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Carbapenemase challenges: mechanisms of action, transmission and control

In vitro prediction of OXA-48 evolution toward ceftazidime hydrolysis

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Background: Carbapenemase OXA-48 is of major clinical concern, as this enzyme is increasingly described among Enterobacteriaceae and confers resistance to carbapenems. OXA-48 is a class D beta-lactamase, hydrolyzing penicillins and carbapenems, but sparing broad-spectrum cephalosporins. This enzyme is not susceptible to b-lactamase inhibitors. Among the OXA-48 variants identified from clinical isolates, OXA-163 and OXA-405, with a 4 amino acids deletion in the C-terminal part, have the ability to hydrolyze the broad-spectrum cephalosporins, i.e. cefotaxime (CTX) and ceftazidime (CAZ). This gain of function was accompanied by the reduced hydrolysis of carbapenems.

Material/methods: In order to further characterize the evolutionary potential of OXA-48 to hydrolyze cephalosporins, it was subjected to several rounds of in vitro-directed evolution with selection on increasing doses of ceftazidime (CAZ).

Results: Mutations leading to an increased ceftazidime hydrolysis were restricted to 3 domains of the protein, namely A, B, and C. Mutations within 1 domain conferred a modest or no increase in resistance to CAZ, while combination of mutations within 2 or 3 domains conferred marked and high resistance to CAZ, respectively (MIC of CAZ up to >256mg/L). For all variants, there was an inverted correlation between the increased resistance to CAZ and resistance to the penicillins. Conversely, there was a correlation between the increased resistance to CAZ and resistance to CTX, but also to ATM. Regarding carbapenems, the variants showing an increased activity to CAZ (MICs of CAZ ≥1mg/L) conversely showed lower activities toward carbapenems, with the exception of few variants where the MIC of IPM remained quite stable. By analyzing the tertiary structure of those variants selected, we observed that domains A, B, and C surrounded the active site, and the selected mutations likely altered this active site, and therefore the enzymatic profile of the enzyme.

Conclusions: We showed here that OXA-48 is prone to evolve into an enzyme able to hydrolyze efficiently CAZ, with some of those variants variants maintaining the ability to hydrolyse carbapenems. This means that use of ceftazidime against ESBL-negative but OXA-48-producing strains exhibiting reduced susceptibility to carbapenems but full susceptibility to broad-spectrum cephalosporins may likely to select OXA-48-like enzymes with ceftazidimase activity.