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Multidrug-resistant *E. coli* in paediatric UTIs in a Chilean hospital

E. coli multifarmacorresistente en ITU pediátricas en un hospital chileno

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ABSTRACT

The purpose of this study was to isolate and molecularly characterize antibiotic-resistant *Escherichia coli* from urine samples of children patients at the Pontifical Catholic University of Chile Clinical Hospital, Chile. The study sought to determine antibiogram profiling and resistance genes in multiple antibiotic-resistant bacterial isolates. A total of 300 urine samples were collected from children patients aged between 3-5 years, with 158 females and 142 males. The samples were transported on ice to the laboratory and analysed bacteriologically. The isolation of *E. coli* and other Gram-negative bacteria was carried out by streaking on Eosin Methylene Blue agar and incubated at 37°C for 24 hours. Identification of isolates was preliminary through colony and morphological characteristics. Further identification was carried out by biochemical tests using Bergey's manual of determinative bacteriology and Analytical Profile Index (API) Kits. Antibiotic susceptibility of isolates was determined using Kirby Bauer's disc diffusion technique. Detection of resistance genes was carried out by Polymerase Chain Reaction (PCR) using appropriate primers. The study found that 40 (13.3%) *E. coli* were recovered from the 300 samples collected, with varying resistance to antibiotics. The highest resistance was observed for Augmentin (35, 87.5%), followed by Ceftriaxone (32, 80%), Gentamycin (30, 75%), Ofloxacin (28, 70%), Cefuroxime (26, 65%), Amoxicillin (16, 40%), Cefixime (11, 27.5%), Cotrimoxazole (10, 25%), Ciprofloxacin (9, 22.5%), Streptomycin (9, 22.5%), Pefloxacin (8, 20%), and Ceftazidime (4, 10%). All the *E. coli* isolates were found to be susceptible to Nitrofurantoin, Erythromycin, and Chloramphenicol. The isolates showed multiple resistance against 3 to 4 different classes of antibiotics, and the study recorded 12 multiple antibiotic resistance patterns. Eight of the twelve selected *E. coli* isolates had blaCTX-M gene, while five harboured the aac3-IV gene. The study concluded that the occurrence of *E. coli* infection is high in the study area, and there is a high level of multiple antibiotic resistance. The recovery of resistance genes (blaCTX-M and aac3-IV) in *E. coli* in the study is of great economic and health consequences. Therefore, antibiotics should only be used when prescribed by physicians to mitigate the problem of multiple antibiotic resistance

Keywords: *E. coli*; resistance; antibiotics; gene

RESUMEN

El propósito de este estudio fue aislar y caracterizar molecularmente *Escherichia coli* resistente a antibióticos a partir de muestras de orina de pacientes infantiles del Hospital Clínico de la Pontificia Universidad Católica de Chile, Chile. El estudio buscó determinar el perfil de antibiograma y los genes de resistencia en múltiples aislados bacterianos resistentes a los antibióticos. Se recogieron un total de 300 muestras de orina de niños de entre 3 y 5 años, 158 mujeres y 142 hombres. Las muestras fueron transportadas en hielo al laboratorio y analizadas bacteriológicamente. El aislamiento de *E. coli* y otras bacterias Gram-negativas se realizó por estría en agar Eosina Azul de Metileno y se incubó a 37°C durante 24 horas. La identificación de los aislamientos fue preliminar a través de la colonia y características morfológicas. La identificación

adicional se llevó a cabo mediante pruebas bioquímicas utilizando el manual de Bergey de bacteriología determinativa y kits de índice de perfil analítico (API). La susceptibilidad a los antibióticos de los aislados se determinó mediante la técnica de difusión en disco de Kirby Bauer. La detección de genes de resistencia se llevó a cabo mediante la reacción en cadena de la polimerasa (PCR) utilizando los cebadores apropiados. El estudio encontró que 40 (13,3%) *E. coli* se recuperaron de las 300 muestras recolectadas, con resistencia variable a los antibióticos. La mayor resistencia se observó para Augmentina (35, 87,5 %), seguida de Ceftriaxona (32, 80 %), Gentamicina (30, 75 %), Ofloxacina (28, 70 %), Cefuroxima (26, 65 %), Amoxicilina (16, 40 %), Cefixima (11, 27,5 %), Cotrimoxazol (10, 25 %), Ciprofloxacina (9, 22,5 %), Estreptomina (9, 22,5 %), Pefloxacina (8, 20 %) y Ceftazidima (4, 10%). Se encontró que todos los aislados de *E. coli* eran susceptibles a la Nitrofurantoína, la Eritromicina y el Cloranfenicol. Los aislamientos mostraron multiresistencia frente a 3 o 4 clases diferentes de antibióticos, y el estudio registró 12 patrones de multiresistencia a antibióticos. Ocho de los doce aislados de *E. coli* seleccionados tenían el gen *bla*CTX-M, mientras que cinco albergaban el gen *aac*3-IV. El estudio concluyó que la incidencia de infección por *E. coli* es alta en el área de estudio y que existe un alto nivel de resistencia a múltiples antibióticos. La recuperación de genes de resistencia (*bla*CTX-M y *aac*3-IV) en *E. coli* en el estudio tiene grandes consecuencias económicas y sanitarias. Por lo tanto, los antibióticos solo deben usarse cuando los prescriben los médicos para mitigar el problema de la resistencia múltiple a los antibióticos.

Palabras clave: *E. coli*; resistencia; antibióticos; gene

INTRODUCTION

Urinary tract infection (UTI) is a bacterial infection that affects one or more parts of the urinary system, such as the urethra, bladder, ureters, and kidneys. The most common cause of UTI is the multiplication of bacteria at the urethra's entrance, which can then move up to the bladder or even the kidneys and spread to the bloodstream [1]. UTIs are a major public health concern in both hospital and community settings, particularly in developing countries, due to their high mortality rate and economic burden [2].

Enterobacteriaceae, specifically those possessing adhesins and hydrolytic enzymes like β -lactamases, are the primary cause of UTIs. β -lactamase production is the most common resistance mechanism used by these bacteria, and their increasing prevalence is a significant complication [2-5]. Children with UTIs can be challenging to diagnose and treat due to the nonspecific symptoms they exhibit, which can result in improper treatment.

The emergence and rapid spread of antibiotic-resistant *Enterobacteriaceae* is a significant public health threat. These bacteria can withstand lethal doses of antibiotics with various mechanisms of action and chemical structures, such as target alteration, decreased uptake, enzyme degradation, and overexpression of efflux proteins. Molecular genotyping and phenotyping techniques can be

used to screen and confirm antimicrobial drug resistance expression in a population.

The prevalence of undiagnosed and improperly treated urinary tract infections (UTIs) in children is a major concern for the public and healthcare professionals due to the difficulty in diagnosing UTIs with nonspecific symptoms in paediatric patients. Although UTI complaints are rare in children, symptoms usually only appear after five years of age, such as fever accompanied by rigors/chills, suprapubic/abdominal pain, and vomiting [6].

CTX-M β -lactamase enzymes are categorized into five groups based on amino acid similarity, with CTX-M-15 and CTX-M-14 being the most prevalent globally. These enzymes are often associated with genes conferring resistance to antibiotics like aminoglycosides and fluoroquinolones. *Escherichia coli*, a Gram-negative bacterium found in the human intestinal tract, is a significant cause of UTIs, bloodstream infections, septicemia, and meningitis. Multi-drug-resistant strains of *E. coli* are becoming increasingly prevalent worldwide, particularly in South American countries with inadequate laboratory diagnostics and surveillance systems.

METHODOLOGY

Sample Collection: The study obtained ethical clearance from the Pontifical Catholic University

of Chile Clinical Hospital, Chile's ethical and research committee in Santiago, Metropolitan Region, Chile, with the reference number UCCHRISTUS/128. Informed consent was obtained from the parents of the children included in the study, and those who declined were not included. Urine samples were collected from a total of 300 subjects using sterile universal bottles, temporarily stored on ice packs at -4 °C, and transported promptly to the laboratory for analysis.

Isolation of Organisms: Using aseptic technique, a loop full of each sample was streaked onto Eosin Methylene blue agar (manufactured by Lab M Ltd, UK), and the agar plates were then incubated at 37 °C for 24 hours. After the incubation period, distinct colonies were subcultured onto sterile nutrient agar plates, which were freshly prepared, and incubated again at 37 °C for 24 hours. The distinctive morphological properties of each pure culture on the agar plates were observed.

Identification of Isolates: The isolates' morphological characteristics were appropriately recorded, and initial identification was conducted using Bergey's Manual of Determinative Bacteriology. To further identify the bacterial isolates, an Analytical Profile Index (API) 20E test kit (manufactured by bioMérieux, Inc.) was utilized.

Antibiotic Susceptibility Test: The Kirby-Bauer's disc diffusion method was utilized to test the susceptibility of the isolates to antibiotics. The antibiotic discs (manufactured by Oxoid Ltd, UK) that were used in the study include Augmentin (30 µg), Ceftriaxone (30 µg), Nitrofurantoin (300 µg), Gentamycin (10 µg), Cotrimoxazole (25 µg), Ofloxacin (10 µg), Amoxicillin (25 µg), Ciprofloxacin (10 µg), Cefotaxime (30 µg), Cefuroxime (30 µg), Cefixime (5 µg), Chloramphenicol (30 µg), Streptomycin (10 µg), Erythromycin (5 µg), and Pefloxacin (5 µg). These discs were placed aseptically onto Mueller-Hinton agar plates, which were previously seeded with a 24-hour-old culture (0.5 McFarland's standard-

107cfu/ml) using sterile forceps. The plates were then incubated at 37°C for 24 hours. The diameter of the zone of inhibition was measured using a transparent calibrated ruler to the nearest millimetre, and the results were interpreted according to the guidelines of the Clinical Laboratory Standards Institute [7]. Multiple antibiotic resistance was defined as resistance to more than two classes of antibiotics.

Molecular characterization of multiple antibiotic resistant *E. coli* isolates: Twelve *E. coli* isolates that were resistant to multiple antibiotics were selected based on their antibiotype and subjected to gene detection analysis. The resistance genes *bla*CTX (585bp) and Tet A (954bp) were detected using appropriate primers as described in Table 1. Bacterial DNA was extracted using the boiling method, whereby the samples were boiled at 100°C for 7 minutes in a water bath and then chilled on ice for 2 minutes. The PCR reaction was carried out using the following thermocycling conditions: initial denaturation at 94°C for 5 minutes, followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 58°C for 30 seconds for *bla*CTX (585bp) gene and 55°C (*aac3-IV*) for 1 minute, extension at 72°C for 1 minute, and a final extension at the same temperature for 5 minutes. The PCR products were visualized using a short wave ultraviolet trans-illuminator and photographed using a gel bioimaging system.

Table 1. Primers for the detection of the resistance genes for *E. coli*

Tabla 1. Iniciaadores para la detección de genes de resistencia en *E. coli*

| Primer | Sequence 5'-3' | Gene | Product Size (bp) | Annealing Temp °C | Reference |
|------------------|-------------------------|------------------|-------------------|-------------------|-----------|
| <i>bla</i> CTX-M | F: CCATGTGCAGTACCAGTAA | <i>bla</i> CTX-M | 585 | 58 | [8] |
| | R: TTAGTGACCAGAATAAGCGG | | | | |
| <i>aac3-IV</i> | F: AGTTGACCCAGGGTGTCCG | <i>aac3-IV</i> | 286 | 55 | [9] |
| | R: GTGTGCTGCTGCCACAGC | | | | |

RESULTS

Occurrence of *E. coli* in Relation to Age and Sex of Children Patients: Out of the total 300 urine samples collected from the cohort of children (158 females and 142 males), 40 (13.3%) yielded *E. coli* isolates. The age and gender distribution of the

participants is presented in Table 2. Among the 300 children, 147 (78 females and 69 males) were in the age range of 3-4 years, whereas 153 (80 females and 73 males) were between the ages of 4-5 years.

One-way ANOVA was performed to determine if there is any significant difference in the occurrence of *E. coli* against the two age groups. The null hypothesis for the one-way ANOVA was that there was no significant difference in growth rates between the two age groups. The alternative hypothesis was that there was a significant difference in growth rates between the two age groups, using a significance level of 0.05. The calculated F-value is 2.08, and the critical F-value is 3.88. Since the calculated F-value is less than the critical F-value, we failed to reject the null hypothesis. This means that there is no significant difference in the occurrence of *E. coli* against the two age groups.

Table 2. Occurrence of *E. coli* in relation to age and sex of the children (n=300)

Tabla 2. Frecuencia de *E. coli* con relación a la edad y sexo de los niños (n=300)

| Age | Number of children sampled | Number of samples with growth n (n %) | Number of females | Number of samples with growth n (n %) | Number of males | Number of samples with growth n (n%) |
|--------------|----------------------------|---------------------------------------|-------------------|---------------------------------------|-----------------|--------------------------------------|
| 3-4 years | 147 | 22(55) | 78 | 13 (59.1) | 69 | 9 (40.9) |
| 4-5 years | 153 | 18 (45) | 80 | 11 (61.1) | 73 | 7 (38.9) |
| Total | 300 | 40 (13.3) | 158 | 24 (15.2) | 142 | 16 (11.3) |

Antibiotic Susceptibility Profile of *E. coli* Isolates Cultured from Urine Samples of Pontifical Catholic University of Chile Clinical Hospital, Chile: The *E. coli* isolates obtained from 40 of the children (13.3% of the total cohort) displayed varying degrees of antibiotic resistance, with the highest resistance rates observed for Augmentin (35 isolates, 87.5%), Ceftriaxone (32 isolates, 80%), and Gentamycin (30 isolates, 75%). In contrast, none of the isolates were resistant to Erythromycin, Chloramphenicol, or Nitrofurantoin. Intermediate resistance was observed in 16 isolates (40%) for Amoxicillin and Cotrimoxazole, while Ceftazidime (32.5%),

Ciprofloxacin (30%), and Streptomycin (25%) showed intermediate resistance in a smaller proportion of isolates. Among the 15 antibiotics tested, Pefloxacin (8 isolates, 20%) and Ceftazidime (4 isolates, 10%) showed the lowest levels of resistance. The percentage resistance of the *E. coli* isolates showed a significant difference ($p < 0.05$). None of the isolates displayed intermediate resistance to Nitrofurantoin, Gentamycin, Ofloxacin, Cefixime, Chloramphenicol, and Erythromycin. The details of the resistance rates and intermediate resistance are provided in Table 3.

Table 3. Antibiotic susceptibility profile of *E. coli* isolates cultured from urine samples of children patients

Tabla 3. Perfil de susceptibilidad a antibióticos de aislados de *E. coli* de muestras de orina de niños

| Antibiotic (µg) | No of Isolates | Number of Isolate Occurrence (n%) | | |
|-------------------------|----------------|-----------------------------------|--------------|-----------|
| | | Susceptibility | Intermediate | Resistant |
| Amoxicillin (25 µg) | 40 | 13 | 16 | 11 |
| Augmentin (30 µg) | 40 | 2 | 3 | 35 |
| Cefixime (5 µg) | 40 | 29 | 0 | 11 |
| Ceftazidime (30 µg) | 40 | 23 | 13 | 4 |
| Ceftriaxone (30 µg) | 40 | 2 | 6 | 32 |
| Cefuroxime (30 µg) | 40 | 9 | 5 | 26 |
| Chloramphenicol (30 µg) | 40 | 40 | 0 | 0 |
| Ciprofloxacin (10 µg) | 40 | 19 | 12 | 9 |
| Cotrimoxazole (25 µg) | 40 | 14 | 16 | 10 |
| Erythromycin (5 µg) | 40 | 40 | 0 | 0 |
| Gentamycin (10 µg) | 40 | 10 | 0 | 30 |
| Nitrofurantoin (300 µg) | 40 | 40 | 0 | 0 |
| Ofloxacin (10 µg) | 40 | 12 | 0 | 28 |
| Pefloxacin (5 µg) | 40 | 30 | 2 | 8 |
| Streptomycin (10 µg) | 40 | 21 | 10 | 9 |

Multiple Antibiotic Resistance Patterns of *E. coli* Isolates from Urine Samples of Pontifical Catholic University of Chile Clinical Hospital, Chile:

The criterion for multiple antibiotic resistance was established as resistance to three or more different classes of antibiotics. The multiple antibiotic resistance profiles of the *E. coli* isolates are provided in Table 3. The antibiotic classes tested comprised Beta lactams (Amoxicillin, Augmentin, Ceftriaxone, Ceftazidime, Cefuroxime, and Cefixime), Fluoroquinolones (Ciprofloxacin, Ofloxacin, and Pefloxacin), Aminoglycosides (Gentamycin and Streptomycin), Sulphonamides/Trimethoprim (Cotrimoxazole), Nitrofurans (Nitrofurantoin), Macrolides (Erythromycin), and Chloramphenicol.

Seventy-five percent (30) of the 40 *E. coli* isolates recovered in this investigation exhibited multiple resistance to at least three antibiotic classes. A total of 12 different multiple antibiotic resistance patterns were identified (Table 4).

Table 4. Multiple antibiotic resistance pattern of *E. coli* cultured from urine samples of children patients

Tabla 4. Patrones de multirresistencia a antibióticos de cultivos de *E. coli* de muestras de orina de niños

| Isolate | No of antibiotic class | Multiple antibiotic resistance pattern* | Frequency, Over all(%) | | | |
|----------------|------------------------|---|------------------------|----------|---------|------|
| <i>E. coli</i> | 4 | AUG, COT, | 5 | 12.5 | 6 (15%) | |
| | | GEN, OFL | 1 | 2.5 | | |
| | 3 | CAZ, COT, OFL, STR | 1 | 2.5 | | 27.5 |
| | | AMX, COT, | | | | |
| | | OFL | | | | |
| | | AUG, COT, GEN | | | | |
| | | AUG, GEN, OFL | | | | |
| | | AUG, GEN, PEF | | | | |
| | | CAZ, PEF, STR, | | | | |
| | | CPX, GEN, OFL | | | | |
| | | CRO, PEF, STR | | | | |
| | | CRX, COT, OFL | | | | |
| CRX, GEN, OFL | | | | | | |
| CRX, OFL, STR | | | | | | |
| | | 1 | 2.5 | 24 (60%) | | |

*COT –Cotrimoxazole 25 µg, CPX –Ciprofloxacin 10 µg, AMX –Amoxicillin 25 µg, OFL –Ofloxacin 5 µg, CRO –Ceftriaxone 30 µg, ERY – 5 µg, GEN –Gentamycin 10 µg, PFX –Pefloxacin 5 µg, AUG –Augmentin 30 µg, NIT- Nitrofurantoin 300 µg, CAZ- Cefazidime 30 µg, CRX–Cefuroxime 30 µg, CXM -Cefixime 5 µg, CHL-Chloramphenicol 30 µg, ERY - Erythromycin 5 µg, STR-Streptomycin 10 µg, PEF –Pefloxacin 5 µg

Molecular Detection of Resistance (*bla*CTX and *aac6'*) Genes in *E. coli*: Eight of the 12 representative isolates that was resistance to beta lactam antibiotics (show *bla*CTX gen). Five of the 12 representative isolates were resistant to gentamycin (*aac6* resistance gene weight of 286bp).

DISCUSSION

E. coli is a well-known bacterial pathogen that is responsible for various infections worldwide. However, the emergence and spread of antibiotic resistance among *E. coli* isolates pose a significant threat to public health globally, leading to major health problems like prolonged hospitalisation and treatment failure [10]. In this study, the researchers investigated the prevalence and patterns of antimicrobial resistance among *E. coli* isolates

obtained from urine samples of children under the age of five in Chile. The study found a significant occurrence of antibiotic resistance among the *E. coli* isolates, indicating a wide spectrum of resistance to all routinely used antibiotics in the region, such as Augmentin, Amoxicillin, Ciprofloxacin, Gentamicin, Cotrimoxazole, and Ofloxacin. The number of *E. coli* isolates with multi-drug resistance was 30 (75%), and all the isolates displayed resistance to one or more antibiotic classes. Moreover, the resistance levels were higher than those reported by previous studies. The researchers suggested that the widespread use of antimicrobial agents for infection treatment in the community could be a contributing factor to this serious antimicrobial resistance problem. Antimicrobial resistance in bacteria of medical importance imposes serious constraints on the treatment options for many infections, which concerns general practitioners and paediatricians in developing countries [12]. According to the study, Augmentin, Gentamycin, Ceftriazone, and Ofloxacin should not be used to treat *E. coli* infections in Chile, due to their high-level resistance. The development of beta-lactamases enzymes may be the cause of Augmentin resistance [13]. Moreover, the presence of extended-spectrum β-lactamase has been identified as the primary source of multiple resistance in *E. coli* [14]. The most common mechanism of Cotrimoxazole resistance is the acquisition of plasmid-encoded, variant diaminopyrimidinefolatereductase enzymes [15], whereas the relatively high rates of gentamycin resistance in this study are most likely due to the antibiotic indiscriminate use. Interestingly, all *E. coli* isolates obtained from urine samples of children under the age of five in Ondo, South West, Nigeria, showed a relatively high rate of sensitivity to nitrofurantoin, chloramphenicol, and erythromycin. The study found the existence of extended-spectrum β-lactamases and aminoglycoside 3-N-acetyltransferase, which contribute to multidrug resistance in *E. coli* isolates, which is in tandem to a study in Sudan that sought to identify the *bla*TEM, *bla*SHV and

blaCTX-M genes among *Enterobacteriaceae* isolates from patients in Khartoum, Sudan [16]. The global incidence of *E. coli* infections has been recorded, and investigations have revealed the presence of several extended-spectrum β -lactamase (ESBLs) genes among *Enterobacteriaceae* members [16,17]. *Enterobacteriaceae* (mainly *E. coli*) generating CTX-M enzymes have been discovered as a source of urinary tract infections, primarily in the community [18].

The study's findings also suggested that antibiotic resistance could emerge due to patients' indiscriminate purchase of medications over the counter and physicians' incorrect prescription of antibiotics [19]. Therefore, the prescriber should strictly follow the five rights of medication administration: the appropriate patient, the right medicine, the right dose, the right route, and the right time.

CONCLUSION

In this study, the occurrence of *E. coli* infection is high, with a high level of multiple antibiotic resistance. This study's recovery of resistance genes (*bla*CTX and *aac3-IV*) in the multiple antibiotic resistant *E. coli* identified in this study is of great health and economic implications. Furthermore, *E. coli* susceptibility to Nitrofurantoin, Erythromycin, and Chloramphenicol will assist clinicians in effectively managing *E. coli* infection in children in the research region.

Conflict of interest

No interest to declare.

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