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Research paper

Considerable rate of putative virulent phylo-groups in fecal carriage of extended-spectrum β-lactamase producing *Escherichia coli*

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ABSTRACT

Extended-Spectrum Beta–lactamase producing Enterobacteriales (ESBL-PE) in fecal carriage have become a global health concern. Detection of putative virulent ESBL-producing *E.coli* (ESBL-EC) isolates among asymptomatic carriers is a threatening issue in public health. The aim of this study was to investigate the intestinal carriage of ESBL-EC, phylo-groups and clonal relatedness among putative virulent groups of ESBL-EC isolated from fecal carriages. A total of 120 rectal swabs; 50.8% (61/120) from inpatients of intensive care unit (ICU) and 49.2% (59/120) from outpatients were collected. The ESBL-EC screening was performed by using MacConkey agar supplemented with cefotaxime. PCR assays were applied for determination of phylo-groups, detection of ESBL and carbapenemase genes. Conjugation experiment, plasmid replicon typing and Multilocus Sequence Typing (MLST) were performed for putative virulent phylo-groups. Totally, of 120 studied individuals, 60.0% (72/120) were carrier for ESBL-EC. The rate of $bla_{CTX\text{-}M\text{-}15}$, bla_{TEM}, bla_{SHV} was 90.2% (65/72), 50.0% (36/72) and 5.5% (4/72), respectively. The frequency of phylo-groups A, B1, B2, C, D, and F were 20.8% (15/72), 6.9% (5/72), 20.8% (15/72), 2.7% (2/72), 13.8 (10/72) and 12.5% (9/72), respectively. In conjugation experiments, of 6 tested isolates, 5 had conjugative plasmids. The most prevalent plasmid types belonged to IncF incompatibility groups. The MLST analysis showed that the main sequence types among ESBL-EC isolates were ST769 and ST472. The current study provides novel information about the presence of the ESBL-EC isolates, particularly putative virulent phylo-groups among fecal carriages in Iran. Our data revealed that there was almost high ST heterogeneity among putative ESBL-EC isolates. In order to implementation of effective infection control program, detection of fecal carriage in appropriate time typically at the beginning of admission to the hospital is recommended.

1. Introduction

Extended-Spectrum Beta-lactamase producing Enterobacteriales (ESBL-PE) notably *E.coli* have been recently detected in both nosocomial and community acquired infections (Popejoy et al., 2016). Along with clinically related ESBL producing isolates, the fecal carriage of ESBL-PE has been recently emerged as a global health concern. Since the majority of carriers are asymptomatic, therefore, it gives rises to expansion of ESBL-PE fecal carriages (Ebrahimi et al., 2016). Uncontrolled use of antibiotics and transmission of resistant bacterial isolates are two main reasons for increasing of fecal carriage rate (de Lastours et al., 2010). Intestinal colonization by ESBL-PE is considerable since ESBL genes are mostly located on conjugative plasmids. This could leads to transmission of resistance genes to other bacteria across species boundaries, horizontal transmission to other persons and infection in the host (Lübbert et al., 2015). Furthermore, they also usually carry other genes that are associated with aminoglycoside or fluoroquinolone resistance (Dolejska and Papagiannitsis, 2018). This situation is of the great concern, as transmission of these plasmids could be resulted in intestinal colonization with multi-drug resistant isolates. These plasmids belong to the diverse incompatibility groups, most, including Inc. A/C, L/M, F, I1, HI2, and N (Solgi et al., 2017a, 2017b). Resistance genes are usually specific to a certain type of plasmids, for example the $bla_{CTX\text{-}M\text{-}15}$ gene is carried on IncFII

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conjugative plasmids (Giedraitienė et al., 2017).

Phylo-grouping study, as a PCR-based typing method, puts *E.coli* into seven major groups including A, B1, B2, C, D, E and F. Determination of the phylo-group types in *E.coli* is important as it could possibly represent the potency of pathogenicity in *E.coli* isolates. Significantly, strains responsible for extra-intestinal infection were far more likely to be members of phylo-groups B2 or D than A or B1. (Clermont et al., 2013).

On the other hand, typing by multi locus sequence typing (MLST) has been proven to be useful technique in order to better understand and to determine the clonal relatedness among isolates. Moreover, analyses of phylogenetic groups, particularly virulent extra-intestinal groups (B2, D and F) (Clermont et al., 2000) are also considerable in fecal carriages (Cornejo-Juárez et al., 2016). Detection of clinically related sequence types of ESBL-EC isolates among asymptomatic carriers is a critical issue in public health since presence of this microorganisms particularly putative virulent phylogroups such as B2 and D in the intestine, could result to increase the risk of infection in fecal carriage.

To the best of our knowledge, a few studies have been carried out about ESBL-PE fecal carriages in Iran (Aghamohammad et al., 2018; Solgi et al., 2017). However, further investigation regarding ESBL-PE carriers is essential for implementation of successful prevention and control program for ESBL-PE. The aim of the present study was to investigate the intestinal carriage of ESBL-EC, determination of phylogroups and clonal relatedness among putative virulent groups of ESBL-EC strains isolated from fecal carriages.

2. Material and method

2.1. Ethical statement and bacterial isolates

The current cross-sectional study was conducted in a university general hospital in Tehran, Iran during January to October of 2016. Totally 120 rectal swabs (RS) were randomly collected from ICU patients and outpatients. All studied patients signed an informed consent form before sampling and declared their willingness to allow the application of their sample and anonymous data for research purposes (IR.PII.REC.1395.44). Rectal swabs were transferred in Tryptic Soy Broth containing a 30-μg cefotaxime (CTX) disk (Mast Group Ltd., Merseyside, UK) and then delivered to the laboratory within 2 h. All swabs were incubated at 37 °C overnight. The MacConkey agar supplemented with cefotaxime (1 mg/L) was used for primary screening and then incubated at 37 °C for overnight (Aghamohammad et al., 2018). In the next step a single colony was sub-cultured in the MacConkey agar supplemented with cefotaxime to make a pure culture. Identification of *E.coli* was confirmed by using biochemical tests (Rasheed et al., 2014).

2.2. Antimicrobial susceptibility test

The susceptibility of cefotaxime resistant *E.coli* isolates was determined by using disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI) guidelines (CLSI, 2017). Eleven antibiotics including ceftazidime (CAZ: 30 μg), ceftazidime /clavulanic acid (CAZ/CLA: 30/10 μg), cefotaxime (CTX: 30 μg), cefotaxime/ clavulanic acid (CTX/CLA: 30/10 μg), cefepime (CPM: 30 μg), amikacin (AK: 30 μg), gentamicin (GM: 30 μg), ciprofloxacin (CIP: 5 μg), levofloxcine (LVX: 5 μg), ertapenem (ETP: 10 μg), imipenem (IMP: 10 μg) (all from MastGroup Ltd., Merseyside, United Kingdom) were tested against studied isolates. *Escherichia coli* ATCC 25922 was used as a control. All isolates were screened for the ESBL production by using double-disk synergy test (DDST) according to the CLSI guidelines. *K. pneumoniae* ATCC 700603 and *E. coli* ATCC25922 were used as positive and negative controls in DDST method, respectively. The Multi Drug Resistant (MDR) strains were defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories (Magiorakos et al., 2012).

2.3. Molecular detection of ESBL and carbapenemase genes

Genomic DNA was extracted by the commercially available DNA extraction kit (Bioneer Company, Korea, AccuPrep Genomic DNA Extraction Kit). ESBL and carbapenemase genes including the bla_{TEM}, bla_{SHV}, $bla_{CTX\text{-}M\text{-}15}$, bla_{VEB}, bla_{PER}, $bla_{NDM\text{-}1}$, $bla_{OXA\text{-}48}$, bla_{VIM}, bla_{IMP} were identified by polymerase chain reaction (PCR) experiments as described previously (Shahcheraghi et al., 2013).

2.4. Phylotyping method and detection of phylo-groups in ESBL-EC isolates

Seven phylog-roups including A, B1, B2, C, D, E and F were recognized by using PCR-based method as described by Clermont et al. Briefly the principal target genes including the chuA, yjaA, TspE4.C2 and arpA were amplified by using PCR. In order to characterization of the phylo-groups C and E, additional allele-specific PCR primers were used (Clermont et al., 2013).

2.5. PCR-based replicon typing and conjugation experiments

Plasmid incompatibility (Inc) types were determined by PCR-based replicon typing (PBRT) method using eighteen pairs of primers (Carattoli et al., 2005). Six lactose negative *E.coli* isolates were selected for conjugation experiments and were analyzed for the $bla_{CTX\text{-}M\text{-}15}$ gene transferring. *E. coli* K12 [F− lac + Nal (r)] was considered as the recipient strain which was previously described (Lyimo et al., 2016). MacConkey agar plates containing 32 μg/mL nalidixic acid and 1 μg/mL cefotaxime was used to select the transconjugants. Plasmid DNA was extracted using the Gene All Exprep Plasmid (GeneAll Biotechnology, Seoul, Korea). The confirmation of putative transconjugants was conducted by the detection of the $bla_{CTX\text{-}M\text{-}15}$ gene with PCR.

2.6. Multi-locus sequence typing (MLST)

Allele typing of eight housekeeping genes (*dinB, icdA pabB, polB, putP, trpA, trpB and uidA*) was performed according to MLST website (https://bigsdb.pasteur.fr/cgi-bin/bigsdb/bigsdb.pl?db=pubmlst_ecoli_seqdef). The allele profiles were compared in Pasteur's webpage (https://bigsdb.pasteur.fr/ecoli/ecoli.html) to identify the sequence type (ST) of each strain. MLST was done for 34 putative virulent ESBL-EC isolates (B2, D and F phylo-groups).

2.7. Statistical analysis

Statistical analyses were performed using SPSS software (version 25; SPSS, Inc., Chicago, IL, USA). The Fisher's exact test or the Chi-square test was applied to analysis categorical data. A p-Values $< .05$ in all experiments were considered statistically significant. Pasteur MLST excel files of isolates were analyzed by PHYLOViZ 2.0 software (Nascimento et al., 2016).

3. Results

3.1. Detection of ESBL-EC in rectal swabs

A total of 120 non-duplicated rectal swabs; 50.8% (61/120) from inpatients of intensive care unit (ICU) and 49.2% (59/120) from outpatients were collected. Based on our results 60.0% (72/120) of studied individuals, including 55.5% (40/72) of outpatients and 45.4% (32/72) of inpatients were carriers of ESBL-EC.

Table 1
antimicrobial resistance profile, Phylogroup typing, clonal relatedness and plasmid Inc. grouping of 72 ESBL-EC isolated from intestinal carriage in Iran.

Strain number	Age	Unit	Non susceptible profile (disk diffusion)	ESBL genes	Carbapenemase genes	Phylogroup type	Sequence Type	Inc group
E1	20	OP	CAZ,CTX,CPM,CIP,LVX	bla_{CTX},bla_{TEM}	–	B2	769	FII
E2	62	OP	CAZ,CTX,CIP,LVX	bla_{TEM},bla_{SHV}	–	F	769	NT
E3	18	G-ICU	CAZ,CTX,CPM,CIP,LVX	bla_{CTX}	–	F	769	FI
E4	52	*E*-ICU	CAZ,CTX,CPM,CIP,LVX	bla_{TEM}	–	F	769	FII
E5	26	OP	CAZ,CTX,CPM,CIP,LVX	bla_{CTX},bla_{TEM}	–	B2	472	FI
E6	74	G-ICU	CAZ,CTX,CIP,LVX,ETP	bla_{CTX}	–	F	472	HI2
E7	65	G-ICU	CAZ,CTX,CPM,GM,CIP,LVX	bla_{CTX}	–	F	472	UT
E8	43	G-ICU	CAZ,CTX,CPM,CIP,LVX	bla_{CTX}	–	D	472	FI
E9	70	G-ICU	CAZ,CTX,CPM,GM	bla_{TEM}	–	B2	8	L/M
E10	37	G-ICU	CAZ,CTX,CPM,CIP	bla_{CTX}	–	B2	8	FII
E11	47	E-ICU	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	D	8	FIV
E12	42	OP	CAZ,CTX,CPM	bla_{CTX}	–	D	771	UT
E13	42	OP	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	F	771	UT
E14	8	OP	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	D	195	FIV
E15	34	E-ICU	CAZ,CTX,CPM,CIP,LVX	bla_{CTX}	–	D	195	FI
E16	58	OP	CTX	bla_{CTX},bla_{TEM}	–	B2	896	UT
E17	34	OP	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	B2	688	UT
E18	35	OP	CAZ,CTX,CPM,GM,CIP,LVX	bla_{CTX}	–	B2	ND	FI
E19	22	OP	CAZ,CTX,CPM,CIP,LVX	bla_{CTX},bla_{TEM}	–	B2	572	UT
E20	26	OP	CAZ,CTX,CPM	bla_{CTX}	–	B2	783	UT
E21	42	OP	CAZ,CTX,CPM,GM,CIP,LVX	bla_{CTX}	–	B2	476	FI
E22	34	OP	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	B2	506	FIV
E23	31	OP	CAZ,CTX,CPM,CIP	bla_{CTX}	–	B2	678	UT
E24	48	OP	CAZ,CTX,CPM,CIP	bla_{CTX},bla_{TEM}	–	B2	580	FII
E25	37	G-ICU	CAZ,CTX,CPM,GM,CIP,LVX	bla_{CTX}	–	B2	527	FI
E26	60	*E*-ICU	CAZ,CTX,CPM,GM,CIP,LVX	bla_{CTX}	–	B2	53	UT
E27	45	OP	CAZ,CTX,CPM	bla_{CTX}	–	D	525	UT
E28	39	OP	CAZ,CTX,CPM	bla_{CTX}	bla_{OXA48}	D	535	FIV
E29	48	OP	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	D	739	W
E30	14	OP	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	D	380	FIV
E31	32	G-ICU	CAZ,CTX,CPM	bla_{CTX}	–	D	ND	FIV
E32	29	OP	CAZ,CTX	bla_{SHV}	–	F	706	FIV
E33	6	OP	CAZ,CTX,CPM	bla_{CTX}	–	F	695	FIV
E34	40	G-ICU	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	F	826	I1
E35	8	OP	CTX,CPM, CIP	bla_{CTX}	–	A	ND	ND
E36	24	OP	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	A	ND	ND
E37	28	OP	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	A	ND	ND
E38	72	OP	CAZ,CTX,CPM,CIP,LVX	bla_{CTX}	–	A	ND	ND
E39	29	OP	CTX,CPM	bla_{CTX}	–	A	ND	ND
E40	21	OP	CAZ,CTX,CPM	bla_{TEM}	–	A	ND	ND
E41	29	OP	CAZ,CTX,CPM,CIP,LVX	bla_{CTX}	–	A	ND	ND
E42	42	OP	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	A	ND	ND
E43	38	OP	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	A	ND	ND
E44	30	OP	CAZ,CTX,CPM	bla_{CTX}	–	A	ND	ND
E45	74	G-ICU	CAZ,CTX,CPM	bla_{CTX}	–	A	ND	ND
E46	40	G-ICU	CAZ,CTX,CPM,CIP,LVX	bla_{CTX}	–	A	ND	ND
E47	61	G-ICU	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	A	ND	ND
E48	30	G-ICU	CAZ,CTX,CPM	bla_{CTX}	–	A	ND	ND
E49	58	*E*-ICU	CAZ,CTX,CPM	bla_{CTX}	–	A	ND	ND
E50	25	OP	CAZ,CTX,CPM	bla_{CTX}	–	B1	ND	ND
E51	15	OP	CAZ,CTX	bla_{CTX}	–	B1	ND	ND
E52	42	OP	CAZ,CTX,CPM	bla_{CTX}	–	B1	ND	ND
E53	36	OP	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	B1	ND	ND
E54	24	*E*-ICU	CAZ,CTX,CPM	bla_{CTX}	–	B1	ND	ND
E55	40	G-ICU	CAZ,CTX,CPM,GM,CIP,LVX	bla_{CTX}	–	C	ND	ND
E56	68	E-ICU	CAZ,CTX,CPM,CIP,LVX,ETP,IMP	bla_{CTX},bla_{TEM}	–	C	ND	ND
E57	34	OP	CTX,CPM	bla_{CTX}	–	UT	ND	ND
E58	40	OP	CTX,CPM	bla_{CTX},bla_{TEM}	–	UT	ND	ND
E59	50	OP	CAZ,CTX,CPM	bla_{CTX}	–	UT	ND	ND
E60	23	OP	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	bla_{NDM-1}	UT	ND	ND
E61	26	OP	CAZ,CTX,CPM,CIP,LVX	bla_{CTX},bla_{TEM}	–	UT	ND	ND
E62	32	G-ICU	CTX,CPM,CIP	bla_{TEM}	–	UT	ND	ND
E63	36	G-ICU	CAZ,CTX,CPM,GM,CIP,LVX	bla_{CTX},bla_{TEM}	–	UT	ND	ND
E64	82	G-ICU	CTX,CPM,CIP,LVX	bla_{CTX},bla_{TEM}	–	UT	ND	ND
E65	61	G-ICU	CAZ,CTX,CPM	bla_{CTX},bla_{SHV}	bla_{NDM-1}, bla_{IMP}	UT	ND	ND
E66	88	G-ICU	CAZ,CTX,CPM,GM	bla_{CTX},bla_{TEM}	–	UT	ND	ND
E67	75	G-ICU	CAZ,CTX,CPM,CIP,LVX	bla_{CTX},bla_{TEM}	–	UT	ND	ND
E68	55	G-ICU	CAZ,CTX,CPM	bla_{TEM}	–	UT	ND	ND
E69	65	G-ICU	CAZ,CTX,CPM,AK,GM,CIP,LVX,ETP,IMP	bla_{CTX},bla_{SHV}	bla_{NDM-1},bla_{OXA48}	UT	ND	ND
E70	39	E-ICU	CAZ,CTX,CPM,GM,CIP,LVX	bla_{CTX},bla_{TEM}	–	UT	ND	ND
E71	64	E-ICU	CAZ,CTX,CPM,CIP,LVX	bla_{CTX},bla_{TEM}	bla_{OXA48}	UT	ND	ND
E72	41	E-ICU	CAZ,CTX,CPM	bla_{CTX},bla_{TEM}	–	UT	ND	ND

ICU, intensive care unit; OP, Outpatient; G-ICU, general intensive care unit; E-ICU, emergency intensive care unit; i; CAZ, ceftazidime; CTX, cefotaxime; CPM, cefepime; AK, amikacin; GM, gentamicin; CIP, ciprofloxacin; LVX, levofloxacine; ETP, ertapenem; IMP, imipenem; ND, Not Determined; UT, Untypable.

3.2. Antimicrobial susceptibility pattern

Disk diffusion method revealed the resistance rates of ESBL-EC isolates against ceftazidime and cefepime were 90.2% (65/72) and 93.0% (67/72), respectively. The rates of resistance to other antimicrobial agents were as follows: ciprofloxacin 44.4% (32/72); levofloxacin 36.1% (26/72); gentamicin, 15.2% (11/72); amikacin, 1.3% (1/72); ertapenem, 4.1% (3/72), imipenem, 2.7% (2/72). 15.2% (11/72) of ESBL-EC isolates were MDR. Generally, resistance to carbapenem was restricted to strains isolated from ICU patients (Table 1).

3.3. Detection of ESBL and carbapenemase genes in ESBL-EC

All ESBL-EC isolates harbored at least one ESBL gene as shown in Table 1. Overall, the rates of the $bla_{\text{CTX-M-15}}$, bla_{TEM}, bla_{SHV} were 90.2% (65/72), 50.0% (36/72) and 5.5% (4/72), respectively. The $bla_{\text{NDM-1}}$, $bla_{\text{OXA-48}}$ and bla_{IMP} genes were detected in 4.1% (3/72), 4.1% (3/72) and 1.3% (1/72) of isolates. None of the isolates were positive for the bla_{KPC}, bla_{VIM}, bla_{VEB} and bla_{PER} genes.

3.4. Detection of phylo-groups in ESBL-EC isolates

According to the Clermont et al. a quadruplex genotype corresponding to the presence/absence of the four genes generated specific profile, e.g. (+, −, +, −) demonstrating arpA (+), chuA(−), yjaA (+), TspE4.C2(−). Based on this method the percentage of phylo-group A, B1, B2, C, D and F were 20.8% (15/72), 6.9% (5/72), 20.8% (15/72), 2.7% (2/72), 13.8 (10/72) and 12.5% (9/72), respectively. None of the isolates were recognized as phylo-group E. Of all isolates, 22.2% (16/72) were classified as untypable. Regarding to our findings, the rate of putative virulent ESBL-EC isolates was 47.2% (34/72) while, the commensal phylo-groups were detected in 30.5% (22/72) of isolates.

3.5. Conjugation assay and replicon typing

The plasmids carrying the $bla_{\text{CTX-M-15}}$ were successfully transferred to *E. coli* K12 in five of six tested isolates using conjugation assay. The presence of the $bla_{\text{CTX-M-15}}$ gene in the transconjugants was confirmed by PCR amplification. The *E. coli* K12 transconjugant of CTX-M-15 displayed resistance against cefotaxime, but remained susceptible to other tested antimicrobial agents, e.g. carbapenem and fluoroquinolones. According to the aim of the present study, 34 putative virulent ESBL-EC isolates were tested for plasmid replicon typing. Regarding Plasmid analysis, the IncF incompatibility groups were the predominant types. Among them, eight isolates belonged to the IncFIV, seven isolates to the IncFI and four isolates to the IncFII type.

3.6. MLST analysis

The MLST analysis of 34 putative virulent ESBL-EC isolates showed high heterogeneity (Fig. 1). The predominant STs were ST769 and ST472 which both were seen in four isolates followed by ST8; three isolates, ST195; two isolates and ST771; two isolates. Some of the isolates shared same ST and same phylo-group. For example as is shown in Table 1, both of strains E2 and E3 with phylo-group F, were ST769.

4. Discussion

Clinical infections caused by ESBL-EC increase worldwide (Pulcini et al., 2018). Several studies determined about phylo-groups among *E.coli* mostly isolated from clinical isolates and fecal carriages (Jørgensen et al., 2017; Johnson et al., 2014).

Intestinal colonization by putative virulent phylo-groups of ESBL-EC such as B2, D and F in fecal carriage is important. The majority of carriers are asymptomatic, however, certain condition such as intestine barrier break, could leads to various infections. In our study sampling was performed among non-infected outpatients and ICU patients without any gastrointestinal symptoms.

From a total of 120 individuals, 60% (72/120) were ESBL-EC carriers. Considering our results, putative virulent phylo-groups including B2, D, and F were 47.2% (34/72). These phylo-groups were more frequent than commensal phylo-groups with 30.5% (22/72) rate. Furthermore, several studies revealed that prolonged carriage is associated with *E. coli* phylo-group B2 (Jørgensen et al., 2017; Titelman et al., 2014), therefore considering current study result, fecal carriage harbor mostly putative virulent phylo-groups particularly B2 which may result in significant health problem both in community and health care settings.

Molecular typing methods such as MLST are accurate techniques for the epidemiological purpose. The MLST results revealed that our studied ESBL-EC isolates were highly heterogeneous in which two sequence types; ST769 and ST472 were predominant. Based on several studies, ST769 and ST472 have been reported from various clinical sources (Steinsland et al., 2010; Suwantarat et al., 2014; Agabou et al., 2014). These studies concluded that ST769 and ST472 were contributed to clinically related isolates such as Enterotoxigenic *Escherichia coli* (ETEC) as a common cause of diarrhea and fluoroquinolone-resistant *E.coli* causing urinary tract infection, respectively. However, detection of these STs in asymptomatic carriers, as found in the present study, is a considerable public health issue as these STs could increase the risk of self-infection.

Our results demonstrated that there were similar profiles in isolates according to MLST results, ESBL genes and types of plasmid within a single ST. For example, E6, E7 and E8 strains, as ST472, had similar resistance profile, ESBL genes and phylo-group types. Furthermore these strains were simultaneously isolated from General-ICU. This indicates that a single strain with similar resistance profile was circulating in General-ICU. Under this circumstance, infection control and prevention program should be more regarded; otherwise the dissemination of pathogenic strains to other wards would be expected. On the other hand, four isolates (E1, E2, E3 and E4) with ST769 shared similar resistance profile and plasmid type (Inc FII) While, they were isolated from different wards (outpatients, General-ICU and Emergency-ICU) and different age groups. The same status was also observed in strains E14 and E15 (ST195). These isolates were recovered from an 8-year-old outpatient child and a 34-year-old woman hospitalized at Emergency-ICU. Moreover, strains with same clonal complex (e.g. ST8 and ST535) were also isolated from different wards. Circulation of single strain or closely related strains among carriers with different ages from both community and hospital settings contributes to the fact that they could disseminate in every setting leading to critical problem for public health.

Analysis of our plasmid replicon typing and ESBL genes revealed that the most prevalent Inc. types was IncF incompatibility group and the most frequent detected ESBL genes were $bla_{\text{CTX-M-15}}$ and bla_{TEM}. Since IncF group are typically carry ESBL genes; $bla_{\text{CTX-M-15}}$ and bla_{TEM} (Villa et al., 2010; Carattoli, 2009), therefor they are mostly associated with the dissemination of antimicrobial resistance. Also several studies have been indicated that IncFII and IncFI are associated with the dissemination of CTX-M-15 (Pitart et al., 2015; Gonullu et al., 2008; Nematzadeh et al., 2015). In the present study, four out of five isolates (E1, E5, E15, E18 and E19) with conjugative plasmids were isolated from outpatients. Presence of ESBL-EC isolates with putative virulent phylo-groups, conjugative plasmids and high antimicrobial resistance in asymptomatic fecal carriage, particularly in outpatients would be critical public health problem as they could easily lead to dissemination in community.

The rate of co-resistance against fluoroquinolones and cephalosporins was nearly high 43% (31/72) and there was a relatedness between the rate of co-resistance and putative virulent phylo-groups (p-Values < .05). There was also statistical relatedness between co-

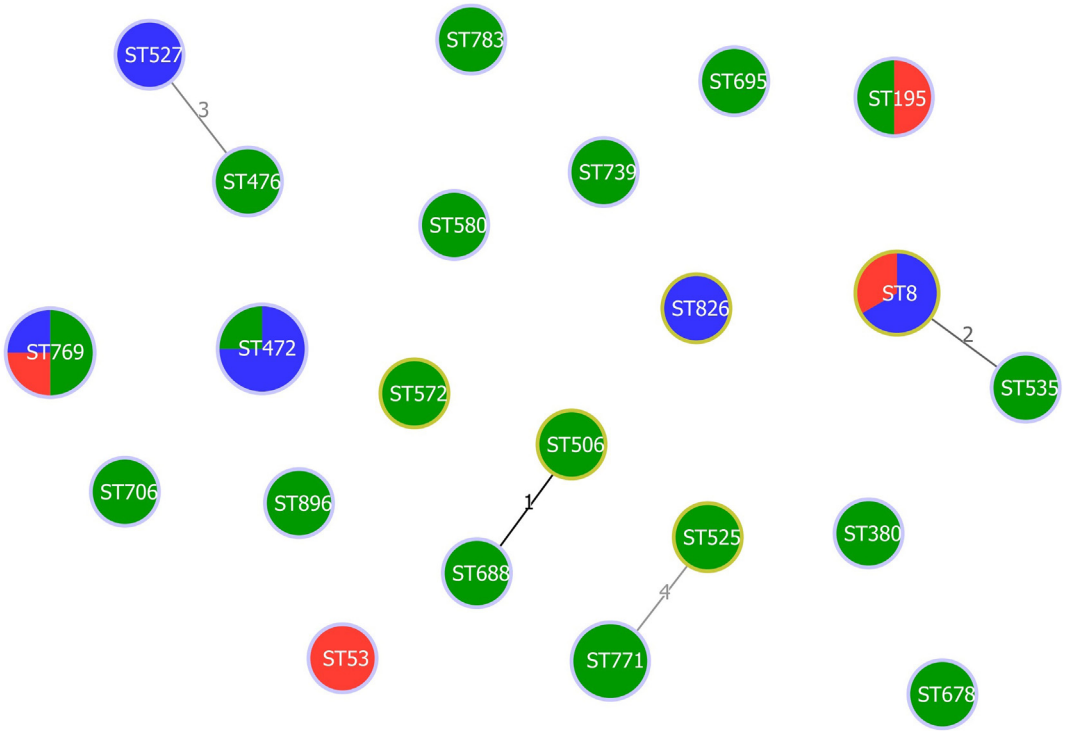

Fig. 1. Genetic relationship of ESBL-EC. Minimum-spanning tree (MST) illustrating STs of ESBL-EC in fecal carriage isolates. STs belonged to outpatient; general intensive care unit and emergency intensive care unit were marked in green, blue and red rectangles, respectively. The number above the line represents the number of allele differences between two sequence types. Pasteur MLST Excel files of isolates were analyzed by PHYLOViZ 2.0 software. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

resistance and ICU isolates compare to outpatients (p-Values < .05). It could be concluded that the presence of resistant putative virulent phylo-groups in intensive care units could be resulted in spreading of resistant strains in hospital and may also cause serious, life-threatening infections.

Besides the putative virulent phylo-groups, co-existence of ESBL and carabapenemase genes or co-resistance of different classes of antibiotics among commensal phylo-groups isolates is important. For instance, E56 with phylo-group C was resistant to cephalosporin, carbapenem and fluoroquinolones. This indicates that even commensal phylo-groups could get resistance genes and become high resistant strains. In such a situation, a carrier could develop a clinical infection without exposing to exogenous source of infection.

In conclusion, the current study provides novel information about the presence of ESBL-EC isolates particularly with putative virulent phylo-groups among fecal carriages in Iran. Our data revealed that there was almost high heterogeneity among ESBL-EC isolates. Our predominant STs from fecal carriage were the same as those isolated from clinically related samples in other studies, indicating increased risk of developing clinical infection among silent reservoir. Low rate of cabapenemase genes in the studied ESBL-EC isolates was one of the important points in our study. This could be resulted in enhancement of treatment options, as in the current study the carbapenem resistant rate was also at a minimum. In order to implementation of effective infection control program, detection of fecal carriage in appropriate time typically at the beginning of admission to the hospital is recommended.

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Ethical statement

This project was done based on ethical guidelines as previously approved by the Pasteur institute of Iran (project no: 180 IR.PII.REC.1397.56).

Conflict of interest

The authors have no conflicts of interest to declare.

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