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

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Background

Biofilm (BF) is the main cause of antibiotic (ATB) failure during device-related infections. ATB failure might be related to persisters (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
DNase 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 log₁₀ CFU/ml including 6,6 log₁₀ CFU/ml R cells, resistance mutation frequency = 5.10^{-3} ;
- Biphasic curve after CIP exposure:
 - initial and rapid 2 log₁₀ 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)

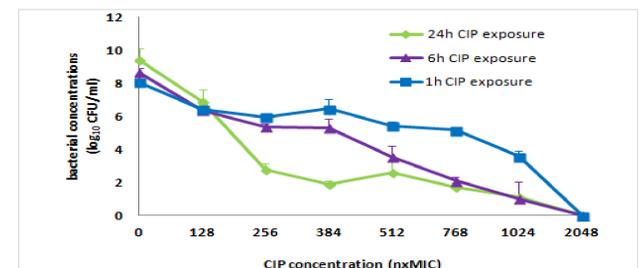


Figure 1: Impact of CIP exposure. Bacterial concentrations after 1h, 6h and 24h CIP exposure at increasing CIP concentrations.

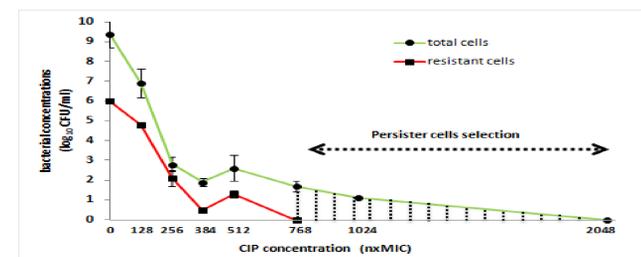


Figure 2: Persister cells selection. Total and resistant cells concentrations after 24h CIP exposure.

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 | | | | |
| <i>spoT</i> | 0.3-0.7* | 0.4-0.4 | 5.0-8.5 | 17.6-50.8 |
| <i>relA</i> | 0.6-0.7 | 0.3-0.4 | 2.4-4.0 | 10.4-31.2 |
| <i>lon</i> | 0.7-0.8 | ND | 4.1-7.6 | ND |
| Sigma factors | | | | |
| <i>rpoS</i> | ND | 0.5-0.6 | 2.0-3.5 | ND |
| <i>rpoN</i> | ND | 0.2-0.5 | 2.2-4.6 | 5.1-14.6 |
| Quorum sensing | | | | |
| <i>lasI</i> | ND | ND | 2.4-4.1 | 6.7-19.5 |

* 2⁻⁴⁴⁶² (control = 1)
ND : no data
P : persister
R : resistant

same CIP concentration
same length of CIP exposure

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 phenotype
- 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.