Identification of genes associated with beta-lactam resistance in clinical isolates of Gram-negative bacteria

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Background: Antibiotic resistance is rising to dangerous levels. New resistance mechanisms are reported every year, threatening the ability to control infectious diseases with available antimicrobial therapies. The characterization of the antibiotic resistance gene pool is therefore crucial for an accurate monitoring and control of high resistant bacteria.

Objectives: The objective of this study was to identify the genes associated with the production of carbapenemases, extended-spectrum \(\beta\)-lactamases (ESBL) and/or AmpC \(\beta\)-lactamases in a collection of gram-negative isolates from a central hospital in the northern region of Portugal.

Methods: Primers to amplify clinically relevant resistance genes were selected from the literature: \textit{blaKPC}, \textit{blaIMP}, \textit{blaVIM}, \textit{blaOXA-48}, \textit{blaOXA-23}, \textit{blaNDM}, \textit{blaSHV}, \textit{blaTEM}, \textit{blaCTX-M}, \textit{blaCMY-2} and \textit{blaDHA}. Two multiplex PCR sets were designed for the detection of carbapenemases, one for the ESBL and one for the AmpC \(\beta\)-lactamases. All assays were validated using 22 control strains containing characterized resistance genes. Subsequently, a set of 52 clinical isolates with antibiotic resistance were evaluated.

Results: PCR screening identified 13 (25.0\%) isolates positives for carbapenemases genes. Of those, 4 (7.7\%), including three \textit{Klebsiella pneumoniae} and one \textit{Escherichia coli}, were positive for \textit{blaKPC} and 9 (17.3\%) \textit{Acinetobacter baumannii} were positive for \textit{blaOXA-23}. ESBL genes were the most prevalent: 14 (26.9\%) isolates were positive for \textit{blaSHV}, 17 (32.7\%) for \textit{blaCTX-M} and 15 (28.8\%) for \textit{blaTEM}. For AmpC \(\beta\)-lactamases only 7 \textit{Enterobacteriaceae} isolates were found positive for \textit{blaCMY-2} or \textit{blaDHA}. Fourteen (26.9\%) isolates did not contain any of the tested genes and 18 (34.6\%) isolates contained two or more resistance genes.