Brief Communication

MDR Escherichia coli carrying CTX-M-24 (IncF[F-:A1:B32]) and KPC-2 (IncX3/IncU) plasmids isolated from community-acquired urinary tract infection in Brazil

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ABSTRACT

Acquired antibiotic resistance in bacteria has become an important worldwide challenge. Currently, several bacteria, including Escherichia coli, have multidrug resistance profiles. Genes such as bla CTX-M-24 and bla KPC-2 (carbapenemase) are widespread. This research letter reports about a genomic surveillance study where multidrug-resistant E. coli containing CTX-M-24 (IncF[F-:A1:B32]) and KPC-2 (IncX3/IncU) plasmids were obtained from community-acquired urinary tract infection in Brazil.

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Acquired antibiotic resistance in bacteria has become an important worldwide challenge. Currently, several bacteria, including Escherichia coli, have multidrug resistance profiles. Genes such as bla CTX-M-24 and bla TEM-1B that encode extended-spectrum beta-lactamase (ESBL) are widespread in E. coli strains. These genes can suppress the action of cephalosporin antibiotics. Besides, the presence of bla KPC-2 (carbapenemase) gene in the bacterial genome may confer a carbapenem resistance profile. During a genomic surveillance study, a multidrug-resistant Escherichia coli containing CTX-M-24 (IncF[F-:A1:B32]) and KPC-2 (IncX3/IncU) plasmids was obtained from community-acquired urinary tract infection in Brazil. This microorganism was isolated in 2016 from a urine sample from a community 79-year-old female patient, without previous hospitalization in the last year, living in the south region (Londrina, state of Paraná, Brazil) and it was associated with ST354 strain after sequencing type analysis.

For genome sequencing, total DNA was extracted using a PureLink™ Quick Gel Extraction Kit (Life Technologies, CA). Libraries were prepared with a NexteraXT library prep kit (Illumina Inc., San Diego, CA). The samples were sequenced via Illumina NextSeq 550 platform (Illumina Inc., San Diego, CA), using 2 x 150-bp paired-end reads. Reads were de novo assembled using Unicycler v0.4.0 software (7). Read with a PHRED quality score below 20 were discarded, and adapters
were trimmed using TrimGalore v0.6.5 (https://github.com/FelixKrueger/TrimGalore). The E. coli ST354 genome was annotated using the Prokaryotic Genome Annotation Pipeline v.3.2 (PGAP/NCBI). Multilocus sequence type (MLST), antimicrobial resistance (AMR) genes, virulence factors and plasmid replications were predicted using the MLST v2.0, pMLST v2.0, ResFinder v4.1, FimTypev1.0, VirulenceFinder v2.0 and PlasmidFinder v2.1 (https://cge.cbs.dtu.dk/services/).

The E. coli ST354 strain exhibited as MDPR profile by the antimicrobial susceptibility testing (Table 1) according to the Clinical and Laboratory Standards Institute guidelines (CLSI, 2020) and by minimum inhibitory concentration (MIC) for colistin using automated Vitek 2 system.

E. coli ST354 is associated with zoonosis and human infections (10). Generally, this strain causes extra-intestinal infections in humans and other animals. Besides, ST354 strain has a resistant profile to fluoroquinolone. The virulome of this isolate from Brazil showed a vast repertoire of virulence consisting of e1A (Salmonella HIA homologue), ipfA (long polar fimbriae), air (enteroaggregative immunoglobulin repeat protein), iss (increased serum survival) and gad (glutamate decarboxylase alphasarcinas). The presence of these virulence factors provides information regarding the high pathogenicity profile of E. coli ST354 lineage isolated from community-acquired urinary tract infection.

Concerning the resistome of E. coli ST354, it contains resistance factors to beta-lactam antibiotics. These factors are TEM-1B, CTX-M-24 and KPC-2. The blaCTX-M-24 gene is present in the IncF [IncF [F::A1:B32]] plasmid while blaKPC-2 was identified within the IncX3/IncU replicons. Additionally, this strain carries the tet(B) gene, which confers resistance to tetracycline. Finally, many point mutations in the parC (Ser80Ile, Glu84Gly, Ser57Thr), parE (Ile355Thr, Leu416Phe) and gyrA (Ser83Leu, Asp87Asn) genes have been identified and are associated with resistance to fluoroquinolones (FQ) drugs. GyrA and ParC proteins are FQ targets. Point alterations in their genes can lead to FQ resistance. The most frequent substitutions are Ser83Leu and Asp87Asn for gyrA and Ser80Ile for parC gene. The presence of resistance factors in replications and chromosomal DNA reinforces the strong, resistant profile of E. coli ST354 strain making it become a potent MDR microorganism.

Many incompatibility group (Inc) plasmids are involved with resistance to several drugs in E. coli lineages. These replications carry various combinations of resistance genes, and they can be transferred by conjugation.13 IncF and IncX are prevalent plasmids type in E. coli. IncX4, for example, is frequently identified as a carrier of resistance genes related to FQ and beta-lactam antibiotics resistance.14,15 Moreover, IncX4 has a high frequency of self-transfer (10⁻¹⁻¹⁰⁻⁶).13 In this study, the IncF [F::A1:B32] plasmid harbors the blaCTX-M-24 gene and IncX3/IncU carry the blaKPC-2 gene.

These findings suggest that the presence of blaCTX-M-24, blaTEM-1B and blaKPC-2 genes in E. coli ST354 could be related to the multidrug resistance profile obtained in the antimicrobial susceptibility test. This strain was mainly resistant to beta-lactam antibiotics such as cephalosporins. Moreover, point mutations in parC, parE and gyrA genes observed in E. coli ST354 could influence the antimicrobial resistance profile to FQ antibiotics observed in this study. In addition to this resistant behavior, this strain contains several virulent factors such as air, gad, e1A, ipfA and iss, which shows its high pathogenic genome content.

Our data could help understand the genetic basis of high pathogenicity of the MDR E. coli ST354 isolated from community-acquired urinary tract infection in Brazil. The presence of blaCTX-M-24, blaTEM-1B and blaKPC-2 genes, as well as incompatibility plasmids such as IncF [F::A1:B32] and point mutations in the parC, parE and gyrA chromosomal genes, may help increase the spectrum of antimicrobial resistance in this microorganism and contribute to its pathogenicity.

Table 1 - Antimicrobial susceptibility profile of E. coli ST354, containing CTX-M-24 and KPC-2 enzymes, obtained from community-acquired urinary tract infection in Brazil.

| Antimicrobials         | Susceptibility profile |
|------------------------|------------------------|
|                        | Sensitive (S) | Resistant (R) |
| Amoxicillin (AMP)      | R           | R           |
| Amoxicillin/clavulanate (AMC) | R | R |
| Trimethoprim-sulfamethoxazole (STX) | R | - |
| Piperacillin-tazobactam (TZP) | R | R |
| Ceftazidime (CAZ)      | R           | R           |
| Cefturoxime (CSS)      | R           | R           |
| Ceftiraxone (CRO)      | R           | R           |
| Cefepime (FEP)         | R           | R           |
| Meropenem (MEM)        | R           | R           |
| Ertapenem (ERF)        | R           | R           |
| Amikacin (AK)          | S           | -           |
| Gentamicin (CN)        | S           | -           |
| Ciprofloxacin (CIP)    | R           | R           |
| Norfloxacin (NOR)      | R           | R           |
| Nitrofurantoin (F)     | S           | R           |
| Trimethoprim-sulfamethoxazole (STX) | S | R |
| Nalidixic acid (NA)    | R           | R           |

Ethical approval

The study was approved by the Ethics and Research Committee of the State University of Londrina CAAE 56869816.0.000.5231.

Data availability

Draft whole-genome assembly was deposited in DDBJ/ENA/GenBank under the SRA accession number SRR10310377.

Conflicts of interest

The authors declare no conflicts of interest.

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REFERENCES

1. Impey RE, Hawkins DA, Sutton JM, da Costa TPS. Overcoming intrinsic and acquired resistance mechanisms associated with the cell wall of gram-negative bacteria. Antibiotics. 2020;9:623–42.

2. Rebbah N, Messai Y, Châtre P, Haenni M, Madec JY, Bakour R. Diversity of CTX-M extended-spectrum β-Lactamases in Escherichia coli isolates from retail raw ground beef: first report of CTX-M-24 and CTX-M-32 in Algeria. Microb Drug Resist. 2018;24:896–908.

3. Singh NS, Singhal N, Virdi JS. Genetic Environment of blaTEM-1, blaCTX-M-15, blaCMY-42 and characterization of integrons of Escherichia coli isolated from an Indian urban aquatic environment. Front Microbiol. 2018;9:382.

4. Galvis F, Moreno LR. Molecular characterization and detection of genes blaCTX-M groups 1 and 9 in Klebsiella pneumoniae resistant to ceftazidime, in a hospital in San José de Cucuta, Colombia. Rev Chil Infectol. 2019;36:304–11.

5. Yauri M, Rodríguez M, Alcocer I. Clonal dissemination of KPC-2 in carbapenem resistant Klebsiella pneumoniae. Infectio. 2020;2024:42–9.

6. Hazen TH, Mettus R, McElheny CL, Bowler SI, Nagaraj S, Doi Y, et al. Diversity among blaKPC-containing plasmids in Escherichia coli and other bacterial species isolated from the same patients. Sci Rep. 2018;8:10291.

7. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. PLoS Comput Biol. 2017;13:1005595.

8. Carattoli A, Zankari E, García-Fernández A, Voldby Larsen M, Lund O, Villa L, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother. 2014;58:3895–903.

9. Performance Standards for Antimicrobial Susceptibility Testing. 30th Ed. Clinical and Laboratory Standards Institute; 2020.

10. Vangchhia B, Abraham S, Bell JM, Cottignon P, Gibson JS, Ingram PR, et al. Phylogenetic diversity, antimicrobial susceptibility and virulence characteristics of phylogroup F Escherichia coli in Australia. Microbiology. 2016;162:1904–12.

11. Johnning A, Kristiansson E, Fick J, Weijdegard B, Larsson DJ. Resistance mutations in gyrA and parC are common in escherichia communities of both fluoroquinolone-polluted and uncontaminated aquatic environments. Front Microbiol. 2015;6:1355.

12. Azargun R, Barbaghi MHS, Kafli HS, Oskouee MA, Sadeghi V, Memar MY, et al. Frequency of DNA gyrase and topoisomerase IV mutations and plasmid-mediated quinolone resistance genes among Escherichia coli and Klebsiella pneumoniae isolated from urinary tract infections in Azerbaijan. Iran J Glob Antimicrob Resist. 2019;17:39–43.

13. Król JE, Wojtowicz AJ, Rogers LM, Heuer H, Smalla K, Krone SM, et al. Invasion of E. coli biofilms by antibiotic resistance plasmids. Plasmid. 2013;70:110–9.

14. Sun J, Fang L, Wu Z, Deng H, Yang RS, Li XP, et al. Genetic analysis of the IncX4 plasmids: implications for a unique pattern in the mcr-1 acquisition. Sci Rep. 2017;7:424.

15. Phan MD, Forde BM, Peters KM, Sarkar S, Hancock S, Stanton-Cook M, et al. Molecular characterization of a multidrug resistance IncF plasmid from the globally disseminated Escherichia coli ST131 Clone. PLoS One. 2015;10:e0122369.