JIDC | THE JOURNAL OF INFECTION IN DEVELOPING COUNTRIES

Original Article

Multidrug-resistant ESBL-producing *Enterobacteriaceae* and associated risk factors in community infants in Lebanon

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Abstract

Introduction: Extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-PE) infections are a growing threat to children, and the treatment of these infections becomes more and more challenging. A huge reservoir for ESBLs in the community is the fecal flora of children. This study investigates the rectal colonization, associated risk factors, antimicrobial susceptibility, and molecular characterization of ESBL-PE in Lebanese community infants.

Methodology: A total of 117 rectal swabs were taken from healthy infants between 1 and 12 months of age. Detection of ESBLs was carried out using the double-disk synergy test, combination-disk method, and multiplex polymerase chain reaction (PCR). A questionnaire about the infant's history and risk factors for carrying ESBL-PE was administered.

Results: In total, 58 (49.6%) of 117 participants were ESBL-PE carriers. Some significant important risk factors for colonization in this study were male gender, hospital birth, caesarean delivery, and being formula-fed. Observed decrease in colonization rate was associated with intimate hygiene habits. Carriers of multiple *bla* genes were the most common. CTX-M type was the major harbored, gene and CTX-M-9 was the most predominant, followed by CTX-M-15 type.

Conclusions: To the best of our knowledge, this is the first available data about the carriage rate of ESBL-PE in community infants in Lebanon and the Middle East, the first study showing that birth in hospital, caesarean delivery, and being formula-fed are all significantly associated risk factors for the high colonization rates in community – not hospitalized – infants, and showing the dominance of multiple resistance gene carriage and wide dissemination of CTX-M-9 ESBL.

Key words: extended-spectrum beta-lactamases; *Enterobacteriaceae*; risk factors; infant; CTX-M-9; Lebanon.

J Infect Dev Ctries 2016; 10(9):947-955. doi:10.3855/jidc.7593

(Received 25 August 2015 – Accepted 08 Dcember 2015)

Introduction

Extended-spectrum beta-lactamase (ESBL)-producing bacteria is a major worldwide threat among drug-resistant bacteria in both hospital and community settings [1]. ESBLs can hydrolyze all penicillins and cephalosporins, including the extended-spectrum cephalosporins such as cefotaxime or ceftazidime [2]. Many ESBL producers are multi-resistant to non-beta-lactam antibiotics, including fluoroquinolones and aminoglycosides [3], trimethoprim, tetracyclines, sulfonamides, and chloramphenicol, and this is often encoded by the gene harbored by the same plasmids that determines the ESBL type [4]. Effective antibiotic therapy for treating these infections is limited to a small number of drugs [5] such as carbapenems; as a result, the chance of resistance to carbapenems among the *Enterobacteriaceae* increases. There are more than 1,600 known beta-lactamases, a list that is rapidly expanding [6]. TEM, SHV, and CTX-M-type of ESBLs are most often found in a wide range of *Enterobacteriaceae*, with increasing frequency [7-9]. However, the majority of ESBL-producing strains are *Escherichia coli* and *Klebsiella pneumoniae* [10]. TEM- and SHV-type beta-lactamases, mainly produced by *K. pneumoniae*, have spread throughout hospital settings, and CTX-M enzymes, mainly produced by *E. coli*, have become predominant in the community [9]. CTX-M enzymes were identified in 1989 from *E. coli* isolates in Germany [11], but they did not become predominant over the other ESBL enzymes until the first decade of the twenty-first century, during which an extraordinary spread of these enzymes was observed [10] in both hospital and community settings [12]. Plasmids encoding $bla_{\text{CTX-M-15}}$ are found mainly in

Enterobacteriaceae and were recently named plasmids of resistance responsible for outbreaks because of their capacity to acquire genes of resistance and to transfer among bacteria [13]. Intestinal colonization by ESBL-producing isolates may thus represent a reservoir for ESBLs in the community not detected in clinical isolates [14]. The clinical impact of ESBL-producing pathogens on morbidity and mortality in infectious diseases in adults, as well as their economic burden, are well documented [15,16]. In young infants, Gram-negative organisms are the most common cause of serious bacterial infection [17,18]. Options for treatment of these infections are generally limited, and given that fewer antibiotics are approved for use in children, the problem is critically important to address [19].

It is critical to better define the prevalence, risk factors, and molecular characterization of ESBL-producing organisms carried by infants to adopt best-practice infection control measures and help in the appropriate choice of empirical antimicrobial coverage for infections in these populations. The aim of this study was to investigate the prevalence and predisposing factors of intestinal carriage of ESBL-producing *Enterobacteriaceae* (ESBL-PE) among healthy Lebanese community infants and to characterize the resistance genes bla_{TEM}, bla_{CTX}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}2}$, $bla_{CTX\text{-}M\text{-}9}$, and $bla_{CTX\text{-}M\text{-}15}$.

Methodology

Sample population and questionnaire

A total of 117 healthy infants between 1 and 12 months of age who were brought to clinics for vaccination were chosen from 3 different clinics in Lebanon for this study. A questionnaire was completed for each participant, which included name, age, gender, hospital or home birth, vaginal or cesarean delivery, premature or low birth weight, extended hospital stay after birth, breast- or formula-fed, previous antibiotic or antacid treatment, previous hospital admission, previous urinary tract infection, contact with pets, and intimate hygiene habits. This investigation was performed between January and May 2013.

Bacterial isolation and ESBL detection and confirmation

From each participant, a rectal swab was taken using a sterile swab moistened with sterile saline and was immediately plated on MacConkey agar plates (Oxoid, Hampshire, UK) supplemented with 2 mg/L ceftazidime (Oxoid, Hampshire, UK) within 5 days of preparation. Selective media showing growth colonies were selected for subsequent characterization. Bacterial identification was performed using Gram staining, biochemical testing (indole, methyl red, Voges-Proskauer, citrate, and urease), and the API 20E system (bioMerieux, Marcy l'Etoile, France).

The isolates were first screened for ESBL production using ceftazidime, cefepime, cefotaxime, cefpodoxime, ceftriaxone, and aztreonam disks (Oxoid, Basingstoke, UK). Next, phenotypic confirmatory tests were carried out by the double-disk synergy test and combination-disk method. The double-disc synergy test was performed on agar with a 30 µg disk of cefotaxime, ceftriaxone, ceftazidime, and a disk of amoxicillin-clavulanate (containing 10 µg of clavulanate) positioned at a distance of 20 mm (center to center). The test was considered positive when a decreased susceptibility to cefotaxime was combined with a clear-cut enhancement of the inhibition zone of cefotaxime in front of the clavulanate-containing disk. The combination-disk method was performed using ceftazidime, ceftazidime-clavulanic acid, cefotaxime, and cefotaxime-clavulanic acid. The organisms were considered to be ESBL producing when a $\geq$ 5 mm increase in a zone diameter for either antimicrobial agent tested in combination with clavulanic acid compared with the zone diameter of the agent when tested alone [20]. Molecular analysis of all positive isolates screened was done.

Antimicrobial susceptibility testing

Antimicrobial susceptibility test was done for all ESBL-producing isolates using the determination of the antibiogram values by agar diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [20]. The following antibiotic disks were used: cefepime (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), gentamicin (30 µg), amikacin (30 µg), tetracycline (30 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), nalidixic acid (30 µg), trimethoprime-sulfamethoxazole (12.5/23.75 µg), and ticarcillin (75 µg) (Oxoid Ltd., Basingstoke, UK) were used to determine the resistance patterns of the isolates.

Characterization of genes encoding ESBLs

Multiplex polymerase chain reaction (PCR) was performed to detect TEM, SHV, and CTX-M genes. Crude genomic DNA was extracted from the isolates by the heat lysis method. One pure colony was suspended in 40 µL of sterile distilled water, and the cells were lysed by heating at 95°C for 5 minutes, then centrifuged, and cooled to 4°C [21]. Suitable primers

[22] (Sigma-Aldrich, Taufkirchen, Germany), each targeting selected regions of the *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ genes were used (Table 1).

Amplification reactions were performed in a final volume of 25 µL containing 12.5 µL PCR Master Mix 2X (Thermo Scientific, Lithunia, EU) and 12.5 µL of DNA, primers, and H_2O. A 12.5 µL PCR master mix reaction buffer, and 0.5 µL TEM F, 0.5 µL TEM R, 1 µL of each remaining primers (10 µM/µL), 2.5 µL H_2O, and 5 µL of the template DNA preparation were added to the reaction mixture. Reactions were performed in a DNA thermal cycler (BIOER, Tokyo, Japan) under the following conditions: denaturation at 94°C for 5 minutes followed by 30 cycles at 94°C for 20 seconds, 61°C for 30 seconds, and 72°C for 1 minute with a final extension of 72°C for 5 minutes [23].

For detection of CTX-M-2-, CTX-M-9-, and CTX-M-15-encoding genes, another multiplex PCR was performed using suitable primers [22] (Table 1).

Reactions were performed in a DNA thermal cycler under the following conditions: denaturation at 94°C for 5 minutes followed by 30 cycles at 94°C for 15 seconds, 56°C for 15 seconds and 72°C for 45 seconds with a final extension of 72°C for 5 minutes.

After PCR amplification, 2.5 µL of each reaction was separated by electrophoresis in 1.5% agarose gel for 30 minutes at 100 V in 0.5 × TBE buffer. DNA was stained with ethidium bromide (1 µg/mL), and the bands were detected using a UV transilluminator (Cleaver Scientific Ltd, Rugby, UK.).

Statistical analysis

The data were analyzed using Yates's corrected χ^2 test. P values < 0.05 were taken as significant. Statistical analysis was performed using Minitab software.

Results

Prevalence of ESBL-PE

Of the 117 subjects who participated in this study, 50 (42.7%) were females and 67 (57.3%) were males, ranging in age from 1 to 12 months. Of these participants, 58 (49.6%) were ESBL-PE carriers, as shown in Table 2. Males had a significantly higher colonization frequency (56.7%) than did females (40%) (p = 0.035).

Factors associated with ESBL-PE carriage

The analysis of risk factors for fecal carriage in healthy infants who participated in this study is shown in Table 2. A significantly high carriage rate was found among infants who were born in hospitals (52.3%) compared with 0% for those who were born at home (p = 0.027). Regarding the type of delivery, caesarean delivery was observed to be associated with an increased risk of carriage, and this was statistically significant (p = 0.036). Formula-fed infants were found to be significantly associated with a higher ESBL-PE carriage rate (60.5%) than those who were breast-fed (p = 0.034). Other associated risk factors were extended hospital stay after birth, low birth weight, previous antibiotic or antacid intake, previous hospital admission, and previous urinary tract infection, although the differences were statistically insignificant. Premature birth and contact with pets were not observed to be associated with ESBL-PE carriage in this study. With respect to intimate hygiene habits, infants whose parents used water or soap and water, and then dried with tissue after each diaper change showed a reduced ESBL-PE carriage rate (43.7%), compared to 56.6% for those whose parents used only dry tissue or wipes (p = 0.08) (Table 2).

Table 1. Primers for the *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, *bla*$_{CTX-M-2}$, *bla*$_{CTX-M-9}$, and *bla*$_{CTX-M-15}$ genes used in this study.

Primers	Primer sequence 5' to 3'	Size (bp)
TEM F	AGT GCT GCC ATA ACC ATG AGT G	431
TEM R	CTG ACT CCC CGT CGT GTA GAT A	
SHV F	GAT GAA CGC TTT CCC ATG ATG	214
SHV R	CGC TGT TAT CGC TCA TGG TAA	
CTX-M- F	ATG TGC AGY ACC AGT AAR GT	593
CTX-M- R	TGG GTR AAR TAR GTS ACC AGA	
CTX-M-2 F	AAA CAG AGC GAG AGC GAT AAG	720
CTX-M-2 R	GGG TAA AGT AGG TCA CCA GAA C	
CTX-M-9 F	GGA TTA ACC GTA TTG GGA GTT T	164
CTX-M-9 R	GAT ACC GCA GAT AAT ACG CAG G	
CTX-M-15 F	CAC GTC AAT GGG ACG ATG T	410
CTX-M-15 R	GAA AGG CAA TAC CAC CGG T	

Table 2. Analysis of the risk factors for extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-PE) fecal colonization in healthy Lebanese infants.

Characteristic	Total	ESBL+	ESBL-	P value
	N (%)	N (%)	N (%)	
	117 (100%)	58 (49.6 %)	59 (50.4%)	
Sex				0.035
Male	67 (57.3%)	38 (56.7%)	29 (43.3%)	
Female	50 (42.7%)	20 (40%)	30 (60%)	
Birth location				0.027
Hospital	111 (94.9%)	58 (52.3%)	53 (47.7%)	
Home	6 (5.1%)	0 (0%)	6 (100%)	
Type of delivery				0.036
Cesarean birth	47 (40.2%)	28 (59.6%)	19 (40.4%)	
Vaginal birth	70 (59.8%)	30 (42.9%)	40 (57.1%)	
Premature birth				0.52
Yes	6 (5.1%)	3 (50%)	3 (50%)	
No	111 (94.9%)	57 (51.3%)	54 (48.6%)	
Low birthweight				0.22
Yes	8 (6.8%)	5 (62.5%)	3 (37.5%)	
No	109 (93.2%)	53 (48.6%)	56 (51.4%)	
Extended hospital stay after birth				0.072
Yes	10 (8.5%)	7 (70%)	3 (30%)	
No	107 (91.5%)	51 (47.7%)	56 (52.3%)	
Milk fed				0.034
Formula	43 (36.8%)	26 (60.5%)	17 (39.5%)	
Breast	74 (63.2%)	32 (43.2%)	42 (56.8%)	
Previous antibiotic intake				0.46
Yes	52 (44.4%)	26 (50%)	26 (50%)	
No	65 (55.6%)	32 (49.2%)	33 (50.8%)	
Previous antacid drug intake				0.87
Yes	14 (12%)	5 (35.7%)	9 (64.3%)	
No	103 (88%)	53 (51.5%)	50 (48.5%)	
Previous hospital admission				0.3
Yes	24 (20.5%)	13 (54.2%)	11 (45.8%)	
No	93 (79.5%)	45 (48.4%)	48 (51.6%)	
Previous UTI				0.76
Yes	8 (6.8%)	3 (37.5%)	5 (62.5%)	
No	109 (93.2%)	55 (50.5%)	54 (49.5%)	
Intimate hygiene habit				0.08
Dry tissue or wipes	53 (45.3%)	30 (56.6%)	23 (43.4%)	
Water + soap or water + tissue	64 (54.7%)	28 (43.7%)	36 (56.3%)	
Contact with pets				0.99
Yes	14 (12%)	3 (21.4%)	11 (78.6%)	
No	103 (88%)	55 (53.4%)	48 (46.6%)	

UTI: urinary tract infection.

Antibiotic susceptibility data

All isolates were resistant to aztreonam, cefepime, cefpodoxime, and ticarcillin. All (100%) were susceptible to imipenem and meropenem, whereas 93.1% and 51.7% were susceptible to amikacin and gentamicin, respectively. Susceptibility to the quinolone antibiotic family (levofloxacin, ciprofloxacin) was 65.5%. Furthermore, 39.7%, 44.8%, and 36.2% of the isolates were susceptible to tetracycline, trimethoprim-sulfamethoxazole, and nalidixic acid, respectively (Figure 1).

Characterization of bla gene encoding ESBL-PE

Molecular characterization of 58 ESBL-PE among the pediatric isolates revealed that multiple gene producers were predominant 65.6% (38/58), whereas CTX-M-type was the most common (91.4%; 53/58), followed by TEM-type and SHV-type genes. Of the 53 isolates harboring the $bla_{\text{CTX-M}}$ gene, 47.2% (25/53) co-produced TEM-type, 20.7% (11/53) co-produced SHV-type and TEM-type, 1.9% (1/53) co-produced SHV-type, and 30.2% (16/53) produced CTX-M-type only. Of the remaining isolates, 3.4% (2/58) harbored the bla_{TEM} gene alone, 3.4% (2/58) isolates harbored only bla_{SHV}, and 1.7% (1/58) harbored both bla_{TEM} and bla_{SHV} (Figure 2).

The majority of ESBL-PE isolates recovered during the study were *E. coli* (n = 45), *K. pneumoniae* (n = 10), *K.* oxytoca (n = 1), and *Enterobacter cloacae* (n = 2), and the majority (79.2%) of the CTX-M-positive isolates were *E. coli* (Figure 3).

Molecular characterization of $bla_{\text{CTX-M}}$ harbored by the 53 isolates using specific primers for CTX-M-2, CTX-M-9, and CTX-M-15 revealed that CTX-M-9 type was predominant 86.8% (46/53), followed by CTX-M-15 and CTX-M-2. Of the 46 CTX-M-9 producers, only 39.1% (18/46) isolates harbored the $bla_{\text{CTX-M-9}}$ gene alone, and the other isolates were co-producers of other types; 39.1% (18/46) were CTX-M-15 co-producers, and 21.7% (10/46) were CTX-M-15

Figure 1. Antimicrobial susceptibility for ESBL-PE isolated from Lebanese community infants

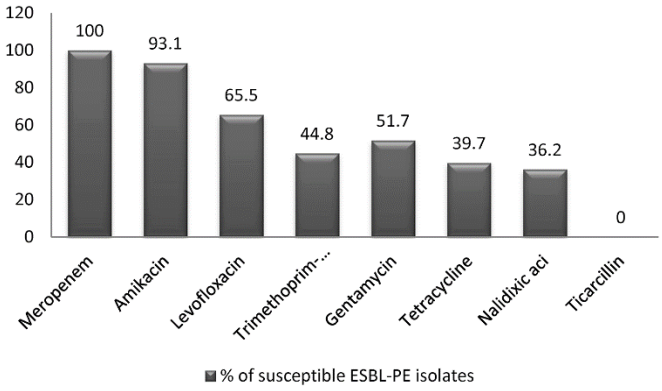

Figure 2. Agarose gel electrophoresis for PCR products obtained from ESBL-PE isolates with TEM, SHV, and CTX-PM primers

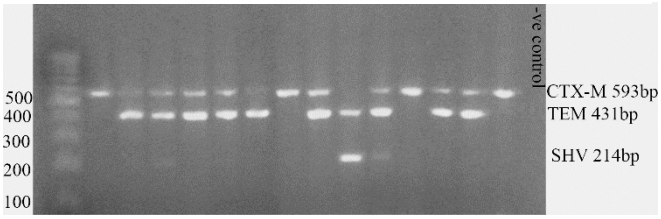

Figure 3. *bla* genes composition of the 58 isolated ESBL-PE from community infants

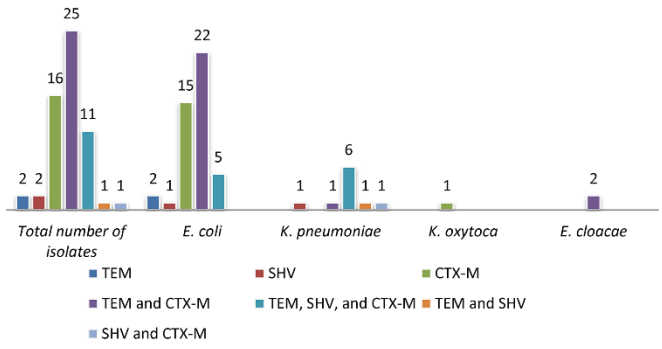

Table 3. Molecular characterization of 53 isolates harboring CTX-M ESBL type in community infants.

CTX-M type	Total number of isolates N = 53	*E. coli* N = 42	*K. pneumoniae* N = 8	*K. oxytoca* N = 1	*E. cloacae* N = 2
CTX-M-9	18	15	3	0	0
CTX-M-9 and CTX-M-15	18	15	1	1	1
CTX-M-9, CTX-M-15, and CTX-M-2	10	8	2	0	0
CTX-M-15	1	0	1	0	0
CTX-M-15 and CTX-M-2	1	0	1	0	0
CTX-M-2	0	0	0	0	0
CTX-M type other than 2, 9, 15	5	4	0	0	1

ESBL: extended-spectrum beta-lactamase.

and CTX-M-2 co-producers. One isolate harbored the *bla*$_{\text{CTX-M-15}}$ gene only, and one isolate harbored both *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{CTX-M-2}}$ genes (Table 3). The remaining five isolates (5/53) did not show any of these three types of CTX-M.

Discussion

Community-acquired ESBLs in healthy children accounted for 22% of infections in the United States [24]. The association between fecal carriage in children and risk for subsequent infection with an ESBL *Enterobacteriaceae* has been reported [25]. To the best of our knowledge, this survey was the first conducted in Lebanon and the Middle East concerning the intestinal carriage of ESBL-PE in the healthy infant community. The prevalence was found to be 49.6% in this age group (1–12 months), which is a high rate compared to other studies among community and hospitalized pediatric patients from different countries and to a previous study in 2005 in an adult population in Lebanon, where the ESBL-PE carriage rate was 2.4% [26]. Intestinal carriage by ESBL-producing Gram-negative bacteria in healthy French and Swedish children ranged from 2.9% to 6.7%, with CTX-M-15, CTX-M-14, and CTX-M-1 constituting the majority of ESBLs [27,28]. Twenty-four percent (30/125) of healthy Spanish children were colonized by ESBL-producing strains, and CTX-M-1 was the most common type [29]. Rare reports are available about carriage in healthy children in Asia, Africa, and the Middle East, and there are very few investigations about carriage in hospitalized children [30]. ESBL-PE strains were detected in 12% (6/50) of fecal samples collected from the inpatients of a Japanese pediatric hospital, and all the ESBLs belonged to the CTX-M-1 group [31]. The carriage rate of ESBL-producing *E. coli* strains isolated from children warded in a Malaysian tertiary hospital was 19.1% (21/110), and CTX-M-15 was the predominant type [32]. ESBLs were identified in 13.4% (18/143) of *E. coli* isolates from Libyan children's stools, and all isolates that produced ESBLs belonged to CTX-M type [33]. Among children living in a very remote Senegalese village, 10% (2/20) were found to be fecal carriers of a multi-resistant *E. coli* clone that produced CTX-M-15 [34]. In a tertiary care center in Turkey, fecal carriage of ESBL-producing *E. coli* and *Klebsiella* spp. was 24% (66/270) in hospitalized pediatric patients and 7.2% (14/194) in ambulatory children [35]. Our investigation showed that the majority of ESBL-PE isolated from healthy Lebanese infants carried multiple *bla* genes and that *bla*$_{\text{CTX-M}}$ was the predominant (91.4%) gene. TEM type was also considered a frequent (67.2%) disseminated beta-lactamase type, as the majority of the CTX-M-producing isolates (69.8%) were found to be co-producers of the TEM and SHV genes; either two or all three genes occurred together. Although epidemiological studies report that CTX-M-type ESBLs are endemic in most European, Asian, and South American countries, with high rates of prevalence ranging from 30% to 90% for *E. coli* [36], TEM enzymes could also spread in community settings.

The mechanisms of CTX-M emergence are more complex than those of TEM- or SHV-type ESBLs, involving not only a clonal spread of bacteria but also the spreading of enzymes encoded by genes harbored by plasmids and/or other mobile genetic elements, which promotes their epidemiological success. Furthermore, all strains of CTX-M-producing *Enterobacteriaceae* are also (most of the time) resistant to other families of antibiotics, especially aminoglycosides and fluoroquinolones, as plasmid-encoded CTX-M often carry other genes of resistance (particularly to aminoglycosides, tetracyclins, sulfamides, and trimethoprim), suggesting co-resistance, co-expression, and co-selection, which makes carbapenem use mandatory [37].

CTX-M-15 is the most frequently found CTX-M-type ESBL in *E. coli* worldwide, involved in community-acquired infections as well in nosocomial infections [38], and has been responsible for various outbreaks, especially in France, Great Britain, Canada, Spain, and Tunisia [38,39]. In the present study, molecular characterization of CTX-M-type ESBL-positive isolates revealed that a greater number of isolates (54.7%) co-harbored either two or three CTX-M-type genes (*bla*$_{\text{CTX-M-9}}$, *bla*$_{\text{CTX-M-15}}$, and *bla*$_{\text{CTX-M-2}}$). CTX-M-9-type was the predominant (86.8%) type, followed by CTX-M-15 (49.1%). Some CTX-M is specific to some countries (*e.g.*, CTX-M-9 and CTX-M-14 in Spain, CTX-M-1 in Italy, or CTX-M-2 in South America and Japan), while CTX-M-15 is distributed worldwide [12]. These data indicate that the epidemiology of colonization with ESBL-PE in the community is complex, and the source of acquisition and transmission of these genes may be from both hospital and community settings; further complex studies are needed.

All ESBL-PE isolated from Lebanese community infants were susceptible (100%) to imipenem, and 93.1% were susceptible to amikacin; this is considered positive regarding absence of metallo-beta-lactamases among these isolates. However, most isolates were resistant to other antimicrobials tested. High resistance to nalidixic acid (63.8%), tetracycline (60.3%),

gentamicin (48.3%), and trimethoprim-sulfamethoxazole (55.2%), was found; this result confirmed the carriage of multidrug-resistant ESBL-PE in asymptomatic healthy infants in Lebanon. This high carriage rate of ESBL-PE and associated resistance to aminoglycosides and trimethoprim-sulfamethoxazole, as well as high frequency of co-existence of fluoroquinolone, increases the risk of infection with multidrug-resistant bacteria, which results in the need for last-resort antibiotics, such as carbapenems and colistin, in the treatment of common infections [5].

Males appeared to have higher colonization rates than did females. This observation is difficult to explain and requires further exploration.

There have been reports on the risk factors for infection or colonization by ESBL-producing bacteria in children from pediatric intensive care units and neonatal intensive care units; the risk factors identified include artificial nails of hospital staff, cockroach infestation as vectors [40,41], younger gestational age, low birth weight, prolonged mechanical ventilation, longer hospital stay, invasive devices, antibiotic use [42-45], and mother-to-child transmission [46].

We investigated the risk factor for fecal carriage among participating infants and found that birth in a hospital and caesarean delivery were significantly associated with an increased risk of ESBL-PE carriage, suggesting that hospital environment or staff may be a source of these isolates. Infants with low birth weight, extended hospital stay after birth, previous antibiotic or antacid intake, previous hospital admission, and previous urinary tract infection were observed to have higher colonization rates, although this was statistically insignificant. On the other hand, we found that infants who were formula-fed had a significantly higher carriage rate (60.4%) than those who were breast-fed (43.2%), and this was statistically significant. This suggested that acquisition of ESBL-PE in this age group may be related to the milk source, water used, or to the practices around of feeding (*e.g.*, hygiene, sterilization of bottles, etc.). Another important risk factor was observed in this investigation was intimate hygiene habits; infants whose intimate areas were cleaned with dry tissue or wipes after each diaper change had higher ESBL-PE colonization rates than their counterparts whose intimate area were washed with water or soap and water. Although this finding was not significant ($p = 0.08$), it could be further studied and explained.

Conclusions

This study sheds light on the ESBL-PE carriage rate in Lebanese community infants and establishes a basis for future surveillance of carriage. A relatively high overall rate compared to some other countries was found. The majority of isolated ESBL-PE harbored multiple genes (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}), where CTX-M was the predominant and CTX-M-9 and CTX-M-15 were the most widespread ESBL types. Significant risk factors for this high carriage were hospital birth, caesarean delivery, and being formula-fed; these findings were observed for the first time. Hygiene measures and protocols are recommended in Lebanese hospitals to reduce transmission of ESBL-PE from staff, equipment, and environment to newborn babies; also, breastfeeding is recommended, as it decreases colonization rates. Further investigations are needed to evaluate the correlation between baby milk formula and ESBL-PE colonization, such as screening infant milk formula in the Lebanese market and screening water used in the preparation of these formulas for ESBL-PE. In addition, the relationship between intimate hygiene habits and decreased level of ESBL-PE carriage rate warrants further investigation. Alarming multidrug-resistant ESBL-PE is of great concern, especially in this age group, where treatment options are limited. Rational use of antibiotics, especially in pediatrics, is required.

Acknowledgements

We thank the pediatricians Maha Abou Alfa, Khalil Osta, and Nussaiba Sheik Ammar, who agreed to participate in this study; Mr. Hassan Abou Zaid for his great cooperation; and nurse Ola Saad for her help in sample collection and questionnaire.

References

1. Bradford PA (2001) Extended-spectrum beta-lactamases in the 21st century: characterization, epidemiology, and detection of this important resistance threat. Clin Microbiol Rev 14: 933-951.
2. Livermore DM (2012) Current epidemiology and growing resistance of Gram-negative pathogens. Korean J Intern Med 27: 128-142.
3. Livermore DM, Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, Ayala J, Coque T M, Kern-Zdanowicz I, Luzzaro F, Poirel L, Woodford N (2007) CTX-M: changing the face of ESBLs in Europe. J Antimicrob Chemother 59: 165-174.
4. Karisik E, Ellington MJ, Pike R, Warren RE, Livermore DM, Woodford N (2006) Molecular characterization of plasmids encoding CTX-M-15 beta-lactamases from *Escherichia coli* strains in the United Kingdom. J Antimicrob Chemother 58: 665-668.
5. Pitout JD (2010) Infections with extended-spectrum beta-lactamase producing *Enterobacteriaceae*: changing epidemiology and drug treatment choices. Drugs 70: 313-333.

6. Bush K (2014) Beta-lactamases: ubiquitous and formidable. In: Interscience Conference on Antimicrobial Agents and Chemotherapy, Washington, DC.
7. Lewis JS, Herrera M, Wickes B, Patterson JE, Jorgensen JH (2007) First report of the emergence of CTX-M-type extended-spectrum beta-lactamases (ESBLs) as the predominant ESBL isolated in a U.S. health care system. Antimicrob Agents Chemother 51: 4015-4021.
8. Paterson DL, Bonomo RA (2005) Extended-spectrum beta-lactamases: a clinical update. Clin Microbiol Rev 18: 657-686.
9. Pitout JDD, Laupland KB (2008) Extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: an emerging public-health concern. Lancet Infect Dis 8: 159-166.
10. Cantón R (2008) Epidemiology and evolution of β-lactamases. In Baquero F, Nombela C, Casslel GH, Gutierrez-Fuentes JA, editors. Evolutionary Biology of Bacterial and Fungal Pathogens. Washington: ASM Press. 249-270.
11. Bauernfeind A, Casellas JM, Goldberg M, Holley M, Jungwirth R, Mangold P, Röhnisch T, Schweighart S, Wilhelm R (1992) A new plasmidic cefotaximase from patients infected with *Salmonella typhimurium*. Infection 20: 158-163.
12. Cantón R, Coque TM (2006) The CTX-M β-lactamase pandemic. Curr Opin Microbiol 9: 466-475.
13. Coque TM, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, Baquero F, Cantón R, Nordmann P (2008) Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum beta-lactamase CTX-M-15. Emerging Infect Dis 14: 195-200.
14. Valverde A, Coque TM, Sánchez-Moreno MP, Rollán A, Baquero F, Cantón R (2004) Dramatic increase in prevalence of fecal carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* during non outbreak situations in Spain. J Clin Microbiol 42: 4769-4775.
15. Schwaber MJ, Carmeli Y (2007) Mortality and delay in effective therapy associated with extended-spectrum beta-lactamase production in *Enterobacteriaceae* bacteraemia: a systematic review and meta-analysis. J Antimicrob Chemother 60: 913-920.
16. Tumbarello M, Spanu T, Di Bidino R, Marchetti M, Ruggeri M, Trecarichi EM, De Pascale G, Proli EM, Cauda R, Cicchetti A, Fadda G (2010) Costs of bloodstream infections caused by *Escherichia coli* and influence of extended-spectrum- β-lactamase production and inadequate initial antibiotic therapy. Antimicrob Agents Chemother 54: 4085-4091.
17. Byington CL, Rittichier KK, Bassett KE, Castillo H, Glasgow TS, Daly J, Pavia AT (2003) Serious bacterial infections in febrile infants younger than 90 days of age: the importance of ampicillin-resistant pathogens. Pediatrics 111: 964-968.
18. Stoll BJ, Hansen N, Fanaroff AA, Wright LL, Carlo WA, Ehrenkranz RA, Lemons JA, Donovan EF, Stark AR, Tyson JE, Oh W, Bauer CR, Korones SB, Shankaran S, Laptook AR, Stevenson DK, Papile LA, Poole WK (2002) Changes in pathogens causing early-onset sepsis in very-low-birth-weight infants. N Engl J Med 347: 240-247.
19. Hsu AJ, Tamma PD (2014) Treatment of multidrug-resistant gram-negative infections in children. Clin Infect Dis 58: 1439-1448.
20. Clinical and Laboratory Standards Institute (2013) Performance standard for antimicrobial disk susceptibility testing: approved standards, 23rd edition. Wayne, PA: CLSI. M100-S20. Available: http//microbiolab-bg.com/CLSI.pdf. Accessed 1 February 2013.
21. Mirhendi H, Diba K, Rezaei A, Jalalizand N, Hosseinpur L, Khodadadi H (2007) Colony-PCR Is a rapid and sensitive method for DNA amplification in Yeasts. Iranian J Publ Health 36: 40-44.
22. Integrated DNA Technologies. Available at: http://eu.idtdna.com/site. Accessed 21 September 2013
23. Kim J, Jeon S, Rhie H, Lee B, Park M, Lee H, Lee J, Kim (2009) Rapid detection of extended spectrum β-lactamase (ESBL) for *Enterobacteriaceae* by use of a multiplex PCR-based method. Infect Chemother 41: 181-184.
24. Qin X, Zerr DM, Weissman SJ, Englund JA, Denno DM, Klein EJ, Tarr PI, Kwong J, Stapp JR, Tulloch LG, Galanakis E (2008) Prevalence and mechanisms of broad-spectrum beta-lactam resistance in *Enterobacteriaceae*: a children's hospital experience. Antimicrob Agents Chemother 52: 3909-2314.
25. Zerr DM, Qin X, Oron AP, Adler AL, Wolter DJ, Berry JE, Hoffman L, Weissman SJ (2014) Pediatric infection and intestinal carriage due to extended-spectrum-cephalosporin-resistant *Enterobacteriaceae*. Antimicrob Agents Chemother 58: 3997-4004.
26. Moubareck C, Daoud Z, Hakime NI, Hamze M, Mangeney N, Matta H, Mokhbat JE, Rohban R, Sarkis DK, Doucet-Populaire F (2005) Countrywide spread of community- and hospital-acquired extended-spectrum beta-lactamase (CTX-M-15)-producing *Enterobacteriaceae* in Lebanon. J Clin Microbiol 43: 3309-3313.
27. Birgy A, Cohen R, Levy C, Bidet P, Courroux C, Benani M, Thollot F, Bingen E (2012) Community faecal carriage of extended spectrum beta-lactamase-producing *Enterobacteriaceae* in French children. BMC Infect Dis 12: 315.
28. Kaarme J, Molin Y, Olsen B, Melhus A (2013) Prevalence of extended spectrum beta-lactamase-producing *Enterobacteriaceae* in healthy Swedish preschool children. Acta Paediatr 102: 655-660.
29. Fernandez-Reyes M, Vicente D, Gomariz M, Esnal O, Landa J, Oñate E, Pérez-Trallero E (2014) High rate of fecal carriage of extended-spectrum-beta-lactamase-producing *Escherichia coli* in healthy children in Gipuzkoa, northern Spain. Antimicrob Agents Chemother 58: 1822-1824.
30. Lukac PJ, Bonomo RA, Logan LK (2015) Extended-Spectrum β-Lactamase-Producing *Enterobacteriaceae* in Children: Old Foe, Emerging Threat. Clin Infect Dis 60: 1389-1397.
31. Minami K, Shoji Y, KasaiM, OgisoY, Tomohiko N, Kawakami Y, Saito Y, Kuzumoto K, Kubota N, Yumoto K, Ishii K (2012) Proportion of rectal carriage of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in the inpatients of a pediatric tertiary care hospital in Japan. Jpn J Infect Dis 65: 548-550.
32. Ho WS, Balan G, Puthucheary S, Kong BH, Lim KT, Tan LK, Koh XP, Yeo CC, Thong KL (2012) Prevalence and characterization of multidrug-resistant and extended-spectrum beta-lactamase-producing *Escherichia coli* from pediatric wards of a Malaysian hospital. Microb Drug Resist 18: 408-416.
33. Ahmed SF, Ali MM, Mohamed ZK, Moussa TA, Klena JD (2014) Fecal carriage of extended-spectrum β-lactamases and AmpC-producing *Escherichia coli* in a Libyan community. Ann Clin Microbiol Antimicrob 13: 22.
34. Ruppe E, Woerther P-L, Diop A, Sene A-M, Costa A D, Arlet G, Andremont A, Rouveix B (2009) Carriage of CTX-M-15-Producing *Escherichia coli* isolates among children living in a

remote village in Senegal. Antimicrob Agents Chemother 53: 3135-3137.
35. Kiremitçi A, Dinleyici EÇ, Yargıç ZA, Durmaz G, Tekin N, Aybey AD, Akşit MA (2011) Prevalence and risk factors of fecal carriage of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae in hospitalized and ambulatory children. J Pediatr Inf 5: 54-58.
36. Lahlaoui H, Ben Haj Khalifa A, Ben Moussa M (2014) Epidemiology of *Enterobacteriaceae* producing CTX-M type extended spectrum β-lactamase (ESBL). Méd Mal Infect Rev 44: 400-404.
37. Pai H, Kim MR, Seo MR, Choi TY, Oh SH (2006) A nosocomial outbreak of *Escherichia coli* producing CTX-M-15 and OXA-30 beta-lactamase. Infect Control Hosp Epidemiol 27: 312-314.
38. Woodford N, Ward ME, Kaufmann ME, Turton J, Fagan EJ, James D, Johnson AP, Pike R, Warner M, Cheasty T, Pearson A, Harry S, Leach JB, Loughrey A, Lowes JA, Warren RE, Livermore DM (2004) Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum beta-lactamases in the UK. J Antimicrob Chemother 54: 735-743.
39. Lavollay M, Mamlouk K, Frank T, Akpabie A, Burghoffer B, Ben Redjeb S, Bercion R, Gautier V, Arlet G (2006) Clonal dissemination of a CTX-M-15 beta lactamase-producing *Escherichia coli* strain in the Paris area, Tunis, and Bangui. Antimicrob Agents Chemother 50: 2433-2438.
40. Cotton MF, Wasserman E, Pieper CH, Theron DC, van Tubbergh D, Campbell G, Fang FC, Barnes J (2000) Invasive disease due to extended spectrum beta-lactamase-producing *Klebsiella pneumoniae* in a neonatal unit: the possible role of cockroaches. J Hosp Infect 44: 13-17.
41. Gupta A, Della-Latta P, Todd B, San Gabriel P, Haas J, Wu F, Rubenstein D, Saiman L (2004) Outbreak of extended-spectrum beta-lactamase–producing *Klebsiella pneumoniae* in a neonatal intensive care unit linked to artificial nails. Infect Control Hosp Epidemiol 25: 210-215.
42. Crivaro V, Bagattini M, Salza MF, Raimondi F, Rossano F, Triassi M, Zarrilli R (2007) Risk factors for extended-spectrum beta-lactamase-producing *Serratia marcescens* and *Klebsiella pneumoniae* acquisition in a neonatal intensive care unit. J Hosp Infect 67: 135-141.
43. Rettedal S, Hoyland Lohr I, Natas O, Sundsfjord A, Oymar K (2013) Risk factors for acquisition of CTX-M-15 extended-spectrum beta-lactamase producing *Klebsiella pneumoniae* during an outbreak in a neonatal intensive care unit in Norway. Scand J Infect Dis 45: 54-58.
44. Shakil S, Akram M, Ali SM, Khan AU (2010) Acquisition of extended-spectrum beta-lactamase producing *Escherichia coli* strains in male and female infants admitted to a neonatal intensive care unit: molecular epidemiology and analysis of risk factors. J Med Microbiol 59: 948-954.
45. Vijayakanthi N, Bahl D, Kaur N, Maria A, Dubey NK (2013) Frequency and characteristics of infections caused by extended-spectrum beta lactamase-producing organisms in neonates: a prospective cohort study. Biomed Res Int 2013: 756209.
46. Denkel LA, Schwab F, Kola A, Leistner R, Garten L, von Weizsäcker K, Geffers C, Gastmeier P, Piening B (2014) The mother as most important risk factor for colonization of very low birth weight (VLBW) infants with extended-spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL-E). J Antimicrob Chemother 69: 2230-2237.

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Conflict of interests: No conflict of interests is declared.