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High incidence of multidrug resistant Escherichia coli producing CTX-M-type ESBLs colonizing the intestine of Jordanian infants

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Abstract

Background: This prospective study investigated major characteristics of *E.coli* colonizing the intestine of out-and in-patient infants, especially their association with CTX-M-type extended spectrum β-Lactamases (ESBLs), integrons and fluoroquinolones-resistance.

Methods: A total of 288 stool samples were collected from infants aged less than 1 year which were admitted at the Pediatric Department, Jordan University Hospital, Amman. The *E.coli* isolates were investigated using antimicrobial susceptibility tests and PCR for detection of CTX-M genes and integrons.

Results: A total of 170 (59%) infants were colonized with *E.coli*. These included 73 (42.9%) females and 97 (57.1%) males. A significant difference was observed between gender and age groups according to the presence or absence of *E.coli* (P= 0.001). Multidrug resistant (MDR) accounted for 52 (30.6%) of the isolates and all these were ESBL producers. The detection rate of CTX-M genes among MDR *E.coli* isolates was 49 (94.2%), CTX-M group 1 accounted for 41 (87.8%) of the isolates, and 30 (73.2%) were CTX-M-15 producers. Only Class I integron was detected in 20/52 (38.5%) of MDR *E. coli* isolates, and resistance to fluoroquinolones accounted for 45/52(86.5%) of these isolates . A total of 33/52 (63.5%) of MDR *E.coli* isolates were resistant to potential quinolone resistance genes *(parC)* and *(gyrA)* in association with CTX-M group.

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Conclusion: This study demonstrates that formula-fed infants, vaginal delivery and old age of infants were higher and significantly associated with colonization of *E .coli*. High incidence of CTX-M ESBL-producing *E. coli* was found in association with fluoroquinolones-resistance and Class I integrons colonizing the intestine of Jordanian infants.

Key words: ESBL, *E.coli*, Class I integrons, Jordanian Infants.

Introduction

In recent years, there is increasing incidence of CTX-M type ESBLs producing *Enterobacteriaceae* in causing serious infections associated with community and hospitalized patients (1,2,3). For empirical treatment of the *E.coli* serious infections in infants, beta-lactam drugs including carbapenems are widely used despite the fact that increasing rates of *E.coli* are becoming resistance to carbapenemase and causing outbreaks of nosocomial infections (2,3).

It has been observed that bacterial strains with the ESBL phenotype are often multidrug resistant in association with plasmids which carry other genes responsible for resistance to aminoglycosides, fluoroquinolones and trimethoprim-sulfamethoxazole. In particular, CTX-M-15 is among the commonest CTX-M variants, discovered in many genera *Enterobacteriaceae* across the world, and it was the most reported variant in Europe (1,2). In addition, *E.coli* strains carrying the CTX-M-15 were more increasingly detected in community acquired infections in both developed and developing countries (1,4).

Therefore, the global spread of carbapenemase-producing *Enterobacteriaceae* constitutes a significant threat for treatment of patients (3,5).

The objectives of this study were to detect the incidence of CTX-M ESBL-producing *E. coli* in association with fluoroquinolones-resistance and Class I integrons colonizing the intestine of Jordanian infants.

Patients and Materials

Patients & Data collection

This prospective study included a total of 288 infants aged less than 1 year old who were admitted to the Pediatric Department, Jordan University Hospital in Amman, Jordan. Feces samples were collected from all infants over the period March-July 2012. A biographical data about each infant was obtained and registered on special form. Data included age, gender, name, duration of hospitalization, disease history, and taking of antibiotic at the time of sampling and before 2 week of sampling, type of feeding. Ethical approval was obtained from the Institutional Ethical Committee at Jordan University Hospital and the Deanship of Scientific Research at the University of Jordan.

Collection of feces samples

A total of 288 infants were investigated, of these 154 (53.5%) were male infants with a mean age of 2.43 ± 3.13 months; and 134 (46.5%) were female

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infants with a mean age of 2.69 ± 3.45months. A sample from stool or rectum from each infant was taken using a sterile cotton swab and then placed and transported in Amies transport media (Advanced trading agency, Co., Jordan) to the microbiology research laboratory for culture .

Culture and identification *E.coli*

All collected samples were cultured within 1-3 hours on MacConkey agar (Oxoid, England) and incubated for 24 hr in 37°C. Five colonies that morphologically resemble lactose-fermented *E. coli* growth were selected and sub-cultured on MacConkey agar again to obtain pure *E.coli* isolates. Pure growth of all sub-cultured *E.coli* isolates were confirmed by the following biochemical standard tests; including citrate and urease utilization, lactose and glucose fermentation in tube Kligler iron agar and indole production, and later representative of isolates were confirmed as *E.coli* by commercial RemelRapID ONE test (Remel INC, USA). Only one single *E.coli* isolates was included for each patients, and all *E.coli* isolates were stored in cryotubes containing brain-heart infusion agar (Oxoid, England) with 15% glycerol at -70 °C.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed on Mueller-Hinton agar (Oxoid, England) according to the recommendation of the Clinical Laboratory and Standards Institute (CLSI, 2012).[6] The results were interpreted according to the guidelines of CLSI. *E. coli* ATCC 25922 strain was included as a control. The antimicrobials disks (Mast group ltd, England) used in our study (µg/disk) were: augmentin (amoxicillin/clavulanic acid 20/10), cefuroxime (30), cotrimoxazole (trimethoprim-sulfamethoxazole 1.25/23.75), gentamicin (10), nalidxic acid (30), nitrofurantoin (300), ciprofloxacin (5),amikacin (30), cefoxitin (30), ceftazidime (30), ceftriaxone (30) ,cefotaxime (30) and gentamicin (10). In addition, minimum inhibitory concentration (MICs) of *E.coli* isolates to cefotaxime, ceftriaxone, imipenem, gentamicin and ciprofloxacin were determined using E-test susceptibility (Oxoid, England).

DNA extraction

E.coli DNA was extracted using the G-spin™ Total DNA Extraction Kit (Intron, Korea) according to the manufacturer's protocol for isolation of bacterial DNA. Multiplex PCR (mPCR) was used for the detection of major CTX-m phylogenetic groups in *E. coli* isolates which were resistant to >3 antibiotic classes (52 isolates), including resistance to cefotaxime and ceftriaxone. Four pairs of primers were used to detect 5 major CTX-m phylogenetic groups (CTX-M-1, CTX-M-2, CTX-M-8 and 25/26, CTX-M-9) as described by Mirzaee *et al.*(7) and CTX-M-15 as reported by Leflon-Guibout *e tal.*[8] Also, potential fluoroquinolones-resistance genes (*parC*) and (*gyrA*) were detected as reported by Leflon-Guibout *et a*l (8).

PCR detection of integrons

Detection of class 1, 2 ,3 integrons in 52 multiresistant *E. coli* isolates was performed using primer pairs (Invitrogen, USA) as described by Dillon *et al.*(9) .

Results

Table 1 shows that 170/288 (59%) of the infants carried *E.coli* in their intestine; of these 73 emales (42.9%) and 97 males (57.1%) .The association between *E. coli* colonization and other potential contributing factors such as gender, taking antibiotic before two week of sampling, taking antibiotic at time of sampling and presence of maternal infection within 4 weeks before delivery, was not significant, but a significant difference was found in relation

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Table 1. Univariate analysis of potential contributing factors associated with 170 *E .coli* isolates from intestines of Jordanian infants.

Contributing Factor		*Escherichia coli*		p-value	OR (95% CI)
		Positive N (%)	Negative N (%)		
Age	(1day - 30 days)	61(44.2)	77(55.8)	0.001*	0.298 (0.182-0.487)
	(>1 month –1 year)	109(72.7)	41(27.3)		
Gender	Male	97(63)	57(73)	0.143	1.422(0.887-2.279)
	Female	73(54.5)	61(45.5)		
Type of feeding	Breast milk	56(47.9)	61(52.1)	0.004*	0.363(0.186-0.708)
	Milk formula	43(71.7)	17(28.3)		
	mixed	71(64)	40(36)		
Antibiotic treatment at time of sampling stool specimens	Yes	15(60)	10(40)	0.918	1.045(0.453-2.414)
	No	155(58.9)	108(41.1)		
Previous antibiotic treatment before 2 weeks of sampling stool specimens	Yes	27(54)	23(46)	0.426	0.780(0.422-1.441)
	No	143(60.1)	95(39.9)		
Presence of maternal infection within 4 weeks before delivery	Yes	25(61)	16(39)	0.784	0.910(0.462-1.790)
	No	145(58.7)	102(41.3)		
Mode of delivery before sampling stool specimens	urgent	80(51.6)	75(48.4)	0.006*	0.510(0.315-0.824)
	vaginal	90(67.7)	43(32.3)		

* Significant.

to age (P=0.000), type of feeding (P= 0.004) and mode of delivery (p= 0.006) as proved by Pearson Chi- square test . The antimicrobial susceptibility patterns of 52/170 (30.6%) *E. coli* isolates are shown in **Table 2.** All these 52 *E.coli* isolate were multidrug resistant to (> 3 drugs) and all were ESBL producers. The detection rate of CTX-M genes among MDR *E.coli* isolates was 49 (94.2%), CTX-M group 1 accounted for 41 (87.8%) of these isolates and 30 (73.2%) were CTX-M-15 producers. Only Class I integron was detected in 20/52 (38.5%) of MDR *E. coli* isolates, and resistance to fluoroquinolones accounted for 45/52(86.5%) of these MDR isolates. A total of 33/52 (63.5%) of MDR *E.coli* isolates were also positive for both *gyrA* and *parC* genes in association with CTX-M group.

Discussion

This study demonstrates that *E.coli* strains colonized 59% of the Jordanian infants aged less one year. The intestines of infants aged less than one month have the lowest rate of colonization (44.2%) with *E.coli* (**Table 1**),and other studies Sweden and Brazil have showed also to some extent similar results

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(19,11). The present study has shown that vaginal delivery of infants was associated with higher and significant rates of *E.coli* colonization (P= 0.006) compared with cesarean delivery (**Table 1**). The study of Penders et al.(12) in Netherlands, has reported that infants acquired similar prevalence rates of *E.coli* colonization (85-91%) when infants were born through natural vaginal delivery at home, hospital or through caesarean section at hospital.

The present study has shown that there was no statistically significant relationship between *E.coli* colonization of infants who have been treated with antibiotics at the time of sampling (60%; P=0.918) or before two week of sampling (54%; P=0.426), respectively. While breastfed infants have less significant rates of *E.coli* colonization (P=0.004) than other feeding types. Previous studies have also reported that formula-fed infants were more often associated with colonization of *E .coli*, *C difficile*, *Bacteroides*, and lactobacilli when compared with breastfed infants (12,13).

This study shows the occurrence of high rate of MDR *E. coli* isolates (30.6%), and about 40% of these were associated with Class 1 integrons (**Table 2**). In addition, the majority of these *E. coli* isolates (94.2%) were producers of CTX-M , and CTX-M-15 type accounted for 73.2% of the isolates (**Table 3**). This result is comparable to the rates (≥ 30%) reported in East and Southeast Asia, Latin America, and Southern Europe, but less rates were found in Australia, Northern Europe, and North America. However, the highest ESBL rates of *E. coli* were re-

Table 2. Distribution of Class 1 integrons genes in 52 MDR *E.coli* isolates (≥ 3 drugs).

Antibiotics	No. (%) of total resistant isolates	MIC 90%	MIC 50%	No. (%) of resistant isolates associated with class 1 integrons	P value
Ceftriaxone	51(98)	>32	>32	20/51 (39)	1.000
Cefotaxime	50(96)	>256	128	18/50 (36)	0.143
Cefixime	49(94)	-	-	18/49(37)	0.551
Augmentin	49(94)	-	-	19/49(39)	1.000
Naldixic acid	42(81)	-	-	17/42(41)	1.000
Ceftazidime	41(79)	-	-	16/41 (39)	0.176
Cortimoxazole	39(75)	-	-	19/39(49)	0.009*
Nitrofuronation	28(54)	-	-	11/28(39)	1.000
Ciprofloxacin	19 (37)	>32	0.25	7/19(37)	0.871
Gentamicin	18(35)	64	1	7/18(39)	1.000
Imipenem	1	0.25	0.25	1/1 (100)	0.385

* Significant value

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Table 3. Distribution of CTX-M genes among 52 MDR of *E .coli* isolates

ESBL phenotypes	No. (%) E.coli isolates
CTX-M group I*	24(46.1%)
CTX-M group I and CTX-M group II	12(23.1%)
CTX-M group II and CTX-M group 9	5(9.6%)
CTX-M group 9	3(5.8%)
CTX-M group I and CTX-M group 9	3(5.8%)
CTX-M group I, CTX-M group II and CTX-M group 9	2(3.8%)
Non-typed	3(5.8%)
Total	52(100%)

*A total of 41/52*E.coli* isolates (87.8%) were identified producing CTX-M group I alone or in association with other CTX-M groups, of these 30/41(73.2%) were CTX-M-15 producers.

ported in India (≥ 80%) and China (≥ 60%), and almost all their isolates were susceptible to imipenem (MIC 0.12 – 0.5 µg/ml) (14,15).

Studies performed few years ago in Jordan, have reported less prevalence rates of MDR *E.coli* isolates (19-25%) from clinical cases, healthy persons and effluent water, including incidence of co-resistance to second and third generations of cephalosporins (16,17). This fact illustrate how rapidly MDR *E.coli* strains can emerge due to their carrying CTX-M-type ESBLs genes. The association of co-resistance in our isolates to antimicrobials such as aminoglycosides, tetracyclines, chloramphenicol, trimethoprim, and quinolones may be considered an important factor in the spread and maintenance of ESBL-producing *E.coli* (18). However, the frequency of resistance to non-beta lactam antibiotics in this study is lower than that was reported in most other Middle East countries (4).

It has also been reported that CTX-M-15 is a major enzyme of the CTX-M -1 group and was associated with epidemic outbreak and plasmids, and it is playing a role in conferring carbapenem resistance (19). CTX-M-15 could be associated with other ESBLs encoding genes and resistance to other antimicrobial agents such as fluoroquinolones, and aminoglycosides (1,20). Recent studies have also reported that ESBL-producing *E. coli* is a major reservoir for *bla*CTX-M-15 gene, and it has rapidly spread in *E.coli* strains isolated from human, animal and environment worldwide (2,21).

The present study demonstrated that 38.5% of MDR *E.coli* isolates carried Class I integrons and were positive for one or both potential fluroquinolone resistance genes (*gyrA* and *parC*). In particular, co-trimoxazole resistance was statically significant (P=0.009) with the presence of Class I integrons. Previous studies from Jordan indicated also higher incidence rates of Class 1 integrons in *E. coli* isolated from water (48%) and stool of normal adult population (74%) especially in association with transferable plasmids (16,22). It has been shown that the integron system has the ability to capture various

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resistance genes and to create novel combinations of resistance genes which may contribute for the evolution of MDR Gram-negative bacteria (23-26).

In conclusion, the high rate of *E. coli* strains in feces of infants carried CTX-M-type ESBLs and associated with Class I integrons and fluoroquinolone-resistant should be considered alarming sign since they may cause serious infection which can't be easily treatable.

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Declaration of interest

Nothing to declare.

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