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CLINICAL AND EPIDEMIOLOGICAL CHARACTERISTICS OF URINARY TRACT INFECTIONS IN FEBRILE CHILDREN AND ANTIMICROBIAL RESISTANCE GENOTYPES OF *ESCHERICHIA COLI*

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PhD. DISSERTATION ABSTRACT

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LIST OF SCIENTIFIC PUBLICATIONS RELATED TO THE THESIS

1. Current status of urinary tract infections in febrile children aged 2 months to 15 years at Nghe An Obstetrics and Pediatrics Hospital. Vietnam Medical Journal, August – Special Issue, 2019, pp. 148-154.
2. Clinical epidemiological and laboratory characteristics of 71 cases of urinary tract infections caused by E. coli at Nghe An Obstetrics and Pediatrics Hospital. Vietnam Medical Journal, December – Thematic Issue, 2021, pp. 124-130.
3. Clinical Epidemiology Characteristics and Antibiotic Resistance Associated with Urinary Tract Infections Caused by *E. coli*. International Journal of Nephrology, Volume 2022, Article ID 2552990, 5 pages, https://doi.org/10.1155/2022/2552990.
4. Evaluation of antibiotic resistance and ESBL-encoding gene characteristics of *E. coli* causing urinary tract infections in children at Nghe An Obstetrics and Pediatrics Hospital. Vietnam Medical Journal, June – Special Issue Part 2, 2022, pp. 398-405.
5. Whole-Genome Sequencing Reveals Temporal Trends in Antibiotic Resistance Genes in *Escherichia coli* Causing Pediatric Urinary Tract Infections in Central Vietnam. Antibiotics, 2024, 13(9), 830, https://doi.org/10.3390/antibiotics13090830.
6. A novel gene linked to Imipenem resistance in E. *coli* isolate lacking known Imipenem-resistance genes. Scientific Reports, (2025) 15:9065, https://doi.org/10.1038/s41598-025-93587-0.
7. Clinical epidemiological and bacteriological characteristics of urinary tract infections in pediatric patients aged 2 months to 15 years at Nghe An Obstetrics and Pediatrics Hospital. Vietnam Medical Journal, June – Special Issue, 2025, Volume 551, pp. 185-193.

INTRODUCTION

Urinary tract infection (UTI) is one of the most common infectious diseases in children. According to the World Health Organization (WHO), it is estimated that there are about 19 episodes of UTI per 1,000 children annually. The prevalence of UTI ranges from approximately 1.2% to 29%. In Vietnam, the prevalence of UTI recorded in hospital-based studies varies from 7.95% to 22.3%. The most common causative pathogens are bacteria, among which *Escherichia coli (E. coli)* ranks first. A challenging and highly topical issue in the treatment of infectious diseases in general, and UTIs in particular, is the increasing antibiotic resistance among causative strains. Bacterial antibiotic resistance is fundamentally driven by genes and the emergence of more complex bacterial phylogroups, meaning that bacteria inherently generate resistance genes within their cells, thereby diversifying into complex phylogroups. The resistance capacity of E. coli is directly encoded by resistance genes (such as *bla*CTX-M, *bla*TEM, *bla*NDM, mcr-1, etc.) located on the chromosome or mobile genetic elements like plasmids. These genes can undergo horizontal gene transfer to other bacteria, causing outbreaks of drug-resistant epidemiological waves that are difficult to control.

Nghe An Obstetrics and Pediatrics Hospital is a major hospital in the North Central region, with a capacity of over 1,000 beds, and the patients are predominantly children. Therefore, what are the clinical epidemiological characteristics of febrile UTIs in children aged 2 months to 15 years at Nghe An Obstetrics and Pediatrics Hospital? What are the bacterial etiologies and their current antibiotic resistance status? What are the antibiotic resistance genes of *E. coli*? These questions urgently require answers. Thus, we conducted this study with the following objectives:

1. To describe the clinical epidemiological characteristics of urinary tract infections in febrile children aged 2 months to 15 years at Nghe An Obstetrics and Pediatrics Hospital from 2018 to 2024.
2. To determine the bacterial etiologies and the current status of antibiotic resistance of bacteria causing urinary tract infections in the aforementioned patients.
3. To identify several antibiotic resistance genotypes of *E. coli* bacteria isolated from these patients.

NEW CONTRIBUTIONS OF THE THESIS

1. Provides additional epidemiological, clinical, and paraclinical data on urinary tract infections in febrile children in the North Central region of Vietnam.
2. Clarifies the microbiological etiology and antimicrobial resistance characteristics of *Escherichia coli* causing urinary tract infections in children.

3. Applies whole-genome sequencing to characterize the antimicrobial resistance gene profile in *E. coli*, revealing an increase in several important resistance genes, including *blaNDM-5*.
4. Identification of a novel resistance gene: the ILR-VIN gene may provide a new avenue for understanding carbapenem resistance mechanisms in *E. coli*, particularly in the context of the increasing emergence of strains resistant to antibiotics such as imipenem..

STRUCTURE OF THE THESIS

The main body of the thesis is 149 pages long, including the following sections: Introduction: 3 pages; Chapter 1 - Literature Review: 38 pages; Chapter 2 - Subjects and Research Methods: 23 pages; Chapter 3 - Research Results: 38 pages; Chapter 4 - Discussion: 43 pages; Conclusion: 2 pages; New contributions: 1 page; Recommendations: 1 page. The thesis contains 38 tables, 23 figures, and 192 references, of which 51 are in Vietnamese and 141 are in English.

CHAPTER 1
LITERATURE REVIEW

1.1. Epidemiological Characteristics of Urinary Tract Infections in Children

1.1.1. Prevalence

- Globally, the prevalence of UTI varies widely depending on the country-specific studies and whether they are community- or hospital-based, fluctuating from 1.2% to 29%.

- Hospital-based studies in Vietnam indicate that the UTI prevalence ranges from 7.95% to 22.3%.

1.1.2. Prevalence of Urinary Tract Infection by Age

- The prevalence of UTI is highest in children < 2 years old, followed by the 2–5 years age group, and is less common in children > 5 years old. The mean age of UTI onset is 5.6±4.2 years.

1.1.3. Prevalence of Urinary Tract Infection by Sex

In Vietnam, authors also note that prior to 1 year of age, UTIs are more common in boys, whereas after this age, a distinct female predominance is observed. UTIs are more frequent in girls due to the anatomical structure of the urinary tract, where the urethral meatus is located close to the anus, an area consistently harboring UTI-causing bacteria originating from the terminal colon.

1.1.4. Prevalence of Urinary Tract Infection According to Place of Residence

The prevalence of urinary tract infection (UTI) differed between rural and urban areas, though findings were not fully consistent across studies. Several reports showed higher rates in rural populations. Dang Van Chuc (2010) found lower UTI prevalence in urban than in rural and coastal areas of Hai Phong, while Nguyen Thi Ly and Nguyen Ngoc Sang (2024) reported that 59.9% of pediatric UTI cases were from rural areas compared with 40.1% from urban areas.

1.1.5. Prevalence of urinary tract infection by season

Urinary tract infections (UTIs) in children tend to increase in summer and early autumn (April–July). In Vietnam, studies have shown a clearly higher incidence during this period, with 45.2% of cases occurring in April–June and an increasing trend in May–July. International studies also report an association between temperature, rainfall, and sunshine duration and UTI rates, with warm and humid climates favoring bacterial growth.

1.2. Bacterial Etiology and Antibiotic Resistance Status of Bacteria Causing Urinary Tract Infections in Children

1.2.1. Bacterial Etiology

The causative pathogens can be bacteria, viruses, fungi, or parasites. Among these, bacteria are the most common cause, with gastrointestinal-derived bacteria remaining the primary agents. Both domestic and international studies show that the predominant pathogens are Gram-negative bacilli, led by *E. coli*, followed by *Proteus spp.*, *Klebsiella spp.*, etc.

1.2.2. Antibiotic Resistance Status of Bacteria

In Vietnam, according to the Ministry of Health, *E. coli* has shown decreased susceptibility to third-generation cephalosporins and a high resistance rate to co-trimoxazole ranging from 60% to 80%. Concurrently, the rate of extended-spectrum β-lactamase (ESBL)-producing bacteria is at an alarming level, with the National Children's Hospital reporting a rate of 37.6% for *E. coli* and 51.3% for *Klebsiella spp.*

Numerous domestic and international studies have observed that the antibiotic resistance of pathogenic bacteria is increasing; specifically, *E. coli* has exhibited resistance rates of 92.5-100% to ampicillin, 74.1-96.3% to co-trimoxazole, 46.3-52.1% to ceftriaxone, and 57.8-81.84% to chloramphenicol.

1.3. Overview of Genes Related to Bacterial Antibiotic Resistance

1.3.1. Role of Extended-Spectrum β-lactamases (ESBLs) in Bacterial Antibiotic Resistance

In the early 1960s, for the first time in history, scientists discovered the TEM-1 enzyme, a plasmid-mediated β-lactamase isolated from a Greek patient named Temoniera who suffered from E. coli septicemia. Approximately 20 years later, many β-lactam antibiotics were developed to counteract the hydrolytic activity of β-lactamases. However, as each new generation of antibiotics was introduced into clinical treatment, a new β-lactamase emerged, resulting in drug resistance. Currently, around 150 different ESBLs have been described. Most ESBLs originate from the TEM, CTX-M, and SHV enzymes, which are considered widespread and highly influential; these ESBL enzymes constantly evolve, increasing in number and becoming more complex in their characteristics.

The application of molecular biology techniques for the rapid identification of E. coli strains carrying antibiotic resistance genes is crucial. It assists

clinicians in making early prognoses regarding antibiotic-resistant bacteria and selecting appropriate antibiotics, thereby reducing treatment costs and saving patient lives.

1.3.2. Next-Generation Sequencing (NGS) Technology

Gene sequencing (DNA sequencing) is the process of determining the order of nucleotide bases (As, Ts, Cs, and Gs) within a segment of a DNA molecule. Whole-genome sequencing of organisms with large DNA lengths is highly complex. This process requires fragmenting the genomic DNA molecule into smaller pieces, which are then sequenced and assembled into a longer sequence or an entire chromosome. Consequently, whole-genome sequencing previously consumed massive amounts of time and effort.

The launch of the first high-throughput sequencing platform in the mid-2000s reduced the cost of gene sequencing by 50,000-fold compared to the prior cost of the Human Genome Project, and was named Next-Generation Sequencing (NGS). The first wave of sequencing technology occurred around 2005, known as second-generation sequencing technology. These technologies, including 454, Illumina, and Ion Torrent, generated short but highly accurate (>99%) sequence reads.

Third-generation sequencing technologies, notably Pacific Biosciences (PacBio) and Oxford Nanopore, emerged in the early 2010s, providing complementary sequencing data with much longer read lengths (averaging over 50 Kb) but lower accuracy (85–95%).

Additionally, there are sequencing platforms with affordable pricing for microbiology laboratories or hospitals. Many microbiology laboratories in Vietnam have been equipped with sequencers such as Illumina MiSeq and Oxford Nanopore MinION, which are capable of bacterial whole-genome sequencing. The number of sequenced bacterial genomes is growing exponentially. Sequencing the genetic blueprint of these bacteria could be the key to understanding the evolution of resistance in superbugs and identifying genetic markers for the rapid diagnosis of bacterial infections.

The NGS technique helps preliminarily determine the bacterial genome sequence within 1 to 2 days from bacterial isolation at an affordable cost suitable for clinical practice. The outstanding advantage of NGS over legacy sequencing techniques lies in its value for the epidemiological study of infectious diseases. A major advantage in the clinical application of NGS compared to legacy methods is its broad applicability to a wide variety of different bacterial pathogens.

Illumina sequencing technology, which utilizes Sequencing by Synthesis (SBS), is the most widely adopted next-generation sequencing technology worldwide, generating over 90% of the world's sequencing data. SBS uses four fluorescently labeled nucleotides to sequence tens of millions of clusters simultaneously on a flow-cell surface. During each sequencing cycle, a single labeled deoxynucleoside triphosphate (dNTP) is added to the nucleic acid chain.

The fluorescent label of the nucleotide acts as a reversible terminator for the polymerization reaction; thus, after each dNTP is integrated, the fluorescent dye is imaged to identify the nucleotide and is subsequently cleaved to allow the synthesis of the next nucleotide. Because all four dNTPs with reversible terminators are present simultaneously as single molecules, natural competition minimizes incorporation bias. Base calling is determined by the signal intensities measured in each cycle, reducing raw errors compared to other technologies. The end result is a highly accurate base-by-base sequence readout that eliminates sequence-specific errors, enabling robust sequencing across the entire genome, including repetitive sequences.

CHAPTER 2
SUBJECTS AND METHODS

2.1. Subjects, Timeframe, and Study Location

Study population: 8,791 pediatric patients aged 2 months to 15 years presenting with fever examined at Nghe An Obstetrics and Pediatrics Hospital from June 1, 2018, to May 31, 2024, among whom 916 pediatric patients were diagnosed with and treated for UTI at Nghe An Obstetrics and Pediatrics Hospital during the same period.

2.1.1. Inclusion Criteria

- Diagnostic criteria for fever followed the Integrated Management of Childhood Illness (IMCI) guidelines, defined as an axillary temperature ≥37.5∘C measured using a mercury thermometer.
- Diagnosis of UTI followed the 2015 European Association of Paediatric Urology (EAPU) guidelines, which incorporate clinical symptoms and urinalysis parameters.

2.1.2. Exclusion Criteria

- Patients diagnosed with UTI who had concurrent comorbidities or severe conditions requiring intensive care unit (ICU) resuscitation.
- Children with hospital-acquired (nosocomial) UTIs developing during hospital stay.
- Families refusing to consent to participate in the study.
- Patients whose urine culture yielded more than one pathogenic bacterial species (polymicrobial culture).

2.2. Research Methods

2.2.1. Study Design

- Cross-sectional descriptive study (for Objectives 1 and 2).
- Experimental and cross-sectional descriptive study applying molecular biology techniques (for Objective 3).

2.2.2. Sample Size and Sampling Method

- Objectives 1 & 2: Exhaustive sampling, including all children who met the diagnostic criteria for fever and UTI within the study timeframe.

- Objective 3: Purposive sampling, selecting 47 out of 186 E. coli strains for whole-genome sequencing (WGS).

2.2.3. Research Indices and Variables

Objective 1: To describe the clinical epidemiological characteristics of urinary tract infections in febrile children aged 2 months to 15 years at Nghe An Obstetrics and Pediatrics Hospital from 2018 to 2024.

- Determine the UTI rate, age of onset, sex, and geographical distribution.

- Clinical factors: Chief complaint upon admission, clinical symptoms, urinalysis, complete blood count (CBC), C-reactive protein (CRP), and urinary tract imaging.

Objective 2: To determine the bacterial etiology and current antibiotic resistance status of bacteria causing urinary tract infections in children.

- Determine the positive urine culture rate, distribution of UTI-causing bacterial strains, and the proportion of ESBL-producing *E. coli*.

- Antibiogram (antibiotic susceptibility testing - AST) profiles of select UTI-causing bacteria.

Objective 3: To identify several antibiotic resistance genes of *E. coli* bacteria isolated from these patients.

- Sequence types (ST) of *E. coli* strains.

- Prevalence of antibiotic resistance genes: TEM gene, CTX-M gene, *bla*EC gene, dfrA17 gene, sul1 gene, sul2 gene, gyrA gene, parC gene, etc.

- Distribution of genotypes conferring resistance to β-lactams, aminoglycosides, trimethoprim, sulfonamides, quinolones, macrolides, and tetracyclines; carbapenem-resistant *E. coli* carrying the carbapenemase-encoding gene *bla*NDM-5.

- Correlation between phenotypic resistance on antibiograms and the presence of resistance genes.

- Identification of a novel resistance gene: the ILR-VIN gene conferring Imipenem resistance.

2.2.4. Techniques and Materials Used in the Study

- Urine Collection Technique: Midstream clean-catch urine collection.

- **Urinalysis Technique**: Urinalysis performed using the Clinitek Novus Automated Urine Analyzer (Siemens) with the Clinitek Novus cassette kit.

- Urine Culture for Bacterial Identification:

+ Chemicals/Media: Blood agar, UTI agar, Gram stain kit, immersion oil, identification cards, and antibiotic discs.

+ Microscopy: Screening for the presence of bacteria, polymorphonuclear leukocytes (PMNs), and epithelial cells.

- Bacterial Identification and Antibiotic Susceptibility Testing (AST): Automated identification, AST, and ESBL detection performed via the VITEK®2 Compact system.

- **Bacterial Strain Collection and Preservation Technique**: Antibiotic-resistant *E. coli* strains were preserved in Brain Heart Infusion (BHI) broth containing 20% glycerol (cryotubes), stored at -80∘C, and transferred to the Department of Gene Technology and Genetics, Military Medical Research Center, Military Medical Academy, for antibiotic resistance gene analysis.

- **Next-Generation Sequencing (NGS) Technique**: Total DNA was extracted from the isolated E. coli strains, quantified, and concentration-normalized for sequencing. Whole-genome sequencing of E. coli was executed using Illumina NGS technology with universal primers provided in the manufacturer's kit (MiSeq v2 Reagent Kit 300 cycles, Ref: 15033624). Labeled DNA was subjected to paired-end sequencing. Sequencing data were downloaded and analyzed using the AMROMICS bioinformatics platform (https://platform.dev. amromics.org). This platform was developed under the project "Solving the Problem of Antibiotic-Resistant Bacteria Using DNA Sequencing Technology and Big Data Analysis" (Code: VINIF.2019.DA11), funded by the Vingroup Innovation Foundation (VINIF).

- The AMROMICS platform integrates state-of-the-art and highly reliable bacterial whole-genome analysis tools, including sequence quality control via FastQC, sequence trimming via Trimmomatic, genome assembly via Shovill, gene annotation via Prokka, and screening for antibiotic resistance and virulence genes via Abricate against databases such as CARD, Megares, Resfinder, Argannot, NCBI, and Plasmidfinder. Concurrently, the platform utilizes PubMLST data for species Sequence Type (ST) determination and Kraken2 for taxonomic identification. Following single-sample profiling, core and accessory genome analyses across the sample cohort were performed using Roary. For each coding gene cluster, nucleotide sequences were translated into protein sequences to execute multiple sequence alignments via MAFFT, identifying amino acid alterations and nucleotide mutations. Finally, IQ-TREE was utilized to construct phylogenetic trees for each gene cluster. This pipeline was implemented in Python 3.6 and the analysis steps are hosted on GitHub (https://github.com/amromics/amrviz).

2.2.5. Data Collection Tools and Statistical Processing

Data were entered using Microsoft Excel. Statistical analyses were performed using SPSS 20.0 software.

2.2.6. Research Ethics

The study protocol strictly adhered to established medical research ethics guidelines.

CHAPTER 3
RESEARCH RESULTS

3.1. Clinical Epidemiological Characteristics of Urinary Tract Infections in Febrile Children Aged 2 Months to 15 Years at Nghe An Obstetrics and Pediatrics Hospital from 2018 to 2024

3.1.1. Epidemiological Characteristics

- The prevalence of UTI among febrile children aged 2 months to 15 years at Nghe An Obstetrics and Pediatrics Hospital was 10.4% (916 patients / 8,791 febrile patients).

- Sex distribution: Of the 916 children with UTI, 470 were girls (51.3%) and 446 were boys (48.7%). The female-to-male ratio was 1.05:1.

- Age distribution: The mean age was 41.37±39.28 months. The 2–24 months group accounted for the highest proportion at 46.4%, followed by the 25–60 months group at 28.5%, and the > 60 months group at 25.1%. In the 2–24 months range, UTIs predominated in boys (27.3%), and the sex variation across age groups was statistically significant ($p<0.05$).

- Geographical distribution: The majority of pediatric UTI patients resided in rural areas, accounting for 74.0%.

- Seasonal distribution: UTI cases occurred sporadically throughout the year, but the highest incidence was recorded from May to August.

3.1.2. Clinical Characteristics of Urinary Tract Infections

Table 3.1. Reasons for Admission

Reason for Admission		Quantity (*n*)	Percentage (%)
Isolated fever		267	29.1
High fever with convulsions		8	0.9
High fever with chills		19	2.1
Fever accompanied by:	Gastrointestinaldisturbances	13	1.4
	Urinary disturbances	511	55.8
	Abdominal pain	98	10.7
Total		**916**	**100**

Observation: Children were admitted for various reasons, with over 50% presenting with fever accompanied by urinary disturbances. A minority presented with fever alongside gastrointestinal disturbances or abdominal pain.

Table 3.2. Body Temperature Distribution

Temperature	Number of Patients (n)	Percentage (%)
37.5−38.4∘C	580	63.3
38.5−39.5∘C	172	18.8
>39.5∘C	164	17.9
Total	916	100

Observation: The majority of children with UTI had a fever ranging from 37.5 to 38.4∘C (63.3%), followed by 38.5 to 39.5∘C (18.8%). The remaining 17.9% presented with temperatures above 39.5∘C

Table 3.3. Distribution of Localized Urinary Tract Symptoms

Urinary Tract Symptom	Number of Patients (*n*)	Percentage (%)
Pollakiuria (Urinary frequency)	469	51.2
Turbid urine (Cloudy urine)	357	39.0
Dysuria (Painful urination)	284	31.0
Crying during urination / Painful voiding	179	19.5
Urinary hesitancy / Difficulty voiding	134	14.6
Hematuria (Blood in urine)	82	9.0

Observation: The most common urinary tract symptom was pollakiuria (51.2%), followed by turbid urine (39%) and dysuria (31%).

Table 3.4. Distribution of Extra-Urinary Clinical Symptoms

Extra-Urinary Symptom	Number of Patients (*n*)	Percentage (%)
Irritability / Crying	186	20.3
Abdominal pain	165	18.0
Diarrhea	80	8.7
Vomiting	64	7.0
Flank pain / Low back pain	22	2.4

Observation: Extra-urinary clinical manifestations such as abdominal pain, vomiting, and diarrhea can occur in pediatric patients with UTI.

Hematological profiles: The mean white blood cell (WBC) count was 14.6±7.0×109/L (range: 3.3−46.4×109/L). Leukocytosis was present in 70.2% of cases, and neutrophilia was observed in 47.3%.

Serum CRP: The mean serum C-reactive protein (CRP) level was 44.4±2.1 mg/L (*SD*=80.9 mg/L, median = 13.8 mg/L, range: 0.1−400.6 mg/L). Serum CRP ranged between 10−40 mg/L in 21.4% of patients, and exceeded 40 mg/L in 33.5% of cases.

Table 3.5. Distribution of Hematuria, Leukocyturia, and Nitrituria

Test Parameter		Number of Patients (*n*)	Percentage (%)
Leukocyturia (*n*=916)	151	16.5	16,5
	330	36.0	36,0
	310	33.8	33,8
	125	13.7	13,7
Hematuria (*n*=916)	373	40.7	40,7
	286	31.2	31,2
	167	18.3	18,3
	90	9.8	9,8
Nitrituria (*n*=916)	707	77.2	77,2
	209	22.8	22,8

***Observation**:* Leukocyturia was predominantly scored at ++ and +++ levels (36.0% and 33.8%), indicating pronounced inflammation. Hematuria was positive in 59.2% of cases, whereas nitrituria was positive in only 22.8%.

Imaging findings: Among 186 pediatric patients evaluated via urinary tract ultrasound, X-ray, or CT scan, structural abnormalities were detected: the most common lesion was pelvicalyceal ectasia and hydronephrosis (54.8%), followed by urinary bladder wall thickening (16.7%), renal-ureteral malformations (14.0%), and duplex collecting systems (4.3%). Less common lesions included vesicoureteral reflux (VUR), neurogenic bladder, ureteropelvic junction (UPJ) obstruction, unilateral renal ectasia with contralateral renal atrophy, and nephrolithiasis.

3.2. Bacterial Etiology and Current Antibiotic Resistance Status of Bacteria Causing Urinary Tract Infections in Children

Of the 916 UTI patients, 735 (80.2%) underwent urine culture. Among these, 285 samples (38.8%) demonstrated significant pathogen growth. Gram-negative bacteria constituted the highest proportion with 244 samples (85.6%). Gram-positive bacteria and fungi were recovered at lower frequencies, representing 26 samples (9.1%) and 15 samples (5.3%), respectively.

Table 3.6. Distribution of Bacteriuria Isolates

Bacteria Species	2018–2021 *n* (%)	2021–2024 *n* (%)	Total *n* (%)
E. coli	71 (84.5)	115 (61.8)	186 (68.9)
Enterococcus spp.	1 (1.2)	25 (13.4)	26 (9.6)
Klebsiella spp.	2 (2.4)	14 (7.5)	16 (5.9)
Proteus spp.	3 (3.6)	13 (7.0)	16 (5.9)
P. aeruginosa	3 (3.6)	7 (3.8)	10 (3.7)
Acinetobacter spp.	2 (2.4)	5 (2.7)	7 (2.6)
Others[1]	2 (2.4)	7 (3.8)	9 (3.3)
Total	**84 (100)**	**186 (100)**	**270 (100)**

[1] *Includes: E. cloacae complex, C. freundii, M. morganii, E. asburiae, P. agglomerans*

Observation: The number of isolates partitioned in the 2021–2024 period more than doubled compared to 2018–2021. *E. coli* remained the primary agent, though its relative proportion decreased in the later phase. Emerging or increasing pathogens in 2021–2024 included *Enterococcus spp.*, *Klebsiella spp.*, and *Proteus spp*.

Table 3.7. Antibiotic Resistance Rates of Enterococcus spp. Strains (*n*=26)

Antibiotic	Tested Cases (n)	Resistant Cases (n)	Resistance Rate (%)
Ampicillin	9	9	100.0
Benzylpenicillin	8	8	100.0
Gentamicin	15	15	100.0
Amoxicillin/Clavulanic acid	14	10	71.4
Clarithromycin	14	6	42.9
Linezolid	22	5	22.7
Vancomycin	8	1	12.5

Observation: *Enterococcus spp.* isolates exhibited 100% resistance to ampicillin, gentamicin, and benzylpenicillin; high resistance to piperacillin, ertapenem, amoxicillin/clavulanic acid, and streptomycin; while maintaining susceptibility to vancomycin, clarithromycin, and linezolid.

Table 3.8. Antibiotic Resistance Rates of *E. coli* Strains (*n*=186)

Antibiotic	Tested Cases (*n*)	Resistant Cases (*n*)	Resistance Rate (%)
Ampicillin	91	87	95.6
Amoxicillin/Clavulanic acid	162	100	61.7
Piperacillin/Tazobactam	107	16	15.0
Cefotaxime	88	55	62.5
Ceftazidime	88	33	37.5
Gentamicin	164	101	61.6
Amikacin	105	14	13.3
Meropenem	93	4	4.3
Imipenem	92	5	5.4
Ciprofloxacin	92	40	43.5
Levofloxacin	78	12	15.4
Norfloxacin	91	30	33.0
Fosfomycin	104	2	1.9
Co-trimoxazole	93	76	81.7
Aztreonam	59	3	5.1
Colistin	17	9	52.9
Tetracycline	18	15	83.3
ESBL (+)	50%		

Observation: *E. coli* strains demonstrated high resistance levels to ampicillin (95.6%), co-trimoxazole (81.7%), tetracycline (83.3%), cefotaxime (62.5%),

amoxicillin/clavulanic acid (61.7%), and gentamicin (61.6%). The phenotypic ESBL-positive rate among *E. coli* isolates was 50%. However, *E. coli* strains remained highly susceptible to fosfomycin, carbapenem, amikacin, and levofloxacin.

Table 3.9. Antibiotic Resistance Rates of Klebsiella spp. Strains (*n*=16)

Antibiotic	Tested Cases (*n*)	Resistant Cases (*n*)	Resistance Rate (%)
Ampicillin	3	3	100.0
Amoxicillin/Clavulanic acid	12	10	83.3
Piperacillin/Tazobactam	6	3	50.0
Cefotaxime	3	2	66.7
Ceftazidime	3	2	66.7
Gentamicin	12	11	91.7
Amikacin	6	4	66.7
Meropenem	3	0	0.0
Imipenem	3	0	0.0
Ciprofloxacin	3	2	66.7
Levofloxacin	9	3	33.3
Norfloxacin	3	1	33.3
Co-trimoxazole	3	3	100.0

Observation: *Klebsiella spp.* displayed elevated resistance to multiple drugs: 100% to ampicillin and co-trimoxazole, followed by gentamicin (91.7%) and amoxicillin/clavulanic acid (83.3%). Resistance to third-generation cephalosporins, amikacin, and ciprofloxacin was also high (66.7%). Susceptibility to the carbapenem group was completely preserved.

Table 3.10. Antibiotic Resistance Rates of Proteus spp. Strains (*n*=16)

Antibiotic	Tested Cases (*n*)	Resistant Cases (*n*)	Resistance Rate (%)
Ampicillin	4	1	25.0
Amoxicillin/Clavulanic acid	11	6	55.5
Piperacillin/Tazobactam	5	0	0.0
Cefotaxime	4	1	25.0
Ceftazidime	4	0	0.0
Gentamicin	10	7	70.0
Amikacin	9	3	33.0
Meropenem	4	0	0.0
Imipenem	4	3	75.0
Ciprofloxacin	4	1	25.0
Levofloxacin	7	7	100.0
Norfloxacin	4	1	25.0
Co-trimoxazole	4	2	50.0

Observation: *Proteus spp.* exhibited 100% resistance to levofloxacin, paired with elevated resistance to imipenem (75.0%) and gentamicin (70.0%). Susceptibility was preserved for piperacillin/ tazobactam, ceftazidime, and meropenem.

3.3. Identification of Several Antibiotic Resistance Genotypes of *E. coli* Causing Urinary Tract Infections in Children

Table 3.11. Distribution of *β*-Lactam Resistance Genes in *E. coli*

Resistance Genotype	Examined Strains	Carrying Strains	Strain Carriage Rate (%)
*bla*TEM-1	47	24	51.10
*bla*EC-5	47	14	29.79
*bla*OXA-1	47	4	8.51
*bla*CMY-2	47	1	2.13
*bla*CMY-42	47	2	4.26
*bla*CTX-M-15	47	5	10.64
*bla*CTX-M-55	47	5	10.64
*bla*CTX-M-27	47	24	51.06
*bla*DHA-1	47	8	17.02
*bla*NDM-5	47	7	14.89

Observation: The carriage rates of the TEM and *bla*CTX-M-27 genes were the highest at 51.1%. Seven carbapenem-resistant *E. coli* strains harboring the carbapenemase gene *bla*NDM-5 were detected.

Table 3.12. Distribution of TEM and CTX-M Genotype Combinations in *E. coli*

Resistance Genotype	Examined Strains	Carrying Strains	Strain Carriage Rate (%)
TEM	47	24	51.60
CTX-M	47	31	65.90
TEM + CTX-M	47	12	25.53

Observation: The distribution of resistance genes showed TEM at 51.6%, CTX-M at 65.9%, and co-carriage of both TEM and CTX-M genes at 25.53%.

Table 3.13. Distribution of Aminoglycoside Resistance Genes in *E. coli*

Resistance Genotype	Examined Strains	Carrying Strains	Strain Carriage Rate (%)
aph(3")-Ib	47	31	66.0
aph(6)-Id	47	31	66.0
aadA5	47	22	46.0
aadA16	47	4	8.5
aadA2	47	8	17.0
aadA1	47	1	2.1
aac(6")-Ib-cr5	47	9	19.1
rmtB1	47	6	12.8
aac(3)-IId	47	14	22.8
aac(3)-IIe	47	4	8.5

Observation: The most frequently encountered aminoglycoside resistance genotypes in *E. coli* were aph(3")-Ib (66%), aph(6)-Id (66%), and aadA5 (46%).

Table 3.14. Distribution of Quinolone Resistance Mutations /Genes in *E. coli*

Resistance Genotype	Examined Strains	Carrying Strains	Strain Carriage Rate (%)
qnrB4	47	8	17.0
gyrA S83L	47	30	63.8
gyrA D87N	47	16	34.0
parC S80I	47	19	40.1
parC E84V	47	4	8.5

Observation: The predominant quinolone resistance markers were the gyrA S83L mutation (63.8%), followed by the parC S80I mutation (40.1%) and the gyrA D87N mutation (34%).

Table 3.15. Distribution of Sulfonamide/Trimethoprim Resistance Genes in *E. coli*

Antibiotic Family	Resistance Genotype	Examined Strains	Carrying Strains	Strain Carriage Rate (%)
Sulfonamides	sul1	47	38	80.90
	sul2	47	31	66.00
	sul1 + sul2	47	24	51.06
Trimethoprim	dfrA17	47	30	63.80
	dfrA12	47	8	17.00
	dfrA27	47	5	10.60
	dfrA14	47	3	6.40
	dfrA7	47	2	4.20
	dfrA8	47	1	2.10

Observation: High prevalence was found for sul1 (80.9%), sul2 (66.0%), and dfrA17 (63.8%), with dual carriage of sul1 and sul2 established at 51.06%.

Table 3.16. Distribution of Macrolide Resistance Genes in *E. coli*

Resistance Genotype	Examined Strains	Carrying Strains	Strain Carriage Rate (%)
mph(A)	47	39	83.0
mph(B)	47	22	46.8

Observation*:* Macrolide resistance genotypes in *E. coli* comprised mph(A) at 83% and mph(B) at 46.8%.

Table 3.17. Distribution of Tetracycline Resistance Genes in *E. coli*

Resistance Genotype	Examined Strains	Carrying Strains	Strain Carriage Rate (%)
tet(A)	47	29	61.7
tet(B)	47	3	6.4
tet(D)	47	1	2.1

Observation: Tetracycline resistance determinants were distributed as tet(A) (61.7%), tet(B) (6.4%), and tet(D) (2.1%).

- Among the 47 *E. coli* isolates, 5 were phenotypically resistant to Imipenem. Four harbored the known carbapenemase gene *bla*NDM-5, whereas strain ECV219 demonstrated Imipenem resistance but lacked any annotated carbapenem resistance genes. This index case strongly suggested an uncharacterized novel mechanism of resistance.

- Comparative genomic profiling among isolates sharing the ST43 sequence type revealed that ECV219 harbored an auxiliary four-gene cluster (aadA5, mph(A), sul1, and dfrA17) absent in susceptible counterparts, signaling a specialized DNA region tied to Imipenem resistance.

- Relative to 10 susceptible strains carrying the same four-gene suite, ECV219 possessed an elongated DNA sequence block exceeding theirs by roughly 600 bp. This structure supported the integration of an insert element mediating Imipenem resistance.

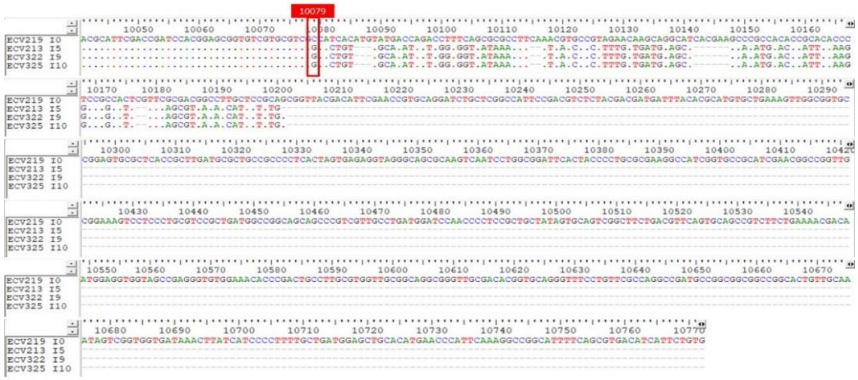

Figure 3.1. Alignment of the distinct sequence region among strains containing the four specific genes (aadA5, mph(A), sul1, dfrA17).

Observation: ECV219 possesses a unique DNA segment absent in susceptible isolates. Within this block, the research team identified a 399-bp open reading frame encoding a hypothetical protein and designated it ILR-VIN. This locus represents the primary candidate for functional validation regarding Imipenem resistance.

Table 3.18. Statistical Correlation Between the ILR-VIN Locus and Carbapenem Phenotypic Resistance

Antibiotic	Gene Marker	*p*-value (Fisher's Exact Test)
Carbapenem	ILR-VIN	0.012637008
Imipenem		**0.001011122**
Meropenem		0.012637008

Observation: The ILR-VIN locus exhibits a statistically significant association with carbapenem resistance, particularly Imipenem (p=0.001). Querying the NCBI public databases confirmed that 7 out of 7 international strains carrying the ILR-VIN sequence were clinically categorized as carbapenem-resistant, substantiating its functional role.

(A) (B)

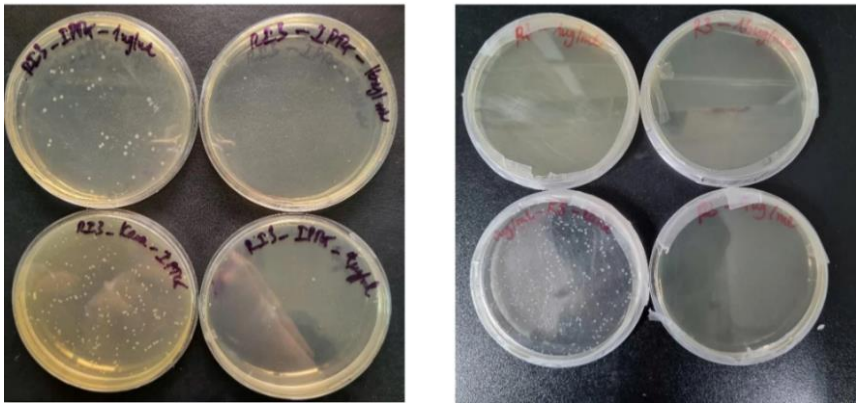

Figure 3.2. Functional confirmation of the role of ILR-VIN in Imipenem resistance.

Observation: In-vitro gain-of-function validation verified that *E. coli* expression hosts driven by the ILR-VIN construct survived under selective Imipenem pressure, whereas non-expressing controls were completely inhibited. This confirms ILR-VIN as a direct functional driver of Imipenem resistance.*E. coli.*

CHAPTER 4. DISCUSSION

4.1. Clinical Epidemiological Characteristics of Urinary Tract Infections in Children Aged 2 Months to 15 Years at Nghe An Obstetrics and Pediatrics Hospital

4.1.1. Epidemiological Characteristics

4.1.1.1. Overall Prevalence of Urinary Tract Infection in Febrile Children

Among 8,791 febrile children aged 2 months to 15 years evaluated via clinical examinations, urinalysis, urine cultures, CBC panels, and imaging modalities at Nghe An Obstetrics and Pediatrics Hospital, 916 met the uniform criteria for UTI diagnosis, representing a prevalence of 10.4%. Our finding is slightly lower than the 11.6% rate reported by Le Quang Phuong and Nguyen Thị Quynh Huong (2016) at the National Children's Hospital. Conversely, it is higher than the data from Dang Van Chuc in Hai Phong, which recorded a 3.6% rate among febrile children under 5 years and a 2.8% community rate. On a global scale, pediatric UTI prevalence varies significantly depending on the country, region, and target demographic. According to a meta-analysis by N. Shaikh et al. (2008), the prevalence among febrile children is approximately 7% for girls and 2% for boys, though it can climb to 20% in uncircumcised boys. Doley A. documented a hospital-based prevalence of 10.7% in children aged 2

to 10 years. Notably, a retrospective study conducted in Poland by A. Zmyslowska reported a UTI rate as high as 29%.

Thus, UTI prevalence is highly localized and context-dependent, reminding clinicians to suspect UTIs in febrile pediatric cohorts and implement routine urinalysis screening.

4.1.1.2. Distribution by Sex

Male patients constituted 48.7% (*n*=446) of the study cohort, which was lower than female patients at 51.3% (*n*=470), establishing a female-to-male ratio of 1.05:1. This pattern aligns with findings by Le Quang Phuong (2016), Tran Van Son (2024), and Vu Thi Phuong Hoa (2025), as well as international studies by Hilal Ünsal (2019, Germany) and Jiangdong Lu (2022, China), confirming a baseline female predominance in pediatric UTIs.

4.1.1.3. Distribution by Age Group

Children aged 2 to 24 months comprised the largest segment at 46.4%, followed by the 25–60 months group at 28.5%, and children older than 60 months at 25.1%. This mirrors data by Nguyen Thi Tuyet Minh (2024), where the 2–24 months cluster similarly peaked at 47.5%, followed by 27.3% (25–60 months) and 25.2% (> 60 months). Parallel studies by Tran Van Son (2024), C. V. Echeverri in Spain, and Jiangdong Lu (2022) uniformly conclude that children under 2 years bear the highest burden of disease, demonstrating consistency between our results and broader scientific data.

4.1.1.4. Geographical Distribution

Pediatric patients living in rural settings accounted for 74% of the cohort. Le Quang Phuong, Dang Van Chuc, and Nguyen Ngoc Sang also reported a higher concentration of rural patients over urban ones in hospital admissions.

4.1.1.5. Seasonal/Monthly Distribution

While cases appeared throughout the year, a clear peak occurred from May to August (summer months), with 91 cases in May, 93 in June, 96 in July, and 89 in August. Le Quang Phuong similarly documented that summer admissions (April to June) accounted for a prominent share (45.2%). A study by Li et al. (2025) in China revealed a positive correlation between mean monthly temperature, rainfall, sunshine hours, and the isolation rates of UTI pathogens in children, particularly E. coli and Enterococcus faecalis. Our findings, congruent with Le Quang Phuong and Li et al. (2025), support the hypothesis that elevated environmental temperatures favor the proliferation and transmission of UTI-causing bacteria, especially *E. coli.*

4.1.2. Clinical Characteristics

4.1.2.1. Reason for Admission

Because this study focused exclusively on febrile patients, fever was present in 100% of cases. However, the diagnostic entries varied; the most common reason for admission was fever paired with urinary tract disturbances, accounting for 55.8% (*n*=511) of the 916 patients. This represents more than half

of the study population, highlighting the predictive value of localized urinary signs when assessing febrile children.

4.1.2.2. Signs and Symptoms

- Urinary localized signs: Pollakiuria was recorded in 51.2% (*n*=469), turbid urine in 39% (*n*=357), dysuria in 31% (*n*=284), and crying during voiding in 19.5% (*n*=179), indicating a predominance of bladder and urethral irritation signs.

- Extra-urinary signs: Irritability/unprovoked crying occurred in 20.3% (*n*=186), abdominal pain in 18% (*n*=165), diarrhea in 8.7% (*n*=80), and vomiting in 7% (*n*=64).

Clinical presentations of UTIs in children are frequently non-specific and atypical compared to adult cohorts. The signs vary substantially across locations and age brackets. Frequently, gastrointestinal signs like vomiting, diarrhea, or abdominal pain mask the underlying pathology, and characteristic urinary disturbances may be absent, leading to misdiagnoses or delayed intervention.

4.1.3. Laboratory and Imaging Profiles

- Leukocytosis (>10×109/L) occurred in 70.2% of patients, with a mean WBC count of 14.6±7.0×109/L, while elevated neutrophils were present in 47.3%.

- The mean serum CRP was 44.4±2.1 mg/L (*SD*=80.9 mg/L).

- Leukocyturia was heavily concentrated at the ++ and +++ tiers (36% and 33.8%), signaling intense local inflammation. Positive hematuria was detected in 59.2%, whereas nitrituria registered a low positive rate of 22.8%.

- A subgroup of 186 patients (20.3% of the total cohort) exhibited abnormalities on imaging: pelvicalyceal ectasia and hydronephrosis (54.8%), urinary bladder wall thickening (16.7%), and congenital renal-ureteral malformations (14%) were the main findings.

4.2. Bacterial Etiology and Antibiotic Resistance Status of Pathogens Causing Urinary Tract Infections in Children

4.2.1. Bacterial Etiology

Gram-negative organisms dominated the culture profiles at 85.6%, compared to Gram-positive lines (9.1%) and fungal isolates (5.3%). *E. coli* maintained its role as the principal pathogen at 68.9% overall (*n*=186). However, a temporal decline was observed, moving from 84.5% in the 2018–2021 window to 61.8% in the 2021–2024 window. This shift was accompanied by an increase in Gram-positive Enterococcus spp., which surged from 1.2% to 13.4% to become the second most common isolate (9.6% cumulative). Gram-negative Klebsiella spp. (rising from 2.4% to 7.5%) and Proteus spp. (rising from 3.6% to 7.0%) also demonstrated an increasing trend. These metrics align with standard baseline domestic and international indices, confirming the primary role of Gram-negative rods, led by *E. coli*, in pediatric UTIs.

4.2.2. Antibiotic Resistance Status

- Enterococcus spp. strains exhibited a 100% resistance profile against ampicillin, gentamicin, and benzylpenicillin, alongside elevated resistance to amoxicillin/clavulanic acid (71.4%), while remaining uniformly sensitive to vancomycin, clarithromycin, and linezolid.

- *E. coli* isolates showed high resistance to ampicillin (95.6%), co-trimoxazole (81.7%), tetracycline (83.3%), amoxicillin/clavulanic acid (61.7%), gentamicin (61.6%), and cefotaxime (62.5%). The phenotypic ESBL production rate reached 50%. Crucially, these strains retained high susceptibility to fosfomycin, carbapenems, amikacin, and levofloxacin.

- *Klebsiella spp*. displayed severe resistance profiles, reaching 100% for ampicillin and co-trimoxazole, followed by gentamicin (91.7%) and amoxicillin/clavulanic acid (83.3%). Resistance to third-generation cephalosporins, amikacin, and ciprofloxacin was also high (66.7%), whereas carbapenem susceptibility remained at 100%.

- Proteus spp. isolates presented critical resistance to fluoroquinolones and aminoglycosides, with 100% resistance to levofloxacin, 75% to imipenem, and 70% to gentamicin. Conversely, susceptibility was well preserved for piperacillin/tazobactam, ceftazidime, and meropenem.

Our results correspond closely with data published by Dang Van Chuc, Nguyen Thi Quynh Huong, Nguyen Ngoc Sang, Luong Thi Phuong, and Tran Quoc Huy. The Ministry of Health's 2020 national surveillance report highlighted that 47.5% of E. coli strains were resistant to ceftazidime, 67.9% to ceftriaxone, and 10% to carbapenems. For Klebsiella pneumoniae, the report indicated a 58.8% resistance rate to ceftazidime, 73.9% to ceftriaxone, and nearly 50% to carbapenems.

A 2016 global meta-analysis regarding antibiotic resistance in pediatric *E. coli* UTIs showed that in OECD nations, resistance stood at 53.4% for ampicillin, 23.6% for trimethoprim, 8.2% for amoxicillin/clavulanic acid, and 2.1% for ciprofloxacin, with nitrofurantoin lowest at 1.3%. In non-OECD countries, the rates were significantly higher: 79.8% for ampicillin, 60.3% for amoxicillin/ clavulanic acid, 26.8% for ciprofloxacin, and 17.0% for nitrofurantoin. Furthermore, Esmatullah Esmat (2025) recorded that 62% of Gram-negative rods produced ESBLs, led by Proteus (90%), followed by Klebsiella (78%) and *E. coli* (59%). *Enterobacteriaceae* showed a 94% resistance rate to ampicillin and augmentin. *E. coli* resistance to ceftazidime and ceftriaxone reached 98% and 82%, respectively, whereas Klebsiella recorded 80% and 77%. In contrast, *E. coli* maintained high susceptibility to nitrofurantoin (99%), fosfomycin (97%), piperacillin/tazobactam (96%), and imipenem (96%).

4.3. Identification of Antibiotic Resistance Genotypes of *E. coli* Causing Urinary Tract Infections in Children

4.3.1. Distribution of β-Lactam Resistance Genes

Among the 47 whole-genome sequenced *E. coli* isolates, the prevalence of TEM was 51.1%, *bla*CTX-M-27 was 51.1%, *bla*CTX-M-15 was 10.64%, and *bla*CTX-M-55 was 10.64% (accumulating to a 65.95% total carriage rate for the CTX-M family). Co-carriage of TEM and CTX-M genes was observed in 25.53% of isolates. Notably, seven carbapenem-resistant strains possessed the carbapenemase-encoding gene *bla*NDM-5. Additionally, *β*-lactamase genes (CTX-M, TEM, OXA) were significantly more enriched in phenotypically ESBL-producing isolates compared to non-producers.

Nguyen Thi Tuyen and Le Nu Xuan Thanh previously reported 7 carbapenem-resistant E. coli strains harboring carbapenemase genes: 3 with *bla*KPC-2, 2 with *bla*NDM-4, and 2 with *bla*OXA-48. Le Van Nam recorded a 100% carriage rate for TEM, 43% for CTX-M, and 16% for SHV. Tran Thi Mai Hung found that TEM was present in 88.2% of isolates and CTX-M in 52.9%, with dual carriage peaking in diarrheic samples (52.8%), followed by pneumonia (25%), skin infections (20%), and UTIs (16.7%), concluding that TEM is the most pervasive resistance gene in community acquired infections in Vietnam. Internationally, Rania Y. Shash et al. in Egypt reported CTX-M as the most prevalent determinant. Conversely, Mojtaba Moosavian et al. in Iran found TEM predominated at 65.5%, while SHV was present in 15% and CTX-M was completely absent. Mohammad in Northern Iran documented TEM as the most common gene (49%), followed by SHV (44%) and CTX-M (28%). While TEM and SHV historically dominated the scientific literature, recent surveillance indicates a global shift toward CTX-M dominance, a trend mirrored in our study where CTX-M surpassed TEM and *bla*NDM-5.

4.3.2. Distribution of Non-β-Lactam Resistance Genotypes

- **Aminoglycosides**: Gene carriage was elevated, with aph(3")-Ib at 66%, aph(6)-Id at 66%, and aadA5 at 46%. However, looking at the genotype-phenotype correlation, only 6.5% of strains carrying aph(3")-Ib or aph(6)-Id displayed phenotypic resistance to amikacin, and 32.3% were resistant to gentamicin.

- **Quinolones**: Chromosomal target mutations were highly prevalent, led by gyrA S83L (63.8%), followed by parC S80I (40.1%) and gyrA D87N (34%). Isolates carrying concurrent mutations in both gyrA and parC exhibited significantly higher phenotypic resistance rates compared to single-mutation carriers ($p<0.05$).

- **Sulfonamides/Trimethoprim**: The target determinants sul1 (80.9%), sul2 (66.0%), and dfrA17 (63.8%) were widespread. Strains harboring dual sul1 and sul2 configurations (51.02%) demonstrated significantly higher co-trimoxazole phenotypic resistance rates than those carrying a single sul gene ($p<0.05$).

- Macrolides and Tetracyclines: We observed mph(A) in 83% and mph(B) in 46.8% of isolates, while tetracycline resistance genes were distributed as tet(A) (61%), tet(B) (6.4%), and tet(D) (2.1%).

4.3.3. Identification of the Novel Imipenem-Resistance Locus ILR-VIN

Of the 47 sequenced *E. coli* isolates, 7 carried established carbapenemase genes, and 5 exhibited phenotypic resistance to Imipenem. While 4 of these resistant strains possessed known resistance genes, one Imipenem-resistant isolate lacked any previously described carbapenemase genes or resistance elements. Comparative genomic analysis revealed that this specific strain contained a unique sequence region absent in susceptible isolates, indicating a potentially novel resistance mechanism. To evaluate its function, cloning and expression experiments were performed using E. coli Rosetta™ (DE3)pLysS expression hosts. Bioinformatics characterization (BLASTp, InterProScan, Pfam) confirmed that this 399-bp open reading frame significantly enhanced host cell survival under selective Imipenem pressure. This novel locus was designated **Imipenem-Linked Resistance Gene VIN (ILR-VIN)**. Notably, ILR-VIN was not detected in susceptible control strains or other local bacterial species, indicating it is an unannotated mobile genetic element associated with Imipenem resistance in Vietnamese E. coli isolates.

CONCLUSIONS

1. Clinical Epidemiological Characteristics of Urinary Tract Infections in Febrile Children Aged 2 Months to 15 Years at Nghe An Obstetrics and Pediatrics Hospital

- The prevalence of UTIs among febrile pediatric patients was 10.4%, with a higher frequency observed in girls. The 2–24 months age group constituted the largest proportion of cases (46.4%). Geographically, 74% of the affected children lived in rural areas. While cases occurred throughout the year, a distinct seasonal peak was observed between May and August.

- Clinical presentation: The primary localized urinary symptoms included pollakiuria (51.2%), turbid urine (39%), and dysuria (31%). Extra-urinary manifestations included irritability/crying (20.3%), abdominal pain (18%), diarrhea (8.7%), and vomiting (7.0%).

- Urinalysis: Leukocyturia was predominantly scored at the ++ and +++ levels (36.0% and 33.8%). Hematuria was positive in 59.2% of cases, while nitrituria demonstrated a low positive rate of 22.8%.

- Hematological profiles: Leukocytosis was observed in 70.2% of cases, with a mean WBC count of 14.6±7.0×109/L and neutrophilia in 47.3%. The mean serum CRP level was 44.4±2.1 mg/L, and 33.5% of patients had a CRP level exceeding 40 mg/L.

- Imaging findings: Structural abnormalities were detected in 20.3% of the patients, with pelvicalyceal ectasia and hydronephrosis being the most common (54.8%), followed by bladder wall thickening (16.7%), renal-ureteral malformations (14.0%), and duplex collecting systems (4.3%).

2. Bacterial Etiology and Antibiotic Resistance Status of Pathogens Causing Urinary Tract Infections in Children

- Gram-negative bacteria were the predominant pathogens (85.6%), while Gram-positive bacteria and fungi accounted for 9.1% and 5.3%, respectively. *E. coli* remained the primary causative agent (68.9%), followed by *Enterococcus spp*. (9.6%), *Klebsiella spp*. (5.9%), and *Proteus spp.* (5.9%).

- The bacterial isolates exhibited high resistance rates to commonly used antibiotics, particularly ampicillin, co-trimoxazole, amoxicillin/ clavulanic acid, gentamicin, and third-generation cephalosporins. Conversely, fosfomycin, carbapenem, and piperacillin/tazobactam maintained high antibacterial efficacy against the majority of the isolated strains.

3. Antibiotic Resistance Genotypes of *E. coli* Causing Urinary Tract Infections in Children

- *β*-lactam resistance genes: The CTX-M family was the most prevalent (65.95%), followed by the TEM gene (51.1%). Co-carriage of both TEM and CTX-M was observed in 25.5% of isolates. Carbapenem resistance was primarily mediated by the carbapenemase-encoding gene *bla*NDM-5.

- Co-trimoxazole resistance genes: Resistance was primarily driven by sul1 (80.9%), sul2 (66.0%), and dfrA17 (63.8%). Strains with dual carriage of sul1 and sul2 (51.02%) demonstrated significantly higher phenotypic resistance rates ($p<0.05$).

- Quinolone resistance mutations: The predominant mutations were located in parC S80I and gyrA D87N. Strains harboring concurrent mutations in both gyrA and parC exhibited significantly higher phenotypic resistance rates than single-mutation strains ($p<0.05$).

- Macrolide and tetracycline resistance genes: Macrolide determinants included mph(A) (83%) and mph(B) (46.8%). Tetracycline determinants were distributed as tet(A) (61%), tet(B) (6.4%), and tet(D) (2.1%).

- Novel resistance mechanism: A novel gene associated with Imipenem resistance was identified in an *E. coli* isolate and designated ILR-VIN (Imipenem-Linked Resistance Gene VIN).

STUDY LIMITATIONS

This study is limited by the relatively small number of *E. coli* strains that underwent whole-genome sequencing, which may not fully reflect the genetic diversity of the broader bacterial population. Furthermore, in-depth analyses correlating specific genotypes with distinct clinical phenotypes and clinical outcomes were not performed. Finally, because the study was conducted at a single medical center, the generalizability of the findings to other geographical regions may be limited.

RECOMMENDATIONS

1. Routine urinalysis screening should be performed in pediatric practice, particularly for children presenting with a fever of unknown origin, to facilitate early detection of urinary tract infections and minimize long-term complications.
2. Healthcare facilities should establish systematic surveillance programs to monitor local antibiotic resistance trends, ensure the rational use of antimicrobial agents, and guide empirical therapy updates.
3. Further research is warranted to characterize bacterial resistance profiles, identify resistance genes, and elucidate the transmission dynamics of these genetic determinants in pathogens causing urinary tract infections, thereby improving the control of multidrug-resistant bacteria.