RESEARCH ARTICLE

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# Whole-Genome Analysis of Multidrug-Resistant *Escherichia coli* from Bloodstream Infections in Iraqi Cancer Patients

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## Abstract

**Background:** In Iraq, oncology patients with bloodstream infections face escalating treatment challenges due to rising antimicrobial resistance in *Escherichia coli*, compounded by limited diagnostic capacity and restricted therapeutic options. However, data from oncology settings in Iraq are limited. This study aimed to characterize and compare the antimicrobial resistance gene profiles of multidrug-resistant and antibiotic-sensitive *E. coli* isolates from cancer patients, to inform infection control and stewardship strategies. **Methods:** A prospective, multicenter investigation was conducted in three oncology hospitals in Baghdad. Fifty-five *Escherichia coli* bloodstream isolates, 36 multidrug-resistant (MDR) (65.5%) and 19 antibiotic-sensitive (34.5%), underwent phenotypic susceptibility testing using VITEK® 2 and disk diffusion. Whole-genome sequencing (Illumina MiSeq) was performed, and antimicrobial resistance genes (ARGs) were identified using AMRFinderPlus and classified by functional category. **Results:** MDR isolates showed a broad resistome dominated by efflux systems. Across all isolates, a total of 36 unique antimicrobial resistance genes were identified, underscoring substantial resistome diversity., efflux pump genes were detected in 100%, β-lactamase genes in 88.9%, macrolide resistance genes in 66.7%, tetracycline resistance genes in 55.6%, and aminoglycoside resistance genes in 41.7%. Representative determinants included AcrAB-TolC/EmrAB-TolC/MdtABC-TolC (efflux) and BlaEC family/CMY-42/TEM types (β-lactams). Among sensitive isolates, only 11 antibiotic-associated genes, mainly efflux or regulators (e.g., acrF, emrD, emrR, emrY, tolC; regulators marR, evgA; target parC; others baeS, cpxA, cysB), overlapped with MDR, suggesting a shared core that is insufficient alone for phenotypic resistance without additional high-level mechanisms. **Conclusions:** Multidrug-resistant *E. coli* from oncology patients harbor dense, efflux-driven resistomes supplemented by diverse β-lactamases and other resistance determinants, while sensitive isolates retain a limited core set of genes. Genomic surveillance in cancer centers is critical for anticipating resistance emergence and informing targeted antimicrobial strategies.

**Keywords:** *Escherichia coli*- whole-genome sequencing- antimicrobial resistance- cancer patients

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## Introduction

Antibiotic resistance poses a critical and escalating challenge to modern medicine, with profound implications for the management of infectious diseases in immunocompromised populations. Among the most vulnerable are cancer patients, who frequently develop infections as a consequence of immunosuppression induced by chemotherapy, radiotherapy, and disease progression. Globally, infections remain a leading cause of morbidity and mortality in oncology care, with bloodstream infections representing one of the most life-threatening complications in this group [1, 2]. The emergence and spread of multidrug-resistant (MDR) pathogens in oncology settings have further complicated treatment outcomes, often limiting therapeutic options, prolonging hospital stays, and increasing healthcare costs. Alarmingly, bloodstream infections in cancer patients are increasingly attributed to antibiotic-resistant bacteria, undermining the efficacy of standard antimicrobial therapy and threatening patient survival rates [1, 2]. In Iraqi oncology hospitals, these challenges are further

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magnified by delayed access to effective antimicrobials, limited availability of last-line agents, and high early mortality rates associated with bloodstream infections in immunocompromised patients [3].

The situation in Iraq and other low- and middle-income countries (LMICs) is exacerbated by a combination of structural and systemic challenges, including limited microbiological diagnostic capacity, the unregulated availability of antibiotics, over-the-counter antimicrobial sales without prescription, and inadequate implementation of antimicrobial stewardship policies [3]. These factors contribute to both the overuse and misuse of antibiotics, accelerating the development and dissemination of resistance. Gram-negative organisms such as *Escherichia coli* have emerged as major pathogens in oncologic infections in these regions, with resistance increasingly observed against key therapeutic agents, including third-generation cephalosporins, carbapenems, and fluoroquinolones [4, 5]. The clinical consequences of such resistance patterns are particularly severe in oncology care, where delays in effective treatment can rapidly lead to septic shock and death.

Whole-genome sequencing (WGS) has transformed the field of antimicrobial resistance (AMR) research by providing high-resolution insights into the genetic architecture of resistance. Beyond phenotypic susceptibility profiles, WGS enables comprehensive mapping of resistance determinants, including specific genes, plasmids, integrons, and point mutations that contribute to antimicrobial resistance. This technology allows for the detection of both well-characterized and novel resistance mechanisms, facilitates tracking of clonal lineages, and supports epidemiological surveillance at local, regional, and global levels [6, 7]. Compared to traditional susceptibility testing methods, WGS offers a more detailed understanding of resistance evolution, enabling the identification of potential transmission pathways and informing targeted infection control interventions.

While previous studies have explored resistance gene profiles in *E. coli* from environmental or animal sources [8], there remains a significant paucity of data on clinical isolates obtained from human cancer patients in the Middle East, particularly in Iraq. Given the rising incidence of AMR in hospital settings and the high mortality associated with bloodstream infections in oncology care, such data are urgently needed. Insights into the molecular epidemiology of *E. coli* in this patient population are essential not only for optimizing empiric antimicrobial therapy but also for informing the development of evidence-based AMR surveillance frameworks and infection prevention strategies tailored to oncology care in resource-limited settings. While earlier investigations from Egypt and the Kurdistan region primarily reported phenotypic resistance patterns or focused on limited genomic markers, none have performed a full whole-genome characterization of *E. coli* bloodstream isolates specifically in oncology patients [3, 5]. Furthermore, no prior work in the region has directly compared the resistomes of MDR and antibiotic-sensitive isolates to elucidate the presence of shared core genes versus expanded resistance determinants [2]. This study therefore fills a critical gap by providing the first comprehensive genomic analysis of *E. coli* bloodstream infections in Iraqi cancer patients. The present study aims to address this gap by conducting a whole-genome analysis of *E. coli* isolates obtained from bloodstream infections in Iraqi cancer patients. We compared the genetic characteristics of antibiotic-resistant and antibiotic-sensitive strains, with a specific focus on identifying key resistance genes, efflux pump systems, and integrons. By integrating genomic and phenotypic data, our findings provide a foundation for understanding the molecular epidemiology of AMR in cancer patients in Iraq and offer actionable insights for strengthening antimicrobial stewardship and improving patient outcomes in oncology settings.

## Materials and Methods

*Study design and setting*

This was a prospective, observational, multicenter study conducted at three major cancer treatment centers in Baghdad, Iraq, between January 1st, 2024, and March 1st, 2025. A prospective approach was chosen to ensure standardized sample collection, reduce recall bias, and allow for precise follow-up of antimicrobial susceptibility and genetic characteristics. The participating hospitals follow the same microbiological protocols based on the Clinical and Laboratory Standards Institute (CLSI) 2022 guidelines, ensuring methodological consistency across sites [9].

*Study population and inclusion rationale*

The study focused on adult Iraqi patients diagnosed with cancer and receiving chemotherapy who developed bloodstream infections due to bacterial pathogens. Although various Gram-negative organisms were isolated, including Klebsiella pneumoniae and Pseudomonas aeruginosa, the analysis in this paper is limited to *Escherichia coli*, due to its high frequency, clinical significance, and genomic relevance in antimicrobial resistance research.

*E. coli* was selected because it is a leading cause of bloodstream infections (BSIs) in immunocompromised populations, particularly cancer patients, and is a globally recognized contributor to multidrug resistance [10]. In addition, *E. coli* is frequently isolated in hospital microbiology laboratories and serves as a model organism for resistance studies owing to its genomic plasticity and the availability of curated resistance gene databases. Understanding resistance mechanisms in this organism has direct clinical relevance and may improve targeted antimicrobial therapy and stewardship in oncology care [11]. From January 2024 to March 2025, a total of 125 bloodstream bacterial isolates were collected across three oncology hospitals in Baghdad. Of these, 100 isolates were phenotypically classified as multidrug-resistant (MDR) and 25 as antibiotic-sensitive using CLSI 2022 criteria. Among these, 55 isolates were identified as *E. coli* and included for genomic and phenotypic analysis, comprising 36 MDR *E. coli* isolates and 19 sensitive *E. coli* isolates. Other species were excluded from analysis to maintain a

focused, comparative investigation of resistance patterns in E. coli.

Eligible participants were adult Iraqi nationals aged 18 years or older with a histologically confirmed diagnosis of either solid tumors or hematological malignancies. All patients were undergoing chemotherapy at the time of bloodstream infection, as confirmed by positive blood cultures. Patients receiving radiotherapy were excluded to minimize confounding effects on mucosal immunity. Those with co-existing viral or fungal infections, chronic inflammatory diseases (e.g., asthma, COPD), or immunosuppressive therapy unrelated to chemotherapy were also excluded. Only patients with monomicrobial *E. coli* infections who provided informed consent were included in the final analysis.

*Sample size and sampling method*

The sample size was calculated based on the study's comparative objective, to detect differences in resistance gene prevalence between multidrug-resistant (MDR) and antibiotic-sensitive *E. coli* isolates in cancer patients. Using Cohen's formula for comparing two independent proportions [12], and referencing prior genomic surveillance studies that report 60–80% of MDR isolates [13-15] and 20–30% of sensitive isolates carry key resistance genes (e.g., ampC, aadA6, AcrAB-TolC) [16], the estimated required sample size ranged from 18 to 72 isolates per group for 95% confidence and 95% power. To accommodate subgroup analyses, sequencing quality control, and possible data loss, we aimed for a total of 125 isolates, with 100 from MDR *E. coli* bloodstream infections and 25 from antibiotic-sensitive cases, which reflects the real-world distribution and supports the study's primary genomic and clinical objectives

*Antimicrobial susceptibility testing (AST)*

Bacterial isolates were identified and tested for susceptibility using both the VITEK® 2 compact system and the disc diffusion method, following CLSI 2022 guidelines [9]. Susceptibility testing was performed against commonly used antibiotics including cefepime, imipenem, piperacillin/tazobactam, ceftazidime, levofloxacin, amikacin, and others. Isolates were classified as multidrug-resistant (MDR) if resistant to at least one agent in three or more antimicrobial categories [17].

*Whole genome sequencing (WGS) and data processing*

DNA was extracted using the QIAamp DNA Mini Kit. Sequencing libraries were prepared with the Nextera XT DNA Library Preparation Kit, and paired-end sequencing (2 × 300 bp) was carried out on the Illumina MiSeq platform. Whole-genome sequencing was chosen over PCR-based methods to allow comprehensive detection of both known and novel antimicrobial resistance genes (ARGs), plasmids, efflux systems, and chromosomal mutations. Raw reads were quality-checked using FastQC, and low-quality reads were trimmed using PRINSEQ-lite. Adapter sequences were removed using Cutadapt. De novo assembly was performed using the CLC Genomics Workbench version 22 with a minimum contig size threshold of 1,000 base pairs [17].

*Identification of resistance genes*

Annotated assemblies were screened for antimicrobial resistance genes using AMRFinderPlus, a high-throughput tool developed by the NCBI that identifies acquired resistance genes, stress response genes, virulence factors, and point mutations associated with resistance [18]. The analysis was restricted to hits with high identity and coverage values, using the 2022 reference gene catalog. Resistance genes were classified by antibiotic class and mechanism, including efflux systems, enzymatic inactivation, and target modification.

*Clinical data and classification*

Demographic and clinical data were extracted from hospital records, including patient age, sex, cancer type, chemotherapy regimen, and prior antibiotic exposure. Antibiotics were categorized according to the World Health Organization (WHO) AWaRe classification as "Access," "Watch," or "Reserve" [19].

*Data Analysis*

Statistical analyses were performed using IBM SPSS Statistics version 26. The analysis included 55 patients with *Escherichia coli* bloodstream infections, comprising 36 multidrug-resistant (MDR) and 19 antibiotic-sensitive isolates. Descriptive statistics (frequencies and percentages) were used to summarize clinical characteristics, cancer types, antimicrobial susceptibility profiles, and the distribution of resistance genes. Resistance determinants were categorized according to functional classes, such as β-lactamases, efflux pumps, target site modifications, and aminoglycoside resistance mechanisms.

## Results

*Baseline Characteristics of Participants with E. coli Bloodstream Infections*

Among the 55 E. coli-positive bloodstream infection cases, 36 isolates (65.5%) were classified as multidrug-resistant (MDR) and 19 (34.5%) were antibiotic-sensitive (Table 1) . MDR *E. coli* was more frequently isolated from hematological cancer patients (66.7%) than from those with solid tumors (33.3%), with acute myeloid leukemia (AML, 27.8%) and acute lymphoblastic leukemia (ALL, 16.7%) being the most common subtypes. In solid tumors, breast cancer accounted for the highest proportion of MDR cases (19.4%). Among sensitive *E. coli* isolates, 68.4% were from hematological cancer patients and 31.6% from solid tumor patients, with the majority exhibiting partial rather than full sensitivity to the tested antibiotics.

*Whole-genome sequencing findings*

Whole-genome sequencing of the 36 multidrug-resistant (*E. coli*) isolates revealed a wide range of antimicrobial resistance (AMR) determinants across multiple functional categories (Table 2). Efflux pump genes were detected in 100% of isolates, β-lactamase genes in 88.9%, macrolide resistance genes in 66.7%, tetracycline resistance genes in 55.6%, and aminoglycoside resistance genes in 41.7%. The MDR resistome was

Table 1. Baseline Characteristics of Participants with *Escherichia coli*-Positive Bloodstream Infections, Stratified by Resistance Status

| Resistance Status | Total *E. coli* Isolates (n) | Hematological Cancer n (%) | Solid Cancer n (%) | Main Hematological Subtypes n (%) | Main Solid Cancer Subtypes n (%) |
|---|---|---|---|---|---|
| MDR (Resistant) | 36 | 24 (66.7%) | 12 (33.3%) | AML: 10 (27.8%)<br>ALL: 6 (16.7%)<br>NHL: 4 (11.1%)<br>MDS: 2 (5.6%)<br>CML: 1 (2.8%)<br>Aplastic anemia: 1 (2.8%) | Breast: 7 (19.4%)<br>Pancreatic: 3 (8.3%)<br>Thyroid: 2 (5.6%) |
| Sensitive | 19 | 13 (68.4%) | 6 (31.6%) | 4 fully sensitive, 9 partially sensitive | 5 fully sensitive, 1 partially sensitive |

AML, acute myeloid leukemia; ALL, acute lymphoblastic leukemia; NHL, non-Hodgkin lymphoma; MDS, myelodysplastic syndrome; CML, chronic myeloid leukemia.

Table 2. Frequency of Antimicrobial Resistance Genes (ARGs) Detected in Multidrug-Resistant *Escherichia coli* Isolates by Whole-Genome Sequencing (N = 36)

| Functional Category | Resistance Genes |
|---|---|
| Efflux Pumps | acrA, AcrAB-TolC, acrB, MexAB-OprM, acrD, acrE, AcrEF-TolC, AcrEF-TolC 1, acrR, acrS, AcrZ, emrA, EmrAB-TolC, EmrAB-TolC 1, EmrAB-TolC 2, emrB, EmrD 1, EmrD 2, EmrD 3, EmrE, emrE1, emrK, EmrKY-TolC, EmrKY-TolC 1, MacA, MacB, mdfA, MdfA/Cmr, mdtA, MdtABC-TolC, MdtABC-TolC 1, MdtABC-TolC 2, mdtB, mdtC, mdtD, mdtE, MdtEF-TolC, MdtEF-TolC 1, mdtF, mdtG, mdtH, mdtK, MdtL, mdtL 1, MdtM, mdtM 1, mdtN, mdtO, mdtP, TolC/OpmH, YojI |
| Beta-lactam Resistance | BlaEC family, CMY-42, TEM family, TEM-1 |
| Macrolide Resistance | mphA, Erm(B), ErmB |
| Tetracycline Resistance | tetA |
| Aminoglycoside Resistance | APH(3")-Ib, APH(6)-Id |
| Regulatory Proteins | adiY, alaS, arnA, baeR, cpxR, CRP, gadE, GadE 1, gadW, gadX, kdpE, leuO, MarA, marA 1, MarB, MarR 1, mfd, mgrB, patA, PhoP, soxR, soxS |
| Target Site Modifications | EF-G, EF-Tu, EF-Tu 1, EF-Tu 2, gyrA, gyrB, H-NS, parE, PmrB, PmrC, PmrF, rpoB |
| Other Resistance Mechanisms | bacA, GlpT, msbA, murA, nfsA, ompF, SugE, SugE 1, UhpT, QAC |

dominated by multidrug efflux systems (e.g., AcrAB-TolC, EmrAB-TolC, MdtABC-TolC), accompanied by β-lactamases (BlaEC family, CMY-42, TEM types), macrolide resistance determinants (mphA, ErmB), and other mechanisms including target site modification and membrane permeability changes.

Moreover, whole-genome sequencing revealed 11 antibiotic-associated genes that were present in both MDR and antibiotic-sensitive *E. coli* isolates (Table 3). Efflux pump–related genes formed the largest category, including acrF, emrD, emrR, emrY, and tolC, all of which are known to facilitate multidrug efflux. Regulatory proteins (baeS, cpxA, evgA, marR) and the target site modification gene parC were also identified, along with the metabolic regulator cysB. The detection of these genes in sensitive isolates suggests the presence of a shared core resistome, which may serve as a reservoir for the development of resistance under selective antimicrobial pressure.

## Discussion

This study provides a genomic snapshot of *Escherichia coli* bloodstream isolates in cancer patients, highlighting a resistome dominated by TolC-dependent multidrug efflux systems and β-lactamase determinants in MDR strains, with a small "core" set of antibiotic-associated genes also present in sensitive isolates. The centrality of efflux-mediated resistance in *E. coli* is consistent with

Table 3. Genes Detected in Both Multidrug-Resistant (MDR) and Antibiotic-Sensitive *Escherichia coli* Isolates by Whole-Genome Sequencing

| Functional Category | Resistance Genes Present in Both MDR and Sensitive Isolates |
|---|---|
| Efflux Pumps | acrF, emrD, emrR, emrY, tolC |
| Regulatory Proteins | baeS, cpxA, evgA, marR |
| Target Site Modifications | parC |
| Other Resistance Mechanisms | cysB |

prior work: AcrAB-TolC and related pumps are repeatedly implicated in broad drug extrusion and stress survival, and can be upregulated through mar/sox regulons or local repressors [20, 21]. Structural and functional studies further show that interactions within the RND complexes (e.g., AcrB with the small protein AcrZ) modulate substrate specificity, offering a mechanistic basis for the wide phenotype we observed [22]. Molecular dynamics and experimental data likewise support direct AcrAB-TolC–antibiotic interactions and their contribution to non-susceptibility patterns [23].

Beyond AcrAB-TolC, additional TolC-dependent assemblies (EmrAB-TolC, EmrKY-TolC, MdtABC-TolC, MdtEF-TolC, MacAB-TolC) can substitute or augment efflux capacity, particularly when overproduced or when AcrAB-TolC is impaired [21]. For example, EmrAB-TolC has been linked to resistance to quinolones and uncouplers [24, 25], while EmrD (MFS) contributes to tolerance to multiple agents and is controlled by two-component signaling (BasSR), tying envelope stress to efflux expression [26]. The CpxRA system can also lift the MDR cascade; CpxR is a known activator of efflux-related responses, while cpxA mutations (or gentamicin stress) rewire the envelope stress network toward increased tolerance [27-29]. Together, these pathways help explain why our MDR isolates carried dense clusters of efflux and regulator genes.

Enzymatic inactivation remains a parallel pillar of resistance in our cohort. Class C β-lactamases of the BlaEC family and TEM-type enzymes are established drivers of β-lactam non-susceptibility, with documented clinical prevalence and limited inhibition by classic β-lactamase blockers [30, 31]. These findings parallel genomic reports from other Middle Eastern settings, including Kuwait and Saudi Arabia, where MDR *E. coli* similarly demonstrated dense efflux-dominated resistomes and high prevalence of TEM and CMY-type β-lactamases. Studies from Iran further report comparable enrichment of aminoglycoside-modifying enzymes and macrolide resistance determinants. Taken together, the resistome architecture identified in this cohort is consistent with broader regional trends, although the clinical impact may be more pronounced in oncology patients due to their immunocompromised status [15, 18]. Co-carriage of disinfectant/biocide resistance loci (e.g., QAC-related) alongside ESBL determinants has been reported in patient isolates and may facilitate co-selection in hospital environments, relevant to oncology wards with heavy antiseptic use [32].

Moreover, this study also included tetracycline and aminoglycoside resistance genes typical of clinical and veterinary E. coli, consistent with the durability of these determinants under diverse selective pressures [33]. For fluoroquinolones, resistance hinges on QRDR alterations in gyrA/parC; mechanistic reviews and mutation surveys align with our finding that target-site genes are detectable across isolates, but phenotypic resistance depends on specific mutations and efflux coupling [34].

Notably, we detected a limited "shared core" among sensitive isolates 11 genes largely tied to efflux (e.g., acrF, emrD, emrR, emrY, tolC), regulation (marR, evgA), and a target enzyme (parC). Prior work shows that basal presence of efflux/regulatory loci does not guarantee resistance; expression control, activator networks (MarA/SoxS/Rob), and assembly with outer-membrane channels are decisive [21, 35]. For instance, TolC is constitutively open but requires inner-membrane partners (AcrB/AcrA) and proper gating; inactivation of tolC sensitizes cells, while pump opening depends on concerted conformational changes [36]. Likewise, EvgS/EvgA contributes to multidrug tolerance in E. coli, yet incomplete pathway components (e.g., evgA without evgS) may blunt functional impact—mirroring why sensitive isolates can harbor "resistance-related" genes without expressing an MDR phenotype [37].

Taken together, our data reinforce a model where MDR in oncology-associated *E. coli* emerges from the convergence of high-capacity efflux (TolC-dependent systems), β-lactamase enzymes, and (where present) target-site mutations, while sensitive isolates may retain a latent scaffold of efflux/regulatory genes that require activation, upregulation, or cassette acquisition to manifest as clinical resistance.The resistome profiles identified in this study have direct implications for antimicrobial stewardship in Iraqi oncology hospitals. Routine incorporation of genomic data could enable earlier detection of emerging β-lactamase variants, clustering of efflux-mediated MDR phenotypes, and real-time tracking of high-risk clones. Integrating genomic surveillance with existing stewardship frameworks may help refine empirical therapy, reduce ineffective antibiotic exposure, and improve treatment outcomes in immunocompromised patients.

*Strengths and Limitations*

This study offers a comprehensive genomic analysis of both MDR and antibiotic-sensitive *E. coli* isolates from cancer patients, enabling direct comparison of resistance determinants across distinct phenotypic groups. By focusing on oncology patients, the work addresses a high-risk population where antimicrobial resistance has serious therapeutic implications. The simultaneous evaluation of multiple resistance mechanisms, including efflux pumps, β-lactamases, aminoglycoside-modifying enzymes, macrolide resistance determinants, and integrons, ensures broad coverage and provides a valuable reference point for future surveillance and stewardship efforts.

Several considerations should be noted when interpreting the findings. Phenotypic susceptibility testing was performed using standard culture-based methods; while these are clinically validated, incorporating minimum inhibitory concentration testing in future studies could provide additional resolution. Gene detection was based on genomic presence, which is highly informative for mapping resistance potential, but does not account for expression levels; integrating transcriptomic analysis in subsequent research would further clarify functional activity. These considerations do not diminish the robustness of the present findings but highlight opportunities for further work.

In conclusion, this study highlights the extensive repertoire of resistance determinants in *E. coli* isolates

from cancer patients, with MDR strains harboring diverse efflux pump, β-lactamase, aminoglycoside, and macrolide resistance genes, as well as integrons that facilitate horizontal gene transfer. The detection of resistance-related genes in phenotypically sensitive isolates underscores the potential for rapid phenotypic shifts under selective pressure, reinforcing the need for molecular surveillance alongside conventional antimicrobial susceptibility testing. Tailoring antimicrobial stewardship strategies to include genomic screening could enable earlier identification of emerging threats, optimize empirical therapy, and mitigate the spread of MDR *E. coli* in oncology settings.

## Author Contribution Statement

WMJ, BARH, and ABAM conceptualized the study. WMJ, MFO, AFAA, MKAQ, and MKA performed the laboratory work, data collection, and genomic analysis. WMJ, MFO, and AFAA conducted data curation and formal analysis. BARH and ABAM supervised the project and validated methodology. All authors contributed to manuscript drafting, critical revisions, and final approval of the submitted version.

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*Conflict of Interest*

The authors declare that they have no conflicts of interest regarding the conduct or publication of this research.

*Ethical Approval and Handling of Ethical Issues*

All procedures performed in this study were conducted in accordance with the ethical standards of the Iraq Ministry of Health, Center of Training & Human Development Committee and with the 1964 Helsinki Declaration and its later amendments.

*The ethical approval number for this study is 46119/41247*

Informed consent was obtained from all participating individuals prior to sample collection, and only anonymized, de-identified data were used in the analysis.

*Availability of Data and Materials*

The genomic datasets generated and analyzed during the current study are available from the corresponding author upon reasonable request and in accordance with institutional and ethical regulations.

*Study Registration*

This study was not registered in any clinical trial or research registry, as it involved genomic characterization of bacterial isolates rather than interventional or observational human clinical experimentation.

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