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Paper Poster Session
Deciphering carbapenem resistance

Characterization of a novel variant of KPC with decreased susceptibility to phenylboronic acid

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Background: Phenotypic carbapenemase inhibition tests are frequently used to the characterization of carbapenemases. In the context of a regional spread of ST512 KPC-3-producing *Klebsiella pneumoniae*, two carbapenem-resistant *K. pneumoniae* isolates were recovered from the same patient, which yielded negative with the combined-disk test of meropenem plus phenylboronic acid. The aim of the study was to characterize both isolates, comparing findings with the KPC-3-producing index case of regional spread.

Material and Methods: Screening of carbapenamase genes were carried out by PCR and further sequencing. Genetic relatedness was performed by *XbaI* PFGE analysis with a position tolerance of 0,5%. Plasmid DNA was extracted by the Kieser method. Transformants of *E. coli* DH10 were obtained by electroporation plating on 0,125 mg/l ertapenem. Comparative analysis with the KPC-3 K2:A-:B-plasmid of the index case was carried out by *XhoI* restriction of plasmid DNA from DH10 transformants and hybridization with a KPC probe. Amplicons of bla_{KPC} gene were ligated into zero-Blunt TOPO plasmid and introduced into *E. coli* TOP10. Susceptibility to carbapenems, piperacillin/tazobactam, temocillin and combined meropenem and inhibitors were determined by the disc diffusion method. Specific activity and IC50 value from crude extracts of clinical isolates, DH10 transformants and TOP10 transformants were analyzed using 0,5 mg/l of protein concentration.

Results: Both isolates differed in 1 band by PFGE and, in turn, they differed with the index case of the regional ST512 spread in 1 band. KPC PCR was positive in both isolates and bla_{KPC} sequence differed from KPC-3 by a single (W104R) aminoacid substitution . Both plasmid DNA *XhoI* restriction patterns were identical as well as to that of the plasmid from index case. bla_{KPC} gene was located in the same 3,5 kb fragment and was flanked by identical sequences in the variants and in the index case. Both DH10 and TOP10 transformants showed no synergy with phenylboronic acid by using combined meropenem and inhibitors discs. Clinical and transformants of the variants yielded reduced inhibition zone for temocillin than the respectively index case, but greater inhibition zone for carbapenems. The addition of boronic acid (400 μM) reduced the specific activity of crude extracts for the index case (from 0.38 to 0.171 UE/mg of protein for imipenem; a similar reduction was observed for meropenem and ertapenem) while no change was found for the variant on this parameter. Boronic IC50 was increased for this new KPC variant (550 μM compared to 350 μM for the KPC-3 for imipenem).

Conclusions: 1) A novel KPC variant was detected within the spread of ST512 KPC-3-producing *K. pneumoniae* in our region; 2) The aminoacid substitution appeared to influence the affinity to boronic acid, and carbapenems and temocillin susceptibility.