International Journal of Infectious Diseases 45 (2016) 24–31

Contents lists available at ScienceDirect

International Journal of Infectious Diseases

journal homepage: www.elsevier.com/locate/ijid

Carriage of beta-lactamase-producing *Enterobacteriaceae* among nursing home residents in north Lebanon

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ARTICLE INFO

Article history:
Received 15 December 2015
Received in revised form 18 January 2016
Accepted 10 February 2016

Corresponding Editor: Eskild Petersen, Aarhus, Denmark.

Keywords:
Carriage
Nursing homes
Resistance
Carbapenemases
ESBLs

SUMMARY

Background: Multidrug-resistant (MDR) *Enterobacteriaceae* can cause severe infections with high morbidity, mortality, and health care costs. Individuals can be fecal carriers of these resistant organisms. Data on the extent of MDR *Enterobacteriaceae* fecal carriage in the community setting in Lebanon are very scarce. The aim of this study was to investigate the fecal carriage of MDR *Enterobacteriaceae* among the elderly residents of two nursing homes located in north Lebanon.

Methods: Over a period of 4 months, five fecal swab samples were collected from each of 68 elderly persons at regular intervals of 3–4 weeks. Fecal swabs were subcultured on selective media for the screening of resistant organisms. The phenotypic detection of extended-spectrum beta-lactamase (ESBL), AmpC, metallo-beta-lactamase (MBL), and *Klebsiella pneumoniae* carbapenemase (KPC) production was performed using the beta-lactamase inhibitors ethylenediaminetetraacetic acid, phenylboronic acid, and cloxacillin. A temocillin disk was used for OXA-48. Multiplex PCRs were used for the genotypic detection of ESBL and carbapenemase genes, and sequencing was performed to identify CTX-M-15. The medical records of each subject were reviewed on a regular basis in order to assess the risk factors associated with MDR *Enterobacteriaceae* fecal carriage.

Results: Over the study period, 76.5% of the recruited elderly persons were at least one-time carriers. A total of 178 isolates were obtained. Phenotypic testing revealed that 91.5% of them were ESBL producers, 4% were AmpC producers, 2.8% were co-producers of ESBL and AmpC, and 1.7% were co-producers of OXA-48 and ESBL. Recent antibiotic intake was found to be the only independent risk factor associated with the fecal carriage of MDR *Enterobacteriaceae*.

Conclusions: The high prevalence of MDR *Enterobacteriaceae* detected in this study and the emergence of carbapenem resistance is alarming. Efficient infection control measures and antibiotic stewardship programs are urgently needed in these settings in order to limit the spread of resistant strains.

1. Introduction

Multidrug-resistant (MDR) *Enterobacteriaceae* are currently considered a major public health concern worldwide.[1,2] They can be transmitted easily among patients and healthy persons.[3] Studies have shown that after being selected by antibiotics, the cross-transmission of these organisms occurs frequently in the health care setting.[4] This dissemination will eventually lead to increased rates of MDR *Enterobacteriaceae* carriage. This carriage is often unrecognized and has been known to increase the risk of contracting infections caused by resistant agents.[5] The treatment of these cases is often challenging due to the limited therapeutic options; the antibiotic pipeline is drying up and no new antimicrobial agents targeted against MDR *Enterobacteriaceae* are foreseen in the near future.[6]

There is increasing evidence that nursing homes in the community are important reservoirs for MDR *Enterobacteriaceae*.[4,7] This is in major part due to the inappropriate use of antimicrobial agents in these facilities,[8] in addition to the difficulties particularly faced when establishing antibiotic stewardship and infection control programs.[9,10] The prevalence of MDR *Enterobacteriaceae* colonization in nursing homes varies according to the geographical location, patient population, and the level of care provided.[10]

In the Middle East, although several studies have been conducted to assess the prevalence of MDR *Enterobacteriaceae* in

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http://dx.doi.org/10.1016/j.ijid.2016.02.007

the hospital ward,[11] data on the prevalence of MDR *Enterobacteriaceae* among nursing home residents in these countries are very scarce. In Lebanon, clinical investigations have shown that the prevalence of MDR *Enterobacteriaceae* is on a continuous rise.[12] Local data reported in the form of flyers summarizing the susceptibility of bacteria at the Centre Hospitalier Du Nord in the north of Lebanon, show that between 2011 and 2013 the rate of extended-spectrum beta-lactamase (ESBL) production among clinical isolates increased from 24.6% to 30.4% and from 26.5% to 31.7% in *Escherichia coli* and *Klebsiella pneumoniae*, respectively (Ziad Daoud). Another recent study reported an increase of 1.2% in resistance and decreased susceptibility to ertapenem in clinical isolates of *Enterobacteriaceae*. This resistance was mainly attributed to the production of OXA-48 beta-lactamase.[12]

In an attempt to understand the situation of carriage in the nursing homes of the country and to shed light on this important issue, the present research group conducted a study in Lebanon in which it was found that 71.6% of the recruited elderly subjects were at least one-time carriers during the study period.[13] The plan was to study the situation in the north of Lebanon, where the extent of the spread of bacterial resistance in the community is not well documented. The socio-cultural as well as economic and educational levels in the north of Lebanon are also very particular to this area of the country. These include the level of poverty and the absence of basic governmental services such as public sanitation and infrastructure, as most of the services are concentrated in the capital Beirut. Unfortunately, all of these data are anecdotal and based on impressions, since official statistics are not available in the country.

The aim of this study was thus to investigate the fecal carriage of MDR *Enterobacteriaceae* among the residents of two major nursing homes located in the north of Lebanon through the determination of the prevalence, dynamics, and risk factors for MDR *Enterobacteriaceae* fecal carriage among elderly subjects. In addition, it was sought to determine whether CTX-M-15, the predominant ESBL gene in the Lebanese population,[14] was also the major ESBL genotype carried among these elderly people.

2. Materials and methods

2.1. Ethics, consent, and permissions

The Research Committee of the University of Balamand and the Project Management Unit at the Lebanese Ministry of Agriculture approved this study. The patient or his/her legal guardian or family member signed a consent form for their participation in the study. The privacy of participants and transparency of the ethical process were guaranteed.

2.2. Study design and population

This was a cross-sectional study conducted in two major nursing homes located in Tripoli in the north of Lebanon. Candidates for this study were elderly residents aged >60 years. A total of 68 individuals were recruited. Fifty-seven were chosen randomly from nursing home 1. This facility has around 60 rooms and a capacity of 200 beds. Eleven elderly persons were recruited randomly from nursing home 2. This facility offers around 20 rooms with a capacity of 50 beds.

2.3. Data collection

The medical records of each elderly person were reviewed with the help of the nurse responsible. Age, sex, number of roommates, mobility status (ambulant/in a wheelchair or bedridden), and the date of admission were all recorded. In addition, urinary/fecal incontinence, the presence of wounds or ulcers, and the previous or current use of a urinary catheter were also reported. Furthermore, the recruited elderly persons were checked for comorbidities (MDR bacterial infections, diabetes, cancer, pulmonary, cardiovascular, renal, or neurological diseases, and urogenital pathologies), hospital admission during the last year, and whether they had undergone any surgeries, as well as their antibiotic intake during the last 3 months.

2.4. Collection of fecal swabs and isolation of resistant Enterobacteriaceae

Between December 2013 and April 2014, five samples (fecal swabs) were obtained from each of 68 elderly persons at regular intervals of 3–4 weeks. A total of 262 samples were collected: 59 at collection 1, 51 at collection 2, 57 at collection 3, 51 at collection 4, and 44 at collection 5. The fecal swabs were subcultured on MacConkey agar supplemented with cefotaxime (2 μg/ml) for the screening of MDR *Enterobacteriaceae*. From each selective plate, different colonies presenting with different morphologies were picked up separately and suspended in Luria broth. After overnight incubation, each bacterial suspension was subcultured again on a selective plate. The following day, if the plate contained colonies with single morphologies, the isolate was preserved in 20% glycerol aliquots at −20 °C for further testing; if more than one type was observed, a re-isolation was performed for further purification.

An elderly subject was defined as a carrier if an MDR *Enterobacteriaceae* was isolated from his/her fecal sample. If the patient was found to be a carrier at all five collections, he/she was considered a 'permanent carrier'. If the resistant bacterium was isolated at fewer than five collections, the subject was defined as an 'intermittent carrier'. Finally, if no MDR *Enterobacteriaceae* was isolated during the five collections, the subject was considered a 'never carrier'. All isolates were identified using biochemical gallery tests (API 20E; bioMérieux).

2.5. Phenotypic tests

Antimicrobial susceptibility testing was performed for 178 isolates using the Kirby–Bauer disk diffusion method. Interpretation of the results was performed in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines 2014.[15] Fifteen antimicrobial agents were tested (Table 1). The amoxicillin–clavulanic acid disk was placed in the center between cefepime, ceftazidime, and aztreonam, in order to detect a possible 'keyhole effect'. AmpC beta-lactamase and carbapenemase production was suspected when resistance to cefoxitin and ertapenem, respectively, was observed. Unfortunately, resistance to cefoxitin is not sufficient to distinguish between constitutive and plasmid-mediated AmpC, therefore it was considered that both types of AmpC were detected by this test. In order to confirm these phenotypically, ethylenediaminetetraacetic acid (EDTA), phenylboronic acid (PBA), and cloxacillin were used as beta-lactamase inhibitors.[16–18] In addition, temocillin susceptibility testing was performed as a presumptive test for the detection of the OXA-48 enzyme.[19]

2.6. Detection of ESBL type using multiplex PCR

In order to identify the type(s) of ESBL present in the clinical isolates, multiplex PCR was performed on a representative sample of 18 isolates chosen based on their profile of resistance. Bacterial DNA was prepared by suspending one or two colonies of each test isolate in 200 μl of distilled water and heating the solution at 95 °C for 10 min. The presence of $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{TEM}, and bla_{OXA} genes was tested using previously published primer sets and

Table 1
Rates of susceptibility of different *Enterobacteriaceae* isolates

Antimicrobial agent	Number of susceptible isolates (%)			
	Escherichia coli (*n* = 159)	*Klebsiella pneumoniae* (*n* = 5)	*Klebsiella oxytoca* (*n* = 9)	*Citrobacter diversus* (*n* = 5)
Ampicillin	0 (0)	0 (0)	0 (0)	0 (0)
Aztreonam	7 (4.4)	0 (0)	2 (22.2)	1 (20)
Cefoxitin	138 (86.8)	5 (100)	8 (88.8)	4 (80)
Cefotaxime	0 (0)	0 (0)	0 (0)	0 (0)
Ceftazidime	16 (10)	0 (0)	1 (11.1)	0 (0)
Cefepime	9 (5.6)	0 (0)	0 (0)	0 (0)
Amoxicillin–clavulanic acid	61 (38.3)	2 (40)	1 (11.1)	3 (60)
Piperacillin–tazobactam	68 (42.7)	1 (20)	4 (44.4)	0 (0)
Meropenem	156 (98.1)	5 (100)	9 (100)	5 (100)
Imipenem	156 (98.1)	5 (100)	9 (100)	5 (100)
Ertapenem	156 (98.1)	5 (100)	9 (100)	5 (100)
Tigecycline	118 (100)[a]	3 (100)[a]	6 (100)[a]	5 (100)
Trimethoprim–sulfamethoxazole	59 (37.1)	0 (0)	1 (11.1)	0 (0)
Ciprofloxacin	65 (40.8)	1 (20)	2 (22.2)	0 (0)
Gentamicin	83 (52.2)	1 (20)	4 (44.4)	2 (40)

[a] Only 118 *E. coli*, three *K. pneumoniae*, and six *K. oxytoca* isolates were tested for tigecycline susceptibility.

conditions.[20] Each reaction tube contained 10 μl of master Mix (Qiagen), 4 μl of primers, and 1 μl of DNA, and was made up to a total volume of 20 μl with sterile distilled water. The PCR reaction conditions consisted of a 15 min denaturation step at 95 °C, followed by 30 amplification cycles of 30 s at 94 °C, 90 s at 62 °C, and 60 s at 72 °C, with a final extension step of 10 min at 72 °C.[20]

The primer sequences and expected amplicon sizes of the target ESBL genes were as follows: for bla_{SHV}: F-CTTTATCGGCCCTCACTCAA, R-AGGTGCTCATCATGGGAAAG (327 bp); bla_{TEM}: F-CGCCGCATACAC-TATTCTCAGAATGA, R-ACGCTCACCGGCTCCAGATTTAT (445 bp); bla_{CTX-M}: F-ATGTGCAGYACCAGTAARGTKATGGC, R-TGGGTRAAR-TARGTSACCAGAAYCAGCGG (593 bp); bla_{OXA}: F-ACACAATACATAT-CAACTTCGC, R-AGTGTGTTTAGAATGGTGATC (813 bp).

In order to visualize the PCR amplicons, samples were mixed with 4 μl of Thermo Scientific loading dye and loaded into the wells of a 1.5% agarose gel in 1× Tris–acetate–EDTA (TAE) buffer. The gel was run at 130 V for 60 min. Amplicons were visualized using an ultraviolet transilluminator system (DIGI DOC-IT System) for analysis. The gel had one well containing a DNA ladder (100 bp; Thermo Scientific) in order to be able to estimate the size of the DNA amplicons.

2.7. Plasmid sequencing and analysis

Plasmid DNA was extracted as described above, quantified using Qubit, and sequenced using the Illumina NGS platform. The sequence data were downloaded from the GenBank database and each sequence file was compared to a number of reference plasmid replicon sequences present in the Plasmid Finder database using BLASTn. Circular representations of the plasmid sequence were created using Unipro UGENE software, and the sequenced plasmids were aligned and compared to reference replicon sequences using BioEdit software.

2.8. Detection of carbapenemase genes using PCR

Another multiplex PCR was conducted for the detection of the carbapenem resistance genes *Klebsiella pneumoniae* carbapenemase (KPC), New Delhi metallo-beta-lactamase (NDM), OXA-48, IMP, SPM, and VIM. DNA extraction was performed as described in the previous section. The presence of the carbapenem resistance genes was tested using universal primers.[21] PCR amplification reactions were performed in a volume of 20 μl containing 10 μl of Taq PCR Master Mix, 5 μl of sterile water, 4 μl of the primer mix, and 1 μl of the extracted DNA. The conditions of the PCR reaction were as follow: 94 °C for 10 min, then 36 cycles of 30 s at 94 °C, 40 s at 52 °C, and 50 s at 72 °C for amplification, then 5 min at 72 °C for the final extension.[21] Amplified DNA products were subjected to electrophoresis on a 1.5% agarose gel in 1× TAE buffer. The gel was run at 130 V for 1 h. The visualization of amplicons was performed using an ultraviolet transilluminator system (DIGI DOC-IT System) for analysis.

The primer sequences and expected amplicon sizes of target carbapenemase genes were as follows: bla_{KPC}: F-CGTCTAGTTCTGCTGTCTTG, R-CTTGTCATCCTTGTTAGGCG (798 bp); bla_{NDM}: F-GGTTTGGCGATCTGGTTTTC, R-CGGAATGGCTCATCACGATC (621 bp); bla_{OXA-48}: F-GCGTGGTTAAGGATGAACAC, R-CATCAAGTT-CAACCCAACCG (438 bp); bla_{IMP}: F-GGAATAGAGTGGCTTAAYTCTC, R-GGTTTAAYAAAACAACCACC (232 bp); bla_{SPM}: F-AAAATCTGGG-TACGCAAACG, R-ACATTATCCGCTGGAACAGG (271 bp); bla_{VIM}: F-GATGGTGTTTGGTCGCATA, R-CGAATGCGCAGCACCAG (390 bp).

2.9. Statistics and data analysis

For univariate analysis, classical descriptive methods were used according to each site separately. Furthermore, the distributions of variables according to carriage status were compared by conducting a bivariate analysis. A p-value of ≤ 0.05 was considered statistically significant. Furthermore, risk factors with a p-value of ≤ 0.15 were subjected to multivariate analysis. IBM SPSS Statistics version 20.0 (IBM Corp., Armonk, NY, USA) was used for all statistical calculations.

3. Results

3.1. Demographics and prevalence of MDR Enterobacteriaceae fecal carriage

The demographic characteristics of the elderly subjects are presented in Table 2. For both nursing homes, the prevalence of fecal carriage was as follow: 32 elderly subjects (54.2%) were fecal carriers at the first collection, 33 (64.7%) at the second collection, 24 (42.1%) at the third collection, 24 (47%) at the fourth collection, and 25 (56.8%) at the fifth collection. Overall, 76.5% of the recruited residents were at least one-time carriers, while 23.5% of them were never carriers.

3.2. Dynamics of MDR Enterobacteriaceae fecal carriage

In this study, 262 samples were collected, of which 138 were positive for MDR *Enterobacteriaceae* (52.6%). From these

Table 2
Characteristics of nursing home residents recruited in this study[a]

	NH1	NH2
Total number	57	11
Sex		
Male	19 (33.3)	5 (45.5)
Female	38 (66.7)	6 (54.5)
Age, years, mean (SD)	78 (7.8)	75.82 (9.3)
LOS, days, mean (median)	1016 (818)	402.36 (598)
Room accommodation		
Single	3 (5.3)	10 (90.9)
Double	13 (22.8)	1 (9.1)
Triple	1 (1.8)	0 (0)
Quadruple	4 (7)	0 (0)
More than 4 beds/room	36 (63.2)	0 (0)
Mobility status		
Ambulant	11 (19.3)	4 (36.4)
Wheelchair	46 (80.7)	4 (36.4)
Bedridden	0 (0)	3 (27.3)
Urinary catheter	4 (7)	4 (36)
Urinary/fecal incontinence	43 (75.4)	7 (63.6)
Wounds/ulcers	6 (10.5)	2 (18.2)
Recent surgery during last 3 months	2 (3.5)	3 (27.3)
Recent hospitalization during last year	8 (14)	4 (36.4)
Recent antibiotic intake during last 3 months	27 (47.4)	7 (63.6)
Multidrug-resistant bacterial infections	2 (3.5)	0 (0)
Diabetes	6 (10.5)	4 (36.4)
Cancer	2 (3.5)	0 (0)
Pulmonary diseases	6 (10.5)	3 (27.3)
Cardiovascular diseases	24 (42.1)	4 (36.4)
Neurological diseases	24 (42.1)	2 (18.2)
Urogenital pathologies	11 (19.3)	4 (36.4)
Renal diseases	1 (1.8)	0 (0)

NH, nursing home; SD, standard deviation; LOS, length of stay.
[a] All data are presented as the number (%) unless stated otherwise.

138 positive samples, 178 isolates were obtained. The number of elderly subjects versus the number of isolates was not 1 to 1, since more than one isolate was obtained for some residents. Overall, 159 isolates (89%) were identified as *E. coli*, 14 (8%) as *Klebsiella spp*, and five (3%) as *Citrobacter spp*.

The fecal carriage among elderly subjects varied from one collection to another (Figure 1). From collection 1 to 2, the carriage of MDR *Enterobacteriaceae* disappeared for four subjects (12.5%), while it appeared in 10 (37%). Between collections 2 and 3, the carriage disappeared in 13 subjects (38.2%), while it appeared in two (11.8%). Between collections 3 and 4, six carriers (25%) became non-carriers, while eight non-carriers (24.2%) became carriers. Between collections 4 and 5, the carriage disappeared in six subjects (25%), while it appeared in 10 (37%). Overall, out of the 52 elderly subjects who were at least one-time carriers, eight (15.4%) were permanent carriers, while 44 (84.6%) were

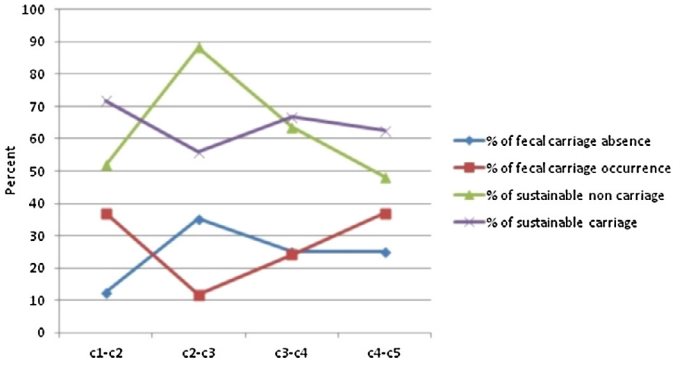

Figure 1. Dynamics and stability of multidrug-resistant *Enterobacteriaceae* fecal carriage.

intermittent carriers. *E. coli* was the most stable resistant colonizer isolated at the five collections, while *Klebsiella spp* and *Citrobacter spp* were only isolated at four and three of the collections, respectively.

3.3. ESBL, AmpC, and OXA-48 detection

The antimicrobial susceptibility testing results are summarized in Table 3. Phenotypic testing revealed that out of 178 isolates, 163 (91.5%) were ESBL producers. Five isolates (2.8%) were found to be co-producers of ESBL and AmpC. Seven isolates (4%) were considered AmpC producers. Furthermore, 46% of the isolated ESBL and/or AmpC producers were co-resistant to at least two other non-beta-lactam antimicrobial agents, 38% were co-resistant to only one non-beta-lactam, and 16% showed no co-resistance. The detailed susceptibility rates for each category are presented in Table 3. As an average of the five collections, 89.5% of ESBL production was detected in *E. coli*, while only 8.5% and 1.8% were detected in *Klebsiella spp* and *Citrobacter spp*, respectively. A 71.4% AmpC production was observed in *E. coli*; however, the methodology used does not distinguish between the constitutive and plasmid-mediated resistance due to AmpC. The simultaneous production of ESBL and AmpC, as well as ESBL and OXA-48, was observed at only the first and second collections; in both cases these were produced by isolates of *E. coli* (Table 4). Three isolates of *E. coli* were carbapenem-non-susceptible. Two of these were isolated from the same patient during the first and second collections, while the third was isolated from another patient during the first collection. In the subsequent collections, no carbapenem-resistant isolates were detected. Phenotypic tests suggested an OXA-48 probably co-produced with ESBL. In this regard, temocillin disks were used for the three isolates (Figure 2).

3.4. Genotypic detection of resistance and occurrence of CTX-M-15

Multiplex PCR analysis performed on 18 isolates revealed the presence of the TEM gene in 17 of them, CTX-M in 16, OXA in four, and SHV in two. Eleven isolates showed coexistence of CTX-M and TEM genes, four showed coexistence of three or four genes, and three isolates harbored only one gene (Figure 3). The 16 isolates harboring the CTX-M gene were all positive for CTX-M-15 after

Table 3
Rates of susceptibility of MDR *Enterobacteriaceae* isolates

Antimicrobial agent	Number of susceptible isolates (%)		
	ESBL producers (n = 163)	ESBL and AmpC co-producers (n = 5)	AmpC producers (n = 7)
Ampicillin	0 (0)	0 (0)	0 (0)
Aztreonam	7 (4.2)	0 (0)	3 (42.8)
Cefoxitin	152 (93.2)	0 (0)	0 (0)
Cefotaxime	0 (0)	0 (0)	0 (0)
Ceftazidime	14 (8.5)	0 (0)	0 (0)
Cefepime	2 (1.2)	0 (0)	7 (100)
Amoxicillin–clavulanic acid	67 (41.1)	0 (0)	0 (0)
Piperacillin–tazobactam	68 (41.7)	2 (40)	3 (42.8)
Meropenem	163 (100)	5 (100)	7 (100)
Imipenem	163 (100)	5 (100)	7 (100)
Ertapenem	163 (100)	5 (100)	7 (100)
Tigecycline	122 (100)[a]	3 (100)[a]	7 (100)
Trimethoprim–sulfamethoxazole	55 (33.74)	3 (60)	2 (28.5)
Ciprofloxacin	61 (37.4)	2 (40)	2 (28.5)
Gentamicin	83 (50.9)	1 (20)	6 (85.7)

MDR, multidrug-resistant; ESBL, extended-spectrum beta-lactamase.
[a] Only 122 ESBL producers and three ESBL and AmpC co-producers were tested for tigecycline susceptibility.

Table 4
Prevalence of MDR *Enterobacteriaceae* in different species over the five collections

	Species	Number of isolates	Phenotypic mechanism of resistance
Collection 1	*Escherichia coli*	35	ESBL
		3	ESBL/AmpC
		1	AmpC
		2	OXA-48/ESBL
	Klebsiella oxytoca	5	ESBL
Collection 2	*Escherichia coli*	36	ESBL
		2	ESBL/AmpC
		1	AmpC
		1	OXA-48/ESBL
	Klebsiella oxytoca	3	ESBL
	Klebsiella pneumoniae	3	ESBL
Collection 3	*Escherichia coli*	25	ESBL
		1	AmpC
	Citrobacter diversus	1	ESBL
Collection 4	*Escherichia coli*	26	ESBL
		1	AmpC
	Klebsiella pneumoniae	1	ESBL
	Citrobacter diversus	1	ESBL
Collection 5	*Escherichia coli*	24	ESBL
		1	AmpC
	Klebsiella oxytoca	1	AmpC
	Klebsiella pneumoniae	1	ESBL
	Citrobacter diversus	2	ESBL
		1	AmpC

MDR, multidrug-resistant; ESBL, extended-spectrum beta-lactamase.

DNA extraction and sequencing, therefore showing a high occurrence of this enzyme in the ESBL population. In the phenotypic testing, 17 out of the 18 isolates showed a keyhole effect and were therefore identified as ESBL producers (Table 5).

Regarding the three carbapenem-resistant isolates, multiplex PCR analysis showed that all of them harbored an OXA-48 gene (Figure 4), thereby confirming the phenotypic results.

In view of the low number of isolates selected for genotypic testing, these results cannot be generalized, and tests addressing a larger number of isolates should be performed in the future to confirm that this is true on a larger scale.

3.5. Risk factors associated with fecal carriage of MDR Enterobacteriaceae

The associations between MDR *Enterobacteriaceae* fecal carriage and different factors are presented in Table 6. Univariate analysis revealed that recent antibiotic intake during the last 3 months and urogenital pathologies were the only risk factors associated with the fecal carriage of MDR *Enterobacteriaceae* ($p = 0.03$ and $p = 0.015$, respectively). The percentage of residents who had a recent antibiotic intake was 59.6% (31/52) among the at least one-time carriers and 18.8% (3/16) among the never carriers. For urogenital pathologies, the prevalence was 28.8% (15/52) in carriers versus 0% (0/16) in never carriers. In the multivariate analysis, three factors were included: recent antibiotic intake ($p = 0.03$), urogenital pathologies ($p = 0.015$), and diabetes ($p = 0.102$). This final analysis revealed that recent antibiotic

Figure 2. Temocillin test for the phenotypic detection of OXA-48 production. (A) Negative results (sensitivity) with non-OXA-48 producing isolates. (B) Positive results (resistance) with the three carbapenem-resistant *Enterobacteriaceae* isolates producing OXA-48 isolated in this study.

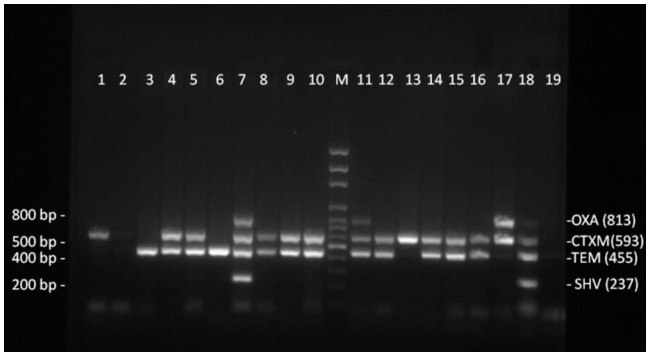

Figure 3. Detection of the beta-lactamase genes SHV, TEM, CTX-M, and OXA in multidrug-resistant *Enterobacteriaceae* isolates obtained from nursing home residents, using multiplex PCR. Lanes 1–18 represent the multidrug-resistant *Enterobacteriaceae* isolates tested. Lane 19 corresponds to the positive control (TEM 455 bp). Lane M is a 1.2-kb DNA ladder. The molecular size of the band in question is indicated in parentheses on the right of the image.

Table 5
Genotypic detection of beta-lactamase genes versus phenotypic identification

Species	Number of isolates	Phenotypic mechanism of resistance	Genes harbored
Escherichia coli	10	ESBL	TEM, CTX-M
Escherichia coli	2	ESBL	TEM, CTX-M,OXA
Escherichia coli	2	ESBL	TEM
Escherichia coli	1	ESBL	CTX-M
Citrobacter diversus	2	ESBL	TEM, SHV, CTX-M, OXA
Citrobacter diversus	1	AmpC	TEM, CTX-M

ESBL, extended-spectrum beta-lactamase.

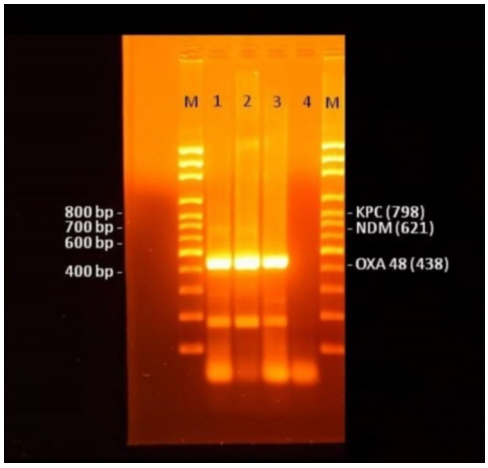

Figure 4. Detection of carbapenemase genes OXA-48, NDM, and KPC in carbapenem-resistant *Enterobacteriaceae* isolates obtained from nursing home residents, using multiplex PCR. Lanes 1–3 represent the carbapenem-resistant *Enterobacteriaceae* isolates. Lane 4 corresponds to the negative control. Lane M is a 1.2-kb DNA ladder. The molecular size of the band in question is indicated in parentheses on the right of the image.

intake was the only independent risk factor associated with MDR *Enterobacteriaceae* fecal carriage.

4. Discussion

Although several studies have addressed the issue of MDR *Enterobacteriaceae* in Lebanon, data on the spread of bacterial resistance in the community are very scarce. Only one recent study has been carried out in nursing homes in Beirut, and that study was performed by the present research group. In that study, it was found that 71.6% of the recruited elderly subjects were at least one-time carriers.[13] Similar results were found in the present study implemented in the north of Lebanon (76.5%). These results, however, are relatively high when compared to those from similar studies conducted in long-term care facilities worldwide: 70.3% in Italy,[22] 41.3% in Japan,[23] and 14.7% in Australia.[24] Differences in sample size, medical care, and hand hygiene practices at each site, in addition to differences in the microbiological screening methods used in each study might have influenced the results and therefore have yielded some variations.[25] Another important factor to consider when comparing these results is that the majority of the studies were conducted at one time-point only.

As shown in the present study, the carriage status of an elderly person should not be assumed on the basis of only one fecal sampling; rather, multiple screening samples are needed. According to Filius et al., differences in colonization rates could arise as a result of antibiotic consumption that has decreased the number of MDR *Enterobacteriaceae* to an undetectable level in the stool sample.[26]

Table 6
Association between different factors and MDR *Enterobacteriaceae* fecal carriage[a]

	At least one-time carrier	Never carrier
Total number	52 (76.5)	16 (23.5)
Sex		
Male	19 (36.5)	5 (31.2)
Female	33 (63.5)	11 (68.8)
Age, years, mean (SD)	77.81 (7.7)	77.63 (9.3)
LOS, days, mean (median)	900.7 (610)	1629 (829.5)
Room accommodation		
Single/double	22 (42.3)	5 (31.2)
Triple and more	30 (57.7)	11 (68.8)
Mobility status		
Ambulant	12 (23)	3 (18.8)
Wheelchair/bedridden	40 (77)	13 (81.2)
Urinary catheter	7 (13.5)	1 (6.2)
Urinary/fecal incontinence	37 (71.2)	13 (81.2)
Wounds/ulcers	7 (13.5)	1 (6.2)
Recent surgery during last 3 months	5 (9.6)	0 (0)
Recent hospitalization during last year	11 (21)	1 (6.2)
Recent antibiotic intake during last 3 months	31 (59.6)[b]	3 (18.8)[b]
Multidrug-resistant bacterial infections	2 (3.8)	0 (0)
Diabetes	10 (19.2)[c]	0 (0)[c]
Cancer	2 (3.8)	0 (0)
Pulmonary diseases	7 (13.5)	2 (12.5)
Cardiovascular diseases	23 (44.2)	5 (312)
Neurological diseases	19 (36.5)	7 (43.8)
Urogenital pathologies	15 (28.8)[b]	0 (0)[b]
Renal diseases	1 (1.9)	0 (0)

MDR, multidrug-resistant; SD, standard deviation; LOS, length of stay.
[a] All data are presented as the number (%) unless stated otherwise.
[b] *p*-Value ≤0.05.
[c] *p*-Value ≤0.15.

The fecal carriage of AmpC producers among the recruited residents is an important finding in this study. AmpC-producing *Enterobacteriaceae* strains have previously been reported in clinical samples from Lebanon.[27,28] However, the present study appears to be the first to report the prevalence of these MDR bacteria in a community setting. AmpC beta-lactamases are cephalosporinases that can be chromosomally mediated with inducible expression or plasmid-mediated with constitutive expression.[29,30] Along with ESBLs, the non-recognition of these mechanisms by clinical laboratory personnel leads to inappropriate reporting of the antibiogram to the physician responsible. This in many cases may lead to therapeutic failures.[31] Nevertheless, the present study might have suffered some limitations due to the use of phenotypic tests to incriminate the corresponding mechanisms of resistance. As is well known, these tests are very helpful for clinical microbiology laboratories; however, their specificities and sensitivities are questionable.

The detection of OXA-48 producers is a major and alarming issue. These beta-lactamases are plasmid-mediated class D oxacillinases that convey resistance to penicillins and have moderate hydrolyzing activity to carbapenems.[32] In this study, the phenotypic confirmation of OXA-48 production was performed using temocillin disks. High-level resistance to temocillin is not restricted to OXA-48 producers; metallo-beta-lactamases (MBLs) and KPCs can also be highly resistant to temocillin.[33] Therefore, temocillin resistance is considered a phenotypic confirmation of OXA-48 only in cases where other carbapenem resistance mechanisms are excluded.[34] It is important to note that the three ertapenem-resistant isolates in this study were intermediate to meropenem and imipenem and were isolated from two different elderly subjects who had no history of recent hospitalization; however, recent antibiotic treatment with amoxicillin–clavulanic acid was reported for one of them.

Of interest, it was found that in spite of the considerable socio-economic and cultural differences between Beirut and Tripoli, the results of this study were, to a certain extent, similar to those obtained in the study previously undertaken by this research group in Beirut.[13] In this context, there is agreement between these two studies on the frequency of carriage of ESBL-producing organisms (*E. coli* 82.7% in Beirut and 89% in Tripoli, *K. pneumoniae* 9.7% in Beirut and 8% in Tripoli). In addition, 80.7% of elderly subjects in Beirut were at least one-time carriers and 19.3% never carriers, while these percentages were found to be 76.5% and 23.5%, respectively, in elderly persons in Tripoli. However, although both studies agree that recent antibiotic intake is a significant risk factor, it was found that recent urinary tract pathologies and diabetes were risk factors only among Tripoli nursing homes residents. In addition, carbapenem-resistant *Enterobacteriaceae* were not isolated from the Beirut population.

Obviously other factors played a role in this relatively high prevalence. One possibility is the cross-transmission with resistant bacteria, since 38.5% of elderly subjects who were at least a one-time carrier had no history of recent antibiotic intake. In nursing homes, modes of transmission of MDR *Enterobacteriaceae* usually result from non-adherence to infection control measures; environmental surfaces are not frequently decontaminated, waste is often disposed of incorrectly, and hand hygiene practices are far from optimal in these settings.[10] In 2011, a randomized controlled trial was undertaken in Hong Kong long-term care facilities in order to determine the effectiveness of a hand hygiene infection control program. During the study period, adherence to hand hygiene increased significantly and the occurrence of serious infections decreased from 1.42 cases to 0.65 cases per 1000 resident-days.[35]

In conclusion, this study demonstrated that the prevalence of fecal carriage of MDR *Enterobacteriaceae* in north Lebanon is high and shows different patterns (one-time carriage, constant carriage, never carriage, etc.). The screening of newly admitted residents for the fecal carriage of MDR *Enterobacteriaceae* becomes a crucial task. The emergence of carbapenem resistance in the community is alarming; training of clinical laboratory technologists on the appropriate detection of the different mechanisms of resistance is essential. The prevalence of MDR *Enterobacteriaceae* fecal carriage among elderly nursing home residents (76.5%) is noteworthy and underlines the importance of nursing homes as reservoirs of resistance in the Lebanese community. The fecal carriage of MDR *Enterobacteriaceae* is dynamic and changes with time. In the majority of the isolates obtained, multidrug resistance was mediated by ESBL production. CTX-M-15 was present in 16 out of the 18 tested ESBL-producing isolates. This does not differ from the average CTX-M-15 in the Lebanese population, although the number of genotypically tested isolates in this study was relatively low. It is well known that phenotypic tests are not as accurate as genotypic methods; however, these are the best available way to detect resistance and incriminate the corresponding mechanism of resistance with an acceptable level of certainty in the clinical laboratories of the country.

Acknowledgements

We are grateful to Dr Jihad Irani (Faculty of Medicine, University of Balamand) for his assistance in the statistics and data analysis of this work. This study was funded by the Lebanese Council for Research. The latter had no role in the study design, in the collection, analysis, and interpretation of data, in the writing of the manuscript, or in the decision to submit the manuscript for publication.

Conflict of interest: The authors declare that no conflict of interest exists.

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