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Acquired Resistance Mechanisms to Broad-Spectrum β-lactams in Gram-negative Bacteria; Characterisation and Strategies to Control their Spread

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Bacteria resistant to multiple antibiotics represent a great public health concern around the world. Treatment of bacterial infections, even by using the last therapeutic options, is being compromised due to the accumulation of multiple resistance mechanisms, with β-lactamases (enzymes that inactivate the β-lactams) playing a crucial role. Although those resistances have been classically attributed to chromosomal mutations, they are now commonly associated to non-chromosomal elements acquired from other bacteria by horizontal gene transfer (HGT).

In the first part of this thesis, A biochemical characterization for the most common Extended-spectrum β-lactamases (ESBLs) as well as two evolutive β-lactamase (GES-6 and CTX-M-33) as a source of last resort antibiotic resistance were performed. Some ESBLs genes such as PER-1, GES-6, or CTX-M-33 were shown to compromised the activity of the ceftazidime-avibactam and ceftolozane-tazobactam combinations or carbapenems.

An epidemiological study describing the evolution of carbapenem-resistant *Klebsiella pneumoniae* over time in a hospital in Portugal, including contemporary isolates recovered after 2014 was also performed. KPC-3 was the most common carbapenemase identified in *K. pneumoniae* isolated, followed by OXA-181. Interestingly, our data described for the first time in Portugal the emergence and spread of *K. pneumoniae* isolates co-producing KPC-3 and GES-5, drastically diminishing the options for treatment. Moreover, *in vivo* transfer of a $bla_{\text{NDM-1}}$ positive-IncC plasmid between different species was also described in two clinical strain from Portugal.

Then, the efficacy of new phage therapy approach was successfully tested against a colistin- and carbapenem-resistant *K. pneumoniae* clone co-producing NDM-1 and OXA-48 and being responsible for an outbreak in Germany.

In the second part of this thesis, the factors increasing the dissemination of resistance genes have been evaluated, showing that antibiotics may significantly induce the transfer of resistance genes through increased plasmid conjugation rate. Then, we demonstrated that antioxidant molecules could be used as mitigators of that induction, thus showing that such molecules could be useful for prevention of the undesired effects of antibiotics with respect to plasmid dissemination.

This work contributed to the overall knowledge on the dissemination and evolution of the resistance genes in multidrug resistant Gram-negative bacteria, and to the evaluation of novel strategies to mitigate this dissemination.

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