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Phylogenetic and antibiotic resistance analysis of *Escherichia coli* isolates from septicemia patients

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Abstract

Escherichia coli is a major bacterium impacting human health, widely present in the environment and the human body. Its persistence challenges the immune system and facilitates opportunistic infections across all age groups. A critical strategy of E. coli is EMDR, which exacerbates chronic diseases and complicates treatment, contributing to the global crisis of antibiotic-resistant epidemics. This study investigated bacteremia caused by E. coli. A total of 100 blood samples were collected from patients exhibiting clinical symptoms such as fever, chills, and weakness. Samples were cultured anaerobically at 37°C for 24–72 hours, then subcultured on selective media. Identification use Vitek ID system, antibiotic sensitivity testing with the Vitek AST card, and 16S rRNA sequencing. Phylogenetic analysis revealed evolutionary relationships between E. coli isolates and members of the Enterobacteriaceae family. **Results**: showed that 75 isolates (75%) were identified as E. coli, confirming its significant role in bloodstream infections (BSI). The evolutionary tree highlighted close genetic links with resistant Enterobacteriaceae strains. To explore alternative therapies, the antibacterial activity of green tea extracts was assessed using agar well diffusion. The aqueous extract exhibited dose-dependent inhibition zones of 25, 20, 15, and 10 mm at concentrations of 40, 30, 20, and 10 mg/mL, respectively. Ethanol extracts showed stronger activity, with inhibition zones of 35, 30, 25, and 22 mm at the same concentrations.

Keywords: E. coli, BSI, EMDR, PCR, phylogenetic tree, green tea extract

Introduction

Escherichia coli is a common pathogen associated with bloodstream infections (BSIs), representing approximately 27% of all bacterial BSIs in high-income countries (1). Global trends in the increase in BSIs are attributable to aging populations, an increased prevalence of comorbidities, and the dissemination of ESBL-producing multidrug-resistant strains (1,2). These resistant strains are linked to increased morbidity and mortality, especially among patients with a 3GCR infection (3,4). ESBL-producing *Enterobacterales* have been

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involved in nosocomial and community-acquired infections (1,5,6).

Patients with *E. coli* expressing β-lactamases have increased in-hospital mortality as compared to those infected with non-expressing strains. Nevertheless, the existing evidence is still restricted due to small sample sizes and the intricate interaction of disease progression-affecting factors (7,8). Extraintestinal pathogenic *E. coli* (ExPEC) strains are leading causes of invasive diseases, including bacteremia and urinary tract infections with complications, particularly among elderly individuals (1,9).

In England (2013–17), epidemiological data demonstrated a significant increase in patients ≥ 60 years of age who suffered from invasive BSI, with chronic UTI as the most common comorbidity and mortality rates up to 15% (10). The *E. coli* genome harbours a wealth of resistance genes that are transferred horizontally, such as blaTEM for ESBLs and AmpC, conferring resistance to quinolones, aminoglycosides, or compounds containing the beta-lactam ring. by means of membrane efflux pumps, altered membrane permeability, and mutations in drug target sites (11-14).

Although highly resistant, aminoglycosides (e.g., amikacin and especially in combination regimens) still show some activity against certain *E. coli* strains; however, the side effects and resistance development limit clinical efficacy (15-17). The extensive use of tetracyclines and penicillins has exerted selective pressure on, resulting in substantial resistance mediated by active efflux and ribosome protection mechanisms (16,18,19). Knowledge of the phylogeny, virulence, and resistance factors of *E. coli* is necessary for surveillance of strain dissemination and development of interventions.

E. coli is one of the common pathogens associated with bacteremias, and the extensive use of antibiotics adds complexity in treatment strategy. Natural extracts, particularly those from plants such as Camellia sinensis (green tea), have been increasingly considered for their potent antimicrobial activity. Catechins and phenolic compounds in green tea shred bacterial membranes and interrupt DNA replication processes. The evaluation of antibacterial effects of green tea extracts against *E. coli*, one causative agent of bacteremia, can be a novel complementary or alternative therapeutic option (20).

The present study highlights the importance of constant surveillance to follow up the distribution and spread of MDR E. coli isolates in Iraq and determine their evolutionary relationships that can help in designing an effective therapeutic approach for controlling a future epidemic.

Material and methods

Sample Collection

One hundred patients were recruited from Al-Salam Hospital during the period of April 2025 and August 2025 according to patients who presented clinically with symptoms (fever, chills, shivering). The study population was composed of 32 elderly men (70–85 yr), 28 elderly women (70–85 yr), and 40 children (1–8 yr-old).

Bacterial Isolation and Culture

Collected samples were initially inoculated in Brain Heart Infusion (BHI) broth and incubated at 37°C for 24 hours or longer when necessary. Positive cultures were sub-cultured on BHI agar using quadrant streaking to obtain pure isolates. Selective and differential media, including Eosin Methylene Blue (EMB), MacConkey agar, and blood agar, were employed for the identification of *Escherichia coli* (21).

Microscopic Examination and Identification

Gram staining was performed on culture-positive isolates. Bacterial identification was carried out using the Vitek ID card system following the manufacturer's instructions.

Antibiotic Susceptibility Testing

Antibiotic susceptibility of isolates was determined using the Vitek AST card system, following the Clinical and Laboratory Standards Institute (CLSI) guidelines. The system evaluates bacterial growth in the presence of varying concentrations of antibiotics to determine the minimum inhibitory concentration (MIC) and classify isolates as sensitive, intermediate, or resistant (22) .

Molecular Analysis

Genomic DNA was extracted using the Geneid kit following the manufacturer's protocol, and DNA purity was assessed with a Nanodrop spectrophotometer. PCR reactions were prepared in a final volume of 20 µL, containing 10 µL of PCR Master Mix, 2 µL of forward primer, 2 µL of reverse primer, 2 µL of template DNA, and 4 µL of distilled water. The PCR cycling conditions included a pre-denaturation step at 95°C for 10 minutes, denaturation at 95°C for 15 seconds, and annealing/extension at 60°C for 1 minute, followed by melting curve analysis with sequential steps at 95°C and 60°C. The amplified PCR products were then electrophoresed on 2% agarose gels, stained with Red Safe dye, and visualized under UV illumination (23).

Sequence Analysis and Phylogenetic Tree Construction

PCR-amplified sequences were analyzed using BLAST (Basic Local Alignment Search Tool) against the NCBI GenBank database to determine sequence homology. Phylogenetic trees were constructed using MEGA-11 software with 100 bootstrap replicates to assess the evolutionary relationships among isolates.

Preparation of Green Tea Extracts

Green tea leaves were used to obtain both aqueous and ethanolic extracts using the method described by (24). The aqueous extract was prepared by dissolving 30 g of green tea leaves in 100 mL of distilled water. The solution was left for 30 minutes and filtered through Whatman filter paper. The filtrate was then dried in an incubator at 37 °C for 48 hours until a dry powder was obtained. The powder obtained was later used to make the appropriate dilutions of 10, 20, 30, and 40 mg/mL. For the ethanolic extract, 30 g of green tea leaves were dissolved in 100 mL of ethanol and left for 30 minutes. The solution was filtered, dried under the same conditions, and used to prepare the desired concentrations.

Effect of Aqueous and Ethanolic Green Tea Extracts on the Growth of Escherichia coli

In this regard, the agar well diffusion method was used to evaluate the antibacterial activity. Bacterial suspensions of E. coli isolates were prepared and adjusted to the turbidity of the 0.5 McFarland standard. Twenty-four-hour-old bacterial cultures were swabbed on Mueller-Hinton agar plates using sterile swabs. The agar medium was pierced to create wells 5 mm in diameter using a sterile cork borer. Using a micropipette, 0.05 mL of each extract concentration was added individually into the wells. One well in each plate contained sterile distilled water as a control. The plates were left for 30 minutes to allow for the diffusion of extracts through the medium at room temperature before being incubated at 37 °C for 24 hours. The determination of the antibacterial activity of both aqueous and ethanolic extracts of green tea was based on the measurement of the diameter of the inhibition zones around each well.

Preparation of Bacterial Suspension

Nutrient broth cultures of the isolates were incubated at 37 °C for 24 hours. After incubation, bacterial suspensions were prepared and adjusted by comparing their turbidity with that of the 0.5

McFarland standard, equivalent to approximately 1 × 10^8 CFU/mL.

Results and Discussion

Detection of Bacterial Pathogens in Blood Samples

All 100 were culture positive for bacterial infection. *Escherichia coli* was the most common causative organism, isolated from 75 (75%) of patients. Of these, 28 were seen in children (37.3% of *E. coli* infections), 26 in elderly males (34.7%), and 21 in elderly females (28%). The remaining 25 cases were caused by the following other bacterial species: *Pseudomonas* aeruginosa in 6 (6%); *Staphylococcus aureus* in 4 (4%); Streptococcus, n =8 (8%), and Neisseria, n =7 (7%). Taken together, the results point to *E. coli* as the most consistent cause of bloodstream infections in the population studied and suggest that other pathogens are contributing less frequently but with similar distribution across age-groups. Microscopy (Gram staining). The strains were subcultured in BHI medium and examined microscopically. Among the isolates. Morphological identification was corroborated by means of selective and differential culture media. Coli grew on MacConkey agar, forming pink colonies with -lactose fermenters in EMB agar, also with metallic franchises (Fig. 1a-b-c). The Staphylococcus isolates formed yellow colonies on Mannitol Salt Agar and β-haemolysis on Blood Agar (Fig. 1 d), and Pseudomonas as black colonies on Blood Agar (Fig. 1e). These identifications were confirmed using the Vitek system as shown in Table 1. The data are consistent with those from earlier studies in Bangladesh that used the Vitek method for bacterial identification of patients with septicemia (30-33).

These findings are consistent with the results reported by (21), who conducted a similar study on the diagnosis of bloodstream infections and observed comparable outcomes. The culture analysis in the present study revealed a positive rate of 72%, with 18 out of 25 samples testing positive for bacterial growth. This relatively high detection rate is consistent with the general trend reported in several recent studies worldwide, although the positivity rate in this study appears higher than in most published reports. For example, (25) conducted a five-year surveillance study of pediatric bloodstream infections in a referral hospital in Tehran and isolated 3,179 pathogens, of which 2,824 were bacterial. Their findings showed a balanced distribution between Gram-negative (54%) and Gram-positive (46%) bacteria, with Pseudomonas spp., *Klebsiella pneumoniae*, and *Stenotrophomonas maltophilia* being the most common Gram-negative isolates, while *Staphylococcus aureus*, Enterococcus spp., and coagulase-negative staphylococci (CONS) predominated among Gram-positive isolates.

Likewise, (26) obtained single-center data and a multicenter study consisting of 3,397 bloodstream isolates in South Korea, with 949 belonging to multidrug-resistant organisms. Their analysis pointed out the increasing trend of resistance against last-resort antibiotics, including carbapenem-resistant *Enterobacterales* and vancomycin-resistant *Enterococcus*, representing the interest of the therapy in bloodstream infections under the era of antimicrobial resistance.

Similar results were found in Lebanon, where (27) studied 76 positive blood cultures received from September 2023 to March 2024. Their results indicated that Gram-positive and Gram-negative bacteria were nearly equally distributed. The Gram-negative group was dominated by *Escherichia coli*, with a considerable rate of extended-spectrum β-lactamases (ESBL) and carbapenem resistance; in the Gram-positive group, *Staphylococcus aureus* and CONS prevailed, including methicillin-resistant S. aureus (MRSA). In a large case series from China, (28) examined over 37,000 blood cultures from a dedicated cardiac hospital in the years 2018 through 2024, which

resulted in 1,055 positive isolates. Their positivity rate was significantly lower than that in the present study, with 2.8%. The spectrum of bacteria identified was comparable, with a high proportion of *Staphylococcus epidermidis*, *S. hominis,* S. *aureus*, *Enterococcus faecium*, *Klebsiella pneumoniae,* and *E. coli,* and alarming carbapenem resistance levels reported for selected isolates.

Sub-Saharan Africa data also highlight the worldwide spread of bacteremia. (29) from Ethiopia in a prospective cross-sectional study of 214 patients with suspected septicemia, a bacterial pathogen was recovered in 21% of confirmed cases. The most frequently identified organisms were *Staphylococcus aureus*, *Klebsiella pneumoniae*, and *Escherichia coli*, a number of which had resistance to multiple classes of antibiotics, notably the β-lactams. In view of these investigations, the current study's positivity rate (72%) is significantly higher. This discrepancy could be related to sample size, inclusion criteria, patient selection, or differences in the method of blood culture processing. However, the distribution of bacterial agents in our data is consistent with global trends; Gram-positive cocci and *Enterobacterales* remain responsible for the majority of BSIs.

Together, these comparisons underscore the clinical significance and the challenge of BSI. The proportions of such detections may differ in different geographic areas and healthcare institutions, but overall, the domination of these organisms (*S. aureus*, CONS*, E. coli*, *K. pneumoniae*) has become internationally uniform. Moreover, the increasing prevalence of antimicrobial resistance, such as MRSA, ESBL producers, and CR Enterobacterales, has become a serious problem for therapy measures. These results support the importance of ongoing surveillance and antimicrobial stewardship programs in order to reduce the spread of MDROs and improve patient outcomes.

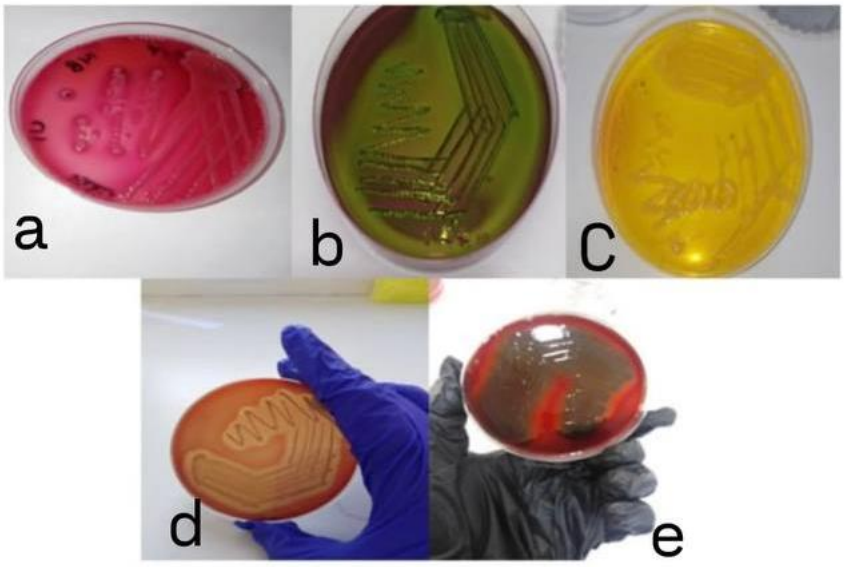

Fig. 1. (a) *E.coli* on MacConkey; (b) *E.coli* on EMB; (c) *Staphylococcus* on manitol; (d) *Staphylococcus* on B.A; (e) *Pseudomonas* on B.A

Table (1). Viteck ID card diagnosis result

Bacterial species	Number of isolates	Percentage%
E.coli	75	75%
Pseudomonas	6	6%
Staphylococcus	4	4%
Streptococcus	8	8%
Neisseria	7	7%
Total	100	100%

Identification and Antibiotic Resistance of Bacterial Isolates

Results: Antimicrobial susceptibility testing of 75 *E. coli* isolates demonstrated varying resistance profiles against different classes of antibiotics. Isolates frequently showed a lag of slug development despite their MDR phenotype – resistance to three or more antimicrobial classes (Table 2).

β-lactams (e.g., ampicillin/sulbactam, piperacillin/tazobactam, cefazolin, cefuroxime, ceftriaxone, cefepime, and ceftazidime), except imipenem and meropenem, were highly resistant drugs with > 70% (approximately 53 out of 75) lower susceptible isolates. This resistance is largely due to the production of β-lactamases, including extended-spectrum β-lactamases (ESBLs) that hydrolyze penicillins and cephalosporins. However, the data demonstrated that a small portion of isolates still were susceptible to β-lactam/β-lactamase inhibitor combinations, including piperacillin-tazobactam, due to partial inhibitory effects against ESBL-producers. Carbapenems (imipenem, meropenem, and ertapenem) had the highest level of susceptibility (85%, 64/75). Merely 15% (11 isolates) displayed intermediate or resistant phenotypes, probably as a result of carbapenemase production, porin deficiency, or efflux pump operation. These results advocated the use of carbapenems as sensitive antibiotics for MDR E. coli in our setting, but it's known that emergent resistance is still a problem.

Moderate activity of aminoglycosides (amikacin, gentamicin): 60% sensitivity (45 of 75). The rest of the isolates showed intermediate or resistant levels, likely mediated by aminoglycoside-modifying enzymes or defective drug uptake.

Fluoroquinolones (ciprofloxacin) showed high resistance, with over 75% of isolates (56/75) having low susceptibility. This is consistent with global surveillance data that correlates resistance with mutations, and can help to define the empirical use of fluoroquinolones in E coli infections. Trimethoprim/sulfamethoxazole, a folate synthesis inhibitor, similarly had increased rates of resistance involving about 80% (60/75) of strains.

Polymyxins (colistin) performed poorly, with 30 out of 75 isolates (40%) resistant (presumed to be *mcr* gene acquisition). Because colistin is a last-ditch antibiotic, this resistance represents a major challenge for treatment.

Both nitrofurantoin and fosfomycin, which are frequently used in UTIs, showed good activity with susceptibility rates of over 70% (none of the 75 isolates had susceptibility rates of less than 53). These agents remain appropriate options for the treatment of uncomplicated UTIs due to *E. coli*.

These results are similar to previously observed studies of *E. coli* resistance profiles (30-32). Isolates that were resistant to more than three classes of antibiotics were considered MDR.

A report from (34) indicated a prevalence of 77.7% *E. coli* in broiler chickens, Sylhet, Bangladesh, showing serious resistance profiles to commonly used antibiotics. Similarly, Barua et al. (2024) observed significant resistance of *E. coli* isolated from goats with respiratory tract infections to multiple antibiotics. These are additional evidence for the prevalence of antibiotic resistance in Bangladesh.

In addition, (35) also studied the concomitance of multidrug resistance with virulence in nosocomial isolates of *Pseudomonas aeruginosa*. Their results emphasized the growing problem of MDR P. aeruginosa in hospitals. These results are in agreement with the present study in P. aeruginosa.

The bacterial isolates in this study were of a similar profile, or comparable to some of the recent works in Bangladesh, in terms of both identification and antibiotic resistance characteristics. The extensive resistance among *E. coli* and P. aeruginosa isolates is a strong signal for the importance of antimicrobial stewardship and infection control strategies. Ongoing surveillance and ITU are important in order to challenge the threat of antimicrobial resistance.

Table (2): Antimicrobial type and MIC

Antimicrobial	MIC (µg/mL)
Amoxicillin/Clavulanic acid	8
Cefazolin	<=8
Cefuroxime	<=1
Cefuroxime axetil	<=0.12
Cefixime	<=0.12
Ceftriaxone	<=0.25
Cefepime	<=0.25
Ertapenem	<=0.25
Imipenem	<=0.25
Meropenem	<=0.25
Amikacin	<=4
Gentamicin	<=4
Ciprofloxacin	>=4
Fosfomycin	<=16
Nitrofurantoin	64
Trimethoprim/Sulfamethoxazole	>=320

Molecular Detection of E. coli via PCR

Molecular detection of *E. coli* was carried out by polymerase chain reaction (PCR), and the PCR test result was positive. Gel electrophoresis of PCR products demonstrated single strong bands in all lanes for samples, approximately 550 bp according to a comparison with a 1000 bp DNA ladder. Figure 2 shows that each of the *E. coli* DNAs gave a neat band of about the same size, indicating that the target gene was really present.

Recent developments have been improving PCR-based procedures for detection. For example, a new multiple-slot-PCR assay was developed by (36), which allows the amplification of three genes, cydA, lacY, and ydiV, which may help to improve rapid and accurate *E. coli* detection. This method decreases the procedure steps and is time-saving for laboratory diagnosis.

Moreover, a new approach based on droplet digital PCR (ddPCR) has been developed for the clinical

detection of *E. coli*. In a study by (37), ddPCR identified the DNA of *E. coli* in 82.7% of bloodstream infections, and showed a specificity that 100%. Importantly, the turnaround time of ddPCR was much shorter than gold standard methods like blood culture, suggesting that it has potential in rapid diagnosis. These findings demonstrate that increasingly, molecular methods are of great importance for the rapid and reliable detection of *E. coli*. The uniformity in banding observed throughout this study correlates with the data obtained from the recent literature review and confirms that PCR is a reliable diagnostic method. Additionally, the application of advanced tools such as multiplex-PCR and ddPCR leads to a better sensitivity and specificity profile, which will help in advancing diagnostics, especially toward both clinical and environmental microbiology.

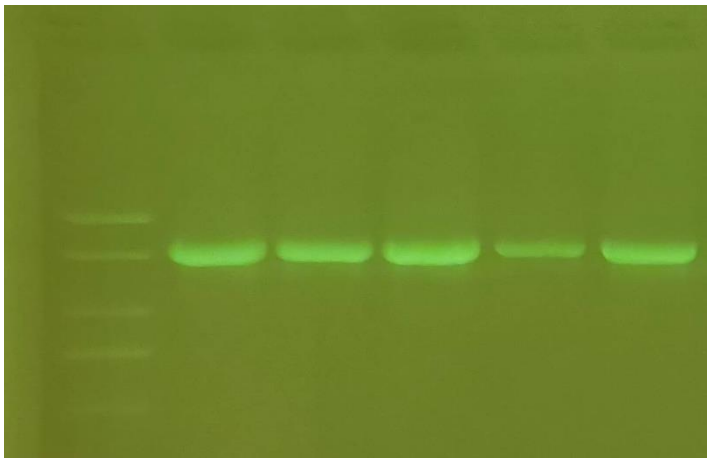

Fig. 2 shows the gel electrophoresis of E. coli DNA samples, PCR

Phylogenetic Analysis of Local *E. coli* Isolates

In comparison with related strains, the local E. coli RANJM isolate showed high homology to closely related strains, as determined by 16S rRNA gene Sanger sequencing. The Neighbor-Joining phylogenetic tree (Fig. 3) shows the association of *E. coli* RANJM (in a black circle) with local and international isolates, which is consistent with the notion that antibiotic resistance in these bacteria poses a threat to the world over all. The tree demonstrates substantial convergence between the local isolates and international reference strains (Table 3) and is therefore of considerable public health concern. Genetic similarity evaluation shows a high degree of evolutionary relationship between the studied *Escherichia coli* and other members of the genus Escherichia. The highest identity was observed with *E. coli* ATCC 11775 (99.88%), followed closely by *E. fergusonii* (99.78%), *E. hominis* (99.7%), *E. albertii* (99.65%), *E. whittamii* (99.53%), and *E. ruysiae* (99.5%). These values suggest that these species share a recent common ancestor and are part of a closely related phylogenetic cluster. The inclusion of *Shigella flexneri* (99.32%) further supports the well-established notion that *Shigella* species are genomically embedded within the *E. coli* lineage, having diverged through the acquisition of virulence factors.

Other species such as *Citrobacter koseri*, *Enterobacter asburiae,* and *Serratia nematodiphila* share lower identity values (from 97 % to 98 %), implying more distant evolutionary relatedness,

although within the same family, *Enterobacteriaceae*. The existence of genera such as *Intestinirhabdus*, *Kosakonia, Cronobacter,* and *Kalamiella* with less than 98% ID indicates more profound phylogenetic divergence and implies that although they possess conserved genetic components, the ecological/pathogenic traits have been separately formed during evolution.

In general, the data supports an obvious evolutionary gradient (with *E. coli* and closely related strains in a tight focus overall, distinct as a growing branch for other genera) and our results match those of earlier works (38,39) aspects (38,39), with University Hospital Prague or Basrah Iraq indicating extensive commonality between local & national Buckeye *E. coli*'s with divergent properties in one international strain. Phylogenetic analysis showed three well-defined clades, and more than 80% of isolates shared only 81%, which highlights the need to know local bacterial populations for an appropriate treatment.

More recent reports have classified *E. coli* isolates into approximately three clades, each with about 81% homology to most strains, that may behave as different serotypes and show essentially distinct patterns of dissemination via contaminated food (5). This is an important reminder that local bacterial populations must be taken into consideration when determining treatment. For example, a recent study (40) analyzed uropathogenic *E. coli* strains and their resistance phenotypes in children and revealed the worldwide distribution of these AMR genes.

Consistent with these results, we reinforce the increasing surveillance of AMR dissemination in *E. coli* worldwide. The unique clades identified and the close relationship between local isolates and international strains highlight the importance of a global strategy for systematic surveillance and prevention of resistant isolates.

Table 3: Most related bacterial species with their accession numbers that show homology with *Escherichia coli* RANJM retrieved from the NCBI database based on the 16S rRNA gene.

Species name	Strain name	Accession No.	Similarity (%)
Escherichia coli	ATCC 11775	X80725	99.88
Escherichia ergusonii	ATCC 35469	CU928158	99.78
Escherichia hominis	NSJ-73	MT905223	99.7
Escherichia albertii	TW07627	ABKX0100030	99.65
Escherichia whittamii	Sa2BVA5	JACSQI01000025	99.53
Escherichia ruysiae	OPT1704	LR745848	99.5
Shigella flexneri	ATCC 29903	X96963	99.32
Intestinirhabdus alba	BIT-B35	MK734184	98.45
Citrobacter koseri	LMG 5519	HQ992945	98.26
Kosakonia sacchari	SP1	CP007215	98.05
Cronobacter turicensis	z3032	FN543093	97.89
Dryocola clanedunensis	H11S18	OM971055	97.55
Enterobacter asburiae	JCM 6051	BBED01000197	97.34
Cedecea lapagei	JCM 1684	LC036260	97.24
Serratia nematodiphila	DSM 21420	JPUX0100001	97.08
Pantoea beijingensis	LMG 27579	KC846071	96.82
Winslowiella iniecta	B120	JRXE0100057	96.65
Kalamiella piersonii	IIIF1SW-P2	RARB0100003	96.39
Erwinia mediterraneensis	Marseille-P5165	LR026978	96.09

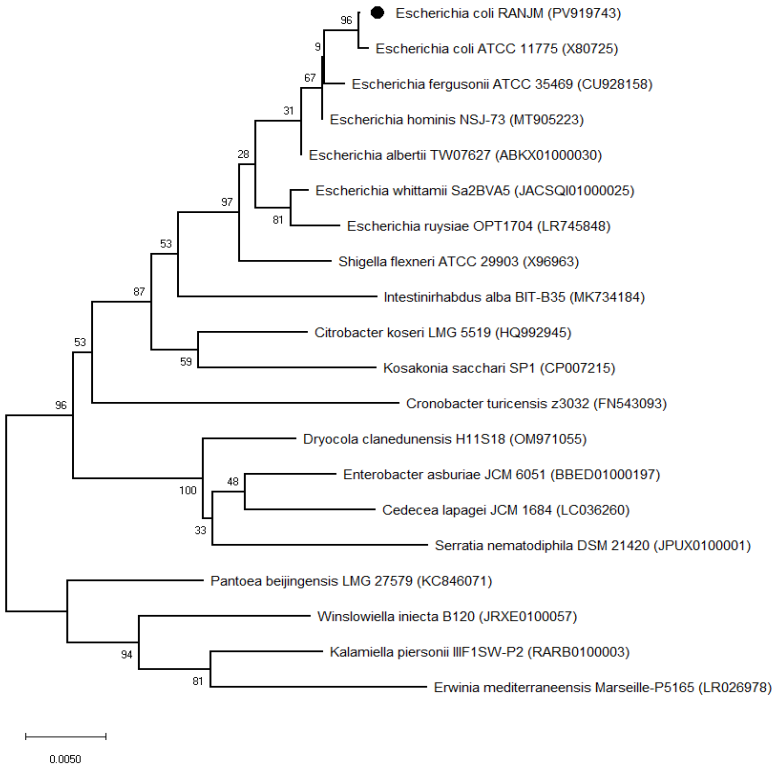

Fig. 3. Neighbor=Joining= phylogenetic= trees showing the relationship between *Escherichia coli* RANJM (indicated in a black circle) =and the closely =related strains= based= on= 16S rRNA gene sequences= using MEGA-11 software =with a= scale= length of 0.005.

Results of green tea extracts

The aqueous and ethanolic extracts of green tea exerted a strong antibacterial effect on E. coli strains (isolated from urinary tract infections). The zones of inhibition by the aqueous extract at 40, 30, 20, and 10 mg/mL were found to be at a mean value of 25,15, and mm, respectively. The ethanolic extract displayed more activity with inhibition zones at 35, 30, 25, and 22 mm in the same concentrations. The results suggest that the antibacterial effects of the green tea extracts are dose-dependent Table 4,5 and Fig 4,5**.**

The results validate that green tea extracts are highly antibacterial against E. coli, which is consistent with recent research highlighting catechins, tannins, and polyphenols involved in the damage to bacterial cell membranes and biofilm formation inhibition. The catechins, in particular those from which EGCG is derived, cause disruption of the membranes and interfere with DNA replication as well as diminishing bacterial adhesion to epithelial cells.

This is of great importance in terms of antibiotic resistance and bacteremia. Due to the increasing incidence of urinary tract and bloodstream infections caused by MDR uropathogenic *E. coli*, there are important public health issues associated with these infections. And that is where natural ingredients from plants, including green tea catechins, serve as a nice complement or replacement for traditional antibiotics. And, of course, it has now been shown that Green tea

extracts can work synergistically with antibiotics to inhibit biofilm and slow down the rate of resistance.

Furthermore, polyphenols in green tea have been shown to inhibit virulence factors, including adhesion and toxin production during the pathogenesis of bacteremia. Antiadhesive actions of green tea extracts against urinary isolates may interfere with bacterial adhesion and invasion, leading to a decrease in the frequency of systemic infection by uropathogens. (41-43).

Table (4) Effect of Aqueous Green Tea Extract on *Escherichia coli*

E. coli Isolates	Inhibition Zone Diameter (mm)	Concentration of Aqueous Extract (mg/mL) + Control
E. coli Isolates1	25	40
E. coli Isolates2	20	30
E. coli Isolates3	15	20
E. coli Isolates4	10	10
E. coli Isolates5	0	Sterile distilled water (Control)

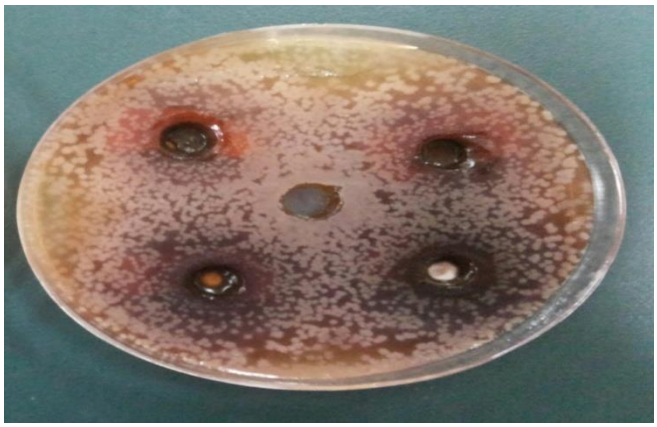

Fig 4. Effect of the aqueous green tea extract on *Escherichia coli.*

Table 5. Effect of Ethanolic Green Tea Extract on *Escherichia coli*

E. coli Isolates	Inhibition Zone Diameter (mm)	Concentration of Ethanolic Extract (mg/mL) + Control
E. coli Isolates1	34	40
E. coli Isolates2	30	30
E. coli Isolates3	25	20
E. coli Isolates4	22	10
E. coli Isolates5	0	Sterile distilled water (Control)

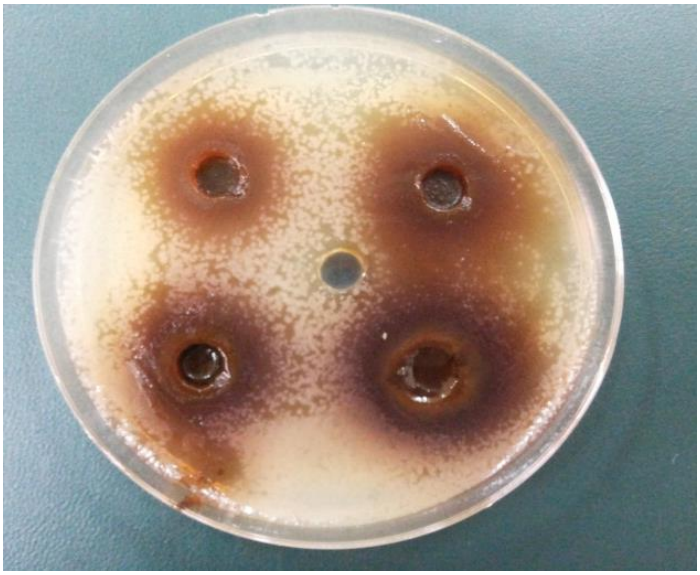

Fig 5. Effect of the ethanolic green tea extract on *Escherichia coli*

Conclusion

This investigation shows the clinical impact and molecular significance of *Escherichia coli* as the predominant pathogen in bacteraemia (75% confirmed cases). The broad dissemination of multidrug-resistant *E. coli*, namely resistance to β-lactams, fluoroquinolones, and sulfonamides, is a matter caught in the therapeutic deadlock, emphasizing an imperative demand for implementation and reinforcement of AMS programs in hospitals.

The therapeutic effectiveness of carbapenems and fosfomycin was high despite challenge, suggesting these to be crucial options in cases of resistance. Furthermore, nitrofurantoin was found to be effective for uncomplicated UTI, supporting its use in uncomplicated UTI as a first-choice agent.

Diagnostically, PCR was highly sensitive; all isolates consistently produced 550 bp bands when identical genomic regions were targeted.

In phylogenetic analysis RANJM isolate of *E. coli* and the other Escherichia species together with Shigella flexneri comprised a closely related genetic cluster. The delineation of a set of three major clades in local and international isolates, with minor divergence in one isolate from abroad, emphasizes the need for knowledge about the genetic structure of regional bacterial populations to inform directed treatment decisions.

These results underscore the importance of ongoing surveillance for AMR, implementation of molecular diagnostics in clinical laboratories, and investment in genomic research. The genetic and resistance patterns of local strains must be known to enhance the quality of medical care and limit infection by resistant bacteria.

The results from this experiment demonstrated that both aqueous and ethanolic extracts of green tea showed significant antibacterial activity against clinical isolates of *Escherichia coli* isolated from UTI. The inhibition zones were in a dose-dependent fashion, with the ethanolic extract demonstrating higher potency than the aqueous extract. These observations highlight the potential contribution of green tea phytochemicals, which are primarily in the form of catechins, tannins, and phenolic compounds, to the disturbance of bacterial cell

membrane integrity as well as to the inhibition of bacterial multiplication and adhesion to epithelial cells, all of which are important for the pathogenesis of urinary tract infections and bacteremia. Importantly, the ability of green tea extracts in this study has evidence to use as a natural antimicrobial agent and can be used along with the conventional antibiotics, leading towards minimizing multidrug resistance and offering more safe user friendly, side effect free drug alternative.

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References

1- Bonten, M., Johnson, J. R., van den Biggelaar, A. H., Georgalis, L., Geurtsen, J., de Palacios, P. I., ... & Poolman, J. T. (2021). Epidemiology of Escherichia coli bacteremia: a systematic literature review. *Clinical Infectious Diseases*, *72*(7), 1211-1219.

2- de Laroche, M., Fellous, L., Salomon, E., Saadeh, D., Duran, C., Bouchand, F., ... & Dinh, A. (2021). Bloodstream infections in older population: epidemiology, outcome, and impact of multidrug resistance. *European Journal of Clinical Microbiology & Infectious Diseases*, *40*(8), 1665-1672.

3- Geurtsen, J., de Been, M., Weerdenburg, E., Zomer, A., McNally, A., & Poolman, J. (2022). Genomics and pathotypes of the many faces of Escherichia coli. *FEMS microbiology reviews*, *46*(6), fuac031.

4- Doua, J., Geurtsen, J., Rodriguez-Baño, J., Cornely, O. A., Go, O., Gomila-Grange, A., ... & Sarnecki, M. (2023, February). Epidemiology, clinical features, and antimicrobial resistance of invasive Escherichia coli disease in patients admitted in tertiary care hospitals. In *Open Forum Infectious Diseases* (Vol. 10, No. 2, p. ofad026). US: Oxford University Press.

5- Laupland, K. B., & Church, D. L. (2014). Population-based epidemiology and microbiology of community-onset bloodstream infections. *Clinical microbiology reviews*, *27*(4), 647-664.

6- Fischer, J., Rodríguez, I., Schmoger, S., Friese, A., Roesler, U., Helmuth, R., & Guerra, B. (2012). Escherichia coli producing VIM-1 carbapenemase isolated on a pig farm. *Journal of Antimicrobial Chemotherapy*, *67*(7), 1793-1795.

7- Bhattacharya, A., Nsonwu, O., Johnson, A. P., & Hope, R. (2018). Estimating the incidence and 30-day all-cause mortality rate of Escherichia coli bacteraemia in England by 2020/21. *Journal of Hospital Infection*, *98*(3), 228-231.

8- Blandy, O., Honeyford, K., Gharbi, M., Thomas, A., Ramzan, F., Ellington, M. J., ... & Sriskandan, S. (2019). Factors that impact on the burden of Escherichia coli bacteraemia: multivariable regression analysis of 2011–2015 data from West London. *Journal of Hospital Infection*, *101*(2), 120-128.

9- Blum, M., Geurtsen, J., Herweijer, E., Sarnecki, M., Spiessens, B., Diogo, G. R., ... & Hope, R. (2025). Epidemiology of invasive Escherichia coli disease in adults in England, 2013–2017. *Epidemiology & Infection*, *153*, e4.

10- Gerver, S. M., Mihalkova, M., Bion, J. F., Wilson, A. P. R., Chudasama, D., Johnson, A. P., & Hope, R. (2020). Surveillance of bloodstream infections in intensive care units in England, May 2016–April 2017: epidemiology and ecology. *Journal of Hospital Infection*, *106*(1), 1-9.

11- Hossain, A. Z., & Chowdhury, A. M. A. (2024). Understanding the evolution and transmission dynamics of antibiotic resistance genes: a comprehensive review. *Journal of Basic Microbiology*, *64*(10), e2400259.

12- Casella, T., Nogueira, M. C. L., Saras, E., Haenni, M., & Madec, J. Y. (2017). High prevalence of ESBLs in retail chicken meat

despite reduced use of antimicrobials in chicken production, France. *International journal of food microbiology*, *257*, 271-275.

13- Van Duijkeren, E., Schink, A. K., Roberts, M. C., Wang, Y., & Schwarz, S. (2018). Mechanisms of bacterial resistance to antimicrobial agents. *Antimicrobial Resistance in Bacteria from Livestock and Companion Animals*, 51-82.

14- Munita, J. M., & Arias, C. A. (2016). Mechanisms of antibiotic resistance. *Virulence mechanisms of bacterial pathogens*, 481-511.

15- Krause, K. M., Serio, A. W., Kane, T. R., & Connolly, L. E. (2016). Aminoglycosides: an overview. *Cold Spring Harbor perspectives in medicine*, *6*(6), a027029.

16- Zhong, L., Li, Y., Xiong, L., Wang, W., Wu, M., Yuan, T., ... & Yang, S. (2021). Small molecules in targeted cancer therapy: advances, challenges, and future perspectives. *Signal transduction and targeted therapy*, *6*(1), 201.

17- Wang, N., Luo, J., Deng, F., Huang, Y., & Zhou, H. (2022). Antibiotic combination therapy: A strategy to overcome bacterial resistance to aminoglycoside antibiotics. *Frontiers in Pharmacology*, *13*, 839808.

18- Poirel, L., Madec, J. Y., Lupo, A., Schink, A. K., Kieffer, N., Nordmann, P., & Schwarz, S. (2018). Antimicrobial resistance in Escherichia coli. *Microbiology spectrum*, *6*(4), 10-1128.

19- Ero, R., Yan, X. F., & Gao, Y. G. (2021). Ribosome protection proteins—"New" players in the global arms race with antibiotic-resistant pathogens. *International Journal of Molecular Sciences*, *22*(10), 5356.

20- Zhang, Q., Zhang, J. I. N., Zhang, J., Xu, D. U. O., Li, Y., Liu, Y., ... & Weng, P. (2021). Antimicrobial effect of tea polyphenols against foodborne pathogens: A review. *Journal of Food Protection*, *84*(10), 1801-1808.

21- Umemura, H., Nishiyama, H., Tanimichi, Y., Seino, K., Nakajima, M., Tsuchida, S., & Nakayama, T. (2025). Impact of direct identification of bacteria in blood culture–positive specimens by matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry on physician selection of antimicrobial therapy. *Journal of Infection and Chemotherapy*, *31*(2), 102548.

22- De Cueto, M., Ceballos, E., Martinez-Martinez, L., Perea, E. J., & Pascual, A. (2004). Use of positive blood cultures for direct identification and susceptibility testing with the Vitek 2 system. *Journal of Clinical Microbiology*, *42*(8), 3734-3738.

23- Olie, S. E., Andersen, C. Ø., van de Beek, D., & Brouwer, M. C. (2024). Molecular diagnostics in cerebrospinal fluid for the diagnosis of central nervous system infections. *Clinical Microbiology Reviews*, *37*(4), e00021-24.

24- Al-Dhaher, Z. A. (2013). Evaluation of antibacterial activity of aqueous extracts of pomegranate peels, green tea leaves, and bay leaves against Vibrio cholera. *The Iraqi Journal of Veterinary Medicine*, *37*(1), 90-95.

25- Sajedi Moghaddam, S., Mamishi, S., Pourakbari, B., & Mahmoudi, S. (2024). Bacterial etiology and antimicrobial resistance pattern of pediatric bloodstream infections: a 5-year experience in an Iranian referral hospital. *BMC Infectious Diseases*, *24*(1), 373.

26- Yoo, J. S., Yu, H. J., Park, K., Lee, W. G., & Shin, B. M. (2024). Emergence of resistance to last-resort antimicrobials in bacteremia patients: A multicenter analysis of bloodstream pathogens in Korea. *Plos one*, *19*(10), e0309969.

27- Hnaineh, Z., & Sokhn, E. S. (2025). Prevalence of bacteremia and antimicrobial resistance pattern among patients in South Lebanon. *American Journal of Infection Control*, *53*(1), 139-143.

28- Wang, P., Jiang, Z., Liao, H., Zhang, S., Que, W., Wang, C., ... & Zhong, L. (2024). Epidemiology, antimicrobial resistance, and risk factors of infection among liver transplant patients in East China: a retrospective study, 2010 to 2023.

29- Belew, H., Tamir, W., Dilnessa, T., & Mengist, A. (2023). Phenotypic bacterial isolates, antimicrobial susceptibility pattern and associated factors among septicemia suspected patients at a hospital, in northwest Ethiopia: Prospective cross-sectional study. *Annals of Clinical Microbiology and Antimicrobials*, *22*(1), 47.

30- Nakib, F. F. (2024). *Study on bacteriological profile and antimicrobial susceptibility pattern in septicemia suspected patients* (Doctoral dissertation, Brac University).

31- Chmel, M., Ježek, P., Šafránková, R., Ileninová, Z., Vlasatá, V., & Mališová, L. (2025). Escherichia marmotae: a multidrug-resistant opportunistic human pathogen—first clinical isolation in the Czech Republic. *Folia Microbiologica*, 1-8.

32- Daood, N. W., & Al-Hamdani, M. A. (2025). The molecular identification of airborne bacteria in Basrah, Iraq. *Iraqi Journal of Science*.

33- Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular biology and evolution*, *38*(7), 3022-3027.

34- Roy, M., Islam, O., Rahman, M. A., Misty, S. S., Kurmi, R., Islam, M. A., ... & Islam, M. S. (2025). Prevalence and antimicrobial resistance patterns of Escherichia coli isolated from broiler chickens in Sylhet district of Bangladesh. *Veterinary Medicine and Science*, *11*(5), e70576.

35- Atakishizada, S., Uckayabasi, A., & Nağıyev, T. (2025). Antimicrobial resistance and inducible beta-lactamase synthesis in Pseudomonas aeruginosa strains isolated from nosocomial infections of various localizations. *Journal of Research in Pharmacy*, *29*(2), 667-672.

36- Zimoń, B., Psujek, M., Matczak, J., Guziński, A., Wójcik, E., & Dastych, J. (2024). Novel multiplex-PCR test for Escherichia coli detection. *Microbiology Spectrum*, *12*(6), e03773-23.

37- Kitagawa, H., Kojima, M., Tadera, K., Kogasaki, S., Omori, K., Nomura, T., ... & Ohge, H. (2025). Clinical diagnostic performance of droplet digital PCR for pathogen detection in patients with Escherichia coli bloodstream infection: a prospective observational study. *BMC Infectious Diseases*, *25*(1), 22.

38- Chen, Y., Yan, A., Zhang, L., Hu, X., Chen, L., Cui, J., ... & Li, Y. (2025). Comparative analysis of inflammatory biomarkers for the diagnosis of neonatal sepsis: IL-6, IL-8, SAA, CRP, and PCT. *Open Life Sciences*, *20*(1), 20221005.

39- Al-Musawi, H., Al-Abdullah, A., & Al-Saad, L. (2025). Identification of Phylogenetic Groups and Antibiotic Resistance in Escherichia coli Isolated from Urinary Tract Infections in Basrah, Iraq. *Medical Journal of Babylon*, *22*(1), 168-174.

40- Farfan, A. B. P., de Barrón, Y. L. M., Yarihuamán, M. M. M., Laines, F. M. P., Pérez, M. B. P., Choque, J. S. C., & Pastor, H. J. B. (2025). Phylogenetic Analysis of Escherichia coli according to Phenotypic Resistance in Urinary Tract Infections in Children, Lima, Peru. *Infection & Chemotherapy*, *57*(1), 93.

41- Teppabut, W., Tragoolpua, Y., & Kaewkod, T. (2025). Antimicrobial and Cytoprotective Effects of Tea Extracts Against Escherichia coli-Producing Colibactin Toxin Infections. *Antibiotics*, *14*(9), 886.

42- Radeva-Ilieva, M., Stoeva, S., Hvarchanova, N., & Georgiev, K. D. (2025). Green Tea: Current Knowledge and Issues. *Foods*, *14*(5), 745.

43- Barbarossa, A., Rosato, A., Tardugno, R., Carrieri, A., Corbo, F., Limongelli, F., ... & Carocci, A. (2025). Antibiofilm Effects of Plant Extracts Against Staphylococcus aureus. *Microorganisms*, *13*(2), 454.