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An integrated view of bacterial resistance in the antibiotic era: efflux pumps biofilms and plasmids

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ABSTRACT

Antibiotic resistance is a widespread problem that reduces the effectiveness of antibiotics, leading the rise of multidrug resistance (MDR). Efflux pump, biofilm formation and plasmid based gene transfer together form the basis of co-adaptive mechanisms providing fleeting tolerance resistant overtime. Genes encoding efflux systems are alternative efflux-pumping systems that remove some antimicrobial agents, so that over extremely low intracellular concentrations are required for the bacterium to survive in the biofilm matrix. These, in turn, contribute to the structural and functional coherence of biofilms, which serve as protected niches favoring horizontal gene transfer and the propagation of plasmid-mediated resistance genes. In this review, we combine recent data from molecular and omics-based studies to demonstrate how these systems are co-regulated to control resistance in bacteria, with an emphasis on cross-talk between regulatory pathways including quorum-sensing, stress-response signalling and global transcriptional regulators. Insights into this interplay can pave the way for innovative therapeutic strategies, such as efflux pump inhibitors, biofilm dispersal agents and plasmid-curing strategies. Comprehensive knowledge of these interrelated processes may influence the development of future antimicrobial agents and their use, and may reduce the emergence of pan-resistant species of bacterial pathogens.

Keywords: Antimicrobial resistance, Bacterial resistance, Efflux pumps, Biofilms, Plasmids, Horizontal gene transfer.

1. Introduction

The increasing problem of antimicrobial resistance (AMR) is now considered one of the most important global health crises and a systematic analysis from 2019 reported that bacterial AMR was linked to approximately 4.9 million deaths, and remains one of the main causes of death due to infectious diseases worldwide, with large regional differences of disease burden and increasing figures projected if trends continue[1].Classic, heritable resistance arises from mutations in chromosomal genes or via horizontal transfer of resistance determinants while bacterial resistance is a complementary, phenotypic survival mechanism driven by a small group of cells or Resistance allowing them to survive brief exposure to otherwise lethal antibiotic concentrations without developing genetic resistance[2].

Resistance emerge through physiological changes (for instance, reduced metabolism, switching on of toxin-antitoxin mechanisms, and stress reactions) that make many bactericidal antibiotics resistant; rejuvenation of these cells once antibiotic pressure is lifted brings dissemination and re-establishment of the infection, promoting chronicity, relapse and promoting the subsequent development of fixed

resistance[3].

Based on some recent mechanistic and systems-level studies, we identified three interrelated determinants that synergistically mediate resistance in many clinically relevant bacteria:

1. Efflux pumps that lower intracellular drug concentrations by actively extruding a structurally diverse spectrum of antimicrobials[4].
2. Biofilms wherein cells embedded within an extracellular polymeric matrix are frequently found to experience limited penetration of antibiotics, metabolic heterogeneity and elevated fraction of Resistance[5].
3. Plasmid-mediated horizontal gene transfer resulting in rapid horizontal transmission of resistance genes across the same and distinct species, with biofilms being commonly shown to be hotspots for widespread conjugation[6].

Growing transcriptomic, proteomic and conjugation data add support for the functional coupling of these elements: sessile communities are associated with upregulation of efflux, biofilm matrices localize donor–recipient cell contacts and free DNA, and antibiotic-induced stress responses can elicit both effux expression and plasmid mobilization [7].

Together, this integrated network both protects antibiotic-stressed bacterial communities under acute assault, and promotes the transition from transient tolerance to stable, heritable resistance — an invitation for a combination therapy targeting both efflux activity, biofilm dispersal and plasmid spread.

2. Selection Criteria and Literature Sources

A systematic search of PubMed, Scopus and Web of Science databases was conducted for the current review. The search covered studies conducted in the litreture pubhilshed between 2018–2025 years and disclose antibiotic resistance mechanisms in bacteria related to efflux pump, biofilm production and plasmid fertility genes. Inclusion/Exclusion Criteria: All articles were peer-reviewed and published in English, original articles and review papers,and Studies that provided molecular, genomic, transcriptomic, or proteomic evidence of the integrated pathways of bacterial resistance were emphasized.

3. Efflux Pumps

Efflux pumps are membrane proteins that conduct an active transport of a wide range of different molecular structures such as antibiotics, heavy metals, and metabolic by-products from bacteria [8]. Efflux pumps permit the survival of bacteria in hostile environments, even those containing antibiotics, as they act to reduce the intracellular concentration of toxic compounds. These systems are centrally involved in both intrinsic and acquired multidrug resistance (MDR), and are regarded a major contributor to bacterial resistance[9],as shown in Figure 1.

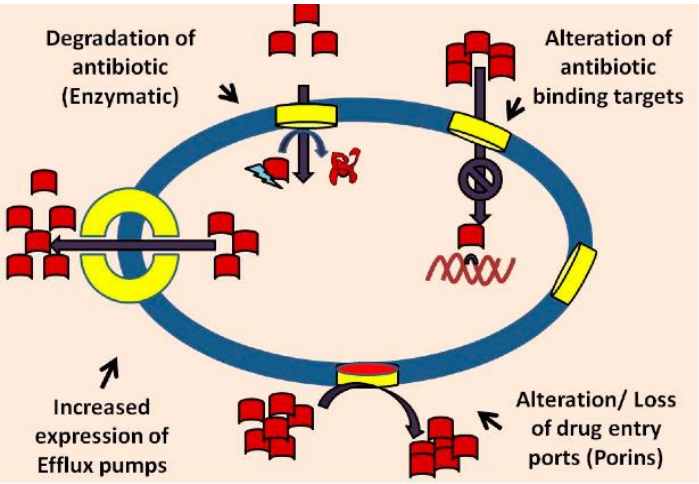

Figure 1. A schematic diagram illustrating the integrated roles in bacterial resistance[10]

Based on structure, energy source, and substrate specificity, bacterial efflux pumps are categorized into five broad families:

- Resistance-Nodulation-Division (RND) family– These pumps are mainly present in Gram-negative bacteria, and typically form tripartite complexes that span the inner membrane, periplasm and outer membrane. This complex uses proton motive force to efflux the broad spectrum of antibiotics such as β-lactams, fluoroquinolones, and aminoglycosides. Classic examples are AcrAB–TolC in Escherichia coli and MexAB–OprM in Pseudomonas aeruginosa[11].
- Major defensive mechanism Superfamily (MFS) – A class of ubiquitous transporters that typically belongs to drug/H^+ antiporters and those able to export tetracyclines, macrolides as well as chloramphenicol. Staphylococcus aureus NorA pump is a well-studied MFS transporter[12].
- Small Multidrug Resistance (SMR) family – Usually small, homodimeric transporters responsible for the export of quaternary ammonium compounds and other cationic toxicants[13].
- MATE–Sodium-dependent antiporters that extrude fluoroquinolones, aminoglycosides, and other substrates (multidrug and toxic compound extrusion [MATE] family) [3].
- ATP-Binding Cassette (ABC) transporters – Export a range of substrates such as macrolides and vancomycin, relying on ATP hydrolysis for energy. Basis of Drug Resistance in Bacterial Cells: ABC PumpsABC pumps are prevalent in both Gram-positive and Gram-negative bacteria [14].

Efflux pumps expression is under complex regulation on various levels. Under non-stress conditions, local repressors like AcrR in the E. coli AcrAB-TolC system prevent basal expression of the pump [15]. Transcriptional upregulation of efflux genes due to genetic plasticity or the action of global transcriptional regulators — for example, MarA, SoxS, Rob and RamA — can also direct these genes in such processes, where activity is deactivated by oxidative stress, exposure to antibiotics, or environmental factors, to

establish a multidrug-resistant (MDR) phenotype [16]. Moreover, antibiotic stress response pathways in bacteria, such as the SOS response and stringent response, also upregulate efflux pump expression and increase survival under this pressure[17].

Due to their pivotal role in resistance, efflux pump inhibitors (EPIs) have been evaluated as potential adjuvant therapies to recover antibiotic susceptibility[18]. In vitro, many small molecules, such as phenylalanine-arginine β-naphthylamide (PAβN), and carbonyl cyanide m-chlorophenylhydrazone (CCCP) inhibit RND pumps and increase the susceptibility of bacteria to antibiotics[19]. Nonetheless, it is faced with several hurdles for clinical translation such as cytotoxicity, low specificity, unfavorable pharmacokinetics, and redundancy of efflux systems in many bacterial species. Hence, combinatorial strategies against several pumps or combining epigenetic modifier simultaneously with biofilm dispersal agents and traditional antibiotics are being studied to circumvent these obstacles[20].

Efflux pumps are also responsible for the resistance of bacteria besides classical mechanism of antibiotic resistance. Pumps export sub-lethal concentrations of antibiotics, relieving intracellular stress and enabling a subset of cells to enter a dormant states[21]. Biofilm-associated bacteria frequently upregulate efflux activity, connecting efflux-mediated detoxification with both biofilm formation and plasmid maintenance to further strengthen the resilience of resistant populations to antibiotic therapy[22].

4. Biofilms

Biofilms are complex structures of densely packed microbial communities in which individual bacterial cells are surrounded by a self-synthesized matrix of extracellular polymeric substance (EPS) material consisting of polysaccharides, proteins, nucleic acids and lipids[23]. It provides structural integrity for the cell and a protective microenvironment to promote bacterial survival during various stress conditions, including exposure to antibiotics, host immune responses, and environmental perturbations[24].

The development of a biofilm is dynamic and conducted in sequence. Initial adhesion to a surface is mediated by surface appendages such as pili, fimbriae, and flagella[25]. The process begins with the adhesion of bacterial cells, followed by the development of microcolonies, eventually forming complex three-dimension structures with a network of fluid-filled channels that can transport nutrients and waste[26]. The third stage is dispersion, where the bacteria starts dissociating and establishing in other niches, contributing to dissemination[27].

The biofilm imparts this antibiotic tolerance through multiple mechanisms. The EPS matrix acts like a barrier that prevents drugs from entering it. Moreover, the formation of biofilms results in nutrient and oxygen gradients that create metabolic heterogeneity, with biofilms containing subpopulations of slow-growing or dormant cells (resistant cells) that are naturally more resistant to antibiotics targeting fast-growing cells[28]. These stress responses, particularly those responsive to a variety of environmental and antibiotic stressors, underscore the role of biofilms as a predominant site for chronic pathogens[29].

The main molecular circuits controlling biofilm development are quorum sensing (QS) and secondary messenger signaling as cyclic-di-GMP (c-di-GMP)[30]. Quorum sensing thus enables the bacteria to monitor their own cell-density and together orchestrate group behaviors (explanation autoinducers, EPS production and biofilm maturation) in strains that produce an autoinducer (AI)[31]. High levels of intracellular c-di-GMP promote adhesion, matrix production and biofilm stability, while low c-di-GMP levels favour motility and dispersion. Importantly, such signalling pathways also adjust efflux pump expression, linking biofilms to faster ejection and antibiotic tolerance, and multidrug tolerance[32].

Another consequence of biofilms is that they facilitate horizontal gene switch (HGT)[33]. Cell density of biofilms is higher, in addition biofilms contain extracellular DNA in the EPS which facilitate the

conjugation and uptake of plasmids which contain genes of antibiotic resistance[29]. These events propagate the transmission of resistance in bacterial populations and contribute to the growing burden of multidrug-resistant infections[34]. The physical protection combined with metabolic heterogeneity and increased transfer of genetic material leads to biofilm-associated infections on implanted medical devices, chronic wounds, and pulmonary surfaces (e.g., cystic fibrosis patients) to be particularly challenging to treat in the clinic[35].

Finally the show of biofilms with efflux pumps and plasmids helps in settlement of bacteria. Because biofilm microenvironments induce efflux pump activity leading to higher levels of antibiotic pumping, and plasmid-borne resistance genes are quickly transferred between cells in these high density environments[36]. This triad creates a self-reinforcing network of resistance: bacteria are both protected from anti-biofilm agents, enriched in tolerant populations and poised for rapid genetic change. Inhibition of biofilm dispersal is a promising strategy to complement the use of antibiotics and efflux pump inhibitors in chronic infections[37]. Design of biofilm-disrupting agents targeting the major pathways for biofilm dispersal — including quorum sensing inhibitors, c-di-GMP modulators or matrix-degrading enzymes — in combination with biofilm-specific effectors may be highly beneficial[38].

5. Plasmids and Horizontal Gene Transfer

Plasmids are described as extrachromosomal self-replicating DNAs that act as vectors for genes conferring beneficial traits to bacteria, most famously determinants of antimicrobial resistance [39]. Plasmids, unlike chromosomal mutations, plow through horizontal gene transfer (HGT) between bacterial cells, spreading resistance features across species and even genera in a matter of days, we surveyed [40]. Conjugation, which is enabled by plasmids encoding their own transfer machinery, is highly effective in delivering extended-spectrum β-lactamases (ESBLs), carbapenemases, mcr genes conferring resistance to colistin, and various other clinically relevant determinants to new bacterial hosts[41].

Even in the absence of selective antibiotic pressure, plasmid maintenance systems comprising partitioning systems, toxin-antitoxin modules, and multimer resolution systems ensure plasmid stability within bacterial populations[42]. Such systems prevent plasmid loss during cell division, enabling the transfer of resistomes among diverse bacterial communities across generations. This leads to enrichment of plasmid-bearers and concomitant higher frequency of resistance determinants in a given population under selective pressures, such as exposure to antibiotics[43].

Plasmid mobilization is Limited but can increase in response to Environmental and cellular stress responses E.g., the SOS response induced by DNA damage and/or antibiotic stress has been shown to activate the expression genes involved in the conjugation and transfer of plasmids[44]. Similarly, it was shown that oxidative stress also increased the horizontal plasmid transfer rate; a phenomenon implicated in rapid dissemination of resistance determinants that may be selected upon exposure to unfavourable environment[45]. These mechanisms highlight the relationship of the adaptation to stress and the transmission of antimicrobial resistance.

Such a dense structure of cells, together with the detection of extracellular DNA in EPS matrix, reinforces hip to hip contact and efficiency of conjugation. The presence of plasmids bearing resistance genes can therefore disseminate very quickly across biofilm communities and thus increase the reservoir of AMR [46]. In clinical scenarios, this is worrisome since biofilm-associated infections on a medical device, catheter, or in a chronic wound serve as a long-term reservoir of multidrug-resistant bacteria [47].

In addition to conjugation (discussed above), plasmids can be spread by transformation, where competent cells take up extracellular plasmid DNA, and, less commonly, transduction, which involves bacteriophages (viruses that infect bacteria)[48]. Although these alternative mechanisms of HGT are less

commonly employed than conjugation, they facilitate HGT of resistance determinants and promote genetic mobility, a third significant aspect of adaptability in bacteria[44].

Together, the interactive dynamics of plasmids with efflux pumps and biofilms form a strong ensemble of networks for bacterial survival. Stress associated with biofilm increases efflux pump activity and plasmid transfer is facilitated in the protected biofilm environment[49]. Thus, IF plasmids are capable of disseminating resistance genes as well as sustain bacterial resistance and tolerance capacity to overcome HCDM, the two-hit mechanism will render infections more difficult to treat[50]. More knowledge on this molecular control and the environmental cues that trigger plasmid-mediated horizontal gene transfer is essential to rationally design effective measures for controlling the resistance and spread of antibiotic resistance in clinical and environmental reservoirs.

6. Integrated Interplay

The genetic basis of bacterial resistance is under the control of an interconnected network of regulons involving efflux pumps, biofilm formation, and plasmid-mediated horizontal gene transfer, rather than independent mechanisms [51]. Such systems are linked by several shared regulatory pathways such as global transcriptional regulators (e.g. MarA, SoxS, Rob, RamA), quorum sensing (QS), and second messengers like cyclic-di-GMP (c-di-GMP). Such orchestrators enable expression in a manner designed to enhance survival, maintain fitness of resistant alleles, and optimize cooperative activities when subjected to similar environmental stressors[52].

Microbiology studies in the omics era — e.g. transcriptomics, proteomics and metabolomics — have revealed that, during antibiotic stress, bacteria often coordinately upregulate efflux, biofilm and plasmid maintenance genes [53]. For example, decreased susceptibility to β-lactams after long-term antibiotic exposure in Pseudomonas aeruginosa is mediated by subinhibitory doses of the antibiotic that induce expression of RND efflux pumps and increase levels of c-di-GMP, resulting in biofilm formation and enhanced horizontal plasmid transfer[54]. Similarly, the SOS response induced by DNA-damaging antibiotics in Escherichia coli promotes plasmid conjugation and expression of efflux genes in a case of cooperative adaptive evolution to antimicrobial challenge[55].

Functional synergy of this triad yields several implications:

- Enhanced tolerance to antibiotic-induced stress — efflux pumps reduce intracellular drug concentrations; biofilms create shielded microenvironments and plasmid-encoded resistance genes provide a genetic buffer[56].
- Encouragement of horizontal gene transfer — biofilm architecture promotes cell–cell contact and increase in conjugation rates, thus conferring a selective advantage with regard to the rapid spread of resistance genes[51].
- The system promotes chronic and relapsing infection — enables bacterial subpopulations to resist treatment, persist in host tissues, and reinstate infections within hours after antibiotics are removed [57].

This consideration of the interconnected interaction is needed for informing the design of multi-target strategies against chronic infections. Compensatory responses within the network render therapeutic approaches targeting a single mechanism almost invariably insufficient [58]. Recent approaches have turned towards combination therapies:

a simultaneous blockade of efflux pump inhibitors

- biofilm-disrupting agents

- plasmid transfer blockers to impair the synergism circuitry whilst also restoring the antimicrobial activity of the antibiotics [59].

Similarly novel systems-level analyses and omics multi-plex profiling are revealing new regulatory nodes governing resistance pathways which may represent exciting molecular targets for next-generation anti-infective therapies [60].

7. Emerging Therapeutic Strategies

The phenomenon of bacterial resistance is one of the major problems of modern medicine because persistent cells can outlive any antibiotic treatment without being genetically resistant so that we can have chronic and continual infections[61]. To counter this phenomenon, new therapeutic strategies are being explored, specifically targeting bacterial resistance. These approaches primarily target aspects of the resistance triad (efflux pumps, biofilm and mobile genetic elements carrying resistance genes)[62].

Efflux Pump Inhibitors (EPIs) aim to inhibit the function of existing bacterial efflux pumps, which are transport proteins present in the cell that pump out antibiotics. EPIs restore the previously inadequate concentration of antibiotics inside the bacterial cells by inhibiting these pumps, making antibiotics effective even against persistent as well as growing bacterial cells[63].

Biofilm dispersal agents act directly on the extracellular polymeric substance (EPS) matrix of biofilms, which is a protective layer and keeps the antibiotics from reaching bacteria cells in biofilm[64]. Dispersal agents can metabolically degrade the biofilm matrix or disrupt biofilm regulatory pathways, facilitating drug penetration and bacterial eradication[65].

Reduction of resistance genes carriage within bacteria — Plasmid-Curing and CRISPR-Based Strategies Plasmid-curing agents remove plasmids containing antibiotic resistance determinants and CRISPR-Cas systems can be designed to specifically recognize and cleave resistance genes[66]. These strategies reduce the risk of transferring the genes horizontally and minimize the dissemination of resistance. Combination Therapies Are a New Hope against Resistance Dosing antibiotics in combination with an EPI, biofilm dispersal agent, or bacteriophage may represent a plausible option in this context, since these mechanisms of action are nearly always non-overlapping resistance phenotypes and thus the probability of bacteria surviving simultaneous exposure (with the dose of an antibiotic) and/or developing resistance to both approaches is very low[67].

Finally, for reducing bacterial resistance, they underline the need for population level preventative measures, environmental interventions, and One-Health approaches. In conclusion preventive measures that control antibiotic use in agriculture, optimize sanitation and monitor natural reservoirs of resistant bacteria contribute to prevent the spread of resistance genes and the establishment of environmental bacterial communities. Conclusion: An integrated strategy to overcome bacterial resistance through concurrent molecular-targeted therapies with ecological and population-level interventions will achieve superior control of chronic and recalcitrant infections[68].

8. Conclusions

Bacterial resistance represents one of the primary challenges associated with effective treatment of infectious diseases due to synergy of efflux pumps, biofilm, and mobile genetic elements located on plasmids [69]. While efflux pumps directly pump the antibiotics from the cells, biofilms provide a shelter for bacteria which make it challengeable for the immune system to kill the bacteria and antimicrobial to penetrate the biofilms, and plasmids also allow the horizontal transfer resistance genes among bacterial populations[70]. The combined activity of these processes allows bacteria to withstand antibiotic therapy

and results in chronic or relapsing infections.

An integrated understanding of these resistance mechanisms will be essential to facilitate multi-target therapeutic strategies. New strategies to simultaneously inhibit efflux systems, disturb biofilms or prevent colonization of resistance genes can enhance the elimination of bacteria that are difficult to eradicate and prevent the evolution of MDROs[71].Studies which relate molecular information to clinical relevance should be the focus of future research. This includes: testing the efficacy of combinations in clinical populations; into the regimens adding phage therapy, biofilm dispersion agents, and efflux pump inhibitors; and by One-Health approaches reducing environmental LRB- reservoirs of resistant bacteria RRB-[72]. These multidisciplinary approaches are essential tools for combating the deteriorating global challenge of antimicrobial-resistant pathogens and for the improved treatment of chronic and recurrent infections[73].

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