Introduction

The global spread of carbapenem-resistant Acinetobacter baumannii has led to an emerging worldwide healthcare problem. The carbapenem-hydrolysing oxacillinas (OXA) are the most commonly reported carbapenem-resistance determinants in Acinetobacter spp., particularly in A. baumannii. There are six OXA-subgroups associated with carbapenem-resistance in A. baumannii: the intrinsic OXA-51-like and the acquired OXA-23-like, OXA-58-like, OXA-40-like, OXA-143-like and OXA-235-like. Of these, OXA-23, OXA-40 and OXA-58 are the most prevalent carbapenem-resistance determinants among isolates worldwide. The lack of effective and reliable tests to detect OXA-mediated carbapenem-resistance is a serious challenge to modern medicine. There is an unmet medical need for reliable and rapid diagnostic tools to detect OXA-23/40/58-like producing strains to ensure a successful treatment of patients and prevent the spread of carbapenemase-producers.

The aim of this work is to expand OXA-detection abilities in the OXA-23 K-SetT to OXA-40/OXA-58-like carbapenemases in A. baumannii clinical isolates.

Methods

1. Generation of antibody producing hybridoma cell clones.
2. Screening for specific antibodies against recombinant OXA-40 and OXA-58 by ELISA.
3. Screening for specific antibodies against native OXA-40 and OXA-58 (A. baumannii lysate) and irrelevant His-Tag protein by ELISA.
4. Determination of antibody cross-reactivity to other OXA-subclases.
5. Cultivation and securing of selected hybridoma clones.
6. Antibody purification.
7. Identification of suitable antibody pairs able to detect recombinant OXA-40 and OXA-58 (ELISA and ICT).

Development of OXA-23 K-SetT – antibody-based detection assay (ICT)

Antibody screening

Hybridoma cell culture supernatants were screened for moabs binding to immobilized recombinant OXA-40/OXA-58 carrying a His-Tag. A. baumannii lysate and irrelevant His-Tag protein by ELISA.

Antibody cross-reactivity

Selected hybridoma cell culture supernatants were screened for antibody cross-reactivity to other OXA-subclases by ELISA.

Results

Antibody-purification

Hybridoma cell culture supernatants were collected and antibodies were purified by Protein G affinity chromatography. Purified antibodies were analysed by SDS-PAGE.

Conclusion

- Specific anti-OXA-40 and anti-OXA-58 antibodies were obtained
- Anti-OXA-40 antibodies show cross-reactivity with OXA-143
- Purified antibodies will be delivered to cooperation partner Cos-BioConcept to generate various ICT prototypes for validation