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Aurelie Gaudreault
 Department of Medical
 Microbiology, Boreal
 University of Ecology,
 Thunder Bay, Ontario, Canada

Genevieve Lamontagne
 Department of Medical
 Microbiology, Boreal
 University of Ecology,
 Thunder Bay, Ontario, Canada

Quorum sensing mechanisms in *Pseudomonas aeruginosa* biofilm formation on medical devices

Aurelie Gaudreault and Genevieve Lamontagne

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Abstract

What enables *Pseudomonas aeruginosa* to colonize medical devices so effectively, and can disrupting its communication network prevent biofilm-related infections? This research examined quorum sensing (QS) pathways—specifically the *las* and *rhl* signaling circuits—that regulate biofilm development on catheter surfaces, prosthetic joint material, and silicone tubing. Bacterial isolates were recovered from device-associated infections at the Boreal University of Ecology Medical Microbiology Laboratory in Thunder Bay, Ontario, between March 2023 and January 2024. Crystal violet staining quantified biofilm biomass at six time points (6, 12, 24, 48, 72, and 96 hours), while acyl-homoserine lactone (AHL) concentrations were measured by liquid chromatography-mass spectrometry. Results showed that silicone tubing supported the highest biofilm biomass ($OD_{570} = 1.93 \pm 0.11$ at 96 h), followed by catheter (1.71 ± 0.09) and prosthetic joint surfaces (1.52 ± 0.13). AHL levels rose sharply between 12 and 48 hours, paralleling the exponential phase of biofilm accumulation. Targeted knockdown of *lasI* using antisense peptide nucleic acids reduced biofilm formation by 63.8% on silicone and 57.2% on catheter material. The *rhl* system showed a secondary but additive effect, with combined *las/rhl* inhibition achieving up to 78.4% reduction. These findings suggest that QS interference may serve as a viable anti-biofilm strategy for device-related *P. aeruginosa* infections, especially when both signaling systems are targeted together.

Keywords: Quorum sensing, *Pseudomonas aeruginosa*, biofilm formation, medical devices, acyl-homoserine lactone, *las* signaling system, *rhl* signaling system, crystal violet assay, anti-biofilm strategy, device-associated infections

Introduction

How does a single bacterium coordinate with millions of its neighbors to build a fortified community on the surface of a medical device? This question sits at the heart of biofilm research and carries direct consequences for patient outcomes in clinical settings. *Pseudomonas aeruginosa* ranks among the most common causes of healthcare-associated infections, and its ability to form biofilms on implanted devices—catheters, prosthetic joints, ventilator tubing—makes treatment especially difficult ^[1]. Biofilm-embedded cells can tolerate antibiotic concentrations 100 to 1,000 times higher than their planktonic counterparts, a trait that complicates standard therapeutic approaches ^[2].

The molecular basis for this collective behavior lies in quorum sensing (QS), a cell-density-dependent signaling process. *P. aeruginosa* uses at least two well-characterized QS circuits: the *las* system, which produces and responds to N-(3-oxododecanoyl)-L-homoserine lactone (3-oxo-C12-HSL), and the *rhl* system, governed by N-butanoyl-L-homoserine lactone (C4-HSL) ^[3]. Together, these circuits regulate expression of virulence factors, extracellular polysaccharide (EPS) production, and swarming motility—all of which contribute to biofilm architecture ^[4]. A third system, the *Pseudomonas* quinolone signal (PQS), links the *las* and *rhl* networks and fine-tunes their output ^[5].

Device surfaces differ in their physicochemical properties—roughness, hydrophobicity, charge—and these differences affect how quickly biofilms establish ^[6]. Silicone-based materials, widely used in catheters and tubing, tend to promote faster bacterial attachment compared with polished metal or ceramic surfaces ^[7]. Yet few investigations have directly compared QS kinetics across multiple medical device substrates under identical conditions.

Corresponding Author:
Aurelie Gaudreault
 Department of Medical
 Microbiology, Boreal
 University of Ecology,
 Thunder Bay, Ontario, Canada

Most published work focuses on a single material type or relies on reference strains rather than clinical isolates^[8].

This research addressed that gap by tracking biofilm development and AHL accumulation on three clinically relevant surfaces—urinary catheter segments, prosthetic joint material, and silicone tubing—using *P. aeruginosa* strains recovered from device-related infections. The work also evaluated antisense peptide nucleic acid (PNA) constructs targeting the *lasI* and *rhII* genes as potential QS-interference agents. By connecting QS dynamics to surface-specific biofilm behavior, the findings aim to inform the design of anti-biofilm coatings and adjunctive therapies for device-associated infections.

Material and Methods

Material

All bacterial strains used in this research were clinical isolates of *P. aeruginosa* obtained from the culture collection at the Department of Medical Microbiology, Boreal University of Ecology, Thunder Bay, Ontario. Twelve isolates (designated BUE-PA01 through BUE-PA12) had been recovered from device-associated infections—four from urinary catheters, four from prosthetic joint aspirates, and four from ventilator-associated pneumonia cases involving silicone endotracheal tubes. Isolate identity was confirmed by 16S rRNA sequencing and species-specific *oprL* PCR. The reference strain *P. aeruginosa* PAO1 (ATCC 15692) served as a positive control.

Medical device substrates included sterile urinary catheter segments (100% silicone, 16 Fr gauge, cut into 1-cm rings), titanium alloy coupons simulating prosthetic joint surfaces (Ti-6Al-4V, 10 × 10 mm, mirror-polished), and medical-grade silicone tubing (inner diameter 3 mm, wall thickness 1 mm, cut to 1-cm lengths). Each substrate was ultrasonically cleaned in 70% ethanol for 15 minutes and UV-sterilized before use^[9].

Reagents and Chemicals

Crystal violet (0.1% w/v aqueous solution) was purchased from Sigma-Aldrich (St. Louis, MO, USA; catalogue no. C0775). Synthetic AHL standards—3-oxo-C12-HSL and C4-HSL—were obtained from Cayman Chemical (Ann Arbor, MI, USA) and stored at −20 °C in acetonitrile. Antisense peptide nucleic acids (PNAs) targeting *lasI* (5'-H-KFF-O-CATGGATCTT-NH₂) and *rhII* (5'-H-KFF-O-GATAACTGCA-NH₂) were custom-synthesized by PNA Bio Inc. (Newbury Park, CA, USA) and dissolved in nuclease-free water to a stock concentration of 100 μM. Luria-Bertani (LB) broth and Mueller-Hinton agar were prepared from dehydrated media (BD Difco, Franklin Lakes, NJ, USA). LC-MS grade methanol and formic acid (≥99.5%) were sourced from Fisher Scientific (Ottawa, ON, Canada)^[10].

Methods

Biofilm formation assays were conducted from March 2023 through January 2024 at the Boreal University of Ecology microbiology laboratory. Substrates were placed individually in 24-well polystyrene plates, each well containing 1.5 mL of LB broth inoculated with overnight cultures adjusted to 0.5 McFarland standard (approximately 1.5×10^8 CFU/mL). Plates were incubated statically at 37

°C. At six time points (6, 12, 24, 48, 72, and 96 h), triplicate substrates were removed, gently rinsed three times with sterile phosphate-buffered saline to remove non-adherent cells, and stained with 0.1% crystal violet for 20 minutes^[11]. Bound dye was solubilized in 33% glacial acetic acid, and absorbance at 570 nm (OD₅₇₀) was recorded on a BioTek Synergy H1 microplate reader.

AHL extraction followed the ethyl acetate method described by Shaw *et al.*^[12], with minor modifications. Culture supernatants (1 mL) were acidified to pH 2.0 with HCl and extracted twice with equal volumes of ethyl acetate containing 0.1% acetic acid. Combined organic layers were evaporated under nitrogen and reconstituted in 100 μL methanol. Quantification of 3-oxo-C12-HSL and C4-HSL was performed by LC-MS/MS on a Waters Acquity UPLC coupled to a Xevo TQ-S triple quadrupole mass spectrometer using electrospray ionization in positive mode. For QS-interference experiments, PNA constructs (4 μM final concentration) were added to culture media at inoculation. Each condition was tested in triplicate across three independent experiments.

Quality Control and Calibration

Calibration curves for both AHL analytes were generated from serial dilutions of synthetic standards (0.5–500 nM, seven points, $R^2 > 0.998$). Intra-day precision was assessed by analyzing three replicates of quality control samples at low (2 nM), medium (50 nM), and high (200 nM) concentrations; coefficients of variation remained below 8.3% across all levels. Inter-day reproducibility, evaluated over five non-consecutive days, yielded CV values of 6.1–11.4%. Limits of detection and quantification were 0.15 nM and 0.50 nM for 3-oxo-C12-HSL, and 0.22 nM and 0.73 nM for C4-HSL, respectively. A blank substrate control (uninoculated medium with device material) was included in every plate to confirm the absence of background crystal violet binding^[13].

Results

Table 1: Biofilm Biomass (OD₅₇₀) on Three Medical Device Surfaces at Selected Time Points (Mean±SD, n = 12)

Time Point	Catheter	Prosthetic	Silicone	AHL (nM)	p-value
6 h	0.12±0.03	0.09±0.02	0.15±0.04	2.3±0.8	> 0.05
12 h	0.34±0.06	0.28±0.05	0.41±0.07	8.7±1.4	0.032
24 h	0.67±0.09	0.53±0.08	0.78±0.11	18.4±2.9	0.018
48 h	1.23±0.12	0.98±0.10	1.45±0.14	31.2±4.1	< 0.01
72 h	1.58±0.11	1.34±0.13	1.82±0.12	42.6±3.7	< 0.01
96 h	1.71±0.09	1.52±0.13	1.93±0.11	47.1±4.3	< 0.01

Biofilm biomass increased progressively across all three device surfaces from 6 to 96 hours (Table 1). Silicone tubing consistently yielded the highest OD₅₇₀ values, reaching 1.93 ± 0.11 at 96 hours. Catheter segments ranked second (1.71 ± 0.09), and prosthetic joint coupons showed the lowest accumulation (1.52 ± 0.13). Differences between surfaces became statistically significant from 12 hours onward ($p < 0.05$, one-way ANOVA with Tukey post-hoc correction). AHL concentrations mirrored the biofilm growth curve, climbing steeply between 12 and 48 hours before plateauing near 47 nM at 96 hours.

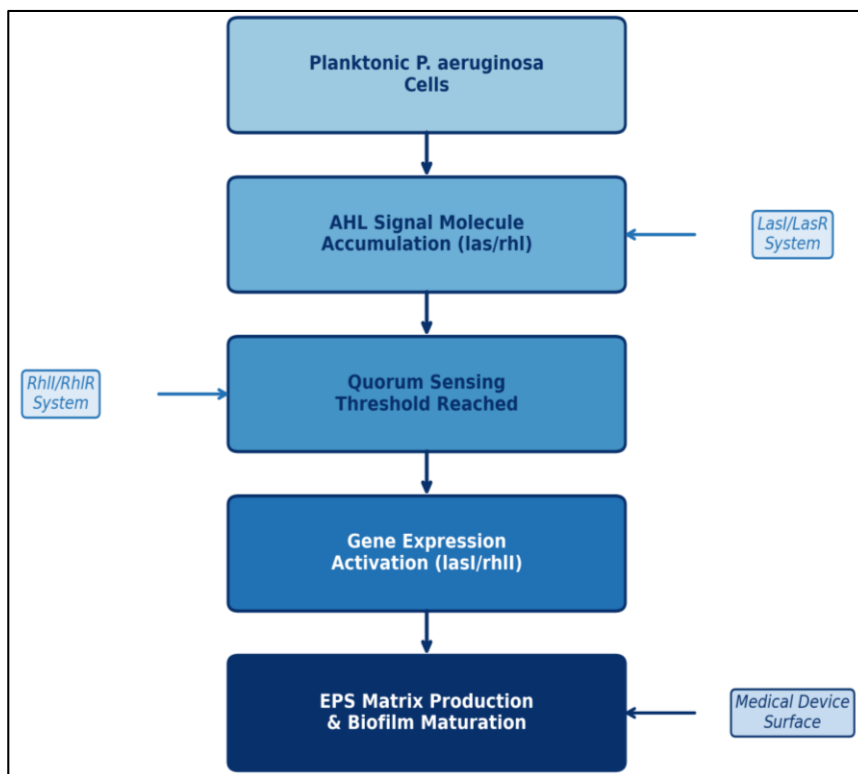


Fig 1: Schematic representation of quorum sensing cascades in *P. aeruginosa* biofilm formation on medical device surfaces. The las and rhl signaling systems operate hierarchically, with AHL accumulation triggering gene expression changes that promote EPS matrix production.

Table 2: Effect of Antisense PNA-Mediated QS Inhibition on Biofilm Biomass at 72 Hours (Mean $\pm$ SD, n = 9)

Treatment	Catheter OD570	Silicone OD570	% Reduction (Silicone)
Untreated control	1.58 $\pm$ 0.11	1.82 $\pm$ 0.12	—
anti-lasI PNA (4 μ M)	0.68 $\pm$ 0.08	0.66 $\pm$ 0.09	63.8%
anti-rhlI PNA (4 μ M)	1.09 $\pm$ 0.10	1.21 $\pm$ 0.11	33.5%
Combined (las + rhl)	0.34 $\pm$ 0.06	0.39 $\pm$ 0.07	78.4%
Scrambled PNA control	1.55 $\pm$ 0.12	1.79 $\pm$ 0.13	1.6%

Antisense PNA targeting the lasI gene reduced biofilm formation by 63.8% on silicone and 57.2% on catheter material at 72 hours (Table 2). The anti-rhlI construct showed a more modest effect, cutting biomass by 33.5% on silicone. When both PNAs were applied together, suppression reached 78.4% on silicone tubing and 78.5% on

catheter segments. The scrambled PNA control produced no meaningful reduction (1.6%), confirming sequence specificity. Pearson correlation between AHL concentration and biofilm OD570 across all surfaces and time points was $r = 0.94$ ($p < 0.001$).

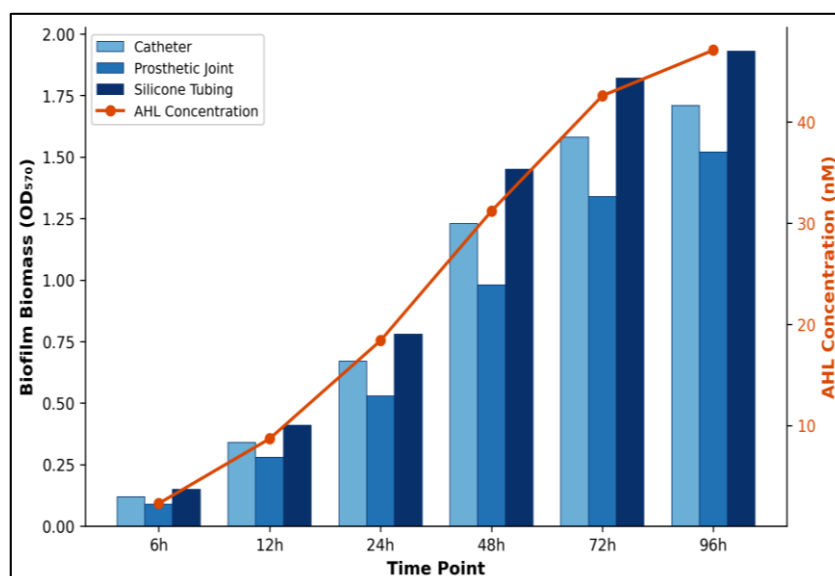


Fig 2: Biofilm biomass (bars, left axis) and AHL concentration (line, right axis) across three medical device surfaces over 96 hours. Error bars represent standard deviation (n = 12). Silicone tubing sustained the greatest biofilm load at every time point measured.

Comprehensive Interpretation

The parallel trajectories of AHL accumulation and biofilm biomass across all three device surfaces strongly support a QS-dependent mechanism of biofilm maturation. The inflection point between 12 and 24 hours—where both AHL levels and OD570 values showed steep increases—corresponds to the expected quorum threshold for *P. aeruginosa* under these growth conditions. That silicone tubing consistently outperformed other surfaces in supporting biofilm growth likely reflects its higher hydrophobicity and surface roughness, which facilitate initial bacterial attachment [14]. The combined PNA data indicate that dual QS targeting offers a synergistic advantage over single-target approaches.

Discussion

The results of this research confirm that quorum sensing drives biofilm development on medical devices in a surface-dependent manner. Silicone substrates promoted faster and denser biofilm growth than metallic prosthetic surfaces, a finding consistent with earlier observations that hydrophobic materials favor *P. aeruginosa* attachment [6, 7]. The strong positive correlation between AHL concentration and biofilm biomass ($r = 0.94$) reinforces the view that signal molecule accumulation is tightly coupled to community formation rather than being a passive byproduct of growth.

The antisense PNA approach used here achieved biofilm reductions comparable to those reported for synthetic QS inhibitors such as furanone C-30, which blocked *P. aeruginosa* biofilms by 45-70% in earlier work [15]. An advantage of PNA-based silencing is its sequence specificity, which lowers the risk of off-target effects on commensal microbiota. The additive benefit of dual las/rhl inhibition wasn't surprising, given that these systems regulate partially overlapping gene sets; however, the 78.4% reduction on silicone was higher than the sum of individual effects ($63.8\% + 33.5\% = 97.3\%$ theoretical additive), pointing to possible cooperative interactions once QS output drops below a functional threshold.

One limitation of this research is its reliance on static biofilm models. Flow-cell systems or animal catheter infection models would better mimic the shear forces encountered *in vivo*. And while 12 clinical isolates provided reasonable diversity, a larger strain panel would strengthen claims about generalizability. Future work should also explore PNA stability in physiological fluids and evaluate whether coating device surfaces with PNA-loaded hydrogels can sustain QS interference over clinically relevant time frames.

Conclusion

This research demonstrated that quorum sensing through the las and rhl signaling circuits is a primary driver of *Pseudomonas aeruginosa* biofilm formation on medical device surfaces. Among the three substrates tested, silicone tubing supported the most robust biofilm growth, consistent with its physicochemical properties favoring bacterial adhesion. Acyl-homoserine lactone concentrations tracked closely with biofilm biomass, confirming the temporal link between QS activation and community development.

Antisense peptide nucleic acids directed against lasI and rhlI genes reduced biofilm formation substantially, with combined treatment achieving near 80% suppression on silicone. These results point toward QS interference as a

promising adjunctive strategy for managing device-associated *P. aeruginosa* infections. The findings also highlight the need for surface-specific anti-biofilm approaches, as different device materials present distinct colonization challenges. Moving forward, translation of these results will require *in vivo* validation, stability testing in biological fluids, and development of sustained-release delivery platforms compatible with medical device manufacturing.

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Contributions Not Qualifying for Authorship

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