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Research Article

Uncovering Hidden Partners: Detecting Co-Microbial Urinary Tract Infections among Middle-Aged and Elderly Patients

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Abstract

Background: Co-microbial infection occupies a big portion of the urinary tract infections (UTI), especially in the elderly. Such infections are hidden, because of the dominance of one species over the other species.

Objectives: This study aims to detect polymicrobial urinary tract infections in middle-aged and elderly patients using both culture-based and molecular methods. It also investigates the presence of aminoglycoside resistance genes, specifically *armA* and *rmtG*, to improve diagnostic precision and support targeted therapeutic decisions.

Methods: In the current study, 100 clinical samples of urine were collected from middle-aged and elderly patients suffering from urinary tract infections. The study was conducted in two hospitals in Baghdad between October 2023 and April 2024. The age of patients was 45+ years equally divided between males and females. These samples were cultured on selective and differential culture media. The grown isolates were further purified and identified using the Vitek system. The antibiotic susceptibility test was done to evaluate the antibiotic resistance pattern. Bacterial DNA was extracted and specific primers were designated for this study, to amplify the resistance coding genes *armA* and *rmtG* by the polymerase chain reaction (PCR) technique.

Results: *Klebsiella pneumoniae* and *Escherichia coli* were the co-existing microbes causing UTI in middle-aged and elderly patients. Significant gender differences were found in the co-infection rates and antibiotic susceptibility tests and genetic analysis identified resistance genes *armA* and *rmtG*. These co-microbes were resistant to aminoglycoside antibiotics. Both *K. pneumoniae* and *E. coli* carried the *armA* gene, while the *rmtG* gene was predominant in *K. pneumoniae* only. *K. pneumoniae* and *E. coli* were found in 42% of the samples. The *armA* gene was detected in 46% of isolates (10/15 E. coli, 4/15 *K. pneumoniae*), while the *rmtG* gene was detected in 26.6% of *K. pneumoniae* isolates and not found in E. coli

Conclusion: This study highlights the occurrence of polymicrobial urinary tract infections among middle-aged and elderly patients, especially females. The detection of resistance genes underscores the importance of molecular diagnostics and gender-specific treatment strategies in managing UTIs.

Keywords: Co-Microbial infection; Mixed UTI; Elderly patients; *E. coli*; *K. pneumoniae; Antibiotic resistance*

Introduction

Urinary tract infections (UTIs) are among the most common bacterial infections in elderly individuals and represent a significant clinical concern worldwide (1). The aging process is associated with anatomical and physiological changes, including a weakened immune system, hormonal alterations, and an increased prevalence of comorbid conditions, all of which predispose older adults to UTIs (1,2). Additionally, the use of urinary catheters in hospital settings and long-term care facilities further increases the risk of infection, particularly among those who are bedridden or have neurogenic bladder disorders (3). UTIs in the elderly range in severity from asymptomatic bacteriuria to life-threatening urosepsis requiring hospitalization (1,4). Asymptomatic bacteriuria, defined as the presence of bacteria in the urine without signs or symptoms of a urinary tract infection, is notably more prevalent in older adults. It is estimated to affect between 4% and 19% of elderly individuals in good health and up to 50% of those who are institutionalized (2). While often benign, distinguishing asymptomatic bacteriuria from a true UTI is challenging, especially in older patients who may present with vague or atypical symptoms. Confusion, fatigue, or a sudden decline in functional status are sometimes the only indicators of a UTI in this population (4). The clinical presentation and risk factors for UTIs in elderly patients differ markedly between men and women. In men, prostatic hypertrophy and urinary retention are major contributors, while in women, estrogen deficiency and pelvic floor dysfunction predominate (1). Both genders, however, share common predispositions including comorbidities, urodynamic abnormalities, immunosenescence, and poor treatment adherence (5). Distinguishing uncomplicated UTIs, which typically affect healthy

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non-pregnant women, from complicated UTIs that can occur in patients of any age or sex and are often linked to structural or functional abnormalities of the urinary tract, is critical for appropriate management (6).

Hospital-acquired infections (HAIs) are defined as infections that develop 48 hours or more after hospital admission (3). Among HAIs, urinary tract infections are among the most frequently encountered, especially in patients with urological interventions (7). *Escherichia coli* remains the most commonly isolated pathogen in both community-acquired and healthcare-associated UTIs (HAUTIs), followed by other members of the Enterobacterales family, including *Klebsiella pneumoniae*, *Proteus* species, and *Citrobacter* species (7,8). Additionally, other pathogens such as *Enterococcus faecalis*, *Pseudomonas aeruginosa*, and *Staphylococcus* aureus are reported to play significant roles in complicated infections (8).

Recent attention has been given to polymicrobial UTIs, particularly in hospitalized or catheterized patients with weakened immune systems. These infections involve more than one microbial agent and often go underdiagnosed due to standard culture techniques favoring the growth of dominant species, such as *E. coli*, while masking secondary pathogens (9). Bacterial combinations such as *E. coli* with *Klebsiella pneumoniae*, *Proteus* species, or *Citrobacter* species are commonly observed in polymicrobial UTIs (7,9). Understanding the individual virulence profiles of these pathogens is essential for deciphering their synergistic behavior in polymicrobial settings. The interaction between these species may enhance their virulence, promote biofilm formation, and increase resistance to treatment (9,10).

E. coli is considered the primary uropathogen, particularly the uropathogenic strains (UPECs), which possess a wide variety of virulence factors that enable adherence to uroepithelial cells, iron acquisition, immune evasion, and tissue damage (11,12,13). Other bacteria, like *Klebsiella pneumoniae*, are frequently associated with healthcare-associated infections and are of growing concern due to their ability to acquire and transmit multidrug resistance (MDR) traits (14,15,16). The polysaccharide capsule of *K. pneumoniae* is its most significant virulence factor, aiding in immune evasion and survival in hostile environments (15,16). There are more than 77 known capsular types, with less virulent strains typically lacking a capsule (15). In addition to the capsule, lipopolysaccharides (LPS) on the bacterial outer membrane are major contributors to inflammation and septic responses (17).

The rise of antimicrobial resistance (AMR) among uropathogens is a global threat. Particularly concerning is resistance to aminoglycoside antibiotics, which are often used in the treatment of complicated UTIs. Genes such as *armA* and *rmtG*, found among Enterobacterales, encode 16S rRNA methyltransferases, which protect bacterial ribosomes from aminoglycoside binding, leading to high-level resistance and treatment failure (18). These resistance genes are increasingly detected in HAIs, posing serious therapeutic challenges, especially in elderly patients with recurrent or complicated UTIs (5,19). The *Enterobacteriaceae* family, to which many of these pathogens belong, consists of Gram-negative, glucose-fermenting, catalase-positive, and oxidase-negative organisms that convert nitrates to nitrites (20,21). *K. pneumoniae* in particular has been implicated in a wide array of infections including pneumonia, sepsis, liver abscesses, and UTIs, with elderly patients representing one of the most at-risk groups (22,16). Genetic studies have revealed various host susceptibility factors that may predispose individuals to upper UTIs (10).

This study aimed to detect polymicrobial urinary tract infections in elderly patients using both culture-based and molecular methods. It tried to investigate the increasing occurrence of co-resistant bacterial isolates, particularly among elderly patients a population known to exhibit higher infection rates due to immunosenescence, chronic comorbidities, and reduced mucosal immunity. This is largely attributed to immune system decline, in addition to age-related comorbidities and frequent exposure to healthcare environments. Elderly individuals, especially those with catheters or chronic conditions, represent a particularly vulnerable group for recurrent and complicated urinary tract infections (UTIs) and aims to detect polymicrobial urinary tract infections in elderly patients using both culture-based and molecular methods. It also seeks to investigate the presence of aminoglycoside resistance genes such as *armA* and *rmtG* was also investigated, with the goal of improving diagnostic precision and guiding appropriate therapeutic decisions for this vulnerable population.

Material and Methods:

Samples: One hundred clinical samples were analyzed in this study, taken from patients at AL-Karama Teaching Hospital and Mohammed Baqer AL Hakeem Hospital in Baghdad, Iraq. The patients' ages ranged from 55–75 years, the ratio of males to female was 1:1. The study was conducted between October 2023 and April 2024.

The exclusion criteria included: Recent antibiotic use, catheterized patients, and patients with chronic kidney disease.

The study was approved by the Ethical Committee under approval number CSEC/1023/0096.

Collection of urine samples: The clinical samples were obtained from patients with UTI from two hospitals in Baghdad. They were taken in sterile cups, transferred to the laboratory and had to be plated within two hours of being collected because there was a chance of bacterial overgrowth that was not typical of the patient's first specimens. Urine samples where plating is postponed — particularly for more than 24 hours — can be considered ineffective (23).

Isolation and identification of co-existing bacterial isolates: Clinical specimens were cultured on selective and differential media and incubated overnight at 37°C. MacConkey agar was used for the

primary isolation of Gram-negative bacteria and to differentiate lactose fermenters from non-lactose fermenters. Lactose-fermenting colonies appeared pink, whereas non-lactose fermenters appeared pale.
Presumptive *Escherichia coli* colonies were identified as small, flat, pink colonies on MacConkey agar and were further sub cultured on eosin methylene blue (EMB) agar for confirmation. A positive identification of *E. coli* was indicated by the appearance of colonies with a characteristic green metallic sheen on EMB agar.
Klebsiella pneumoniae colonies were distinguished on MacConkey agar by their large, mucoid, and convex morphology with a pink coloration. The obtained *Klebsiella* isolates were purified on MacConkey agar, and colony morphology, including margin, elevation, and size, was examined. Colony size was measured using a calibrated scale, and colony color was assessed by microscopic observation.
For further confirmation, thirty isolates of *E. coli* and *Klebsiella pneumoniae* were cultured on chromogenic media according to the manufacturer's instructions. In addition, the Vitek system was used to confirm the identification of the isolated bacteria.

Antimicrobial Susceptibility Testing: Thirty isolates of *E. coli* and *K. pneumoniae* from elderly patients were selected for antibiotic susceptibility testing. The isolates were screened on Mueller–Hinton agar using the disk diffusion method. Results were interpreted according to CLSI guidelines (24), and the isolates were classified as susceptible or resistant to the tested antibiotics. The subsequent antibiotics were utilized: Gentamicin (10 µg), Oflaxacin (5 µg), Cefixime (5µg), Amoxy-clav (30 µg), Nitrofurantoin (100 µg), Ceftazidine (30 µg), Amikacin (10 µg), Carbenicillin (25 µg), Meropenem (10 µg), Tobramycin (10 µg). The source of all the antibiotic discs was from Hi-media / India.

Molecular study

DNA Extraction: DNA extraction using the Genomic DNA micro kit (Geneaid, Thailand) for gram negative.

PCR Amplification of Aminoglycoside resistance coding genes: The primers used to detect *armA and rmtG* in *Escherichia coli and Klebsiella pneumoniae* were anew designated in this study table (1):

Table 1: The primers used in the study

Genes	Sequence 5'-3'	Size (bp)
ArmA-F	ATTCTGCCTATCCTAATTGGG	429bp
ArmA-R	AAAGCTGTAGGAAATCCAAGA	
rmtG-F	AAGAAACAGATGCCGTGTAT	454 bp
rmtG-R	TATAGCTCGTTATCCGTTTCC	

Lyophilized primers were dissolved in sterile deionized water to a final concentration of 100 pmol/µL. After thawing, DNA, primers, and the PCR premix were vortexed and centrifuged. The PCR mixture included 25µL of PCR premix, 1.5µL of primers, and 4µL template DNA. The negative control was replaced by distilled water. The DNA was amplified using a thermocycler PCR device (Germany Eppendorf) that included a program of: One cycle of initial denaturation at 95°C for one minute, 35 cycles of denaturation at 95°C for 30 seconds, annealing at 50°C for 45 seconds, and extension at 72°C for 30 seconds, and one cycle of final denaturation.

Determination of PCR Product Specificity: The specificity of PCR results was determined using agarose gel electrophoresis. A 1.5% concentration of agarose gel was prepared by dissolving 1.5g of agarose powder in 1X TBE buffer, boiling, and cooling. Red safe dye was added, and the gel was cooled. The wells of the gel were filled 10 µL of each PCR result and the first well was filled with 10 µL of 100 bp DNA ladder. The gel documentation system was used to visualize the red safe stained bands. Electrophoresis (Cleaver, United Kingdom) was done for one hour (25).

Statistical Analysis:

The Statistical Analysis System- SAS (2018) program was used to detect the effect of difference factors in study parameters. Chi-square test was used to significant compare between percentage (0.05 and 0.01 probability in this study.

Results

It was found that female subjects in the current study recorded a significantly higher prevalence of co-infection compared to males. The age distribution of co-infection cases among females was predominant in the 45–60 years age group (68%), followed by 20% in the 61–66 years group, and 12% in the 67+ years group. In contrast, males showed an equal prevalence of co-infection in the 55–60 years and 67–75 years age groups, (40%) each, with the remaining 20% in the 61–66 years group, Table (1) and Figure (1).

Table 2: Distribution of the clinical specimens by co-infection, age and gender

Gender	No.	Co-infection	Age group (years)		
			(45–60)	(61–66)	(67+)
Male	50	10 (20%)	4 (40%)	2 (20%)	4 (40%)
Female	50	25 (50%)	17 (68%)	5 (20%)	3 (12%)
P-value	100		0.0001 **		

** ($P \leq 0.01$). NS: Non-Significant.

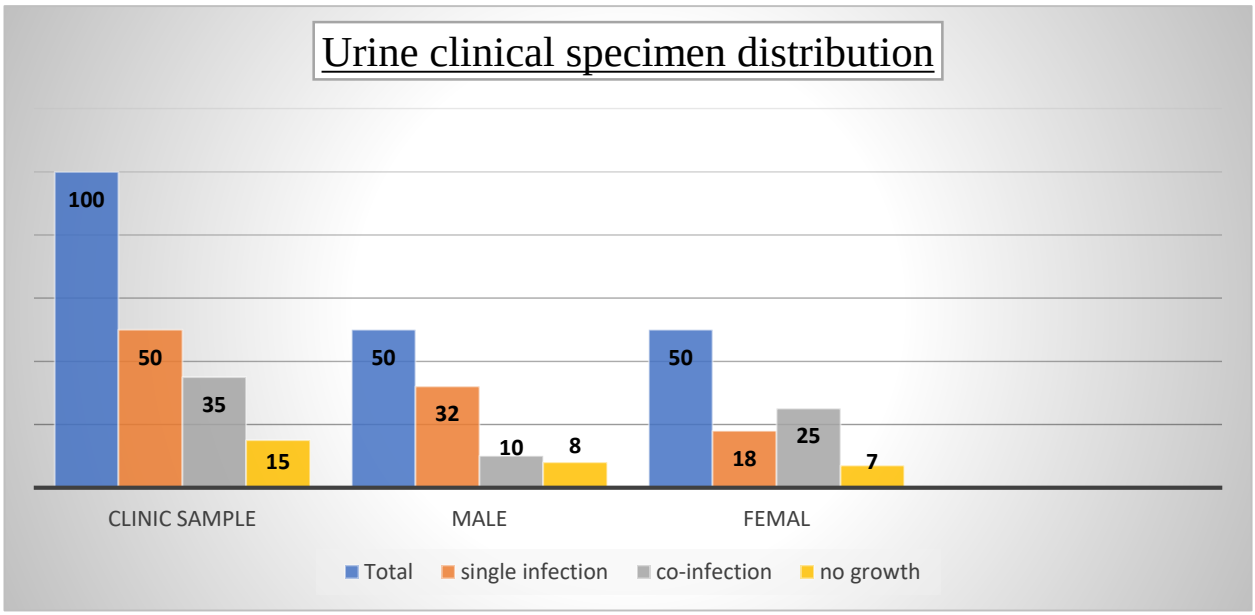

Figure 1: Distribution of the urine samples by gender and bacterial growth

The isolates were further purified on MacConkey medium to ensure colony purity and aid in phenotypic characterization. Among all clinical specimens for microbial infections combined, five bacterial genera were detected and isolates were identified as *Escherichia coli, Klebseilla spp., Citrobacter spp.,* and *Proteus spp.*

Predominance of co-microbial infection in bacterial isolates The study analyzing co-microbial isolates in 35 clinical specimens revealed that lactose-fermenting Gram-negative bacteria were more prevalent than the non-lactose-fermenting bacteria. Specifically, among the isolates, *Escherichia coli* and *Klebsiella pneumoniae* were identified as the two most common bacteria, accounting for 30/70 (42%) of the cases, Figure (2).

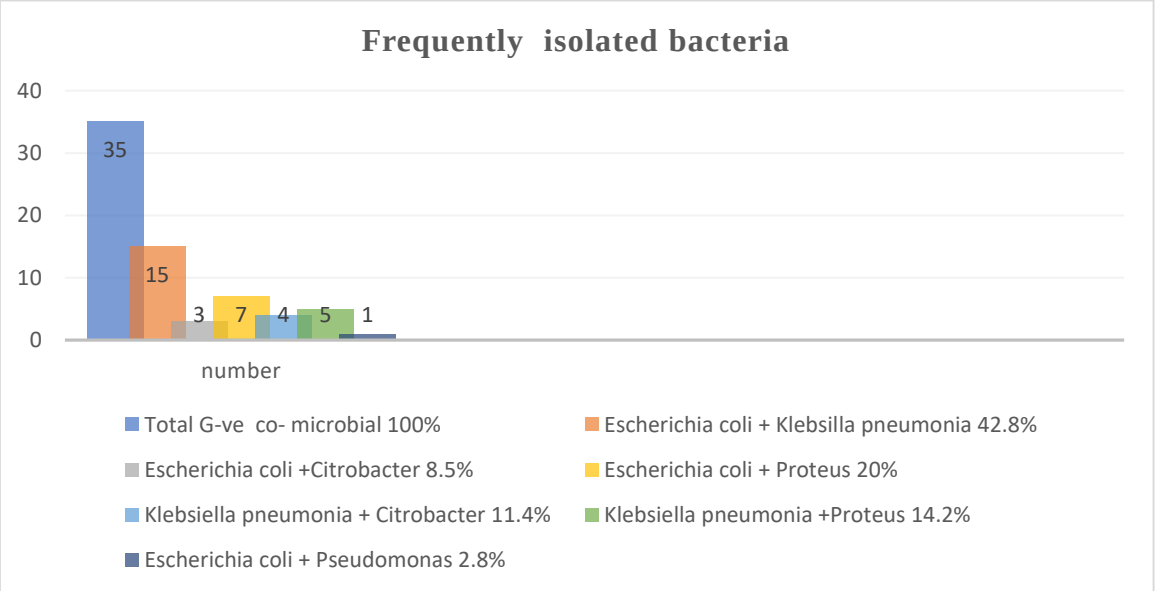

Figure (2): Distribution of the types of frequently occurring co-microbial Gram-negative bacteria

Phenotypic confirmation of *E. coli* and *Klebsiella pneumonia* On chromogenic media, *E. coli* isolates appeared as pink colonies, whereas Klebsiella pneumoniae isolates showed greenish colonies. These colony colors were consistent with the expected chromogenic characteristics provided by the manufacturer, confirming the phenotypic identification of the isolates (Figure 3).

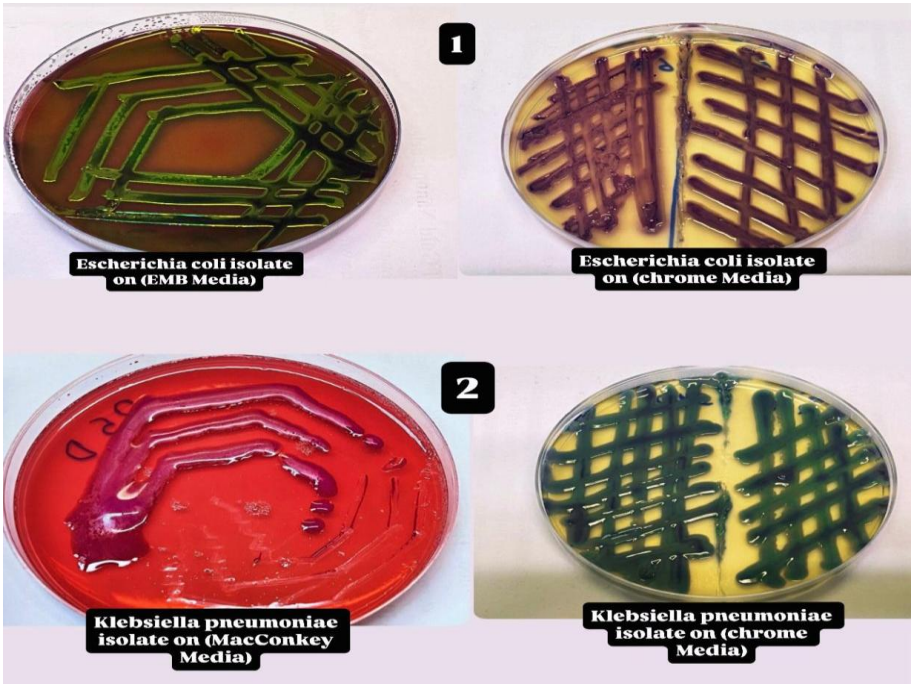

Figure (3): Image obtained from the actual culture work conducted as part of this study
1 – *Escherichia coli* isolate on (EMB and chrome) Media
2 - *Klebsiella pneumoniae* isolate on (MacConkey and chrome) Media (1 and 2) were incubated for 24 hours at 37°C

Antibiotic susceptibility of Uropathogenic *E. coli* and *K. pneumoniae*

Thirty isolates were tested for antibiotic sensitivity using 10 types of antibiotics, utilizing the Kirby-Bauer disk diffusion test according to CLSI 2021, CLSI 2022, and CLSI 2023 (Figure 4).

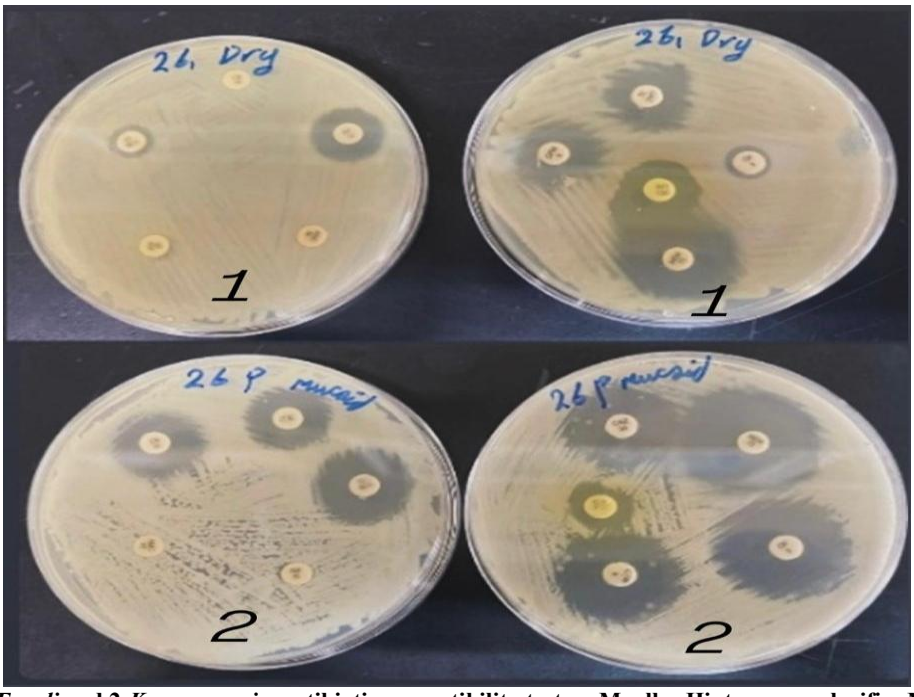

Figure (4): 1- *E. coli* and 2-*K. pneumonia* antibiotic susceptibility test on Mueller Hinton agar, clarifies MDR isolates
Resistance: ≤ 14 mm Intermediate: 15–17 mm Sensitive: ≥ 18 mm

The results of this study revealed a significant presence of multidrug-resistant (MDR) isolates. Antimicrobial susceptibility testing was performed on 30 isolates of *Escherichia coli* and *Klebsiella pneumoniae*, using a panel of commonly used antibiotics. The highest resistance rates were observed for Carbenicillin and Amoxicillin-clavulanic acid, with 28 out of 30 isolates (93%) demonstrating resistance. This was followed by Gentamicin and Ofloxacin, with resistance reported in 20 isolates (66%). In contrast, Meropenem and Nitrofurantoin showed the lowest resistance levels among the tested drugs. Moderate resistance was detected against Tobramycin, Cefixime, Ceftazidime,

and Amikacin, with 14 isolates (46%) exhibiting resistance.

Results of molecular study DNA concentration and purity After DNA extraction using the Genomic DNA Mini Bacteria Kit, DNA concentration was found to range between 76 and 91 ng/µl.the purity ratio (A260/A280) was approximately 1.89, indicating good quality DNA suitable for PCR amplification.

Result of *armA* and *rmtG* coding genes, detection by PCR The molecular analysis in this study demonstrated that the armA gene was detected in 14 out of 30 (46%) of the *E. coli* and *Klebsiella pneumoniae* isolates recovered from urinary tract co-infections. These isolates were phenotypically resistant to both Gentamicin and Amikacin. The rmtG gene, which amplifies at approximately 454 base pairs (bp), was detected in 4 out of 15 (26.6%) *K. pneumoniae* isolates, but was not detected in any of the *E. coli* isolates included in this study. The polymerase chain reaction (PCR) results for both *rmtG* (454 bp) and *armA* (429 bp) genes are presented in Figures 8 and 9, respectively.

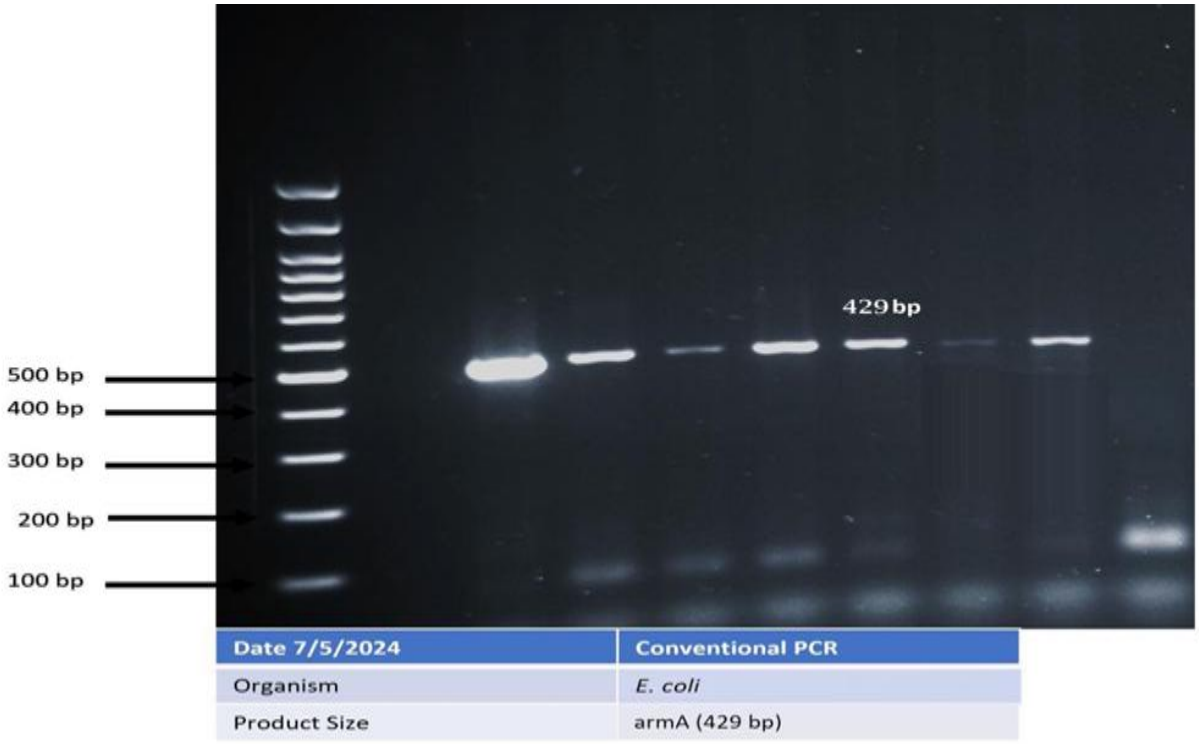

Figure (5): Agarose gel electrophoresis of PCR product of *armA* gene (429 bp) in *E. coli*

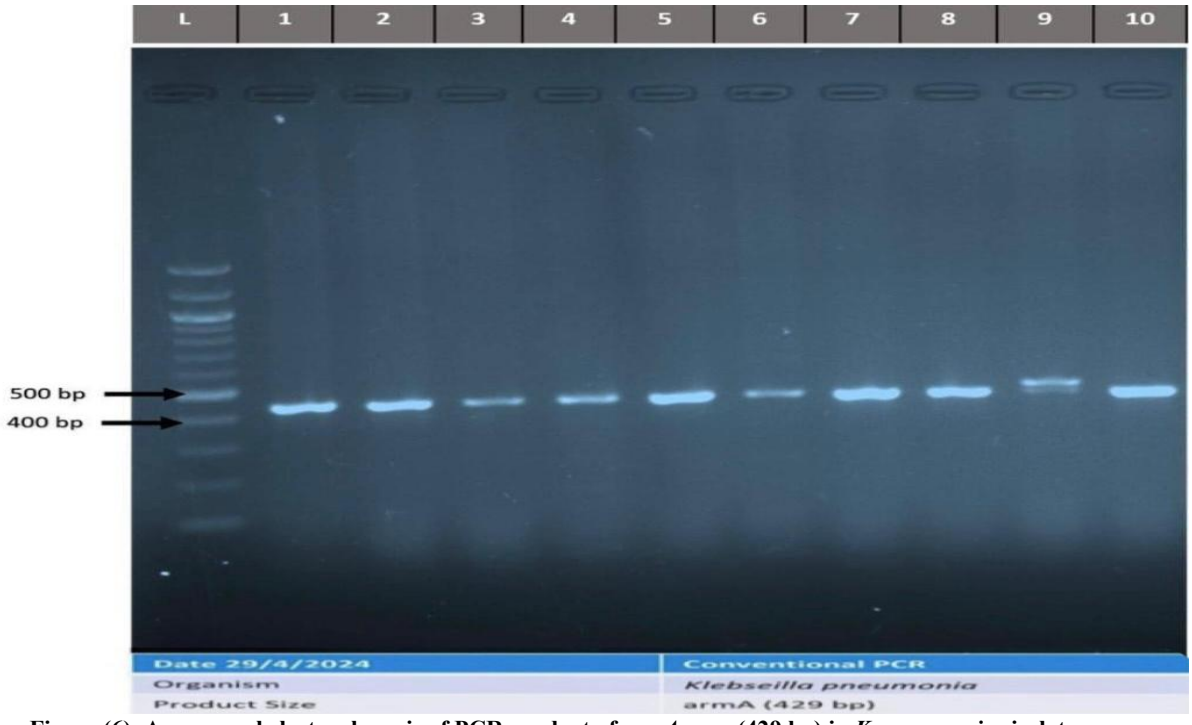

Figure (6): Agarose gel electrophoresis of PCR product of *armA* gene (429 bp) in *K. pneumoniae* isolate

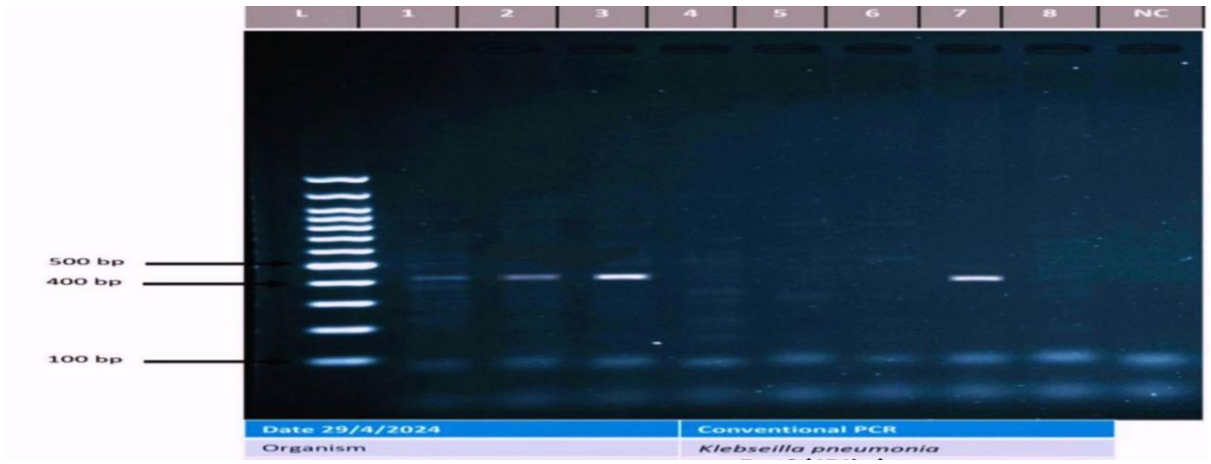

Figure (7): Agarose gel electrophoresis of PCR product of Weak *RmtG* gene (454 bp) in *K. pneumoniae* isolate

Table 3: PCR Amplification of Aminoglycoside resistance *armA* gene by PCR

Bacteria	Total	Positive *armA*	%	Negative *armA*	%	*P*-value
E. coli	15	10	66.0%	5	33.0%	0.084 NS
Klebsiella pneumonia	15	4	26.6%	11	73.4%	0.0497 *
P-value	30	---	0.044 *	---	0.047 *	---

* ($P \leq 0.05$), NS: Non-Significant.

Table 4: PCR Amplification of Aminoglycoside resistance *rmtG* gene by PCR

	Total	Positive *rmtG*	%	Negative *rmtG*	%	*P*-value
E. coli	15	0	0%	15	100%	0.0001 **
K.pneumonia	15	4	26.6%	11	73.4%	0.0497 *
P-value	30	---	0.0278 *	---	0.082 NS	---

* ($P \leq 0.05$), ** ($P \leq 0.01$).

For tables 2 and 3, statistical analysis was performed using the Statistical Analysis System (SAS), version 9.4 (SAS Institute, 2018). The Chi-square test (χ^2) was used to compare differences in proportions and percentages among study groups. Results were considered statistically significant at $P < 0.05$ and highly significant at $P< 0.01$.

Discussion

The results of the current study indicate a higher occurrence of lactose-fermenting bacteria in the clinical specimens examined. *Escherichia coli* remains the most frequently isolated pathogen in UTIs, accounting for approximately 70–80% of community-acquired cases. However, its prevalence drops below 50% in healthcare-associated UTIs (26). The frequency of antimicrobial resistance among UTI-causing microbes has become a global concern and a key factor in antibiotic selection. Aminoglycosides remain a vital class of antimicrobial agents used to treat a variety of severe Gram-negative bacterial infections. Resistance mechanisms to aminoglycosides include reduced outer membrane permeability, increased efflux activity, enzymatic drug modification, and alteration of the aminoglycoside-binding site. Resistance genes include the phosphoethanolamine transferase gene mcr-9.1 (linked to polymyxin resistance) and the 16S rRNA methylase gene *rmtG*, which confers strong resistance to nearly all aminoglycosides (27). *armA* is the most predominant among the 16S rRNA methyltransferases (16S-RMTases). High-level resistance to aminoglycosides mediated by 16S-RMTases is increasingly reported in various Gram-negative pathogens (28). These mechanisms may explain the high resistance observed to aminoglycosides in our study, the obtained purity ratio (A260/A280 ≈ 1.89) falls within the generally accepted range of 1.8–2.0, which is considered an indicator of pure DNA and minimal contamination with proteins or phenol (29). Particularly, the presence of the armA and rmtG genes, which are known to encode 16S rRNA methyltransferases and are associated with such resistance patterns. Other study focused on the treatment of recurrent urinary tract infections in women, highlighted that the prevalence of co-infections is particularly relevant in elderly female patients, who may be at higher risk for recurrent infections and antibiotic resistance, which is in agreement with the finding in the current study (30). In other Study, on MacConkey agar, (67.5%) isolates of lactose fermenters and (32.5%) isolates of non-lactose fermenters (31).

Co-infections can facilitate genetic recombination and the emergence of novel antigenic variants, which may compromise the effectiveness of current drugs, treatment protocols, and vaccines. Moreover, understanding co-infection biology is essential for evaluating responses to pharmacological interventions. When patients with co-infections

undergo antimicrobial therapy, the risk of promoting multidrug-resistant organisms and accelerating antimicrobial resistance (AMR) increases (33). 16S-RMTases are frequently identified in *Enterobacteriaceae* worldwide and include *armA* and *rmtG*. The *rmtG* gene was first reported in *K. pneumoniae* producing KPC-2 in Brazil (34). In the current study, the prevalence of *armA* and *rmtG* genes in Iraqi *E. coli* and *K. pneumoniae* isolates was noteworthy. The *armA* gene was dominant in 66% of *E. coli* isolates, while it was nearly absent in *K. pneumoniae*, appearing in only 26.6% of the isolates. It is important to distinguish between correlation and statistical significance when interpreting these findings. Correlation refers to a relationship or association between two variables (e.g., age and infection rate), without necessarily indicating causation. Statistical significance, on the other hand, offers mathematical evidence that an observed relationship is unlikely due to chance. While the study observed notable associations between bacterial resistance and patient demographics, further statistical testing is required to determine whether these associations are statistically significant.

In contrast, our study found that the *rmtG* gene, also associated with aminoglycoside resistance, was weakly present in *K. pneumoniae* (26.6%) and entirely absent in *E. coli*. These results suggest a distinct distribution pattern of resistance genes between the two species. A comparable study from India reported *rmtB* in 31.7% of multidrug-resistant *E. coli* isolates, with 95% of those co-harboring carbapenem resistance genes (34,35). This pattern of co-resistance underlines the growing threat posed by strains carrying multiple resistance mechanisms. The higher prevalence of aminoglycoside resistance genes in *E. coli* compared to *K. pneumoniae* in Iraq may be attributed to inappropriate antibiotic use, the absence of stewardship programs, and reliance on empirical treatment. Another study noted rising aminoglycoside resistance among *K. pneumoniae* isolates, with 13.7% identified as high-level aminoglycoside-resistant (HLAR), and 15 aminoglycoside resistance genes detected (36). This growing resistance is concerning, especially in settings with limited therapeutic alternatives. The absence of *rmtG* in *E. coli* aligns with earlier studies reporting that this gene is rarely found in that species (37). Aminoglycoside resistance in bacteria is primarily mediated through enzymatic modification by aminoglycoside-modifying enzymes (AMEs). These enzymes are classified into three main groups: Acetyltransferases (AACs), phosphotransferases (APHs), and nucleotidyl transferases (ANTs), A stands for Aminoglycoside in all abbreviations: APH (Aminoglycoside Phosphotransferases) and ANT (Aminoglycoside Nucleotidyltransferases). as described by Cox in 2015 (38). In response to this challenge, semi-synthetic aminoglycosides were developed in the 1970s. Among these, Amikacin emerged as the most widely used agent due to its resistance to the majority of AMEs, according to Ramirez (2017) (39). However, resistance to Amikacin still occurs, often attributed to the overproduction of bacterial alginate, which interacts with the positively charged aminoglycosides and impairs their ability to penetrate bacterial cells (40). This mechanism has been observed particularly in *K. pneumoniae*, which demonstrated reduced sensitivity to a wide range of antibiotics, including beta-lactams, fluoroquinolones, and aminoglycosides (41).

These observations highlight species-specific variations in the acquisition and maintenance of resistance determinants. The presence of *armA* in *E. coli*, but not as frequently in *K. pneumoniae*, may reflect differences in genetic mobility, selective pressures, and patterns of local antimicrobial usage. Although this study successfully identified key resistance genes in clinical isolates, further exploration of quantitative resistance thresholds (e.g., MIC values) in future studies could strengthen clinical interpretation and support antimicrobial decision-making. Tackling these challenges demands robust diagnostic strategies and well-designed antimicrobial stewardship programs to limit the spread of resistant pathogens.

Limitations

This study offers meaningful insights into resistance gene patterns within a real clinical context. While the focus was intentionally kept on a specific setting and sample size to allow for detailed analysis, future studies involving larger and more diverse datasets could build on these results and help translate them into broader clinical applications.

Conclusion

This study highlights the occurrence of polymicrobial urinary tract infections among middle-aged and elderly patients, especially females. The detection of resistance genes underscores the importance of molecular diagnostics and gender-specific treatment strategies in managing UTIs.

Authors' Declaration

We confirm that all the tables and figures in the manuscript contain the results of the current study.

The authors have signed on the ethical considerations of the approval for Ethical Clearance: Accepted by the Researcher Ethical Committee and Scientific Committee designated by the Biology Department, College of Science, and University of Baghdad, under the reference number (CSEC/1023/0096).

Conflict of interest

The authors declare that they have no conflicts of interest relevant to this work.

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Data availability: Upon reasonable request, the corresponding author will make the data sets generated and/or analyzed during the current work available.

Authors' contributions

Study conception & design: (Mazin Y.Z. AL-Khairo & Marwa H. Alkhafaji). Literature search: (Mazin Y.Z. AL-Khairo & Marwa H. Alkhafaji). Data acquisition & Data analysis: (Mazin Y.Z. AL-Khairo & Marwa H. Alkhafaji). Manuscript preparation, editing & review: (Mazin Y.Z. AL-Khairo & Marwa H. Alkhafaji)

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كشف الشركاء الخفيّين:تشخيص العدوى البولية المشتركة متعددة الميكروبات لدى المرضى متوسطي وكبار السن

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الخلاصة
الخلفية: تشغل العدوى المشتركة بالميكروبات جزءًا كبيرًا من التهابات المسالك البولية، ولا سيّما لدى كبار السن. وتكون مثل هذه العدوى خفيّة بسبب التداخل وهيمنة نوع واحد على الأنواع الأخرى.
الأهداف: تهدف هذه الدراسة إلى كشف التهابات المسالك البولية متعدّدة الميكروبات لدى المرضى متوسّطي العمر وكبار السن باستخدام طرائق قائمة على الزرع وطرائق جزيئية معًا. كما تتحرّى وجود جينات مقاومة الأمينوغليكوزيد، وتحديدًا armA وrmtG ، لتحسين دقّة التشخيص ودعم القرارات العلاجية الموجّهة.
المنهجية: في الدراسة الحالية، جُمعت 100 عيّنة بول سريرية من مرضى كبار سن يعانون من التهابات المسالك البولية. أُجريت هذه الدراسة في مستشفى في بغداد عام 2024. تراوحت أعمار المرضى بين متوسّطي العمر وكبار السن 45+ سنة، وقُسّموا بالتساوي بين الذكور والإناث. زُرعت هذه العينات على أوساط زرعية انتقائية وتفريقية. ونُقّيت العزلات النامية لاحقًا وتمّ تعريفها باستخدام نظام .Vitek أُجري اختبار الحساسية للمضادات الحيوية لتقييم نمط مقاومة المضادات الحيوية. استُخلص DNA جرثومي، ووُضعت بادئات (Primers) نوعية لهذه الدراسة لتضخيم جينات ترميز المقاومة armA و rmtG بتقنية تفاعل البوليميراز المتسلسل.(PCR)
النتائج: كانت Klebsiella pneumoniae و *Escherichia coli* هما الميكروبان المتعايشان المسبّبان لالتهاب المسالك البولية لدى المرضى متوسّطي العمر وكبار السن. وُجدت فروق معنوية بين الجنسين في معدلات العدوى المشتركة، كما حدّدت اختبارات الحساسية للمضادات الحيوية والتحليل الجيني جينات المقاومة armA و *rmtG*. وكانت هذه الميكروبات المشتركة مقاومة لمضادات الأمينوغليكوزيد. حمل كلٌّ من *K. pneumoniae* و*E. coli* جين*armA*، في حين كان جين *rmtG* سائدًا في *K. pneumoniae* فقط. وُجدت *K. pneumoniae* و *E. coli* في 42% من العينات. كُشف عن جين *armA* في 46% من العزلات (10/15 من *E. coli*، و15/4 من *K. pneumoniae*، بينما كُشف عن جين *rmtG* في 26.6% من عزلات *K. pneumoniae* ولم يُعثر عليه في *E. coli*.
الاستنتاج: تُبرز هذه الدراسة حدوث التهابات المسالك البولية متعدّدة الميكروبات بين المرضى متوسّطي العمر وكبار السن، ولا سيّما الإناث. ويؤكّد كشف جينات المقاومة أهمية التشخيص الجزيئي واستراتيجيات العلاج الخاصة بكل جنس في تدبير التهابات المسالك البولية.
الكلمات المفتاحية: العدوى المشتركة بالميكروبات؛ التهاب مسالك بولية مختلط؛ مرضى كبار السن؛*E. coli*؛*K. pneumoniae* ؛ مقاومة المضادات الحيوية