Understanding the Mechanisms of Bacterial Antimicrobial Resistance within Biofilms

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ABSTRACT

Biofilm is known as a community of single or multi species of microorganisms, including bacteria. Bacteria in biofilms have increased resistance to antimicrobials that may reach 10 to 1000 times more than the minimal inhibitory concentrations required for free-living bacteria. The increased antimicrobial resistance is thought to be due to pathways other than the conventional resistance mechanisms seen in free-living (planktonic) bacteria. There is no single general mechanism that explains biofilm resistance to antimicrobials; it is rather a complex process that involves a growing list of many factors. Several studies support the increasing number of resistance mechanisms, which are categorized in six classes: extracellular matrix, altered microenvironment, stress response, quorum-sensing, presence of persisters, and bacterial outer membrane proteins; in addition to other mechanisms that do not fall under these six general categories. A full understanding of biofilm resistance mechanisms will help researchers to develop effective treatment strategies for eradicating biofilm-based infections in the future.

Key Words: *Bacteria, Biofilms, Multi-drug resistance, Antimicrobials*

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INTRODUCTION

Most bacteria in our environment are found as aggregates rather than free-floating bacteria. These aggregates encase themselves in a polymer of extracellular matrix composed mainly of exopolysaccharide (EPS) [1, 2]. Such aggregates of microorganisms are known as biofilms that are composed of either single or multiple species, which may reach > 500 different species [3]. The formation of biofilms starts when planktonic bacteria attach to almost any solid surface, biotic or abiotic [4]. Then, adherent bacteria secrete cell-to-cell signaling molecules and upon reaching a certain threshold, depending on attached cells density, they cause alterations in bacterial gene expression that favor biofilm formation [5]. Presence in a community benefits bacteria in several aspects. As bacteria grow in a biofilm, they form an increasing mass of microorganisms within the EPS that prevents the bacteria from being phagocytized by host immune cells. At the same time, the extracellular polymer prevents complement-mediated lysis as well as opsonization. While encapsulation in a spore-like structure helps bacteria to cope with adverse environmental effects such as starvation, low pH, and mechanical damage [1, 6].

Bacteria in biofilms become resistant to antimicrobial drugs, which may reach up to 1000 times higher concentrations than the minimal inhibitory concentrations (MICs) required for its free-floating counterpart [7, 8]. This resistance begins as early as the organism attaches to a surface and gradually increases as the biofilm grows up in age and can be seen almost in any organism capable of forming biofilm [9, 10]. Due to increased antimicrobial resistance, biofilms can cause persistent and difficult to treat infections that are associated with many medical conditions especially cystic fibrosis, periodontitis, osteomyelitis, prosthetic joint, and indwelling medical devices infections, as well as many other nosocomial

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infections [11]. There are about 1 million nosocomial infection cases related to biofilm in indwelling medical devices in the United States each year [11, 12]. Treatment of such infections costs a tremendous amount of effort and money that is estimated to be 6 billion dollars a year in the United States [13]. In addition, they compromise patients' health and increase their trauma, especially in the case of implanted medical devices where the most successful treatment is the removal of the implant [14].

Conventional antibiotic resistance mechanisms of planktonic bacteria do not seem to play a major role in biofilm resistance to antibiotics such as target site mutations, reduced membrane permeability, enzymatic breakdown of antibiotics, efflux pumps, and antibiotic neutralization and modification [15]. Most of these mechanisms are usually irreversible. However, resistant bacteria within biofilms retain their sensitivity to antibiotics when they switch to the free-floating stage [8]. For example, the role of the efflux pump was investigated by studying the role of multiple drug resistance (mar) loci. It has been found that the genes encoding for the pump were not induced during the biofilm phase of growth; furthermore, deletion of the mar gene in *Escherichia coli* showed relatively the same resistance pattern to ciprofloxacin in biofilm as wild-type bacteria [16]. In another study, *Pseudomonas aeruginosa* strain that lack MexAB-OprM multi-drug resistance pump remained resistant to ciprofloxacin [17]. Similarly, the biofilm formed by *Klebsiella pneumoniae* mutant that lacked ß-lactamase activity was still able to show reduced susceptibility to ampicillin [18]. Antibiotic resistance in biofilms is not attributed to one mechanism solely. It is usually a complicated process that includes many factors and may vary in different organisms that could utilize more than one mechanism of resistance at the same time [8, 19]. To date, the hypothesized mechanisms for biofilms resistance are related to the external matrix barrier, altered microenvironment, stress response, quorum-sensing, persisters population, and bacterial outer membrane proteins. Furthermore, additional resistance mechanisms have been identified that do not belong to these different categories. Each one of these mechanisms is discussed in this review.

Resistance mechanisms

Extracellular matrix

The first mechanism that may explain biofilm antibiotic resistance is the mechanical barrier served by EPS that could prevent or slow down the diffusion of antimicrobial drugs [20, 21]. de Beer *et al*. [22] have measured the concentration of chlorine using a microelectrode in biofilm formed by *P. aeruginosa* and *K. pneumoniae*. They revealed that the concentration of chlorine within the biofilms was 80% less than that found in the bulk solution [22]. Furthermore, diffusion of piperacillin was impaired in biofilms formed by *P. aeruginosa* grown on dialysis membranes [23]. One reason for the reduced penetration could be due to the stimulation of EPS production by antibiotics, which has been seen with other antimicrobial agents [24]. Increasing the mass of EPS will make it more difficult for the antibiotic to penetrate the biofilm and reach bacteria. In return, this results in the exposure to sublethal concentrations of the antibiotics' inner bacteria within the biofilm, which promotes the development of mutational resistance [25]. Alternatively, it has been proposed that the negatively charged matrix could bind and prevent the penetration of positively charged antibiotics [26]. For example, alginate, an anionic EPS produced by *P. aeruginosa*, was shown to trap and slow down the diffusion of cationic antimicrobials including tobramycin and antimicrobial peptides in biofilms, which protects the bacteria from antimicrobial killing action [27, 28]. Decreasing the rate of penetration may give more time for enzymes produced by bacteria within biofilms to neutralize and break down the antimicrobial agents [7], which may explain the deactivation of ampicillin in biofilms of ß-lactamase-negative *K. pneumoniae* strain compared to wild-type [29]. However, the mechanical barrier does not seem to be the only factor for resistance since a wider range of antibiotics including ampicillin, ciprofloxacin, tetracycline, and rifampin can efficiently penetrate biofilms [30].

Altered microenvironment

Within biofilms, there is a concentration gradient of nutrients, which could affect the rate of bacterial growth and the shape of biofilm [31]; depletion of nutrients and accumulation of waste products in the inner part of the biofilm forces the inner bacteria to slow down their metabolic activity to a point that some bacteria become metabolically inactive [32]. Since many antibiotics' action depends on the growth rate and metabolic activity of bacteria (such as penicillins), and their bactericidal effect is directly proportional to bacterial growth rate, slowing down the growth rate may render these antimicrobial agents ineffective [33]. For example, the susceptibility of *P. aeruginosa*, *E. coli*, and *Staphylococcus epidermidis* to ciprofloxacin was shown to be dependent on growth rate in which increasing the growth rate resulted in higher susceptibility [34-36]. In addition to nutrient gradient, oxygen was found to be abundant at the biofilm surface and decreased gradually towards the center of the biofilm, forming an anaerobic zone at the inner portions of biofilm [37, 38]. Oxygen availability can alter the effectiveness of some antibiotics such as aminoglycosides, which work optimally under aerobic conditions [39, 40]. Furthermore, the accumulation of acidic waste products in the inner area of the biofilm can introduce pH differences that can modify

the action of antibiotics [41, 42]. However, additional factors must be involved in the resistance mechanism other than slow growth due to nutrient limitation and different oxygen gradients, which is supported by increased resistance of *Burkholderia cepacia* biofilms to ciprofloxacin with the increased growth rate of the bacteria within the biofilm [43].

Altered microenvironment

Both planktonic and sessile (attached) bacteria have induced responses to environmental stresses such as temperature fluctuation, pH changes, antimicrobial compounds, and DNA damage [44, 45]. However, sessile bacteria may have a better chance to express these responses than planktonic bacteria due to the delayed transportation of antibiotics or reduced metabolic activity, as described earlier. The first stress response system that shows a link to resistance in biofilms is the RpoS system [46]. Its importance in biofilms has been illustrated by the failure of Δ*rpoS E. coli* mutant strain to form a mature biofilm, which showed a 50% reduction in size compared to wild-type [47]. Also, *rpoS* transcript was detected in sputa of cystic fibrosis patients [48], which was thought to be a key molecule in the survival of bacteria in this harsh environment [49]. It has been recently postulated that the slow growth of bacteria in biofilms is not a result of reduced nutrient concentrations; but rather an outcome of stress responses in mature biofilm [49]. A second stress response sigma factor, named AlgT, has been identified in *P. aeruginosa* [50]. The role of AlgT and RpoS in mediating *P. aeruginosa* resistance to hydrogen peroxide (H_2O_2) and monochloramine has been studied by Cochran *et al*. [51] They have constructed two knockout mutant strains in which each strain lacked one sigma factor. Both mutants were susceptible to H_2O_2 and resistant to monochloramine in 24-hour old biofilm formed on alginate beads compared with the wild-type strain that was resistant to both compounds [51]. Although AlgT and RpoS stress response systems have an association with biofilm formation and resistance, their exact mechanism in biofilm resistance to antimicrobial is still unknown. On the contrary, *P. aeruginosa rpoS* mutants formed biofilms that were approximately 4 times thicker than the wild-type strain and had much higher resistance to tobramycin and other antibiotics compared to wild-type bacteria [26, 52]. Taken together, these observations highlight the involvement of more factors in biofilms' resistance to antimicrobials.

Quorum-sensing

Quorum-sensing (QS) compounds are small signaling molecules that are secreted by bacteria in biofilms to enable them to communicate with each other and alter the expression of the number of genes involved in virulence, motility, biofilm formation, and maintenance in both Gram-positive and negative bacteria [53, 54]. Their role in biofilms resistance to antimicrobials was first described by Davies *et al*. [55] who showed that *P. aeruginosa* that lacks one of the two quorum-sensing genes, Δ*LasI*, formed biofilms that were flat and more sensitive to sodium dodecyl sulfate (SDS) compared to the wild-type strain. Upon supplying these biofilms with synthetic signaling molecules, mutant bacteria retained both normal biofilm architecture and resistance to SDS [55]. Furthermore, blocking QS by different drugs renders biofilms more susceptible to several antimicrobials [7, 56-60]. Interestingly, it has been shown that *P. aeruginosa* biofilm was able to kill polymorphonuclear leukocytes (PMNs) by rhamnolipid B; a detergent that mediates necrosis of PMNs. Rhamnolipid B expression is under the control of QS, which represents an indirect role of QS molecules in avoiding immune system-mediated killing [61]. Again, the overall contribution of QS in biofilm-mediated resistance to antimicrobial is not fully understood.

Role of persisters

Persisters are a subpopulation of bacteria that enter a non-heritable and reversible high resistance state to antimicrobials and chemical disinfectants even with prolonged treatment [62]. They exist in both biofilm embedded and planktonic bacteria. However, they are found in much higher numbers in biofilms [63]. It has been demonstrated that exposure of *E. coli* in biofilms to imipenem and ciprofloxacin resulted in the eradication of most of the biofilms bacteria, but a small population remained resistant to further higher concentrations of these antibiotics [64]. Based on these findings and others, a model for the role of persisters in biofilm-based infections has been proposed [65], in which administration of antimicrobial agents to patients with the biofilm-based infection will eradicate the free-floating and most biofilm bacteria, but they will fail to remove persisters and the biofilm mass. As a result, persisters embedded in biofilm mass are protected from antimicrobial-mediated killing, and recurrence is always seen when treatment is stopped [66]. Yet, how bacteria enter this state is still elusive. Switching to this phenotype could be triggered by high cell density, limited nutrition, environmental stress, or amalgamation of these factors. Nevertheless, the development of persisters is accompanied by low expression of genes coding for metabolic activities (i.e. the bacteria are metabolically inactive), overexpression of stress response elements and toxin/antitoxin systems, and high expression of *glpD* and *plsB* (potential persisters genes) [67-71].

Bacterial outer membrane proteins

Alteration of bacterial outer membrane proteins affects antibiotics permeability and has been proposed as an important mechanism for increased biofilms tolerance to antibiotics [72-74]. In *E. coli* K-12, the permeability of low molecular weight hydrophilic compounds is regulated via the outer membrane porin proteins such as OmpF and OmpC. Mutants in ompB, a gene that controls the expression of OmpF and OmpC, and OmpF have been linked to increased resistance to ß-lactams and cefoxitin [75]. Furthermore, mutant *E. coli* that lacked OmpF outer membrane protein, which is under the regulation of respiratory quinol oxidase cytochrome bd [76], showed increased resistance to chloramphenicol and tetracycline [77]. Recently, a link between free DNA in biofilms extracellular matrix has been described where it was found to work as a cation chelating agent which reduces the level of cations in the surrounding environment that will ultimately affect the bacterial outer membrane and reduce the permeability of antibiotics, including cationic antimicrobial peptides and aminoglycosides [78, 79]. The disruption of the extracellular DNA results in increased susceptibility to antimicrobials [80]. Taking together all the previous findings, change in permeability of bacterial outer membrane proteins is likely to occur in biofilms and is involved in high biofilm resistance to antimicrobials.

Other mechanisms

Additional biofilm resistance mechanisms that do not fall under the previously outlined categories have also been described. One example is circular glucans. Mah *et al*. [81] demonstrated that mutation in the *ndvB* gene, which is essential for the production of periplasmic glucans, in *P. aeruginosa* resulted in bacterial strains that were capable of producing similar biofilm architecture to the wild-type but were less resistant to tobramycin. This observation suggests that circular glucans expression works by sequestering tobramycin, rendering the bacteria resistant to tobramycin [41]. Another resistance mechanism was discovered while screening *P. aeruginosa* transposon mutants that lost that antibiotic resistance in biofilm [82]. This study identified two genes, PA1874 and PA1877, that formed a novel type of efflux bump highly expressed in biofilms and involved in high resistance of *P. aeruginosa* biofilm to several antibiotics including tobramycin [83]. Finally, a recent report demonstrated the coexistence of pyocyanin-sensitive bacteria with pyocyanin-producing bacteria within the same biofilm, only when the biofilm was established with both strains together. This observation suggests that pyocyanin-sensitive bacteria encase themselves with pyocyanin-resistant bacteria, which results in protecting the former from antibiotic action [84].

CONCLUSION

The mechanisms that render biofilms resistant to antibiotics are complicated and multi-factorial. If we imagined a community of microorganisms, we would realize that each organism within this community could be under different conditions and may be exposed to different stress factors. For example, bacteria that occupy the inner part of the biofilm may have limited nutrition and a higher concentration of cell-to-cell communication molecules. On the other hand, if we moved from the biofilm center, bacteria at the periphery will be exposed to a higher concentration of antimicrobials than the central area, which may trigger the stress response mediated resistance. Moreover, bacteria that are in direct contact with the surface that the biofilm is attached to may be exposed to different factors (s) that could trigger other mechanisms. The overall picture of biofilm-mediated resistance to antibiotics will continue to expand and more resistance mechanisms are likely to be discovered in the future.

We should also notice that most of the experiments that have been conducted to elucidate these mechanisms are based on in vitro models using a single microorganism, which is different from what is seen in real life. Biofilm-based infections are usually composed of more than one species that cooperatively interact with each other and survive better than single-species biofilm in the presence of different antibacterial agents [38, 85, 86]. Furthermore, bacteria in biofilms interact with the surface that they are formed on. Therefore, in vivo approach may provide more realistic findings that may further confirm or contradict the in vitro results. All these factors will add more complication to the understanding of the overall resistance mechanisms in biofilm.

The obscurity of the biofilm resistance is an open sea for future research. In the beginning, there is a strong demand to shift from in vitro experiments to in vivo systems by finding a suitable animal model. Such experiments may lead to discoveries of new findings and mechanisms. Furthermore, the development of standard procedures to grow biofilms and test their resistance may reduce the size of contradiction and increase the consistency of results. Finally, metatranscriptomics and metaproteomic studies have shown several alterations in gene expression of global proteins when the bacteria switch to biofilm formation. These differentially expressed genes and proteins may hide valuable information that could further broaden our knowledge about biofilms; thus, they could be excellent targets for future work that could aid in the discovery of new anti-biofilm drugs for eradicating biofilm-based infections.

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REFERENCES

[1] Gloag ES, Fabbri S, Wozniak DJ, Stoodley P. Biofilm mechanics: implications in infection and survival. Biofilm 2019;2:100017.

[2] Costa OYA, Raaijmakers JM, Kuramae EE. Microbial extracellular polymeric substances: ecological function and impact on soil aggregation. Front Microbiol. 2018;9:1636.

[3] Rao Y, Shang W, Yang Y, Zhou R, Rao X. Fighting mixed-species microbial biofilms with cold atmospheric plasma. Front Microbiol. 2020;11:1000.

[4] Muhammad MH, Idris AL, Fan X, Guo Y, Yu Y, Jin X, et al. Beyond Risk: Bacterial Biofilms and Their Regulating Approaches. Front Microbiol. 2020;11:928.

[5] Grinberg M, Orevi T, Kashtan N. Bacterial surface colonization, preferential attachment and fitness under periodic stress. Plos Comput Biol. 2019;15(3):e1006815.

[6] Yin W, Wang Y, Liu L, He J. Biofilms: The Microbial "Protective Clothing" in Extreme Environments. Int J Mol Sci. 2019;20(14):3423.

[7] Sharma D, Misba L, Khan AU. Antibiotics versus biofilm: an emerging battleground in microbial communities. Antimicrob Resist Infect Control. 2019;8(1):76.

[8] Dincer S, Uslu FM, Delik A. Antibiotic Resistance in Biofilm. In: Dincer S, Özdenefe MS, Arkut A. Bacterial Biofilms. IntechOpen; 2020. 168 p.

[9] Flemming HC, Wingender J, Szewzyk U, Steinberg P, Rice SA, Kjelleberg S. Biofilms: an emergent form of bacterial life. Nat Rev Microbiol. 2016;14(9):563-75.

[10] Wong GCL, Antani JD, Lele PP, Chen J, Nan B, Kühn MJ, et al. Roadmap on emerging concepts in the physical biology of bacterial biofilms: from surface sensing to community formation. Phys Biol. 2021;18(5):051501.

[11] Khatoon Z, McTiernan CD, Suuronen EJ, Mah TF, Alarcon EI. Bacterial biofilm formation on implantable devices and approaches to its treatment and prevention. Heliyon. 2018;4(12):e01067.

[12] Jass J, Surman S, Walker J. Medical Biofilms: Detection, Prevention and Control. Wiley; 2019. 291 p.

[13] Dadgostar P. Antimicrobial Resistance: Implications and Costs. Infect Drug Resist. 2019;12:3903-10.

[14] Oliva A, Miele MC, Ismail DA, Timoteo FD, Angelis MD, Rosa L, et al. Challenges in the Microbiological Diagnosis of Implant-Associated Infections: A Summary of the Current Knowledge. Front Microbiol. 2021;12:750460.

[15] Hasan CM, Dutta D, Nguyen ANT. Revisiting Antibiotic Resistance: Mechanistic Foundations to Evolutionary Outlook. Antibiotics. 2021;11(1):40.

[16] Singh S, Singh SK, Chowdhury I, Singh R. Understanding the Mechanism of Bacterial Biofilms Resistance to Antimicrobial Agents. Open Microbiol J. 2017;11:53-62.

[17] Alav I, Sutton JM, Rahman KM. Role of bacterial efflux pumps in biofilm formation. J Antimicrob Chemother. 2018;73(8):2003-20.

[18] Ragupathi NKD, Sethuvel DPM, Dwarakanathan HT, Murugan D, Umashankar Y, Monk PN, et al. The Influence of Biofilms on Carbapenem Susceptibility and Patient Outcome in Device Associated K. pneumoniae Infections: Insights into Phenotype Vs Genome-Wide Analysis and Correlation. Front Microbiol. 2020;11:591679.

[19] Koo H, Allan RN, Howlin RP, Stoodley P, Hall-Stoodley L. Targeting microbial biofilms: current and prospective therapeutic strategies. Nat Rev Microbiol. 2017;15(12):740-55.

[20] Pinto RM, Lopes-de-Campos D, Martins MCL, Dijck PV, Nunes C, Reis S. Impact of nanosystems in Staphylococcus aureus biofilms treatment. Fems Microbiol Rev. 2019;43(6):622-41.

[21] Karygianni L, Ren Z, Koo H, Thurnheer T. Biofilm Matrixome: Extracellular Components in Structured Microbial Communities. Trends Microbiol. 2020;28(8):668-81.

[22] Beer DD, Srinivasan R, Stewart PS. Direct measurement of chlorine penetration into biofilms during disinfection. Appl Environ Microb. 1994;60(12):4339-44.

[23] Hoyle BD, Alcantara J, Costerton JW. Pseudomonas aeruginosa biofilm as a diffusion barrier to piperacillin. Antimicrob Agents Chemother. 1992;36(9):2054-6.

[24] Singh S, Datta S, Narayanan KB, Rajnish KN. Bacterial exo-polysaccharides in biofilms: role in antimicrobial resistance and treatments. J Genetic Eng Biotechnol. 2021;19(1):140.

[25] Ahmed MN, Porse A, Sommer MOA, Høiby N, Ciofu O. Evolution of Antibiotic Resistance in Biofilm and Planktonic Pseudomonas aeruginosa Populations Exposed to Subinhibitory Levels of Ciprofloxacin. Antimicrob Agents Chemother. 2018;62(8):e00320-18.

[26] Ciofu O, Tolker-Nielsen T. Tolerance and Resistance of Pseudomonas aeruginosa Biofilms to Antimicrobial

Agents-How P. aeruginosa Can Escape Antibiotics. Front Microbiol. 2019;10:913.
[27] Kamali E, Jamali A, Ardebili A, Ezadi F, Mohebbi A. Evaluation of antimicrobial resistance, biofilm forming potential, and the presence of biofilm-related genes among clinical isolates of Pseudomonas aeruginosa. BMC Res Notes. 2020;13(1):27.
[28] Yasir M, Willcox M, Dutta D. Action of Antimicrobial Peptides against Bacterial Biofilms. Materials. 2018;11(12):2468.
[29] Kundukad B, Udayakumar G, Grela E, Kaur D, Rice SA, Kjelleberg S, et al. Weak acids as an alternative anti-microbial therapy. Biofilm. 2020;2:100019.
[30] Verderosa AD, Totsika M, Fairfull-Smith KE. Bacterial Biofilm Eradication Agents: A Current Review. Front Chem. 2019;7:824.
[31] Salgar-Chaparro SJ, Lepkova K, Pojtanabuntoeng T, Darwin A, Machuca LL. Nutrient Level Determines Biofilm Characteristics and Subsequent Impact on Microbial Corrosion and Biocide Effectiveness. Appl Environ Microb. 2020;86(7):e02885-19.
[32] Achinas S, Charalampogiannis N, Euverink GJW. A Brief Recap of Microbial Adhesion and Biofilms. Appl Sci. 2019;9(14):2801.
[33] Li J, Xie S, Ahmed S, Wang F, Gu Y, Zhang C, et al. Antimicrobial Activity and Resistance: Influencing Factors. Front Pharmacol. 2017;8:364.
[34] Duguid IG, Evans E, Brown MRW, Gilbert P. Growth-rate-independent killing by ciprofloxacin of biofilm-derived Staphylococcus epidermidis evidence for cell-cycle dependency. J Antimicrob Chemother. 1992;30(6):791-802.
[35] Evans DJ, Allison DG, Brown MRW, Gilbert P. Susceptibility of Pseudomonas aeruginosa and Escherichia coli biofilms towards ciprofloxacin: effect of specific growth rate. J Antimicrob Chemother. 1991;27(2):177-84.
[36] Ghanbari A, Dehghany J, Schwebs T, Müsken M, Häussler S, Meyer-Hermann M. Inoculation density and nutrient level determine the formation of mushroom-shaped structures in Pseudomonas aeruginosa biofilms. Sci Rep-Uk. 2016;6(1):32097.
[37] Klementiev AD, Jin Z, Whiteley M. Micron Scale Spatial Measurement of the O 2 Gradient Surrounding a Bacterial Biofilm in Real Time. Mbio. 2020;11.
[38] Penesyan A, Paulsen IT, Kjelleberg S, Gillings MR. Three faces of biofilms: a microbial lifestyle, a nascent multicellular organism, and an incubator for diversity. Npj Biofilms Microbiomes. 2021;7(1):80.
[39] Jadhav RW, Kobaisi MA, Jones LA, Vinu A, Bhosale SV. The Supramolecular Self-Assembly of Aminoglycoside Antibiotics and their Applications. Chemistryopen. 2019;8(9):1154-66.
[40] Yang JH, Bening SC, Collins JJ. Antibiotic efficacy-context matters. Curr Opin Microbiol. 2017;39:73-80.
[41] Hall CW, Mah TF. Molecular mechanisms of biofilm-based antibiotic resistance and tolerance in pathogenic bacteria. Fems Microbiol Rev. 2017;41(3):276-301.
[42] Hughes G, Webber MA. Novel approaches to the treatment of bacterial biofilm infections. Brit J Pharmacol. 2017;174(14):2237-46.
[43] Desai M, Bühler T, Weller PH, Brown MR. Increasing resistance of planktonic and biofilm cultures of Burkholderia cepacia to ciprofloxacin and ceftazidime during exponential growth. J Antimicrob Chemother. 1998;42(2):153-60.
[44] Mah TF, O'Toole GA. Mechanisms of biofilm resistance to antimicrobial agents. Trends Microbiol. 2001;9(1):34-9.
[45] Holden ER, Webber MA. MarA, RamA, and SoxS as Mediators of the Stress Response: Survival at a Cost. Front Microbiol. 2020;11:828.
[46] Rode DKH, Singh PK, Drescher K. Multicellular and unicellular responses of microbial biofilms to stress. Biol Chem. 2020;401(12):1365-74.
[47] Amores GR, Heras A de L, Sanches-Medeiros A, Elfick A, Silva-Rocha R. Systematic identification of novel regulatory interactions controlling biofilm formation in the bacterium Escherichia coli. Sci Rep. 2017;7(1):16768.
[48] Foley I, Marsh P, Wellington EMH, Smith AW, Brown MRW. General stress response master regulator rpoS is expressed in human infection: a possible role in chronicity. J Antimicrob Chemother. 1999;43(1):164-5.
[49] Uruén C, Chopo-Escuin G, Tommassen J, Mainar-Jaime RC, Arenas J. Biofilms as Promoters of Bacterial Antibiotic Resistance and Tolerance. Antibiotics. 2020;10(1):3.
[50] Moradali MF, Ghods S, Rehm BHA. Pseudomonas aeruginosa Lifestyle: A Paradigm for Adaptation, Survival, and Persistence. Front Cell Infect Microbiol. 2017;7:39.
[51] Cochran WL, Suh SJ, McFeters GA, Stewart PS. Role of RpoS and AlgT in Pseudomonas aeruginosa biofilm resistance to hydrogen peroxide and monochloramine. J Appl Microbiol. 2000;88(3):546-53.
[52] Duan X, Pan Y, Cai Z, Liu Y, Zhang Y, Liu M, et al. rpoS-mutation variants are selected in Pseudomonas aeruginosa biofilms under imipenem pressure. Cell Biosci. 2021;11(1):138.
[53] Subramani R, Jayaprakashvel M. Implication of Quorum Sensing and Biofilm Formation in Medicine, Agriculture and Food Industry. Springer. 2019:21-37.
[54] Pena RT, Blasco L, Ambroa A, González-Pedrajo B, Fernández-García L, López M, et al. Relationship

Between Quorum Sensing and Secretion Systems. Front Microbiol. 2019;10:1100.

[55] Davies DG, Parsek MR, Pearson JP, Iglewski BH, Costerton JW, Greenberg EP. The Involvement of Cell-to-Cell Signals in the Development of a Bacterial Biofilm. Science. 1998;280(5361):295-8.

[56] Bjarnsholt T, Givskov M. Quorum sensing inhibitory drugs as next generation antimicrobials: Worth the effort? Curr Infect Dis Rep. 2008;10(1):22-8.

[57] Bjarnsholt T, Kirketerp-Møller K, Kristiansen S, Phipps R, Nielsen AK, Jensen PØ, et al. Silver against Pseudomonas aeruginosa biofilms. Apmis. 2007;115(8):921-8.

[58] Srinivasan R, Santhakumari S, Poonguzhali P, Geetha M, Dyavaiah M, Xiangmin L. Bacterial Biofilm Inhibition: A Focused Review on Recent Therapeutic Strategies for Combating the Biofilm Mediated Infections. Front Microbiol. 2021;12:676458.

[59] Langendonk RF, Neill DR, Fothergill JL. The Building Blocks of Antimicrobial Resistance in Pseudomonas aeruginosa: Implications for Current Resistance-Breaking Therapies. Front Cell Infect Microbiol. 2021;11:665759.

[60] Martin I, Waters V, Grasemann H. Approaches to Targeting Bacterial Biofilms in Cystic Fibrosis Airways. Int J Mol Sci. 2021;22(4):2155.

[61] Jurado-Martín I, Sainz-Mejías M, McClean S. Pseudomonas aeruginosa: An Audacious Pathogen with an Adaptable Arsenal of Virulence Factors. Int J Mol Sci. 2021;22(6):3128.

[62] Singla M, Chaudhary V, Ghosh A. Bacterial Multidrug Tolerance and Persisters: Understanding the Mechanisms, Clinical Implications, and Treatment Strategies. Antimicrob Resist. 2022:29-69.

[63] Carvalho G, Balestrino D, Forestier C, Mathias JD. How do environment-dependent switching rates between susceptible and persister cells affect the dynamics of biofilms faced with antibiotics? Npj Biofilms Microbiomes. 2018;4(1):6.

[64] Ashby MJ, Neale JE, Knott SJ, Critchley IA. Effect of antibiotics on non-growing planktonic cells and biofilms of Escherichia coli. J Antimicrob Chemother. 1994;33(3):443-52.

[65] Miyaue S, Suzuki E, Komiyama Y, Kondo Y, Morikawa M, Maeda S. Bacterial Memory of Persisters: Bacterial Persister Cells Can Retain Their Phenotype for Days or Weeks After Withdrawal from Colony–Biofilm Culture. Front Microbiol. 2018;9:1396.

[66] Jiang Y, Geng M, Bai L. Targeting Biofilms Therapy: Current Research Strategies and Development Hurdles. Microorganisms. 2020;8(8):1222.

[67] Kaldalu N, Mei R, Lewis K. Killing by Ampicillin and Ofloxacin Induces Overlapping Changes in Escherichia coli Transcription Profile. Antimicrob Agents Chemother. 2004;48(3):890-6.

[68] Keren I, Shah D, Spoering A, Kaldalu N, Lewis K. Specialized Persister Cells and the Mechanism of Multidrug Tolerance in Escherichia coli. J Bacteriol. 2004;186(24):8172-80.

[69] Shah D, Zhang Z, Khodursky AB, Kaldalu N, Kurg K, Lewis K. Persisters: a distinct physiological state of E. coli. BMC Microbiol. 2006;6(1):53.

[70] Spoering AL, Vulić M, Lewis K. GlpD and PlsB Participate in Persister Cell Formation in Escherichia coli. J Bacteriol. 2006;188(14):5136-44.

[71] Eisenreich W, Rudel T, Heesemann J, Goebel W. Persistence of Intracellular Bacterial Pathogens—With a Focus on the Metabolic Perspective. Front Cell Infect Microbiol. 2021;10:615450.

[72] Cattelan N, Villalba MI, Parisi G, Arnal L, Serra DO, Aguilar M, et al. Outer membrane protein OmpQ of Bordetella bronchiseptica is required for mature biofilm formation. Microbiology. 2016;162(2):351-63.

[73] Li B, Huang Q, Cui A, Liu X, Hou B, Zhang L, et al. Overexpression of Outer Membrane Protein X (OmpX) Compensates for the Effect of TolC Inactivation on Biofilm Formation and Curli Production in Extraintestinal Pathogenic Escherichia coli (ExPEC). Front Cell Infect Microbiol. 2018;8:208.

[74] Peterson E, Kaur P. Antibiotic Resistance Mechanisms in Bacteria: Relationships Between Resistance Determinants of Antibiotic Producers, Environmental Bacteria, and Clinical Pathogens. Front Microbiol. 2018;9:2928.

[75] Jaffe A, Chabbert YA, Semonin O. Role of porin proteins OmpF and OmpC in the permeation of beta-lactams. Antimicrob Agents Chemother. 1982;22(6):942-8.

[76] Beebout CJ, Sominsky LA, Eberly AR, Horn GTV, Hadjifrangiskou M. Cytochrome bd promotes Escherichia coli biofilm antibiotic tolerance by regulating accumulation of noxious chemicals. Npj Biofilms Microbiomes. 2021;7(1):35.

[77] Moore JP, Li H, Engmann ML, Bischof KM, Kunka KS, Harris ME, et al. Inverted Regulation of Multidrug Efflux Pumps, Acid Resistance, and Porins in Benzoate-Evolved Escherichia coli K-12. Appl Environ Microb. 2019;85(16):e00966-19.

[78] Li Y, Xiao P, Wang Y, Hao Y. Mechanisms and Control Measures of Mature Biofilm Resistance to Antimicrobial Agents in the Clinical Context. ACS Omega. 2020;5(36):22684-90.

[79] Buzzo JR, Devaraj A, Gloag ES, Jurcisek JA, Robledo-Avila F, Kesler T, et al. Z-form extracellular

DNA is a structural component of the bacterial biofilm matrix. Cell. 2021;184(23):5740-58.

[80] Powell LC, Pritchard MF, Ferguson EL, Powell KA, Patel SU, Rye PD, et al. Targeted disruption of the extracellular polymeric network of Pseudomonas aeruginosa biofilms by alginate oligosaccharides. Npj Biofilms Microbiomes. 2018;4(1):13.

[81] Mah TF, Pitts B, Pellock B, Walker GC, Stewart PS, O'Toole GA. A genetic basis for Pseudomonas aeruginosa biofilm antibiotic resistance. Nature. 2003;426(6964):306-10.

[82] Varadarajan AR, Allan RN, Valentin JDP, Ocampo OEC, Somerville V, Pietsch F, et al. An integrated model system to gain mechanistic insights into biofilm-associated antimicrobial resistance in Pseudomonas aeruginosa MPAO1. Npj Biofilms Microbiomes. 2020;6(1):46.

[83] Poudyal B, Sauer K. The ABC of Biofilm Drug Tolerance: the MerR-Like Regulator BrlR Is an Activator of ABC Transport Systems, with PA1874-77 Contributing to the Tolerance of Pseudomonas aeruginosa Biofilms to Tobramycin. Antimicrob Agents Chemother. 2018;62(2):e01981-17.

[84] Thi MTT, Wibowo D, Rehm BHA. Pseudomonas aeruginosa Biofilms. Int J Mol Sci. 2020;21(22):8671.

[85] Cepas V, López Y, Muñoz E, Rolo D, Ardanuy C, Martí S, et al. Relationship Between Biofilm Formation and Antimicrobial Resistance in Gram-Negative Bacteria. Microb Drug Resist. 2019;25(1):72-9.

[86] Li Q, Liu L, Guo A, Zhang X, Liu W, Ruan Y. Formation of Multispecies Biofilms and Their Resistance to Disinfectants in Food Processing Environments: A Review. J Food Protect. 2021;84(12):2071-83.

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