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Abstract

Persister Cell Formation in Clinical Isolates of *Pseudomonas aeruginosa* †

Hiral Patel, Kranti Bhamnote and Devarshi Gajjar *

The Department of Microbiology and Biotechnology Centre, The Maharaja Sayajirao University of Baroda, Vadodara 390002, Gujarat, India; patelhiral1317@gmail.com (H.P.); krantibhamnote20@gmail.com (K.B.)

* Correspondence: devarshimistry@yahoo.com; Tel.: +91-9898933299

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Persister cells (PC) are non-growing and metabolically inactive cells, which lack transcription, translation, and proton motive force. PC formation was reported to occur by (i) stochastically (ii) antibiotic treatment (iii) nutrient deprivation and (iv) other biofilm-related conditions. PCs are responsible for chronic and relapse of biofilm infections as well as bacterial infections. *Pseudomonas aeruginosa* (Pa) is an opportunistic pathogen frequently causing chronic airway infections in patients with cystic fibrosis (CF), chronic urinary tract infection (UTI), and ventilator-associated pneumonia. For this study, Pa isolates; TP-10, ST-13, and POA1 were used. The Minimum Inhibitory Concentration (MIC) was determined and a time-kill assay was carried out for PC isolation in the planktonic stage. Further, Flow Cytometry (FC) and qRT-PCR for stringent response genes (*rel A*, *spo T*, and *lon*) and toxin-antitoxin (*hig B* and *hig A*) were done. A biphasic kill curve pattern was observed which varied across the antibiotic treatment. Starting from 12–13 $\log_{10}$ cfu/mL mean inoculum, after ceftazidime treatment 9–10 $\log_{10}$ cfu/mL lasted, while on gentamicin and ciprofloxacin treatment 5–6 $\log_{10}$ cfu/mL lasted and formed a biphasic pattern. The PCs were analyzed through FC using Redox sensor green (RSG) and propidium iodide (PI) staining. It was observed that ceftazidime treatment has high redox activities followed by ciprofloxacin and gentamicin treatment compared to untreated isolate. Whereas ST-13 isolate had high redox activities on ceftazidime treatment followed by gentamicin and ciprofloxacin. The *rel A*, *spo T*, *lon*, *hig B*, and *hig A* genes were upregulated on ceftazidime and gentamicin treatment compared to untreated in PAO1 and TP-10 isolates. Whereas in ST-13 isolate, genes were upregulated on gentamicin treatment. From the above study, it can be concluded that gentamicin and ceftazidime lead to PC formation in *P. aeruginosa* planktonic stage.

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