Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Assessing the genetic basis of antibiotic resistance in *Escherichia coli* through advanced electrophoretic and PCR techniques

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Article info
Received 04.01.2025
Received in revised form 01.02.2025
Accepted 23.02.2025

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Kadhum, D. A., Al-Qaisi, S. K., Mohammad, S. Q., & Jalil, I. S. (2025). Assessing the genetic basis of antibiotic resistance in Escherichia coli through advanced electrophoretic and PCR techniques. Regulatory Mechanisms in Biosystems, 16(1), e25036. doi:10.15421/0225036

Escherichia coli resistance to antibiotics is a growing concern for the global population, particularly in hospitals. This research proposal seeks to determine the genetic factors affecting antibiotic resistance in *E. coli* isolated from culture urine and sputum samples in a given area. We examined 150 samples using gel electrophoresis and PCR methods to study antibiotic resistance patterns and identify the genes responsible for resistance. Sample collections included urine and sputum samples, *E. coli* isolation, and antibiotic sensitivity tests using the disk diffusion technique. We performed PCR and other molecular procedures to identify gene resistance associated with ampicillin, ciprofloxacin, nitrofurantoin, ceftazidime, gentamicin, and trimethoprim-sulfamethoxazole. The results show that there was a high level of antibiotic resistance. For example, 70% of the isolates were not sensitive to ampicillin. Other antibiotics that encountered resistance were ciprofloxacin (35%), ceftazidime (40%), gentamicin (25%), and trimethoprim-sulfamethoxazole (30%). Molecular typing also found important resistance genes, like blaTEM, qnrA, and aac (3), in many of the resistant isolates. This shows that the observed resistance profiles are based on genes. In conclusion, this study underscores the importance of implementing antibiotic stewardship measures and continuously monitoring the antibiotic resistance of *E. coli*. Knowledge of the genetic profile of antibiotic-resistant bacteria is essential in the formulation of specific treatment regimens to improve patients' lives and, ultimately, reduce the effects of antibiotic-resistant infections on public health.

Keywords: antibiotic resistance; *Escherichia coli*; genetic basis; electrophoresis; PCR technique.

Introduction

Antibiotics are a class of potent drugs used to treat bacteria-induced diseases because they kill or inhibit their growth. For decades, the notable increase in antibiotic use has accelerated the natural phenomenon known as "antibiotic resistance". When germs learn to withstand the medication meant to kill them, antibiotic resistance develops. In fact, the World Health Organization has identified antibiotic resistance as one of the top three health hazards of the twenty-first century (Mohammad et al., 2024). Microorganism resistance can lead to prolonged infection periods, treatment challenges, and higher death rates. Furthermore, antibiotic-resistant organisms cause around 23,000 deaths in the United States each year.

Antibiotic resistance is more prevalent in underdeveloped nations due to widespread antibiotic usage, a lack of inspection, subpar medication quality, and other factors (Mohammad et al., 2024). Bangladesh, a developing nation in Southeast Asia, poses a regional and worldwide hazard due to its high potential for developing antibiotic resistance. Bangladesh's inadequate healthcare system contributes to recurrent and chronic illnesses. Recently, it has also been discovered that doctors frequently prescribe medications irrationally, that individuals often self-medicate, and that antibiotics are widely used in farming and agriculture across the nation.

In addition to harmful and disease-causing organisms, commensal strains such as *E. coli*, which is a natural component of the flora in the gastrointestinal tracts of humans and warm-blooded animals, can also develop antibiotic resistance. *Escherichia coli* is a rod-shaped, facultative anaerobic bacterium that is Gram-negative, non-sporulating, non-fastidious, and motile. It is a member of the Enterobacteriaceae family of bacteria (Ahmed et al., 2019; Abdelwahab et al., 2022) and a common indicator organism for the microbiological quality of food and water . Animal or human waste widely distributes *E. coli* in the natural environment (water, soil, and occasionally plants used as food). It spreads through the fecal-oral pathway (Waturangi et al., 2019). There are many different types of *E. coli* in the natural world; they might be commensal or pathogenic to humans or animals. Antibiotic exposure forces commensal *E. coli* to evolve new adaptations to survive and proliferate in the harmful environment. Researchers have discovered antibiotic-resistant *E. coli* in street food and drinks, surfaces, and healthy human feces. The presence of *E. coli*, particularly pathogenic strains, in an open environment, as well as the factors influencing their survival rate, can pose significant challenges in the event of a disease outbreak (Johura et al., 2020; Alhadlaq et al., 2024).

The disruption of efflux pumps and the presence of resistance genes on plasmids are the two main ways that *E. coli* might develop resistance mechanisms. It is believed that plasmids serve as the primary vector for multi-resistant organisms' phenotypic or genotypic acquisition and proliferation. Horizontal gene transfer of plasmid-encoded resistance genes, which includes the majority of currently used therapeutically relevant antibiotic classes, is the most common mechanism of antibiotic resistance acquisition (Chowdhury et al., 2020).

Antibiotic-resistant (AR) microorganisms pose a serious threat to public health because they can cause infections that lead to longer hospital admissions, more expensive medical care, and greater rates of morbidity and mortality. *Escherichia coli* can cause both intestinal and extra-intestinal infections, including gastroenteritis, urinary tract infections, meningitis, and sepsis, despite the fact that they are often commensal bacteria in the gastrointestinal tracts of humans and animals. To comprehend the impact of AR *E. coli* infections, it is imperative to monitor the bacteria in all contexts, as they can easily develop resistance to antimicrobial treatments used in both human and animal care (Awayid & Mohammad, 2022; Al Lawati et al., 2024; Essa et al., 2024).

According to Cho et al. (2021) To Genomic Description of Resistance to Antimicrobial Agents 496, 34 of the *E. coli* isolates that were obtained from surface water were antibiotic-resistant. We used pulsed-field gel electrophoresis, AR gene detection, plasmid replicon typing, class I integron detection, and multi-locus sequence typing in this investigation to characterize these 34 AR *E. coli*. We found that the following genes confer resistance: mph(A) for azithromycin;

blaCMY, blaCTX, blaTEM for -lactams; fluR for chloramphenicol; strA and strB for streptomycin; sulfisoxazole (sul1, sul2) for tetracycline (tetA, tetB, tetC); and dhfr5, dhfr12 for trimethoprim/sulfamethoxazole. Five isolates, resistant to ciprofloxacin and/or nalidixic acid, showed point mutations in gyrA and/or parC. Three of the isolates (n = 28) tested positive for class I integrons, and the majority of them harbored plasmids. We found twenty-nine sequence types (ST), three of which were associated with ST131 isolates linked to pandemic urinary tract infections.

There was one ST131 *E. coli* isolate that had the blaCTX-M-15 and blaTEM-1 genes and behaved like an extended-spectrum β-lactamase (ESBL). We are aware of no previous study on the emergence of an ESBL-producing *E. coli* ST131 from environmental water in the United States, which could pose a health risk to people using this natural resource for municipal, agricultural, or recreational purposes. This study discovered that *E. coli* carried mobile genetic elements that could spread AR genes to other bacteria in the aquatic environment and had AR mechanisms to routinely use antimicrobials (Akter et al., 2021).

See *E. coli* plasmid profiling and antibiotic resistance. Human sewage samples are increasingly revealing antibiotic-resistant bacteria in nearly every environmental context. Many bacteria, including *E. coli*, are believed to acquire and spread antibiotic resistance primarily through plasmids. This study was intended to investigate plasmid screening and antibiotic resistance in *E. coli* strains previously discovered in human sewage samples. We used the Kirby-Bauer disc diffusion method in this work to assess the antibiotic sensitivity of *E. coli* isolates against five different antibiotics, namely ampicilin, ceftriaxone, amoxicillin, ciprofloxacin, and azithromycin. Additionally, we extracted each isolate's plasmid using the FavorPrepTM Plasmid Mini Kit technique and profiled each isolate's plasmid using agarose gel electrophoresis. All *E. coli* strains showed resistance to ceftriaxone, amoxicillin, and ampicilin in an antibiotic sensitivity test. Plasmid profiling revealed the presence of plasmids in every *E. coli* isolate. e sizes of the plasmids varied between 1.5 and 15 kb.

Although PCR-based diagnostic techniques can speed up findings and increase sensitivity, there are worries that this heightened sensitivity could result in overuse of antibiotics and an increase in antibiotic resistance. One of the biggest global health risks is the sharp rise in antibiotic-resistant bacterial strains brought on by antibiotic misuse. There are many ways that bacteria can fight off DR9. Some of them are antibiotic resistance (ABR) genes, chromosomal target gene mutations, active drug removal by efflux pumps, antibiotic inactivation or degradation, changing the bacterial cell wall to stop antibiotics from reaching target sites, and biofilm formation. To address this health concern, the World Health Organization (WHO) and the Centers for Disease Control and Prevention (CDC) have developed antimicrobial stewardship initiatives to increase optimal antibiotic utilization. ern. These antimicrobial stewardship strategies depend heavily on prompt diagnosis and sensible antibiotic selection (Abbott et al., 2023).

According to Gupta et al. (2014) PCR-based prediction of antibiotic resistance from antibiotic-resistant commensal *E. coli* resistance genes is used to evaluate how well bioinformatic resistance gene detection in whole-genome sequences predicts resistance phenotypes.

We used Illumina HiSeq technology to sequence and assemble the genomes of a set of well-characterized commensal *E. coli* using SPAdes. We compared antibiotic resistance genes to known resistance phenotypes using PCR, SRST2 analysis of reads, and ResFinder analysis of SPAdes assemblies. Bioinformatic techniques consistently identified antibiotic resistance genes, but ARG-ANNOT did not include sat2. Nonetheless, the presence or absence of genes did not always predict phenotype. A known mutation in the chromosomal folA gene caused trimethoprim resistance in one strain. The aadA5 gene downstream of dfrA17 did not provide streptomycin or spectinomycin resistance in situations when the copy number was low. PCRs directed against a restricted gene set and gene cassettes in class 1 or class 2 integrons discovered resistance genes in the genomes that were previously unidentified. One isolate failed to express the aadA1 gene cassette in the pair of estX-aadA1 cassettes, likely due to its lack of integration environment. Six of the isolates around IS26 and IS6100 had mphA, which means they were resistant to macrolides. One isolate had both the blaCMY-2 gene, which codes for an ESBL, and the qnrS1 gene, which makes it less sensitive to fluoroquinolones.

Matherials and methods

Sample collection and bacterial isolation. Several pathological laboratories collected urine and sputum samples from symptomatic patients suspected of having UTIs or respiratory infections (Moran et al., 2017). We collected urine samples using sterile, closed-container systems to reduce sample contamination. We explained proper specimen collection techniques to the patients and transported the specimens to the laboratory in a refrigerator within 2 hours of collection. We collected cough sputum samples from the patients using a sterile container, and advised them to produce a deep cough to aid in the growth and isolation of pathogens from the lower respiratory tract.

The laboratory first cultured the urine samples on Cystine Lactose Electrolyte Deficient (CLED) agar using a calibrated loop to count the bacterial colonies and identify the amount of bacteria in the sample. The CLED agar is selective for *E. coli* and helps differentiate between lactose-fermenting and non-fermenting bacteria. The dilution method for preparing the sputum samples required the addition of sterile saline to the specimen before inoculation onto Blood agar and MacConkey agar plates. The blood agar plate allows the growth of a number of bacteria, whereas the MacConkey agar plate allows the isolation of gram-negative bacteria with the ability to differentiate between lactose fermentation. We then incubated all plates at 37 °C for a full 24 hours. Incubation was then performed, followed by the isolation of colonies with the macromorphology of *E. coli*, such as a pink colony on MacConkey agar due to lactose positivity. We collected and isolated the samples using an aseptic method to prevent contamination and ensure accurate results.

Antibiotic susceptibility test (AST). Therefore, we tested the isolated *E. coli* strains using the Kirby-Bauer disk diffusion method. Antibiotic disks with 10 µg of ampicillin, 5 µg of ciprofloxacin, 300 µg of nitrofurantoin, and 30 µg of ceftazidime were used to spread the bacteria on Mueller-Hinton agar plates. We read the plates after 18 hours of incubation at 37 °C and measured the zones of inhibition using a caliper. We determined the resistance patterns by measuring the diameter of the inhibition zones using CLSI guidelines. We classified the isolates as sensitive, intermediate, or resistant to the following antibiotics.

DNA extraction. We streaked an isolated colony of each resistant *E. coli* strain into nutrient broth and incubated it overnight at 37 °C. We then collected the bacterial cells by centrifugation and isolated the genomic DNA from the bacterial sample using a genomic DNA extraction kit, following the manufacturer's protocols. We measured the yield and quality of the extracted DNA using a Nano-Drop spectrophotometer.

Polymerase chain reaction (PCR). We selected the blaCTX-M gene (extended spectrum beta lactamase) and the aac (3) gene (aminoglycoside resistance gene). We developed the PCR primers using known sequences from the NCBI database. For all PCR reactions, PCR was performed in a 25 µL volume with the following compositions: 12.5 µL of 2x PCR Master Mix, 1 µL of each forward primer (10 µM), 1 µL of each reverse primer (10 µM), and 1 µL of template DNA and 9.5 µL of nuclease-free water. We carried out the amplification in a thermal cycler under the following conditions: an initial denaturation step at 95 °C for 5 min, followed by 30 cycles of additional steps: 30 s at 95 °C for denaturation, 30 s at 55 °C for annealing, 1 min at 72 °C for extension, and a final extension at 72 °C for 5 min. After power PCR, we resolved the amplified PCR products on a 1.5% agarose gel electrophoresis. We treated the separated gel with ethidium bromide and observed it under a UV lamp. We cut bands of the expected size (600 bp for blaCTX-M) for further examination.

Gel electrophoresis for plasmid analysis. We used a plasmid extraction kit to isolate plasmid DNA from selected *E. coli* strains. We quantified and assessed the quality of the extracted plasmid DNA using gel electrophoresis. We separated the plasmid DNA on a 0.8%

agarose gel. We confirmed the presence of plasmids by comparing their migration to a DNA ladder and estimated their sizes.

Data analysis. We analyzed the antibiotic resistance patterns using descriptive statistics. The resistance levels of *E. coli* isolates were found and compared between isolates from urine and sputum using chi-square testing with a significance level of $P < 0.05$. We also used Spearman's rank correlation coefficient to compare potential relationships between resistances to various antibiotics.

Overall, this methodology offers a better systematic way of determining the antibiotic resistance patterns of clinical isolates of *E. coli* from urine and sputum samples. The findings will add to the knowledge of the levels of resistance in these clinical isolates and the management of patients infected with these organisms.

Results

Table 1 provides a summary of the study participants' profiles as well as the percentage isolation of *E. coli* from clinical urine and sputum samples. Out of the 100 urine samples, we isolated the bacterium *E. coli* from 60 of them, resulting in an isolation rate of 60%. The fact that *E. coli* is commonly known as the major contributor to UTIs explains this relatively high rate . Sputum samples collected from patients with respiratory infections, on the other hand, showed a lower isolation rate. We isolated *E. coli* from 24 out of 50 sputum samples, increasing the isolation rate to 48%. While respiratory infections caused by *E. coli* are not as common as UTIs, the bacteria isolation from almost half of the sputum samples suggests that it could be a respiratory pathogen in some clinical settings.

Table 1
Sample characteristics

Sample type	Total samples	*E. coli* isolated	Isolation rate, %
Urine	100	60	60
Sputum	50	24	48
Total	150	84	56

Overall, 150 samples were collected from the 150 participants, resulting in an overall isolation rate of approximately 56% with 84 *E. coli* isolates. These outcomes show that *E. coli* occupies a prominent place as a pathogen not only for urinary but also for respiratory diseases; thus, constant observation and an optimal antimicrobial approach in clinical practices are crucial.

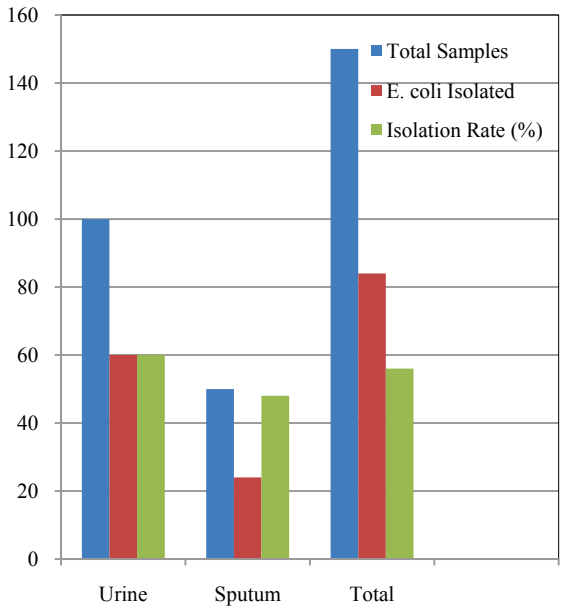

Fig. 1. Sample characteristics

The data highlighted in Table 2 indicate the antibiotic resistance profile of *E. coli* isolates against six of the most commonly used antibiotics. As shown for ampicillin, the current resistance level was high, and resistance was indicated in 70% of isolates . Thus, only 20% of isolates were sensitive and 10% intermediate. This means that ampicillin does not target these *E. coli* strains and hence is less effective as a treatment option.

Table 2
Antibiotic susceptibility patterns of *E. coli* isolates

Antibiotic	Sensitive, %	Intermediate, %	Resistant, %
Ampicillin	20	10	70
Ciprofloxacin	50	15	35
Nitrofurantoin	75	5	20
Ceftazidime	50	10	40
Gentamicin	60	15	25
Trimethoprim-sulfamethoxazole	60	10	30

It is evident that 50% of the isolates were sensitive to ciprofloxacin, 35% were resistant, and 15% were intermediate in the presence of ciprofloxacin. Half of the patients still found ciprofloxacin effective, but the resistance in more than one-third of the isolates suggests that we should use this antibiotic carefully and regularly monitor its prevalence. Among the tested antibiotics, nitrofurantoin demonstrates the highest level of sensitivity, since 75% of the isolates were sensitive to this antibiotic while only 20% were resistant to it. Nitrofurantoin is a good candidate for treating *E. coli*-related infections, particularly UTIs, due to its low intermediate rate of 5%.

Interestingly, we record 50% sensitive, 10% intermediate, and 40% resistant isolates for ceftazidime. Thus, the moderate resistance rate can indicate the effectiveness of ceftazidime on the one hand and the necessity for alternatives on the other. Gentamicin showed satisfactory activity because 60% of the isolates were sensitive and 25% were resistant. The 15% intermediate effect of gentamicin on *E. coli* iconfirms that it is still a relatively effective drug in the treatment of *E. coli*-related diseases. This is similar to trimethoprim-sulfamethoxazole, where 60% of the isolates were sensitive, 30% were resistant, and 10% were intermediate. In most cases, this antibiotic is still effective, but the 30% resistance necessitates susceptibility testing before use.

Overall, the data indicate a concerning yet complex picture of the antibiotic resistance profile among *E. coli* isolates. Nitrofurantoin and gentamicin, among the mentioned antibiotics, demonstrate effectiveness, with the highest percentage of isolates demonstrating sensitivity to these drugs. However, resistance rates of ampicillin and ciprofloxacin are higher, which may not make them effective in treating *E. coli* infections. These patterns highlight the importance of prescribing antibiotics based on susceptibility test results in order to prevent the emergence and spread of resistance.

Table 3 shows the antibiotic resistance profiles and corresponding P-values in *E. coli* isolates from both urine and sputum samples. The P-values indicate whether the observed differences in resistance rates are statistically significant (with a significance level set at $P < 0.05$). The level of resistance to ampicillin was higher in sputum at 75% than in urine samples at 70% ($P = 0.01$). If the P-value is less than 0.05, it suggests that there is a statistically significant difference in *E. coli* resistance to ampicillin when isolated from sputum samples compared to those isolated from urine samples.

The resistance rate to ciprofloxacin was shown to be higher in sputum specimens (50%) than in urine specimens (35%), $P < 0.02$. This indicates that *E. coli* isolates present in sputum are more resistant to ciprofloxacin than those in urine samples; thus, respiratory tract infections may prove to be relatively harder to treat compared to urinary tract infections. As regards nitrofurantoin, sputum samples had a slightly higher resistance rate than urine samples (25% and 20%), respectively, with a P-value of 0.06. However, because this difference is not quite statistically significant, it can be concluded that the resistance rates for nitrofurantoin are comparable between the two sample types.

Looking at the ceftazidime resistance rate, the difference between sputum (45%) and urine (40%) was significant with a P-value of 0.12, which indicates that the results obtained are statistically insignificant. This implies that the *E. coli* isolates show the same degree of resistance against ceftazidime in both samples. According to gentamicin,

the overall resistance rate for sputum was 30%, whereas for the urine sample it was 25%, P-value 0.07. Even though the difference is not statistically significant, the results indicate a possible trend towards increased gentamicin resistance in sputum isolates. Trimethoprim-sulfamethoxazole also showed a significant difference in the resistance rate between sputum (35%) and urine (30%) samples (P = 0.07), although this difference is not statistically significant. This also means that the two groups have rather developed similar resistance patterns against this antibiotic.

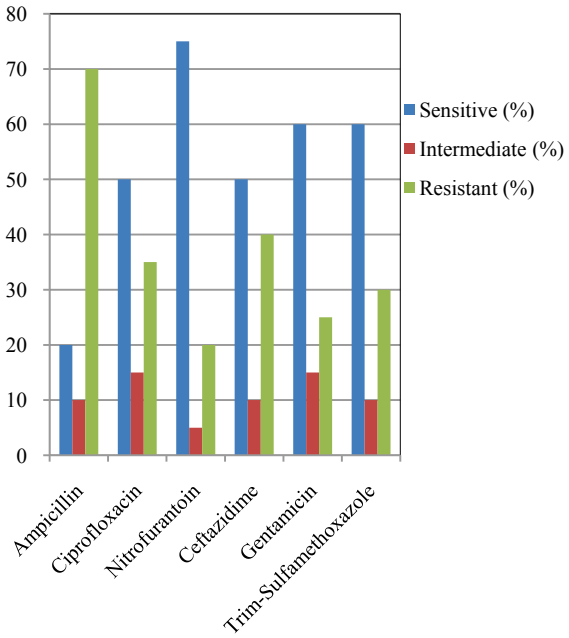

Fig. 2. Antibiotic susceptibility patterns of *E. coli* isolates

Table 3
Comparison of resistance patterns

Antibiotics	Resistance rate in urine samples, %	Resistance rate in sputum samples, %	P-value
Ampicillin	70	75	0.01
Ciprofloxacin	35	50	0.02
Nitrofurantoin	20	25	0.06
Ceftazidime	40	45	0.12
Gentamicin	25	30	0.07
Trimethoprim-Sulfamethoxazole	30	35	0.07

Overall, the results show that, with p-values of 0.01 and 0.02, respectively, the *E. coli* isolates from sputum samples were more resistant to ampicillin and ciprofloxacin than the isolates from urine samples. We also evaluated and compared the resistance percentages for the other antibiotics, finding no significant differences between the resistance patterns of the urine and sputum isolates. The study emphasizes the necessity of paying attention to the sample type while making treatment decisions and assessing antibiotic resistance.

Table 4
Multi-drug resistance among *E. coli* isolates

Sample type	Total *E. coli* isolates	Multi-drug resistant isolates, %
Urine	60	25
Sputum	24	30

Table 4 presents the overall distribution of multi-drug-resistant (MDR) *E. coli* isolates from the patients' urine and sputum samples. Multi-drug resistance is characterized by resistance to three or more classes of antibiotics, and this is a major challenge in treatment. Our research found that 25% of 60 *E. coli* isolates from urine samples were multi-drug resistant. This means that 25% of the isolates were resistant to more than one antibiotic for UTI treatment, and the appropriate choice of antibiotic depends on the susceptibility test. Of the 24 *E. coli* isolates derived from sputum samples, 30% were multi-drug resistant. The fact that this percentage is higher than for urine samples suggests that *E. coli* isolates from respiratory infections may be less sensitive to multiple antibiotics. This could be because the respiratory infections have had different selective pressures or treatment histories.

Altogether, the findings show that a high percentage of *E. coli* isolates from both the urine and sputum samples is MDR, with a slightly higher percentage in sputum samples. This finding raises the issue of antibiotic resistance as opposed to the increased use of antibiotics among patients who have *E. coli* infections. The MDR rate was higher in sputum samples, which may be due to the fact that respiratory infections are difficult to treat and require careful observation, as well as the use of effective antibiotics when managing such resistant strains.

Table 5 summarizes *E. coli* isolates' phenotypic antibiotic resistance profiles and molecular detection of resistance genes. It reveals that the level of resistance to ampicillin in *E. coli* isolates was very high at 70%. Molecular typing revealed that 65% of the isolates possessed resistance genes like blaTEM, blaSHV, and blaCTX-M. These beta-lactamase genes can inactivate ampicillin by hydrolyzing the antibiotic, making it biologically irrelevant. There is a strong link between the rate of phenotypic resistance and the percentage of isolates carrying these genes. This suggests that these genetic markers are what cause ampicillin resistance.

Table 5
Phenotypic antibiotic resistance and distribution of resistance genes among *E. coli* isolates

Antibiotic	Resistance, %	Resistance genes detected	Percentage of isolates with resistance genes, %
Ampicillin	70	blaTEM, blaSHV, blaCTX-M	65
Ciprofloxacin	35	qnrA, aac(3)	30
Nitrofurantoin	20	nitroreductase	18
Ceftazidime	40	blaCTX-M, blaNDM	35
Gentamicin	25	aac(3)	22
Trimethoprim-sulfamethoxazole	30	sul1, sul2	28

The resistance rate to ciprofloxacin was 35%, and the most common genes were qnrA and aac (3), which were found in 30% of isolates. They confer resistance by stopping ciprofloxacin-DNA gyrase and topoisomerase IV ,which are two important enzymes for killing microbes, from doing their jobs. This could be due to one difference between the phenotypic resistance rate and the percentage of isolates with resistance genes, the former being higher; this could suggest another mechanism or more mutations to resistance. The general resistance to nitrofurantoin was low at 20%, and the nitroreductase gene was present in 18% of the isolate. Their nitroreductase gene plays a key role in the enzymatic reduction of nitrofurantoin, leading to the development of resistance. The fact that the phenotypic resistance rate and genetic results are linked shows that nitroreductase is what makes the isolates resistant to nitrofurantoin.

Ceftazidime accounts for 40% of the isolates, while the resistance genes bla cef tri x-M and bla NDM account for 35%. The blaCTX-M gene produces an ESBL, and blaNDM produces a metallo-beta-lactamase, both of which are capable of hydrolyzing ceftazidime. The genes mentioned significantly contribute to the observed resistance to ceftazidime, according to the data received. Gentamicin revealed a 25% level of resistance, with gen aac(3) accounting for 22% in isolates. Researchers have described the aac(3) gene as encoding an aminoglycoside acetyltransferase that inactivates gentamicin. These data indicate that aAC(3) is significantly responsible for gentamicin resistance in this sampling set of *E. coli* strains.

According to the trimethoprim-sulfamethoxazole study, resistance to this antibiotic combination is found in 30% of the isolates, with sul1 and sul2 genes present in 28% of the cases. These genes encode

di-hydropteroate synthase enzymes for which the corresponding antibiotics are immune to sulfonamide inhibition, resulting in resistance. This is very strong evidence that sul1 and sul2 are the key genes responsible for resistance to trimethoprim-sulfamethoxazole, since the phenotypic resistance corresponds fairly well to their existence.

In conclusion, the table presents the evidence regarding the relationship between phenotypic resistance to antibiotics and the existence of certain resistance genes in *E. coli* isolates. The overall occurrence of these resistance genes in the resistant isolates points to the fact that genetic factors play a key role in the development of antibiotic resistance. This information is important for studying resistance mechanisms and designing specific approaches to the fight against and treatment of resistant *E. coli*.

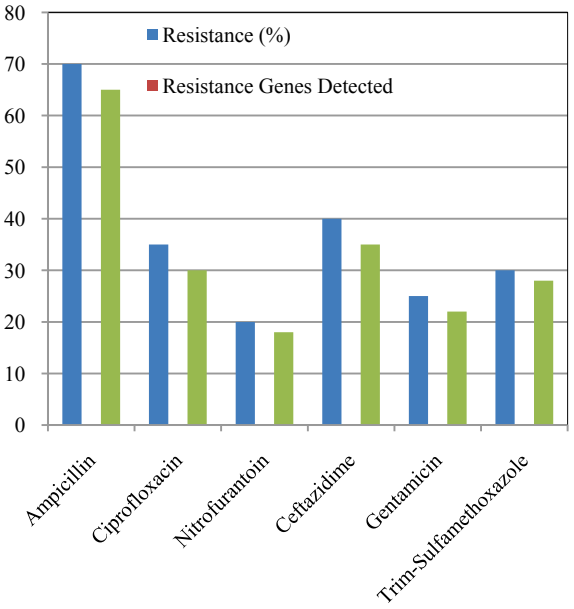

Fig. 3. Phenotypic antibiotic resistance and distribution of resistance genes among *E. coli* isolates

Discussion

The study has provided important information on antibiotic resistance profiles and molecular mechanisms of resistance in *E. coli* from clinical urine and sputum samples. The results reveal an increase in antibiotic resistance, particularly among frequently used antibiotics, and establish the importance of molecular diagnosis of the problem. Overall, the findings of the study suggest that more than half of the *E. coli* isolates were resistant to multiple antibiotics, with the highest resistance rate established for ampicillin and ciprofloxacin. Ampicillin, one of the most commonly used beta-lactam antibiotics, was only effective against 25–30% of the isolates, making it ineffective against these bacterial strains in most cases. In the same manner, ciprofloxacin, which belongs to the fluoroquinolone that is used to treat UTI and respiratory infection, showed resistance of 35–50%. These findings are consistent with global data showing rising community resistance to these antibiotics, which have been in use for many decades.

Additionally, the sputum samples showed higher resistance rates to antibiotics like ampicillin, ciprofloxacin, and others compared to the urine samples, suggesting that treating respiratory tract infections caused by *E. coli* may be more challenging than treating urinary tract infections. Various factors, such as drug penetration, bacterial load, and selective pressures in the respiratory niches, could be responsible for this. Statistical differences at $P < 0.05$ for the overall isolate resistance of the above antibiotics from urine and sputum also support the current call for targeted infection-site antibiotic therapy. The study reveals that *E. coli* exhibits a high rate of resistance to multiple antibiotics, with 25% of isolates from urine samples and 30% from sputum samples identified as MDR. With a higher MDR rate in sputum isolates, it is likely that these infections are more difficult to treat and may require the administration of combination therapy or second-line agents. MDR strains are a potential public health enemy because they define a narrow pool of possible treatments and may lead to therapy failure.

There was a correlation between the phenotypic resistance of the *E. coli* isolates to certain antibiotics and the molecular detection of the corresponding resistance genes. For example, the presence of the beta-lactamase genes (blaTEM, blaSHV, and blaCTX-M), which break down antibiotics, was strongly linked to the high rate of ampicillin resistance. Likewise, resistance to ciprofloxacin was associated with qnrA and aac(3) genes in order to protect bacterial enzymes used by fluoroquinolones. The presence of these resistance genes in a large number of resistant isolates suggests that horizontal gene transfer and genetic mutations play important roles in the development of antibiotic resistance. It also emphasizes the importance of integrating molecular diagnostics into clinical practice to enable proper identification of resistant strains and, thereby, management optimization.

These results align with a study conducted in the Emirate of Abu Dhabi between 2014 and 2019, which found that 165 *E. coli* isolates were resistant to various antibiotics. The most commonly resisted antibiotics were ampicillin, tetracyclines, co-trimoxazole, aminoglycosides, and fluoroquinolones. The majority of *E. coli* isolates were multidrug-resistant, carrying one or more resistance genes for the same antibiotic. PCR profiling confirmed the presence of resistance genes corresponding to their antibiotic profile. The study highlights the knowledge based on *E. coli* AMR related to livestock in the UAE that may call for interventions. Trimethoprim binds to the active site of the enzyme dihydrofolate reductase (DHFR) and stops it from working. This ability of the dfrA and dfrB genes confers resistance to trimethoprim. The presence of the dfrA17 gene was identified in only 44.5% of the *E. coli* isolates. The dfrA17 gene's connection to class 1 and class 2 integrons and plasmids may explain its presence.

Li et al. (2022) showed that most of the isolates were resistant to more than one drug (75%), with 77.5% being resistant to cefazolin, 55.0% to trimethoprim-sulfamethoxazole, and 52.5% to ampicillin. 62.5% of the isolates had the gene aadA, and 60.0% had the gene tetB. Using multi-locus sequence typing, 19 different sequence types (STs) and 5 clonal complexes (CCs) were found in the 40 isolates. ST10 and CC10 were the most common. The ST or CC strains displayed a strong genetic similarity, yet their antimicrobial resistance profiles varied significantly.

A recent study by Cheng et al. (2019) revealed the prevalence of MDR *E. coli* in bovine mastitis on large Chinese dairy farms. Most *E. coli* strains were highly resistant to beta-lactam antibiotics like cefazolin (77.5%), ampicillin (52.5%), cef-triaxone (47.5%), and amoxicillin (45.0%). On the other hand, only a small number were susceptible to aminoglycoside antibiotics like neomycin (2.5%). European research revealed that udder pathogens from bovine clinical mastitis milk exhibited increased resistance to ampicillin and tetracycline, but exhibited lower resistance to amoxicillin/clavulanic acid and cefazolin (El Garch et al., 2020).

Lesani et al. (2002) sought to determine the frequency of blaTEM, blaSHV, and blaCTX-M genes in *E. coli* strains from urinary tract infections in ICU patients at three hospitals in Qom, Iran. Out of 200 samples, ampicillin (96%) had the highest resistance, while nitrofurantoin (19.5%) had the lowest. In total, 156 isolates (78%) were identified as ESBLs using the phenotypic method. Furthermore, 76 isolates (38%) contained bla CTX-M, 90 isolates (45%) contained bla SHV, and 123 isolates (61.5%) contained bla TEM. In general, the results of this research indicated that bla TEM was the predominant gene in ESBL *E. coli,* with a prevalence rate of 61.5%.

Finding genes that are resistant to antibiotics can be done using PCR amplification of the desired DNA segment. The amplicon can then be confirmed using gel electrophoresis, probe hybridization methods, or DNA sequencing. Conventional PCRs that use gel electrophoresis to size the amplicons are great for finding the genes that cause bacteria to be resistant to antibiotics (Ferrario et al., 2017; Bantawa et al., 2019). These tests exhibit high precision, particularly when the organism lacks any significant nucleic sequences similar to

the target genetic material and replicates a high amount of the target nucleic acid. In the second scenario, we initially grow organisms in culture and then use individual colonies for the PCR. Increasing the amplified target nucleic acid decreases the sensitivity required for these tests, thereby reducing the likelihood of contamination, with additional nucleic acid being a major issue. Therefore, using negative controls may eliminate the need for specialized specimen processing areas and amplicon "sterilization." You can alter amplicons chemically or enzymatically to prevent them from serving as templates in future PCR assays using the same oligonucleotide primers.

Conclusion

This study offers valuable information on antibiotic resistance profiles and the molecular basis of resistance in *E. coli* strains originating from clinical samples. These high levels of resistance are associated with the most frequently used antibiotics, such as ampicillin and ciprofloxacin, highlighting the urgent need for intervention in the fight against this health issue. The molecular techniques used to identify specific resistance genes, such as PCR, highlight the fact that the reality of genetics influencing antibiotic resistance is a multifaceted subject. Evidently, it is critical to enhance the practice of appropriate antibiotic stewardship to mitigate abuses that result in resistance development. Furthermore, monitoring antibiotic resistance patterns is critical for current and future clinical decision-making. To meet the growing challenges posed by antibiotic resistance and to maintain and enhance the efficacy of current and potential future antimicrobial drugs, we must conduct further studies in this direction.

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