

## Case Report

# Detection of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* producing NDM-1 in Lebanon

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### Abstract

Carbapenem resistance has been encountered globally with poor outcome of infected patients. NDM-1 (New Delhi metallo-beta-lactamase) gene containing organisms have emerged and are now spreading in all continents. This is the first report of Iraqi patients referred to Lebanon from whom carbapenem resistant *Enterobacteriaceae* were recovered. The genes involved in carbapenem resistance were *bla*-OXA-48 and the novel NDM-1. This report highlights the alarming introduction of such resistance among *Enterobacteriaceae* to this country.

**Key words:** carbapenem; *Enterobacteriaceae*; carbapenemases; New Delhi metallo-beta-lactamase; resistant; Lebanon

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### Introduction

Carbapenem antibiotics are considered the drugs of choice for the treatment of extended spectrum beta-lactamase (ESBL)-producing *Enterobacteriaceae* and other multidrug resistant bacteria [1]. The emergence of bacterial strains that produce carbapenemases further limits the therapeutic options available to clinicians.

The increasing incidence of carbapenem resistant *Enterobacteriaceae* has been described in different countries around the world. In the Middle East, including Lebanon, such reports are very scarce [2,3]. Herein, we give an account of three patients from Iraq presenting to our institution, and from whom carbapenemase-producing *Escherichia coli* or *Klebsiella pneumoniae* were recovered. Three patients were referred from Iraq for management of different medical conditions at the American University of Beirut Medical Center (AUB-MC) in July 2010. The first two patients had leukemia diagnosed upon arrival and developed febrile neutropenia one week later. The third patient presented for meatoplasty and excision of a distal glandular burn scar of the glans resulting from a complicated transurethral resection of the prostate.

### Methodology

Blood cultures (BactAlert, BioMerieux, Marcy-L'Étoile, France) and urine culture were performed for the detection of pathogens. Isolates showing a disc diffusion diameter of  $\leq 19$  mm for ertapenem were considered carbapenem resistant, and were selected for further screening tests. DNA extraction of the isolates was performed using the Genomic DNA Extraction and Purification Kit (GE Healthcare, UK Limited, Little Chalfont Buckinghamshire, UK) according to the manufacturer's specifications. Minimum inhibitory concentrations (MICs) for ertapenem, imipenem, meropenem and aztreonam were determined using E-test strips (AB Biodisk, Solna, Sweden). As recommended by the CLSI [4], isolates with an ertapenem, imipenem or meropenem MIC  $\geq 2$   $\mu\text{g/ml}$  were considered potential carbapenemase producers and were selected for further testing. PCR was then used to amplify the ESBLs encoding genes *bla*-TEM-1, *bla*-CTX-M 15, *bla*-SHV, and *bla*-OXA-1; the AMP-C gene *bla*-CMY-2; the carbapenemases encoding genes GES (Guiana extended-spectrum), IMI (imipenem-hydrolyzing), NMC (not metalloenzyme carbapenemase), GIM (German imipenemase), SPM (Sao Paulo MBL), IMP (active on imipenem), VIM (Verona integron-encoded MLB), SME (*Serratia marsecens* enzyme),

SIM (Seoul Imipenemase), KPC (*Klebsiella pneumoniae* carbapenemase), *bla*<sub>OXA-23</sub>, *bla*<sub>OXA-48</sub>, the novel *NDM-1* encoding gene; and the fluoroquinolone resistance encoding gene, *qnrS*. PCR amplification was also performed to detect the outer membrane porin encoding genes *OmpF* and *OmpC*. Sequencing reactions were performed using the Dynamic ET terminator cycle sequencing kit (GE Healthcare, UK) and the PrepEase Sequencing Dye Clean-Up Kit (Affymetrix, Santa Clara, CA, USA). Purified products were sequenced by the ABI 3100 genetic analyzer (Applied Biosystems, Foster City, CA, USA) and analyzed using the BioEdit Sequence Alignment Editor (BioEdit, Carlsbad, CA, USA). Conjugation experiments were performed using sodium azide resistant J53 *E. coli* cells as the recipient strain. Transconjugants were grown on Mueller Hinton agar plates containing 150 mg/L sodium azide and 1 mg/L ceftazidime or 20 mg/L cefoxitin to select for recipients that had acquired resistance. Antibiotic susceptibility testing for the transconjugants was performed as described above.

## Results

The blood cultures from the first two patients (BactAlert, BioMerieux, Marcy-L'Etoile, France) grew *E. coli* from one and *K. pneumoniae* from the other, and both were carbapenem resistant. The urine culture of the third patient upon admission grew carbapenem resistant *K. pneumoniae*. According to the CLSI guidelines [4], all three isolates were found to be resistant to carbapenems, all cephalosporins, aztreonam, aminoglycosides and quinolones. All the isolates were susceptible to tigecycline and colistin (Table 1). Only the third isolate was susceptible to fosfomycin. The first isolate produced the enzyme OXA-48, the second produced both OXA-48 and NDM-1, and the third harbored only the NDM-1 carbapenemases responsible for the resistance. These isolates also harbored OXA-1, TEM-1 and CTXM-15 with outer membrane porin mutations (*OmpF* and *OmpC*) that contribute to carbapenem resistance. *OmpF* and *OmpC* are outer membrane protein porin that form passive diffusion pores which allow small molecular weight hydrophilic materials across the outer membrane, including antibiotics. The mutation of these porins together with ESBLs and AmpC production confers carbapenem-resistance phenotype.

The genes responsible for carbapenem resistance and MICs of the different carbapenems are detailed in Table 2. Testing for MIC of tigecycline and colistin was not performed. None of the isolates

harbored the fluoroquinolone resistance encoding gene, *qnrS* (Table 2). Conjugation experiments showed the transfer of cephalosporin resistance to the recipient *E. coli* J53 after conjugation with the *E. coli* isolate of the first patient only, indicating that the resistance gene is transferable.

## Discussion

This is the first report from Lebanon on carbapenem resistant *Enterobacteriaceae* isolates imported from Iraq. The introduction of such resistant strains, particularly those carrying the *NDM-1* gene, constitutes an alarming threat. Reports on carbapenem resistant *E. coli* and *K. pneumoniae* have been published from different countries in the region including Turkey [1,5-7], Greece [8-10], Egypt [11], Saudi Arabia [12], Oman [13], Israel [14], and Iraq [15]. However, *NDM-1* producing *Enterobacteriaceae* have not yet been reported from any of these neighboring countries except for one report from Oman [13] and recently one from Iraq [15]. The *NDM-1* gene raised global concern since its first recognition in Pakistan [16] and spread to many other countries via medical tourists [13,16,17,18].

The genes involved in carbapenem resistance among *Enterobacteriaceae* vary greatly among different countries. In Lebanon, these carbapenemases are OXA-48 from both *K. pneumoniae* and *E. coli*. In 2009 a study among ESBL isolates showed that 2.5% of *E. coli* and 7.84% of *K. pneumoniae* were carbapenem resistant [2,3]. The carbapenemases described in Turkey were the OXA-48 and VIM-5 [1,6-7]. One case of a *K. pneumoniae* producing OXA-48 was reported in an Egyptian patient hospitalized in France [11]. Reports from Greece revealed class B, VIM types -1, -2, -12, -19; class A, KPC-2 and class D, OXA-48 [8-10]. In Israel, reports described the presence of KPC-2 and KPC-3 [14]. The latter was also spread from Israel to Columbia through a liver transplant patient hospitalized initially in Israel [19]. Recently, one report on carbapenem resistance was published from Saudi Arabia describing a *Pseudomonas aeruginosa* strain harboring the *bla*<sub>VIM-2</sub> gene from a Saudi patient hospitalized in France [12].

**Table 1.** Antimicrobial susceptibility pattern of the three isolates according to CLSI

Organism	Antimicrobial susceptibility profile by disc diffusion in mm													
	ERT	IMP	MER	CTX	CPD	CAZ	FEP	CXM	ATM	SXT	CIP	NN	TIG	COL
Patient 1 <i>E. coli</i>	R	R	R	R	R	R	R	R	R	R	R	R	S	S
Patient 2 <i>K. pneumoniae</i>	R	R	R	R	R	R	R	R	R	R	R	R	S	S
Patient 3 <i>K. pneumoniae</i>	R	R	R	R	R	R	R	R	R	R	R	R	S	S

CTX: Cefotaxime, CPD: Cefpodoxime; CAZ: Ceftazidime; FEP: Cefepime; CXM: cefuroxime; ATM: Aztreonam; SXT: sulfamethoxazole-Trimetoprim; CIP: Ciprofloxacin; NN: Tobramycin; TIG: Tigecycline; COL: Colistin; R: indicates a resistant profile

**Table 2.** MIC results of carbapenems, and carbapenem-resistant genes recovered from the three patients

	Patient N°1 <i>E. coli</i>	Patient N°2 <i>K. pneumoniae</i>	Patient N°3 <i>K. pneumoniae</i>
Antimicrobial (MIC µg/ml)			
Ertapenem	>32	>32	>32
Imipenem	>32	32	32
Meropenem	12	16	16
Doripenem	4	>32	>32
Genes detected			
OmpF	+	-	-
OmpC	+	-	-
OXA-1	+	+	+
OXA-48	+	+	-
CTXM-15	+	-	+
TEM-1	+	-	-
NDM-1	-	+	+
Qnrs	-	-	-

Treatment of patients infected with carbapenem resistant *Enterobacteriaceae* is challenging due to the currently limited options. Such isolates also show resistance to all beta-lactam antibiotics and very often carry on the same transposon the genes responsible for resistance to trimethoprim-sulfamethoxazole, aminoglycosides and fluoroquinolones [20]. Only tigecycline, colistin and fosfomycin can be effective but these also have limitations. Tigecycline has limited use in urine and primary blood-stream infection despite *in vitro* susceptibility. In other clinical scenarios, better results were obtained when tigecycline was used in combination [21,22]. Colistin is being reused in the era of antibiotic resistance to treat multidrug resistant strains as either monotherapy or preferably as part of combination therapy [21,23,24]. In addition, *Enterobacteriaceae* resistant to colistin have been recently described [20]. Fosfomycin, not widely available, shows excellent activity *in vitro* against strains resistant to both colistin and tigecycline [25]. These limitations warrant the search for new alternative treatments that so far remain under research and development.

In conclusion, Lebanon, like other countries, is now facing a dangerous threat with the emergence of carbapenem-resistant *Enterobacteriaceae* and the imported *NDM-1* strains to this country. Since Lebanon has recently become a common destination for Iraqi patients seeking advanced medical care, physicians caring for such patients should be aware of these reports to screen patients and institute proper infection control practices when needed. As well, foreign patients (American soldiers or others) with risk factors who have resided in Iraq and had sought medical care in Iraqi hospitals should be screened for the presence of such resistant isolates when seeking further medical care in their home countries to avoid the spread of these multidrug resistant strains. A multidisciplinary approach to limit the spread of such organisms is essential. Proper prevention, detection, antimicrobial stewardship and adequate infection control measures should help in limiting the spread of these organisms.

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