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Plasmid profile of Extended Spectrum β -lactamases (ESBL)-producing Enterobacteriaceae in Jordan

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Background: Extended spectrum β -lactamases (ESBL)-producing organisms pose unique challenges to clinical microbiologists, clinicians, infection control professionals and antibacterial-discovery scientists. Members of family Enterobacteriaceae can acquire resistance to extended spectrum beta lactams by a number of mechanisms; most important being the plasmid encoded extended spectrum beta lactamase (ESBL) and AmpC beta lactamase. The purpose of this study was to determine the presence of plasmids and their correlation with drug resistance of ESBL-producing *K. pneumoniae* and *E. coli* and to investigate the prevalence of these plasmid profiles in North of Jordan. **Methods:** ESBL production was studied in *K. pneumoniae* and *E. coli* and susceptibility testing of ESBL positive isolates was done for various beta lactams, cephalosporins, monobactams and other commonly used drugs against them. Plasmid DNA isolation of all the ESBL positive strains was done by alkalilysis method. The presence of plasmid was correlated with susceptibility to beta lactam drugs. **Results:** *E. coli*, *K. pneumoniae*, harbored multiple plasmids. The number of plasmids ranged from 1 to 9. Five plasmid profiles (A, B, C, D and E) were found in *E. coli* isolates and three plasmid profiles (A, B and C) were found in *K. pneumoniae* isolates. Among the different plasmid profiles profile C and D in *E. coli* and profile C in *K. pneumoniae* were the most resistant to antibiotics. One plasmid (M.W greater than 23 kbp) was present in 90% of all isolates. Also, the results showed that about 96% of the *E. coli* and *K. pneumoniae* isolates are multidrug resistant. **Conclusion:** There is a strong correlation between the number of plasmids harbored by an isolate and resistance to various drugs tested.

Polyclonal KPC-3-producing Enterobacteriaceae in Portugal

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Introduction: The emergence and rapid dissemination of carbapenemase (CARB)-producing Enterobacteriaceae has become an important therapeutic and infection control problem in Europe. In Portugal, according to the European Antimicrobial Resistance Surveillance Network (EARS-Net) surveillance study, there was an increase in carbapenem nonsusceptibility of *K. pneumoniae* isolates from 0.72% in 2008 to 1.58% in 2010. However, little is known on CARB-producing Enterobacteriaceae. KPC enzymes have not been described in Portuguese clinical Enterobacteriaceae isolates, but a KPC-2-producing *E. coli* isolate was recently found in the aquatic environment.

Methods: In this study, we report 6 KPC-3-producing Enterobacteriaceae isolates (5 *K. pneumoniae* and 1 *Enterobacter cloacae*), collected between March 2010 and December 2011 and sent to the NIH-Lisbon for carbapenem susceptibility confirmation. Antimicrobial susceptibility of clinical isolates was performed by disk diffusion method. PCR and sequencing were applied to detect and identify CARB-encoding genes as well as *bla*_{ESBL} and *bla*_{P_{MA β}} (plasmid-mediated AmpC β -lactamases); the respective genetic environment was revealed by sequencing using PCR mapping, after standard cloning experiments. Direct transfer of the carbapenem resistance phenotype was attempted by mating-out assays. Antibiotics susceptibility (MIC) of transconjugants and respective isolates were tested by microdilution and the results were interpreted according to EUCAST breakpoints. The plasmids obtained for all isolates were characterized by PCR-based replicon typing (PBRT). Clonal relatedness of the 5 *K. pneumoniae* isolates was investigated by multilocus sequence typing (MLST), using the protocol developed by the Institute Pasteur (www.pasteur.fr/mlst/Kpneumoniae.html).

Results: The majority of isolates were collected from the urine (42.8%) of elderly (≥ 65 years old) male patients (85.7%), admitted at four geographic distant Portuguese hospitals. The KPC-3 producers displayed a MDR phenotype with consistent susceptibility only to colistin and tigecycline. All isolates demonstrated a positive combined disk test with meropenem and meropenem-boronic acid, indicative of serine CARB production. Overall, the *bla*_{KPC-3} gene was confirmed in all isolates, alone or in combination with other *bla* genes, namely *bla*_{SHV-26}, an inhibitor-resistant SHV; *bla*_{CTX-M-15}, the most frequent ESBL-type found worldwide; and the *bla*_{SHV-164} (HE981194), here firstly described. No co-expression of KPC-3 with other CARB or PMA β was detected. Recombinant plasmids with a 4,110 bp insert conferring resistance to carbapenems were obtained after cloning experiments. Genetic mapping around the *bla*_{KPC-3} gene identified the Tn3-like Tn4401 transposon. In this study, we detected, in all isolates, the 68-bp deletion isoform, which ends upstream of the -35 region of the promoter. In our study, the majority of the *bla*_{KPC-3}-harboring plasmids appear to be nonconjugative, since only one conjugant [EcK12 C600 (INSRA14159-KPC-3)] and one transformant [EcDH5a (INSRA12267-KPC-3)] were obtained. In general, both transformants had antibiotic resistance profiles similar to those of their parental clinical isolates. All but two KPC-3-producing isolates were positively typed by the PBRT method: IncF_{repB} + IncFIIIs (n=3), IncF_{repB} + IncP (n=1) and IncF_{repB} (n=1). Both conjugant and transformant had only the IncF_{repB}, suggesting that this Inc group is associated with KPC-3-harboring plasmids. The KPC-3-producing *K. pneumoniae* were from distinct sequence types, namely ST14, which have been associated with NDM-producing *K. pneumoniae*, ST34, ST59, ST416 and the novel ST960.

Conclusion: This study provides new data regarding the molecular epidemiology of CARB-producing Enterobacteriaceae in Portugal, which includes KPC-3-harboring IncF_{repB} plasmids that are shared by polyclonal *K. pneumoniae* and *E. cloacae* clinical strains. Overall, our results emphasize the need of a concerted action to manage carbapenem use.

Keywords Tn4401-KPC-3; IncF_{repB}