

BRIEF REPORT

MICROBIOLOGICAL AND MOLECULAR CHARACTERIZATION OF ANTIMICROBIAL RESISTANCE IN UROPATHOGENIC *Escherichia coli* FROM PERUVIAN PUBLIC HOSPITALS

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ABSTRACT

We characterized the antimicrobial resistance of 70 *Escherichia coli* isolates obtained from patients with a urinary tract infection (UTI) from 8 public hospitals in Peru. Resistance profiles were identified using the automated MicroScan® system. A standard polymerase chain reaction was used for the detection of the *bla*_{TEM}, *bla*_{CTX-M}, *bla*_{SHV} and *bla*_{PER} genes. The 65.7% (46/70) of the isolates presented a multidrug-resistant phenotype and 55.7% (39/70) were extended-spectrum beta-lactamases producers. High levels of resistance were detected for ampicillin (77,1%), ciprofloxacin (74,3%), trimethoprim/sulfamethoxazole (62,9%), cefepime (57,1%), and cefuroxime (57,1%). The *bla*_{TEM} gene was the most frequent (31,4%), followed by *bla*_{CTX-M} (18,6%) and *bla*_{SHV} (2,9%) genes. These results show high resistance levels to antimicrobials of clinical use in *E. coli* isolates from hospital UTI patients in Peru.

Keywords: *Escherichia coli*, Drug Resistance, beta-Lactam Resistance, Urologic Diseases, Peru (source: MeSH NLM).

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INTRODUCTION

Urinary tract infections (UTI) have a high incidence worldwide and cause high treatment costs for healthcare systems⁽¹⁾. *Escherichia coli* is the etiological agent most frequently found in UTIs and is mainly treated with antimicrobials. However, the lack of regulation of these treatments favored the emergence of multidrug-resistant (MDR) strains worldwide⁽²⁾ and the emergence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* strains with the ability to hydrolyze penicillins, cephalosporins and monobactams. Genes encoding ESBL production are frequently found in plasmids and are usually accompanied by other genes for resistance to cephalosporins, sulfonamides, fluoroquinolones and aminoglycosides⁽³⁾. There are several genes coding for ESBL, the most frequent being those of the TEM, SHV and

CTX-M families^(4,5). More than 400 types of these enzymes have been reported and CTX-M are the most frequent worldwide⁽⁶⁾.

In Peru, the detection of antimicrobial resistance in bacteria causing UTI is not included in the epidemiological surveillance system and no updated data are available⁽⁷⁾. Knowing the levels of resistance and genes associated with ESBL production in *E. coli* isolates of UTI will allow the establishment of effective empirical therapies and control programs. Therefore, the aim of this study was to characterize by phenotypic and molecular tests the antimicrobial resistance and prevalence of ESBL in *E. coli* isolates from patients with UTI from eight public hospitals in different departments of Peru.

THE STUDY

A descriptive study was conducted, based on obtaining bacterial isolates from eight public hospitals located in the departments of Cusco, Huancavelica, La Libertad, Loreto, Madre de Dios, Puno, San Martín and Tumbes. A total of 70 *E. coli* isolates obtained from outpatients with clinical diagnosis compatible with UTI collected during 2018 were used.

The isolates were characterized at the Molecular Biology Research Laboratory of the Universidad Peruana Unión. Species confirmation and determination of resistance profiles was carried out using the MicroScan® automated system (AutoScan-4) and gram-negative panels (Dade MicroScan®) following the manufacturer's instructions. Fifteen antimicrobials from different families were included: ampicillin (AMP), ampicillin with sulbactam (AMP/SUL), amoxicillin with clavulanic acid (AMC), piperacillin with tazobactam (PIP/ TZ), aztreonam (ATM), cefepime (FEP), cefuroxime (CFX), ceftazidime (CAZ), cefotaxime (FOX), tobramycin (TOB), gentamicin (GEN), ciprofloxacin (CIP), trimethoprim/sulfamethoxazole (SXT), colistin (COL) and tigecycline (TIG). In addition, we detected the presence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli*. The results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) recommendations⁽⁸⁾.

DNA extraction was carried out with the innuPREP kit following the manufacturer's instructions (Analytik Jena, Germany). For gene identification, we used a conventional polymerase chain reaction (PCR) designed for each gene *bla*_{CTX-M}⁽⁹⁾, *bla*_{TEM}⁽¹⁰⁾, *bla*_{SHV}⁽¹¹⁾ and *bla*_{PER}⁽¹²⁾. The primer sequences used are detailed in Table 1.

KEY MESSAGES

Motivation for the study: It is important to update data on levels and patterns of antimicrobial resistance in uropathogenic *Escherichia coli*. Determining which are the resistance patterns allows guiding adequate therapeutics for this type of infections.

Main findings: High levels of resistance were detected for ampicillin, ciprofloxacin, trimethoprim/ sulfamethoxazole, cefepime and cefuroxime. We identified 55.7% of isolates as extended spectrum beta-lactamase (ESBL) producers with the presence of *bla*_{TEM}, *bla*_{CTX-M} and *bla*_{SHV} genes.

Implications: The high frequency of multidrug-resistant ESBL-producing *E. coli* strains is alarming and should raise awareness about the appropriate use of antimicrobials in the treatment of urinary tract infections.

Multidrug resistance was defined as the detection of a resistant phenotype for at least one antimicrobial in three or more classes. The proportion of resistant isolates was stratified according to patient sex. Bivariate analysis was developed using Fisher's exact test with a 95% confidence level. Data was managed with the Stata 16 statistical package (StataCorp, College Station, Texas, USA).

The study was evaluated and approved by the Ethics Committee of the Universidad Peruana Unión (N2019-CEUPeU-0001) and by the Dirección Universitaria de Investigación, Ciencia y Tecnología of the Universidad Peruana Cayetano Heredia.

FINDINGS

Seventy *Escherichia coli* isolates were obtained from outpatients with diagnosis compatible with urinary tract infection (UTI) from public hospitals in Huancavelica (n=15), Loreto (n=14), Tumbes (n=13), Madre de Dios (n=12), La Libertad (n=8), Puno (n=4), Cusco (n=3) and San Martín (n=1). The mean age was 38.8 years and 80% (n=56) of the patients were female. Of the isolates, 65.7% (46/70) had a multidrug-resistant (MDR) phenotype, which was more frequent in male patients (78.6%, 11/14) compared to female patients (62.5%, 35/56). A total of 39/70 (55.7%) isolates were identified as extended-spectrum beta-lactamase (ESBL) producers, with a higher frequency in male patients (64.3%, 9/14) compared to female patients (53.6%, 30/56). However, these differences were not

Table 1. Primers used for gene amplification.

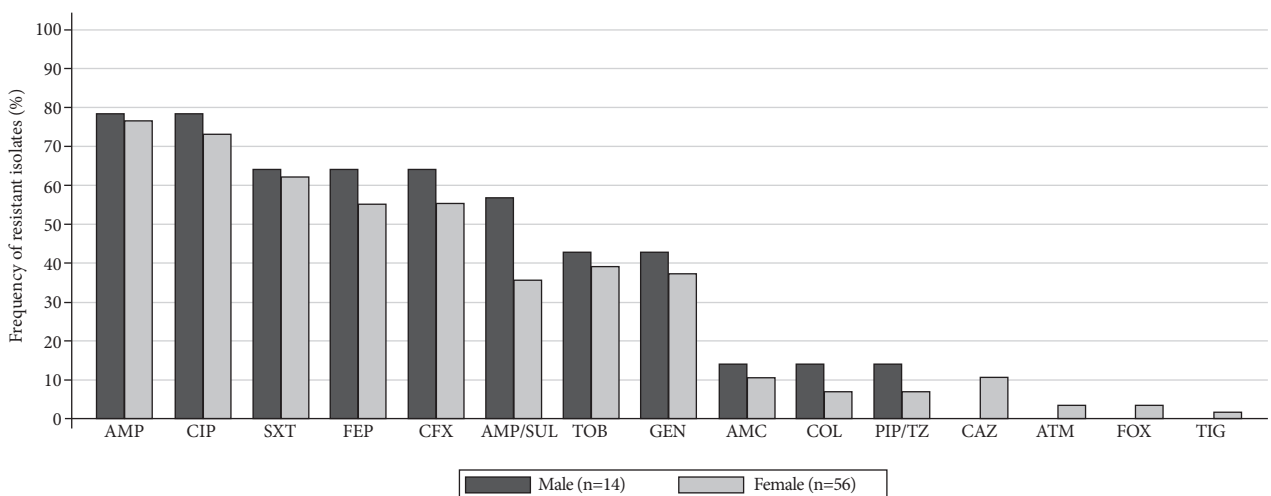
Genes	Amplicon (bp)	Primer	Sequences	Reference
<i>bla</i> _{CTX-M}	544	CTX/F	TTTGCGATGTGCAGTACCAGTAA	9
		CTX/R	CGATATCGTTGGTGGTGCCAT	
<i>bla</i> _{TEM}	504	TEM/F	TTGGGTGCACGAGTGGGTTA	10
		TEM/R	TAATTGTTGCCGGGAAGCTA	
<i>bla</i> _{SHV}	865	SHV/F	ATGCGTTATATTCGCCTGTG	11
		SHV/R	GTTAGCGTTGCCAGTGCTCG	
<i>bla</i> _{PER}	927	PER/F	ATGAATGTCATCACAAAATG	12
		PER/R	TCAATCCGGACTCACT	

statistically significant ($p > 0.05$). Of the isolates identified as ESBL producers, 92.3% (36/39) had an MDR phenotype including resistance to aminoglycosides, fluoroquinolones, beta-lactams and sulfonamides.

High percentages of resistance were detected against ampicillin (77.1%), ciprofloxacin (74.3%), trimethoprim/sulfamethoxazole (62.9%), cefepime (57.1%), cefuroxime (57.1%) and ampicillin with sulbactam (40%). Isolates from male patients had the highest levels of resistance (Figure 1). However, only isolates from female patients showed resistance to ceftazidime (10.7%, $n=6$), aztreonam (3.6%, $n=2$), cefotaxime (3.6%, $n=2$), tigecycline (1.8%, $n=1$). These differences were not statistically significant ($p > 0.05$). No

isolates resistant to amikacin, ertapenem, imipenem and meropenem were detected.

The *bla*_{TEM} gene family was detected by PCR in 31.4% (22/70), followed by *bla*_{CTX-M} (18.6%, $n=13$) and *bla*_{SHV} (2.9%, $n=2$), in both ESBL-producing and non-ESBL-producing isolates. Of the total ESBL isolates, 56.4% (22/39) were positive for at least one of the genes evaluated. The *bla*_{CTX-M} gene was the most common (28.2%, 11/39), and only one isolate presented it in combination with the *bla*_{SHV} gene. The *bla*_{TEM} gene was found in 25.6% (10/39) of the ESBL-producing isolates. There was no evidence of the joint presence of the *bla*_{CTX-M} and *bla*_{TEM} genes. Of the total non-ESBL-producing isolates, 54.8% (17/31) did not present any of the genes evaluated; 38% (12/31) had the *bla*_{TEM} gene and

**Figure 1.** *Escherichia coli* resistance profiles according to patient sex.

AMP: ampicillin, CIP: ciprofloxacin, SXT: trimethoprim/sulfamethoxazole, FEP: cefepime, CFX: cefuroxime, AMP/SUL: ampicillin with sulbactam, TOB: tobramycin, GEN: gentamicin, AMC: amoxicillin with clavulanic acid, COL: colistin, PIP/TZ: piperacillin with tazobactam, CAZ: ceftazidime, ATM: aztreonam, FOX: cefotaxime, TIG: tigecycline.

Table 2. Detection of genes related to extended-spectrum beta-lactamase (ESBL) production in uropathogenic *Escherichia coli*.

Phenotype	n	<i>bla</i> _{CTX-M}	<i>bla</i> _{SHV}	<i>bla</i> _{TEM}	<i>bla</i> _{PER}
ESBL (n=39)	17	-	-	-	-
	10	-	-	+	-
	10	+	-	-	-
	1	-	+	-	-
	1	+	+	-	-
ESBL negative (n=31)	17	-	-	-	-
	12	-	-	+	-
	2	+	-	-	-

+/-: presence/absence of the gene according to the results of the polymerase chain reaction.

only 6.5% (n=2) had the *bla*_{CTX-M} gene. Of the total number of ESBL-producing and non-ESBL-producing isolates, we did not detect the presence of the *bla*_{PER} gene (Table 2).

DISCUSSION

E. coli isolates from patients with UTI had high levels of resistance to clinically important antimicrobials. More than 50% of the isolates were classified as MDR and ESBL-producing. Isolates were mostly resistant to ampicillin, ciprofloxacin and trimethoprim/sulfamethoxazole, which are antimicrobials frequently used for the treatment of UTI⁽¹³⁾. In addition, we found isolates resistant to colistin, a drug of last resort used in complicated UTI caused by MDR bacteria,⁽¹⁴⁾ Isolates resistant to imipenem, meropenem, and ertapenem were not detected. However, the detection of ESBL-producing uropathogenic *E. coli* isolates represents a potential risk of resistance development, due to their ability to carry resistance genes to other antimicrobials⁽¹⁵⁾.

Isolates from male patients showed higher levels of resistance to ampicillin, ciprofloxacin, trimethoprim/sulfamethoxazole, cefepime, cefuroxime, ampicillin with sulbactam, tobramycin, gentamicin, amoxicillin with clavulanic acid, colistin and piperacillin with tazobactam. The differences found according to the sex of the patients were not significant, so we cannot evidence that being a male patient would be an indicator for the empirical selection of antimicrobials for the treatment of UTIs. However, our results show a high prevalence of *E. coli* resistant to several commonly used antimicrobials in these infections. Greater care should be considered in the selection of therapeutic options by treating physicians to avoid selective pressure leading to the emergence of MDR strains.

The *bla*_{TEM} gene family was the most frequent, followed by *bla*_{CTX-M} and *bla*_{SHV}. These results contrast with those found by other authors, where the *bla*_{CTX-M} gene was found to be the most frequent in patients with UTI in Peru⁽¹⁶⁻¹⁸⁾. A total of 17 ESBL-producing isolates were negative for all the genes evaluated. Even the presence of *bla*_{CTX-M} (n=2) and *bla*_{TEM} (n=12) genes was detected in isolates identified as non-ESBL producers. The differences observed in the detection of ESBL isolates by phenotypic and genotypic methods reflect the low sensitivity of the phenotypic method and the possible influence of external factors in the occurrence of resistance. Possibly, many isolates did not produce ESBL at levels detectable by the phenotypic method, which could explain the presence of ESBL-negative isolates, but with genes related to the production of these enzymes. In contrast to the phenotypic method, gene detection by PCR amplification has better levels of specificity and sensitivity. However, these values are closely correlated to the quality and design of primers. Even non-ESBL variants of *bla*_{TEM} or *bla*_{SHV} genes have been described, so sequencing methods are essential for their identification and characterization⁽¹⁹⁾.

In conclusion, the results show high levels of resistance in *E. coli* isolates carrying *bla*_{TEM}, *bla*_{CTX-M} and *bla*_{SHV} genes recovered from outpatients diagnosed with UTI in different regions of Peru. Peruvian public hospitals conduct antimicrobial resistance monitoring using mainly phenotypic methods and to a lesser extent, molecular methods. However, these methodologies may not correctly reflect the status of antimicrobial resistance in UTIs and other types of infections. Therefore, the implementation of cutting-edge methods for genomic surveillance of antimicrobial resistance in hospitals is necessary.

The results obtained correspond to a first phase of the study. We will use whole genome sequencing methods and bioinformatic analysis for the study of antimicrobial resistance in these bacterial isolates. The information generated will serve as an epidemiological tool to determine the distribution of resistance genes and also as a guide to evaluate the trend and possible changes in therapeutic schemes by treating physicians.

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Authorship contributions: PM and PT participated in the conception and design of the study. PM, GS and PT conducted data analysis and interpretation. PM, JY, GS and PT carried out the drafting and critical revision of the article. JY, JP, NV, PD, IM, PA, CP, CH, AB, MR, NL, AL and LA provided data, strain collection and processing. GS provided statistical advice. All authors approved the final version and are responsible for the contents of the manuscript.

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