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Rapid detection and discrimination of plasmid- and chromosome-mediated resistance to polymyxins in Enterobacteriaceae using MALDI-TOF

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Background: Carbapenem-resistant Enterobacteriaceae (mostly carbapenemase-producing Enterobacteriaceae (CPE)), became more and more prevalent during the last decade worldwide. This phenomenon led to the accelerate use of polymyxins in CPE endemic, and subsequently increase resistance to polymyxins. In Enterobacteriaceae, acquired resistance to polymyxins results mostly from modifications of the polymyxin target, the lipopolysaccharide (LPS). These modifications correspond to addition(s) of cationic groups such as phosphoethanolamine (pETN) and/or 4-amino-L-arabinose (L-Ara4N) on the lipid A part of the LPS. These modifications are associated with chromosome-encoded mechanisms (e.g. mutations in two-component systems or alterations of the mgrB gene) or may result in the expression of the plasmid-encoded phosphoethanolamine transferase, MCR-1. Firstly reported in 2016, MCR-1-producing Enterobacteriaceae have been described worldwide, mostly in *Escherichia coli*.
To contribute to the containment of polymixin resistance dissemination in Enterobacteriaceae, there is an urgent need for a test that enables (i) rapid detection of polymyxin-resistance and (ii) discrimination between plasmid- and chromosome-encoded resistance.

Material/methods: Here, we develop a cost-effective tool based on MALDI-TOF mass-spectrometry that aims to detect polymyxin resistance directly on intact Enterobacteriaceae in less than 15 min. The MALDI-TOF acquisition was performed on a single colony grown on Luria-Bertani agar using a new designed atypical matrix. From the obtained spectra, peaks corresponding to intact lipid A and modified lipid A were analysed manually.
This novel patented technique as been validated on a collection of (i) 53 *E. coli* including 9 polymyxin-resistant strains (within 6 *mcr-1* positive strains) and (ii) 81 *Klebsiella pneumoniae* including 49 polymyxin-resistant strains (within 6 *mcr-1* positive strains). Polymyxin susceptible strains (44 *E. coli* + 32 *K. pneumoniae*) were of various phenotypes (from wild-type to carbapenemase production). Colistin susceptibility testing has been performed by broth-microdilution according to EUCAST guidelines.

Results: Only the peak(s) corresponding to the intact lipid A (1796 m/z for *E. coli*, 1796 m/z and 1840 m/z for *K. pneumoniae*) has(ve) been detected for polymyxin-susceptible isolates. Independently of the resistance mechanism involved (chromosome- or plasmid-encoded), a peak at 1919 m/z corresponding to the addition of pETN to the 1796 m/z non-modified lipid A has been detected for all polymyxin-resistant isolates. Of note, no addition of pETN could be detected on the 1840 m/z lipid A of *K. pneumoniae*.
In case of resistance to polymyxin related to the plasmid-encoded *mcr-1* gene, an additional peak of high intensity was observed at 1821 m/z. Also, this peak was not characterized yet, it seems to be specific for the expression of a phosphoethanolamine transferase as it was observed only in MCR-1-producing isolates independently of the considered species.

Conclusions: This rapid and cost-effective technique offers a reliable tool for identification of plasmid-mediated polymyxin resistance in Enterobacteriaceae and therefore a very useful tool for preventing their spread.