



ISSN : 2347 - 2243

Indo - American Journal of Life Sciences and Biotechnology



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Biofilm Formation by Clinical Isolates of *Pseudomonas aeruginosa* and Its Correlation With Antibiotic Resistance

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Received: March 24, 2014 Accepted: August 6, 2014 Published: December 28, 2014

DOI: [10.62644/iajlb.2014.v11.i4.pp1-8](https://doi.org/10.62644/iajlb.2014.v11.i4.pp1-8)

ABSTRACT

Pseudomonas aeruginosa is an opportunist that causes stubborn infections in hospitals and in people with damaged airways, and two of its habits are blamed for much of that stubbornness: it forms biofilms, and it resists many antibiotics. It is often assumed that the isolates which build the thickest biofilms are also the most resistant. This narrative review asks how far that assumption can be defended. It draws on published work on biofilm development, on the genetics of resistance, and on the laboratory methods used to measure both, and it reports no new isolates and no new data. Biofilm growth protects cells through slow diffusion, oxygen limitation, dormant subpopulations, matrix components and biofilm-specific genes, while conventional resistance rests on efflux, enzymes, target changes and reduced permeability. The two sets of mechanisms overlap but are not the same, so a correlation across a collection of isolates is possible without being necessary, and its direction can depend on who is sampled. Differences in assay design, growth medium, plate surface and definitions of resistance make published comparisons hard to line up. The review concludes that biofilm capacity and resistance should be reported together but interpreted separately, and it sets out what a convincing comparison of clinical isolates would need to include.

Keywords: *Pseudomonas aeruginosa*; Biofilm; Antibiotic resistance; Clinical isolates; Antibiotic tolerance; Crystal violet assay; Narrative review

Citation: Cifuentes, Kesselring and Tornatore [2014]. Biofilm Formation by Clinical Isolates of *Pseudomonas aeruginosa* and Its Correlation With Antibiotic Resistance. *Indo-Am. J. Life Sci. Biotechnol.* 11(4):1-8. DOI: [10.62644/iajlb.2014.v11.i4.pp1-8](https://doi.org/10.62644/iajlb.2014.v11.i4.pp1-8)

Article Class: Narrative Review

1. Introduction

Pseudomonas aeruginosa is a Gram-negative rod of soil, water and wet hospital surfaces. It seldom troubles healthy people, yet it is a leading cause of infection in those with burns, ventilated

lungs, indwelling devices or cystic fibrosis (1, 2). Its reputation among clinicians rests on a stubborn double character: it is intrinsically unresponsive to many antibiotics, and it readily grows as a biofilm, a surface-attached community



embedded in a self-made matrix (3, 4).

Cells inside biofilms survive drug concentrations that would clear the same cells growing freely, and biofilm infections tend to relapse after treatment appears to have worked (5, 6). It is therefore tempting to assume that clinical isolates which form the heaviest biofilms in the laboratory must also be the most resistant on a susceptibility report.

This review sets out how biofilm formation, resistance and tolerance arise in *P. aeruginosa*, how they are measured in clinical isolates, and what the published work says about a correlation between them. We report no isolates of our own and no local surveillance data; the question is treated through the published literature in general. The aim is to be clear about which parts of the story are settled and which are only repeated.

2. Literature Review

2.1 *Pseudomonas aeruginosa* as a Pathogen

Its success owes much to a large, flexible genome and many virulence factors (1). It is also one of the group of pathogens, often abbreviated ESKAPE, singled out as a threat because treatment options are narrowing (7).

2.2 How a Biofilm Forms

Biofilm development is usually described as a sequence. Single cells attach to a surface, a step in which flagella and type IV pili play a part (8); they multiply and gather into microcolonies; a matrix accumulates around them; the community matures into a thick, structured form; and cells eventually disperse to start again, as outlined in Figure 1 and Table 1. A biofilm is thus not one physiological state but several side by side (9).

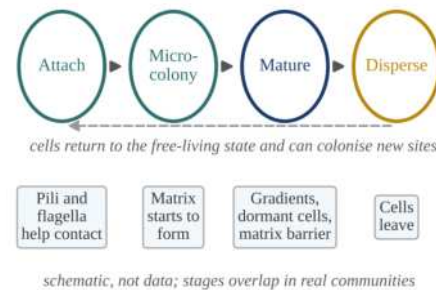


Figure 1. Development cycle of a *Pseudomonas aeruginosa* biofilm, with the points at which tolerance is thought to arise (schematic, not data).

Table 1. Stages of *Pseudomonas aeruginosa* biofilm development and their relevance to antibiotic response.

Stage	What happens	Relevance to resistance
Attachment	Cells settle on a surface, aided by flagella and pili (8)	Little protection yet
Microcolony	Growth and aggregation; matrix production begins (9)	Gradients start to form
Maturation	Thick, structured community in a matrix (10, 11)	Strongest tolerance reported (5)
Dispersal	Cells leave and may seed new sites (9)	Released cells may seed new sites

Note: Qualitative summary of the cited reviews; stages overlap and their timing varies with strain and conditions.

The matrix is a mixture of exopolysaccharides, extracellular DNA, proteins and lipids that holds the community together and shapes its chemistry (10). Extracellular DNA is needed for early biofilm formation in this species (12), and the polysaccharides called Pel and Psl contribute to matrix structure and attachment in laboratory strains (11, 13). Strains differ in which components they emphasise, so one laboratory model cannot stand for every clinical isolate. This helps to explain why the same organism can look tolerant in one



report and ordinary in the next.

2.3 Signals and Switches

Two regulatory systems recur in this literature. Cell-to-cell signalling, often called quorum sensing, links gene expression to population density, and mutants lacking one of its signals were reported to form flat, abnormal biofilms (14). The second is the intracellular messenger cyclic di-GMP, whose high levels generally favour a sessile lifestyle and whose low levels favour motility; a chemosensory system in *P. aeruginosa* has been shown to change those levels in response to surface contact (15, 16).

2.4 Resistance and Tolerance

Conventional resistance in *P. aeruginosa* comes from low outer-membrane permeability, multidrug efflux pumps, an inducible chromosomal beta-lactamase, target mutations and aminoglycoside-modifying enzymes (17, 18, 19). These mechanisms are measured in planktonic cells, usually by determining the lowest concentration of a drug that stops growth.

Biofilm tolerance is a different thing, and the vocabulary matters. A drug that fails to clear a biofilm may do so because of poor penetration, local oxygen limitation, slow growth, a small population of dormant persister cells (20), or a handful of biofilm-specific genes, with several acting at once (21, 22, 23). Table 2 and Figure 2 set these beside the conventional mechanisms. Experiments with this species suggest that oxygen limitation and low metabolic activity matter more than simple failure of a drug to enter (24, 25). A mechanism that works only in a deep, crowded community is not one that a standard susceptibility test would ever reveal. Separating these explanations in a clinical isolate is rarely straightforward.

Table 2. Resistance mechanisms in planktonic cells compared with those proposed for biofilms.

Feature	Planktonic cells	Biofilm cells
Efflux	Pumps with broad substrate range (18)	Pumps plus biofilm-linked systems (26, 27)
Enzymes	Chromosomal beta-lactamase (19)	Induction under drug exposure (28)
Permeability	Porin changes and altered uptake (17)	Matrix may slow entry (24)
Growth state	Mostly dividing	Slow or dormant fractions (20, 25)
Genetic basis	Mutation or acquired genes (19)	Mostly expression changes (29)

Note: The mechanisms overlap in practice; the table separates them for clarity only.

Some biofilm-specific mechanisms have been characterised at gene level. Periplasmic glucans encoded by one locus were found to bind aminoglycosides and to contribute to biofilm antibiotic resistance (29), and a separate efflux system has been implicated in resistance seen only in the biofilm state (26). Exposure to a beta-lactam was reported to increase beta-lactamase and alginate production in biofilms (28), and tolerance to colistin was linked to metabolically active subpopulations and to genes also involved in conventional resistance (27). Extracellular DNA can chelate cations and so induce resistance to some drugs (30).

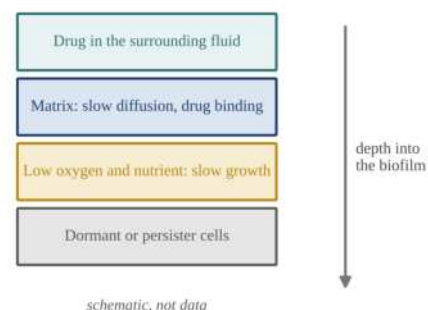


Figure 2. Layers of protection proposed for biofilm cells, from outer matrix to inner dormant core (schematic of a conceptual model, not data).



2.5 Measuring Biofilm and Resistance in Isolates

In diagnostic and research laboratories, biofilm formation is most often judged with a microtitre plate stained with crystal violet, a method adapted from work on staphylococci and later standardised in a protocol for *Pseudomonas* (31, 32). Other approaches include confocal imaging with image analysis software that reports thickness and structure (33), and devices that grow biofilms on pegs so that their drug susceptibility can be tested directly (34, 35). Table 3 summarises these methods.

Table 3. Methods used to measure biofilm formation and what each one leaves out.

Method	What it reports	Main limitation
Crystal violet, microtitre plate	Total attached biomass (31, 32)	Stains dead cells and matrix alike
Confocal imaging and COMSTAT	Structure and thickness (33)	Few isolates per run
Calgary device	Biofilm susceptibility to drugs (34)	Pegs differ from tissue
Biofilm susceptibility for clinical isolates	Practical drug testing (35, 36)	Not standardised across laboratories

Note: Descriptions are of the methods as published; none gives a clinically validated cut-off.

The choice of method has consequences. A crystal violet reading reflects total attached material, living or dead, and does not say how tolerant the community is. When biofilm susceptibility has been compared with planktonic susceptibility in isolates from patients with cystic fibrosis, results under biofilm conditions differed from the standard report for some drugs (35, 36). Such differences look small on a single plate, but they decide whether two studies can be compared at all, so the method belongs in any honest summary of the

result.

3. Materials and Methods

3.1 Scope of the Review

This is a narrative review. Its question is whether biofilm formation by clinical isolates of *P. aeruginosa* tracks their antibiotic resistance, and by what mechanisms such a link might arise. It does not present new laboratory work. None of these checks is hard, but each one is easy to leave out.

3.2 Literature Assembly

Sources were gathered by reading and by following citations from reviews and key papers, not by a formal systematic search, and no search string, database list or screening protocol was applied. Selection therefore reflects the judgement of the authors, and relevant studies may have been missed. Only work published by the end of 2014 is cited.

3.3 Appraisal Framework

Each source was read for four things: whether the isolates were clinical or laboratory strains, how biofilm was measured, how resistance was defined, and whether the conclusions went beyond what the design allowed. No numerical values from the studies are pooled or re-analysed here.

4. Results

4.1 What the Literature Shows About the Link

The central finding of this review is that no simple rule has been established. Mechanistically there are reasons to expect a positive association: a regulator that promotes matrix production may also change drug permeability or efflux, and a strain that has spent years under antibiotic pressure may have accumulated both resistance mutations and altered biofilm behaviour. One study of phenotypic



variants tied biofilm formation and antibiotic resistance to the same variation (37).

There are equally good reasons to expect no association. Conventional resistance determinants and biofilm determinants are mostly different genes, and tolerance in the biofilm state is largely a property of the community, not of the cell (5, 38). An isolate that is susceptible in a standard test can still be hard to eradicate on a surface, and a highly resistant one may be a poor biofilm former. That is a modest conclusion, and it is the one the published work can bear.

4.2 Why Collections of Isolates Disagree

Where studies of clinical collections disagree, the explanation is probably methodological and ecological. The sign of an association in a collection depends on who has been sampled. Unrelated hospital isolates mix lineages with different histories, chronic airway isolates mix adaptation with treatment, and a single outbreak clone offers little variation to correlate (Figure 3). A population that has lived for years in one airway behaves differently from one sampled at admission.

Collection of isolates	What may blur a link
Mixed hospital isolates	Many unrelated lineages
Chronic airway isolates	Adapted strains, prior therapy
Single outbreak clone	Little variation to compare
Device-related isolates	Surface and exposure differ

qualitative, not data

Figure 3. Collections of isolates in which a link between biofilm capacity and resistance may be blurred (qualitative matrix, not data).

Technical choices add noise: medium, plate surface, incubation time, washing and the threshold for calling an isolate a strong or weak former all differ between

laboratories. Definitions of resistance differ too, since a single drug, a drug class or a multidrug category may be used (39). The point is not that an assay is poor, only that its result travels less far than its summary suggests.

4.3 Clinical Settings

In cystic fibrosis the lung is thought to hold the bacterium as a biofilm, since signalling molecules typical of surface-attached communities have been detected in sputum (40), and tests under biofilm conditions give a different picture from standard reports (35, 36). In device-associated infection, the surface itself is part of the problem, and removal of the device is often more decisive than the choice of drug (41, 42). In both settings the link between a laboratory biofilm score and the response of a patient has not been shown in a way that supports routine use of the score (43).

A clinician given a biofilm score alongside a susceptibility result would like to use both, yet the literature offers no validated threshold and no agreed way to combine them. Until it does, the two reports are best treated as separate pieces of information about the same organism. A clinician reading a laboratory report wants to know whether a strong biofilm former is also likely to be a difficult one to treat.

5. Discussion

5.1 Interpretation

The main message is conditional. Biofilm capacity and antibiotic resistance are not the same property, even though they overlap in mechanism and in clinical consequence. A correlation may be found in some collections and not in others, and either result is compatible with the underlying biology. Neither a positive correlation as proof of causation nor a null result as proof of independence is justified (3, 41). Read this way, the absence of a



correlation in a small collection is not evidence that none exists.

5.2 Limitations

This review has the limitations of its kind. The literature was not searched systematically, so selection bias is possible, and the weight given to individual studies reflects our judgement. Much of the mechanistic work uses a few laboratory strains under conditions that may not reflect infected tissue (11, 38). Reviews of this kind are more useful when they mark where the evidence stops than when they smooth it over.

5.3 Future Work

A convincing comparison of clinical isolates would need several features. Isolates should come from a defined source, with relatedness known. Biofilm should be measured by more than one method, including one that reports tolerance. Resistance criteria and assay conditions should be stated in full. Analysis should allow for related isolates being counted more than once. It is a habit worth keeping even when the answer seems obvious.

6. Conclusion

Pseudomonas aeruginosa forms biofilms and resists antibiotics, and both help to explain why its infections persist. Whether the isolates that do the first also do the second remains unsettled. The published work supplies mechanisms for a positive association, mechanisms for none, and enough methodological variation to account for the mixed picture. Reports of correlation should therefore be read in the light of how isolates were chosen and how each trait was defined and measured.

For the practitioner, a biofilm score is additional information and not a substitute for susceptibility testing or clinical judgement. For the researcher, the

opportunity lies in comparisons that report both traits together and interpret them separately. These cautions apply to any organism in which tolerance and resistance are studied together, not only to this one.

7. Acknowledgements

The authors thank colleagues who commented on earlier drafts of this review.

8. Funding

This work received no specific grant from any funding agency in the public, commercial or not-for-profit sectors.

9. Conflict of Interest

The authors declare no conflict of interest.

10. Author Contributions

N.C. conceived the review and wrote the sections on biofilm biology. W.K. wrote the sections on resistance and tolerance. G.T. wrote the sections on laboratory methods and clinical settings. All authors revised the manuscript and approved the final version.

11. Ethics Statement

This is a review of published literature. No human or animal subjects were involved and no ethical approval was required.

12. Data Availability

No new data were generated. All sources are cited in the reference list.

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