PREVALENCE OF ANTIBIOTIC RESISTANCE IN CLINICAL ISOLATES OF E. COLI

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Abstract

Antimicrobial resistance (AMR) represents one of the most pressing global public health challenges of the 21st century. Among Gram-negative pathogens, Escherichia coli (E. coli) plays a central role due to its dual nature as a commensal organism and a frequent opportunistic pathogen. This review critically evaluates the prevalence, molecular mechanisms, and epidemiological distribution of antibiotic resistance in clinical isolates of E. coli. Evidence from multiple peer-reviewed studies demonstrates a significant rise in multidrug-resistant (MDR) and extended-spectrum beta-lactamase (ESBL)-producing strains, particularly in hospital environments. Resistance to first-line antibiotics such as ampicillin, fluoroquinolones, and third-generation cephalosporins is highly prevalent, while reduced susceptibility to carbapenems has emerged as an alarming trend. The findings emphasize the urgent necessity for antimicrobial stewardship programs, strengthened surveillance systems, and global coordinated interventions.

Keywords: Escherichia coli, antibiotic resistance, multidrug-resistant (MDR), ESBL, clinical isolates, antimicrobial resistance.

Introduction

Escherichia coli (E. coli) is a Gram-negative, facultative anaerobic bacterium belonging to the family Enterobacteriaceae. It is a normal inhabitant of the human gastrointestinal tract and plays an important role in maintaining intestinal homeostasis. However, certain pathogenic strains of E. coli are responsible for a wide range of infections, including urinary tract infections (UTIs), bloodstream infections (bacteremia), neonatal meningitis, and gastrointestinal diseases. Urinary tract infections represent the most common clinical manifestation of E. coli infection, accounting for approximately 70–90% of community-acquired cases. In addition, E. coli is frequently isolated in hospital-acquired (nosocomial) infections, particularly in intensive care units (ICUs), where immunocompromised patients are at higher risk. (Flores-Mireles)

In recent decades, the therapeutic management of E. coli infections has become increasingly challenging due to the rapid emergence and spread of antimicrobial resistance (AMR). According to the World Health Organization, antimicrobial resistance is among the top ten global public health threats facing humanity, with significant implications for morbidity, mortality, and healthcare costs. (Organization, 2017)

Antibiotic resistance in E. coli develops through multiple mechanisms, including the production of beta-lactamase enzymes, extended-spectrum beta-lactamases (ESBLs), efflux pump activation, and genetic mutations affecting antibiotic target sites. Of particular concern is the horizontal transfer of resistance genes via plasmids, which accelerates the dissemination of multidrug-resistant (MDR) strains across healthcare and community settings.The increasing prevalence of resistant E. coli strains has significantly reduced the effectiveness of commonly used antibiotics such as ampicillin, fluoroquinolones, and third-generation cephalosporins. In some regions, carbapenem-resistant E. coli strains have also been reported, further limiting last-line treatment options.

This growing resistance trend has been extensively documented in clinical studies indexed in PubMed and other scientific databases, highlighting a global and rapidly evolving public health crisis that requires urgent intervention through antimicrobial stewardship, infection control strategies, and continuous surveillance systems. (Ventola, 2015)

Mechanisms of Antibiotic Resistance

Antimicrobial resistance (AMR) occurs when microorganisms such as bacteria, viruses, fungi, and parasites no longer respond to antimicrobial agents. As a result, standard treatments become ineffective, and infections become increasingly difficult to treat, leading to a higher risk of disease spread, severe illness, disability, and death. AMR is a natural evolutionary process that occurs over time through genetic changes in microorganisms. However, its emergence and spread are significantly accelerated by human activities, particularly the misuse and overuse of antimicrobial agents in human medicine, agriculture, and animal production. Antimicrobial drugs are essential to modern medicine, and the rise of resistant pathogens threatens the effectiveness of life-saving procedures such as chemotherapy, organ transplantation, and major surgical interventions. (World Health Organization (2023). Antimicrobial resistance.)

In Escherichia coli, antibiotic resistance is mediated through multiple mechanisms. One of the most important is the production of β-lactamase enzymes, including extended-spectrum β-lactamases (ESBLs), which hydrolyze and inactivate β-lactam antibiotics such as penicillins and cephalosporins. This significantly reduces available treatment options for infections caused by ESBL-producing strains. The emergence of carbapenem-resistant E. coli (CRE) strains represents a major clinical concern, as these isolates often exhibit resistance to multiple antibiotic classes, including fluoroquinolones, trimethoprim-sulfamethoxazole, and broad-spectrum cephalosporins. Consequently, treatment options are limited to last-resort antibiotics such as polymyxins, tigecycline, fosfomycin, and aminoglycosides, which may be used alone or in combination.In addition to enzymatic degradation, E. coli exhibits other resistance mechanisms, including reduced membrane permeability, which limits antibiotic entry into the bacterial cell, and the activation of efflux pump systems that actively expel antimicrobial agents. Furthermore, enzymatic modification and target site mutations contribute to reduced susceptibility to various antibiotic classes. (Davies)

Biofilm formation represents an important survival strategy that contributes to antibiotic resistance. Biofilms are structured communities of bacteria embedded in a self-produced extracellular matrix composed of exopolysaccharides, proteins, and extracellular DNA. This matrix protects bacterial cells from antimicrobial agents and host immune responses, making infections persistent and difficult to eradicate. Biofilm development occurs in several stages, including bacterial adhesion, quorum sensing, and biofilm maturation. Within biofilms, bacterial cells exhibit altered metabolic activity and reduced growth rates, which further decrease antibiotic susceptibility. Additionally, biofilms facilitate resistance by preventing antibiotic penetration and protecting bacteria from immune-mediated clearance.

Horizontal gene transfer plays a crucial role in the spread of antibiotic resistance in E. coli. Mobile genetic elements such as plasmids, transposons, and integrons carry multiple antibiotic resistance genes, allowing rapid dissemination of multidrug-resistant (MDR) and extensively drug-resistant (XDR) strains across bacterial populations. (Blair, 2015)

The widespread use of β-lactam antibiotics, including penicillins, cephalosporins, carbapenems, and monobactams, has contributed to the global expansion of β-lactamase-mediated resistance. In response, β-lactamase inhibitors such as clavulanic acid, sulbactam, and tazobactam have been developed and are commonly used in combination with β-lactam antibiotics to restore their effectiveness. However, these inhibitors are not effective against all classes of β-lactamases, particularly carbapenemases and metallo-β-lactamases, which continue to pose a significant therapeutic challenge.Overall, the combination of multiple resistance mechanisms in E. coli significantly limits treatment options, prolongs hospitalization, increases healthcare costs, and contributes to higher mortality rates, making antibiotic resistance a major global public health concern. (Sina Nasrollahian)

Methodology

This study was conducted as a literature-based and data-supported analysis of antibiotic resistance in clinical and environmental isolates of Escherichia coli. Data were obtained from published scientific articles, clinical reports, and global health databases, including sources such as the World Health Organization and PubMed.In addition, antibiotic susceptibility data were analyzed for E. coli isolates obtained from two sources: clinical urinary tract infection (UTI) samples and municipal wastewater samples. Antimicrobial susceptibility testing was performed using the disk diffusion (Kirby-Bauer) method, following standard microbiological guidelines. The antibiotics tested, along with their concentrations and classifications, are presented in Table 2 and 3. Resistance patterns were interpreted based on established criteria and categorized according to the WHO AWaRe (Access, Watch, Reserve) classification and the CIA (Critically Important Antimicrobials) list. Multidrug resistance (MDR) was defined as resistance to three or more classes of antibiotics. Comparative analysis was conducted to evaluate differences in resistance patterns between clinical and environmental isolates. (Magiorakos, 2012)

Table 1. Antibiotics used in this study for susceptibility testing. (Makeda Matthew-Bernard)

Antibiotic	Abbreviation/Concentration µg	Class of Antibiotic
Amoxicillin	AMC 30	Penicillin + beta-lactamase inhibitor
Ampicillin	AM 10	Penicillin (Beta-lactam)
Aztreonam	ATM 30	Monobactam
Cefazolin	CZ 30	First generation cephalosporin
Cefoxitin	FOX 30	Second generation cephalosporin
Ceftriaxone	CRO 30	Third generation cephalosporin
Cefuroxime	CXM 30	Second generation cephalosporin
Ciprofloxacin	CIP 5	Fluoroquinolone
Gentamicin	GM 10	Aminoglycosides
Nalidixic Acid	NA 30	Quinolones
Nitrofurantoin	FM 300	Nitrofuran-derivative
Sulfamethoxazole-trimethoprim	SXT 25	Folate pathway antagonist

Results

Antibiotic Resistance Patterns Based on CIA Classification

A total of 120 Escherichia coli isolates were analyzed, including 34 clinical isolates from urinary tract infections (UTIs) and 86 isolates from municipal wastewater samples. Among clinical isolates, 52.9% (18/34) were resistant to at least one tested antibiotic, whereas 15.1% (13/86) of wastewater isolates showed resistance. According to the CIA classification, similar resistance patterns were observed between clinical and wastewater isolates. Among critically important antibiotics, ampicillin exhibited the highest resistance rates, followed by amoxicillin/clavulanic acid and nalidixic acid. Resistance to sulfamethoxazole-trimethoprim was observed in both groups within the highly important category, while nitrofurantoin showed low resistance levels in both sample types.

For wastewater isolates, resistance rates were relatively low, with ampicillin showing 9.3% resistance, followed by amoxicillin/clavulanic acid (3.5%), nalidixic acid (3.5%), and gentamicin (2.3%). Notably, all isolates were susceptible to ceftriaxone and ciprofloxacin.

In contrast, clinical UTI isolates demonstrated significantly higher resistance rates. Ampicillin resistance reached 38.2%, followed by amoxicillin/clavulanic acid (23.5%), nalidixic acid (14.7%), and gentamicin (11.8%). Resistance to ceftriaxone (5.9%) and ciprofloxacin (2.9%) remained relatively low. (BMC Infectious Diseases (2020). Prevalence of antibiotic resistance in E. coli isolates.)

Table 2. Patterns of antibiotic resistance (R), intermediate (I), and susceptibility (S) among Escherichia coli isolates from wastewater samples determined by disk diffusion method. (Makeda Matthew-Bernard)

Antibiotic	**R-resistance**		**I-intermediate**		**S-susceptibility**	
	N	**%**	**N**	**%**	**N**	**%**
Amoxicillin	3	3.5	3	3.5	80	93.0
Ampicillin	8	9.3	12	14.0	66	76.7
Aztreonam	0	0	0	0.0	86	100.0
Cefazolin	2	2.3	0	0.0	84	97.7
Cefoxitin	2	2.3	1	1.2	83	96.5
Ceftriaxone	0	0	1	1.2	85	98.8
Cefuroxime	2	2.3	0	0.0	84	97.7
Ciprofloxacin	0	0	3	3.5	83	96.5
Gentamicin	2	2.3	9	10.5	75	87.2
Nalidixic Acid	3	3.5	1	1.2	82	95.3
Nitrofurantoin	1	1.2	4	4.7	81	94.2
Sulfamethoxazole-trimethoprim	3	3.5	1	1.2	82	95.3

Table 3. Patterns of resistance, determined by disk diffusion, for E. coli isolates from clinical UTI samples. (Makeda Matthew-Bernard)

Antibiotic	**R-resistance**		**I-intermediate**		**S-susceptibility**	
	N	**%**	**N**	**%**	**N**	**%**
Amoxicillin	8	23.5	6	17.6	20	58.8
Ampicillin	13	38.2	1	2.9	21	61.8
Cefazolin	3	8.8	1	2.9	30	88.2
Cefoxitin	1	2.9	0	0	33	97.1
Ceftriaxone	2	5.9	0	0	32	94.1
Cefuroxime	2	5.9	7	20.6	25	73.5
Ciprofloxacin	1	2.9	2	5.9	32	94.1
Gentamicin	4	11.8	0	0	30	88.2
Nalidixic Acid	5	14	3	8.8	26	76.5
Nitrofurantoin	1	2.9	0	0	33	97.1
Sulfamethoxazole-trimethoprim	9	26.5	0	0	25	73.5

Antibiotic Resistance Based on AWaRe Classification

Based on the AWaRe classification, the highest resistance levels were observed among antibiotics in the ACCESS group, particularly ampicillin, with resistance rates of 9.3% in wastewater isolates and 38.2% in clinical isolates. In contrast, nitrofurantoin demonstrated the lowest resistance rates (1.2% and 2.9%, respectively).
Antibiotics in the WATCH group, such as cefuroxime and cefoxitin, showed similar resistance patterns across both sample types, whereas ciprofloxacin and ceftriaxone exhibited minimal resistance in wastewater isolates. Aztreonam, categorized under the RESERVE group, demonstrated complete susceptibility (100%) in wastewater isolates.

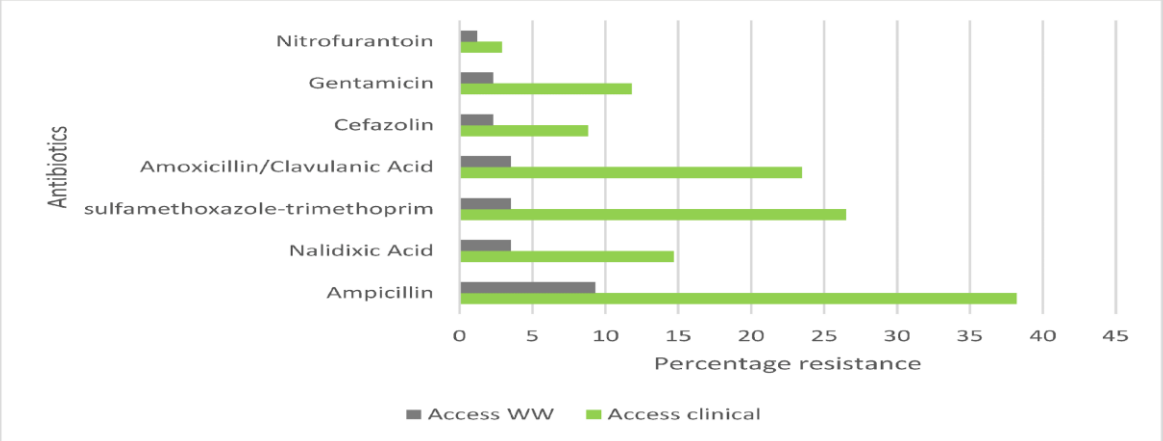

Figure 4. Percentage of antibiotic resistance among Escherichia coli isolates from clinical UTI samples and municipal wastewater, based on AWaRe (ACCESS) classification. (Makeda Matthew-Bernard)

Multidrug Resistance (MDR) Patterns

A total of nine multidrug resistance (MDR) patterns were identified, including three from wastewater isolates and seven from clinical isolates. The most frequently observed MDR combinations included AMC/AM, AMC/AM/CZ, AMC/AM/CZ/CXM, and AMC/AM/CZ/CXM/FOX.
These findings indicate a higher prevalence and diversity of MDR patterns among clinical isolates compared to environmental samples.

Table 5. Phenotypic antibiotic resistance profile of E. coli isolates from wastewater and clinical UTI samples. (Makeda Matthew-Bernard)

Pattern	Number of isolates	Source	Phenotypic Resistance Profile
A	2	Clinical UTI	AMC AM SXT
B	1		AM GM SXT
C	1	Clinical UTI	AMC AM FOX
D	1	Wastewater	AMC AM CZ CXM NA
E	1		AMC AM CZ CXM FOX
F	1	Wastewater	AMC AM CZ CXM FOX CRO GM
E	1	Wastewater	AMC AM CZ GM SXT CIP NA
F	1	Clinical UTI	AMC AM CZ CXM CRO GM SXT
		Clinical UTI	
		Clinical UTI	

Discussion

The present study demonstrates a significant level of antimicrobial resistance among Escherichia coli isolates, with notable differences observed between clinical urinary tract infection (UTI) samples and municipal wastewater isolates. Clinical isolates exhibited substantially higher resistance rates compared to environmental isolates, highlighting the greater selective pressure present in clinical settings. The high resistance rates observed for ampicillin and trimethoprim-sulfamethoxazole in clinical UTI isolates are likely associated with their widespread empirical use in the treatment of urinary tract infections. In contrast, wastewater isolates showed lower resistance levels, although the presence of resistant strains in environmental samples indicates that wastewater may serve as a potential reservoir for antimicrobial resistance. (Paterson, 2005) Reduced susceptibility to fluoroquinolones among clinical isolates is particularly concerning, given their frequent use in treating more complicated infections. Resistance mechanisms, including mutations in DNA gyrase and topoisomerase IV as well as increased efflux pump activity, contribute to decreased antibiotic effectiveness and facilitate the spread of resistant strains in both hospital and community settings. Although carbapenems remained highly effective against both clinical and wastewater isolates, the emergence of resistant strains reported globally emphasizes the need for careful stewardship of these last-resort antibiotics. The detection of multidrug-resistant (MDR) patterns, particularly among clinical isolates, further underlines the clinical importance of resistance surveillance. Overall, the comparative findings of this study highlight the critical role of both clinical and environmental sources in the epidemiology of antimicrobial resistance.

Continuous monitoring, rational antibiotic use, and effective infection control strategies are essential to limit the spread of resistant E. coli strains. (Pitout, 2008)

Conclusion

The findings of this study confirm that antibiotic resistance in Escherichia coli is a significant and growing public health concern, particularly in clinical settings. Clinical UTI isolates demonstrated higher resistance rates compared to wastewater isolates, reflecting the impact of antibiotic exposure and selective pressure in healthcare environments. While environmental isolates exhibited lower resistance levels, their role as potential reservoirs of resistant bacteria should not be underestimated. The presence of multidrug-resistant (MDR) strains in both clinical and environmental samples highlights the interconnected nature of antimicrobial resistance between human and environmental sources. Although certain antibiotics, such as carbapenems, remain largely effective, increasing resistance to commonly used agents limits treatment options and complicates infection management. These findings emphasize the importance of continuous surveillance, antimicrobial stewardship, and integrated approaches that address both clinical and environmental pathways of resistance spread. Future research should focus on monitoring resistance trends across different settings and developing strategies to reduce the dissemination of resistant E. coli strains.

References

[1] Blair, J. M. (2015). Molecular mechanisms of antibiotic resistance. Nature Reviews Microbiology.
[2] BMC Infectious Diseases (2020). Prevalence of antibiotic resistance in E. coli isolates.
[3] Davies, J. a. Origins and evolution of antibiotic resistance. Microbiology and Molecular Biology Reviews.
[4] Flores-Mireles, A. L. Urinary tract infections: epidemiology, mechanisms and treatment options. Nature Reviews Microbiology.
[5] Magiorakos, A. P. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria. Clinical Microbiology and Infection.
[6] Makeda Matthew-Bernard, K. F.-D.-B.-B. (n.d.). Phenotypic Antibiotic Resistance Patterns of Escherichia coli Isolates from Clinical UTI Samples and Municipal Wastewater in a Grenadian Community.
[7] World Health Organization (2017). Global priority list of antibiotic-resistant bacteria.
[8] Paterson, D. L. (2005). Extended-spectrum beta-lactamases: a clinical update. Clinical Microbiology Reviews.
[9] Pitout, J. D. (2008). Extended-spectrum beta-lactamase-producing Enterobacteriaceae. Clinical Microbiology Reviews.
[10] Sina Nasrollahian, J. P. A review of the mechanisms that confer antibiotic resistance in pathotypes of E. coli.
[11] Ventola, C. L. (2015). The antibiotic resistance crisis: causes and threats. Pharmacy and Therapeutics.
[12] World Health Organization (2023). Antimicrobial resistance.