ORIGINAL ARTICLE

Chromosomal mutations in commensal *Escherichia coli* genomes: drivers of antibiotic resistance among children in a community in Lima, Peru

Brenda Ayzanoa[1], Diego Cuicapuza[1,2], Pablo Tsukayama[1,2,3,4]

[1] Laboratorio de Genómica Microbiana, Departamento de Ciencias Celulares y Moleculares, Facultad de Ciencias y Filosofía, Universidad Peruana Cayetano Heredia, Lima, Peru
[2] Emerge (Emerging Diseases and Climate Change Research Unit), Facultad de Salud Pública y Administración, Universidad Peruana Cayetano Heredia, Lima, Peru
[3] Instituto de Medicina Tropical Alexander von Humboldt, Universidad Peruana Cayetano Heredia, Lima, Peru
[4] Parasites and Microbes Program, Wellcome Sanger Institute, Hinxton, United Kingdom

ABSTRACT

Escherichia coli is an enterobacterium that is part of the intestinal microbiome of mammals and is capable of causing various diseases, especially in vulnerable populations. Additionally, the emergence of antibiotic-resistant variants of *E. coli* poses a growing global threat to public health. This resistance is usually encoded by multiple genes, which code for the expression of enzymes, membrane proteins, porins, efflux pumps, or target molecule mutations. Recent research has reported specific resistance-associated mutations, such as *qnr*, *pmrB*, *glpT*, and the bla variant (C32T). The aim of this study was to identify the frequency of chromosomal mutations that confer antibiotic resistance in *E. coli* genomes from children in the district of Villa El Salvador in Lima, Peru. A total of 19 complete *E. coli* genomes were downloaded from Bioproject PRJNA633873 located at NCBI GenBank. After converting and assessing the quality of the reads with FastQC, assembly was performed using SPAdes v3.15.2 and contig evaluation through QUAST v5.0.2. Multilocus sequence type (MLST) genomic profiles were identified with PubMLST, and we searched for resistance genes using AMRFinderPlus. Finally, we analyzed gene patterns and gene absence/presence by MCA using Stata v17 and R studio. A total of 11 genomes had a total of seven mutations in genes associated with resistance to four antibiotic families, including *glpT*(E448) for fosfomycin, *pmrB* (Y358) for colistin, *gyrA*(S83L) and *parC*_S57T for quinolones, bla_{TEM} (C32T) for amoxicillin with clavulanic acid and piperacillin-tazobactam, and *cyaA*(S352T) for fosmidomycin. Proximal relationships were evaluated for the presence/absence of genes that included bla_{TEM} , *catA1*, *sul1*, *qnrB19*, *tetA*, and *mphA* genes. Our study is the first to describe gene mutations associated with antimicrobial resistance in *E. coli* genomes from a pediatric population in a community in Lima, Peru.

Keywords: Antibiotic Resistance; Chromosome Structures; *Escherichia coli*; Child (Source: MeSH)

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Corresponding author:
Pablo Tsukayama
E-mail: pablo.tsukayama@upch.pe

ORCID iDs
Brenda Ayzanoa
https://orcid.org/0000-0001-9059-9544
Diego Cuicapuza
https://orcid.org/0000-0002-5735-4614
Pablo Tsukayama
https://orcid.org/0000-0002-1669-2553

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Resistencia a antibióticos generada mediante mutaciones cromosómicas en aislados de *Escherichia coli* provenientes de coprocultivos de niños de una comunidad de Lima, Perú

RESUMEN

Escherichia coli es una enterobacteria que forma parte del microbioma intestinal de los mamíferos y es capaz de causar diversas enfermedades, especialmente en poblaciones vulnerables. Adicionalmente, la emergencia de variantes de *E. coli* resistentes a los antibióticos supone una creciente amenaza global para la salud pública. Esta resistencia, usualmente es codificada por múltiples genes, que codifican para la expresión de enzimas, proteínas de membrana, porinas, bombas de flujo o mutaciones de la molécula diana. Investigaciones recientes han reportado mutaciones específicas asociadas a resistencia, como *qnr*, *pmrB*, *glpT*, y la variante bla_{TEM} (C32T). El objetivo de este estudio fue identificar la frecuencia de mutaciones cromosómicas que otorgan resistencia antibiótica en genomas de *E. coli* provenientes de niños en el distrito de Villa El Salvador en Lima, Perú. Un total de 19 genomas completos de *E. coli* fueron descargados a partir del Bioproyecto PRJNA633873 ubicado en GenBank de NCBI. Después de

convertir y evaluar la calidad de las lecturas con FastQC, se realizó un ensamblaje mediante SPAdes v3.15.2 y evaluación de contigs a través de QUAST v5.0.2. Se identificaron perfiles genómicos de tipo de secuencia multilocus (MLST) con PubMLST y buscamos genes de resistencia con AMRFinderPlus. Finalmente, analizamos los patrones de genes y la ausencia/presencia de estos mediante MCA, usando Stata v17 y R studio. Un total de 11 genomas presentaron un total de siete mutaciones en genes asociados a resistencia a cuatro familias de antibióticos, incluyendo *glpT* (E448) para fosfomicina, *pmrB* (Y358) para colistina, *gyrA* (S83L) y *parC*_S57T para quinolonas, bla_{TEM} (C32T) para amoxicilina con ácido clavulánico y piperacilina-tazobactam, y *cyaA* (S352T) para fosmidomicina. Se evaluaron las relaciones proximales para la presencia/ausencia de genes que incluyó los genes bla_{TEM}, *catA1*, *sul1*, *qnrB19*, *tetA* y *mphA*. Nuestro estudio describe por primera vez las mutaciones en genes asociados a la resistencia antimicrobiana en genomas de *E. coli* provenientes de población pediátrica de una comunidad en Lima, Perú.

Palabras clave: Resistencia a Antibióticos; Estructuras Cromosómicas; *Escherichia coli*; Niño (Fuente: DeCS)

INTRODUCTION

Antimicrobial resistance represents a growing threat to global public health in recent years. The World Health Organization has reported enterobacteria such as *Escherichia coli* as a priority group of high public health risk (1), due to numerous reports of genes conferring antibiotic resistance (2). *E. coli* is a commensal bacterium that inhabits the intestines of almost all living beings. Although most strains are harmless and play a fundamental role in digestion, some variants can cause intra- and extraintestinal diseases, such as urinary tract infections (UTI), bacteremia, diarrhea, and pneumonia, among others (3,4). It is common for infants to suffer from several conditions such as diarrhea, meningitis, and pneumonia (5).

Studies have shown that *E. coli* has several mutations in specific genes and regions that play a crucial role in antibiotic resistance (6,7). Among these, qnr, *pmrB* and *glpT* mutations have been identified as significant determinants of resistance (8-10). Point mutation of the *gyrA* gene, which encodes topoisomerase II, has been associated with resistance to quinolones (11), whereas pmrB, a regulator of polymyxin resistance, has been shown to influence the ability of *E. coli* to resist polymyxin antibiotics. *pmrB* gene receives much more attention because the polymyxin group, known as a "drug of last resort," is used as a last resort for clinical treatments (12). On the other hand, *glpT*, a glycerol-3-phosphate transporter, has been associated with fosfomycin resistance (13). Furthermore, the bla_{TEM}–C32T variant of the bla_{TEM} gene, responsible for beta-lactamase production, has been associated with beta-lactamase resistance due to a variant in promoter 3 (14).

Considering that genomic studies of *E. coli* in the Peruvian infant population have been scarce in recent years, the aim of this study was to identify the presence of mutations that confer antibiotic resistance in *E. coli* genomes from children in a community in Lima, Peru. The importance of this approach lies in the need to understand the frequency and diversity of mutations that contribute to antibiotic resistance in *E. coli* from a specific population that has been little studied so that this information can then be used for decision-making in patient management.

METHODS

Nineteen sequence read archives (SRA) containing *E. coli* data were obtained from fecal samples collected from clinically healthy children under 24 months of age in a community in Lima. These archives were accessed using the SRA-Tools module for further analysis. The study, published by Murray *et al.* (15), aimed to monitor the levels of phenotypic resistance and resistance genes present in the *E. coli* genome from different human and animal sources. For this study, the SRA files deposited in the NCBI (National Center for Biotechnology Information) GenBank Bioproject PRJNA633873 were used.

For analysis, the files were converted to FASTQ format using fasterq-dump, and the quality of the reads was assessed using FastQC v0.11.5. Then, adapters were trimmed, and low-quality sequences were removed using Trimmomatic v.0.39. De novo assembly was performed with SPAdes v3.15.2 using the default parameters. Finally, contigs were assessed for quality using QUAST v5.0.2. Multilocus sequence type (MLST) genomic profiles were identified using PubMLST (https://pubmlst.org/organisms/escherichia-spp) and antimicrobial resistance genes (ARGs) and mutations were searched for using AMRFinderPlus with a coverage length ≥ 90 %, nucleotide identity ≥ 90 %. Genotypic patterns of bla_{TEM}, *catA1*, *sul1*, *qnrB19*, *tetA* and *mphA* genes were analyzed using multiple correspondence analysis (MCA) to explain presence/absence relationships of extended-spectrum beta-lactamase (ESBL) genes among isolates using two-dimensional derivation. The MCA analysis was performed using Stata v17 (StataCorp, College Station, TX, USA) and R studio.

RESULTS

Of the 19 *E. coli* sequences obtained from healthy children in one community, 11 had a total of 7 chromosomal mutations in genes associated with resistance to four families of antibiotics. All 11 genomes had the *glpT*(E448) mutation, which is associated with fosfomycin resistance, while six of them also had the *pmrB* (Y358) mutation, associated with colistin resistance. Two isolates had the three mutations *glpT*(E448), *pmrB* (Y358), and *gyrA*(S83L), the latter associated with quinolone resistance. Other mutational resistance patterns were observed, highlighting the mutation pattern *glpT*(E448), bla_{TEM}(C32T), which confers resistance to amoxicillin-clavulanic acid and piperacillin-tazobactam, and *cyaA*(S352T), which provides resistance to fosmidomycin. The mutation pattern *glpT*(E448), bla_{TEM}(C32T), *cyaA*(S352T), *parC*_S57T, which confers resistance to fosfomycin, and *uhpT*_E350Q, responsible for resistance to quinolones, were also identified (Figure 1).

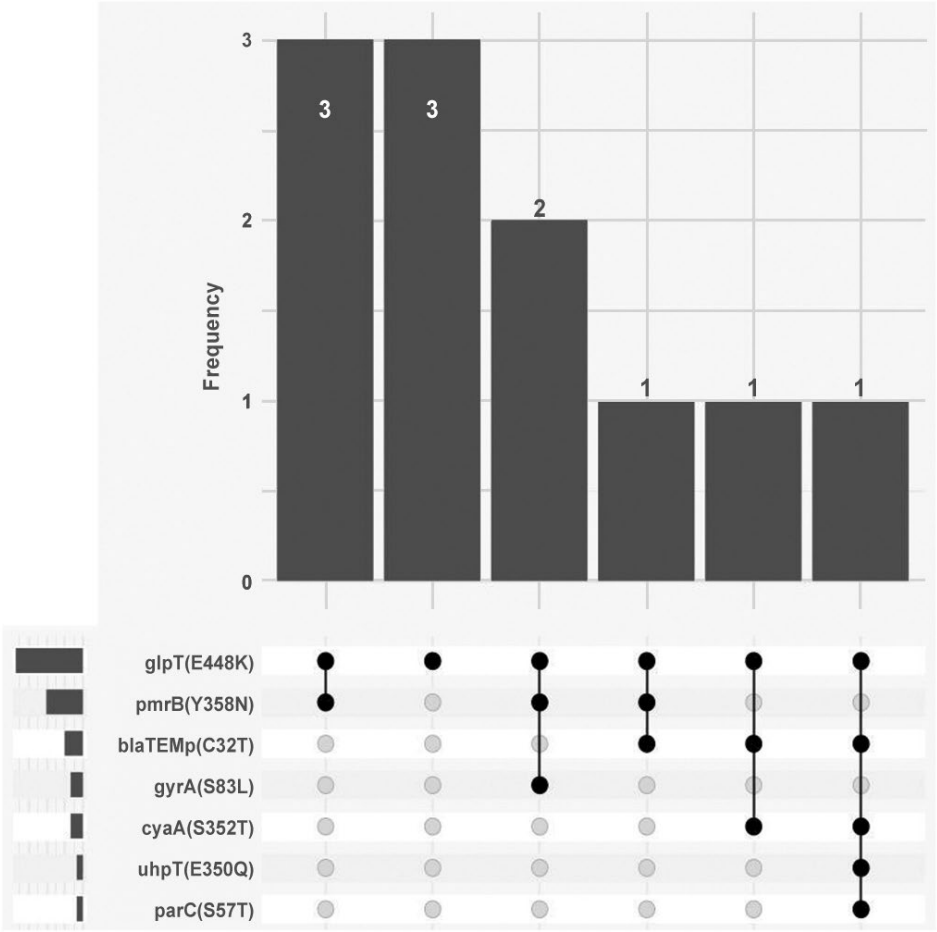

Figure 1. Frequency of chromosomal mutations identified in *E. coli* genomes.

The MCA performed to evaluate the proximal relationships for the presence/absence of genes included the genes bla_{TEM}, *catA1*, *sul1*, *qnrB19*, *tetA*, and *mphA*, as they were the most representative genes in our study. The two-dimensional model explained 84.2 % of the total variance of the original variables (first dimension = 58.6 %; second dimension = 24.6 %). Figure 2 highlights the clustering of genomes based on the absence of resistance genes, mostly coinciding. The most related genes were *catA1*, *sul1*, and *mphA* as these were absent by an average of 80.7 %. The percentage match of missing genes for bla_{TEM} and *tetA* was 57.9 % and 73.7 %, respectively.

DISCUSSION

Our study is the first to describe gene mutations associated with antimicrobial resistance in *E. coli* genomes from a pediatric population in a community in Lima, Peru. *E. coli* is a bacterium that causes numerous infections during the pediatric age, such as gastroenteritis, hospital-acquired pneumonia, urinary tract infections, neonatal osteomyelitis, meningitis, and sepsis, among many others (16). Due to the selective pressure exerted from the use of antimicrobials, multiple resistance patterns have been reported in these bacteria, including extended-spectrum beta-lactamases (ESBLs), *AmpC* beta-lactamases, carbapenemases, as well as resistance to fluoroquinolones, aminoglycosides, sulfonamides and polymyxins (17). The distribution of resistant *E. coli* is diverse worldwide and also varies according to the population studied (18-24). Studies in the pediatric population are still limited, and even more so when they involve clinically healthy individuals, as in our study.

Antimicrobial resistance has become a global public health problem in recent years and is a major impediment to the treatment of childhood diseases in developing countries such as Peru (25). Commensal bacteria can play a crucial role in the spread of resistance within a community by acting as an important reservoir of resistance genes (26,27). Exposure of commensals such as *E. coli* to antibiotics increases the carriage of resistant organisms and if plasmid-mediated,

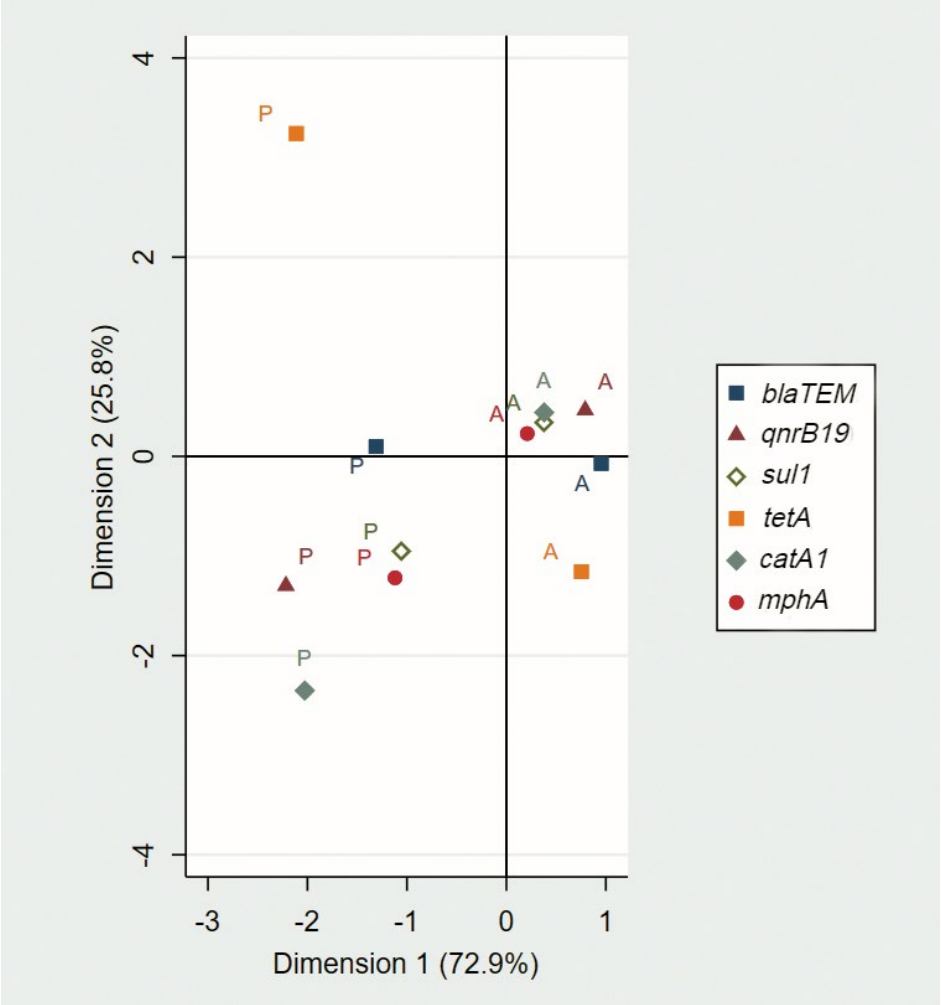

Figure 2. MCA coordinate plot for genotypic characterization of *E. coli*. A, absence; P, presence.

resistance could be transmitted to a more virulent acquired organism (28). The poor regulation of antibiotic use is one of the main factors in the emergence of resistant bacteria in human populations (29,30). This situation is particularly exacerbated in developing countries such as Peru due to overcrowding, poor management of excreta and available water, lack of regulations and policies, and lack of knowledge about antimicrobial resistance (31).

In Peru, high levels of antibiotic resistance in commensal *E. coli* have been reported in children. Antibiotics such as ampicillin, sulfa-trimethoprim, tetracycline, streptomycin, and chloramphenicol have all shown high levels of resistance, with up to 90 % (n=1,080) of the isolates being multidrug-resistant (MDR). Additionally, the resistance genes that were most frequently identified were bla_{TEM}, *tetA*, *dfrA8*, *sul1*, *sul2*, and *cat1* (31). In another study published in Peru (15), the resistance profiles of 60 *E. coli* isolates from a pediatric population aged 0 to 2 years were determined. The isolates also showed high levels of resistance, mainly to tetracyclines, sulfa-trimethoprim, amoxicillin, azithromycin, chloramphenicol, cephalothin, cefotaxime, and gentamicin, with 48 % of MDR isolates (32). The resistance results found were alarming, reporting even an isolate carrying the *mcr-1* gene for colistin resistance (32).

Genomic surveillance of resistance usually focuses on detecting genes acquired by horizontal gene transfer (33). In this study, we explored for the first time the presence of point mutations for detecting resistance mechanisms in commensal *E. coli* from a pediatric population in Peru. The high frequency of resistance-associated mutations found highlights the need to implement genomic surveillance of antimicrobial resistance in patients and commensal bacteria.

Authors' contribution

Conceptualization: BA; data collection, management, and curation: BA, DC; data analysis: BA, DC; visualization: DC original version drafting: BA, DC, PT; interpretation of results: BA, DC; final version drafting and revision: BA, DC, PT.

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Ethical aspects

Not applicable.

Conflicts of interest

The authors have no conflict of interest associated with the material presented in the manuscript.

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