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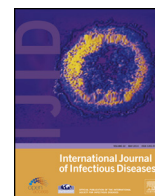
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Countrywide spread of OXA-48 carbapenemase in Lebanon: surveillance and genetic characterization of carbapenem-non-susceptible *Enterobacteriaceae* in 10 hospitals over a one-year period



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SUMMARY

Objectives: To detect, characterize, and assess the genetic clonality of carbapenem-non-susceptible *Enterobacteriaceae* in 10 Lebanese hospitals in 2012.

Methods: Selected *Enterobacteriaceae* isolates with reduced susceptibility to carbapenems were subject to phenotypic study including antibiotic susceptibility, cloxacillin effect, modified Hodge test, and activity of efflux pump inhibitor. Carbapenemase genes were detected using PCR; clonal relatedness was studied by pulsed field gel electrophoresis.

Results: Out of 8717 *Enterobacteriaceae* isolated in 2012, 102 (1.2%) showed reduced susceptibility to carbapenems. Thirty-one (70%) of the 44 studied clinical isolates harbored *bla*_{OXA-48}, including 15 *Klebsiella pneumoniae*, eight *Escherichia coli*, four *Serratia marcescens*, three *Enterobacter cloacae*, and one *Morganella morganii*. The majority of OXA-48 producers co-secreted an extended-spectrum beta-lactamase, while one had an acquired AmpC of the ACC type. In the non-OXA-48 producers, carbapenem resistance was attributed to the production of acquired AmpC cephalosporinases of MOX or CIT type, outer membrane impermeability, and/or efflux pump overproduction. DNA fingerprints revealed that OXA-48 producers were different, except for clonal relatedness among four *K. pneumoniae*, two *E. coli*, two *E. cloacae*, and three *S. marcescens*.

Conclusions: Nosocomial carbapenem-non-susceptible *Enterobacteriaceae* are moderately spread in Lebanon and the predominant mechanism is OXA-48 production.

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1. Introduction

Enterobacteriaceae are Gram-negative rod-shaped bacteria that normally colonize the human intestinal tract and are capable of causing opportunistic infections in both community and hospital settings. They are easily spread among individuals and can acquire

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genetic material through lateral gene transfer, largely mediated by mobile elements like plasmids and transposons.¹ The dissemination of *Enterobacteriaceae* producing extended-spectrum beta-lactamases (ESBLs) has compromised susceptibility to cephalosporins in many areas of the world, including Lebanon, and has increased the consumption of carbapenems.^{2,3} Recently developed antibiotics among the class of beta-lactams, carbapenems (imipenem, meropenem, ertapenem, and doripenem) are not hydrolysable by EBSLs and represent the antimicrobial options of last resort.² In 1993, the first incidence of carbapenem resistance in *Enterobacteriaceae* was described in *Enterobacter cloacae* due to production of carbapenemase NmcA.⁴ Since then, various carbapenemases have been detected in *Enterobacteriaceae* and the majority belong to three Ambler classes: (1) class A, such as KPC, first described in the USA but now disseminated worldwide; (2) class B, such as IMP, VIM, and NDM metallo-beta-lactamases, encountered in Japan, Taiwan, and Greece, as well as many European countries; and (3) class D oxacillinases, such as OXA-48, originating in Turkey and now detected in Europe and the Mediterranean region.¹ Rare acquired cephalosporinases of Ambler class C may also show low carbapenem-hydrolyzing activity.⁵ Carbapenem resistance in *Enterobacteriaceae* may also arise from non-carbapenemase-mediated mechanisms, like permeability defects or the impact of efflux transporters.⁶

In Lebanon, carbapenem-resistant *Enterobacteriaceae* have been detected since 2008, with single case reports of OXA-48, IMP-1, and NDM-1 carbapenemases.^{7–10} The aims of the current study were to evaluate the dissemination of carbapenem-resistant *Enterobacteriaceae* in various Lebanese hospitals, to identify the mechanisms of resistance, to examine the types of carbapenemases involved in resistance, and to analyze the clonal relationship of such isolates collected during the year 2012. Part of this work was presented at the RICAI (Réunion Interdisciplinaire de Chimiothérapie Anti-infectieuse) 2012 congress in Paris, France (November, 22–23, 2012; abstract number 300).

2. Materials and methods

2.1. Bacterial isolates

From January 1, 2012 to December 31, 2012, carbapenem-intermediate/resistant isolates of *Enterobacteriaceae* were collected from the bacteriology laboratories of 10 Lebanese hospitals located in diverse geographic areas: Beirut (Hotel Dieu de France, Saint-George Hospital, Clinique de Levant); Mount Lebanon (Bellevue Medical Center, Arz Hospital); North Lebanon (Monla Hospital); South Lebanon (Secours Populaire Libanais, Labib Medical Center), and Bekaa (Farhat Hospital, Chtaura Hospital). Using standard disk diffusion testing, and following the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST),¹¹ strains with inhibition zone diameters of imipenem smaller than 22 mm, or of ertapenem smaller than 25 mm, were included. These isolates were delivered to the central microbiology laboratory at the Faculty of Pharmacy, Saint-Joseph University, Beirut.

2.2. Phenotypic analysis

2.2.1. Antimicrobial susceptibility testing and detection of ESBLs

Antimicrobial susceptibility testing was performed by disk diffusion on Mueller–Hinton agar plates, according to EUCAST guidelines.¹¹ Amoxicillin/clavulanic acid (AUG), ceftazidime (CAZ), cefotaxime (CTX), ceftazidime (CPM), ceftazidime (FOX), aztreonam (ATM), and imipenem (IMP) disks (Mast Diagnostics, Merseyside, UK) were tested. After overnight incubation, an increase in the

inhibition zone of the third- and/or fourth-generation cephalosporin disk towards the clavulanate-containing disk by at least 5 mm was considered as indicating synergy and the presence of an ESBL.¹² The minimum inhibitory concentration of ertapenem (MIC_{ERT}) was determined using the Etest (Liofilchem, Roseto degli Abruzzi, Italy).

2.2.2. Cloxacillin test for detection of AmpC cephalosporinases

Antibiotic susceptibility testing was performed with cloxacillin (25–500 mg/l) to inhibit AmpC cephalosporinases. An increase in susceptibility to cephalosporins in the presence of cloxacillin was considered to be due to AmpC production. The cloxacillin test was also used to allow better visualization of synergy between: (1) clavulanic acid and third- and/or fourth-generation cephalosporins, indicating the possibility of ESBL production, and (2) clavulanic acid and imipenem, indicating the production of an ESBL with carbapenem-hydrolyzing activity.¹²

2.2.3. Modified Hodge test

A modification of the Hodge test was applied to screen for carbapenemase production.¹³ The indicator organism, *Escherichia coli* ATCC 25922 at a turbidity of 0.5 McFarland, was used to inoculate the surface of Mueller–Hinton agar plates, and a 10 µg meropenem disk (Mast Diagnostics, Merseyside, UK) was placed at the center. The test strain was heavily streaked from the disk to the plate periphery. After overnight incubation, the presence of a cloverleaf-shaped indentation of growth of the test strain versus the indicator strain was interpreted as carbapenemase production, with the highest sensitivity to those belonging to Ambler classes A and D.¹⁴ Heavy streaks of carbapenemase-positive and carbapenemase-negative strains were used as internal controls.

2.2.4. Activity of efflux pump inhibitor

Mueller–Hinton plates containing 100 mg/l of efflux pump inhibitor phenylalanine–arginine β-naphthylamide hydrochloride (PAβN; Sigma-Aldrich, USA) were prepared. Selected isolates were swabbed on plates with and without PAβN, and Etest strips of ertapenem, cefixime, and levofloxacin (Liofilchem, Via Scozia, Italy) were placed on the plates. After overnight incubation at 37 °C, MICs in the presence and the absence of the inhibitor were compared, and a two-fold decrease in MIC in the presence of PAβN was considered as indicative of efflux activity.^{15,16} *E. coli* ATCC 25922 was used as internal control.

2.3. Genotypic analysis

The presence of various types of beta-lactamase genes was screened for by PCR using primers designed by the Institut Pasteur, Unité des Agents Antibactériens, Paris, France.¹⁷ When ESBLs were detected by phenotypic tests, PCR experiments to detect *bla*_{CTX-M group 1} and *bla*_{SHV} genes were performed. When hyperproduction of AmpC was suspected by the cloxacillin test, multiplex PCR for plasmid-encoded AmpC genes was performed, including *bla*_{ACC}, *bla*_{FOX}, *bla*_{MOX}, *bla*_{DHA}, *bla*_{CIT}, and *bla*_{EBC}. To detect carbapenemases, strains were tested for *bla*_{KPC}, *bla*_{GES}, *bla*_{OXA-48}, *bla*_{VIM}, *bla*_{IMP-1}, *bla*_{IMP-2}, and *bla*_{NDM} genes. For OXA-48 producers, the presence of insertion sequence IS1999 of transposon Tn1999 known to carry the *bla*_{OXA-48} gene was investigated, as described by Aubert et al.¹⁸

2.3.1. Conjugation experiments with *Klebsiella pneumoniae*

Conjugation experiments were performed with *K. pneumoniae* Kpd2, using the recipient nalidixic acid-resistant *E. coli* K12, as described previously,¹⁹ but changing the mating broth medium to brain–heart infusion.

Table 1

Numbers and percentages of carbapenem-non-susceptible *Enterobacteriaceae* (CNE) collected from 10 participating hospitals in different areas of Lebanon during the year 2012

Species	Total	Number (%) of CNE ^a
<i>Klebsiella pneumoniae</i>	1826	26 (1.4)
<i>Escherichia coli</i>	6631	51 (0.8)
<i>Enterobacter cloacae</i>	513	12 (2.3)
<i>Serratia spp</i> ^b	199	11 (5.5)
<i>Morganella morganii</i>	229	2 (0.9)
<i>Salmonella spp</i>	87	1 (1.1)
All <i>Enterobacteriaceae</i>	8717	102 (1.2)

^a The percentage for each species indicates the proportion of CNE among total isolated strains within the same species.

^b *Serratia marcescens* and *Serratia odorifera*.

2.3.2. Clonality of the OXA-48-positive strains

Genomic DNA digested with XbaI (Roche, Meylan, France) was subjected to pulsed field gel electrophoresis (PFGE) using a clamped homogeneous electric-field apparatus (CHEF-DRII, Bio-Rad). Bionumerics (Applied Math, Kortrijk, Belgium) was used to establish a similarity matrix for the DNA based on calculation of the Dice coefficient (pairwise comparison of strains). A dendrogram was generated with the unweighted pair group using arithmetic means (UPGMA) hierarchical algorithm. Gels were compared using *Staphylococcus aureus* NCTC 8325 as a reference strain. PFGE patterns were interpreted according to international recommendations.²⁰

3. Results

Out of 8717 *Enterobacteriaceae* isolated in 2012, 102 (1.2%) non-duplicate carbapenem-non-susceptible strains were reported in the records of the participating hospitals (Table 1). The distribution of the 102 strains among hospitals was as follows: 31 were from Hotel Dieu de France, 32 from Saint-George Hospital, six from Clinique de Levant, two from Bellevue Medical Center, two from Arz Hospital, one from Monla Hospital, 27 from Farhat Hospital, and one from Chtaura Hospital. Of these, only 44 isolates were delivered to the central laboratory. The studied species and corresponding hospital sources are shown in Figure 1. Twenty-two specimens were isolated from urine, 10 from sputum, four from pus, four from wound swabs, two from blood, one from stool, and one from pleural fluid. Eighteen isolates out of 44 were nosocomial.

The results of disk diffusion tests and MICs of ertapenem are shown in Table 2. Isolates of *K. pneumoniae* and *E. coli* displayed ertapenem-susceptible ($MIC_{ERT} \leq 0.5$ mg/l), intermediate ($MIC_{ERT} > 0.5-1$ mg/l), or resistant ($MIC_{ERT} > 1$ mg/l) phenotypes, concurrent either with sensitivity or resistance to tested cephalosporins

and aztreonam. All *E. cloacae* were resistant to ertapenem, tested cephalosporins, and aztreonam. *Serratia marcescens*, *Serratia odorifera*, and *Salmonella enterica* subsp. *arizonae* were highly resistant to the beta-lactams tested, while the *Morganella morganii* isolate was resistant to cephalosporins and aztreonam but intermediate to carbapenems.

Synergy between amoxicillin/clavulanic acid and third/fourth-generation cephalosporins was observed for 31 strains including 11 *K. pneumoniae*, six *E. coli*, nine *E. cloacae*, four *S. marcescens*, and one *M. morganii*. These isolates were candidates for ESBL gene detection. None of the isolates displayed synergy between amoxicillin/clavulanic acid and imipenem, indicating the absence of ESBLs with carbapenem-hydrolyzing activity. Moreover, synergy between amoxicillin/clavulanic acid and third/fourth-generation cephalosporins was better visualized on cloxacillin-containing media for the majority of the 31 strains described above.

Inhibition zones of third/fourth-generation cephalosporins (CAZ, CTX, and CPM) on Mueller–Hinton agar plates with 25–500 mg/l of cloxacillin increased for two *K. pneumoniae* and two *E. coli*, indicating the possibility of production of an acquired AmpC cephalosporinase. *E. cloacae*, *S. marcescens*, and *M. morganii* naturally produce chromosomal AmpC beta-lactamases and hyperproduction can be induced by exposure to beta-lactams.²¹ In two out of nine *E. cloacae* strains, three out of five *Serratia* strains, and in *M. morganii*, the cloxacillin test was positive. In the remaining strains, the test was negative, possibly due to a lack of hyperproduction of a chromosomal AmpC, or to masking by co-production of an ESBL or a carbapenemase.

The cloverleaf-like growth pattern in the modified Hodge test was positive for 35 strains including 15 *K. pneumoniae*, eight *E. coli*, seven *E. cloacae*, four *S. marcescens*, and one *M. morganii*.

3.1. OXA-48-producing strains

Out of 44 tested *Enterobacteriaceae*, 31 (70.4%) harbored OXA-48, including 15 *K. pneumoniae*, eight *E. coli*, three *E. cloacae*, four *S. marcescens*, and one *M. morganii*. Of the 31 OXA-48 producers, 28 (90.3%) carried IS1999. In addition to OXA-48, 20 (64.5%) isolates had an ESBL of CTX-M group 1 type, including 11 *K. pneumoniae*, five *E. coli*, three *E. cloacae*, and one *M. morganii*. In *Serratia*, the four (12.9%) OXA-48-positive isolates were also positive for an ESBL of the SHV type. In all these strains with both carbapenemase and ESBL enzymes, there was non-susceptibility to third-generation cephalosporins in addition to ertapenem. One *K. pneumoniae* harbored OXA-48 with an acquired AmpC of the ACC type. In conjugation experiments, OXA-48 was not transferable to recipient *E. coli* K12.

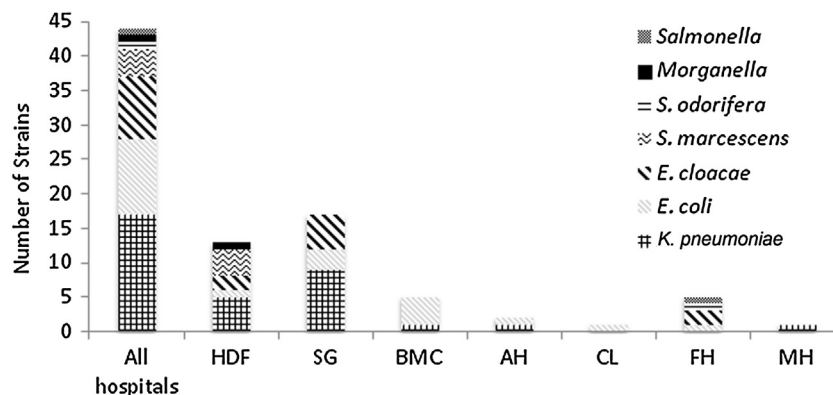


Figure 1. Distribution of the tested carbapenem-non-susceptible *Enterobacteriaceae* strains collected during the study by species and hospital source (HDF, Hotel Dieu de France; SG, Saint-George Hospital; BMC, Bellevue Medical Center; AH, Arz Hospital; CL, Clinique de Levant; FH, Farhat Hospital; MH, Monla Hospital.).

Table 2
Antimicrobial susceptibility patterns and genotypic profiles of tested carbapenem-non-susceptible strains

Species ^a	Tested strains	Genotype (number of strains)	ESBL	Acquired AmpC	Carbapenemase	Hodge test	AMC	FOX	CTX	FEP	CAZ	ATM	IMP	MIC _{ERT} (mg/l)	
<i>K. pneumoniae</i>	17	I (1)	Neg	MOX	Neg	Neg	R	R	R	S	R	R	I	0.25	
		II (1)	Neg	ACC	OXA-48	Pos	R	R	I	I	S	S	R	>32	
		III (11)	CTX-M1	Neg	OXA-48	Pos	R	R	R	R	R	R	R	R	>32
		IV (3)	Neg	Neg	OXA-48	Pos	R	I/R	I	S	S/I	S/I	I/R	R	(0.75–32)
		V (1)	Neg	Neg	Neg	Neg	R	R	R	R	R	R	R	R	>32
<i>E. coli</i>	11	I (1)	Neg	CIT	Neg	Neg	R	R	R	I	R	R	I	0.5	
		II (1)	CTX-M1	CIT	Neg	Neg	R	R	R	I	R	R	I	>32	
		III (5)	CTX-M1	Neg	OXA-48	Pos	R	I/R	R	R	I/R	R	I/R	R	(1–>32)
		IV (3)	Neg	Neg	OXA48	Pos	R	I	S	S	S/I	S	I	R	(0.5–1)
		V (1)	Neg	Neg	Neg	Neg	R	R	R	R	R	R	R	R	>32
<i>E. cloacae</i>	9	I (3)	CTX-M1	Neg	OXA48	Pos	R	R	R	R	R	R	R	>32	
		II (6)	1 CTX-M1 1 SHV 4 Neg	Neg	Neg	4 Pos 2 Neg	R	R	R	R	R	R	R	I/ R	(2–>32)
<i>S. marcescens</i>	4	I (4)	SHV	Neg	OXA-48	Pos	R	R	R	R	R	R	R	>32	
<i>S. odorifera</i>	1	I (1)	Neg	Neg	Neg	Neg	R	R	R	R	R	R	R	>32	
<i>M. morgani</i>	1	I (1)	CTX-M1	Neg	OXA-48	Pos	R	R	R	R	R	R	I	0.75	
<i>S. enterica</i> subsp. <i>arizonae</i>	1	I (1)	Neg	Neg	Neg	Neg	R	R	R	R	R	R	R	>32	

ESBL, extended-spectrum beta-lactamase; AMC, amoxicillin/clavulanic acid; FOX, ceftoxitin; CTX, cefotaxime; FEP, cefepime; CAZ, ceftazidime; ATM, aztreonam; IMP, imipenem; MIC_{ERT}, minimum inhibitory concentration of ertapenem; S, sensitive; I, intermediate; R, resistant.

^a *K. pneumoniae* = *Klebsiella pneumoniae*; *E. coli* = *Escherichia coli*; *E. cloacae* = *Enterobacter cloacae*; *S. marcescens* = *Serratia marcescens*; *S. odorifera* = *Serratia odorifera*; *M. morgani* = *Morganella morgani*; *S. enterica* = *Salmonella enterica*.

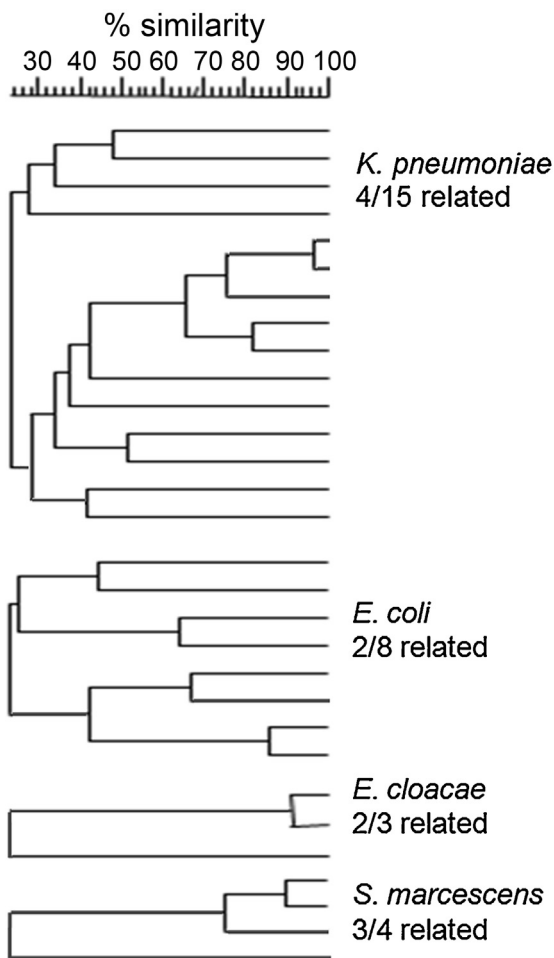


Figure 2. Dendrogram of DNA macrorestriction patterns of OXA-48-producing *Enterobacteriaceae* with XbaI. Strains were clustered by the unweighted pair-group method of arithmetic averages (UGPMA).

The XbaI-digestion PFGE patterns of the OXA-48-producing strains are presented in Figure 2. Data show the existence of clonally related strains among *K. pneumoniae* (4/15 isolates), *E. coli* (2/8 isolates), *E. cloacae* (2/3 isolates), and *S. marcescens* (3/4 isolates). The clonally related *K. pneumoniae* and *E. coli* were isolated from different hospitals.

3.2. Acquired AmpC-producing strains

Various acquired AmpC enzymes belonging to the groups MOX, ACC, and CIT were detected. One *K. pneumoniae* harbored MOX and another harbored ACC along with carbapenemase OXA-48. CIT was detected in two *E. coli* isolates; one of these also expressed an ESBL of CTX-M group 1 type.

3.3. Ertapenem resistance and efflux activity

Eleven isolates with a high level of resistance to ertapenem were subjected to the efflux inhibition test. These included one *K. pneumoniae* genotype V, two *E. coli* genotypes II and V, six *E. cloacae* genotype II, *S. odorifera* genotype II, and *S. enterica* subsp. *arizonae*. Ertapenem MICs were reduced by two-fold in six isolates that revealed a negative modified Hodge test, indicating the possibility of efflux pump activity in the absence of carbapenemase. These included one *K. pneumoniae* genotype V, four *E. cloacae* genotype II, and *S. odorifera* genotype II. The efflux test, however, was negative in three isolates (one *E. coli* genotype V, one *E. cloacae* genotype II, and *S. enterica* subsp. *arizonae*) eliminating efflux as a mechanism of ertapenem resistance in these strains and suggesting the possibility of outer membrane protein mutation. The two remaining isolates, one *E. coli* genotype II and one *E. cloacae* genotype II, did not grow in the presence of the inhibitor; the test was inconclusive.

4. Discussion

Although several studies have addressed the issue of the emergence of carbapenem-non-susceptible pathogens worldwide, no epidemiological survey has been carried out in Lebanon,

stressing the need for a nationwide investigation. The present study represents the first broad surveillance of carbapenem-non-susceptible *Enterobacteriaceae* isolated from Lebanese hospitals. It also represents the first report of acquired AmpC enzymes in Lebanon.

The overall prevalence of carbapenem-non-susceptible *Enterobacteriaceae* in Lebanon during 2012 was 1.2%. If compared to the 4.2% reported by the Centers for Disease Control and Prevention (CDC) in the year 2011, carbapenem resistance in *Enterobacteriaceae* in Lebanon appears modest.²² Concerning the distribution of carbapenem resistance among enterobacterial species, the European network on carbapenemases reported in 2012 that carbapenemase producers in Europe are mainly identified among *K. pneumoniae* and *E. coli*; the prevalence for *K. pneumoniae* was found to vary significantly, from very high in Greece (59.9%) to low in Germany (0.2%).²³ In this study, carbapenem resistance was disseminated among different enterobacterial species including *K. pneumoniae*, *E. coli*, *E. cloacae*, *S. marcescens*, and *M. morgani*. With the exception of *Serratia spp.*, resistance ranged from 0.8% for *E. coli* to 2.3% for *E. cloacae*. With regard to *Serratia spp.*, 10 out of the 11 non-susceptible strains isolated during 2012 originated from a single hospital, likely indicating a limited epidemic.

OXA-48 carbapenemase was the prominent mediator of carbapenem resistance in Lebanese hospitals. The detection of OXA-48 among *K. pneumoniae* and *E. coli* is in agreement with other data reported in Lebanon.^{7,10} Since the first description of OXA-48-producing *K. pneumoniae* in Turkey, numerous reports have indicated its dissemination to Mediterranean countries including France, Egypt, Tunisia, and Morocco.^{8,24–27} No other carbapenemases were detected among *Enterobacteriaceae* strains in this study. According to our results, the metallo-beta-lactamases NDM-1 and IMP-1 previously detected in case reports in Lebanon did not propagate to Lebanese patients.^{9,10} Also, although KPC is now endemic in Israel, it was not detected in this study.²⁸ This may indicate that the spread of carbapenemases of classes A and B among isolates of *Enterobacteriaceae* in Lebanon is still restricted, unlike that of OXA-48.

All isolates producing OXA-48 were associated with a positive modified Hodge test. This is consistent with previous data indicating that the test shows excellent sensitivity for the detection of carbapenem resistance mediated via the production of class D carbapenemases.²⁹ However, the test may lack sensitivity in *Enterobacter* species;²⁵ this may explain the false-positive results in four *E. cloacae* of genotype II, which were OXA-48-negative but modified Hodge test-positive. In this study, the majority of OXA-48 producers harbored insertion sequence IS1999 suggesting horizontal transfer of the *bla*_{OXA-48} gene. However, conjugation experiments were not efficient, which is indicative of probable *bla*_{OXA-48} chromosomal integration, as reported previously.^{30,31}

To test the genetic relatedness of the strains, PFGE allowed the comparison of the genome of the *bla*_{OXA-48}-positive *Enterobacteriaceae* obtained from different hospitals. For each species, certain strains were clonally related, indicating that clonal dissemination played a role in the overall spread of *bla*_{OXA-48}. The results of PFGE for OXA-48-producing *K. pneumoniae* and *E. coli* strains revealed high genetic diversity. OXA-48-producing *E. cloacae* and *S. marcescens* strains demonstrating genetic relatedness originated from the same hospitals, revealing small institutional-level outbreaks. In order to obtain a better analysis of clonality in these two genera, greater sampling from different hospital sources is needed.

In non-OXA-48 producers, acquired AmpC enzyme alone (*K. pneumoniae* genotype I and *E. coli* genotype I) conferred only intermediate resistance to imipenem. Like chromosomal AmpC, the plasmid-encoded AmpC enzymes slightly affect carbapenem susceptibility.⁵ However, carbapenem resistance can arise in

clinical isolates where AmpC enzymes or ESBLs exist together with lower permeability, like in *E. coli* genotype II.³² Indeed, the emergence of resistance to carbapenems can, in part, be due to the loss or alteration of outer membrane porins.³³ This could be the case in samples in which no investigated carbapenems of classes A, B, or D have been detected, like *E. coli* genotype V. In *K. pneumoniae* genotype V and *S. odorifera*, highly resistant to both cephalosporins and carbapenems, no beta-lactamase genes were detected. Efflux pump inhibitor tests were positive, indicating a beta-lactamase-independent resistance mechanism. In contrast, in *S. enterica* subsp. *arizonae*, neither beta-lactamase genes nor the efflux inhibition test were positive, suggesting the existence of mutant outer membrane proteins.

In conclusion, carbapenem-non-susceptible *Enterobacteriaceae* are moderately spread in Lebanese hospitals and resistance depends predominantly on OXA-48 production. A combination of AmpC production, efflux pump overexpression, and/or mutant porin proteins may also play a role in carbapenem resistance. As the reservoirs of OXA-48 producers are growing worldwide, concerted surveillance and infection control measures are needed for the containment of further dissemination in Lebanon.

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References

- Nordmann P, Naas T, Poirel L. Global spread of carbapenemase-producing *Enterobacteriaceae*. *Emerg Infect Dis* 2011;**17**:1791–8.
- Nordmann P, Dortet L, Poirel L. Carbapenem resistance in *Enterobacteriaceae*: here is the storm! *Trends Mol Med* 2012;**18**:263–72.
- Moubareck C, Daoud Z, Hakime NI, Hamze M, Mangeny N, Matta H, et al. Countrywide spread of community- and hospital-acquired extended-spectrum beta-lactamase (CTX-M-15)-producing *Enterobacteriaceae* in Lebanon. *J Clin Microbiol* 2005;**43**:3309–13.
- Naas T, Nordmann P. Analysis of a carbapenem-hydrolyzing class A beta-lactamase from *Enterobacter cloacae* and of its *lysR*-type regulatory protein. *Proc Natl Acad Sci U S A* 1994;**91**:7693–7.
- Jacoby GA. AmpC beta-lactamases. *Clin Microbiol Rev* 2009;**22**:161–82.
- Fernandez L, Hancock RE. Adaptive and mutational resistance: role of porins and efflux pumps in drug resistance. *Clin Microbiol Rev* 2012;**25**:661–81.
- Matar GM, Dandache I, Carrer A, Khairallah MT, Nordmann P, Sabra A, et al. Spread of OXA-48-mediated resistance to carbapenems in Lebanese *Klebsiella pneumoniae* and *Escherichia coli* that produce extended spectrum beta-lactamase. *Ann Trop Med Parasitol* 2010;**104**:271–4.
- Carrer A, Poirel L, Yilmaz M, Akan OA, Feriha C, Cuzon G, et al. Spread of OXA-48-encoding plasmid in Turkey and beyond. *Antimicrob Agents Chemother* 2010;**54**:1369–73.
- Daoud Z, Hobeika E, Choucair A, Rohban R. Isolation of the first metallo-beta-lactamase producing *Klebsiella pneumoniae* in Lebanon. *Rev Esp Quimioter* 2008;**21**:123–6.
- Baroud M, Dandache I, Araj GF, Wakim R, Kanj S, Kanafani Z, et al. Underlying mechanisms of carbapenem resistance in extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* and *Escherichia coli* isolates at a tertiary care centre in Lebanon: role of OXA-48 and NDM-1 carbapenemases. *Int J Antimicrob Agents* 2013;**41**:75–9.
- The European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters. Version 2.0. EUCAST; 2012. Available at: <http://www.eucast.org> (accessed as of 01/01/2012).
- Thomson KS. Extended-spectrum beta-lactamase, AmpC, and carbapenemase issues. *J Clin Microbiol* 2010;**48**:1019–25.
- Miriagou V, Cornaglia G, Edelstein M, Galani I, Giske CG, Gniadkowski M, et al. Acquired carbapenemases in Gram-negative bacterial pathogens: detection and surveillance issues. *Clin Microbiol Infect* 2010;**16**:112–22.
- Girlich D, Poirel L, Nordmann P. Value of the modified Hodge test for detection of emerging carbapenemases in *Enterobacteriaceae*. *J Clin Microbiol* 2012;**50**:477–9.

15. Doumith M, Ellington MJ, Livermore DM, Woodford N. Molecular mechanisms disrupting porin expression in ertapenem-resistant *Klebsiella* and *Enterobacter* spp. clinical isolates from the UK. *J Antimicrob Chemother* 2009;**63**:659–67.
16. Grobner S, Linke D, Schutz W, Fladerer C, Madlung J, Autenrieth IB, et al. Emergence of carbapenem-non-susceptible extended-spectrum beta-lactamase-producing *Klebsiella pneumoniae* isolates at the university hospital of Tübingen, Germany. *J Med Microbiol* 2009;**58**(Pt 7):912–22.
17. Antibiotic resistance techniques, Sixth ed., Paris, France: Institut Pasteur Unité des Agent Antibactériens; 2011.
18. Aubert D, Naas T, Heritier C, Poirel L, Nordmann P. Functional characterization of IS1999, an IS4 family element involved in mobilization and expression of beta-lactam resistance genes. *J Bacteriol* 2006;**188**:6506–14.
19. Moubareck C, Lecso M, Pinloche E, Butel MJ, Doucet-Populaire F. Inhibitory impact of bifidobacteria on the transfer of beta-lactam resistance among *Enterobacteriaceae* in the gnotobiotic mouse digestive tract. *Appl Environ Microbiol* 2007;**73**:855–60.
20. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* 1995;**33**:2233–9.
21. Paterson DL. Resistance in Gram-negative bacteria: *Enterobacteriaceae*. *Am J Infect Control* 2006;**34**(5 Suppl 1):S20–8. discussion S64–73.
22. Centers for Disease Control and Prevention (CDC). Vital signs: carbapenem-resistant *Enterobacteriaceae*. *MMWR Morb Mortal Wkly Rep* 2013;**62**:165–70.
23. Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, et al. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. *Clin Microbiol Infect* 2012;**18**:413–31.
24. Poirel L, Potron A, Nordmann P. OXA-48-like carbapenemases: the phantom menace. *J Antimicrob Chemother* 2012;**67**:1597–606.
25. Nordmann P, Poirel L. Strategies for identification of carbapenemase-producing *Enterobacteriaceae*. *J Antimicrob Chemother* 2013;**68**:487–9.
26. Ktari S, Mnif B, Louati F, Rekik S, Mezghani S, Mahjoubi F, et al. Spread of *Klebsiella pneumoniae* isolates producing OXA-48 beta-lactamase in a Tunisian university hospital. *J Antimicrob Chemother* 2011;**66**:1644–6.
27. Hays C, Benouda A, Poirel L, Elouennass M, Nordmann P. Nosocomial occurrence of OXA-48-producing enterobacterial isolates in a Moroccan hospital. *Int J Antimicrob Agents* 2012;**39**:545–7.
28. Warburg G, Hidalgo-Grass C, Partridge SR, Tolmasky ME, Temper V, Moses AE, et al. A carbapenem-resistant *Klebsiella pneumoniae* epidemic clone in Jerusalem: sequence type 512 carrying a plasmid encoding aac(6′)-Ib. *J Antimicrob Chemother* 2012;**67**:898–901.
29. Lee K, Chong Y, Shin HB, Kim YA, Yong D, Yum JH. Modified Hodge and EDTA-disk synergy tests to screen metallo-beta-lactamase-producing strains of *Pseudomonas* and *Acinetobacter* species. *Clin Microbiol Infect* 2001;**7**:88–91.
30. Poirel L, Abdelaziz MO, Bernabeu S, Nordmann P. Occurrence of OXA-48 and VIM-1 carbapenemase-producing *Enterobacteriaceae* in Egypt. *Int J Antimicrob Agents* 2013;**41**:90–1.
31. Gulmez D, Woodford N, Palepou MF, Mushtaq S, Metan G, Yakupogullari Y, et al. Carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* isolates from Turkey with OXA-48-like carbapenemases and outer membrane protein loss. *Int J Antimicrob Agents* 2008;**31**:523–6.
32. Livermore DM, Woodford N. The beta-lactamase threat in *Enterobacteriaceae*, *Pseudomonas* and *Acinetobacter*. *Trends Microbiol* 2006;**14**:413–20.
33. Dahmen S, Mansour W, Charfi K, Boujaafar N, Arlet G, Bouallegue O. Imipenem resistance in *Klebsiella pneumoniae* is associated to the combination of plasmid-mediated CMY-4 AmpC beta-lactamase and loss of an outer membrane protein. *Microb Drug Resist* 2012;**18**:479–83.