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# Phenotypic and Genotypic Detection of Gram-Negative Bacteria Isolated from Urinary Tract Infections in Intensive Care Unit Patients in Duhok City.

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**Abstract:** Urinary tract infections are among the most prevalent hospital-acquired infections, particularly in intensive care unit patients, and are often linked to increased morbidity, prolonged hospitalization, and rising antimicrobial resistance. This study aimed to identify Gram-negative bacteria from intensive care unit urine samples using both phenotypic and genotypic methods to enhance diagnostic accuracy and evaluate antimicrobial resistance patterns. The cross-sectional study was conducted in Duhok City, involving 230 urine samples collected from 628 intensive care unit patients. Conventional microbiological techniques were used for initial culture, and the VITEK 2 system for identification and antibiotic susceptibility testing. Genotypic confirmation was then applied to verify the identity of Gram-negative isolates. Out of the 230 samples, 69 (30%) showed positive cultures, with 40 identified as Gram-negative. Phenotypically, *Escherichia coli* was the most frequent (65%), followed by *Klebsiella pneumoniae* (25%). Polymerase chain reaction analysis confirmed *E. coli* in 70% of isolates, while *Pseudomonas aeruginosa* was not confirmed genotypically. Resistance analysis revealed that 60% of the isolates were multidrug-resistant, 30% were susceptible, and 5% each showed extensive or pan-drug resistance. The results showed notable discrepancies between phenotypic and genotypic identification, particularly in species misclassification, indicating that phenotypic methods alone may be insufficient. The predominance of *E. coli* and *K. pneumoniae* highlights their role as key uropathogens in intensive care unit patients. These findings stress the importance of combining phenotypic and genotypic approaches to improve diagnostic precision and guide effective antimicrobial therapy in critical care settings.

**Keywords:** Gram-negative bacteria; Uropathogenic *Escherichia coli*; Intensive Care Unit; 16S rRNA.

## 1. Introduction

Urinary tract infections (UTIs) are still among the most common healthcare-associated infections, especially in intensive care units (ICUs), where patients often undergo invasive interventions, prolonged catheter use, and experience compromised immunity [1]. These infections significantly raise the risk of complications, extend hospitalization, and hinder efforts in antibiotic stewardship and infection control [2,3]. On a global scale, UTIs are estimated to represent 35–40% of all hospital-acquired infections, with ICU patients facing a particularly elevated risk of developing such infections [4].

In Iraq and throughout much of the Middle East, Gram-negative organisms such as *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are the primary causative agents of ICU-related urinary tract infections [5,6]. Research from regions like Duhok and Basra has reported a notably high incidence of multidrug-resistant (MDR) strains, posing challenges to empirical antibiotic therapy [7,8]. Furthermore, the emergence of extended-spectrum β-lactamase (ESBL)-producing and carbapenem-resistant pathogens is becoming more frequent, underscoring the need for accurate

and rapid diagnostic approaches [9]. Automated platforms such as the VITEK 2 system (bioMérieux) are widely used in clinical microbiology laboratories for the rapid identification of bacteria and evaluation of their antibiotic susceptibility. This system relies on biochemical pattern analysis through a database-driven algorithm and has shown strong performance for frequently encountered clinical isolates [10]. Nonetheless, studies have highlighted its limitations in accurately identifying uncommon Enterobacterales and non-fermenting organisms [11,12]. In direct urine analyses, concordance with standard reference methods typically ranges between 80% and 90% [13].

To minimize phenotypic misidentification, molecular methods like polymerase chain reaction (PCR) are increasingly employed for confirming bacterial species. Although PCR is widely recognized for detecting antimicrobial resistance genes, it also plays a valuable role in validating species-level identification, particularly in critical care settings like ICUs [14,15]. Comparative analyses have revealed inconsistencies between molecular diagnostics and phenotypic tools such as VITEK 2, especially when atypical biochemical characteristics or rare urinary pathogens are involved [16].

Although PCR offers significant benefits for confirming bacterial species, its routine application remains limited in many Iraqi healthcare facilities. Challenges such as limited resources, insufficient technical expertise, and inadequate laboratory infrastructure have restricted its broader implementation [17]. Molecular confirmation tools are essential for enhancing the precision of pathogen identification and supporting more targeted antimicrobial therapies. In settings with limited diagnostic capacity, the absence of such methods often leads to reliance on empirical treatment, which can accelerate antimicrobial resistance. Therefore, expanding access to confirmatory diagnostics is crucial for advancing antimicrobial stewardship and lowering resistance rates globally [18].

Currently, no documented research from Duhok systematically compares species identification using the VITEK 2 system with PCR-based confirmation. In this study, PCR was not employed for detecting resistance genes, but rather to validate the identity of bacterial species identified phenotypically. By conducting this comparative evaluation, we aim to examine the consistency between methods, highlight any recurrent misidentifications, and offer actionable recommendations to improve diagnostic accuracy in ICU microbiology laboratories.

## 2. Methodology

### *2.1. Sample Collection and Bacterial Isolation*

Between November 2024 and January 2025, a total of 230 urine specimens were obtained from patients clinically suspected of urinary tract infections (UTIs) and admitted to the Intensive Care Units (ICUs) of various healthcare facilities in Duhok City, Kurdistan Region. These included Azadi Teaching Hospital, Hivi Teaching Hospital, Wan-Global Private Hospital, Veen Private Hospital, Vajeen Private Hospital, Shiryan Private Hospital, Dr. Ghazi Abdulla Private Hospital, Shilan Private Hospital, and Kurdistan Private Hospital. Samples were collected under aseptic conditions from individuals presenting with typical UTI symptoms. Following collection, standard microbiological protocols were applied for culture and isolation. Each sample was inoculated on blood agar, MacConkey agar, and Mannitol Salt Agar (MSA), then incubated aerobically at 37°C for 18 to 24 hours to promote bacterial growth.

### *2.2. Phenotypic Identification and Antibiotic Susceptibility Testing*

Bacterial identification and antimicrobial susceptibility testing (AST) were performed using the VITEK® 2 Compact automated system (bioMérieux, France), following the manufacturer's instructions. Gram-negative isolates were identified using the GN ID Card (code 21341), which allows for the identification of up to 170 species of Gram-negative bacilli. Antibiotic susceptibility testing was conducted using the AST-N419 card, which includes 15 antimicrobial agents relevant to Gram-negative uropathogens, including Ampicillin/Sulbactam, Piperacillin/Tazobactam, Cefotaxime, Ceftazidime, Ceftazidime/Avibactam, Ceftolozane/Tazobactam, Cefepime, Imipenem, Meropenem, Amikacin, Gentamicin, Ciprofloxacin, Tigecycline, Colistin, and Trimethoprim/Sulfamethoxazole.

The classification of antimicrobial resistance was based on internationally accepted definitions. Multidrug-resistant (MDR) isolates were defined as those that were non-susceptible to at least one agent in three or more antimicrobial categories. Extensively drug-resistant (XDR) organisms were those showing non-susceptibility to all but two or fewer antimicrobial classes, meaning that XDR isolates are also considered MDR. Pan drug-resistant (PDR) isolates were identified as non-susceptible to all tested antibiotics across all antimicrobial categories. These definitions were applied to determine the resistance profiles of the isolated pathogens and to evaluate their clinical impact [19].

### *2.3. Genotypic Analysis Using PCR*

#### 2.3.1. DNA Extraction

Genomic DNA was isolated from purified Gram-negative bacterial isolates using a commercial DNA extraction kit (Addbio, Korea) according to the manufacturer's recommended procedure. In summary, individual bacterial colonies were grown overnight on nutrient agar, and a portion of the fresh culture was transferred into the lysis buffer supplied with the kit. The extraction process included bacterial cell lysis, elimination of proteins and other cellular impurities, followed by purification of the DNA using spin-column chromatography. The resulting DNA was eluted in nuclease-free water and its concentration and purity were measured using a NanoDrop spectrophotometer (Thermo Fisher Scientific, USA). The absorbance ratio at 260/280 nm was used to evaluate DNA quality, and samples with concentrations between 50 and 100 ng/μL were selected for subsequent PCR amplification.

#### 2.3.2. PCR Amplification of the 16S rRNA Gene

Polymerase Chain Reaction (PCR) was utilized to amplify the conserved region of the 16S rRNA gene using the universal primers 27F (5'AGAGTTTGATCCTGGCTCAG3') and 1492R (5'TACGGYTACCTTGTTACGACTT3'), which are commonly applied for bacterial identification at the species level. The reaction mixture had a final volume of 25 μL. It included 12.5 μL of 2X AddTaq™ Master Mix (Addbio, Korea), 2 μL of extracted genomic DNA at a concentration of 50–100 ng/μL, 1 μL of each primer at 10 pmol/μL [20], and 8.5 μL of nuclease-free distilled water.

Thermal cycling conditions were optimized to ensure specific amplification. The protocol began with an initial denaturation at 94 °C for 5 minutes, followed by 35 cycles consisting of denaturation at 94 °C for 45 seconds, primer annealing at 55 °C for 45 seconds, and extension at 72 °C for 45 seconds. A final elongation step was performed at 72 °C for 7 minutes to complete the amplification process. The resulting PCR products were separated by electrophoresis on a 1.5% agarose gel stained with Safe Red dye (Addbio, Korea), and the bands were visualized under UV light using a gel documentation system [20].

#### 2.3.3. 16S rRNA Gene Sequencing

PCR products that displayed clear single bands of the expected 1600 bp were selected for subsequent sequencing analysis. The amplified DNA fragments were purified using a commercial PCR purification kit, such as the QIAquick PCR Purification Kit (Qiagen, Germany), and were then submitted to Macrogen Inc. (Korea) for Sanger sequencing. The resulting nucleotide sequences were compared against reference sequences available in the GenBank database of the National Center for Biotechnology Information (NCBI) using the Basic Local Alignment Search Tool (BLAST). Identification at the species level was confirmed only for sequences exhibiting greater than 99% similarity to known bacterial strains in the database.

### *2.4. Ethical Approval*

This study was ethically approved by the Research Ethics Committee of the Duhok Directorate General of Health, Ministry of Health, Kurdistan Region of Iraq. Approval was granted under the reference number 30102024-9-56 on October 30, 2024.

### *2.5. Statistical Analysis*

All collected data were entered, organized, and analyzed using Microsoft Excel 2019 and IBM SPSS Statistics Version 26. Descriptive statistics were used to summarize categorical variables, including the frequency and percentage of positive urine cultures, types of Gram-negative bacterial isolates, and antibiotic resistance patterns (MDR, XDR, PDR, and non-resistant). The results were presented in tables and graphs. The Chi-square test ($\chi^2$) was employed to assess the association between categorical variables such as bacterial species and resistance profiles, and between phenotypic and genotypic identification results. A p-value < 0.05 was considered statistically significant. Where applicable, cross-tabulation was used to explore the relationship between demographic characteristics (e.g., gender, age group, hospital unit) and bacterial resistance categories. The overall agreement between phenotypic and PCR-based genotypic identification was evaluated using the correlation coefficient, to determine the consistency of diagnostic methods.

## 3. Results

Among the 230 patients included in this study, selection was based on the clinical judgment of the attending physicians and formal medical requests for urine culture analysis. Comprised of 85 (36.96%) males and 145 (63.04%) females, the positivity of urine culture was found to be 69/230 (30%), as depicted in Table 1. All isolates included in the analysis were obtained from pure cultures. No mixed infections were detected among the studied samples.

Gram-negative bacteria isolates constituted 40/69 (57.97%), and Gram-positive bacteria were 29/69 (42.03%). *Escherichia coli* was the predominant isolate at 26/40 (65%), followed by *Klebsiella pneumoniae* at 10/40 (25%), *Pseudomonas aeruginosa* at 2/40 (5%), and Proteus mirabilis at 2/40 (5%), as depicted in Table 2.

A statistically significant association was observed between age and gender in relation to GNB-UTI cases (p = 0.009), indicating that the distribution of infections varied meaningfully across demographic groups. This suggests that certain age or gender groups may be more susceptible to Gram-negative urinary tract infections.

Distribution of Gram-Negative UTI Cases by Comorbidities and Gender is detailed in Table 3, which reveals that females had higher prevalence rates of cardiovascular diseases, and type 2 diabetes. At the same time, males exhibited greater frequencies of asthma and chronic liver disease. The chi-square analysis of comorbidities and gender among GNB-UTI patients revealed no statistically significant association (p = 0.783). This indicates that the distribution of Gram-negative urinary tract infections across different comorbid conditions does not vary meaningfully between males and females in the studied population. In other words, the presence of specific underlying diseases was not strongly linked to gender-based differences in infection rates.

**Table 1.** Age and Gender-Based Distribution of UTI Cases Among Study Participants.

| **Age and Gender** | | | | |
|---|---|---|---|---|
| | | **Count** | | |
| | | **Gender** | | |
| | | **UTI / Male** | **UTI / Female** | **UTI / Total** |
| **Age** | 1- 20 | 6 / 15 | 17 / 33 | **23 / 48** |
| | 21-40 | 1 / 10 | 5 / 27 | **6 / 37** |
| | 41-60 | 8 / 30 | 15 / 54 | **23 / 84** |
| | More than 60 | 8 / 30 | 9 / 31 | **17 / 61** |
| | **Total** | **23 / 85** | **46 / 145** | **69 / 230** |

**Table 2.** Age and Gender Distribution of UTI Cases Caused by Gram-Negative Bacteria.

| **Age and Gender** | | | | |
|---|---|---|---|---|
| | | **Count** | | |
| | | **Gender** | | |
| | | **GNB-UTI/ Male** | **GNB-UTI/ Female** | **GNB-UTI/ Total** |
| **Age** | 1- 20 | 0 / 15 | 12 / 33 | **12 / 48** |
| | 21-40 | 1 / 10 | 0 / 27 | **1 / 37** |
| | 41-60 | 5 / 30 | 10 / 54 | **15 / 84** |
| | More than 60 | 7 / 30 | 5 / 31 | **12 / 61** |
| | **Total** | **13 / 85** | **27 / 145** | **40 / 230** |

**Table 3.** Distribution of Gram-Negative UTI Cases by Comorbidities and Gender.

| **Comorbidities** | Gender | | **GNB-UTI/ Total** |
|---|---|---|---|
| | **GNB-UTI/ Male** | **GNB-UTI/ Female** | |
| Chronic Kidney Disease | 1 / 4 | 5 / 17 | **6 / 21** |
| Hypertension | 3 / 12 | 6 / 20 | **9 / 32** |
| Cardiovascular Diseases | 0 / 3 | 0 / 18 | **0 / 21** |
| Congenital heart disease | 0 / 0 | 0 / 1 | **0 / 1** |
| Asthma | 4 / 21 | 4 / 14 | **8 / 35** |
| Asthma, Hypertension | 0 / 0 | 1 / 1 | **1 / 1** |
| Chronic Obstructive Pulmonary Disease | 1 / 7 | 1 / 14 | **2 / 21** |
| Cancer | 0 / 2 | 0 / 3 | **0 / 5** |
| Type 1 diabetes | 0 / 4 | 0 / 6 | **0 / 10** |

| | | | |
|---|---|---|---|
| Type 2 diabetes | 3 / 13 | 4 / 23 | **7 / 36** |
| Hemorrhage | 0 / 0 | 1 / 1 | **1 / 1** |
| Epilepsy | 0 / 0 | 0 / 1 | **0 / 1** |
| Trauma | 1 / 3 | 0 / 2 | **1 / 5** |
| Chronic liver disease | 0 / 2 | 0 / 0 | **0 / 2** |
| Burn | 0 / 1 | 0 / 3 | **0 / 4** |
| Meningitis | 0 / 0 | 1 / 4 | **1 / 4** |
| Pneumonia | 0 / 3 | 2 / 7 | **2 / 10** |
| Post-operative complications | 0 / 8 | 1 / 7 | **1 / 15** |
| Sepsis | 0 / 2 | 1 / 1 | **1 / 3** |
| Severe Dehydration | 0 / 0 | 0 / 2 | **0 / 2** |
| **Total** | **13 / 85** | **27 / 145** | **40 / 230** |

### *3.1. Phenotypic Identification Results*

Out of the 40 Gram-negative bacterial isolates obtained from ICU urine samples, phenotypic identification using the VITEK 2 system revealed the presence of four main species. The most frequently identified organism was *Escherichia coli*, accounting for 26 isolates (65%), followed by *Klebsiella pneumoniae* in 10 samples (25%), and both *Pseudomonas aeruginosa* and *Proteus mirabilis* in 2 samples each (5%), as illustrated in Figure 1.

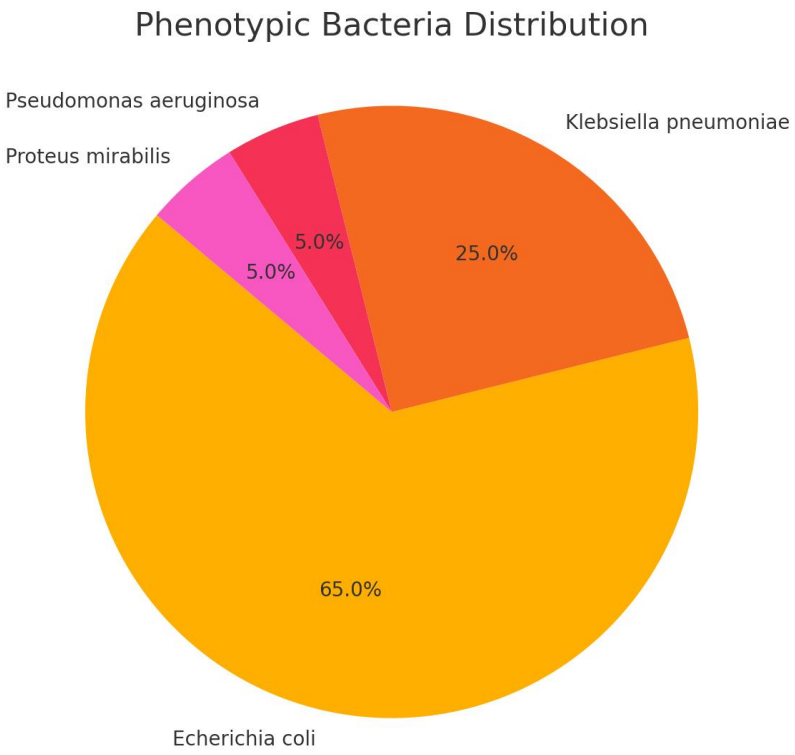


**Figure 1.** Phenotypic Distribution of Gram-negative bacteria based on VITEK Identification System.

### *3.2. Genotypic Identification by Sequencing*

Genotypic identification was performed by sequencing a specific region of the 16S rRNA gene using universal primers (1600 bP band) as shown in Figure 2. The result showed a different distribution profile. *Escherichia coli* was confirmed in 28 samples (70%), while *Klebsiella pneumoniae* was confirmed in 6 samples (15%). In addition, *Morganella morganii* was identified in 3 samples (7.5%), *Proteus mirabilis* in 2 samples (5%), and *Acinetobacter baumannii* in 1 sample (2.5%), as illustrated in Figure 3. Notably, *Pseudomonas aeruginosa* was not confirmed in any of the samples using genotypic methods, as shown in Table 4.

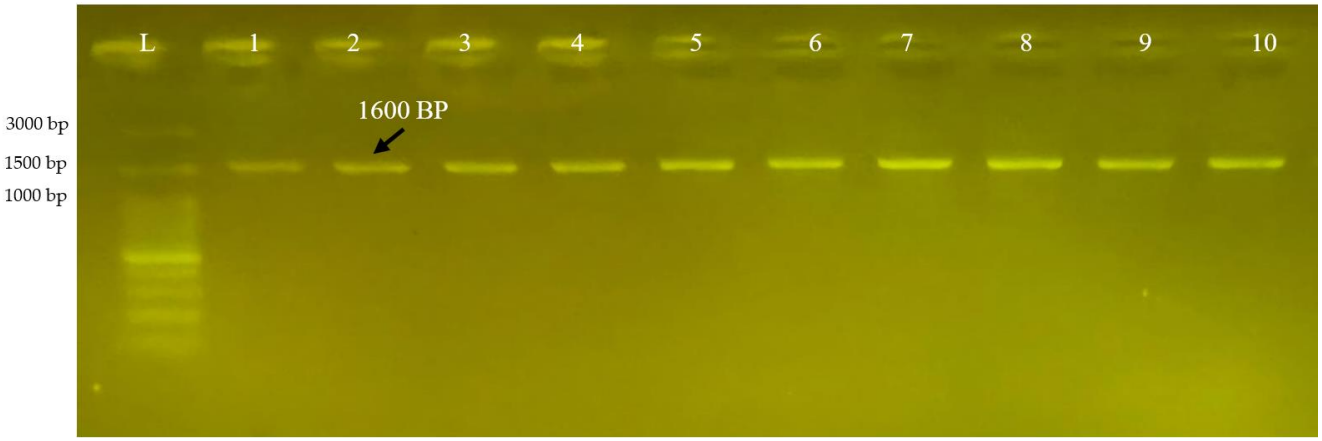


**Figure 2.** PCR Product of 16S RNA Gene, Lane L Ladder (100 bp DNA Ladder) Lane 1–10 Samples (1600 bp Band).

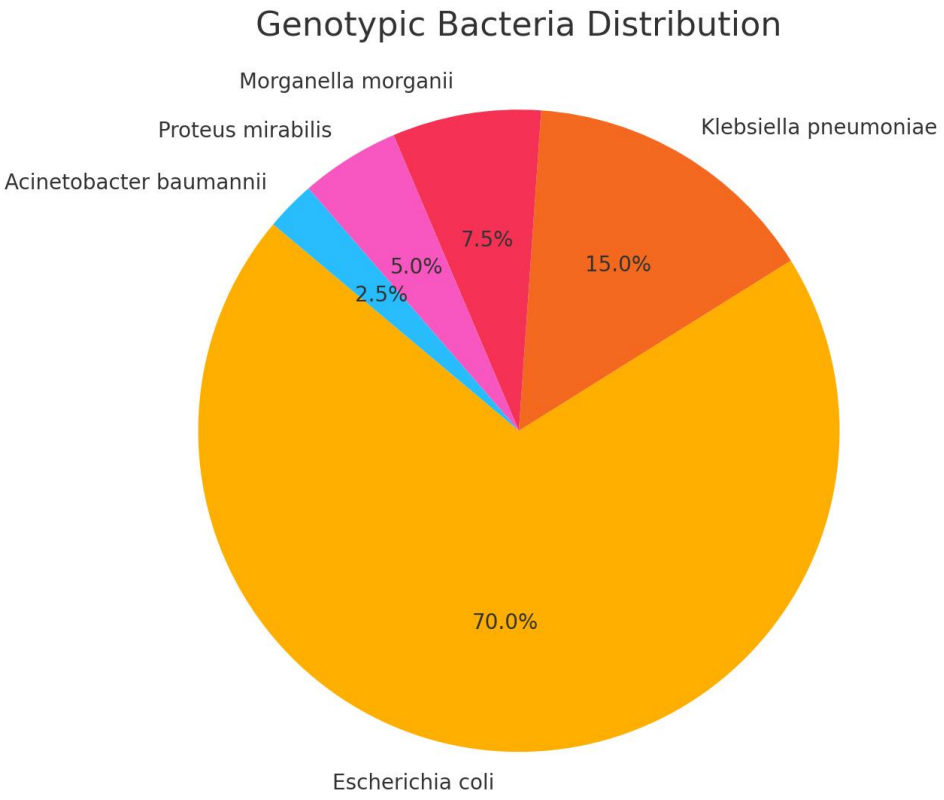


**Figure 3.** Genotypic Distribution of Gram-negative Bacteria based on 16S rRNA Gene Sequencing.

**Table 4.** Distribution of Bacterial Species Identified Through Sequencing of 40 Gram-negative Isolates.

| No. | Samples ID | Similarity | Cover % | E Value | NCBI Sequences Acc. No. | Bacteria |
|---|---|---|---|---|---|---|
| 1. | 10 | 100 | 100 | 0 | CP083492 | *Escherichia coli* |
| 2. | 15 | 99.75 | 100 | 0 | CP021175 | *Escherichia coli* |
| 3. | 26 | 99.93 | 100 | 0 | GU586133 | *Klebsiella pneumoniae* |
| 4. | 30 | 99.78 | 100 | 0 | CP133106 | *Escherichia coli* |
| 5. | 34 | 100 | 100 | 0 | CP163901 | *Escherichia coli* |
| 6. | 44 | 100 | 100 | 0 | CP008697 | *Escherichia coli* |
| 7. | 50 | 100 | 100 | 0 | LN558632 | *Morganella morganii* |
| 8. | 53 | 100 | 100 | 0 | MT845092 | *Escherichia coli* |
| 9. | 59 | 99.78 | 100 | 0 | MH396737 | *Escherichia coli* |

| | | | | | | |
|---|---|---|---|---|---|---|
| 10. | 65 | 100 | 100 | 0 | CP139231 | *Klebsiella pneumoniae* |
| 11. | 73 | 100 | 100 | 0 | MN795608 | *Proteus mirabilis* |
| 12. | 89 | 100 | 100 | 0 | MN208223 | *Escherichia coli* |
| 13. | 96 | 100 | 100 | 0 | MF428899 | *Escherichia coli* |
| 14. | 104 | 99.84 | 100 | 0 | AP028310 | *Escherichia coli* |
| 15. | 112 | 99.71 | 100 | 0 | OR563961 | *Escherichia coli* |
| 16. | 122 | 100 | 100 | 0 | PQ725651 | *Escherichia coli* |
| 17. | 127 | 100 | 100 | 0 | CP153718 | *Klebsiella pneumoniae* |
| 18. | 129 | 100 | 100 | 0 | CP173596 | *Escherichia coli* |
| 19. | 134 | 100 | 100 | 0 | CP019243 | *Escherichia coli* |
| 20. | 144 | 100 | 100 | 0 | PQ725651 | *Escherichia coli* |
| 21. | 154 | 100 | 100 | 0 | MK560859 | *Escherichia coli* |
| 22. | 161 | 100 | 100 | 0 | KJ803898 | *Escherichia coli* |
| 23. | 163 | 100 | 100 | 0 | CP083492 | *Escherichia coli* |
| 24. | 170 | 100 | 100 | 0 | OM066746 | *Escherichia coli* |
| 25. | 172 | 100 | 100 | 0 | MH718833 | *Proteus mirabilis* |
| 26. | 179 | 100 | 100 | 0 | OK271862 | *Escherichia coli* |
| 27. | 181 | 100 | 100 | 0 | PP188096 | *Escherichia coli* |
| 28. | 183 | 100 | 100 | 0 | MK951734 | *Escherichia coli* |
| 29. | 185 | 99.93 | 100 | 0 | CP029328 | *Escherichia coli* |
| 30. | 186 | 100 | 100 | 0 | CP077816 | *Klebsiella pneumoniae* |
| 31. | 193 | 99.18 | 100 | 0 | PP177461 | *Acinetobacter baumannii* |
| 32. | 202 | 99.55 | 100 | 0 | AP028310 | *Escherichia coli* |
| 33. | 209 | 100 | 100 | 0 | CP042945 | *Escherichia coli* |
| 34. | 212 | 100 | 100 | 0 | KY780346 | *Escherichia coli* |
| 35. | 215 | 100 | 100 | 0 | CP028750 | *Escherichia coli* |
| 36. | 217 | 100 | 100 | 0 | KJ803927 | *Klebsiella pneumoniae* |
| 37. | 221 | 100 | 100 | 0 | CP059477 | *Morganella morganii* |
| 38. | 227 | 100 | 100 | 0 | KX350022 | *Klebsiella pneumoniae* |
| 39. | 228 | 100 | 100 | 0 | KP790053 | *Morganella morganii* |
| 40. | 230 | 100 | 100 | 0 | CP164151 | *Escherichia coli* |

### *3.3. Comparison Between Phenotypic and Genotypic Methods*

A comparative analysis between phenotypic identification using the VITEK 2 system and genotypic confirmation via PCR revealed a high degree of concordance. Out of a total of 40 Gram-negative isolates from ICU urine samples, 33 cases (82.5%) showed complete agreement between the phenotypic and genotypic methods. Only seven isolates (17.5%) exhibited discrepancies between the two identification approaches.

The mismatched cases included samples where the phenotypic result identified the organism as *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*. At the same time, genotypic confirmation revealed the presence of other species such as *Escherichia coli, Morganella morganii,* or *Acinetobacter baumannii,* as shown in Table 5. This shows that Escherichia coli had the highest concordance between phenotypic and genotypic results (25 out of 28 genotypically identified E. coli), while significant mismatches were observed in other species, as shown in Figure 4. The statistical association was tested using the Chi-Square test, yielding a highly significant result ($\chi^2 = 72.14$, $p < 0.0000000002$), indicating a strong discrepancy between the two identification methods.

**Table 5.** Cross-tabulation between Phenotypic and Genotypic Identification Results of Gram-negative Bacterial Isolates(n=40).

| PHENOTYPIC * GENOTYPIC | | | | | | | |
|---|---|---|---|---|---|---|---|
| | | GENOTYPIC | | | | | |
| | | *Escherichia coli* | *Klebsiella Pneumoniae* | *Morganella Morganii* | *Proteus Mirabilis* | *Acinetobacter Baumannii* | **Total** |
| PHENOTYPIC | *Escherichia Coli* | 25 | 0 | 1 | 0 | 0 | 26 |
| | *Klebsiella Pneumoniae* | 2 | 6 | 1 | 0 | 1 | 10 |
| | *Pseudomonas Aeruginosa* | 1 | 0 | 1 | 0 | 0 | 2 |
| | *Proteus Mirabilis* | 0 | 0 | 0 | 2 | 0 | 2 |
| | **Total** | **28** | **6** | **3** | **2** | **1** | **40** |

**Figure 4.** Phenotypic-genotypic Discrepancy: Colonies on MacConkey Agar Suspected *E. coli* but identified by VITEK as *Pseudomonas Aeruginosa*, but Confirmed Genotypically as *Escherichia coli*.

*3.4. Antibiotic Susceptibility Profile of Gram-Negative Isolates*

The analysis of antibiotic susceptibility for the 40 Gram-negative bacterial isolates included in this study, as presented in Table 6 and visually supported by Figure 5, reveals a broad range of responses to the 15 antimicrobial agents tested using the VITEK 2 Compact system. The variability in these responses underscores the complex resistance landscape among Gram-negative organisms, particularly in the context of urinary tract infections in intensive care settings.

Among the tested antibiotics, the carbapenem class—specifically Meropenem and Imipenem—demonstrated the highest level of efficacy. Meropenem exhibited a sensitivity rate of 85.0%, while Imipenem closely followed at 82.5%, with both showing relatively low resistance levels (15.0% each). Similarly, Amikacin, an aminoglycoside antibiotic, showed strong antibacterial activity, with 82.5% of isolates reported as sensitive, 5.0% as intermediate, and only 12.5% resistant.

Tigecycline also demonstrated high in vitro effectiveness, with a sensitivity rate of 85.0% and no intermediate resistance noted. Piperacillin/Tazobactam, on the other hand, achieved a moderate sensitivity rate of 67.5%, with resistance recorded in 25.0% of isolates. In contrast, the cephalosporin group—including Cefotaxime, Ceftazidime, and Cefepime—performed poorly, each exhibiting a resistance rate of 72.5%, while sensitivity rates remained under 30.0%.

Ampicillin/Sulbactam showed an almost equal distribution across the three categories, with 30.0% of isolates classified as sensitive, 35.0% as intermediate, and 35.0% as resistant. Ciprofloxacin demonstrated a sensitivity rate of only 30.0%, with more than half of the isolates (52.5%) being resistant and 17.5% falling in the intermediate range. Likewise, Trimethoprim/Sulfamethoxazole (TMP-SMX) was associated with a resistance rate of 65.0%, confirming the limited utility of these agents in treating Gram-negative infections, particularly in ICU settings.

An interesting and clinically important finding was observed in the susceptibility profile of Colistin. While only 30.0% of isolates were fully sensitive, a significant proportion (57.5%) fell into the intermediate category, and only 12.5% were resistant.

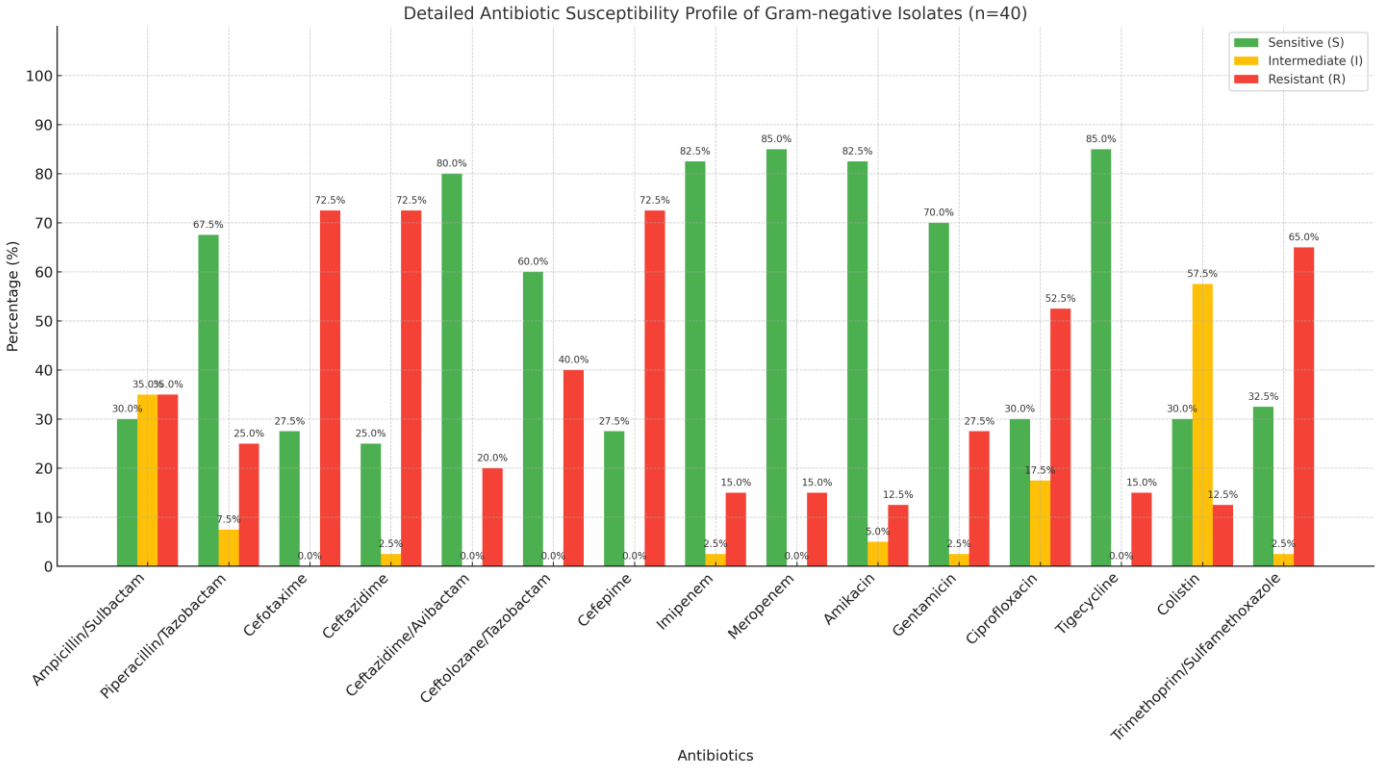


**Figure 5.** Antibiotic Susceptibility Profile of Gram-Negative Bacterial Isolates.

**Table 6:** Antibiotic Susceptibility Patterns of Gram-negative Bacterial Isolates (n = 40) Based on VITEK 2 Compact System.

| No. | Antibiotic | S (n, %) | I (n, %) | R (n, %) | Total (n, %) |
|---|---|---|---|---|---|
| 1. | Ampicillin/Sulbactam | 12 (30.0%) | 14 (35.0%) | 14 (35.0%) | 40 / 100 % |
| 2. | Piperacillin/Tazobactam | 27 (67.5%) | 3 (7.5%) | 10 (25.0%) | 40 / 100 % |
| 3. | Cefotaxime | 11 (27.5%) | 0 (0.0%) | 29 (72.5%) | 40 / 100 % |
| 4. | Ceftazidime | 10 (25.0%) | 1 (2.5%) | 29 (72.5%) | 40 / 100 % |
| 5. | Ceftazidime/Avibactam | 32 (80.0%) | 0 (0.0%) | 8 (20.0%) | 40 / 100 % |
| 6. | Ceftolozane/Tazobactam | 24 (60.0%) | 0 (0.0%) | 16 (40.0%) | 40 / 100 % |
| 7. | Cefepime | 11 (27.5%) | 0 (0.0%) | 29 (72.5%) | 40 / 100 % |
| 8. | Imipenem | 33 (82.5%) | 1 (2.5%) | 6 (15.0%) | 40 / 100 % |
| 9. | Meropenem | 34 (85.0%) | 0 (0.0%) | 6 (15.0%) | 40 / 100 % |
| 10. | Amikacin | 33 (82.5%) | 2 (5.0%) | 5 (12.5%) | 40 / 100 % |
| 11. | Gentamicin | 28 (70.0%) | 1 (2.5%) | 11 (27.5%) | 40 / 100 % |

| | | | | | |
|---|---|---|---|---|---|
| 12. | Ciprofloxacin | 12 (30.0%) | 7 (17.5%) | 21 (52.5%) | 40 / 100 % |
| 13. | Tigecycline | 34 (85.0%) | 0 (0.0%) | 6 (15.0%) | 40 / 100 % |
| 14. | Colistin | 12 (30.0%) | 23 (57.5%) | 5 (12.5%) | 40 / 100 % |
| 15. | Trimethoprim/Sulfamethoxazole | 13 (32.5%) | 1 (2.5%) | 26 (65.0%) | 40 / 100 % |

*3.5. Drug Resistance Phenotypes Among Gram-Negative Isolates*

Table 7 presents the distribution of antimicrobial resistance phenotypes—namely, Non-Drug-Resistant (NDR), Multidrug-Resistant (MDR), Extensively Drug-Resistant (XDR), and Pan Drug-Resistant (PDR)—among 40 Gram-negative bacterial isolates obtained from urine samples of ICU patients. The data reflect a concerning resistance trend, with MDR strains accounting for 60% of the isolates, while XDR and PDR phenotypes were each identified in 5% of cases. Only 30% of the isolates were fully susceptible to the antibiotics tested.

*Escherichia coli*, the most prevalent isolate (n = 28), contributed substantially to the resistance profile. Approximately 64.3% of *E. coli* isolates were MDR, while XDR and PDR phenotypes were observed in 3.6% of the cases each.

*Klebsiella pneumoniae* (n = 6) showed a similarly problematic pattern, with two isolates classified as MDR, and one isolate each demonstrating XDR and PDR characteristics.

Less frequently isolated species such as *Morganella morganii* and *Proteus mirabilis* also exhibited high resistance. All *P. mirabilis* isolates were MDR, and two-thirds of *M. morganii* showed a similar resistance phenotype. Although the single *Acinetobacter baumannii* isolate was not MDR.

Overall, the findings indicate a significant burden of antimicrobial resistance among Gram-negative uropathogens in ICU settings. The presence of XDR and PDR strains, though limited in number, is clinically alarming due to the narrowing range of effective treatment options.

**Table 7:** Prevalence of Multidrug-Resistant, Extensively Drug-Resistant, Pan Drug-Resistant, Non-Drug-Resistant Among Gram Negative Bacteria.

| Bacteria | NONE | MDR | XDR | PDR | Total |
|---|---|---|---|---|---|
| *Escherichia coli* | 8 | 18 | 1 | 1 | 28 |
| *Klebsiella Pneumoniae* | 2 | 2 | 1 | 1 | 6 |
| *Morganella Morganii* | 1 | 2 | 0 | 0 | 3 |
| *Proteus Mirabilis* | 0 | 2 | 0 | 0 | 2 |
| *Acinetobacter Baumannii* | 1 | 0 | 0 | 0 | 1 |
| **Total** | 12 | 24 | 2 | 2 | 40 |
| **Percentage** | 30% | 60% | 5% | 5% | 100% |

## 4. Discussion

The results revealed that Gram-negative bacteria accounted for 40 out of 69 positive cultures, representing 57.97%, while Gram-positive isolates constituted 29 cases (42.03%). Among the Gram-negative pathogens identified by VITEK, Escherichia coli was the most frequently detected species, accounting for 26 out of 40 isolates (65%). Klebsiella pneumoniae followed this in 10 cases (25%), and both *Pseudomonas aeruginosa* and *Proteus mirabilis*, each found in 2 isolates (5%).

However, the genotypic results revealed that *Escherichia coli* was the most predominant uropathogen, accounting for 70% (28 out of 40) of the confirmed isolates. This was followed by *Klebsiella pneumoniae* (15%), *Morganella morganii* (7.5%), *Proteus mirabilis* (5%), and *Acinetobacter baumannii* (2.5%). These findings indicate a clear dominance of *E. coli* among ICU UTI cases at the molecular level. The results underscore the importance of genotypic confirmation to ensure accurate identification of the causative pathogens.

A related study conducted by Al-Naqshbandi et al. (2019) at Rizgary Teaching Hospital in Erbil, Iraq, analyzed 2692 urine samples. Of these, 450 samples (16.72%) yielded pathogenic bacterial growth. Among the pathogens isolated, 371 isolates (82.44%) were identified as Gram-negative bacteria, while the remaining 17.56% were Gram-positive. *Escherichia coli* was reported as the most prevalent Gram-negative uropathogen, indicating its dominant role in urinary tract

infections. In contrast, *Acinetobacter baumannii* was recognized as the most resistant Gram-negative species, highlighting a serious concern for antimicrobial resistance in ICU-related infections [21].

The outcomes of this study emphasize the vital role of precise bacterial identification in intensive care unit (ICU) environments, particularly concerning urinary tract infections (UTIs) caused by Gram-negative pathogens. The observed concordance rate of 33/40 (82.5%) between phenotypic and genotypic identification methods suggests that the VITEK 2 system generally performs well in routine clinical diagnostics. Nevertheless, the remaining 7/40 (17.5%) mismatch highlights the inherent limitations of relying solely on phenotypic techniques, especially when dealing with rare, atypical, or fastidious bacterial species that may not be accurately identified by biochemical profiling alone [22,23].

In this study, seven bacterial isolates were identified differently when comparing phenotypic and molecular approaches. Misidentification was most commonly observed with *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, whereas *Morganella morganii* and *Acinetobacter baumannii* were exclusively detected through PCR-based methods. These findings reinforce the value of 16S rRNA gene sequencing as a reliable molecular tool for confirming bacterial species, particularly in cases where automated phenotypic systems yield ambiguous or inaccurate results [24].

The findings of this study are consistent with those reported by reference [25], which indicated that although VITEK systems achieve an identification accuracy of 85–90% for commonly encountered bacterial species, their performance tends to decline in ICU-derived samples. This reduction in reliability is attributed to resistance-associated genetic mutations, atypical biochemical profiles, and the presence of complex microbial communities in critically ill patients, all of which can interfere with accurate phenotypic interpretation.

Additional evidence is provided by previous research, which reported that as many as 18% of bacterial isolates obtained from ICU settings were inaccurately identified by phenotypic systems and required genotypic confirmation to establish correct species identification [26].

Similar observation was reported in Sulaymaniyah, where significant discrepancies were found between phenotypic and molecular methods in the identification of *Klebsiella pneumoniae* isolates from clinical specimens [27].

A study conducted in Baghdad reported over 90% concordance between the VITEK 2 Compact system and molecular identification techniques. However, a portion of the isolates still required reclassification following molecular confirmation, highlighting occasional limitations of phenotypic methods in clinical diagnostics [28].

In a study conducted in Hamadan, Iran, Alikhanzadeh et al. (2021) compared phenotypic identification methods with PCR-based detection for carbapenemase-producing *Pseudomonas aeruginosa*. The authors reported that molecular testing enhanced diagnostic accuracy by approximately 18%, particularly in cases where phenotypic approaches failed to provide reliable results [29]. Similar outcomes were observed in a regional study by Alkhulaifi and Mohammed (2023) in Basra, where manual methods, the VITEK 2 system, and PCR-based confirmation were compared for clinical *Pseudomonas aeruginosa* isolates. Their findings revealed notable inconsistencies between methods and demonstrated that molecular confirmation frequently altered the initial identifications produced by phenotypic systems [30].

Al Tememe and Abbas (2022) conducted a study in Basrah to evaluate the identification of *Pseudomonas aeruginosa* using both conventional methods, including the VITEK 2 system, and molecular techniques such as 16S rRNA and *aroE* gene sequencing via PCR. Among the examined isolates, ten were confirmed through ~1500 bp 16S rRNA sequencing. The findings revealed that reliance on phenotypic methods alone was associated with a misidentification rate ranging from 10% to 20%, particularly in non-fermenting Gram-negative bacilli. These results emphasize the limitations of automated phenotypic platforms like VITEK in accurately distinguishing between closely related bacterial species [31].

Several studies have indicated that *Morganella morganii* is often misidentified or overlooked by phenotypic identification systems, primarily due to its inconsistent biochemical characteristics. In contrast, PCR-based molecular techniques have demonstrated higher accuracy in confirming its presence. These observations reinforce the growing recognition that genotypic methods are crucial for the precise identification of Gram-negative pathogens in clinical microbiology [32].

A multicenter study published in *Antibiotics* in 2022 reported that although the VITEK 2 system provides rapid identification and susceptibility testing, species-level discrepancies were observed in approximately 12% of cases, particularly involving *Pseudomonas* and *Proteus* species [33]. Complementary findings from the *Annals of Laboratory Medicine* highlighted the value of incorporating PCR-based confirmation alongside automated phenotypic platforms to minimize

misidentification rates [34]. Supporting this perspective, a study featured in *Scientific Reports* demonstrated that molecular diagnostic approaches, including PCR, achieved significantly higher positive predictive agreement (PPA) compared to traditional biochemical identification systems [35].

Collectively, the findings of this study not only align with both regional and international research, but also represent the first molecular confirmation-based investigation of urinary tract pathogens among ICU patients in Duhok. The notable prevalence of *Escherichia coli* and *Klebsiella pneumoniae*, along with the genotypic detection of *Morganella morganii* and *Acinetobacter baumannii*, highlights the diagnostic value of integrating molecular methods into routine laboratory practices across ICU settings in Iraq. These results reinforce the critical role of genotypic tools in supporting traditional phenotypic systems, particularly in high-risk clinical environments where accurate pathogen identification is essential for guiding effective treatment and infection control strategies.

## 5. Conclusions

This study emphasizes the importance of accurate identification of Gram-negative pathogens causing ICU-acquired urinary tract infections through a combination of phenotypic and genotypic methods. While PCR-based genotypic techniques are highly precise and play a critical role in confirming ambiguous or misidentified isolates—particularly in detecting less common organisms such as *Morganella morganii* and *Acinetobacter baumannii*—the routine use of molecular diagnostics in all clinical laboratories remains unrealistic due to high cost, technical complexity, and long processing times.

Given these practical limitations, it becomes essential to enhance the reliability of automated phenotypic systems such as the VITEK. Our findings highlight that although the VITEK system provides rapid preliminary identification, it still exhibits a notable error rate and may fail to identify certain clinically relevant bacteria, leading to potential misclassification.

Therefore, we recommend that manufacturers focus on improving the sensitivity and accuracy of automated identification platforms. Meanwhile, clinical laboratories should consider molecular confirmation selectively, especially in cases with unclear results or unusual resistance patterns. This balanced approach can improve diagnostic outcomes, ensure better-targeted antimicrobial therapies, and support antimicrobial stewardship efforts in critical care settings. Further research is needed to validate these observations and guide the development of more reliable and cost-effective diagnostic protocols.

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