Abstract

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Title: Characterisation of multidrug resistant *Klebsiella pneumoniae* and *Enterococcus faecium* isolates by spectroscopic and genotypic methods.

Ever increasing antimicrobial resistance is currently a worldwide problem, traditionally addressed by DNA-based approaches. This study aimed to evaluate the potential of Fourier transform infrared spectroscopy with attenuated total reflectance (FTIR-ATR) for the characterization of multidrug resistant carbapenemase-producing *K. pneumoniae* and *E. faecium* isolates. We analysed 20 clinical *K. pneumoniae* isolates obtained from different community laboratories from Portugal between March 2014 and September 2015 and 143 previously characterized vancomycin-resistant *E. faecium* isolates obtained from humans, animals, and the environment in 26 countries between 1992 and 2015. Isolates were primarily characterized by genotypic methods including antimicrobial susceptibility testing, detection of carbapenemases and extended-spectrum β-lactamases (ESBLs), identification of antibiotic resistance coding transposons (Tn) and plasmids and genetic relatedness of isolates multi-locus sequence typing (MLST) and subsequently by FTIR and comparison of spectra by multivariate data analysis. *K. pneumoniae* isolates produced KPC-3 and ESBLs (SHV or CTX-M types) and were resistant to aminoglycosides (76 %), carbapenems (70 %) or nitrofurantoin (55 %). Bla_{KPC-3} was identified within Tn *4401* variant "d" and IncFIA and IncN plasmids. Using FTIR analysis, we were able in less than 48 hours to distinguish five clones that perfectly matched results obtained by MLST identifying sequence types ST147, ST15, ST231, ST348, and ST109. *E. faecium* showed 24 sequence types belonging to 6 BAPS (Bayesian Analysis of Population Structure) subgroups (2.1a, 2.1b, 3.1, 3.2, 3.3a1, 3.3a2) which were discriminated by FTIR analysis. FTIR-ATR coupled with multivariate data analysis was shown to be a promising money and time saving alternative tool for fast assessments of clonal relationships among clinically relevant *K. pneumoniae* and *E. faecium* isolates.