C, Donelli G. Healthcare-associated infections, medical devices and biofilms: risk, tolerance and control. *J Med Microbiol*. 2015;64(Pt 4):323-334. doi:10.1099/jmm.0.000032