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Molecular Typing by RAPD-PCR of Gram-Negative Bacteria Isolated from Urinary Tract Infections in Samarra, Iraq

Noor Khalid Ismael[1,*]; Wahran Khudhair Abbas[2]; Heba Tallah Mahfoodh Khaleel[3]; Duaa Ahmed Hassan Al-Kailani[3]; Ahmed Suhail Hussein[3]; Wisam Ibrahim Al-Darraji[3]; Younis Waleed Younis[3]; Zeyad Taha Hussein[4]

[1] University of Samarra. Education of Pure Science faculty. Department of Biology, Iraq.
[2] University of Samarra. Applied Sciences faculty. Department of Pathological analysis, Iraq.
[3] University of Samarra. Applied Sciences faculty. Department of Biotechnology, Iraq.
[4] University of Tikrit. Veterinary medicine faculty. Department of Microbiology, Iraq.

*Corresponding author: Noor Khalid Ismael.
University of Samarra. Education of Pure Science faculty.
Department of Biology, Iraq.
E-mail: noor.k.i@uosamarra.edu.ig
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Abstract

Introduction: Urinary tract infections (UTIs) are among the most common bacterial infections worldwide. Gram-negative bacteria represent the main causative agents and contribute significantly to the clinical burden of UTIs. Molecular typing methods are increasingly used to explore genetic diversity among bacterial isolates and to support preliminary epidemiological investigations, particularly in settings with limited access to high-resolution genomic tools. **Objective:** This study aimed to assess the genetic diversity of Gram-negative bacterial isolates recovered from patients with urinary tract infections at Samarra Hospital using the Random Amplified Polymorphic DNA Polymerase Chain Reaction (RAPD-PCR) technique during the period from April to August 2024. **Methods:** A descriptive laboratory-based study was conducted on eighteen Gram-negative bacterial isolates obtained from urine samples of UTI patients. All isolates were subjected to RAPD-PCR analysis, and banding patterns were evaluated using agarose gel electrophoresis to identify distinct genetic profiles. **Results:** RAPD-PCR amplification was successfully obtained in sixteen of the eighteen analyzed isolates. Analysis of banding patterns revealed three distinct RAPD profiles, designated as patterns A, B, and C, indicating detectable genetic heterogeneity among the isolates. The observed patterns reflected the coexistence of genetically diverse profiles within the studied clinical setting. **Conclusion:** The findings demonstrate the presence of genetic diversity among Gram-negative uropathogenic isolates at Samarra Hospital. RAPD-PCR proved to be a practical and exploratory molecular typing tool for the preliminary assessment of genetic variability in a resource-limited context. Further studies using larger sample sizes and higher-resolution molecular techniques are recommended to clarify the clinical and epidemiological significance of the observed genetic diversity.

Keywords: Urinary Tract Infections (UTIs). Gram-negative bacteria (GNB). RAPD-PCR.

Introduction

Urinary tract infections (UTIs) continue to stand out as one of the most frequent bacterial infections worldwide, and each year they place a noticeable strain on both patients and healthcare systems [1,2]. Their impact is not limited to discomfort; they contribute substantially to illness among women, young children, and older adults, and they remain one of the most common reasons for outpatient visits and antibiotic prescriptions. Clinically, UTIs may involve only the lower urinary tract, as in cystitis, or progress to the upper tract, resulting in pyelonephritis. Because

many of the symptoms overlap with other conditions, achieving a clear diagnosis is not always straightforward, yet it is essential to avoid delays in treatment and prevent complications [3].

This challenge becomes even more pressing in areas where antimicrobial resistance is widespread. In such settings, empirical treatment often fails, increasing the need for diagnostic tools that can quickly and accurately identify the responsible pathogens [4]. Gram-negative bacteria (GNB) are widely recognized as the primary cause of UTIs and continue to pose a major global health concern, largely because their resistance to many antibiotics has been rising steadily [5]. Beyond UTIs, these organisms are implicated in a broad spectrum of infections—such as otitis media, wound infections, and bacteremia—and they are frequently associated with hospital-acquired cases [6,7].

Many GNB strains possess specific virulence factors that promote firm adhesion to the epithelial lining of the urinary tract, allowing them to evade host defenses and persist within the urinary environment [8]. These characteristics make them difficult pathogens to manage and highlight the urgent need for reliable diagnostic approaches, particularly in regions with limited laboratory resources.

The growing complexity of UTI etiology and the rapid emergence of resistant strains have increased the need for molecular epidemiological tools capable of distinguishing closely related bacterial isolates. Molecular screening techniques have significantly improved pathogen identification by offering high sensitivity, improved accuracy, and rapid processing, while also providing valuable insights into genetic diversity, transmission dynamics, and strain-level relationships [9,10].

These molecular approaches play a pivotal role in surveillance programs and infection control, especially in hospital environments where recurrent or clustered infections may signal clonal spread. Multiple molecular typing techniques have been developed to assess genetic variation among bacterial populations, including hybridization-based approaches such as Restriction Fragment Length Polymorphism (RFLP) and PCR-based approaches such as Random Amplified Polymorphic DNA (RAPD), Simple Sequence Repeats (SSR), Single Nucleotide Polymorphism (SNP), and Amplified Fragment Length Polymorphism (AFLP) [11]. Among these, RAPD-PCR has gained widespread importance because of its technical simplicity, low cost, minimal DNA requirement, and its ability to generate high levels of polymorphism. RAPD also enables the detection of subtle genomic variations, making it a practical tool for epidemiological investigations, strain differentiation, and preliminary genetic studies.

Despite the substantial burden of UTIs in Iraq, there remains a limited number of molecular typing studies examining the genetic diversity of Gram-negative uropathogens, particularly in regions such as Samarra and Tikrit. Understanding the genomic patterns of circulating strains is essential for guiding infection control, tracing transmission pathways, and supporting early detection of emerging clones.

Therefore, this study aimed to characterize the genetic diversity of Gram-negative bacteria isolated from patients with urinary tract infections in Samarra, Iraq, during April– August 2024 using the RAPD-PCR technique, with the objective of identifying distinct genomic patterns to support local epidemiological surveillance.

Materials and Methods

Study Design and Setting

This work was designed as a descriptive cross-sectional laboratory study based on urine samples obtained from adult patients who presented with symptoms suggestive of urinary tract infection. This study adhered to the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines for cross-sectional observational research. All specimens were collected from different departments within Samarra Hospital over a five-month period extending from April 2024 to August 2024. The laboratory procedures, including bacterial isolation, DNA extraction, and molecular analysis, were carried out in the Department of Biotechnology, College of Applied Sciences, University of Samarra.

Ethical Considerations

All ethical guidelines and standards of scientific research were fully followed throughout this study.

Sample Selection and Eligibility Criteria

Samples were obtained from adult patients aged 18 years or older who exhibited one or more clinical features of UTI, including dysuria, increased urinary frequency, fever, suprapubic pain, flank discomfort, or a feeling of incomplete bladder emptying. Samples showing mixed microbial growth, contamination, or yielding Gram-positive organisms were excluded. The final number of isolates (n=18) represented all consecutive Gram-negative bacterial isolates recovered during the study period. Because the study population consisted of all available cases, no formal sample-size calculation was required.

Bacterial Isolation and Preliminary Identification

Urine specimens were cultured immediately upon receipt using MacConkey agar (HiMedia Laboratories, India, Cat. No. M081, Lot No.212123). and incubated at 37°C for 24 hours. Lactose-fermenting and non-fermenting colonies characteristic of Gram-negative bacteria were selected for further analysis. From each primary culture, three to five well-isolated colonies were transferred to nutrient broth(Nutrient broth (HiMedia Laboratories, India; Cat.No. M002; Lot No. 165830).)and incubated overnight at 37°C to generate sufficient biomass for DNA extraction.

Genomic DNA Extraction

Following overnight incubation, 1.5 mL of each broth culture was transferred to an Eppendorf microcentrifuge tube and centrifuged at 12,000 rpm for five minutes by microcentrifuge (Eppendorf 5415C, Germany). The supernatant was discarded, and the pellet was resuspended in bacterial lysis buffer and treated with proteinase K using the Microspin system (Biosan 12, Latvia).Genomic DNA was then isolated using the Geneaid bacterial DNA mini kit (Geneaid Biotech Ltd. GBB100, Taiwan), following the manufacturer's protocol for Gram-negative organisms. DNA purity and concentration were assessed using a Bio-Rad spectrophotometer (Bio-Rad Laboratories, USA; Model SmartSpec Plus),and integrity was confirmed through electrophoresis on a 1% agarose gel Figure 1 stained with Red Safe dye, run at 5 V/cm in SB buffer. All extracted DNA samples were stored at –20°C until PCR amplification.

Figure 1. Electrophoresis of genomic DNA (1% agarose gel with Red Safe stain). Source: Own authorship.

RAPD-PCR Amplification

Random Amplified Polymorphic DNA analysis was performed using the primer OPA-06 which has the nucleotide sequence 5′-GGTCCCTGAC-3′ Table 1 was obtained from Macrogen (Korea). For each isolate, a total reaction volume of 25 µL was prepared Table 2, consisting of 12.5 µL of 2× Master Mix (TransGen Biotech, China), 1.5 µL of primer (10 pmol; Macrogen), 3 µL of genomic DNA, and 8 µL of nuclease-free water (Hemidya). Reaction tubes were gently vortexed and briefly centrifuged to collect all components at the bottom before being placed into the thermal cycler.

Table 1. Primer details and company.

Name	Sequence 5>3	Company
OPA-06	GGTCCCTGAC	Macrogen

Source: Own authorship.

Table 2. PCR reaction components and total volume.

Material	Volume / µl	Company
Master mix	12.5	TransGen
Primer	1.5	Macrogen
Free nuclease water	8	Hemidya
DNA	3	
Total	25	

Source: Own authorship.

Electrophoresis of PCR Products and Band Visualization

PCR products were analyzed on a 2% agarose gel prepared with SB buffer and stained with Red Safe dye. Electrophoresis was performed at 5 V/cm, and the gels were visualized under UV illumination using a gel documentation system. A molecular weight ladder ranging from 25 bp to 2000 bp was used as a reference to identify band sizes and compare amplification profiles among isolates. RAPD banding patterns were analyzed using GelJ software version 2.0. Bands were scored manually as present (1) or absent (0), and a binary matrix was generated for all isolates.

PCR Cycling Conditions

The protocol began with an initial denaturation at 95°C for 10 minutes, followed by 40 amplification cycles consisting of denaturation at 95°C for 30 seconds, primer annealing at 37°C for 45 seconds, and extension at 72°C for 30 seconds. A final extension step was carried out at 72°C for 7 minutes, after which the samples were held at 4°C until further processing Table 3.

Table 3. RAPD-PCR protocol for bacterial isolates

Step	Temp °C	Time	Cycles
First denaturation	95	10 min	1
denaturation	95	0.30 sec	
Annealing	37	0.45 sec	40
Extension	72	0.30 sec	
Final extension	72	7	1
Holding	4	∞	

°C: Celsius, min: minute, sec: second, ∞: infinity. Source: Own authorship.

Results

Identification of Clinical Isolates

All eighteen urine samples yielded bacterial growth when cultured on MacConkey agar, confirming the presence of Gram-negative bacteria and their suitability for subsequent molecular analysis.

PCR Amplification and RAPD Analysis

Electrophoretic analysis of RAPD-PCR products demonstrated successful amplification in sixteen of the eighteen analyzed isolates. No amplification was detected in isolates 1 and 18. RAPD-PCR banding patterns revealed three distinct genetic profiles, designated as patterns A, B, and C, as illustrated in Figure 2. Pattern A was observed in ten isolates (samples 2, 3, 5, 6, 9, 11, 13, 14, 15, and 16) and was characterized by a prominent band of approximately 175 bp. Pattern B was identified in two isolates (samples 4 and 10), each displaying a band of approximately 150 bp. Pattern C was detected in two isolates (samples 7 and 12) and exhibited a distinct band of approximately 250 bp.

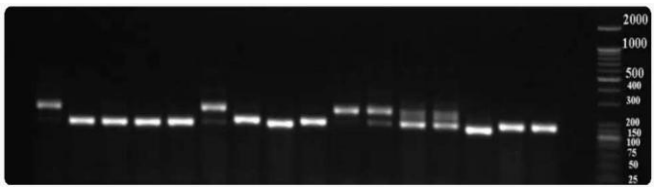

Figure 2: Electrophoresis of RAPD-PCR results (2% agarose gel with Red Safe stain). Source: Own authorship.

Discussion

The present study demonstrates that RAPD-PCR was able to reveal detectable genetic variability among Gram-negative bacterial isolates obtained from patients with urinary tract infections at Samarra Hospital. The identification of three distinct RAPD banding patterns indicates that genetically heterogeneous profiles may coexist within a single clinical setting, even when isolates originate from a similar type of infection. This observation is consistent with previous reports describing genetic diversity among Gram-negative clinical isolates using RAPD-PCR, particularly in hospital-based studies conducted in resource-limited environments. As a PCR-based fingerprinting method, RAPD-PCR relies on random primer annealing across the bacterial genome, allowing the detection of polymorphic regions without prior sequence knowledge. In this context, the technique functions as an exploratory molecular tool that provides an initial overview of genetic diversity rather than a definitive assessment of genetic relatedness or evolutionary distance **[1,7]**.

Several studies have reported similar applications of RAPD-PCR for assessing genetic variation among Gram-negative bacteria isolated from clinical sources. For example, investigations focusing on *Pseudomonas aeruginosa* have shown that RAPD-PCR can generate distinct banding profiles among isolates that are phenotypically similar, highlighting underlying genomic variation that may not be apparent through routine microbiological methods. However, such studies have also emphasized that RAPD-derived patterns should be interpreted cautiously, as they do not establish precise phylogenetic relationships or confirm strain identity. These limitations are well recognized in the broader field of molecular epidemiology, where RAPD-PCR is generally considered a screening-level genotyping approach rather than a high-resolution typing method suitable for outbreak investigation or transmission analysis **[2,6,7]**.

The detection of a predominant RAPD pattern alongside less frequent patterns in the present study suggests the presence of multiple genetic lineages within the sampled bacterial population. While the predominance of a specific pattern may reflect the circulation of certain genetic profiles within the local clinical environment, the present study did not assess bacterial fitness, virulence factors, antimicrobial resistance, or patient-related variables that could explain such distribution patterns. Therefore, no assumptions can be made regarding the biological behavior, pathogenic potential, or clinical relevance of individual RAPD profiles. These findings should be viewed strictly as descriptive representations of genetic diversity revealed by RAPD-PCR analysis, in line with methodological evaluations reported in previous molecular typing studies **[2,6]**.

It is also important to consider that variations in RAPD banding patterns observed across different studies and geographic regions may be influenced by multiple contextual factors, including local antimicrobial usage practices and differences in healthcare settings. Prior investigations have suggested that selective pressures within hospital environments can shape the distribution of bacterial genotypes over time. However, the present study was not designed to evaluate such associations, and no clinical or antimicrobial exposure data were collected. As a result, comparisons with findings from other regions should be interpreted as contextual observations rather than direct epidemiological correlations **[1,5,8]**.

Several limitations of this study should be acknowledged. The relatively small sample size and the single-center design may limit the generalizability of the findings to other settings. In addition, RAPD-PCR is a dominant marker technique with known limitations related to reproducibility and discriminatory resolution, particularly when compared with advanced molecular typing approaches such as multilocus sequence typing or whole-genome sequencing. The use of a single RAPD primer further restricts the depth of genetic characterization achievable in the present analysis.

These methodological constraints have been widely discussed in the literature and underscore the need for cautious interpretation of RAPD-PCR results, especially when used as the sole genotyping method **[2,6,10]**.

Despite these limitations, the present study provides baseline data on the genetic heterogeneity of Gram-negative uropathogens in Samarra, Iraq, and contributes to the limited regional literature on molecular typing of urinary tract infection pathogens. In settings where access to high-resolution genomic technologies is limited, RAPD-PCR may serve as a practical and cost-effective approach for preliminary assessment of genetic diversity and exploratory epidemiological surveillance. Future studies involving larger, multicenter cohorts and the integration of complementary molecular methods, together with relevant phenotypic and clinical data, are required to further clarify the genetic structure and potential clinical implications of Gram-negative uropathogenic populations **[1,6,11]**.

Limitations

This study was conducted in a single center with a limited sample size and used only RAPD-PCR for typing. These factors may limit the generalizability and resolution of the findings compared to multi-centre studies using methods like MLST or PFGE.

Conclusion

This study demonstrates the presence of detectable genetic heterogeneity among Gram-negative bacterial isolates recovered from patients with urinary tract infections at Samarra Hospital, as revealed by RAPD-PCR analysis. The identification of three distinct RAPD banding patterns indicates that genetically diverse strains may coexist within a single clinical setting, even within the same bacterial group. Although RAPD-PCR is a screening-level molecular typing method with recognized limitations in resolution and reproducibility, its application in this study provided baseline information on the genetic diversity of uropathogenic Gram-negative bacteria in a resource-limited context. These findings support the use of RAPD-PCR as an exploratory tool for preliminary molecular epidemiological assessment, while underscoring the need for future investigations that incorporate larger, multicenter cohorts and higher-resolution molecular approaches, integrated with relevant phenotypic data, to better clarify the clinical and epidemiological significance of genetic diversity among uropathogens.

Recommendations

Expand the scope of the study to include a larger number of samples from different hospitals and geographical regions to enhance the robustness and generalizability of the findings. Integrate RAPD-PCR results with antimicrobial susceptibility testing to correlate genetic patterns with resistance profiles, aiding in the development of more targeted therapeutic strategies. Utilize advanced molecular techniques such as Multilocus Sequence Typing (MLST) or Whole Genome Sequencing (WGS) to confirm RAPD results and achieve high-resolution genetic characterization. Strengthen epidemiological surveillance programs in hospitals to monitor the spread of highly virulent and resistant bacterial strains. Promote the rational use of antibiotics to limit the emergence and spread of multidrug-resistant pathogens.

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Author contributions

Noor Khalid Ismael: Sample collection; Wahran khudhair abbas: Manuscript drafting; Heba Tallah Mahfoodh khaleel: Laboratory work; Ahmed Suhail Hussein: Study design and proofreading; Duaa Ahmed Hassan Al-Kailani: Data analysis; Zeyad Taha Hussein**:** Sample collection and Laboratory work; Younis Waleed Younis: Final approval.

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Ethical Approval

All ethical guidelines and standards of scientific research were fully followed throughout this study, and was conducted in accordance with the Declaration of Helsinki, as revised in 2024.

Informed Consent

It was applicable.

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Data Sharing Statement

The datasets generated and analysed during the

current study are not publicly available because of confidentiality of patient information, but are available from the corresponding author on reasonable request, subject to institutional approval and compliance with applicable data-protection regulations.

Conflict of Interest

The authors declare no competing interests.

Similarity Check

It was applied by Ithenticate®.

Application of Artificial Intelligence (AI)

Not applicable.

Peer Review Process

It was performed.

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