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Carbapenemase challenges: mechanisms of action, transmission and control

Class B1 metallo-beta-lactamase NDM-1: preliminary study of structure-activity relashionship

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Background: NDM-1 carbapenemase that went global within a few years, belongs to the B1 metallo β-lactamases (B1-MBLs). The latter share similar structural features: 5 loops (L) and 1 helix (H), surrounding the 2 active Zinc ions, with however quite divergent amino acid (AA), suggesting differences in substrat recognition and specificity. The aim of this work was to deepen our knowledge on the NDM-1 active site.

Material/methods: 10 AA positions known to be crucial for VIM-1 and IMP-1 activity have been replaced in NDM-1 by alanines using a site directed mutageneisis kit (Invitrogen). Random mutagenesis was performed on bla_{NDM-1} gene. Mutants were identified by PCR-sequencing, antibiograms, and MICs according to Eucast guidelines. Specific activity was determined on crude β-lactamase extracts. Some Mutant were cloned in pET41b, over-expressed and purified. Kinetic parameters were determined by UV spectrophotometry.

Results: Site directed mutagenesis in loop 1 (M61A, V65A and G67A) resulted in similar hydrolytic profiles as NDM-1. Mutation in loop 3 (K224A) resulted in an enzyme with very low β-lactam hydrolytic properties. Similarly, the 4 mutants in the heart of the active site (L4, L5; especially D199A and S262A) showing the most altered phenotypes. 19 random mono-mutants were identified: **A)** 11 were located close to the different loops with 3 types of antibiogram profiles : (1) completely susceptible: H196Y (3H site) and S262P (L4) (2) intermediate susceptible as compared to NDM-1, (weak penicillin and/or ceftazidim hydrolytic activity) : C221S (DCH), D267E (adjacent to L4), T115N (adjacent to L5), W87C (L2), L233H (L3) N138T and N138S (Hα3), P62R (L1) and (3) with slightly increased carbapenem MICs as compared to NDM-1: E149V **B)** 8 were located outside of known loops but with similar groups of profile: (1): L3W , L107M and P192A ; profile (2) F40L,W165R, D267E, A270T, A279T ; (3) A110E. Specific activities for carbapenems for E149V and A110E were consistent with MICs. Kinetic data showed that E149V had a lower affinity for all β-lactams as compared to NDM-1, but hydrolyzed carbapenems at a higher level (2 fold) as compared to NDM-1 and NDM-4, but at a lower one than NDM-9.

Conclusions: We confirmed the key role of residues K224 in L3 for substrat recognition and specificity, and F218, K121, D199, S262 for active site stability. In L1, M61, V65, and G67 seem to behave like in VIM-1, with a minor involvement in the substrat recognition. Random mutagenesis identified 14 residues affecting the enzyme's ability to hydrolysis carbapenems. Mutant E149V in Hα3 (same mutated residue as in NDM-9 but with a different substitution) displayed increased hydrolytic activity toward carbapenems, confirming the role of Hα3 in carbapenem hydrolysis.