Systems pharmacology of interactions among *Acinetobacter baumannii*, human lung epithelial cells and polymyxin B

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Introduction

- *Acinetobacter baumannii* can cause life-threatening pneumonia in critically-ill patients and can be resistant to multiple antibiotics [1].
- Treatment of *Acinetobacter* pneumonia may require the use of the 'last-line' polymyxins [2].
- There is a lack of information available for how *A. baumannii* and human lung epithelial cells interact at the molecular level when concomitantly exposed to polymyxin B.

Aims

- To investigate the gene expression changes in *A. baumannii* and A549 cells when both are combined and exposed to polymyxin B.
- To provide better understanding of the interaction of *A. baumannii* with respiratory epithelial cells during treatment with polymyxins.

Materials and methods

- *A. baumannii* ATCC 19606 was co-cultured with A549 cells for 2 h with or without 2 mg/L polymyxin B.
- Extracted bacterial mRNA was enriched with MICROBEnrich kit then quantified with Illumina HiSeq1500.
- Extracted A549 mRNA was quantified with Affymetrix microarray.
- R limma package was employed to analyse the transcriptomic data.
- *A. baumannii* AB5075 transposon insertion mutant library was employed to evaluate differentially expressed genes (DEGs) that are highly upregulated.
- Time-kill and population analysis profile (PAP) studies were used to examine genes related to polymyxin resistance.

Results

- *A. baumannii* exposed to A549 cells upregulated genes that are enriched for arginine and tyrosine degradation pathways (Figure 1) and genes encoding for outer membrane protein (Omp) A and W.

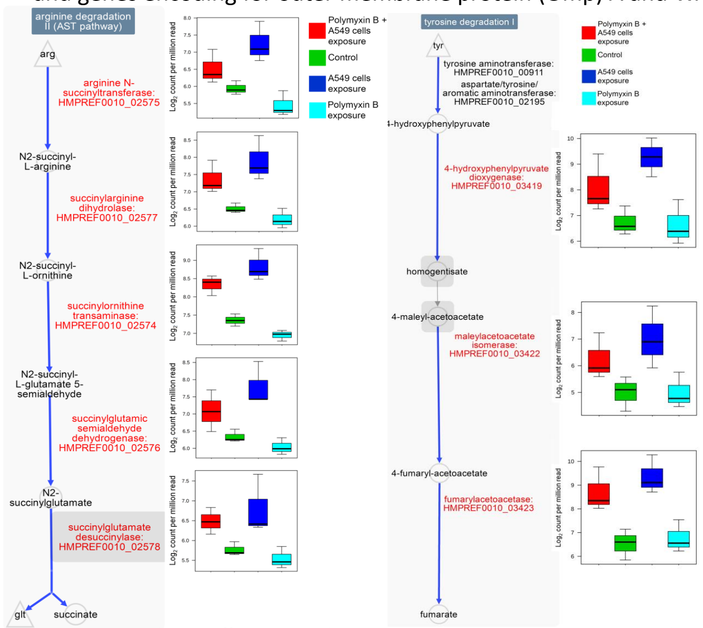

Figure 1. Major pathways affected in *A. baumannii* ATCC 19606 exposed to A549 cells. Significantly upregulated enzymes are highlighted red and their relative expression level in different exposure groups are shown by the box and whiskers plots to their right.

- *A. baumannii* exposed to 2 mg/L polymyxin B upregulated genes that are enriched for the histidine degradation pathway (Table 1) and *rcnB* gene involved in nickel/cobalt homeostasis.
- Time-kill studies showed *rcnB* mutant more susceptible to polymyxin B treatment and PAPs showed higher polymyxin resistant frequency from *rcnB* mutant treated with polymyxin B (Figure 2).

Table 1. Enzymes encoded by the significantly upregulated genes *in A. baumannii* ATCC 19606 at 2 h after exposure to 2 mg/L of polymyxin B.

Histidine degradation II	Log, fold-change	FDR
Histidine ammonia-lyase	1.43	1.01E-03
Imidazolonepropionase	1.24	2.25E-03
Urocanate hydratase	1.08	1.82E-02

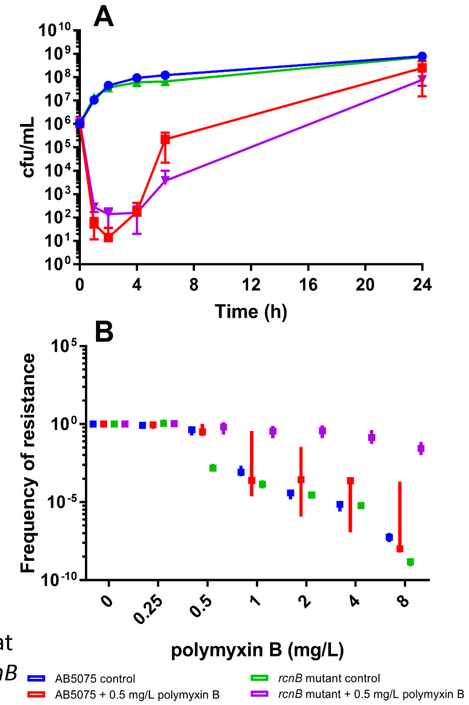

Figure 2. (A) Time-kill assay with 0.5 mg/L polymyxin B against *A. baumannii* AB5075 wildtype and *rcnB* mutant. (B) PAPs of *A. baumannii* AB5075 and *rcnB* mutant with different concentration for polymyxin B (mg/L) at 24 h following the time-kill assay.

- *A. baumannii* already exposed to A549 cells showed significant upregulation of lipoprotein transporter activity (e.g. *lolB*) when further exposed to 2 mg/L polymyxin B.
- A549 cells exposed to *A. baumannii* upregulated genes that are enriched for inflammation responses, specifically TNF signaling pathway (Table 2).

Table 2. Significant pathways and genes upregulated by A549 cells at 2h following exposure to *A. baumannii*.

GO term/KEGG pathway	FDR	Upregulated genes
GO biological process		
GO:0006954~inflammatory response	8.99E-10	IL6, NFKBIZ, NFKBID, TNFAIP3, IL1A, CXCL2, TNF, CCL20, PTX3, CXCL3, CXCL1, IL1B, CXCL8
GO:0006955~immune response	1.66E-06	IL6, CCL20, CSF2, LTB, CXCL3, CXCL1, IL1A, CXCL2, TNF, IL1B, CXCL8
GO:0071222~cellular response to lipopolysaccharide	1.05E-04	IL6, CCL20, CSF2, TNFAIP3, ICAM1, TNF, CXCL8
GO molecular function		
GO:0008009~chemokine activity	0.001966	CCL20, CXCL3, CXCL1, CXCL2, CXCL8
GO:0005125~cytokine activity	0.016727	IL6, CSF2, LTB, IL1A, TNF, IL1B
KEGG pathway		
hsa04668:TNF signaling pathway	8.95E-09	IL6, CCL20, CSF2, CXCL3, CXCL1, TNFAIP3, ICAM1, CXCL2, TNF, IL1B
hsa05323:Rheumatoid arthritis	9.47E-08	IL6, CCL20, CSF2, LTB, IL1A, ICAM1, TNF, IL1B, CXCL8
hsa05132:Salmonella infection	3.01E-06	IL6, CSF2, CXCL3, CXCL1, IL1A, CXCL2, IL1B, CXCL8

- A549 cells exposed to 2 mg/L polymyxin B showed no significant changes in their gene expression profile as the drug concentration is too low.
- A549 cells already exposed to *A. baumannii* showed minimal transcriptomic changes when further exposed to 2 mg/L polymyxin B.

Conclusions

- A systems approach for the host-pathogen-drug interactions provides a better understanding of bacterial infection in the presence of antibiotics.
- We are the first to show that arginine and tyrosine degradation in *A. baumannii* are major pathways involved in its infection, and histidine degradation pathway and *rcnB* gene may be involved in polymyxin resistance in *A. baumannii*.

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References

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