



## Detection of OXA-48-like Carbapenemase-Producing *Klebsiella pneumoniae* in a Tertiary Care Center in Turkey: Molecular Characterization and Epidemiology

Dear Editor,

The first outbreak of OXA-48-producing *Klebsiella pneumoniae* isolates was reported in Turkey in 2008 (1). Two different clones were detected among 39 *K. pneumoniae* isolates in the first published outbreak (1). In addition to the clones of individual hospitals, the plasmid-mediated nature of OXA-48 offers the opportunity for horizontal transfer between strains and genera, resulting in dissemination both on a national and international level (2, 3).

Sixteen carbapenem-resistant strains of *K. pneumoniae* isolated from patients hospitalised at Başkent University Hospital between the years 2007 and 2011 were included in this study. All strains were isolated from patients in intensive care units. Five of the 16 strains were isolated from solid organ transplant recipients. Carbapenem-resistant *K. pneumoniae* isolates were screened by PCR amplification of genes encoding a variety of carbapenemases, using a multiplex real-time PCR assay described by Monteiro et al. (4) with minor modifications. The presence of genes encoding bla<sub>TEM</sub>, bla<sub>SHV</sub> and bla<sub>CTX-M</sub> types of ESBLs was investigated according to the method described by Mostein et al. (5). Pulsed-field gel electrophoresis (PFGE) typing of the *K. pneumoniae* isolates was performed according to a previously described protocol (6).

A total of eight genetic clones were identified in the PFGE analysis. All strains were found to be positive for the gene encoding bla<sub>OXA-48</sub>, but none were positive for the IMP, VIM and NDM types of metalloenzymes, or the GES and KPC types of carbapenemases. DNA sequencing confirmed the presence of the OXA-48 gene in all isolates.

A total of 11 isolates were found to be harbouring at least one of the TEM and CTX-M types of ESBL genes and the remaining five were negative for these enzymes in the PCR analysis. The SHV type ESBL gene was not present in any of the strains. The characteristics of the 16 isolates are shown in Figure 1.

In SDS-PAGE analysis, 13 strains were found to be lacking in at least one of the OmpL35 and OmpK36 porin proteins. A representative gel image of the outer membrane protein profiles of the isolates is shown in Figure 2.

*In vitro* susceptibility test showed that all *K. pneumoniae* isolates were susceptible to amikacin and colistin. Only one isolate was resistant to tigecycline (number 7). Eleven strains were positive for ESBL production in the double disc diffusion

test, and no isolate was positive for MBL production as tested with E-test strips.

Imipenem and meropenem MIC values generally fall into the 'susceptible' category for OXA-48-producing strains, if it is the only resistance mechanism present (7). However, the presence of other resistance mechanisms along with OXA-48, such as outer membrane protein loss, results in high imipenem and meropenem MIC values. All strains in this study had MICs of 32 µg/mL for carbapenems, i.e. they were resistant. This result seems to be related to the presence of accompanying resistance mechanisms, such as outer membrane protein loss. A similar co-presentation, i.e. outer membrane protein loss and OXA-48 positivity, was recently published in Turkey (8).

There seems to be no dominant clone among our isolates. This finding is in accordance with the previously published data emphasising the horizontal dissemination between strains and even species (2, 9). In this study, clonal diversity implicates the plasmid-mediated dissemination of OXA-48-mediated resistance in clonal and non-clonal ways in our country. The accumulation of different resistance mechanisms, such as ESBL production and outer membrane protein loss, makes OXA-48-producing *K. pneumoniae* strains more resistant to available therapeutic agents.

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**Informed Consent:** N/A.

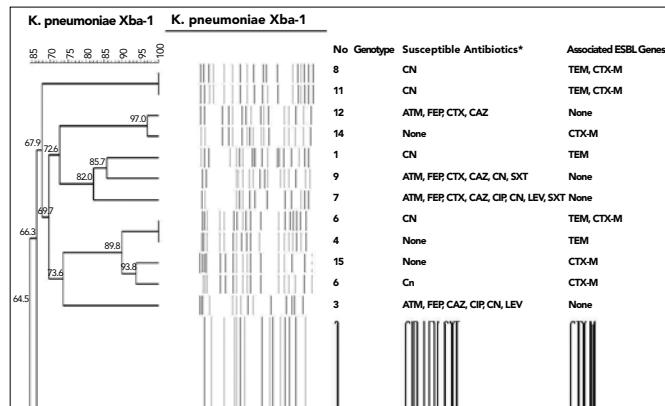
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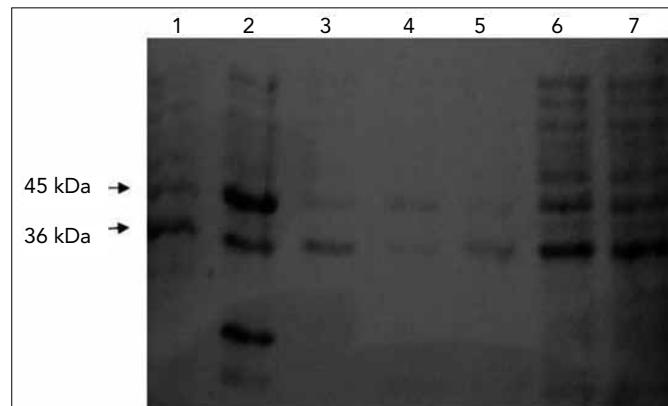




**Figure 1. Pulse field gel electrophoresis patterns of *K. pneumoniae* strains with OXA-48 like enzymes**

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**Figure 2. SDS-PAGE image of outer membrane proteins**

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