O590
2-hour Oral Session
Resistance mechanisms in Gram-negatives

Identification of the molecular support of the intrinsic resistance to colistin in Proteus vulgaris by a genomic comparative approach and in vitro tests

Sophie Baron[1], Jean-Marc Rolain*[2]

[1]*Ihu Méditerranée Infection, Urmite Um 63 Cnrs 7278 Ird 198 Inserm U1095, Marseille, France*

[2]*Ihu Méditerranée Infection, Marseille Cedex 05, France*

Background: For several years, colistin has been used as the last line antimicrobial drug for the treatment of multi-drug resistant Gram negative bacteria infections. Unfortunately, the renewed interest for this antimicrobial peptide led to the emergence of acquired resistance especially in *Enterobacteriaceae*, as the increase of infections due to intrinsic colistin-resistant bacteria. Mechanisms of resistance to colistin are complex and misunderstood, particularly in these bacteria. While all *Proteus* bacteria are naturally resistant, we surprisingly recently isolated a *P. vulgaris* clinical strain susceptible to colistin, and we propose in this work to understand the molecular support of colistin resistance in this species by comparative genomic and phenotypic approach.

Material/methods: Genomes of *P. vulgaris* susceptible strain CSUR P1868_S and a resistant one CSUR P1867_R were sequenced and compared to select groups of interesting genes that were absent, truncated or mutated in the susceptible strain. Among the group of mutated genes, we selected those that were already known to be putatively involved in antimicrobial peptide resistance and we realized a SIFT score to predict if mutation could alter protein function. Use of electronic microscopy and lipid A analysis by mass spectrometry help us to understand pathways involved in colistin susceptibility/resistance.

Results: Genome of CSUR P1868_S was 4.28 Mb in length with a GC content of 37.8% whereas CSUR P1867_R genome was 3.9 Mb and 38.1%. It contained numerous resistant genes carried by various plasmids. Comparative genomic analysis allowed to identify 20 genes that were absent in the susceptible strain. We also highlighted the absence of sugars on the outer membrane by absence of red ruthenium staining, and the absence of amino-4-arabinose on lipopolysaccharide by mass spectrometry analysis. Two genes belonging to *arn* operon, *arnA* and *ArnB,* involved in biosynthesis and fixation of this aminosugar were mutated in the susceptible strain. Study of genes expression by QPCR will allow to show that these genes are not functional anymore, explaining this increased colistin susceptibility in this particular *P. vulgaris.*

Conclusions: 2 genes of *arn* operon was mutated in the susceptible *P. vulgaris.* It is known that sugars fixation on lipid A is a key pathway for polymyxin. However, it is certainly not the only one: bacterial defense system and antimicrobial peptides co-exist for a long time, and lots of enigmas on colistin resistance remain. Other genes that were absent in the susceptible strains could be linked to colistin resistance, including a whole operon involved in sialic acid metabolism and two genes involved in O-antigen biosynthesis. We are currently developing knock-out techniques to study these different hypothetical targets.. This is the first report that describes genomic differences between this particular susceptible strain and a typical *P. vulgaris* opening new research pathway to understand colistin resistance.