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Carbapenem resistance mechanisms in carbapenemase-negative Klebsiella pneumoniae

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Background: *Klebsiella pneumoniae* is a serious nosocomial pathogen. Increasing antibiotic resistance in this species, especially against carbapenems, elicit major concern. Carbapenem resistance is mediated through the presence of carbapenemases or loss of outer membrane channels (porins) linked with the presence of beta-lactamases. Data from the National Reference Centre for Multidrug-resistant Gram-negative Bacteria shows that approximately 50% of carbapenem resistant *K. pneumoniae* strains do not have a carbapenemase.

The aim of this study was to investigate carbapenem resistant but carbapenemase-negative *K. pneumoniae* with regards to porin loss and ß-lactamase presence.

Material/methods: Thirty-three carbapenemase negative *K. pneumoniae* isolates recovered between 2011 and 2015 in Germany were investigated. The carbapenem susceptible reference strain *K. pneumoniae* ATCC 700603 was used for comparison. Antimicrobial susceptibility to ertapenem, imipenem, meropenem and 1st-4th generation cephalosporins was determined by agar dilution using EUCAST breakpoints for interpretation. Whole genome sequencing was performed by MiSeq. Assembled genomes were analyzed using Resfinder to determine the resistome. Alterations in porin encoding genes were investigated by comparison to the reference strain.

Results: All strains were tested non-susceptible to ertapenem and the cephalosporins. Twenty-six isolates were non-susceptible to imipenem and twenty-nine isolates to meropenem. All isolates had at least one and up to six acquired beta-lactamases. Beta-lactamase types detected and their prevalence is shown in Figure 1. DHA-1 and OXA-9 were found to be associated with higher MICs of all carbapenems, and LEN beta-lactamases with higher ertapenem and meropenem MICs.

The amino acid sequence of OMP-K35 differed in 17 isolates from ATCC 700603, whereas the amino acid sequence of OMP-K36 was different in all isolates. The reading frame of OMP-K35 was disrupted by premature stop-codons (n=14) or Insertion-Sequence (IS) -elements (n=1). The reading frame of OMP-K36 was also disrupted by premature stop-codons (n=12), IS-elements (n=13) and a missing start-codon (n=1). The 14 IS-elements belonged to 5 different IS families (IS1, IS4, IS5, IS6 and IS1380).

Conclusions: We did not find a single fundamental mechanism leading to carbapenem resistance and porin disruption in *K. pneumoniae*. A wide spectrum of different acquired beta-lactamases combined with point mutations, deletions or insertions in porin-encoding genes was found, resulting in premature stop-codons. Diverse IS-elements disrupting the porin encoding genes were identified. Generally OMP-K35 was found to be more conserved than OMP-K36. Disruption of the porin genes by an IS-element was mainly observed for OMP-K36 and seems to be a common mechanism for loss of this porin. The presence of the AmpC beta-lactamase DHA-1 combined with porin loss resulted in resistance to all carbapenems.