

ARTICLE ORIGINAL/ORIGINAL ARTICLE

SURVEILLANCE of CARBAPENEM NON-SUSCEPTIBLE GRAM NEGATIVE STRAINS and CHARACTERIZATION of CARBAPENEMASES of CLASSES A, B and D in a LEBANESE HOSPITAL

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Hammoudi D, Ayoub Moubareck C, Kanso A, Nordmann P, Karam Sarkis K. Surveillance des bacilles à Gram négatif de sensibilité diminuée aux carbapénèmes et caractérisation des carbapénémases de classes A, B, et D dans un hôpital libanais. *J Med Liban* 2015 ; 63 (2) : 66-73.

ABSTRACT • The production of carbapenem-hydrolyzing enzymes has been recognized as one of the most currently relevant resistance mechanisms in gram negative bacterial isolates, and is being detected in various countries. In Lebanon, carbapenem resistance was studied among gram negative pathogens collected from a university hospital from January to June of years 2011 and 2012. All isolates were subjected to phenotypic tests including antibiotic susceptibility, cloxacillin effect, modified Hodge test, and Etest[®] for metallo- β -lactamase detection. They were also subjected to genotyping by PCR sequencing to characterize β -lactamases.

Between January and June 2011, 48 carbapenem non-susceptible strains were collected. Of these, one *Klebsiella pneumoniae* harbored OXA-48 and insertion sequence IS1999; four *Acinetobacter baumannii* harbored simultaneously OXA-23 and GES-11, and three *Pseudomonas* harbored VIM-2 carbapenemase.

Between January and June 2012, 100 carbapenem non-susceptible strains were collected. Of these, one *K. pneumoniae* harbored simultaneously OXA-48, IS1999, and an acquired AmpC of the ACC group; four *Serratia marcescens* harbored OXA-48, while among eight *A. baumannii*, one strain co-harbored OXA-23 and GES-11, six harbored OXA-23 and one OXA-24. Fifteen *P. aeruginosa* and two *Pseudomonas* species harbored VIM-2; two *P. aeruginosa* strains produced IMP-1 and two others IMP-2. This epidemiological survey demonstrates the presence of carbapenemases of Ambler classes A, B, and D in a Lebanese hospital and indicates increase in the number and variety of such enzymes.

Keywords: carbapenemases, resistance, OXA-48, OXA-23, GES-11, VIM-2

RÉSUMÉ • La production de carbapénémases correspond à l'un des mécanismes de résistance les plus pertinents chez les bacilles à Gram négatif, mécanisme détecté au fur et à mesure dans de multiple pays. Au Liban, la résistance aux carbapénèmes a été étudiée chez les bacilles à Gram négatif isolés au CHU Hôtel-Dieu de France de janvier à juin en 2011 et 2012. Toutes les souches ont été soumises à des tests phénotypiques incluant l'étude de la sensibilité aux antibiotiques, l'effet de la cloxacilline, l'analyse du Hodge test modifié et l'utilisation des bandelettes Etest[®] pour la détection des métallobactamases. Elles ont également fait l'objet d'une étude génotypique incluant des techniques de PCRs et de séquençage afin de caractériser les β -lactamases produites.

Sur les 48 souches isolées en 2011, une *K. pneumoniae* produisant OXA-48 et la séquence d'insertion IS1999, quatre *Acinetobacter baumannii* produisant OXA-23 et GES-11, ainsi que trois *Pseudomonas* sécrétant VIM-2 ont été mises en évidence.

Sur les 100 souches isolées en 2012, une *K. pneumoniae* produisant OXA-48 et la séquence d'insertion IS1999 ainsi qu'une AmpC plasmidique de type ACC a été décrite. Quatre souches de *Serratia marcescens* résistantes à l'imipénème sécrétaient OXA-48. Parmi les huit *A. baumannii* de sensibilité diminuée aux carbapénèmes, une souche produisait OXA-23 et GES-11, six sécrétaient OXA-23 et une OXA-24. Quinze *Pseudomonas aeruginosa* et deux *Pseudomonas* spp. isolées en 2012 sécrétaient VIM-2, deux *P. aeruginosa* produisaient IMP-1 et deux autres IMP-2. Cette étude épidémiologique a montré la présence de carbapénémases de classes A, B et D à l'Hôtel-Dieu de France et mis en évidence une augmentation dans le nombre et la variabilité de ces enzymes.

INTRODUCTION

Carbapenems such as imipenem, ertapenem, meropenem and doripenem are important therapeutic agents for treating infections caused by multi-drug resistant gram negative bacteria including *Enterobacteriaceae*, *Acinetobacter*, and *Pseudomonas*. Nowadays, the global emergence of a number of carbapenem-hydrolyzing β -lactamases (carbapenemases) challenges the efficacy of carbapenems, and therefore limits the available therapeutic options in both hospital and community settings [1]. Carbapenemases are enzymes that destroy β -lactam antibiotics; some belong to Ambler class A and are inhibited by boronic acid, such as KPC, while some belong to

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Ambler class B and are inhibited by ethylene diamine tetra-acetic acid (EDTA), such as VIM, IMP and NDM. Other carbapenemases belong to Ambler class D and are not affected by clavulanic acid or EDTA, like OXA-48 [2].

Carbapenem-resistant gram negative bacilli have been reported worldwide as a consequence of acquisition of carbapenemase genes, among other resistance mechanisms. Outbreaks caused by carbapenemase producers have been reported in the Middle East and Mediterranean region and carbapenemases are now considered endemic in some countries like OXA-48 in Turkey and VIM-1 in Italy [3]. In Lebanon, literature on carbapenemases is scarce, with three separate case reports describing OXA-48 and IMP-1 in *Enterobacteriaceae* and OXA-58 in *Acinetobacter baumannii* [4-6]. The *bla*_{OXA-48} gene was found to be plasmid borne and located between two identical insertion sequences, IS1999, forming composite transposon Tn1999. The metallo- β -lactamase NDM-1 was reported in 2012 in *Klebsiella pneumoniae* isolates obtained from two Iraqi patients referred to Lebanon for treatment of medical complications, and *Enterobacteriaceae* harboring both OXA-48 and NDM-1 have been described in 2013 [7-8]. The objective of the current study was to evaluate and compare carbapenem resistance mechanisms in *Enterobacteriaceae*, *A. baumannii* and *Pseudomonas* isolated in a Lebanese university hospital, between January and June 2011 and 2012. Part of this work was presented at the RICAI (Réunion Interdisciplinaire de Chimiothérapie Anti-Infectieuse) 2011 congress, Paris, France (December 1-2, 2011, Abstract no. 73), and the RICAI 2012 congress (November, 22-23, 2012, Abstract no. 300), Paris, France.

MATERIALS AND METHODS

Bacterial isolates

Between January and June 2011 and 2012, carbapenem intermediate/resistant gram negative bacterial agents were collected from the Microbiology Laboratory of Hôtel-Dieu de France, a university medical center in Beirut, Lebanon, and run by Saint-Joseph University. It includes 430 beds and receives over 60,000 admissions and emergencies per year. All *Enterobacteriaceae* and *A. baumannii* strains with inhibition zone diameter (IZD) of imipenem ≤ 24 mm, and all *Pseudomonas* strains with IZD of imipenem ≤ 22 mm were included. Presumptive identification of the species was confirmed by standard laboratory methods. Pure isolates were deep frozen at -80°C for future study.

Phenotypic analysis

■ Antimicrobial susceptibility testing and detection of extended spectrum- β -lactamases

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar by disk diffusion method according to the guidelines set by the Comité de l'antibiogramme

de la Société Française de Microbiologie [9]. IZD of amoxicillin/clavulanic acid (AUG), ceftazidime (CAZ), cefotaxime (CTX), cefepime (CPM), ceftiofexim (FOX), aztreonam (ATM), imipenem (IMI), gentamicin (GM), tobramycin (TN), amikacin (AK), ofloxacin (OFX), ciprofloxacin (CIP), and levofloxacin (LEV) disks were measured. Antibiotic disks were from Mast Diagnostics (Merseyside, United Kingdom). After overnight incubation, increase of the IZD centered by a third and/or fourth generation cephalosporin disks toward the clavulanate-containing disk by at least 5 mm indicated the presence of an extended spectrum- β -lactamase (ESBL) [10]. Also, the minimal inhibitory concentration (MIC) of ertapenem was measured against *Enterobacteriaceae* by Etest[®] (Liofilchem, Via Scozia, Italy) on Mueller-Hinton agar.

■ Cloxacillin test

Antibiotic susceptibility testing was also performed using Mueller-Hinton agar supplemented with cloxacillin (25-500 $\mu\text{g/L}$) to inhibit AmpC cephalosporinases. An increase in the IZD of cephalosporins in the presence of cloxacillin was considered positive for AmpC production. The cloxacillin test was also used for better visualization of synergy between clavulanic acid and third and/or fourth generation cephalosporins, indicating ESBL production, while visualization of synergy between clavulanic acid and imipenem, indicated the production of an ESBL with carbapenem-hydrolyzing activity [10].

■ Metallo- β -lactamase detection

To test the production of Ambler class B carbapenemases (metallo- β -lactamases) which are inhibited by metal ion chelators like EDTA, a combined Etest[®] of imipenem/imipenem+EDTA (Liofilchem, Via Scozia, Italy) was used on Mueller-Hinton agar according to the manufacturer's instructions. A ratio of $\text{MIC}_{\text{imipenem}}/\text{MIC}_{\text{imipenem+EDTA}} \geq 8$ was considered to be presumptive of a metallo- β -lactamases [10].

■ Modified Hodge test

A modification of the Hodge test was applied to screen for carbapenemase production in *Enterobacteriaceae*. The indicator organism, *Escherichia coli* ATCC 25922 at a turbidity of 0.5 McFarland standards, was used to inoculate Mueller Hinton agar, and a 10 μg meropenem disc (Mast Diagnostics, Merseyside, United Kingdom) was placed at the center. The test strain was heavily streaked from the disk to the plate periphery. Heavy streaks of carbapenemase-positive and carbapenemase-negative control strains were also made. 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 [11].

Genotypic analysis

Polymerase chain reaction (PCR) experiments were used to detect various types of β -lactamase genes using sets of primers [12]. *Enterobacteriaceae* were tested for *bla*_{KPC}, *bla*_{GES}, *bla*_{OXA-48}, *bla*_{VIM}, *bla*_{IMP-1}, *bla*_{IMP-2} and *bla*_{NDM} genes. *Enterobacteriaceae* demonstrating phenotypic evidence of AmpC cephalosporinases were additionally

Results for *K. pneumoniae*

Strain K1 was isolated from blood of a one-year old Iraqi patient with congenital hydrocephaly, hemophilia A and seizures. Antibiotic susceptibility testing showed resistance to tested cephalosporins, aminoglycosides, fluoroquinolones, and imipenem with synergy between amoxicillin/clavulanic acid and third generation cephalosporins, indicating ESBL production. The cloxacillin test was neg-

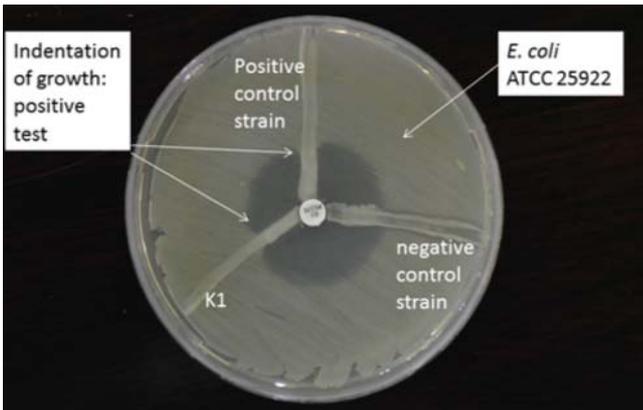


FIGURE 1. Modified Hodge test. A disk of 10 µg of meropenem was placed at the center of Muller-Hinton agar inoculated with 0.5 McFarland dilution of the meropenem-sensitive indicator organism *E. coli* ATCC 25922.

A negative control strain was streaked from the edge of the disc to the periphery of the plate. Positive control organisms and the strain K1 were similarly streaked.

The latter strains produce carbapenemases, hydrolyzing meropenem and allowing the indicator organism to grow in a clover-leaf like manner around their streaks.

ative indicating the absence of an AmpC cephalosporinase; the combined Etest (imipenem/imipenem+EDTA) was negative indicating the absence of metallo-β-lactamase, while the modified Hodge test was positive indicating possibility of carbapenemase production (Figure 1). MIC of ertapenem was greater than the cut-off limit of 32 mg/L. Resistance to imipenem was attributed *bla*_{OXA-48} gene and insertion sequence *IS1999* identified by PCR, while cephalosporin resistance was due to an ESBL of the CTX-M group 1.

Strain Kpd2 was cultured from a vulvar abscess of a female hospitalized for a short time in the infectious disease unit. The combined Etest was negative while the cloxacillin test was positive indicating a plasmid-acquired AmpC (Figure 2), and the modified Hodge test was positive indicating possibility of carbapenemase production. The genes for the carbapenemase OXA-48, *IS1999* and an AmpC of the ACC group were detected by PCR. Attempts to transfer *bla*_{OXA-48} to *E. coli* K12 by conjugation were not successful.

Results for *S. marcescens*

The four *S. marcescens* isolates were obtained from urine specimens of elderly Lebanese males with underlying prostate or urinary tract pathology. All demonstrated low level resistance to tested third generation cephalosporins and amikacin, and synergy of clavulanic acid with at least one third generation cephalosporin, as well as positive modified Hodge test.

The cloxacillin test was weakly positive, possibly due to production of a natural AmpC by *S. marcescens*. The

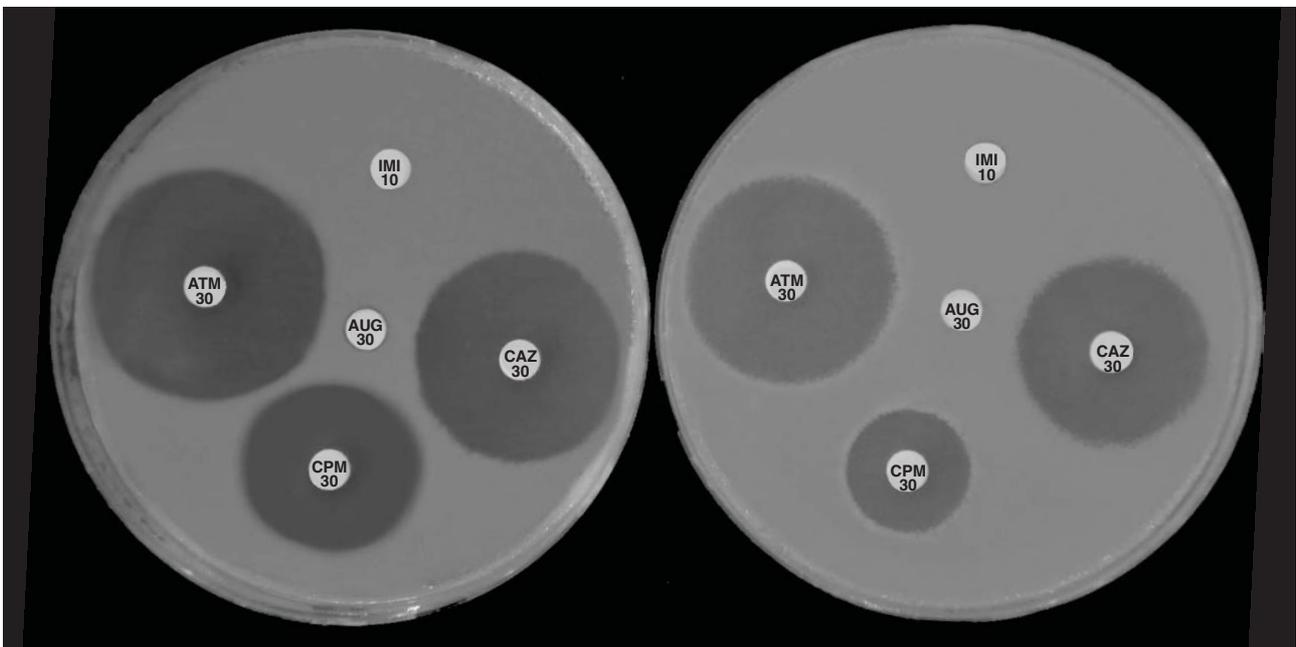


FIGURE 2. Susceptibility of *K. pneumoniae* Kpd2 to β-lactams using the disk diffusion method.

Inhibition zone diameters obtained on Mueller-Hinton agar in absence (right) and in the presence (left) of cloxacillin in the medium: Diameters with/without cloxacillin are 30/37 mm for aztreonam; 17/25 mm for cefepime, and 27/30 mm for ceftazidime. The increase in diameters of third generation cephalosporins in the presence of cloxacillin indicates the production of AmpC by Kpd2.

AUG: amoxicillin/clavulanic acid **ATM:** aztreonam **CAZ:** ceftazidime **CPM:** cefepime **IMI:** imipenem

combined Etest was negative for all isolates indicating the absence of metallo- β -lactamases. MICs of ertapenem against all strains were greater than the cut-off limit of 32 mg/L. In PCR experiments, all *Serratia* were shown to harbor the genes for SHV type ESBL, and an OXA-48 associated with insertion sequence IS1999.

Results for *A. baumannii*

A. baumannii strains were obtained from wound aspirate (n = 4), sputum (n = 3), bronchial aspirate (n = 2), urine (n = 1), blood (n = 1), and hepatic drainage (n = 1). For all *A. baumannii*, while the combined Etest was negative, the cloxacillin test was positive indicating the presence of naturally produced chromosomal AmpC. Moreover, the isolates appeared resistant to all tested antibiotics of the β -lactam, aminoglycoside and fluoroquinolone classes. Four strains collected in 2011 and one in 2012 showed synergy between imipenem and clavulanic acid (Figure 3). The gene for a carbapenem-hydrolyzing ESBL GES-11 was identified in these strains by PCR-sequencing. In addition, all strains were found to harbor the carbapenemase gene *bla*_{OXA-23}. Six strains of *A. baumannii* isolated during 2012 harbored *bla*_{OXA-23} alone, and one strain *bla*_{OXA-24} gene.

Results for *Pseudomonas*

Pseudomonas strains were obtained from urine (n = 10), sputum (n = 6), blood (n = 4), stool (n = 2), wound aspirate (n = 1), and biliary liquid (n = 1). For all *Pseudomonas*, the cloxacillin test was positive indicating the presence of naturally produced chromosomal AmpC, with high resistance towards all antibacterial agents tested. In *P. aeruginosa* and *Pseudomonas* species with low resistance to carbapenems, the decrease susceptibility may arise from impermeability due to mutation in OprD, and/or overexpression of MexAB-OprM efflux pump. *Pseudomonas* isolates with high level resistance to imipenem (MIC > 256 mg/L) were phenotypically selected for further analysis. These strains presented a combined Etest ratio MIC_{imipenem}/MIC_{imipenem+EDTA} greater than 8. They were thus highly suspected to be metallo- β -lacta-

mase producers, and subjected to PCR-sequencing. Two *P. aeruginosa* and one *Pseudomonas* species isolates were genotypically tested during 2011, while 19 *P. aeruginosa* and 2 *Pseudomonas* species were tested in 2012. PCR experiments showed in 2011 two *P. aeruginosa* and 1 *Pseudomonas* species harboring *bla*_{VIM-2}; 19 *P. aeruginosa* strains with carbapenemases were detected in 2012, of which 15 harbored *bla*_{VIM-2}, 2 harbored *bla*_{IMP-1}, and 2 harbored *bla*_{IMP-2}.

DISCUSSION

This study represents a survey carried out in a single Lebanese tertiary care center in order to assess the extent of carbapenemase dissemination among nosocomial gram negative pathogens. The data presented indicate a high prevalence of carbapenem resistance among *Pseudomonas* and *A. baumannii* isolates compared to *Enterobacteriaceae*. While the Centers for Disease Control and Prevention (CDC) report a percentage of carbapenem-resistant *K. pneumoniae* that exceeds 10% [14], the percentages reported in this study are low with 0.8% in 2011 and 0.9% in 2012. Comparison of the results of the 6-month periods over 2011 and 2012 revealed that the number of carbapenem non-susceptible gram negative isolates has increased in 2012 compared to the same period in 2011 (Table I), with 24% of isolates collected in 2011 non-susceptible to imipenem versus 28.7% in 2012. This increase was mainly due to the detection of a higher number of *S. marcescens* resistant to imipenem (3.6% in 2011 and 17.9% in 2012), the consequence of a putative limited epidemic in the hospital. Moreover, additional carbapenemases (OXA-24, IMP-1, IMP-2) were detected in the same Lebanese hospital during the second period of the study.

There are few reports on the prevalence of carbapenemase-producing gram negative pathogens in Lebanon and the Middle East (Figure 4). While some enzymes have been detected in Turkey, Greece, Israel, and Egypt, no carbapenemases were found in Syria or Jordan [15]. Out of 42 carbapenemase-producing isolates recovered in our study, only 4 (about 10%) were isolated from patients presenting from outside Lebanon to receive healthcare at Hôtel-Dieu de France. These included one Iraqi patient (congenital hydrocephaly, hemophilia A and seizures) with OXA-48 producing *K. pneumoniae*, one Syrian patient (encephalopathy and poliomyelitis) and one French patient (cystic fibrosis) with VIM-2 producing *P. aeruginosa*, and one Syrian patient (cardiogenic shock and hemodialysis) with OXA-23 producing *A. baumannii*. Such data suggest that carbapenemases might long have been present in Lebanon, the majority of the patients being Lebanese with underlying malignancy, chronic complications, or nosocomial acquisition. This warrants the need for long-term surveillance studies that may better represent the status of carbapenemase dissemination in Lebanon. Moreover, it is possible that patient/medical tourist transfer from neighboring countries eventually increases the spread of carbapenemase-producers in Lebanon, especially with the demo-

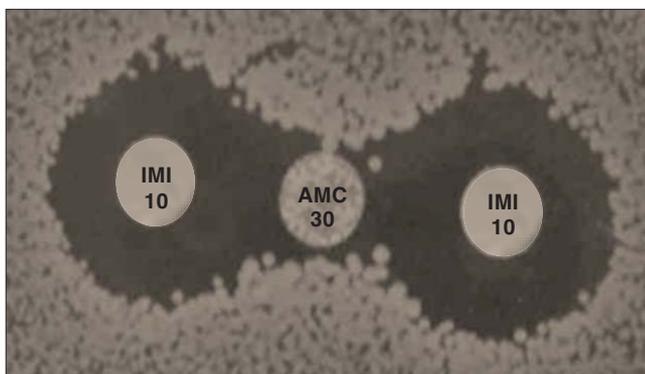


FIGURE 3. Susceptibility of *A. baumannii* ABD4 to β -lactams by disk diffusion. The synergy between imipenem (right and left) and amoxicillin/clavulanic acid (center) indicates GES-11 activity.
AMC: amoxicillin/clavulanic acid **IMI:** imipenem

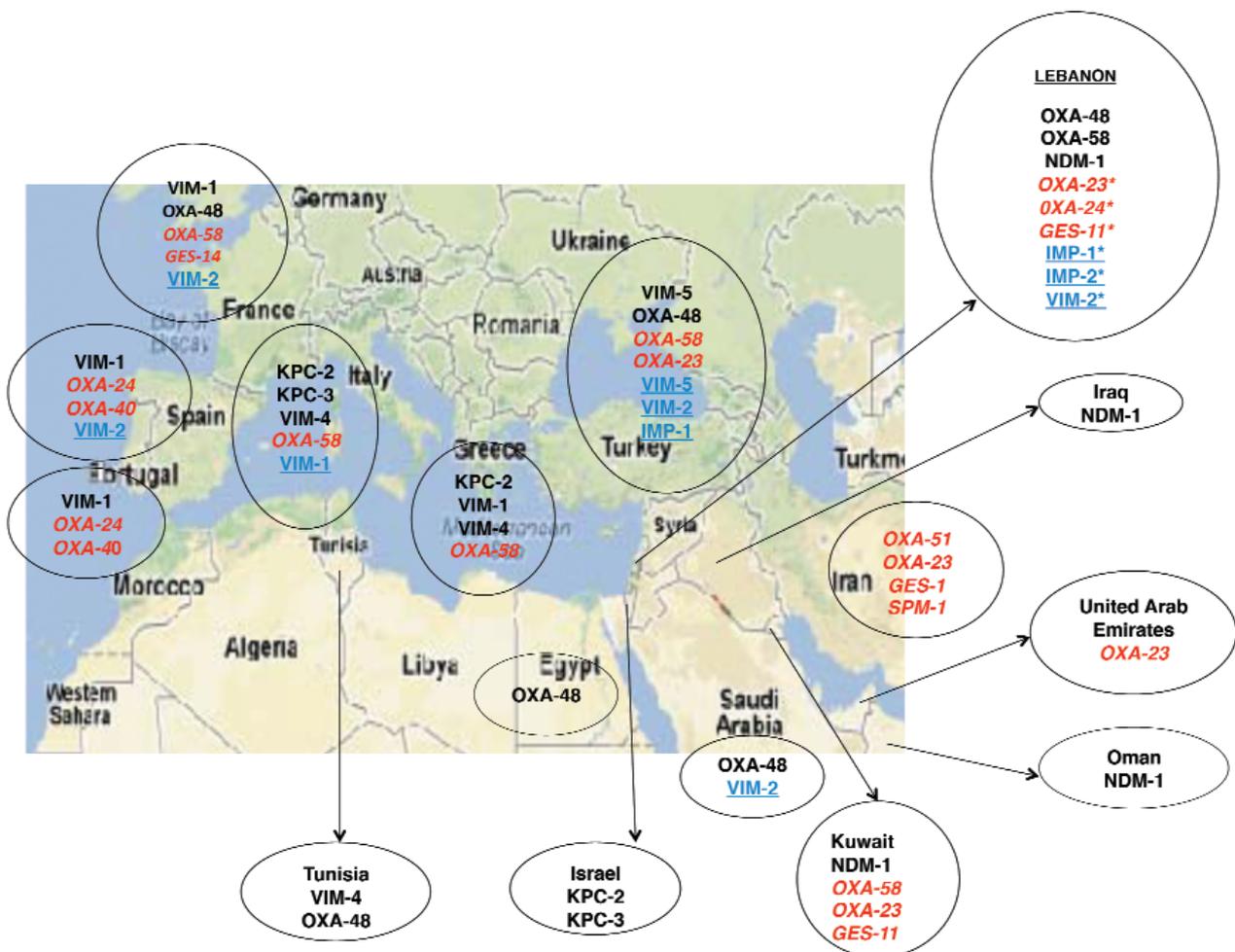


FIGURE 4. Distribution of carbapenemase producers in Lebanon & 15 other countries in the Mediterranean region & the Middle East.^{2,3,6,7,15} Findings from Lebanon include those in this study (* Newly described carbapenemases). Carbapenemases of *Enterobacteriaceae* are shown in black (bold), of *A. baumannii* in red (italics), and of *Pseudomonas* in blue (underlined).

graphic and public health changes occurring in the Middle East countries due to civil wars. Further screening and infection control are needed for containment of such spread.

Regarding *K. pneumoniae*, after the initial threat of *bla_{KPC}* harboring strains detected in New York city, USA, these strains have disseminated widely to some countries of the Mediterranean region like Israel [16]. Apart from KPC, metallo-β-lactamases like VIM-1 have also been described in *Enterobacteriaceae* isolates in Mediterranean regions like Greece [17]. The carbapenemases KPC and VIM-1 had not yet been reported in Lebanon. The class-D carbapenemase OXA-48, identified in *K. pneumoniae* strains from Turkey and Tunisia, has been detected previously in Lebanese strains of *K. pneumoniae* [4]. In the present study, two additional *bla_{OXA-48}* harboring *K. pneumoniae* were detected revealing dissemination of such strains in the country. A prevalence of 0.8 and 0.9% carbapenem non-susceptible *K. pneumoniae* was detected respectively in 2011 and 2012, which is low in this Lebanese hospital if compared to high prevalence in regions like Turkey and North African countries [18]. Transfer of the *bla_{OXA-48}* gene

to *E. coli* K12 via conjugation was not efficient; a similar result was previously reported by Gulmez *et al.* and Poirer *et al.* who explained this by the possibility that *bla_{OXA-48}* gene might have been chromosomally integrated [19-20]. The relationship between IS1999 and the *bla_{OXA-48}* gene in K1 and Kpd2 strains needs further investigation.

In addition to the production of a carbapenemase, *K. pneumoniae* may be multiresistant by accumulating various types of β-lactamases. In this study, *K. pneumoniae* isolate K1 did contain, along with OXA-48, CTX-M group 1 ESBL while Kpd2 produced an acquired AmpC. Plasmid acquired cephalosporinases (AmpCs) are clinically significant for their ability to increase the level of resistance to β-lactam antibiotics in *Enterobacteriaceae* [10]. They have been previously detected in *K. pneumoniae* strains and reported to be responsible for outbreaks in different hospitals like the one described in France [21]. Moreover, the coexistence of two or more carbapenemases in this organism has been recently reported in Morocco where one *K. pneumoniae* strain simultaneously expressed *bla_{OXA-48}*, *bla_{NDM1}*, and *bla_{VIM-1}* [22].

S. marcescens isolates described herein revealed for the first time the presence of *bla*_{OXA-48} gene in this species. Another carbapenemase, SME, has been reported recently in 2011 from strains isolated in the United States [23]. Other mechanisms of resistance to carbapenems in *Serratia* include combined AmpC overproduction and outer membrane protein (OmpF) loss [24]. The coexistence of OXA-48 and IS1999 may indicate acquisition of carbapenemase genes via mobile determinants from other resistant species.

Regarding *A. baumannii*, a previous Lebanese study has described the presence of OXA-58 carbapenemase in 2008 [6]. We report for the first time in Lebanon the detection of an *A. baumannii* harboring *bla*_{OXA-24} and 11 *bla*_{OXA-23} harboring strains, with five (42%) of these co-expressing *bla*_{GES-11}, which is an ESBL with carbapenemase activity [25]. *A. baumannii* harboring *bla*_{OXA-24} were previously detected in Turkey [26]. *A. baumannii* isolates harboring simultaneously *bla*_{OXA-23} and *bla*_{GES-11} have been recently reported in Kuwait with a frequency of 52% [27]. They have also been detected in Sweden with, however, a prevalence not exceeding 8% [28]. The existence of more than one carbapenemase-encoding gene is thought to efficiently promote high level resistance to carbapenems in *A. baumannii* [27].

Detection of carbapenemases was not expected in the majority of *Pseudomonas* isolates. Carbapenem resistance in this organism is associated with other mechanisms including derepressed chromosomal AmpC β -lactamases, energy-dependent multidrug efflux pumps and restricted outer membrane permeability through mutation in the porin OprD [29]. Nevertheless, increase in carbapenem resistant strains mediated by acquired metallo- β -lactamases including IMP and VIM in *Pseudomonas* have been reported from several countries [30]. The metallo- β -lactamase IMP-1, detected in 2 of our *P. aeruginosa* isolates, was previously described in another Lebanese survey in *K. pneumoniae* [5]. This study reports, in addition, IMP-2 and VIM-2 carbapenemases in *Pseudomonas*. The finding of *bla*_{IMP-1}, *bla*_{IMP-2}, and *bla*_{VIM2} metallo- β -lactamase genes in *Pseudomonas* strains in Lebanon is concurrent with other studies in our region, namely from Turkey and Saudi Arabia [31-32].

The OXA-48 positive strains Kpd2 and *S. marcescens* were carbapenem-resistant but retained susceptibility to most tested aminoglycosides and fluoroquinolones. This is in parallel with previous data describing OXA-48 producers [15, 33]. In contrast, all remaining carbapenemase producing isolates in this study were multiresistant to β -lactams, aminoglycosides, and fluoroquinolones, suggesting that resistance genes to these antibiotics are on the same genetic support. This is consistent with previous data indicating that metallo- β -lactamase and KPC producing organisms often carry resistance genes to other antibiotics on the same plasmid, and remain only susceptible to colistin and tigecycline [15, 34]. Among patients with carbapenemase-producing isolates in this study, 11 (26%) were treated with colistin, once in combination with tigecycline, and the survival rate was more than 80%.

This places these antibiotics among the limited therapeutic options currently available for management of such resistant organisms.

In summary, carbapenemase-producing gram negative bacteria have become more widespread and variable in the same setting over two study periods. The carbapenemase genes *bla*_{OXA-23}, *bla*_{OXA-24}, *bla*_{GES-11}, *bla*_{VIM-2}, and *bla*_{IMP-2} and ACC acquired AmpC are reported here for the first time in Lebanon. It is anticipated that a countrywide, long-term study would be more representative of the current status of carbapenemases occurrence. Also, an analysis of the clonality of carbapenemase-producing strains would shed a light on the possibility of intra- and inter-hospital spread. The use of carbapenemase detection techniques whether biochemical like Carba NP test or analytical like matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) may be promising to facilitate screening of carbapenemase-producing bacteria without a significant extent of false positives or false negatives [35]. In Lebanon, proper surveillance of resistance and proper infection control, as well as monitoring of the emergence and spread of resistant strains, are needed to reduce the impact of resistance.

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DISCLOSURE STATEMENT

All authors disclose no competing interests in connection with this study.

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