

Antibacterial Susceptibility Profiling of *Escherichia coli* Isolates from Hospital Clinical Samples

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ABSTRACT: Multidrug-resistant *Escherichia coli* has become a global health threat, with limited effective treatment options. The growing prevalence of resistance poses significant challenges in managing these infections. This study aims to investigate the antibiotic resistance profiles of *E. coli* isolates collected from clinical samples. In this study, we biochemically characterized 48 *E. coli* isolates (using MIU, KIA and Citrate tests) collected from clinical samples at a hospital in Dhaka City, Bangladesh. We then analyzed the antibiotic sensitivity patterns of these isolates against eleven different antibiotics (ten individual antibiotics and one combination), namely Ampicillin, Cefixime, Levofloxacin, Tobramycin, Doxycycline, Aztreonam, Ceftazidime, Nitrofurantoin, Piperacillin, Fosfomycin and the antibiotic combination Co-trimoxazole (Sulfamethoxazole + Trimethoprim). The *E. coli* isolates showed significantly reduced susceptibility to most of the antibiotics tested. Only two antibiotics—Tobramycin (75%) and Fosfomycin (83%)-demonstrated significant effectiveness against the *E. coli* isolates. Necessary steps should be taken to prevent *E. coli* from acquiring further resistance to the remaining effective antibiotics, especially considering that ampicillin showed the highest resistance rate at 96%, whereas Fosfomycin exhibited the lowest resistance at only 17%.

Key words: *Escherichia coli*, Sensitivity, Resistance, Biochemical characterization

INTRODUCTION

Escherichia coli (*E. coli*) is a Gram-negative anaerobic bacterium that is typically found in the intestines of humans and animals. While many *E. coli* strains are benign and part of the normal microbiota, some pathogenic strains are the cause of numerous illnesses, such as urinary tract infections (UTIs), foodborne illnesses, gastroenteritis, as well as life-threatening conditions such as septicemia and hemolytic uremic syndrome (HUS).¹ The ability of *E. coli* to acquire and transfer genetic material, including antimicrobial resistance (AMR) genes, makes it a major risk to public health.²⁻⁴

The global development of multidrug-resistant (MDR) *E. coli* has led to increased treatment failures, extended hospital stays and higher mortality rates, underscoring the need for ongoing surveillance and research on its resistance mechanisms.⁵⁻⁷ The rise in resistance among *E. coli* is largely fuelled by the uncontrolled use of antibiotics in livestock and aquaculture, which creates constant selective pressure in the environment. In addition, easy access to unprescribed antibiotics from community pharmacies accelerates misuse in humans, further driving resistance.

In parallel, antibiotic sensitivity testing is essential in figuring out the effectiveness of commonly used antibiotics against *E. coli* isolates. With the increasing prevalence of antibiotic resistance, it is essential to monitor antibiotic susceptibility characteristics across a broad spectrum of drugs.² Accurate identification of *E. coli* is

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essential prior to resistance analysis. In this study, biochemical tests (citrate, KIA, and MIU) were used to confirm the identity of the isolates before conducting antibiotic susceptibility testing.^{8,9,10} This testing helps identify strains resistant to specific drugs, providing critical data for clinicians to make informed decisions about treatment regimens. Additionally, understanding the mechanisms behind resistance is vital for creating novel therapeutic approaches and curbing the spread of resistant strains.¹¹

This study focuses on the biochemical characterization and antibiotic sensitivity analysis of *E. coli* samples obtained from clinical samples. The objectives of this study are to characterize the biochemical properties of *E. coli* strains, assess their antibiotic resistance profiles, and evaluate the prevalence of multidrug-resistant isolates. These findings aim to improve knowledge of the current resistance patterns of *E. coli* in Dhaka, contributing to more effective treatment protocols and helping inform public health interventions to combat antibiotic resistance.

MATERIALS AND METHODS

Sample collection. Ethical approval for this research was gained from the Ethical Review Committee of the Department of Pharmacy, World University of Bangladesh (Approval No: WUB/PHR/2024/08). With the consent of the authorities at Enam Medical College & Hospital (EMCH), Savar, Dhaka 1340, we collected 80 *E. coli* isolates from clinical samples presenting with infections, including typhoid fever, food poisoning, and diarrhea. All samples were collected from EMCH, with bacterial isolates obtained non-selectively. Most of the samples were obtained from urine (44), while others were from wound swabs (2) and pus specimens (2).

Biochemical characterization. To identify the *E. coli* isolates, we performed three biochemical tests: the Citrate test, Kligler's Iron Agar (KIA) test and the Motility Indole Urease (MIU) test. *E. coli* isolates were citrate-negative. Simmons' citrate agar

remained green (showing no color change) in the presence of *E. coli*, which was unable to metabolize citrate.¹² *E. coli* isolates fermented both lactose and glucose, shown by the yellow color in the slant (aerobic zone) and butt (anaerobic zone) due to acid production. Gas (CO₂) production was indicated by a gap at the bottom of the test tube.¹³ Motility was tested by inoculating MIU agar with a sterile needle and incubating at 35-37°C for 18-24 hrs. Since the bacteria spread outside of the stab line, diffuse, cloudy growth was observed, confirming that the bacteria were motile.¹⁴ Positive and negative controls were run in parallel to validate the tests. *Klebsiella pneumoniae* was used as a citrate-positive control, while *E. coli* ATCC 25922 served as the citrate-negative and indole-positive control in MIU and KIA assays to ensure reliability of the biochemical interpretations.

Antibiotic sensitivity test. Antibiotic susceptibility testing was conducted using the Kirby-Bauer disc diffusion method, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI).¹⁵ Mueller-Hinton Agar (MHA) was used as the medium due to its suitability for antibiotic testing. The antibiotic discs and Mueller-Hinton Agar medium were obtained from Tradesworth Group. A standardized bacterial suspension, adjusted to 0.5 McFarland turbidity, was evenly spread on the MHA plate using a sterile cotton swab. Antibiotic discs were put on the inoculated surface and plates were incubated at around 35-37°C for about 16-18 hours. Then, the inhibition zones around the antibiotic discs were measured in millimeters (mm) using a ruler. Results were interpreted as susceptible (S), intermediate (I), or resistant (R) according to the CLSI guidelines 30. Intermediate values were considered as Resistant (Table 1).

Tables were generated in Microsoft Word, while figures for the MDR (Multi-Drug Resistant) and MAR (Multiple Antibiotic Resistance) indices were created using Microsoft Excel.

Table 1. Critical values for the inhibition zone of antibiotics used.

Antibiotics name	Code name	Family	Dose	Critical inhibition diameter values (mm)	
				Sensitive	Resistant
Ampicillin	AM	Beta lactams	10 µg	≥17	≤13
Cefixime	CFM	Cephalosporin antibiotics	5 µg	≥ 19	≤ 15
Levofloxacin	LE	Quinolone antibiotics	5 µg	≥ 21	≤ 16
Tobramycin	TOB	Aminoglycoside antibiotic	10 µg	≥15	≤12
Doxycycline	DO	Tetracycline antibiotics	30 µg	≥13	≤9
Aztreonam	ATM	Monobactams	30 µg	≥ 21	≤ 17
Co-trimoxazole	COT	Trimethoprim and sulfamethoxazole	25 µg	≥16	≤10
Ceftazidime	CAZ	Cephalosporin beta-lactam antibiotic	30 µg	≥18	≤14
Piperacillin	PI	Beta lactams	100 µg	≥21	≤14
Nitrofurantoin	NIT	Nitrofurans	300 µg	≥17	≤14
Fosfomycin	FO	Phosphonic acids	200 µg	≥16	≤12

*The CLSI guidelines 30 contain critical values.

RESULTS AND DISCUSSION

Sensitivity and resistance profile of *Escherichia coli* isolates. In this study, we observed that only 4% of isolates were susceptible to Ampicillin. These findings are consistent with other studies in Bangladesh — for example, Shahriar et al. (2010) found only ~4% of *E. coli* samples sensitive to ampicillin,¹⁶ and Bakshi et al. (2023) observed ~90.9% resistance of *E. coli* from food samples to the same agent¹⁷ — reflecting a persistently low level of effectiveness of beta-lactams in the region. A study carried out in a private hospital in Bangladesh in 2015 reported that 28.34% of *E. coli* isolates were susceptible to levofloxacin, a broad-spectrum fluoroquinolone antibiotic,¹⁸ which is closely aligned with our study's result of 35%. This similarity suggests a comparable susceptibility rate of *E. coli* isolates to fluoroquinolones across different settings in Bangladesh. Our study also found that 35% of *E. coli* samples were sensitive to Aztreonam, a beta-lactam monobactam antibiotic, which is comparable to Mollick et al. (2016), who reported 48.33% susceptibility among *E. coli* isolates in Bangladesh.¹⁸ This highlights that monobactams like Aztreonam maintain moderate efficacy against *E. coli* strains in the region. In terms of co-trimoxazole, a combination of trimethoprim and sulfamethoxazole, 33% of isolates in our study were sensitive to this antibiotic, which is comparable to previous reports from

Bangladesh showing 20% susceptibility (Shahriar et al., 2010) and 46.66% susceptibility (Mollick et al.,

2016).^{16,18} This suggests a moderate level of resistance among *E. coli* isolates to this commonly used antimicrobial agent.

Interestingly, the sensitivity of *E. coli* samples to Ceftazidime, a third-generation cephalosporin, was found to be 10% in our study, which is somewhat lower than the 16.25% reported in a study from Dhaka City, Bangladesh.¹⁶ This decrease in susceptibility could point to emerging resistance trends, necessitating closer monitoring of cephalosporin resistance in the region. On the other hand, our study revealed a high susceptibility rate of 83% for Fosfomycin, a phosphonic acid derivative, which is slightly lower than the 95% susceptibility reported by Rahman et al. (2025) in Dhaka City.¹⁵ Fosfomycin's high effectiveness suggests its continued potential as a valuable treatment option for *E. coli* infections. This high susceptibility in *E. coli* possibly because it has a unique cell wall-targeting mechanism, is used less frequently (reducing selective pressure), resistance is uncommon and often costly for the bacteria and it achieves high urinary concentrations that enhance its effectiveness. In our study, 10, 75, 54, 6 and 52% of *E. coli* isolates were susceptible to Cefixime, Tobramycin, Doxycycline, Piperacillin, and Nitrofurantoin, respectively. These

susceptibility rates show notable discrepancies when compared to previously conducted studies in Bangladesh.^{15,16,18-22} The observed differences in susceptibility could be attributed to factors such as variations in sample collection, geographical differences, differences in bacterial strains, or variations in antibiotic usage patterns across studies. These inconsistencies highlight the need for further investigation into local resistance trends and suggest that *E. coli* resistance may vary over time and between different settings.

The chi-square test showed a strong correlation between antibiotic type and *Escherichia coli* susceptibility/resistance ($\chi^2= 156.90$, $df= 10$, $p < 0.001$). The low p-value indicates that susceptibility and resistance patterns vary significantly among antibiotics, suggesting differences in their effectiveness against *E. coli* samples (Table 2).

Table 2. Sensitivity and resistance profile of *Escherichia coli* isolates (N=48).

Antibiotics (disc load)	Sensitive (%)	Resistant (%)
Fosfomycin (200 μ g)	40 (83.00)	8 (17.00)
Tobramycin (10 μ g)	36 (75.00)	12 (25.00)
Doxycycline (30 μ g)	26 (54.00)	22 (46.00)
Nitrofurantoin (300 μ g)	25 (52.00)	23 (48.00)
Levofloxacin (5 μ g)	17 (35.00)	31 (65.00)
Aztreonam (30 μ g)	17 (35.00)	31 (65.00)
Co-trimoxazole (23.75 μ g)	16 (33.00)	32 (67.00)
Cefixime (5 μ g)	5 (10.00)	43 (90.00)
Ceftazidime (30 μ g)	5 (10.00)	43 (90.00)
Piperacillin (100 μ g)	3 (6.00)	45 (94.00)
Ampicillin (10 μ g)	2 (4.00)	46 (96.00)

The chi-square test revealed a strong correlation between antibiotic type and *E. coli* susceptibility ($\chi^2 (10, N=48) = 156.90$, $p < 0.001$).

MDR data of *Escherichia coli*. MDR (multidrug-resistant) organisms are defined as microbes that are resistant to three or more antibiotic categories. Out of 48 *E. coli* isolates, 40 (84%) were classified as MDR (Figure-1).¹¹ For Gram-negative bacteria like *E. coli*, two major factors contribute to their intrinsic resistance: the outer membrane and the expression of efflux pumps. A study conducted in Mymensingh city, Bangladesh, found that 92% of *E. coli* isolates collected from urinary tract infection

patients were multidrug-resistant (MDR),²³ which is closely aligned with the findings of our study.

Resistance phenotype of *Escherichia coli*. A total of 48 *E. coli* isolates were tested against 11 antibiotics. The resistance profiles revealed significant variations in susceptibility. Ampicillin showed a high resistance rate of 96% (46/48 isolates), followed by Piperacillin at 94% (45/48 isolates), and Ceftazidime at 90% (43/48 isolates) making these antibiotics largely ineffective against *E. coli* infections. Cefixime demonstrated a resistance rate of 90% (43/48 isolates), further confirming its limited efficacy. Nitrofurantoin was found to be resistant in 48% (23/48 isolates), while other antibiotics like Aztreonam (65%, 31/48), Levofloxacin (65%, 31/48), and Co-trimoxazole (67%, 32/48) also showed moderate resistance. Doxycycline exhibited a resistance rate of 46% (22/48 isolates), which is relatively lower compared to the other antibiotics tested.

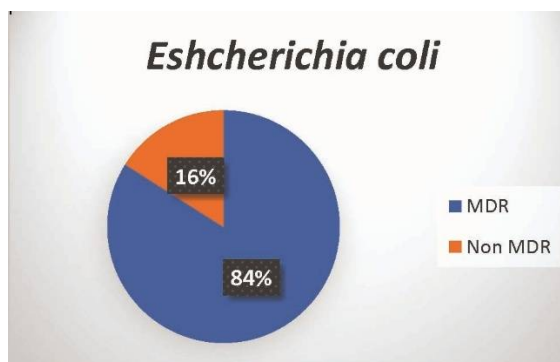


Figure 1. MDR data of *Escherichia coli*.

On the other hand, Fosfomycin demonstrated the highest effectiveness, with only 17% (8/48 isolates) resistance, making it a viable option for treatment. Tobramycin also showed promising results, with 75% (36/48 isolates) susceptibility and 25% resistance, positioning it as another effective alternative for treating *E. coli* infections amidst rising resistance. Although Fosfomycin-resistant mutants can develop easily *in vitro* with high mutation frequencies, the prevalence of Fosfomycin resistance in *E. coli* isolates remains low (usually <2%), even among ESBL-producing isolates (Table-3).²⁴

The chi-square test for independence was carried out to assess whether resistance to different antibiotics was significantly associated. The test yielded a chi-square statistic of 72.48 with 190

degrees of freedom and a p-value of 1.00. This suggests that resistance to one antibiotic does not strongly predict resistance to another, implying independent resistance mechanisms among the *E. coli* samples (Table 3).

Table 3. Resistance phenotype of *Escherichia coli*.

Samples	AM	ATM	COT	DO	CAZ	LE	FO	TOB	CFM	PI	NIT
E coli 1	R	-	-	-	R	-	R	R	R	R	R
E coli 2	R	-	R	-	R	R	-	-	R	R	-
E coli 3	R	R	R	-	R	R	-	-	R	R	-
E coli 4	R	R	R	-	R	-	R	R	R	R	R
E coli 5	R	R	R	-	R	-	-	-	R	R	-
E coli 6	R	R	R	-	R	R	-	-	R	R	-
E coli 7	R	R	R	-	R	-	-	-	R	R	R
E coli 8	R	-	R	-	-	-	-	-	R	R	-
E coli 9	R	R	R	-	R	R	-	-	R	R	-
E coli 10	R	-	R	-	R	-	-	-	R	R	R
E coli 11	R	R	R	-	-	R	R	R	R	R	R
E coli 12	R	-	-	-	-	-	-	-	R	R	-
E coli 13	R	R	R	-	R	-	-	-	R	R	-
E coli 14	R	-	-	-	-	-	-	-	R	R	-
E coli 15	R	-	R	-	-	R	-	-	R	R	R
E coli 16	R	R	R	R	R	R	-	-	R	R	-
E coli 17	R	R	-	-	R	R	-	-	R	R	-
E coli 18	R	R	R	-	R	R	-	-	R	R	R
E coli 19	R	R	R	R	R	R	-	R	R	R	R
E coli 20	R	-	R	R	R	R	R	-	R	R	R
E coli 21	R	R	-	R	R	R	R	-	R	R	R
E coli 22	-	R	-	R	R	-	R	-	R	R	-
E coli 23	R	R	R	R	R	R	-	-	R	R	-
E coli 24	R	R	-	-	R	R	-	R	R	R	-
E coli 25	R	-	-	-	R	-	R	-	R	R	-
E coli 26	R	-	R	R	R	R	-	R	R	R	R
E coli 27	R	R	R	R	R	R	-	R	-	R	R
E coli 28	R	R	R	R	R	-	-	-	-	-	R
E coli 29	R	R	R	R	R	R	-	-	-	-	R
E coli 30	R	R	R	R	R	R	-	-	-	-	R
E coli 31	R	R	R	R	R	R	-	-	-	R	-
E coli 32	R	R	R	R	R	R	-	-	R	R	-
E coli 33	R	-	-	R	R	R	-	-	R	R	-
E coli 34	R	-	-	R	R	R	-	-	R	R	R
E coli 35	R	R	R	R	R	R	-	R	R	R	R
E coli 36	R	R	-	-	R	R	-	R	R	R	R
E coli 37	R	R	R	-	R	R	-	-	R	R	-
E coli 38	R	-	R	-	R	-	-	-	R	R	-
E coli 39	R	-	R	R	R	R	-	-	R	R	-
E coli 40	R	R	-	-	R	-	-	-	R	R	-
E coli 41	R	R	-	R	R	-	-	-	R	R	-
E coli 42	R	R	-	-	R	-	-	-	R	R	-
E coli 43	R	R	-	-	R	R	-	R	R	R	R
E coli 44	-	R	-	-	R	-	-	-	R	R	-
E coli 45	R	-	R	R	R	R	-	-	R	R	R
E coli 46	R	-	R	R	R	R	-	-	R	R	R
E coli 47	R	R	R	R	R	R	R	R	R	R	R
E coli 48	R	R	R	R	R	R	-	R	R	R	R

*Resistant (R), Sensitive (-), Ampicillin (AM), Co-Trimoxazole (COT), Aztreonam (ATM), Doxycycline (DO), Cefazidime (CAZ), Levofloxacin (LE), Fosfomycin (FO), Tobramycin (TOB), Cefixime (CFM), Piperacillin (PI), Nitrofurantoin (NIT).

The correlation analysis examined the relationships between resistance patterns across different antibiotics. Strong positive correlations were observed between Fosfomycin (FO) and Tobramycin (TOB) ($r = 0.69$), Fosfomycin (FO) and Nitrofurantoin (NIT) ($r = 0.55$) and Tobramycin (TOB) and Nitrofurantoin (NIT) ($r = 0.55$), likely reflect co-resistance due to shared mechanisms, such as plasmid-mediated resistance, efflux pumps, or ESBL production. Moderate correlations were noted between Ceftazidime (CAZ) and Aztreonam (ATM) ($r = 0.41$), as well as Levofloxacin (LE) and Aztreonam (ATM) ($r = 0.39$), may result from co-selection in multidrug-exposed environments. However, most other antibiotics exhibited weak or negligible correlations, reinforcing the chi-square results that resistance patterns are largely independent, which suggest independent resistance mechanisms or heterogeneous antibiotic exposure histories among the *E. coli* isolates (Table 3).

MAR Index data. The Multiple Antibiotic Resistance (MAR) index is a numerical value used to quantify the extent of antibiotic resistance in a bacterial isolate. It is calculated as the ratio of the number of antibiotics to which an isolate is resistant, relative to the total number of antibiotics tested. A MAR index greater than 0.2 indicates a high-risk source of contamination, typically associated with environments where antibiotics are frequently used.²⁵ Among the *E. coli* isolates, the MAR index ranged from 0.27 to 1.00. The lowest MAR value was 0.27, with the isolate being resistant to 2 out of 11 antibiotics, while the highest MAR value was 1.00, with the isolate being resistant to all 11 antibiotics tested. These findings highlight the significant variability in antibiotic resistance among the isolates, with the highest MAR value being particularly concerning for controlling *E. coli* infections (Figure 2). Several studies conducted in different regions of Bangladesh have also reported high MAR index values (greater than 0.2).²⁶⁻²⁸

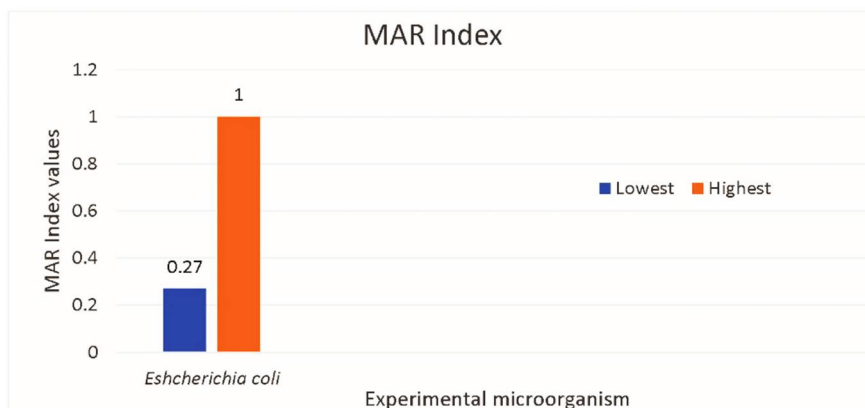


Figure 2. MAR Index data for *Escherichia coli*.

CONCLUSION

The results reveal a significant degree of resistance to several commonly used antibiotics, particularly Cefixime, Piperacillin, Ceftazidime and Ampicillin, which are largely ineffective in treating *E. coli* infections. In contrast, Fosfomycin and Tobramycin demonstrated greater efficacy, with Fosfomycin showing the lowest resistance rate,

positioning it as a potential alternative treatment option. However, moderate resistance to other antibiotics, such as Levofloxacin, Aztreonam, Nitrofurantoin and Co-trimoxazole, suggests that while their clinical utility may be compromised, they may still be considered as secondary treatment options depending on local resistance profiles and clinical judgment. The lack of genotypic data to

corroborate the observed phenotypic resistance and the geographic restriction of isolates to a single hospital were the limitations of this study, which could restrict the findings' applicability.

The study also highlighted a concerning prevalence of multidrug-resistant (MDR) strains, with 84% of *E. coli* isolates classified as MDR. This underscores the growing challenge of managing infections caused by resistant *E. coli* and emphasizes the urgent need for continuous surveillance of antibiotic resistance trends. The MAR index further underscores this concern, with elevated values indicating high resistance levels, particularly in isolates exhibiting the highest MAR scores. In conclusion, these findings emphasize the need for alternative treatment strategies, responsible antibiotic usage and the development of new therapeutic options to combat the rising threat of *E. coli* infections and antibiotic resistance.

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DISCLOSURE STATEMENT

There are no conflicting interests, the authors claim.

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