

Effect of Sub-Inhibitory Antibiotic Concentrations from Pharmaceutical Effluents on Biofilm-Mediated Resistance in *Pseudomonas aeruginosa*

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ABSTRACT

Pharmaceutical effluents are increasingly recognised as significant contributors to environmental antibiotic contamination, creating persistent hotspots for antimicrobial resistance development. Unlike therapeutic exposures, these effluents often expose microbes to chronic, sub-inhibitory antibiotic concentrations that shape bacterial adaptation rather than induce immediate cell death. *Pseudomonas aeruginosa*, a metabolically versatile opportunistic pathogen, exemplifies the public health risks associated with such exposure. This review synthesises open-indexed evidence on how sub-minimum inhibitory concentration (sub-MIC) antibiotics from pharmaceutical discharge can promote biofilm-mediated resistance in *P. aeruginosa*. Biofilms, structured microbial communities encased in a self-produced matrix, provide a protective environment that limits antibiotic penetration and fosters metabolic heterogeneity. Under sub-MIC exposure, *P. aeruginosa* biofilms exhibit enhanced formation, altered gene expression, and increased persistence of tolerant and resistant subpopulations. Repeated low-dose antibiotic challenge not only remodels bacterial behaviour but also selects for heritable resistance traits, especially within biofilm-associated cells. Evidence indicates that industrial effluents containing mixtures of antibiotics can maintain selective pressure in aquatic environments, facilitating the survival and propagation of resistant strains. Moreover, the capacity of biofilms to act as reservoirs for resistance genes amplifies their impact on both environmental and clinical settings. Understanding the interplay between pharmaceutical pollution, sub-inhibitory antibiotic levels, and biofilm biology is crucial for mitigating the spread of resistance. This review underscores the urgent need for improved waste management, environmental monitoring, and policy interventions to address the escalating threat of antibiotic resistance driven by pharmaceutical effluents.

Keywords: Pharmaceutical effluent; Sub-inhibitory concentration; *Pseudomonas aeruginosa*; Biofilm; Antimicrobial resistance

Introduction

Pharmaceutical effluents have become important environmental sources of antibiotic contamination because manufacturing plants and related discharge streams can release active antimicrobial residues, resistant bacteria, and resistance

genes into receiving waters, thereby creating pollution hotspots where selective pressure for antimicrobial resistance can persist beyond the point of drug production, as described by [1]. Unlike short-term therapeutic exposure in patients, environmental contamination from industrial discharge may be chronic, spatially uneven, and chemically complex, so microorganisms in wastewater channels, sediments, and downstream aquatic systems are repeatedly exposed to antibiotic mixtures under conditions that favour adaptation rather than immediate elimination^{1 2}. In microbiological terms, sub-inhibitory or sub-MIC antibiotic concentrations are levels below the minimum concentration required to inhibit visible bacterial growth, yet these doses remain biologically active because they can still influence gene expression, signalling pathways, stress responses, and community behaviour even when they do not kill or stop bacterial cells completely². Such concentrations are especially relevant outside clinical settings because antibiotics released into the environment are often diluted, partially degraded, intermittently replenished, or retained within surface-associated microbial communities, meaning that bacteria are more likely to encounter fluctuating low-dose exposure than the high, uniform concentrations assumed in standard susceptibility testing^{1 2 3}.

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This issue is particularly important for *Pseudomonas aeruginosa*, a metabolically versatile Gram-negative bacterium that survives in diverse environmental niches and also functions as a major opportunistic pathogen in immunocompromised individuals, patients with cystic fibrosis, burn wounds, chronic lung disease, urinary tract infections, and device-associated infections, making it a strong bridge between environmental microbiology and clinical disease^{2 4}. The medical significance of *P. aeruginosa* is amplified by its intrinsic low outer-membrane permeability, multidrug efflux systems, adaptive regulatory networks, and capacity to acquire additional resistance through mutation under antimicrobial stress, which together make this organism difficult to treat once persistent infection is established^{2 3 4}.

Biofilms deepen this problem because they are structured microbial communities attached to surfaces and embedded in a self-produced extracellular matrix, and this lifestyle gives bacterial cells a strong survival advantage by limiting antibiotic penetration, generating metabolic heterogeneity, and protecting subpopulations that would be far more vulnerable in planktonic form, as emphasised by [4]. In *P. aeruginosa*, biofilm growth is closely associated with chronic infection and treatment failure because sessile cells can tolerate antimicrobial challenge through slow growth, stress-adapted physiology, local nutrient gradients, and matrix-mediated protection, allowing resistant or tolerant populations to persist even when standard therapy appears microbiologically appropriate^{2 4}. Importantly, exposure to low antibiotic concentrations may not simply fail to eradicate the organism but may actively remodel its behaviour, since sub-inhibitory carbapenems such as imipenem have been associated with thicker and more robust biofilms, while ciprofloxacin and tobramycin have also been reported to stimulate biofilm formation in *P. aeruginosa*^{2 4}. The consequences extend beyond temporary phenotypic change because repeated exposure to sub-inhibitory concentrations of clinically relevant antibiotics has been shown to select bacterial populations with reduced susceptibility and mutations in resistance-associated genes, including regulators linked to efflux and aminoglycoside resistance, showing that low-dose exposure can drive heritable adaptation rather than merely reversible stress responses³. Environmental antibiotic exposure may therefore influence resistance by maintaining a persistent selective landscape in which biofilm-associated cells are repeatedly challenged at nonlethal levels, efflux systems and stress pathways are induced, tolerant communities are retained on surfaces, and mutants with survival advantages are given opportunities to accumulate and persist over time^{2 3}. When these conditions arise in pharmaceutical effluent-impacted environments, the risk becomes especially significant because industrial antibiotic residues can sustain exactly the kinds of chronic, heterogeneous, and sublethal exposures that favour biofilm-mediated persistence in *P. aeruginosa*, and for this reason the present review aims to synthesize verified open indexed evidence on pharmaceutical effluents as contamination sources, clarify the meaning and significance of sub-MIC exposure, examine *P. aeruginosa* as a clinically important opportunistic pathogen, discuss the role of biofilms in antimicrobial resistance, and define the environmental scope through which low antibiotic concentrations may promote biofilm-mediated resistance development^{1 2 3 4}.

Pharmaceutical Effluents as Sources of Sub-Inhibitory Antibiotics

Pharmaceutical effluents are among the most important point sources of antibiotic contamination because active pharmaceutical ingredients can enter wastewater during manufacturing, washing, synthesis, formulation, quality-control losses, and improper disposal of unused products, allowing antimicrobial residues to move from industrial settings into broader aquatic systems¹. Once released, these compounds may enter collection systems and wastewater treatment plants, but conventional treatment processes are not designed to completely eliminate all antibiotics, so a fraction of the parent compounds and transformation products can remain in the final effluent and continue into receiving waters^{5 6}. This matters because antibiotics do not need to be present at therapeutic doses to exert ecological effects; concentrations below clinical inhibitory thresholds can still influence microbial physiology, favour resistance selection, and reshape community structure in ways that support long-term persistence of antimicrobial traits^{2 3}. In environmental systems, therefore, sub-inhibitory exposure is not an exception but a likely outcome wherever treated or untreated industrial discharges meet rivers, estuaries, sediments, or biofilm-coated surfaces that retain contaminant residues^{7 8}. Pharmaceutical wastewater is especially concerning because it may contain complex mixtures of antibiotic classes rather than single-compound contamination, and mixture exposure can increase the probability that bacteria encounter multiple selective pressures at once, even when each individual antibiotic is present at a low concentration^{9 10}. The environmental relevance of these residues is amplified by the fact that wastewater treatment often lowers concentrations but does not necessarily remove antibiotics fully, so effluent can still contain compounds capable of exerting selective or signalling effects after discharge into surface waters^{10 11}. As a result, pharmaceutical effluents function not merely as waste streams but as ongoing reservoirs of antimicrobial pressure that connect industrial production to downstream ecological and public-health risks^{1 8}.

After treatment, antibiotics that survive wastewater processing are released into the receiving environment, where they may partition between water columns, suspended solids, and sediments depending on compound properties such as hydrophobicity, charge, degradation rate, and sorption affinity^{8 12}. This partitioning is important because sediments can act as long-term sinks and secondary sources, allowing adsorbed antibiotics to persist and intermittently re-enter the water phase under changing pH, redox, or hydrodynamic conditions^{5 13}. The persistence of antibiotics in natural waters is influenced by both environmental and chemical factors, including sunlight, temperature, microbial activity, dilution, organic matter content, and the structural stability of the antibiotic molecule itself, so some compounds degrade relatively quickly while others remain detectable over extended periods^{8 14}. Fluoroquinolones and tetracyclines, for example, tend to persist more strongly in sediments because of their binding behaviour, whereas more labile compounds may decline faster in the water column, although even rapidly degraded compounds can still matter if they are replenished continuously from upstream discharge^{12 13}. Because environmental concentrations are typically far lower than those used in therapy, many exposed bacteria encounter antibiotic levels that are insufficient to kill but sufficient to alter transcriptional regulation, stress responses, and biofilm development, creating the conditions for sub-MIC selection^{7 15}.

In this sense, the pathway from pharmaceutical production to environmental exposure is not linear alone but dynamic: production losses enter wastewater, treatment reduces but does not eliminate residues, effluent reaches rivers and sediments, and microbial communities in these habitats experience low-dose antibiotic pressure that can encourage adaptive responses^{1 10}.

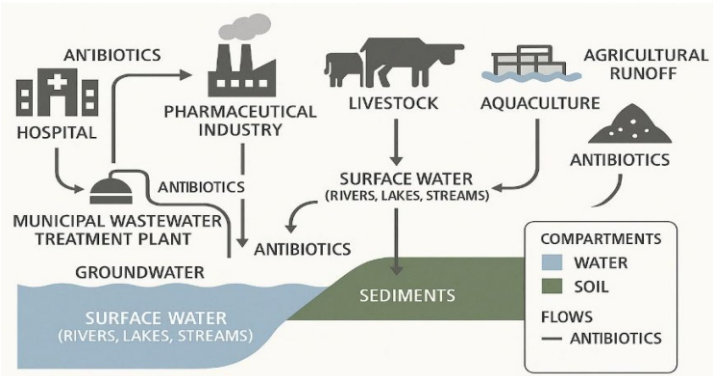


Figure 1. Pharmaceutical effluent contamination pathway
Source: [1]

Figure 1 summarises this contamination pathway: This simple flow captures the key environmental transition that converts industrial antibiotic use into ecological antibiotic pressure, and it highlights why sub-inhibitory exposures deserve attention in AMR research^{17 18}. In practice, the concentrations that reach aquatic systems are shaped by production scale, treatment efficiency, seasonal flow, dilution, sorption, and degradation, so measured residues may vary widely between sites and between compounds^{19 20}. The persistence of these compounds is especially relevant to biofilm-rich habitats, where cells are embedded in extracellular polymeric substances that can slow diffusion, retain pollutants, and create microscale gradients in antibiotic concentration that differ from the surrounding water^{21 4}. From an ecological perspective, this means that environmental antibiotic exposure is not evenly distributed but concentrated in niches where bacteria live attached to surfaces, which increases the chance that low-dose contact will repeatedly affect the same communities over time^{22 21}. The resulting exposure can promote survival strategies such as biofilm enhancement, efflux induction, mutation selection, and exchange of resistance determinants, all of which are especially relevant to *Pseudomonas aeruginosa* and other opportunistic bacteria that tolerate fluctuating environments well^{2 3}.

Table 1: Major antibiotic classes associated with pharmaceutical effluents, their sources, environmental occurrence, and possible microbial effects

Antibiotic class	Representative compounds	Main sources	Environmental occurrence	Possible microbial effects
Fluoroquinolones	Ciprofloxacin	Pharmaceutical and clinical wastewater	Persistent in wastewater and receiving waters	Biofilm stimulation and selection of resistant mutants in <i>P. aeruginosa</i> ¹²
Macrolides	Azithromycin, Clarithromycin	Pharmaceutical and clinical wastewater	Frequently detected after treatment and discharge	Ecological effects under low-level exposure ^{4,5}
Sulfonamides and trimethoprim	Sulfamethoxazole, Trimethoprim	Pharmaceutical and wastewater streams	Often incompletely removed during treatment	Sustained low-level exposure and selection pressure ^{3,4}
Tetracyclines	Tetracycline, Oxytetracycline	Pharmaceutical wastewater and contaminated solids	Strong binding to sediments and suspended solids	Prolonged exposure in benthic biofilms and surface-associated communities ⁴
Overall environmental relevance	—	Pharmaceutical effluent discharge	Influenced by persistence, removal, and environmental accumulation	Sub-inhibitory exposure may contribute to resistance selection and maintenance ^{2,3}

Sub-Inhibitory Antibiotic Exposure and *Pseudomonas aeruginosa*

Sub-inhibitory antibiotic concentrations are levels below the threshold required to stop visible bacterial growth, yet they remain biologically meaningful because bacteria can detect and respond to them rather than simply ignoring them, which makes these concentrations fundamentally different from killing doses that directly eliminate susceptible cells^{22 23}. At higher concentrations, antibiotics act primarily as bactericidal or bacteriostatic agents, but at sub-MIC levels they may function more like environmental signals that reshape physiology, behaviour, and population structure without necessarily causing immediate death^{22 24}. This distinction is important for *Pseudomonas aeruginosa* because the organism frequently experiences variable antibiotic exposure in clinical and environmental settings, where drug levels may fall below inhibitory ranges and still trigger adaptive responses^{24 25}. In such situations, growth may slow, stress responses may be activated, and the cells may alter membrane properties, metabolism, and community behaviour rather than being fully suppressed^{24 23}. Sub-inhibitory exposure can therefore be understood as a transition zone between treatment and selection, where the antibiotic no longer functions only as a killer but also as a cue that changes bacterial decision-making^{22 26}. For *P. aeruginosa*, this transition zone is especially concerning because its innate resistance mechanisms already make intracellular drug accumulation difficult, and even modest

additional adaptation can produce measurable changes in survival and persistence^{27 28}.

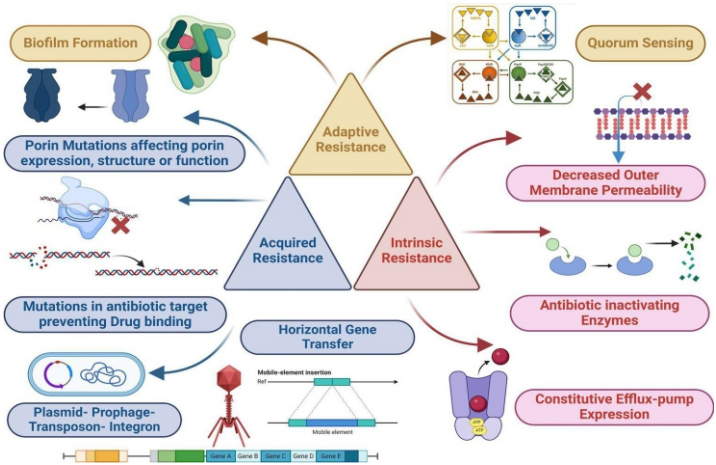


Figure 2. Biological responses of *Pseudomonas aeruginosa* to sub-inhibitory antibiotic exposure
Source: [3]

Figure 2 summarises this biological progression by showing sub-MIC exposure, stress and signalling responses, altered gene expression, biofilm, virulence, and resistance responses. The figure is useful because it visually separates direct killing from phenotypic adaptation and highlights the fact that bacterial response begins well before growth is halted^{21 22}.

In practical terms, this pathway helps explain how residual antibiotics from environmental or clinical sources may prime *P. aeruginosa* for a more persistent state even in the absence of therapeutic doses^{3 23}.

One of the most important effects of sub-MIC exposure is the induction of gene-expression changes that alter stress tolerance and antimicrobial behaviour. *P. aeruginosa* can respond to low antibiotic concentrations by inducing efflux systems, modifying virulence-associated pathways, and activating global stress regulators that increase the likelihood of persistence under repeated exposure^{24 23}. The MexXY multidrug efflux system is a classic example because it is strongly linked to aminoglycoside resistance and can be induced by ribosome-targeting antibiotics or oxidative stress, showing how nonlethal exposure can still push the cell toward a more resistant phenotype^{25 24}. Low-dose exposure can also change virulence-associated expression patterns, meaning that the bacterium may become less sensitive to host defences or more efficient in colonising surfaces even if growth rate is not dramatically increased^{26 24}. These responses are not simply transient noise; repeated sub-inhibitory exposure can create sustained selection pressure that enriches cells carrying mutations or regulatory states associated with reduced susceptibility^{3 23}. In experimental work, passage of *P. aeruginosa* under sub-inhibitory concentrations of ceftazidime, ciprofloxacin, meropenem, or tobramycin produced populations with significantly reduced susceptibility and mutations in genes associated with resistance regulation, demonstrating that low-dose exposure can select heritable change rather than merely reversible stress responses³. This finding is crucial because it shows that environmental and therapeutic residues need not reach inhibitory concentrations to shape the future resistance profile of the organism^{21 3}.

Sub-inhibitory exposure may also influence resistance development through broader ecological and genetic processes, including community interactions and gene transfer. Antibiotics in natural environments are not isolated single-cell stressors; they occur in microbial communities where they can alter signalling, competition, and the movement of resistance determinants among organisms^{21 27}. The environmental resistome is large, and low-dose antibiotic exposure can favour maintenance and spread of resistance genes by allowing partially resistant cells to survive long enough for transfer or mutation to occur^{28 29}. Although the exact contribution of horizontal gene transfer varies by setting, the broader literature supports the view that low-level antibiotic pressure can increase the ecological opportunity for acquisition and dissemination of resistance traits, particularly in dense communities where cells are in close contact^{30 31}. In this sense, *P. aeruginosa* should be viewed not only as a recipient of resistance but also as a participant in a dynamic community in which sublethal antibiotic exposure reshapes the balance between susceptible and adapted subpopulations^{32 24}.

This helps explain why environmental exposure is so consequential: even when the antibiotic concentration is too low to kill, it may still be high enough to alter behaviour, support survival, and enrich resistant lineages over time^{23 3}.

Biofilm Formation and Biofilm-Mediated Resistance

Biofilm formation is a central survival strategy for *P. aeruginosa* and proceeds through a series of coordinated stages that begin with initial attachment to a surface and continue through microcolony formation, maturation into structured three-dimensional communities, and eventual dispersion^{33 34}. During the attachment phase, planktonic cells use flagella, pili, and surface adhesins to anchor themselves to abiotic or biotic surfaces, after which cell division and matrix production lead to the development of microcolonies^{35 36}. These early structures are not passive aggregates; they are actively regulated communities in which cells communicate, alter gene expression, and begin producing extracellular polymeric substances (EPS) that provide the scaffold for mature biofilm architecture^{34 37}. EPS is composed of polysaccharides, proteins, lipids, and extracellular DNA, and it plays a decisive role in maintaining structure, retaining water, binding nutrients, and shielding cells from antimicrobial attack^{38 39}. In *P. aeruginosa*, matrix components such as alginate, Psl, and Pel contribute to cohesion and protection, with differences in matrix composition influencing both adherence and tolerance^{40 37}.

Quorum sensing is another major driver of biofilm development because it coordinates population-level behaviour through chemical signalling and regulates genes involved in adhesin expression, matrix synthesis, virulence factor production, and dispersal^{41 42}. The Las, Rhl, and PQS systems allow *P. aeruginosa* to integrate cell density with environmental conditions, making biofilm maturation a regulated developmental programme rather than a random aggregation of cells^{42 43}. This regulatory logic matters for resistance because quorum sensing influences not only virulence but also tolerance, persistence, and communal survival traits that make antibiotic treatment less effective^{44 45}. As biofilms mature, antibiotic penetration becomes increasingly limited by the matrix and by local physicochemical gradients, so the outer layers may be exposed to higher concentrations than the deeper layers, creating a protective gradient that allows some cells to survive treatment^{46 47}. This heterogeneous exposure is one reason biofilms are so recalcitrant: the antibiotic may reach the community but fail to eliminate every embedded cell^{46 34}. Table 2 can be used to summarise the major mechanisms of biofilm-mediated resistance in *P. aeruginosa*, linking each mechanism to its biological process and its contribution to antibiotic failure: surface attachment and matrix production, quorum-sensing regulation, reduced penetration, persister-cell enrichment, metabolic heterogeneity, efflux activity, and altered outer-membrane permeability.

Table 2: Major mechanisms of biofilm-mediated resistance in *Pseudomonas aeruginosa*, their biological processes, and contributions to antibiotic failure

Mechanism	Biological process	Contribution to antibiotic failure
Surface attachment and matrix production	Initial adhesion to surfaces followed by production of an extracellular polymeric substance (EPS) matrix containing alginate, Pel, Psl and extracellular DNA	Provides structural protection, promotes stable biofilm formation and limits antibiotic access to embedded cells
Quorum-sensing regulation	Cell-to-cell signalling through Las, Rhl and PQS systems regulates biofilm development, matrix production and virulence	Coordinates community behaviour and promotes biofilm maturation and persistence, reducing susceptibility to treatment
Reduced antibiotic penetration	The dense EPS matrix and biofilm architecture restrict diffusion and create concentration gradients within the biofilm	Decreases the amount of active antibiotic reaching cells, allowing bacteria in deeper biofilm layers to survive
Persister-cell enrichment	A subpopulation enters a transient, highly tolerant physiological state without necessarily acquiring genetic resistance	Allows a fraction of the population to survive antibiotic exposure and contributes to relapse after treatment

Metabolic heterogeneity	Oxygen, nutrient and metabolite gradients produce cells with different metabolic and physiological states across the biofilm	Slow-growing or metabolically inactive cells are less susceptible to antibiotics that depend on active cellular processes
Efflux activity	Increased activity of multidrug efflux systems exports antibiotics and other toxic compounds from bacterial cells	Reduces intracellular antibiotic concentrations and contributes to multidrug tolerance and resistance
Altered outer-membrane permeability	Changes in outer-membrane porins and permeability modify the entry of antimicrobial compounds into the cell	Restricts antibiotic uptake and lowers intracellular drug concentrations, reducing antimicrobial effectiveness

Another key feature of biofilm resistance is the presence of persister cells and metabolic heterogeneity. Persisters are phenotypically tolerant subpopulations that survive antimicrobial exposure without necessarily carrying classical resistance mutations, and they are often enriched in biofilm communities where nutrient limitation, oxygen gradients, and stress heterogeneity create distinct physiological niches^{35 36}. Because many antibiotics are most effective against active growth, slow-growing or dormant cells in deeper biofilm layers are less vulnerable and can reseed infection after treatment ends^{34 37}. In parallel, efflux pumps and altered permeability reduce intracellular antibiotic accumulation, further weakening treatment efficacy in *P. aeruginosa* biofilms^{32 38}. The RND family efflux systems, especially MexAB-OprM, MexCD-OprJ, MexEF-OprN, and MexXY, contribute to multidrug tolerance by exporting diverse compounds and limiting intracellular drug levels^{32 39}.

Because these mechanisms operate together, biofilm-mediated resistance is not a single trait but a layered defence system involving matrix protection, signal coordination, reduced penetration, altered metabolism, persister survival, and active efflux^{34 40}.

The clinical and environmental consequence is that biofilms promote both antimicrobial tolerance and true resistance. Tolerance allows the community to survive treatment long enough for recovery, while persistent survival increases the chance that resistant mutants, adaptive expression states, or horizontally acquired genes will accumulate over time^{35 41}. In *P. aeruginosa*, this is especially important because biofilm-associated infections often become chronic and difficult to eradicate, and low-dose antibiotic exposure may actually encourage the transition to this sessile lifestyle rather than prevent it^{4,24}. For this reason, the mechanisms summarised in Table 2 show how surface attachment and matrix production, quorum-sensing regulation, reduced penetration, persister-cell enrichment, metabolic heterogeneity, efflux activity, and altered outer-membrane permeability collectively contribute to antibiotic failure.

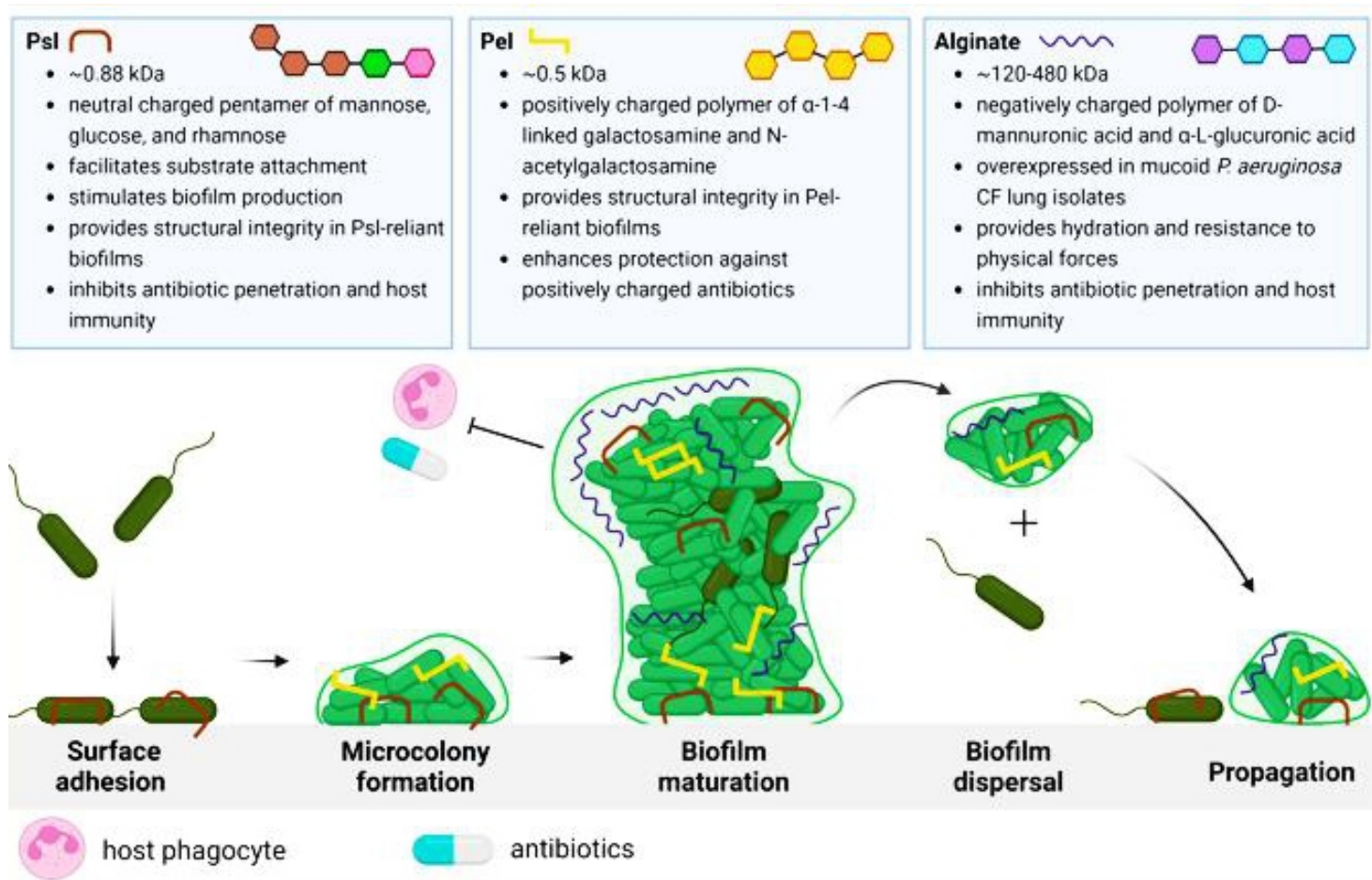


Figure 3. Biofilm development and biofilm-mediated resistance in *Pseudomonas aeruginosa*
Source: [31]

Figure 3 visually depicts the pathway attachment → microcolony → mature biofilm → dispersion, while indicating where EPS, quorum sensing, efflux, and persister cells shape resistance at each stage. Together, these mechanisms explain why biofilms are not simply structural communities but specialised resistance systems that enable *P. aeruginosa* to withstand antimicrobial stress in ways that planktonic cells cannot³⁷⁻⁴¹.

Effects of Sub-Inhibitory Antibiotics on Biofilm Formation

Sub-inhibitory antibiotic exposure plays a central role in shaping how *Pseudomonas aeruginosa* behaves under stress. Concentrations below the minimum inhibitory concentration do not fully prevent visible growth, but they can still alter regulatory pathways, surface behaviour, and community structure in ways that matter for persistence and treatment outcome³⁸.

In *P. aeruginosa*, this is especially important because the organism already combines low outer-membrane permeability, inducible resistance mechanisms, and a strong capacity for biofilm formation, so even modest antibiotic exposure can shift the balance toward survival rather than clearance^{27 32 39 41}. One of the clearest findings across the literature is that sub-MIC exposure can change biofilm biomass, but not in a single predictable direction. The outcome depends on the antibiotic class, the concentration reached, the duration of exposure, and the physiological state of the cells^{38 42}. In *P. aeruginosa*, carbapenems such as imipenem have been associated with thicker and more robust biofilms, while ciprofloxacin and tobramycin have also been reported to induce biofilm formation under sub-inhibitory conditions^{43 44}. These observations show that antibiotic concentrations too low to eradicate the organism may still promote a more protected mode of growth. At the same time, other exposures can reduce selected virulence-associated outputs, which means the biological response is conditional rather than uniform^{45 46}.

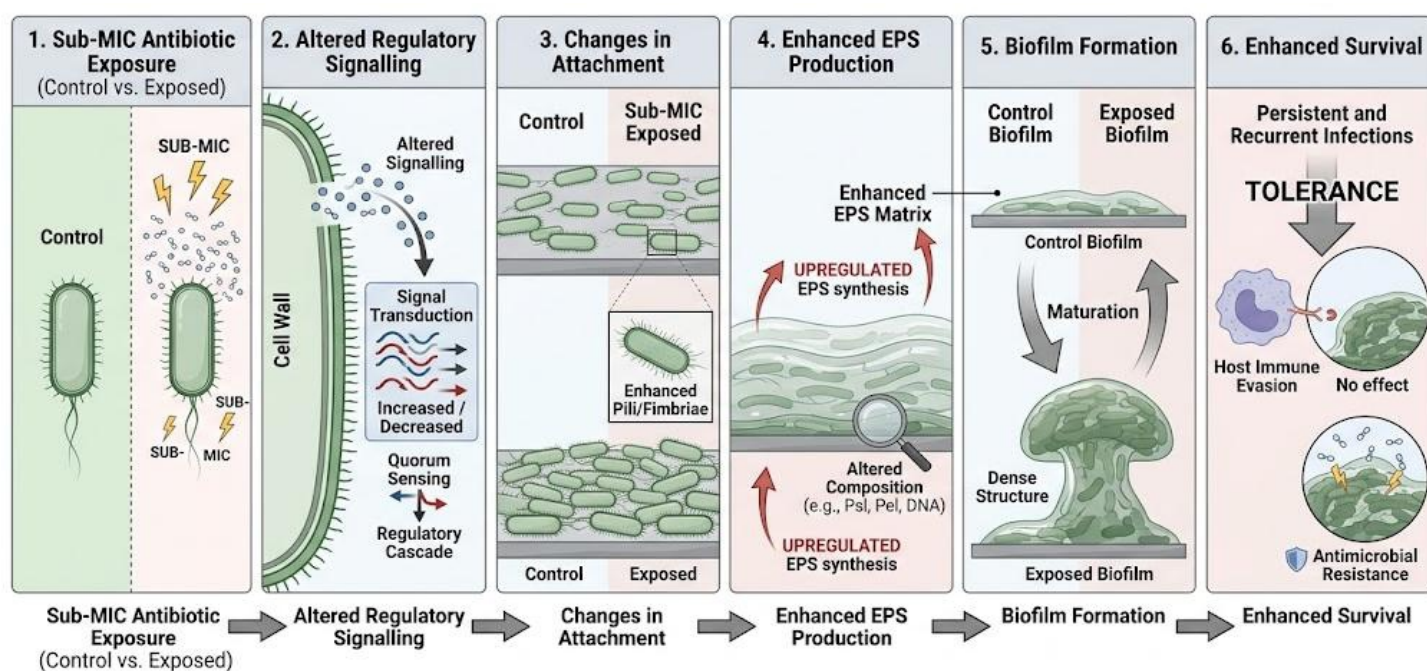


Figure 4. Effect of sub-inhibitory antibiotic exposure on biofilm development in *Pseudomonas aeruginosa*: sub-mic exposure → altered regulatory signalling → changes in attachment and EPS production → biofilm formation and enhanced survival
Source: [31]

Figure 4 illustrates how sub-MIC exposure can alter the regulatory balance between surface attachment, extracellular polymeric substance production, and biofilm development. Changes in biomass are closely linked to changes in the extracellular polymeric substances (EPS) that give the biofilm its structure. The matrix is composed of polysaccharides, proteins, lipids, and extracellular DNA, and it functions as both a scaffold and protective barrier^{40 41 42}. In *P. aeruginosa*, the major matrix-associated components include alginate, Pel, Psl, and extracellular DNA, each of which contributes to cohesion, hydration, and tolerance under stress^{43 44 45}. When sub-inhibitory antibiotics stimulate biofilm development, the effect is therefore not limited to a larger number of attached cells. It can also mean a denser or more organised matrix that slows antibiotic diffusion, creates local concentration gradients, and supports survival of embedded subpopulations⁴⁶. Quorum sensing is another major route through which sub-MIC antibiotics influence biofilm-associated traits.

In *P. aeruginosa*, the Las, Rhl, and PQS systems coordinate behaviours linked to adhesion, matrix production, virulence factor release, and biofilm maturation^{47 48}. Because these systems integrate environmental cues with population density, they are well positioned to respond to antibiotic stress that does not immediately kill the cells. Reviews of sub-inhibitory exposure in *P. aeruginosa* indicate that some antibiotics can suppress quorum-sensing-controlled virulence traits, whereas others may support persistence-related responses that favour biofilm development⁴⁹. Macrolides, particularly azithromycin, are often cited as examples of compounds that reduce production of several virulence factors despite limited direct antipseudomonal activity at standard inhibitory levels⁵⁰. Motility and attachment are also sensitive to low-dose antibiotic exposure. Early biofilm development depends on flagella, type IV pili, and regulated transitions between motile and sessile states⁵¹.

Swarming, swimming, and twitching motility all contribute to surface exploration and microcolony formation, and these behaviours are tied to quorum sensing and broader regulatory networks⁵². Low-dose exposure can therefore perturb the regulatory balance linking movement, attachment, and surface commitment rather than always increasing or suppressing biofilm initiation⁴⁹.

Virulence-associated pathways are altered in parallel. In *P. aeruginosa*, virulence regulation overlaps extensively with biofilm regulation through quorum sensing, stress responses, and global transcriptional control⁴¹. [32] note that tetracycline can induce the type III secretion system and enhance cytotoxicity in vivo, while macrolides can reduce production of several virulence factors. Other work has shown that sub-inhibitory antibiotics may affect pyocyanin, elastase, protease production, alginate-associated traits, and secretion-linked phenotypes, again in a context-dependent manner⁵⁰. These shifts matter because chronic persistence does not require maximal expression of every acute virulence factor. In some settings, reduced motility or altered secretion may coincide with stronger surface association and greater tolerance.

The connection between sub-MIC exposure and increased resistance or tolerance is one of the most important outcomes of this literature. Biofilm induction itself promotes tolerance because matrix-rich communities are harder to eradicate than planktonic populations and contain cells in multiple physiological states⁴⁶. In addition, sub-inhibitory concentrations of protein synthesis inhibitors, including aminoglycosides, tetracycline, and chloramphenicol, can induce the MexXY multidrug efflux system in *P. aeruginosa*²⁵. Morita et al.³² also describe induction of MexCD-OprJ in response to disinfectants such as chlorhexidine and benzalkonium. These responses reduce intracellular drug accumulation and can support survival during later exposure. Ciprofloxacin is especially notable because it has been linked not only to biofilm induction but also to increased frequency of mutation to carbapenem resistance, while longer-term sub-MIC exposure has been associated with adaptive phenotypes involving efflux and altered motility⁴³. Taken together, these findings support the central connection of this section: sub-inhibitory exposure can alter bacterial behaviour in ways that enhance biofilm-associated survival and increase the likelihood of later resistance. The information in Table 3 shows that the effects of sub-inhibitory exposure are class- and context-dependent, but commonly converge on altered biofilm development, signalling, efflux, virulence, and survival.

Table 3: Synthesis of the effects of sub-inhibitory antibiotic exposure on biofilm-associated traits in *Pseudomonas aeruginosa*

Antibiotic/class	Reported response	Major affected trait	Implication
Carbapenems (e.g., imipenem)	Thicker and more robust biofilms	Biofilm biomass and structure	Increased protected growth
Fluoroquinolones (e.g., ciprofloxacin)	Biofilm induction and adaptive changes	Biofilm formation, mutation and resistance	Greater potential for persistence and later resistance
Aminoglycosides (e.g., tobramycin)	Biofilm induction and efflux responses	Biofilm formation and drug export	Reduced intracellular antibiotic accumulation
Tetracycline	Altered virulence and efflux-related responses	Type III secretion and MexXY activity	Enhanced adaptation under exposure
Macrolides (e.g., azithromycin)	Reduced production of several virulence factors	Quorum sensing and virulence	Context-dependent changes in persistence and pathogenic behaviour
Disinfectants	Induction of MexCD-OprJ	Efflux activity	Increased tolerance to subsequent antimicrobial exposure

Source: Ramsay et al.³

Environmental and Clinical Implications

The importance of sub-inhibitory antibiotic exposure extends beyond laboratory culture because *P. aeruginosa* moves readily between environmental and clinical settings. Antibiotics released into wastewater do not need to remain at therapeutic concentrations to matter biologically. At lower levels, they can still shape microbial communities, maintain selective pressure, and favour organisms able to persist through stress adaptation, efflux, and biofilm formation⁴⁸. This is especially relevant for pharmaceutical wastewater, where antibiotic residues may enter treatment systems in mixtures and at fluctuating concentrations that are unlikely to sterilise bacteria but may still alter their behaviour. *P. aeruginosa* is well suited to exploit such environments because it is widely distributed in water, soil, plumbing systems, and other moist built environments, and it tolerates nutrient limitation, oxidative stress, and chemical exposure better than many organisms^{27 32 41}. These traits make wastewater-associated habitats plausible reservoirs for persistence, where repeated exposure to sub-inhibitory antibiotics, disinfectants, and other stressors may favour adaptive responses, including biofilm formation and efflux induction^{24 39}.

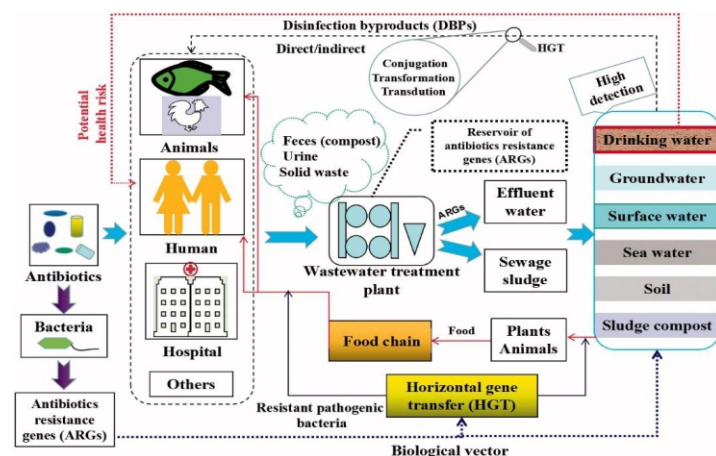


Figure 5. Environmental-to-clinical pathway of sub-inhibitory antibiotic exposure
Source: [14]

Figure 5 illustrates how environmental antibiotic exposure can connect pharmaceutical effluents with clinical consequences. The environmental dissemination of resistant *P. aeruginosa* is concerning because biofilm growth increases the likelihood of long-term survival on surfaces, sediments, pipes, tanks, and treatment infrastructure.

Biofilms protect cells from hydraulic disturbance, nutrient fluctuation, and antimicrobial exposure, while also supporting physiological heterogeneity within the community^{45 48}. Once a resistant or tolerance-prone population becomes established in an environmental biofilm, it may persist even when bulk antibiotic concentrations decline, and cells released from these biofilms can seed downstream waters, contact surfaces, or engineered systems.

The boundary between environmental and clinical settings is therefore porous. *P. aeruginosa* is an opportunistic pathogen, so strains that persist in environmental reservoirs may later reach susceptible hosts through water contact, contaminated surfaces, medical devices, or hospital plumbing systems. The same traits that support environmental persistence can complicate treatment after transmission. Biofilm formation on catheters, ventilator equipment, sinks, drains, and wound surfaces creates reservoirs that are difficult to disinfect and difficult to treat once infection is established^{49 50}. Sub-inhibitory exposure remains relevant in these settings because incomplete antibiotic penetration, residual disinfectants, and fluctuating drug levels during therapy can recreate the same low-dose conditions that favour adaptive responses^{51 52}. These links help explain why *P. aeruginosa* is so often associated with hospital-acquired and difficult-to-treat infections, including chronic wound infection, ventilator-associated pneumonia, catheter-related infection, and persistent lung infection^{47 51}. Inadequate pharmaceutical wastewater treatment increases this risk because it allows both antibiotic residues and resistant bacteria to remain in circulation. If treatment lowers concentrations without removing biologically active compounds, it may still leave behind a selective environment in which susceptible cells are disadvantaged and tolerant or adaptive populations persist^{45 46}. The concern becomes greater when wastewater also contains disinfectants or mixed pollutants capable of inducing additional resistance mechanisms, including efflux responses⁵¹. These patterns support a One Health perspective in which human antibiotic use, industrial discharge, environmental contamination, microbial adaptation, and clinical infection are connected parts of the same system.

Monitoring, Control and Future Perspectives

Because the problem begins before infection, control has to begin upstream. Improved pharmaceutical wastewater treatment is one of the most important interventions because it reduces the chance that biologically active antibiotic residues will enter wider environmental systems. Conventional treatment may lower contaminant loads, but reduction alone is not enough if the remaining concentrations can still alter bacterial behaviour^{45 46 23}. Antibiotic-residue monitoring is therefore essential and should track not only individual antibiotics but also mixtures that may exert combined selective effects. This is particularly relevant for *P. aeruginosa*, whose responses vary by antibiotic class, exposure duration, and growth mode^{51 49}.

Figure 5 presents the control pathway from prevention at the source through wastewater treatment, environmental monitoring, resistance surveillance, and risk management. Surveillance should extend beyond chemistry to include resistant bacteria and resistance genes because wastewater systems contain both the selective agents and the organisms responding to them.

Tracking *P. aeruginosa* occurrence, biofilm-forming capacity, and relevant resistance determinants would provide a more realistic assessment of risk, especially where biofilms have already formed on treatment surfaces or downstream infrastructure^{1 2 3}. This combined approach is more useful than residue monitoring alone because the biological outcome depends on both exposure and community composition.

Advanced treatment approaches deserve attention because they may improve removal of biologically active residues that conventional systems leave behind. Advanced oxidation processes can help degrade persistent antibiotic molecules, while biological treatment and polishing systems may improve transformation and removal under controlled conditions. Even so, these approaches have practical limits⁷. Oxidative treatment can be costly and may generate transformation products whose biological effects still need evaluation, while biological systems may vary in performance depending on operating conditions and influent composition. Source control is equally important. Responsible antibiotic production, better containment of manufacturing losses, and stricter management of waste streams can reduce the burden placed on downstream treatment systems. If fewer active residues enter wastewater in the first place, there is less opportunity for sub-inhibitory exposure to shape environmental bacterial behaviour¹⁰.

Another priority is standardised environmental risk assessment. Many current frameworks focus on toxicity to higher organisms or on broad contamination indicators, but sub-inhibitory antibiotic effects require a more microbiologically focused approach. Risk assessment should consider whether measured concentrations are capable of altering bacterial signalling, biofilm formation, efflux activity, or resistance selection^{4 5 6}. It should also account for repeated exposure, mixture effects, and the fact that biofilm-associated communities may experience local concentrations very different from those measured in bulk water. Further research is still needed, especially on how sub-MIC exposure shapes biofilm responses under realistic environmental conditions. Much of the available evidence comes from controlled laboratory systems, short-term exposures, or single-antibiotic experiments^{5 6}. Future work should therefore connect mechanistic microbiology with environmental monitoring and treatment design by studying not only whether a residue is present, but whether it changes attachment, matrix production, quorum sensing, efflux induction, or persistence in ways that matter outside the laboratory.

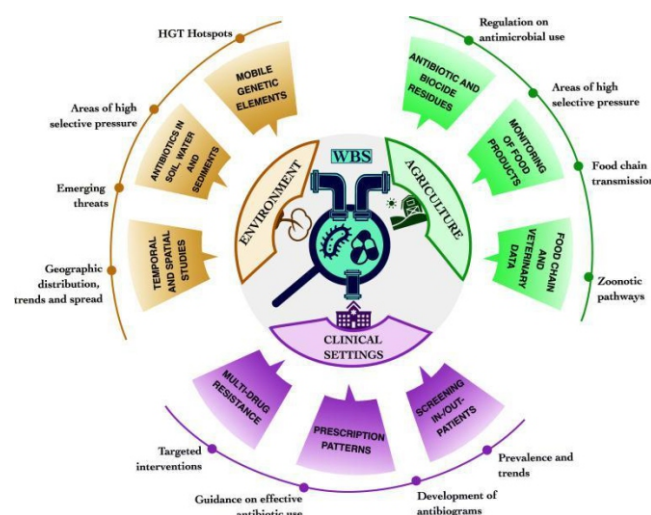


Figure 6. Integrated control framework for sub-inhibitory antibiotic exposure
Source: [31]

Conclusion

Pharmaceutical effluents can expose environmental bacteria to antibiotic concentrations that are too low to kill but still high enough to alter behaviour. In *Pseudomonas aeruginosa*, these exposures can affect attachment, signalling, matrix production, motility, and efflux activity, all of which shape the transition toward biofilm-associated persistence. Once biofilms are established, the organism gains layered protection through the EPS matrix, reduced antibiotic penetration, physiological heterogeneity, and survival of tolerant subpopulations. Taken together, the evidence shows that sub-inhibitory antibiotic exposure can reshape biofilm-associated traits and increase the likelihood of persistence and resistance, although the outcome depends on the antibiotic, concentration, and surrounding conditions. This makes pharmaceutical wastewater an important environmental concern, not only as a source of residues but as a setting in which resistant and biofilm-forming populations may be maintained and dispersed. Better effluent treatment, stronger surveillance, and more realistic mechanistic research are therefore essential if the aim is to limit the environmental conditions that support biofilm-mediated resistance in *P. aeruginosa*.

Authors' Contributions

The authors of this research have significantly contributed to the study's conception, data collection, and manuscript development. All authors were involved in writing the manuscript or critically reviewing it for its intellectual value. They have reviewed and approved the final version for submission and publication and accepted full responsibility for the content and integrity of the work.

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Conflict of Interest

The authors declared that there are no conflicts of interest.

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