Bacterial persistence in biofilms : transcriptional analysis in a model of Pseudomonas catheter-assosciated urinary tract infections

Anaïs Soares, Elise Fiaux, Martine Pestel-Caron, François Caron, Manuel Etienne

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Bacterial persistence in biofilms : transcriptional analysis in a model of *Pseudomonas* catheter-associated urinary tract infections

Soares A[1,2], Fiaux E[1,3], Pestel-Caron M[1,2], Caron F[1,3], Etienne M[1,3]
[1] GRAM EA2656 ; [2] Bacteriology ; [3] Infectious Diseases, Rouen University Hospital, Rouen, France

Background

Biofilm (BF) is the main cause of antibiotic (ATB) failure during device-related infections. ATB failure might be related to persister (P) cells, a subset of bacteria able to survive to high ATB concentrations in BF, though tested fully susceptible (S) with usual minimum inhibitory concentration (MIC) tests in planktonic cultures.
The aim was to develop *in vitro* a model of bacterial persistence to investigate the transcriptional adaptation induced by antibiotic stress.

Methods

Catheter-associated urinary tract infection (CAUTI) model *in vitro*

- A 4 days *Pseudomonas aeruginosa* (**PA14**) BF formed on urinary catheters (UC)
- **1h**, **6h** or **24h** ciprofloxacin (CIP) exposure at different concentrations (from 1 to 2048xMIC)
- **Surviving S and resistant (R)** PA14 numbered on MH2 and MH2+CIP (1xMIC)

→ **Transcriptomic analysis**

On a mix of R and P cells
On pure P cells
1h and 24h CIP exposure
various CIP concentrations

RNA extraction directly from UC
DNAse treatment
DNAc synthesis

Target genes :

spoT, ***relA***, ***lon*** (stringent response)
rpoS, ***rpoN*** (sigma factors)
lasI (quorum sensing)
(reference gene : 16S)

Expression ($2^{-\Delta\Delta Ct}$) compared to untreated UC

Results

Impact of CIP exposure (Figure 1) :

- **Before CIP exposure:** 9 $\log_{10}$ CFU/ml including 6,6 $\log_{10}$ CFU/ml R cells,
  resistance mutation frequency = 5.10^{-3} ;
- **Biphasic curve after CIP exposure:**
  initial and rapid 2 $\log_{10}$ CFU/ml drop of S and R cells in the same proportion
  plateau of bacterial populations despite increasing CIP concentrations ;
- **Lower ATB doses to eradicate R cells than S cells** (768 to 1024xMIC *vs* 2048xMIC);
- **UC Sterilization at ATB concentrations > 1024xMIC** whatever the length of CIP exposure

Surviving S cells highly tolerant to ATB = Persister cells

« Selection window » of pure P population (Figure 2) :

[768 to 2048xMIC] (6h & 24h), [1024 to 2048xMIC] (1h)

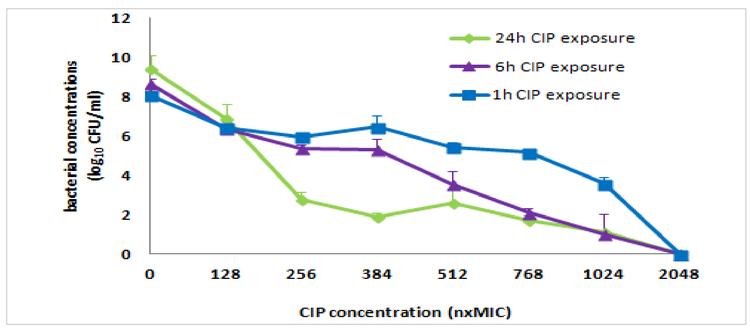

Figure 1 : Impact of CIP exposure. Bacterial concentrations after 1h, 6h and 24h CIP exposure at increasing CIP concentrations.

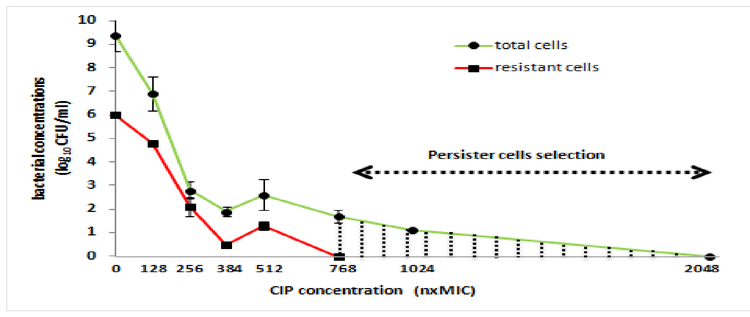

Figure 2: Persister cells selection. Total and resistant cells concentrations after 24h CIP exposure.

Table 1: Quantitative expression of genes involved in stringent response, sigma factors and quorum sensing at different experimental conditions (mean ± standard deviation)

	R and P cells		Pure P cells	
	512xMIC 1h	768xMIC 1h	768xMIC 24h	1024xMIC 1h
Stringent response				
spoT	0.3-0.7*	0.4-0.4	**5.0-8.5**	**17.6-50.8**
relA	0.6-0.7	0.3-0.4	**2.4-4.0**	**10.4-31.2**
lon	0.7-0.8	ND	**4.1-7.6**	ND
Sigma factors				
rpoS	ND	0.5-0.6	**2.0-3.5**	ND
rpoN	ND	0.2-0.5	**2.2-4.6**	**5.1-14.6**
Quorum sensing				
lasI	ND	ND	**2.4-4.1**	**6.7-19.5**

same CIP concentration
same length of CIP exposure

*$2^{-\Delta\Delta Ct}$ (control = 1)
ND : no data
P : persister
R : resistant

Transcriptomic analysis (Table 1) :

In the conditions of CIP exposure associated with mixed R + P cells :

no overexpression whatever CIP exposure

In the conditions of CIP exposure associated with P selection (as compared to untreated UC):

- **early (1h) intense upregulation for *spoT*, *relA*, *rpoN* and *lasI* (x10 to 35)**
- **fading upregulation over time** (1h *vs* 24h) though more intense **(x3 to 6)** at higher CIP concentrations

Discussion

→A catheter-associated urinary tract infection model *in vitro* for persister selection at very high CIP concentrations ; ATB failure not related to R cells, but to P cells;
→Low RNA amounts extracted limiting the number of genes quantified ;
→Early and intense activation of *lasI* potentially for BF consolidation, of *relA* and *spoT* for shutdown of cellular process, tolerance towards CIP and increased survival
➔ **multiple dynamic process of gene expression regulation leading to persister phénotype**
→Overexpression of *spoT* controversial role → synthase or hydrolase activity to be determined

Conclusion

In this ATB–treated BF model of *Pseudomonas* infection, the transcriptional adaptation induced is **highly dynamic** and varies along the ATB exposure and according to BF environment. Eradication failure at high CIP doses was related to **persister phenotype** and associated with **early intense upregulation of stringent response genes.**