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Resistance profile of isolated bacterial urinary tract infections in children in Abidjan from 2018 to 2020

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Urinary infection is a common pathology in children. It constitutes the second cause of pediatric consultation after respiratory infections. It is a public health problem, with 150 million cases of urinary tract infections recorded worldwide every year. The objective of this study was to update data on the prevalence of bacteria isolated from urinary infections in children and determine the evolution of sensitivity to the different antibiotics prescribed in Abidjan, Côte d'Ivoire. 118 cases of urinary tract infection were collected over a period of three years. The analysis was interpreted according to Kass criteria: leukocyturia>10^4/ml and bacteriuria>10^5 germs/ml for single isolated bacteria. The isolated strains were subjected to an antibiogram using the Kirby Bauer agar medium method. The interpretation was made according to the standards of the antibiogram committee of the French Society of Microbiology (CA-SFM). The bacteria isolated were mainly enterobacteria led by *Escherichia coli* (44%) followed by *Klebsiella pneumoniae* (12.7%). The sensitivity study globally showed a high rate of resistance to amoxicillin and amoxicillin-clavulanic acid with 79.6 and 64.4%, respectively. Cephalosporin resistance for cefuroxime, ceftazidime, cefixime, ceftriaxone and cefepime were 49.5, 38.1, 43.2, 44 and 34.7%, respectively. Resistance to third-generation cephalosporins by extended spectrum beta-lactamase (ESBL) production was 44.2%. Resistance to imipenem was 15.2% while that of amikacin was 2.5%.

Key words: Urinary tract infection, pediatrics, antibiotic resistance.

INTRODUCTION

Urinary tract infection is a common pathology in children, with a higher frequency in infants and young children (Karmazyn et al., 2017; Leung et al., 2019; Mattoo et al., 2021; Miron et al., 2021). It is a public health problem, with 150 million cases of urinary tract infections recorded worldwide every year (Alfuraiji et al., 2022). It is the second most common reason for paediatric consultations and antibiotic prescriptions after respiratory infections (Vazouras et al., 2019, Autore et al., 2023). It affects 8% of girls and around 2% of boys during the first eight years of life. In addition, 11% of children will present with an episode of urinary infection or a recurrence before the age of 16 (Nji et al., 2020; Esposito et al., 2021). They are responsible for considerable morbidity which in the long term can lead to complications such as septicaemia, to pyelonephritis or chronic kidney disease (Gunduz and

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Uludağ, 2018; Autore et al., 2023; Tiwari et al., 2023). Despite the frequency of this condition and the risk of irreversible complications in children, diagnosis remains low. In fact, a study carried out in North Carolina in the USA showed that the most common cause of infection in febrile infants with no other specific signs was a urinary tract infection (Antoon et al., 2019). The main bacteria responsible for these infections are members of the Enterobacteriaceae family, led by *Escherichia coli* (Ganesh et al., 2019; Ohnishi et al., 2020). In the management of these infections, it is essential to identify the microorganism responsible and select an effective antibiotic for treatment. However, the paucity of clinical signs often leads to late discovery of the infection, especially in infants and children under 2 years of age (Antoon et al., 2019; Autore et al., 2023). To limit the risk of complications, probabilistic antibiotic therapy is often initiated even before the results of the urine cytobacteriological examination are available (Mitiku, 2018; Autore et al., 2023; Kawalec et al., 2023). But one of the major consequences of the overuse of antibiotics is the risk of the emergence of antibiotic-resistant bacteria (Madhi et al., 2018; Esposito et al., 2022). Bacterial resistance to antibiotics in paediatric urinary tract infections is increasing worldwide, particularly in developing countries where empirical antibiotic therapy is the mainstay of treatment. (Mitiku et al., 2018; Shaaban et al., 2021, Rosado et al., 2022). In Ivory Coast, a study carried out by Kouassi-M'bengue et al. (2008) showed an increase in the resistance of enterobacteria to common antibiotics ranging from 66 to 85% in neonatal urinary tract infections. The aim of this study is to update data on the prevalence of bacteria isolated from urinary tract infections in children and to determine changes in sensitivity to the various antibiotics prescribed in Abidjan.

MATERIALS AND METHODS

Type of study and sampling

Study setting

The Clinical Bacteriology laboratory used for this study is a unit of the Department of Bacteriology and Virology at the Institut Pasteur de Côte d'Ivoire. As well as providing services, the laboratory is responsible for monitoring bacterial pathologies with epidemic potential, such as bacterial meningitis, shigellosis and cholera. The department also advises practitioners on the therapeutic management of patients. It carries out cytobacteriological tests on various biological products. The urine samples analysed came from both outpatients and hospital wards. The laboratory data came from the laboratory's data entry software.

Type and period of study

This is a retrospective cross-sectional study carried out in the clinical bacteriology laboratory of the Institut Pasteur of Côte d'Ivoire (IPCI). The data covered a three-year period from January 2018 to December 2020.

Study population and sampling

The data for this study were collected from patients' microbiological records and from the bacteriology laboratory's data entry software. This study included all urine from children aged 0 to 15 years for whom a request for a urine cytobacteriological examination had been expressed by a medical analysis bulletin.

Cytobacteriological examination of urine

A urine cytobacteriological examination was carried out on the urine samples in accordance with the recommendations of the medical microbiology guidelines (REMIC, 2018). Cytology was performed to look for bacteria and leukocytes, as well as other figurative elements such as red blood cells, crystals and columnar cells. The morphological character of the bacteria was assessed after Gram staining. A 10 µl calibrated loop was used for culturing on Uriselect ®4 chromogenic medium (Biorad-France). The agar plates were incubated in an oven at 37°C for 24 h. Identification of the bacteria was based on morphological, cultural and biochemical characteristics. For bacterial identification, API 20^{E},API NE, API® Staph, API® 20 Strep from BioMerieux™ and the Vitek®2 compact automated identification system (BioMérieux) were used. For biological interpretation, the culture was considered positive when bacterial growth was ≥ 10^5 CFU/ml (colony-forming unit per millimeter). Only culture with a monomicrobial culture were retained (Denis et al., 2016).

Antibiotic susceptibility testing of strains

Internal quality control was carried out in accordance with the recommendations of the Antibiogram Committee of the French Society of Microbiology/European Committee on Antimicrobial Susceptibility Testing antibiogram committee (CA-SFM/EUCAST, 2017) using the following reference strains: *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853 and *S. aureus* ATCC 25923. Antimicrobial susceptibility testing was performed using the Kirby-Bauer agar diffusion method (Bauer, 1966). The following antibiotics were tested: Amoxicillin (AMX: 20 µg), amoxicillin + clavulanic acid (AMC: 20 µg/10 µg); ceftriaxone (CRO: 30 µg), cefotaxime (CTX: 5 µg), cefixime (CFM: 5 µg); cefoxitin (FOX: 30 µg) ; gentamycin (GEN :10 µg) ; Amikacin (AN : 30 µg) ; Trimethoprime-sulfamethoxazole(TMP-SMX :1,25/23,75 µg) ; ciprofloxacin (CIP:5 µg), ceftazidime (CAZ: 10 µg) ;Imipenem (IMP:10 µg). The antibiogram was interpreted according to the recommendations of the Antibiogram Committee of the French Society of Microbiology/European Committee on Antimicrobial Susceptibility Testing depending on the year of the study (CA-SFM/EUCAST, 2017).

Resistance phenotypes

The various resistance profile detected were as follows:

1. ESBL (extended spectrum β-lactamase) profile: Resistance of enterobacteria to β-lactam antibiotics was detected using the synergy detection method between an amoxicillin+clavulanic acid disc and three third-generation cephalosporin discs, looking for a champagne cork image in accordance with the recommendations of the Antibiogram Committee of the French Microbiology Society for the detection of resistance mechanisms (EUCAST/CASFM, 2017).

Table 1. Distribution of isolated bacteria in children from 2018 to 2020.

Microorganism	**n**	**Percent**
Escherichia coli	52	44.0
Klebsiella pneumoniae	15	12.7
Enterobacter aerogenes	10	8.5
Staphylococcus aureus	7	5.9
Enterococcus faecalis	6	5.1
Enterobacter cloacae	5	4.2
Acinetobacter baumannii	5	4.2
Coagulase negative staphylococci	5	4.2
Morganella morganii	4	3.4
Pseudomonas aeruginosa	3	2.5
Providencia stuartii	2	1.7
Citrobacter koseri	1	0.9
Klebsiella oxytoca	1	0.9
Proteus mirabilis	1	0.9
Salmonella sp	1	0.9
Total	118	100.0

2. RFQ profile (fluoroquinolone cross-resistance): This is detected by resistance to ciprofloxacin.
3. BMR (multi-resistant bacteria): An isolate is multi-resistant if it is resistant to at least two or three families of antibiotics (Aruhomukama, 2020).

Data processing and statistical analysis

The data were retrieved in Microsoft Excell format and analysed using Epi Info software version 3.5.4. Quantitative variables were presented in the form of proportions, tables and figures. Qualitative variables were compared using the chi-square test. A value of $p < 0.05$ were considered statistically significant.

RESULTS

Sampling

Over the three years of the study, 118 positive urine samples were collected. The mean age was 3.93 years with extremes ranging from 0 to 14 years. Males accounted for 51.7% of the sample compared with 48.3% for females. The male/female sex ratio was 1.07 translating to 61 boys and 57 girls.

Identification of isolated germs

The bacteriological study summarised in Table 1 showed that Gram-negative bacilli with *Escherichia col*i were the main bacteria isolated from children (44%), followed by *Klebsiella pneumoniae* (12.7%) and *Enterobacter aerogenes* (8.4%). As for Gram-positive cocci, *Staphylococcus aureus* was the most commonly isolated species (5.9%).

Antibiotic sensitivity

Bacterial resistance to beta-lactam antibiotics was 79.6% for amoxicillin and 64.4% for amoxicillin-clavulanic acid. Resistance to cephalosporins was high, with cefuroxime, ceftazidime, cefixime, ceftriaxone and cefepime showing resistance levels of 49.5, 38.1, 43.2, 44 and 34.7%, respectively (Figure 1).

Resistance to imipenem was 15.2%, while resistance to erythromycin and amikacin was 27% and 2.5% respectively (Figure 2).

With regard to the resistance profile of *Escherichia coli* isolated during this study, shown in Figure 3, 100% of strains of this species were resistant to amoxicillin and 76.9% of strains were resistant to the combination of amoxicillin + clavulanic acid. Of *E. coli* strains tested,13.4% showed resistance to imipenem and 34.6%of these isolates expressed resistance to fluoroquinolones.

Resistance phenotype

The resistance phenotypes of the main bacteria isolated in urine are shown in Table 2. Among enterobacteria, 44.2% produced an extended-spectrum β-lactamase (ESBL) and 36.2% were resistant to fluoroquinolones. In 7.6% of cases, multidrug resistance with a combination of an extended-spectrum betalactamase and resisitance of imipenem (ESBL + Imipenem-R) was observed. One strain of *Escherichia coli* was resistant to fluoroquinolones and produced a β-lactamase

DISCUSSION

The purpose of this study was to determine the

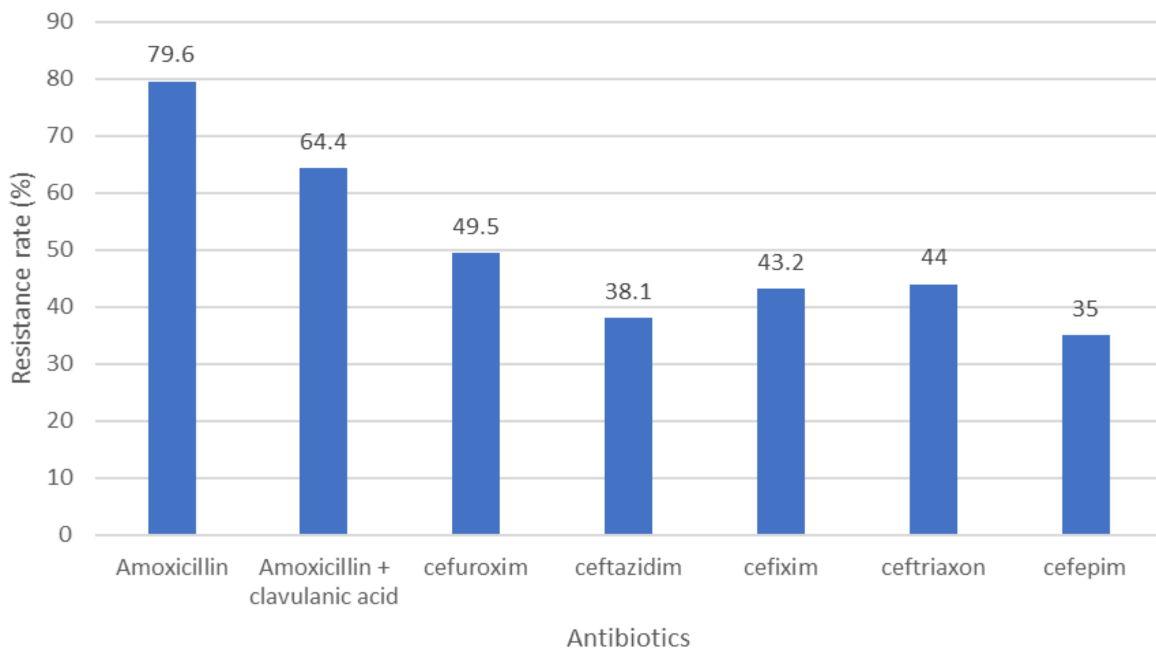

Figure 1. Resistance of bacteria isolated from infections of the urinary tract of children to β-lactams from 2018 to 2020.

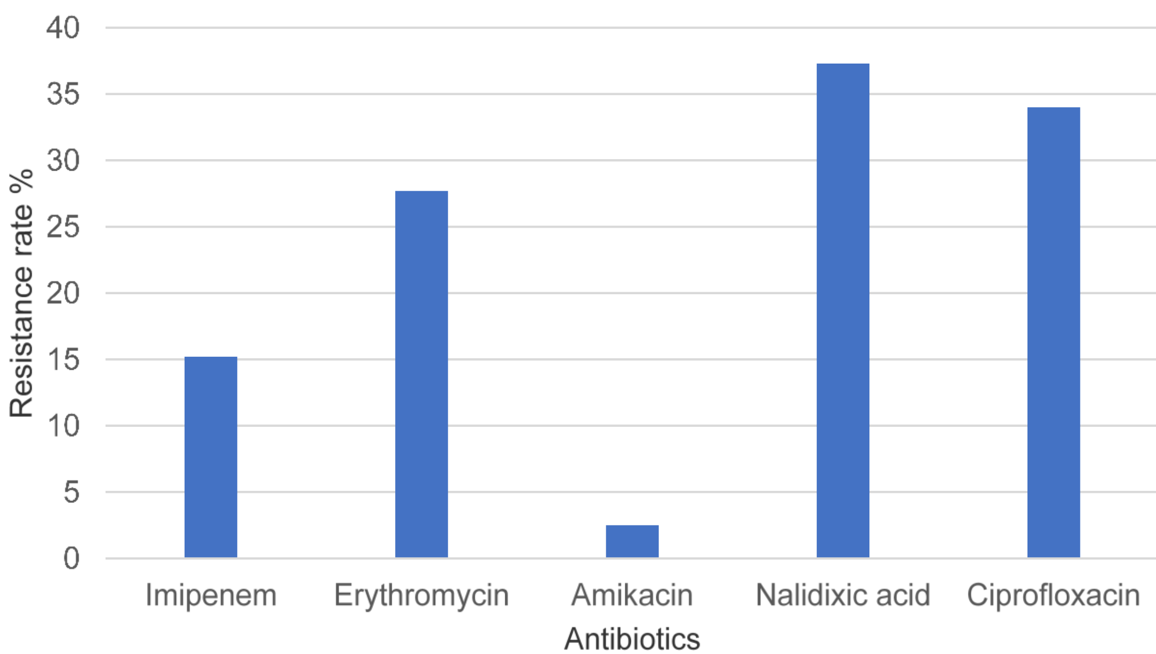

Figure 2. Bacterial resistance to other antibiotic families.

prevalence of bacteria responsible for urinary tract infections in children and the sensitivity of these bacteria to the different antibiotics used to treat these infections.

Urinary tract infection predominantly affected boys, with 61 cases compared with 57 for girls, giving a sex ratio of 1.07. These results are similar to those of Esposito et al. (2021) and Mattoo et al. (2021) who showed that during the first years of life, the incidence of urinary tract infections predominated in uncircumcised male children (Esposito et al., 2021). However, several other studies

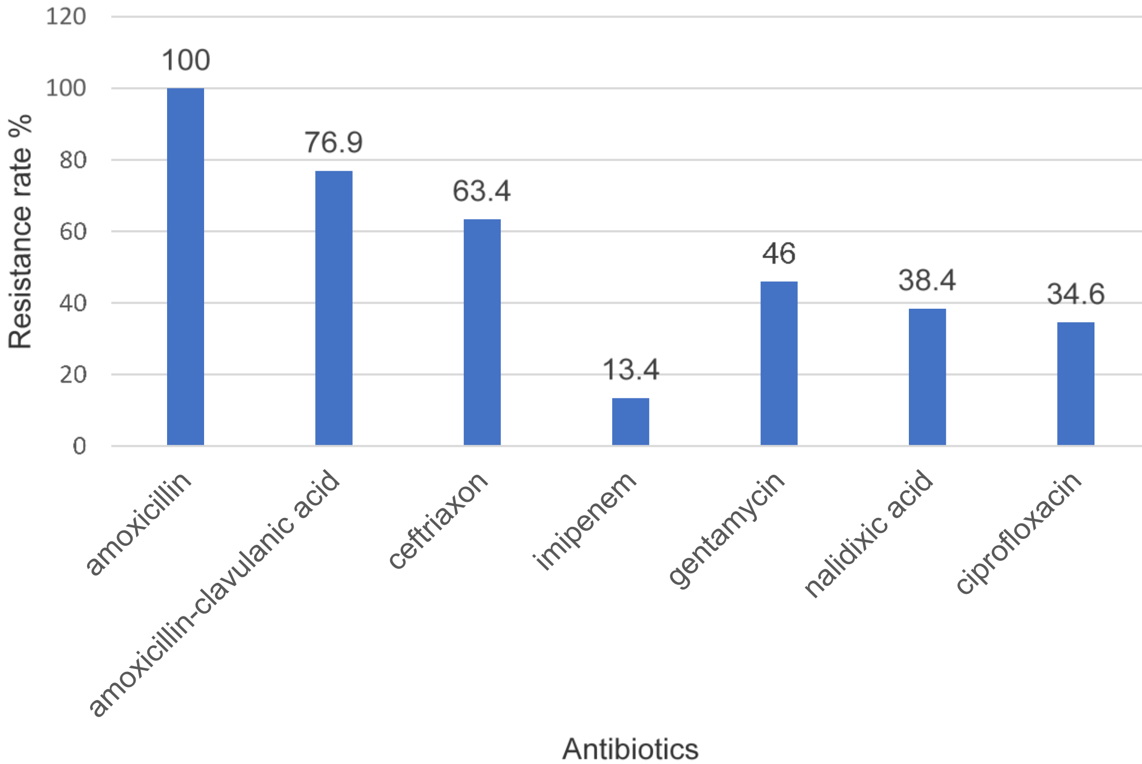

Figure 3. 2018-2020 antibiotic resistance profile of *Escherichia coli* uropathogen.

disagreed with our observations. These studies showed a predominance of urinary tract infections in girls. They explained this fact by the proximity of the girl's urogenital tract to the digestive tract, which can favour migration of bacteria from the digestive tract to the urinary tract (Kassogue, 2023). Another reason would be the existence of significant moisture in the perineum of the girl (Nguyen et al., 2022).

From a bacteriological point of view, the bacteria isolated during this study were dominated by Gram-negative bacilli, with *E. coli* predominating in 44% of cases. A study carried out in India on urinary tract infections in malnourished children showed that *E. coli* was isolated in 54.54% of urinary tract infection cases in this study (Tiwari et al., 2023). Another study carried out in Madagascar stated that E.coli was the main agent responsible for healthcare-associated or community-acquired urinary tract infections (Rakotovao-Ravahatra et al., 2017). This observation has been made by several other authors in similar proportions (Mahony et al., 2020; Iqbal et al., 2021). According to some authors, the predominance of enterobacteria in urinary tract infections is due to the proximity of the digestive tract to the urinary tract. The presence of pili also helps bacteria to attach themselves to the uroepithelial mucosa, enabling them to multiply and invade tissues, ultimately creating invasive infections (Esposito et al., 2022; Zerefaw et al., 2022). In agreement with these authors, the study can conclude that the prevalence of bacteria isolated from urinary tract infections in children is dominated by enterobacteria.

With regard to the susceptibility of isolates to antibiotics, susceptibility tests were used to establish the susceptibility profile of the bacteria isolated. Overall, the strains isolated in this study showed a clear increase in resistance to most of the standard antibiotics used in the treatment of urinary tract infections in children. The percentage of beta-lactam resistance to amoxicillin and amoxicillin-clavulanic acid rose to 79 and 64% respectively; Mekonnen et al., (2023) explained that this was the result of selection pressure linked to overuse of these antibiotics. The rise in resistance to amoxicillin and amoxicillin-clavulanic acid is thought to be due to the empirical prescribing of these drugs, particularly in outpatient clinics while waiting for the results of urine analysis (Hameed et al., 2019; Autore et al., 2023).This resistance could also be explained by a reduction in the activity of the beta-lactamase inhibitor, resulting from hyperproduction of penicillinases, or inactivation of the inhibitor itself (Esposito et al., 2022). Rakotovao-Ravahatra et al. (2017) study revealed that the extent of antibiotic resistance is probably due to the inappropriate use of these molecules, especially penicillins, which are frequently used in the course of any infection. Self-medication is also a real problem, rapidly encouraging the emergence of resistant germs.

The cephalosporin class was also affected by this high

Table 2. Resistance phenotypes of urine-isolated bacterial strains in children, 2018 to 2020.

Phenotypes of resistance	(%)
ESBL	44.2
RFQ	36.5
ESBL + Imipenem R	7.6
ESBL + Gentamycin R	19.2
Ciprofloxacin R + Imipenem R	1

ESBL, Extended Spectrum β-Lactamase phenotype; RFQ, Fluoroquinolone resistance phenotype; ESBL + Imipenem R, extended spectrum β-Lactamase phenotype associate with resistance to imipenem; ESBL + Gentamycin R, extended spectrum β-Lactamase phenotype associate with resistance to gentamycin; Ciprofloxacin R+ Imipenem R, resistance to ciprofloxacine associate with resistance to imipenem.

level of resistance, with 49.5% for cefuroxime, 38% for ceftazidime, 43% for cefixime, 44% for ceftriaxone and 34.7% for cefepime. These results were similar to those of Masika et al. (2017) in Kenya, where resistance was 87 and 74% for the first two molecules, amoxicillin and amoxicillin-clavulanic acid. Resistance to cephalosporins, ranging from 0 to 3.2%, was low compared with our data. According to a study carried out in Vietnam, the rate of *E. coli* resistance to cephalosporins was on the rise, reaching 66.7% in 2020 (Nguyen et al., 2022).

With regard to the sensitivity of *E. coli* to antibiotics, it should be noted that this bacterium, which was naturally sensitive to antibiotics, has seen its percentage of resistance increase over time. In this study, 100% of *E. coli* strains were resistant to amoxicillin and 76% to amoxicillin-clavulanic acid. Resistance to ceftriaxone was 63.4%. Several studies carried out previously in Côte d'Ivoire had already shown this increase in resistance. In 2008, the study by Kouassi-M'Bengue et al., 2008 showed rates of 75 and 25% respectively for amoxicillin and amoxicillin-clavulanic acid. In 2014, Boni-Cissé showed 51.3 and 37.8% resistance to these two molecules (Boni-Cissé, 2014). Elsewhere, in Pakistan, a study also showed significant antibiotic resistance of over 80% (Iqbal et al., 2021). With carbapenems such as imipenem, the proportion of resistance was 13%. According to the Demir and Kazanasmaz (2020) study in Turkey, *E. coli* resistance to imipenem was low at 1.4%. Parenteral use of aminoglycosides is very common in the treatment of infections in hospital patients In the case of gentamycin, the aminoglycoside commonly used in our hospital environment, the resistance rate was 46%. This high rate of resistance could be explained by the frequent use of this molecule in hospitals for the treatment of infections in children (Fenta et al., 2020).

Analysis of the phenotypes of *E. coli* strains isolated from this study showed that 44.2% produced an enlarged-spectrum betalactamase (ESBL) and 36.5% had cross-resistance to fluoroquinolones. In addition, this study observed multiple resistance associations with 7.6% of strains that presented both an ESBL with imipeneme resistant, 19.2% ESBL associated with gentamycin resistance and one strain had both ciprofloxacin and imipenem resistance.

Analysis of the phenotypes of the *E. coli* strains isolated in this study showed that 44.2% produced an extended-spectrum β-lactamase (ESBL) and 36.5% showed resistance to fluoroquinolones. Other authors have shown similar ESBL rates to our study. In Demir and Kazanasmaz (2020)'s study of uropathogens in outpatient and inpatient children in Turkey, the rate of ESBL *E. coli* isolates was 34.8%. Another study conducted by He (2023) showed that the percentage of ESBL-producing Enterobacteriaceae was 21.4% in 2021. In addition, this study revealed multi-drug resistance, with 7.6% of strains producing both β-lactamase and carbapenemase. Multidrug resistance was defined as resistance of a bacterial strain to more than one or two families of antibiotics. In addition, 15.3% of the isolates in our study were resistant to fluoroquinolones associated with the production of β-lactamase enzymes, and one strain was resistant to both ciprofloxacin and imipenem.

Conclusion

Urinary tract infection in children is a problem in diagnosis because it requires rapid management to avoid long-term complications in children. The bacterial ecology of this pathology was dominated by enterobacteria with mainly *E. coli* (44%).

The growing increase in resistance to the usual antibiotics foreshadows the difficulties clinicians will face in managing urinary tract infections in children in the near future. Therefore, the prescription of antibiotics for empirical treatment of these infections should be avoided. The establishment of collaboration between clinicians and biologists to lead to increased surveillance in the prescription and consumption of antibiotics is necessary because of these high rates of resistance. This resistance monitoring must be done by cytobacteriological examination of urine systematically before any antibiotic therapy.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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