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Abstract (poster session)

Inclusion of blaOXA-235-like primers in a multiplex polymerase chain reaction (PCR) detecting prevalent carbapenemase genes in Acinetobacter spp.

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Objectives: OXA-235-like is a novel subclass of acquired carbapenem-hydrolysing class D beta-lactamases (CHDL) in Acinetobacter baumannii, composed of three variants (OXA-235, -236 and -237). Its prevalence is unknown as it was undetectable using the established multiplex PCR to identify the intrinsic OXA-51-like and the acquired OXA-23-like, -40-like, -58-like and -143-like (Woodford et al, IJAA 2006; 27: 351-53 and Higgins et al., IJAA 2010; 35: 305-14). The aim of the present study was to expand the existing OXA-multiplex PCR by addition of primers for blaOXA-235-like. Methods: Because the original multiplex PCR amplified products between 150-600 bp a region of approximately 700 bp was chosen for blaOXA-235-like primer design. Nucleotide sequences of blaOXA-235 and its variants were aligned. Primer pair OXA-235_F (TTGTTGCCTTTACTTAGTTGC) and OXA-235_R (CAAAATTTTAAGACGGATCG) were designed and tested in combination with the original multiplex primers at a final concentration of 0.2 µM. Total DNA of clinical A. baumannii isolates was used as template, including ten isolates that harbored OXA-235-like and 40 isolates that encoded representative genes of each OXA subclass. Results: Primers OXA-235_F and OXA-235_R amplified blaOXA-235 and its variants in the ten tested isolates. Furthermore all OXA controls amplified PCR products specific for their blaOXA type. With a size of 768 bp, the blaOXA-235-like PCR product was easily differentiated from blaOXA-51-like, -23-like, -40-like, -58-like and -143-like products. Conclusion: The OXA-235-F/R primer pair is able to detect blaOXA-235 variants and can be used in combination with the original OXA-multiplex primers. This modified PCR can help to examine the spread of carbapenem-resistant A. baumannii. Therefore we recommend inclusion of the blaOXA-235-like primer pair in the original multiplex PCR.

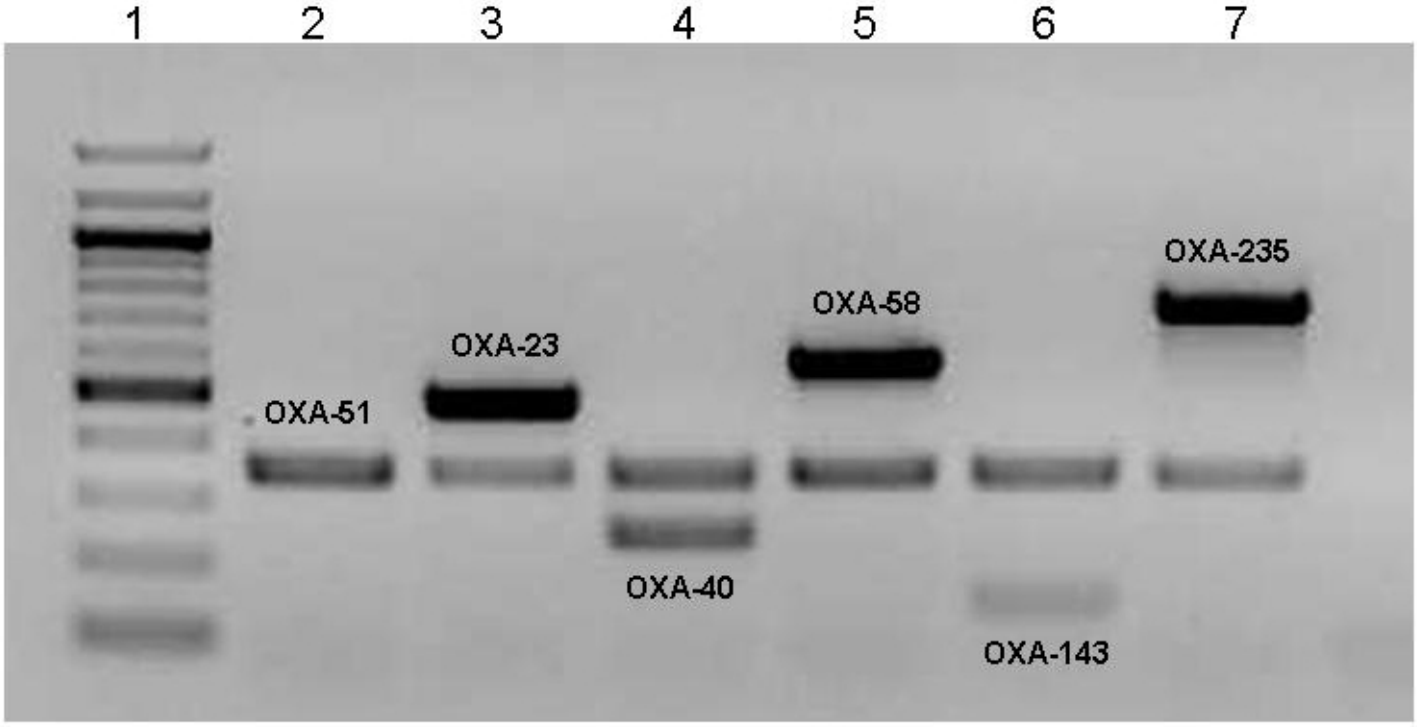

Figure: Detection of blaOXA genes by multiplex PCR. Isolates harbor the intrinsic blaOXA-51-like (lanes 2-7) and the acquired blaOXA-23-like, -40-like, -58-like, -143-like and -235-like (lanes 3-7). The molecular size marker is a 100 bp ladder (NEB, Frankfurt am Main, Germany).