Genetic Characterization of Plasmid-mediated \textit{qepA} Gene Among ESBL-producing \textit{Escherichia Coli} Isolates in Mexico

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Research Article

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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 (blaCTX-M-15), macrolides (mphA), fluoroquinolones (qepA1), trimethoprim (dfbB4) and sulphonamides (sul1). Likewise, the IncF-pEC8020 plasmid carried several insertion sequences including ISCR3B, IS6100 and multiple copies of IS26. 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 cephalosporin, 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 healthcare 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 quinolone 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 qoxAB 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 of quinolone 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 IS26 and ISCR3C 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 Cancerología (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 and 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 (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 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 (ABIPrima 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 microdilution 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) 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.
trimethoprim, sulphonamides and cephalosporin, respectively. The PlasmidFinder analysis identified the replicons IncFIA, IncFIB (pB171) and IncFII with the antimicrobial resistance genes CTX-M-15, SHV-12, TEM-1, TEM-5 and CTX-M-15, respectively.

The complete nucleotide sequence of plasmid pEC8020 obtained from ESBL-producing *E. coli* 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 IS26, 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 ISCR3, a truncated intI1-groEL, the dfrB4 gene, the qacED1-sul1 genes, the chromate ion transporter chrA, the transposase IS6100, 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 IS26. Following this genetic structure, we identified a Tn3 family transposase, the blaCTXM-15 and two IS1 family transposases (Fig. 1b). Particularly, the class 1 integron in the 5’ region and the qepA with the insertion sequence ISCR3C 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 IS26 sequences not yet described. The 5’ region corresponding to the arrangement observed in plasmid pHPA (Fig. 1a) [28], with an IS26, intI1, truncated dfr2 located upstream of qepA and flanked downstream by the ISCR3 and truncated intI1-groEL. Instead of truncated mmtB, blatEM-1, and mphR genes flanking by IS26 downstream of the ISCR3 and intI1-groEL described in plasmid pHPA, we identified the dfrB4, qacED1-sul1, chrA, IS6100, mphR, mrx, mphA and insertion sequences IS26. The arrangement of these genes was previously described as part of the transposon structure Tn6242 flanked by two IS26 sequences [28].

Remarkable, the Tn6242 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 Tn6242, and IncFII type plasmid harbors the conserved qepA gene flanked by the IS26. The mechanism of how this novel structure recruit fragments of plasmid like pHPA and the recombination with Tn6242 will needs further study in the future.

The novel genetic context previously described flanked by the IS26 were identified in the eleven isolates by PCR and sequencing analysis. However, the Tn3, CTXM-15 and IS7 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 (IS26, intI1, dfr2, qepA, ISCR3, intI1-groEL, dfrB4, qacED1-sul1, chrA, IS6100, mphR, mrx, mphA, IS26, Tn3, CTXM-15 and IS7).

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 mmtB genes, flanked by the transposable element IS26 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, chrA 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.

References


**Figures**

![Figure 1](image.png)

**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.