

Characterization of multi-resistant *Pseudomonas aeruginosa* from clinical and municipal wastewater systems of two different cities

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Scientific Background and Objectives

Among the most serious problems our health systems are facing is the increased number of infections involving antibiotic-resistant bacteria, which can no longer be treated with previously potent antimicrobial agents. A reason for the emergence of more and more resistant bacterial species is the increased and more widespread use of antibiotics for both therapeutic and preventive applications. Selective pressure refers to environmental conditions including not only the use of antibiotics but also other environmental factors such as other drugs used or environmental pollutants. Such selective pressures are a general concept that refers to many factors that create an environmental landscape and allow organisms with novel mutations or newly acquired characteristics to survive and proliferate. Evidence has shown that even in sub-inhibitory level concentration, antibiotics may still exert their impact on microbial community by influencing transcription in microbes. Long-term effects of exposure to low concentrations of antibiotics are still unknown.

The study aimed at enriching and identifying multi-drug resistant *P. aeruginosa* strains carrying more than five different clinically relevant antibiotic resistances from hospital and municipal wastewater systems of two German cities. Besides conventional antibiogram testing, the clonal identities of the *P. aeruginosa* strains were studied by Pulsed Field Gel Electrophoresis (PFGE) and subsequent cluster analyses. Such multi-resistant *P. aeruginosa* isolates were also investigated regarding gene expression levels of the four most important efflux pump systems. Over-expression of efflux pumps can result from mutations within local repressor genes or activation of global transcriptional regulators and may contribute to the multi-drug resistant phenotypes.

Results and Discussion

Antibiogram testing, PFGE, and cluster analyses of multi-resistant *P. aeruginosa* isolates

Isolate numbers	GM	CIP	IPM	CAZ	AN	AZ	PT	Genotype
References								
ATCC27853	S	S	S	S	S	S	S	n.d.
Sensitive isolates from clinical wastewaters								
30	S	S	S	S	S	S	S	A
31	I	S	S	S	S	S	S	A
41	S	S	S	S	S	S	S	F
Clinical wastewater of city A								
910	R	R	R	R	S	R	I	G
911	R	R	R	R	S	R	S	G
912	R	R	R	R	S	I	I	G
913	R	R	R	R	S	R	R	E
914	R	R	R	R	S	S	S	J
Municipal wastewater treatment plant of city A (influent)								
49	R	R	R	R	R	R	R	H
55	R	R	R	R	R	R	R	n.d.
56	R	R	R	R	R	R	R	H
57	R	R	R	R	R	R	R	I
59	R	R	R	R	R	R	R	H
Clinical wastewater of the city B								
965	S	R	R	S	S	R	R	C
966	S	R	R	S	S	R	R	C
967	S	R	R	S	S	R	R	n.d.
968	S	R	R	S	S	R	R	C
969	S	R	R	S	S	R	R	C
Municipal wastewater treatment plant of city B (influent)								
987	I	R	R	S	S	R	R	C
988	R	R	R	S	S	R	R	D
989	S	R	R	S	S	R	S	B
990	I	R	R	S	S	R	R	C
991	I	R	R	S	S	R	R	C

- Multi-resistant *P. aeruginosa* were isolated from clinical and downstream wastewater systems carrying up to seven acquired therapeutically relevant antibiotic resistances.
- The isolates could be clustered in different geno-groups of the habitats (no clonal identities).
- Different clusters were identified in city A and B.
- Clinical wastewater isolates were also detected in municipal wastewaters of city B, but not in city A.

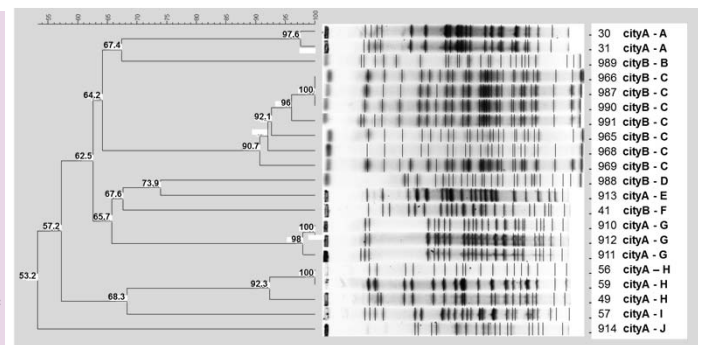


Table 1: List of *P. aeruginosa* strains enriched from different wastewater compartments of two German cities. Agar diffusion testing for gentamicin GM (10 µg), ciprofloxacin CIP (5 µg), imipenem IPM (10 µg), ceftazidim CAZ (10 µg), amikacin AN (20 µg), azlocillin AZ (30 µg), and piperacillin/tazobactam PT (30/10 µg) resistance; S: susceptible; R: resistant; I: intermediate; Genotyping was performed via PFGE; n.d.: not determined.

Figure 1: Pulsed field gel electrophoresis (PFGE) of the different multi-resistant and antibiotic-susceptible *P. aeruginosa* isolates from wastewater systems. Right side: numbers refer to isolates listed in table 1. The group association (A-J) of the isolates derived from the dendrogram of similarities (left side). Similarities are given in percentages.

Expression of efflux pump genes in multi-resistant *P. aeruginosa*

It has been previously shown that in clinical isolates from patients the over-expression of one or more efflux pump systems can contribute significantly to multi-resistance against various antibiotics. The gene expressions of the most important efflux pump operons of *P. aeruginosa* (*mexA-mexB-oprM*, *mexC-mexD-oprJ*, *mexE-mexF-oprN*, and *mexX-mexY-oprM*) were studied via reverse RNA transcription to cDNA followed by quantitative real time PCR. Such gene expression approaches resulted in no absolute transcription product values, but rather result in expression data relative to constantly expressed housekeeping genes, like the ribosomal gene product.

The highest expression rates were detected in antibiotic-susceptible *P. aeruginosa* strains. No over-expressions of efflux pump operons were observed indicating that multi-resistance of the isolated *P. aeruginosa* strains were based on antibiotic-specific resistance mechanisms. This was proved by molecular biology gene detection for imipenem and ciprofloxacin resistance (Volkman *et al.*, 2004; Schwartz *et al.*, 2006). Although this study was not a quantitative approach, over-expressions of efflux pumps seem to be specific for patient's isolates. Therefore, we hypothesise that such genotypes are not fit enough to survive in wastewater habitats.

Literature: Volkman *et al.*, 2004, J. Microbiol. Meth. 56, 277-286; Schwartz *et al.*, 2006, FEMS Microbiol. Ecol. 57, 158-167.

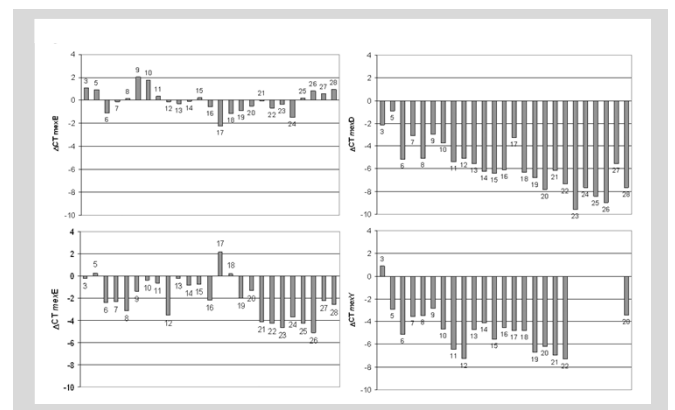


Figure 2: Expression of *mexB*, *mexD*, *mexE*, and *mexY* efflux pump genes of multi-resistant *P. aeruginosa* strains in comparison with a susceptible isolate. Presented are Delta Ct values of the normalised data. The numbers within the histograms indicate the different *P. aeruginosa* strains.

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