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# Detection of OXA-48-like and NDM carbapenemases producing *Klebsiella pneumoniae* in Jordan: A pilot study

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**Summary** Little is known of carbapenemase producing *Klebsiella pneumoniae* (CPK) in Jordan. This study aimed to determine the prevalence of CPK in a major hospital in Amman, Jordan in 2012—2013 and to characterize the isolates and detect the types of carbapenemase(s) they produced.

For the 296 isolates investigated, species identification and antimicrobial susceptibilities were determined (Vitek II, bioMérieux). Isolates with decreased ertapenem susceptibility were tested for carbapenemase production using the Modified Hodge Test. Isolates with a carbapenemase-positive phenotype were characterized further via multiplex PCRs for extended-spectrum β-lactamase and carbapenemase genes and by Pulsed Field Gel Electrophoresis (PFGE).

Seven of 296 *K. pneumoniae* isolated in 2012—2013 (2.4%) were carbapenemase producers, five produced class D carbapenemases (OXA-48-like) and two produced a NDM metallo-beta-lactamase. All seven isolates also encoded CTX-M enzymes; CTX-M-1-like enzymes were detected in five isolates (two co-producing NDM enzymes and

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three co-producing OXA-48-like enzymes), CTX-M-9 was found in the two remaining OXA-48-like producers. PFGE revealed five genetically distinct types amongst the seven carbapenemase producing *K. pneumoniae*, with two pairs of identical isolates associated with patients treated on the same wards.
The emergence of OXA-48-like and NDM carbapenemases associated with multi-drug resistant (MDR) isolates in Jordan is concerning. The strict implementation of infection control practices will help to disrupt the spread of MDR carbapenemase producers in Jordanian hospitals.


## Introduction

Carbapenem antimicrobials are the drugs of choice for severe infections caused by multidrug resistant Gram-negative bacteria [1]. Carbapenem-resistant *Enterobacteriaceae* (CRE) can cause treatment failure and constitute an important public health problem [2]. Resistance due to the production of a carbapenemase is horizontally transferred amongst *Enterobacteriaceae* and the types of carbapenemase are myriad; clavulanic-acid-inhibited β-lactamases (Class A: KPC, NMC, IMI, SME, and GES); metallo-β-lactamases (Class B: IMP, VIM, NDM, GIM, SPM, and SIM) and expanded-spectrum oxacillinases (Class D: OXA-48-like) [3].

European surveillance data have indicated the incidence of carbapenem-resistant *Klebsiella pneumoniae* to be low (less than 0.5%) in many Northern European countries including Finland, Denmark, Ireland and Sweden in 2014. By contrast Italy and Greece had endemic problems with frequencies of 34.3% in Italy and 59.4%, respectively (*EARS-Net interactive database: http://ecdc.europa.eu/en/healthtopics/antimicrobial_resistance/database/Pages/table_reports.aspx) [4]. Elsewhere, carbapenemase-producing Klebsiella (CPK) are considered endemic in the North East of the USA, Puerto Rico, Zhejiang Province of China, Southern Asia, and parts of the Middle East, while very few cases have been reported from Australia and Africa [5]. In Jordan and other Arabian countries the isolation rates for CPK range between 2% and 10% [6—8] and OXA-48 and NDM-1 enzymes are known to predominate amongst *Enterobacteriaceae* in Morocco, Palestine and the Arabian Gulf countries [6,7,9]. While Jordanian hospitals, including our own, do receive medical cases referred from other Arabian countries, little is known of the prevalence of CPK infections, their outcomes and the degree of spread amongst hospital patients [8]. This pilot study aimed to determine the frequency of infections due to CPK and to characterize the isolates and the carbapenemase enzymes they encode in order to determine the molecular epidemiology of CPK amongst effected patients in a major Jordanian teaching hospital.

## Materials and methods

### Bacterial isolates

From March 2012 to April 2013, 296 *K. pneumoniae* were isolated from clinical specimens (blood culture, urine, fluid, wound, tissue and sputum) taken from 296 patients attending Islamic Hospital which is a 450 bed hospital in Amman, Jordan. Isolates were identified and tested for susceptibility to a panel of antimicrobials (Vitek II, bioMérieux), the results for the latter were interpreted according the Clinical and Laboratory Standards Institute (CLSI) guidelines, 2014 [10].

### Screening for carbapenemase producing isolates

Isolates with decreased susceptibility to cefoxitin MIC ≥16 μg/ml, ceftazidime MIC ≥8 μg/ml, cefotaxime MIC ≥2 μg/ml, imipenem MIC >1 mg/l, or meropenem MIC >1 mg/ml were investigated for carbapenemase activity [11]. Also, isolates with decreased susceptibility to ertapenem (zones of 21 mm or less around a 10 μg ertapenem disk) were investigated as possible carbapenemase producers according to CLSI guidelines (2014) [10]. The Modified Hodge Test (MHT) [11] was performed on suspected carbapenemase producers according to CLSI guidelines (2014) [10], with *K. pneumoniae* ATCC BAA-1705 and *K. pneumoniae* ATCC BAA-1706 used as positive and negative control strains, respectively.