Inclusion of *bla*$_{\text{OXA-235-like}}$ primers in a multiplex PCR detecting prevalent carbapenemase genes in *Acinetobacter* spp.

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Introduction and Purpose

- Carbapenem-hydrolysing oxacillinases (OXA) in *Acinetobacter baumannii* include the intrinsic OXA-51-like and four acquired subclasses OXA-23-like, OXA-40-like, OXA-58-like and OXA-143-like.
- The genes encoding these OXAs are easily detected in a multiplex PCR.[1, 2]
- In the literature there are many reports of carbapenem resistant *A. baumannii* but no resistance determinant is identified.
- Recently we identified OXA-235, OXA-236 and OXA-237, three variants of a novel OXA-subclass, that were not detected by the established multiplex PCR.[3]
- OXA-235-like was been detected in 10 carbapenem-resistant *A. baumannii* isolates originating from Mexico and the US.
- The prevalence of OXA-235-like is still unknown.
- The aim of the present study was to expand the existing OXA-multiplex PCR by addition of primers for *bla*$_{\text{OXA-235-like}}$.

Methods

- The original multiplex PCR amplifies products between 150-600 bp. Therefore a PCR product of approximately 700 bp was chosen for *bla*$_{\text{OXA-235-like}}$ primer design.
- DNA sequences of *bla*$_{\text{OXA-235}}$ and its variants were aligned. Primer pair OXA-235_F and OXA-235_R was 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. PCR was performed with DNA of ten isolates that harbored OXA-235-like, as well as DNA of 40 isolates that encoded representative genes of the other subclasses.

Results

- Primers OXA-235_F and OXA-235_R (Table) amplified *bla*$_{\text{OXA-235}}$, *bla*$_{\text{OXA-236}}$ and *bla*$_{\text{OXA-237}}$ in the ten tested isolates.
- Furthermore all OXA controls amplified PCR products specific for their blaOXA type.
- With a size of 768 bp, the *bla*$_{\text{OXA-235-like}}$ PCR product was easily differentiated from *bla*$_{\text{OXA-51-like}}$, *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-40-like}}$, *bla*$_{\text{OXA-58-like}}$ and *bla*$_{\text{OXA-143-like}}$ products (Figure).

Table: Primers to amplify *bla*$_{\text{OXA-235-like}}$, which can be included in the established OXA-multiplex PCR.

Primer	5´-3´sequence
OXA-235_F	TTGTTGCCTTTACTTAGTTGC
OXA-235_R	CAAAATTTTAAGACGGATCG

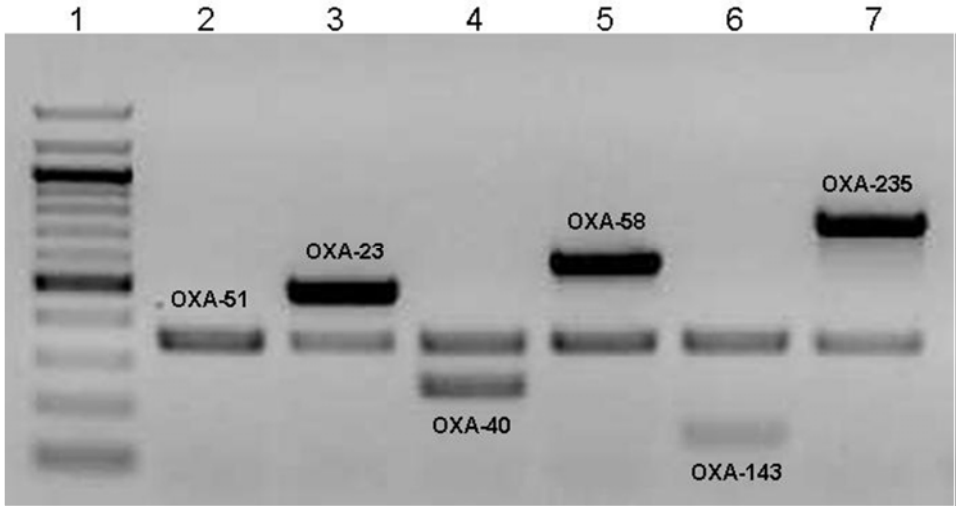

Figure: Detection of *bla*$_{\text{OXA}}$ genes by multiplex PCR. Isolates harbor the intrinsic *bla*$_{\text{OXA-51-like}}$ (lanes 2-7) and the acquired *bla*$_{\text{OXA-23-like}}$, *bla*$_{\text{OXA-40-like}}$, *bla*$_{\text{OXA-58-like}}$, *bla*$_{\text{OXA-143-like}}$ and *bla*$_{\text{OXA-235-like}}$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

Conclusions

- The OXA-235_F/OXA-235_R primer pair is able to detect *bla*$_{\text{OXA-235}}$ variants.
- The primers did not affect the specificity of the original multiplex PCR.
- These primers can be easily added to the existing multiplex PCR.
- This modified PCR can help to examine the spread of carbapenem-resistant *A. baumannii*.
- Therefore we recommend inclusion of the *bla*$_{\text{OXA-235-like}}$ primers to the original multiplex PCR.

References

[1] Woodford et al., IJAA (2006); **27**: 351-3
[2] Higgins et al., IJAA (2010); **35**: 305-14
[3] Higgins et al., AAC (2013); **57**: 2121-6

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