Growth Kinetics of *Pseudomonas aeruginosa* Following Induction of Piperacillin–Tazobactam Resistance

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Background

Pseudomonas aeruginosa is an opportunistic nosocomial pathogen that can acquire antibiotic resistance under selective pressure, complicating treatment[1]. This is particularly concerning in strains harbouring silent resistance genes that are genotypically resistant but phenotypically susceptible[2]. This study compared the growth kinetics of induced-resistant and parent susceptible *P. aeruginosa* strains following piperacillin–tazobactam resistance induction.

Methodology

Three biological replicates of a susceptible *P. aeruginosa* strain carrying a silent piperacillin–tazobactam resistance gene were induced for resistance. The strains were grown in Mueller-Hinton broth and incubated in a 37°C shaking incubator[3]. Growth kinetics of induced-resistant and parent strains were compared using hourly OD_{600} measurements on a nanospectrophotometer (Implen GmbH, Germany), over 24 hours . Area under the curve (AUC) and growth rate (μ) were analysed using GraphPad Prism (v8.0; GraphPad Software, 2019) after confirming data normality with the Shapiro–Wilk test. A p-value of < 0.05 was considered statistically significant.

Results

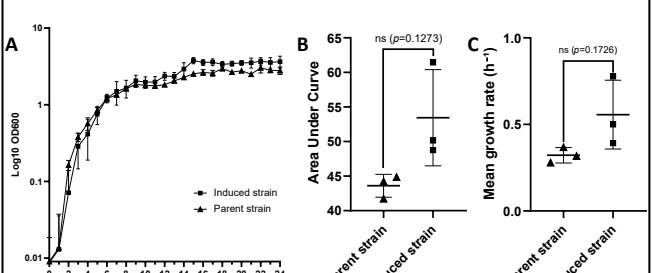

Fig. 1 (A) The growth curves, **(B)** Corresponding area under the curve, and **(C)** Growth rates of *P. aeruginosa* parent and induced resistance strains grown in Mueller-Hinton broth. Error bars are the standard deviation for each data point.

- The minimum inhibitory concentration increased from 2/4 μg/ml to ≥256/4 μg/ml, confirming successful resistance induction.
- Induced-resistant strains showed a slightly higher growth plateau and mean AUC.
- Differences in AUC (p=0.127) and μ (p=0.1726) were not statistically significant.

Conclusion

Induction of piperacillin–tazobactam resistance did not result in a significant alteration in growth kinetics, suggesting no detectable growth-associated fitness cost under the conditions tested, which may facilitate persistence and dissemination in clinical settings. This finding supports the hypothesis that certain resistance mechanisms may be fitness-neutral, contributing to long-term persistence in hospital environments.

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References

[1] Elfadadny A, Ragab RF, AlHarbi M, Badshah F, Ibáñez-Arancibia E, Farag A, Hendawy AO, De Los Ríos-Escalante PR, Aboubakr M, Zakai SA, Nageeb WM. Antimicrobial resistance of *Pseudomonas aeruginosa*: navigating clinical impacts, current resistance trends, and innovations in breaking therapies. *Frontiers in Microbiology*. 2024;15:1374466. https://doi.org/10.3389/fmicb.2024.1374466

[2] Deekshit VK, Srikumar S. "To be, or not to be"—The dilemma of 'silent' antimicrobial resistance genes in bacteria. *Journal of Applied Microbiology*. 2022;133(5):2902–2914. https://doi.org/10.1111/jam.15738

[3] Cummins J, Reen FJ, Baysse C, Mooij MJ, O'Gara F. Subinhibitory concentrations of the cationic antimicrobial peptide colistin induce the pseudomonas quinolone signal in *Pseudomonas aeruginosa*. *Microbiology (Reading)*. 2009;155(Pt 9):2826–2837. https://doi.org/10.1099/mic.0.025643-0