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Paper Poster Session II

Focus: Acinetobacter, Pseudomonas and other nonfermenters

Polymyxin-induced envelope stress response and adaptive resistance in *Pseudomonas aeruginosa*

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Objectives. We recently found that *Pseudomonas aeruginosa* (PA) is able to develop a transient, adaptive response to polymyxins allowing a sub-population to survive and multiply in the presence of bactericidal concentrations of drug. Unexpectedly, the *arn* operon, which promotes the addition of 4-aminoarabinose molecules to the LPS and which contributes to the acquired (stable) resistance to polymyxins in this species, contributes only modestly to this adaptive process. This study reports on a novel and complementary mechanism of adaptation, named hypervesiculation.

Methods. The *arn* operon, the genes modulating its expression (*pmrAB*, *parRS*, *phoPQ* and *cprRS*), as well as *mmsAB* and *algW* were deleted from bioluminescent strain PAO1::*luxCDABE*. Counts of survivors after colistin exposure were assessed by luminometry. Transcriptomic analyses were performed by RNA-Seq. Outer membrane vesicles (OMVs) quantitation was performed according to a standard protocol.

Results. The quintuple mutant PAO1?*arn*-?*pmrAB*-?*parRS*-?*cprRS*-?*phoPQ* exhibited the same adaptive resistance to 8 mg/L colistin (4 x MIC) and regrowth as its wild-type parent PAO1. Comparison of the transcriptomes of the mutant (i) non-exposed, (ii) exposed to 4 mg/L, (iii) and to 8 mg/L colistin revealed that 385 genes were dysregulated (≥ 2-fold) corresponding to 6.9% of the PAO1 genome. Interestingly, we found that many of these genes are under the control of alternative sigma factor AlgU, whose homolog, RpoE, plays a potential role in the adaptation of *Escherichia coli* to envelope stress. AlgU is known to contribute to OMV formation. Consistent with this, the adaptive process was concomitant with the overexpression of genes (*pqsABCDE*, *phnAB*) encoding the *Pseudomonas* quinolone signal (PQS) which is key to OMV formation. Deletion of the *algW* gene, which codes for a membrane serine protease involved in the signal-transduction regulatory cascade that eventually activates AlgU, strongly impaired the tolerance of PA to colistin in an *arn* deleted mutant. The production of OMVs was impacted in a double *algW-arn* mutant compared with wild-type PAO1.

Conclusion

Adaptation to the killing effect of colistin relies upon complex mechanisms some of which imply the outer membrane as a first line of defence. Our results suggest that in addition to the *arn*-mediated decrease of negative charges at the cell surface, the *algU*-dependent formation of extracellular OMVs might prevent cationic peptides from reaching their target by sequestration. Dissecting the pathways that are essential for PA to persist *in vivo* under treatment will provide new molecular insights to tackle infections due to XDR/PDR strains.