Genetic Characterization of Plasmid-mediated *qepA* Gene Among ESBL-producing *Escherichia Coli* Isolates in Mexico

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Abstract

A molecular characterization of a plasmid-born *qepA* gene in (ESBL)-producing *E. coli* clinical isolates were performed. An 2.63% (11/418) were *qepA* positive isolates, of which a 90.0% carried CTX-M-15 (9/11) and SHV-12 (1/11). All isolates showed chromosomal mutations in the *gyrA* and *parC* genes. The clonal groups A, B and C were identified and belonged to, respectively, phylogroups A, B1 and D, as well as the sequence types 205, 405 and 617. Several plasmid profiles were determined with incompatibility groups FIA, FIB and FII. The genetic environment of the *qepA* in plasmid pEC8020 was different from those reported previously. The plasmid sequence included genes conferring resistance to β-lactams (*bla*CTX-M-15), macrolides (*mphA*), fluoroquinolones (*qepA1*), trimethoprim (*dfrB4*) and sulphonamides (*sul1*). Likewise, the IncF-pEC8020 plasmid carried several insertion sequences including IS*CR3*, IS*6100* and multiple copies of IS*26*. This work contributes to the epidemiology and genetics of plasmid-born *qepA* genes of ESBL-producing *E. coli.*

Introduction

Antimicrobial resistance is one of the main global public health problems. In low-income countries, including Latin America, the indiscriminate use of antimicrobials has increased the rates of antimicrobial resistance development. The introduction and subsequent use of broad-spectrum antibiotics, such as quinolone and cephalosporine, are widely and frequently used in healthcare systems [1].

Urinary tract infections (UTI) are the most frequent bacterial infections, that require prescription antibiotics. *Escherichia coli* is the most common agent of UTI and it is found around 70–90% of the cases [2, 3]. The first treatment in patients with UTI is frequently empirical and one of the most widely used antimicrobials are quinolones such as nalidixic acid, ciprofloxacin and fluoroquinolones [4, 5]. They have an efficient and rapid absorption after oral administration and their main route of excretion is at renal level. The increase of multidrug resistant bacteria in community and hospital settings are an important issue for the health care systems [6].

The emergence and increase of quinolone resistant strains and their subsequent spread has been reported in several countries [1]. The main mechanism to quinolones resistance in *E. coli* is the chromosomal mutations in the type II topoisomerase genes encoding GyrA and GyrB proteins. Resistance can also be due to mutations in the genes for the topoisomerase IV ParC and ParE subunit proteins, though less frequently. However, the description of plasmid-mediated quinolone resistance (PMQR) genes that confer reduced susceptibility to quinolones was described [7]. The three main mechanisms of resistance are the *qnr* protein product that protects the binding site in type II DNA topoisomerases, the enzymatic modification of the drug by the *aac(6`)-Ib-cr* product, and the efflux pumps encoded by *qepA* and *oqxAB* genes [8].

These PMQR genes have been found in bacterial isolates worldwide and they reduce bacterial susceptibility to fluoroquinolone, although usually not to the level of clinical non-susceptibility. They do, however, facilitate the bacterial survival and the subsequent generation mutants with a higher level or fluoroquinolone resistance and probability of treatment failure [9]. Several reports describe the in the community and hospital spread of extended-spectrum β-lactamase (ESBL)-producing *E. coli* isolates resistant to both cephalosporins and fluoroquinolones [10]. Clinical isolates with multi-drug and fluoroquinolone resistance mechanisms generate therapeutic failure to both cephalosporin and quinolones and /or fluoroquinolones antibiotics. QepA is an efflux pump that decreases susceptibility to hydrophilic fluoroquinolones, especially ciprofloxacin and norfloxacin [11]. The *qepA* gene has been described on large conjugative IncF group plasmids with the encoding aminoglycoside ribosomal methylase *rmtB* and TEM-1 β-lactamase genes flanked by IS*26* and IS*CR3C* insertion sequences as part of compound transposons [12, 13]. In this work, we determined the prevalence of ESBL-producing *Escherichia coli* isolates having the plasmid-mediated *qepA* gene and used molecular and whole-genome sequencing tools was determined the genetic environment of the *qepA* gene.

Materials And Methods

Clinical isolates included in the study

A total of 418 clinical isolates of ESBL-producing *E. coli* were collected from patients during the period 2005–2018 from five hospitals of Mexico: ISTE-S, ISSSTESON, Hermosillo, Sonora; International Reference Laboratory (CAPERMOR), Ciudad de Mexico; Instituto Nacional de Cancerologia (INCan), Ciudad de México; Sanatorio Durango (SD-DF), Ciudad de Mexico and Hospital de Pediatria, Centro Medico Nacional Siglo XXI, Ciudad de México.

The bacterial species identification and susceptibility pattern were detected by the Dade MicroScan and VITEK 2 compact system (BioMérieux, Durham, USA) [14, 15, 16]. The of ESBL production phenotype was determined using the double-disc synergism method according to guidelines of the Clinical and Laboratory Standards Institute (CLSI) (M100-S21) [17]. The following experiments were carried out only to ESBL-producing *E. coli* isolates positive for the *qepA* gene.

PCR Amplification and DNA sequencing

The *qepA*, *aac(6´)-Ib-cr* and *chrA* genes were screened by single PCR with specific primers for each gene. In the *qepA* positive isolates the mutations in the *gyrA a*nd *parC* chromosomal genes were determined by PCR using specific primers, and confirmed by nucleotide sequencing [14]. The class 1 integrons in the 5´ region were determined with the oligonucleotide *IntI1* (CGTTCCATACAGAAGCTGG) vs *qepA*-R (CTGCAGGTACTGCGTCATG). The relationship of the *qepA* with the insertion sequence ISCR3C was identified with the oligonucleotide *qepA-F* (CGTGTTGCTGGAGTTCTTC) and ISCR3C-F (CCACTGCGGTGGCACCGT). In addition, the SHV-, CTX-M-type and TLA-1 β-lactamases genes were screened by PCR, as were the PMQR *qnrA*, *qnrB*, *qnrS* genes using specific oligonucleotides described previously [14, 18]. The PCR products specified by nucleotide sequencing were purified with the commercial kit from Roche (Roche, USA) and sequenced by the BigDye Terminator v3.1 Cycle Sequencing Kit in the automated system (ABIPrisma 3100, Applied Biosystem, USA). The Translate Tool (http://ca.expasy.org/tools/dna.html) was used for each nucleotides sequence to obtain the amino acid sequences and were compared by BLASTp in the GenBank database (http://www.ncbi.nlm.nih.gov/).

Susceptibility determination

The minimal inhibitory concentration (MIC) of cefotaxime, ceftazidime, nalidixic acid, ciprofloxacin, levofloxacin and gentamicin were determined by micro-dilution in broth according to CLSI [17].

Phylogenetic grouping determination

Phylogenetic grouping of the *E. coli* isolates was performed using the triplex PCR for *chuA* and *yjaA* genes and DNA fragment TSPE4.C2, which allow classification of four different groups, as previously described by Clermont *et al* [19].

Genetic characterization

Random amplified polymorphic DNA (RAPD) analysis was performed to identify the genetic diversity of *qepA* positive *E. coli* isolates. The RAPD was performed using decameric primers P1254 and PCR conditions described by Betancor *et al* [20]. The patterns were considered to be different according to the criteria established by Tenover *et al*. [21] The MultiLocus Sequencing Typing (MLST) was performed in all *qepA* positive isolates using the MLST tools (https://enterobase.warwick.ac.uk) [22].

Plasmid analysis and mating experiments

Plasmid profiles were obtained according to the method described by Kieser [23]. Mating assays for the horizontal transfer of quinolone resistance were performed using the *E. coli* J53-2 (met^-, pro^-, Rif^r) as the recipient strain, in solid-phase mating as described by Miller [24]. Transconjugants were selected on Luria-Bertani (LB) agar supplemented with rifampin (100 µg/ml) and nalidixic acid (8 mg/L), ampicillin (100 mg/L) or cefotaxime (1 mg/L). All transconjugants were verified by their auxotrophic requirements (Pro and Met) and plasmids were analyzed according to the method described by Kieser [23].

Plasmid Typing

Incompatibility groups of transconjugants were detected by PCR replicon typing. Specific primers were used including: HI1, HI2, I1, X, L/M, N, FIA, FIB, W, P, FIC, Y, FIIA, A/C, T, K, B/O, and F previously described by Carattoli *et al*. [25].

Plasmid sequencing and accession number.

Plasmid DNA was obtained from transconjugants pEC8020 and sequenced by pyrosequencing on Platform 454 (Roche). The assembly was obtained using the PHRED-PHRAP-CONSED and Newbler program. The prediction of open reading frames (ORFs) was done with the Glimmer3 and RAST programs and compared with the GenBank nr database. *In silico* plasmid analysis was performed using the Center for Genomic Epidemiology tools (https://cge.cbs.dtu.dk/) to identify antimicrobial resistance genes (ResFinder), plasmid replicons (PlasmidFinder) [26].

Results

In total, eleven (2.63%) ESBL-producing *E. coli* isolates were positive for the *qepA* gene by PCR amplification (Table 1). In addition, the isolates were positive for the chromate resistance *chrA* gene. The ESBL CTX-M-15 gene was identified in 81.8% (9/11) isolates and the SHV-12 gene was identified in 9.0% (1/11) (Table 1). The TLA-1 β-lactamase, the PMQR *qnrA*, *qnrB*, *qnrS* and *aac(6´)-Ib-cr* gene were not identified.

Table 1
Molecular characteristics and antimicrobial resistance of *qepA* gene positive ESBL-producing *E. coli* clinical isolates

Isolate	Year of Isolation	Mexican Region	RAPD	Phylogroup	MLST	Plasmid Profile (kb)[a]	Inc Group[b]	GyrA		ParC	ESBL gene	MIC (μg/ml)			
								Ser83	Asp87	Ser80		CTX	CAZ	NAL	CIP
7530	2006	Estado de Mexico	A	B1	205	130	FIA, FIB, FII	Leu	Asn	Ile	CTXM-15	> 256	16	> 256	> 64
7505	2007	Mexico City	A	B1	205	130	ND	Leu	Asn	Ile	CTXM-15	> 256	32	> 256	> 64
7514	2007	Mexico City	A	B1	205	130	ND	Leu	Asn	Ile	CTXM-15	> 256	64	> 256	> 64
03212	2008	Sonora	A	B1	205	130	ND	Leu	Asn	Ile	CTXM-15	> 256	16	> 256	> 64
03210	2008	Sonora	B	D	405	130	FIA, FIB, FII	Leu	Asn	Ile	SHV-12	> 256	2	> 256	> 64
8020	2009	Mexico City	B	D	405	130	FIA, FIB, FII	Leu	Asn	Ile	CTXM-15	> 256	64	> 256	> 64
8019	2009	Mexico City	B	D	405	100, 130	FIA, FIB, FII	Leu	Asn	Ile	CTXM-15	> 256	64	> 256	> 64
7537	2006	Estado de Mexico	C	A	617	130	FIA, FIB, FII	Leu	Asn	Ile	-	> 256	1	> 256	> 64
7544	2007	Estado de Mexico	C	A	617	130	ND	Leu	Asn	Ile	CTXM-15	> 256	64	> 256	> 64
09220	2008	Mexico City	C	A	617	100, 130	FIA, FIB, FII	Leu	Asn	Ile	CTXM-15	> 256	128	> 256	> 64
10246	2008	Mexico City	C	A	617	60, 100, 130	ND	Leu	Asn	Ile	CTXM-15	> 256	64	> 256	> 64

[a]Incompatibility group of the transconjugants.

[b]The underline plasmid corresponded to plasmid identified in the transconjugant.

Abbreviations: RAPD, Random Amplified Polymorphic DNA; MLST, Multilocus sequence typing; ESBL, extended-spectrum b-lactamase; MIC, minimum inhibitc concentration; ND, not determinate, -, negative; Ser, Serine;

Leu, Leucine; Asp, Asparagine; Asn, aspartic acid; Ile, isoleucine; CTX, cefotaxime; CAZ, ceftazidime; NAL, nalidixic acid; CIP, ciprofloxacin; LEV, levofloxacin; GI gentamicin.

In the GyrA and ParC proteins were identified the following mutations: GyrA, Ser83→Leu and Asp87→Asn; ParC, Ser80→Ile (Table 1). The isolates showed a multidrug resistance phenotype and were resistant to cefotaxime (> 256 μg/ml), nalidixic acid (> 256 μg/ml), ciprofloxacin (> 64 μg/ml) and levofloxacin (8 μg/ml). Of the isolates, 9/11 were resistant to ceftazidime (16 μg/ml) and all were sensitive to gentamicin (Table 1). The susceptibility results for nalidixic acid, ciprofloxacin and levofloxacin were consistent with the identification of substitutions in GyrA and ParC proteins (27).

In order to identify the genetic diversity, phylogenetic group and relationship among *E. coli* ESBL isolates, random amplified polymorphic DNA (RAPD), phylogroup analysis and the MultiLocus Sequencing Typing (MLST) were used, respectively (18,19,21). The isolates 7530, 7505, 7514 and 03212 were grouped in the clone A; followed by the 03210, 8020 and 8019 in the groups B and 7537, 7544, 09220 and 10246 in the group C. The phylogroups B1, D and A were, respectively, identified in the groups A, B and C. Likewise, the sequence types 205, 405 and 617, corresponding to A, B and C groups determined by RAPD (Table 1).

The plasmid DNA was extracted from all the isolates to determine the plasmid profiles. We observed that the isolates contained plasmids of 60,100 and 130-kb, 100 and 130-kb and 130-kb (Table 1). We selected five isolates (7530, 03210, 8019, 8020 and 09220) with ESBLs CTX-M-15 and SHV-12 for the mating experiments. The mating experiments were successful in that in all transconjugants the *qepA1* gene and *chrA* gene determinants were co-transferred along with the ESBL CTX-M-15 in the four mating experiments. The mating experiments with the SHV-12 gene were negative in terms of the ESBL gene transfer. The PCR-based replicon typing showed the presence of FIA, FIB and FII replicons among all *qepA*-positive transconjugants in a plasmid of 130-Kb obtained in this study (Table 1).

The complete nucleotide sequence of plasmid pEC8020 obtained from ESBL-producing *E. coli* 8020 isolate was 127,611-bp. *In silico* analysis by ResFinder showed the antimicrobial resistance genes *qepA1*, *mphA*, *dfrB4*, *sul1* and CTX-M-15, which confer antimicrobial resistance to, fluoroquinolones, macrolide, trimethoprim, sulphonamides and cephalosporin, respectively. The PlasmidFinder analysis identified the replicons IncFIA, IncFIB (pB171) and IncFII with 99.38%, 99.74% and 96.18% of identity, respectively.

The genetic context of *qepA* gene was determined and corresponded to a complete transfer region with an IS*26*, followed by a truncated class 1 integrase and truncated *dfr2* were located upstream *qepA* gene (Fig. 1). Downstream from the *qepA* gene we identified an IS*CR3*, a truncated *intI1-groEL*, the *drfB4* gene, the *qacED1-sul1* genes, the chromate ion transporter *chrA*, the transposase IS*6100*, three macrolide resistant genes (*mphR*, the erythromycin resistance repressor; *mrx*, a transmembrane transport protein of MFS family; and *mphA*, the macrolide-2'-phosphotransferase) and the IS*26*. Following this genetic structure, we identified a Tn3 family transposase, the *bla*CTXM-15 and two IS*1* family transposases (Fig. 1b). Particularity, the class 1 integron in the 5´ region and the *qepA* with the insertion sequence IS*CR3C* was identified in all *qepA* gene positive *E. coli* isolates.

Discussion

The prevalence of *qepA* reported in recent literature fluctuates between 8.3% and 10%, and is still low in any case [29, 30, 31]. In Egypt, *qepA* gene was identified in 10% of a collection of 39 MDR isolates. In the children´s hospital in Doha, *qepA* gene was identified in the 10% of 19 *E. coli* isolates from neonatal intensive care unit. Similar percentage of 8.3% *qepA* gene-positive was reported in a collection of 144 ESBL-producing *E. coli* from Tabriz University in Iran. In addition, the susceptibility results to fluoroquinolone antibiotics were consistent with the identification of substitutions in the GyrA and ParC proteins found in all isolates [27]. As suggested by Jacoby *et al* [32], the horizontal transfer of PMQR determinants accelerates the selection of higher levels of quinolone resistance, which facilitates bacterial survival and subsequent generation of mutants in GyrA and ParC with higher-level to quinolone resistance that produce therapeutic failure [9].

The eleven isolates formed three specific groups; the first corresponds to clone A by RAPD, and belongs to phylogroup B1 and ST205. The members of this clone were isolated in three different years from at least two regions geographically separated from each other (northwest and center of Mexico). A similar characteristic was observed in the second clone, B, which belongs to phylogroup D and ST405. The third clone C belongs to phylogroup A and ST617. The genetic relationship between the members of each clone suggesting a low frequency of identification; however, the spread of resistance of ESBL-producing *E. coli* isolates to fluoroquinolones in Mexican hospitals has remained constant [14, 16].

Plasmid sequence analyses of pEC8020 showed an environment for the *qepA* gene flanked by IS*26* sequences not yet described. The 5´ region corresponding to the arrangement observed in plasmid pHPA (Fig. 1a) [28], with an IS*26*, *Int1*, truncated *dfr2* located upstream of *qepA* and flanked downstream by the IS*CR3*, and truncated *intI1-groEL*. Instead of truncated *rmtB*, *bla*TEM-1, and *tnpR* genes flanking by IS*26* downstream of the IS*CR3* and *intI1-groEL* described in plasmid pHPA, we identified the *drfB4*, *qacED1-sul1*, *chrA*, *IS6100*, *mphR*, *mrx*, *mphA* and insertion sequences IS*26*. The arrangement of these genes was previously described as part of the transposon structure Tn*6242* flanked by two IS*26* sequences [28].

Remarkable, the Tn*6242* was identified after whole genome sequencing as being inserted in the chromosome of one *E. coli* ST405 belong to phylogroup D obtained from a urine sample [32]. Curiously, the plasmid pEC8020 was obtained from an ESBL-producing *E. coli* ST405 belongs to phylogroup D isolated from blood culture. We hypothesize a possible recombination between an *E. coli* ST405 with a chromosomal or plasmidic Tn*6242*, and IncFII type plasmid harbors the conserved *qepA* gene flanked by the IS*26*. The mechanism of how this novel structure recruit fragments of plasmid like pHPA and the recombination with Tn*6242* will needs further study in the future.

The novel genetic context previously described flanked by the IS*26* were identified in the eleven isolates by PCR and sequencing analysis. However, the Tn*3*, CTXM-15 and IS*1* were absent in the isolates with the SHV gene.

The recruitment of multiple resistance mechanisms bordered by diverse insertion sequences was evident in this structure (IS*26*, *Int1*, *dfr2*, *qepA*, IS*CR3*, *intI1-groEL*, *drfB4*, *qacED1-sul1*, *chrA*, *IS6100*, *mphR*, *mrx*, *mphA*, IS*26*, Tn*3*, CTXM-15 and IS*1*).

In the case of *chrA*, the heterologous expression in a plasmid of *chrA* alone from *Shewanella* sp, on *E. coli* and *Pseudomonas aeruginosa* conferred increased chromate resistance [34]. Caballero *et al* (2012), suggested that the use of metal derivatives as antiseptics in hospital as an important factor for the selection of bacteria that acquire genes to confer and spread metal resistance among bacteria in hospitals [35].

The previous identification of *qepA* and *rmtB* genes, flanked by the transposable element IS*26* in a transferable plasmid belong to incompatibility group IncFII, such as pHPA, suggested the efficient dissemination of these genetic structures [11, 33]. The description of a novel genetic structure in this work of *qepA* gene in plasmid pEC8020, which belongs to IncFII type, will be an efficient medium for the dissemination of resistance genes and the constant spread among *Enterobacteriaceae*. The emergence of multidrug-resistant gram-negative bacteria that harbor plasmids bearing *qepA*, *crhA* and CTX-M-15 genes could become a serious clinical concern for all public health care systems.

Declarations

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Transparency declarations

The authors declare no competing interests.

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Figures

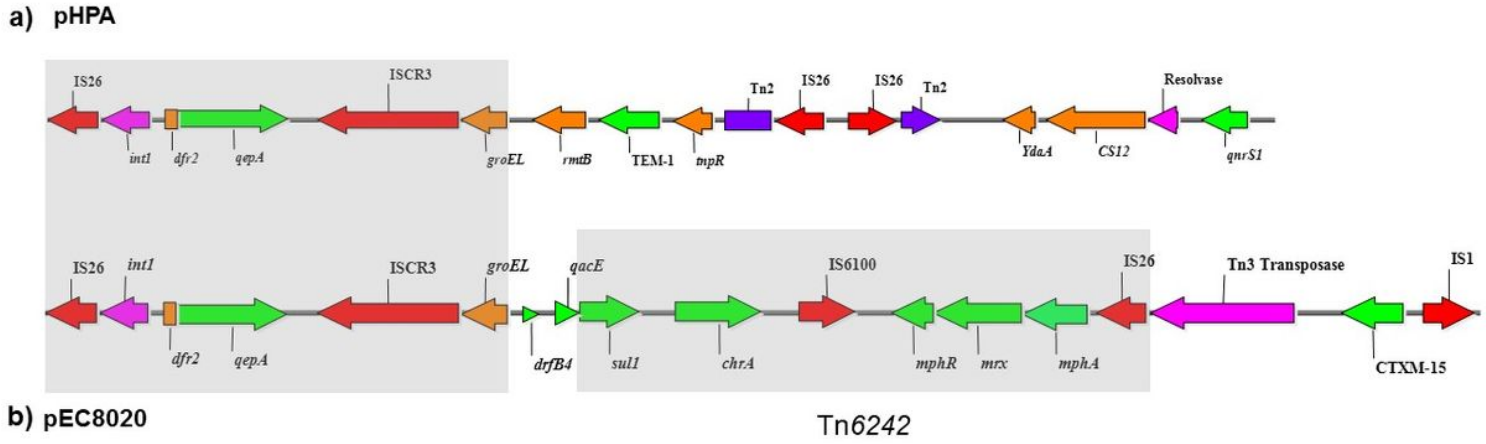

Figure 1

Figure 1

Comparison of the genetic context of the qepA gene in plasmid pHPA and plasmid pT8020 from ESBL-producing E. coli. Open reading frames of resistance genes and genetic mobile elements are indicated in green and red, respectively. Shaded areas show the genetic context shared between the two plasmids structures.