Genome wide analysis of sequential colistin-susceptible and resistant *Acinetobacter baumannii* isolated from Greek patients

Xavier B.B.[1], Dafopoulou K.[1,3], Zarkotou, O.[2], Sabirova J[1], Lammens C[1], Tsakris A[3], Goossens, H.[1], Pournaras S[3], Malhotra-Kumar, S.[1]

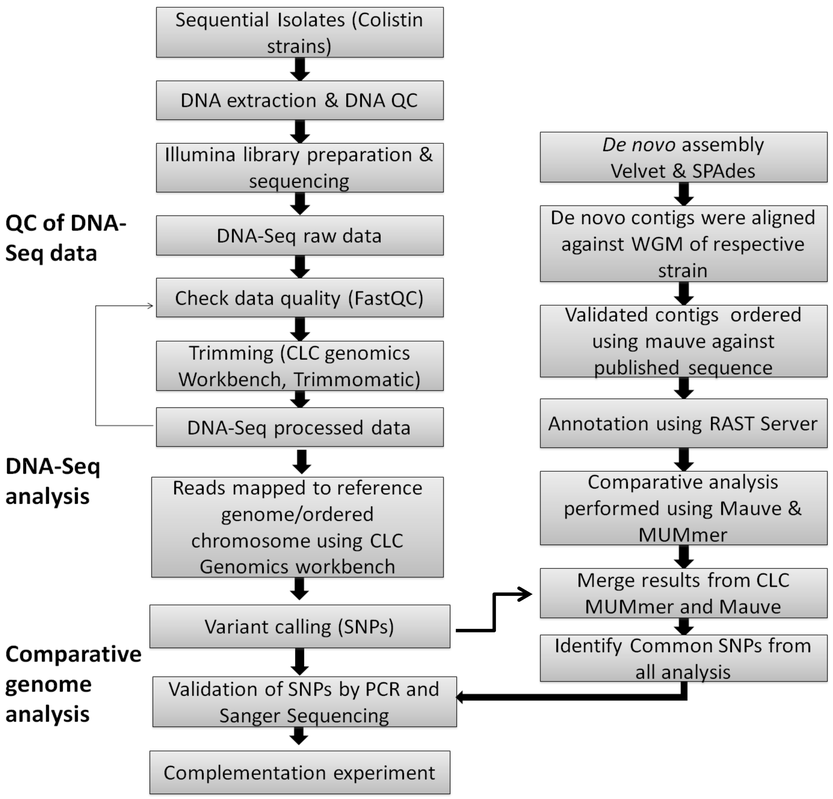
Department of Medical Microbiology, Vaccine & Infectious Disease Institute, University of Antwerp, Antwerp, Belgium[1] - Department of Microbiology, Tzaneio General Hospital, Piraeus[2]
Department of Microbiology, Medical School, University of Athens, Athens, Greece[3]

Introduction

During the last few years, *A. baumannii* resistant to colistin have emerged and caused outbreaks in Greek hospitals[1]. Known colistin resistance mechanisms in *A. baumannii* are due to overexpression of the *PmrCAB* proteins that cause lipopolysaccharide (LPS) modifications or inactivation of the *lpx* genes leading to complete loss of LPS production[2]. However, there are indications of as yet unexplored genes and, possibly, novel mechanisms of colistin resistance existing in *A. baumanii*. We carried out a genome-wide comparison of sequential *A. baumanii* that had evolved to colistin resistance *in vivo* under treatment pressure.

Methods

Two pairs of colistin-susceptible and resistant (CS/CR) (248CS/249CR, and 299CS/347CR) *A. baumannii* recovered sequentially from two Greek patients after prolonged colistin therapy were investigated. Antimicrobial susceptibility testing, MLST, PFGE (*ApaI*), and antibiotic resistance gene profiles (extended spectrum beta-lactamase genes, aac6-Ib and qnr) by PCR and sequencing were performed on all four isolates. Colistin MICs were determined by macrobroth dilution method according to CLSI guidelines. Strains were subjected to Whole Genome Mapping™ (WGM, Argus® system, Opgen Inc, Gaithersburg, USA). Maps were edited and compared using Bionumerics v7.1 (Applied Maths, Belgium). Whole genome sequencing (WGS) of sequential isolates was performed via 2×150b paired end sequencing (Nextera XT sample) preparation kit and Miseq, Illumina) (Workflow Below).



Results

All four clinical strains belonged to ST2. PFGE and antibiogram patterns were same for both pairs except for colistin susceptibility. Colistin MICs for the two pairs were 0.5 µg/ml for 248CS and 299CS, and 128 and 32 µg/ml for 249CR and 347CR, respectively. WGMs showed only 3% difference between 248CS and 249CR and 6% variation between 299CS and 347CR **(Fig. 1A).** However, an inter-pair comparison showed only 57% similarity in genome content between the 248CS/249CR and 299CS/347CR. The level of homology was also observed on whole genome sequencing comparisons **(Fig. 1B).** Intra-pair genomic comparisons showed both pairs have a single amino acid change in their *PmrB* protein, P233S for 248CS/249CR and P170L for 299CS/347CR. Additional changes in the CR strains were in the *lpsB* gene encodes a highly conserved LPS glycosyltransferase involved in biosynthesis of the LPS core. This gene harboured a mutation which converted a stop codon to Lys (*241L). We also identified potential synonymous mutations in the alkyl hydroperoxide reductase, stress related protein and *LysR* regulatory protein (LTTR) domain.

Figure 1A: Whole genome maps of two sequential pairs of CR and CS *A. baumanii*. Green lines indicate identity of restriction pattern among the maps and red horizontal marks represent variations. **B**. Comparison of whole genome sequenced sequential isolates. Pink color indicates highest Similarity. Variations are shown in different colors.

Conclusions

This study highlights a novel set of genes, which are seminal for *A. baumannii* pathogenesis and LPS synthesis, as potential targets of emerging colistin resistance in this pathogen.

References

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CONTACT: Prof. Dr. Surbhi Malhotra-Kumar, email: surbhi.malhotra@uantwerpen.be, phone: +32 265 27 52, fax: +32 3 265 26 63.

Laboratory of Medical Microbiology
Vaccine & Infectious Disease Institute
University of Antwerp