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Isolation and Identification of *Candida* and Bacterial Species from the Blood of Immunocompromised Patients in Kirkuk, Iraq

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Abstract

Bloodstream infections (BSIs) are a major cause of morbidity and mortality among immunocompromised patients, yet local data from Iraq combining hemodialysis and oncology populations remain scarce. This study aimed to isolate and identify *Candida* and bacterial species from the blood of immunocompromised patients in Kirkuk, Iraq, and to characterize their antimicrobial susceptibility profiles. A total of 350 blood samples were collected between November 2024 and December 2025 from 160 hemodialysis patients, 170 oncology patients, and 20 healthy controls, cultured in Brain Heart Infusion Broth, and subjected to phenotypic, biochemical, VITEK 2, and molecular (ITS/16S rRNA) identification. Of 330 patient samples, 200 (60.6%) yielded positive growth, comprising 124 bacterial (62.0%) and 76 fungal (38.0%) isolates. Gram-negative bacteria predominated (87.10%), led by *Escherichia coli* (26.85%), with an unusually high combined proportion of *Proteus mirabilis* and *Morganella morganii* (26.64%); *Staphylococcus aureus* was the leading Gram-positive isolate (10.48%). Among *Candida* isolates, *C. albicans* was most prevalent (39.47%), followed by an unusually elevated proportion of the intrinsically fluconazole-resistant *C. krusei* (38.16%). Imipenem (84.7% susceptibility) and amphotericin B/micafungin (85.5% each) were the most effective antibacterial and antifungal agents, respectively. Molecular confirmation by Sanger sequencing validated species identity, with representative isolates deposited in GenBank. These findings reveal a distinctive local microbial and resistance profile among immunocompromised patients in Kirkuk and underscore the need for continued epidemiological surveillance to guide empirical therapy.

Keywords: Bloodstream infection; Candida; immunocompromised; hemodialysis; antimicrobial resistance; Kirkuk

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1. Introduction

Bloodstream infections (BSIs) remain one of the most serious complications encountered in hospitalized patients, associated with substantial morbidity, mortality, and healthcare costs (1). BSI is defined, in its broadest sense, as the presence of viable microorganisms in the bloodstream, and is distinguished from the narrower term bacteremia, which refers strictly to bacterial isolates, and from fungemia, which denotes viable fungi—most commonly *Candida* species—circulating in the blood (2, 3). Clinically, BSI is confirmed by a positive blood culture accompanied by signs and symptoms of systemic infection (2).

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The pathogenesis of BSI follows three sequential stages common to most pathogens: colonization of a primary site using diverse adhesion factors; dissemination into the bloodstream through toxin-mediated disruption of epithelial and endothelial barriers; and finally, survival against host clearance mechanisms mediated by the liver and spleen (4, 5). Pathogens that evade these defenses trigger a cytokine-driven inflammatory cascade (IL-6, IL-8, TNF-α) that can progress to septic shock and multi-organ failure (2, 6).

This risk is markedly amplified in immunocompromised patients, a growing population rendered vulnerable by malignancy and its chemotherapeutic treatment, chronic renal failure requiring hemodialysis, organ transplantation, or other causes of secondary immunosuppression (7). Chemotherapy-induced neutropenia and hemodialysis-related vascular access both create distinct but converging pathways to bloodstream invasion, and 30-day mortality among immunocompromised patients with sepsis or septic shock in intensive care settings has been reported as high as 48.8% (8), with BSI documented in up to 40.3% of such patients (7).

The microbial spectrum responsible for BSI in this vulnerable population is broad and clinically significant. On the fungal side, candidemia represents the most common form of hospital-acquired invasive fungal infection, contributing to over 1.5 million deaths annually worldwide (9), with a well-documented epidemiological shift toward non-albicans *Candida* (NAC) species that frequently display intrinsic or acquired resistance to available antifungals (10). On the bacterial side, immunocompromised patients face escalating threats from multidrug-resistant organisms, particularly the ESKAPE group (*E. faecium*, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, and *Enterobacter* species), which account for a substantial proportion of hospital-acquired infections (11). This burden is compounded by the global crisis of antimicrobial resistance (AMR), estimated to cause approximately 1.27 million deaths directly and to be associated with nearly 5 million deaths worldwide, with projections of up to 10 million annual deaths by 2050 in the absence of decisive intervention (12, 13).

Despite the well-recognized vulnerability of hemodialysis and oncology patients to opportunistic bloodstream pathogens, local epidemiological data from Iraq remain limited, and few studies have concurrently characterized both the bacterial and fungal spectra of BSI within a single immunocompromised cohort combining these two distinct patient groups. Such combined surveillance is essential, as the divergent immunosuppressive mechanisms in each group—chronic cumulative immune dysfunction in hemodialysis patients versus acute chemotherapy-induced neutropenia in oncology patients—may shape distinct pathogen profiles requiring tailored empirical management. This study therefore aimed to isolate and identify *Candida* and bacterial species from the blood of immunocompromised patients attending the Oncology Hospital and Al-Amal Hemodialysis Center in Kirkuk, Iraq, confirming their identity through morphological, biochemical, and molecular approaches.

2. Materials and Methods

2.1. Ethical Approval and Study Design

Ethical approval for this study was obtained from the Research Ethics Committee, College of Science, University of Kirkuk. Written informed consent was obtained from all patients and healthy participants, or their legal guardians, prior to sample collection. All procedures were conducted in accordance with the ethical principles governing medical research involving human subjects, and participant confidentiality was maintained throughout.

2.2. Study Population and Sample Collection

A total of 350 blood samples were collected between November 2024 and December 2025 from

participants aged 10–70 years attending two specialized centers in Kirkuk Governorate, Iraq: the Oncology Hospital and Al-Amal Hemodialysis Center. Participants comprised 20 healthy controls, 160 hemodialysis patients, and 170 oncology patients. A single 5 mL blood sample was drawn from each participant using a sterile disposable syringe, inoculated directly into Brain Heart Infusion Broth (BHIB), and incubated at 37 °C for up to three weeks with daily monitoring for turbidity and gas production (14). Positive broths were subcultured onto selective/differential solid media for isolation and identification (14).

2.3. Isolation and Presumptive Identification

Positive broth cultures were subcultured onto Blood Agar, MacConkey Agar, and Mannitol Salt Agar for bacterial isolation, and onto Sabouraud Dextrose Agar (SDA) supplemented with chloramphenicol for fungal isolation (14, 15). *Candida* isolates were presumptively identified by colonial morphology on SDA (15), microscopic examination of Lactophenol Cotton Blue- and Gram-stained wet mounts (400×) for pseudohyphae, true hyphae, and budding (16), and colony colour on *Candida* CHROMagar following 24–48 h incubation at 37 °C (17); *C. albicans* was further distinguished by germ tube formation in human serum after 2–3 h at 37 °C (18), chlamydospore formation on Corn Meal Agar with 1% Tween 80 after 48 h at 37 °C (19), and growth at 45 °C for 48–72 h (20). Bacterial isolates were presumptively identified by colonial morphology on Blood Agar and MacConkey Agar (colour, shape, size, texture, elevation, margin, haemolytic pattern, lactose fermentation) (22), Gram staining under oil immersion (1000×) (23), and a standard biochemical panel comprising catalase, oxidase, Mannitol Salt Agar growth, coagulase (slide and tube methods), haemolysin activity, indole, citrate utilization, Voges–Proskauer, methyl red, urease, nitrate reduction, gelatin liquefaction, H_2S production on Kligler Iron Agar, and motility testing (24–26).

2.4. Confirmatory Identification by VITEK 2 Compact System

Isolates were confirmed using the VITEK 2 Compact System (bioMérieux, France). For *Candida* isolates, a suspension was prepared from a 24–48-hour pure colony in 3 mL of sterile 0.45–0.5% NaCl, adjusted to a turbidity of 1.80–2.20 McFarland using the VITEK 2 DensiCHEK device, and loaded onto the YST identification card, with results generated within 18–24 h and reported accuracy exceeding 96% (21). For bacterial isolates, suspensions were prepared to a turbidity of 0.50–0.63 McFarland and identified using GN (Gram-negative) or GP (Gram-positive) cards as appropriate, with results reported within 24 h and reported accuracy of up to 99% (27).

2.5. Molecular Identification

2.5.1. Molecular Identification of Candida spp.

Molecular confirmation was performed by PCR amplification of the ITS (Internal Transcribed Spacer) region using the primers listed in Table 1, following the protocol of White et al. (28). Genomic DNA was extracted from pure yeast colonies using the EZ-10 Spin Column Yeast Genomic DNA Mini-Preps Kit (Bioneer, South Korea) according to the manufacturer's instructions, involving liquid-nitrogen homogenization, Proteinase K lysis (56 °C, 30 min), and spin-column purification; DNA concentration and purity were assessed by NanoDrop spectrophotometry. PCR reactions (25 µL total: 5 µL master mix, 1 µL each primer, 13 µL nuclease-free water, 5 µL genomic DNA) were amplified under the following conditions: initial denaturation at 95 °C for 3 min; 35 cycles of 95 °C for 30 s, 56 °C for 1 min, and 72 °C for 50 s; and a final extension at 72 °C for 5 min. Amplicons were resolved by 1% agarose gel electrophoresis (100 V/80 mA, 1 h) with ethidium bromide staining and visualized under UV transillumination alongside a 100–1000 bp DNA ladder.

2.5.2. Molecular Identification of Bacterial Isolates

Bacterial isolates were confirmed by PCR amplification of the 16S rRNA gene using the universal primers listed in Table 2 (29). Genomic DNA was extracted using a commercial extraction kit (Anotoia, Turkey) with minor modifications (30, 31): briefly, a fresh overnight culture was lysed with Proteinase K (65 °C, 30 min), proteins were precipitated, and DNA was purified via a silica spin column with elution in pre-warmed elution buffer; purity was assessed by NanoDrop spectrophotometry, with an A260/A280 ratio ≥1.8 considered acceptable. PCR reactions (25 µL total: 5 µL master mix, 1 µL each primer, 13 µL nuclease-free water, 5 µL genomic DNA) were amplified under the following conditions: initial denaturation at 95 °C for 5 min; 35 cycles of 95 °C for 30 s, 55 °C for 30 s, and 72 °C for 90 s; and a final extension at 72 °C for 10 min. Amplicons (~1500 bp) were resolved by 1% agarose gel electrophoresis (100 V/80 mA, 1 h) and visualized under UV transillumination.

Table 1. Primers used for molecular identification of *Candida* spp.

Primer	Sequence (5′–3′)
ITS1 (Forward)	TCCGTAGGTGAACCTGCGG
ITS4 (Reverse)	TCCTCCGCTTATTGATATGC

Table 2. Primers used for molecular identification of bacterial isolates.

Primer	Sequence (5′–3′)
27F (Forward)	AGAGTTTGATCMTGGCTCAG
1492R (Reverse)	TACGGYTACCTTGTTACGACTT

2.5.3. DNA Sequencing

Purified PCR products from both *Candida* and bacterial isolates were sent for Sanger sequencing (ABI 3730XL Genetic Analyzer, Macrogen Inc., South Korea). Sequences were analysed against the NCBI-BLAST database, with species-level identification of bacterial isolates accepted at ≥97% similarity to reference sequences (32).

2.6. Antimicrobial and Antifungal Susceptibility Testing

2.6.1. Kirby-Bauer Disk Diffusion Method

Antimicrobial susceptibility testing of all bacterial and fungal isolates was performed by the standard Kirby-Bauer disk diffusion method according to CLSI guidelines (33, 34). Microbial suspensions were prepared and adjusted to 0.5 McFarland turbidity standard (~1.5×10^8 CFU/mL for bacteria; ~1×10^6 CFU/mL for fungi), then swabbed onto Mueller-Hinton Agar (bacterial isolates) or Mueller-Hinton Agar supplemented with 2% glucose and 0.5 µg/mL methylene blue (fungal isolates). Antimicrobial or antifungal disks were applied, and plates were incubated at 37 °C for 24 h (bacteria) or 35 °C for 24–48 h (fungi). Zones of inhibition were measured and interpreted according to CLSI breakpoints (33, 34).

2.6.2. Susceptibility Testing of Resistant Isolates by VITEK 2 Compact System

Isolates identified as resistant by disk diffusion underwent automated minimum inhibitory concentration (MIC) determination using the VITEK 2 Compact System (bioMérieux, France), employing the appropriate bacterial AST card or the AST-YS card for antifungal susceptibility (fluconazole, voriconazole, caspofungin, micafungin, amphotericin B, and flucytosine).

Isolates were categorized as susceptible, intermediate, or resistant according to CLSI criteria (35).

2.7. Statistical Analysis

Data were analyzed using SPSS software (Statistical Package for Social Sciences), version 26. The Chi-square test was used to compare categorical variables, the independent-samples t-test to compare two means, and one-way ANOVA with Duncan's multiple range test for multiple comparisons, at a significance level of $P \leq 0.05$. Quantitative results are expressed as mean ± standard deviation (SD) and, where appropriate, mean ± standard error (SE).

3. Results and Discussion

3.1. Isolation

Results showed that of the 330 immunocompromised patients enrolled (160 hemodialysis, 170 oncology), 200 samples (60.6%) yielded confirmed microbial growth versus 130 (39.4%) culture-negative ($P<0.05$); (Figure 1). Of the 200 positive isolates, 124 (62.0%) were bacterial and 76 (38.0%) fungal (Figure 1). By center, Al-Amal Hemodialysis Center yielded 95/160 positive samples (59.4%: 53 bacterial, 42 fungal), while the Oncology Hospital yielded 105/170 (61.8%: 71 bacterial, 34 fungal) (Figure 2). The fungal-isolate proportion was higher among hemodialysis patients (44.2% of their isolates) than oncology patients (32.4%), whereas bacterial isolates predominated among oncology patients (67.6% vs. 55.8%).

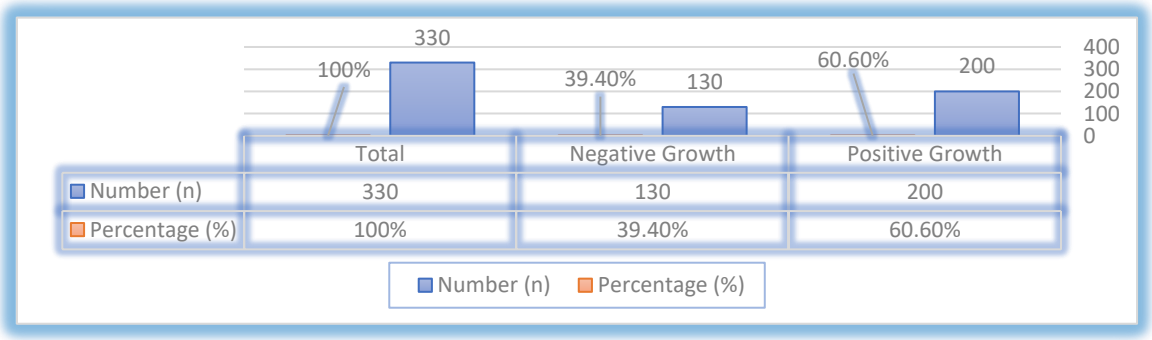

Figure 1. Distribution of microbial isolates by collection center and isolate type (Al-Amal Hemodialysis Center, n = 160; Oncology Hospital, n = 170; total = 330).

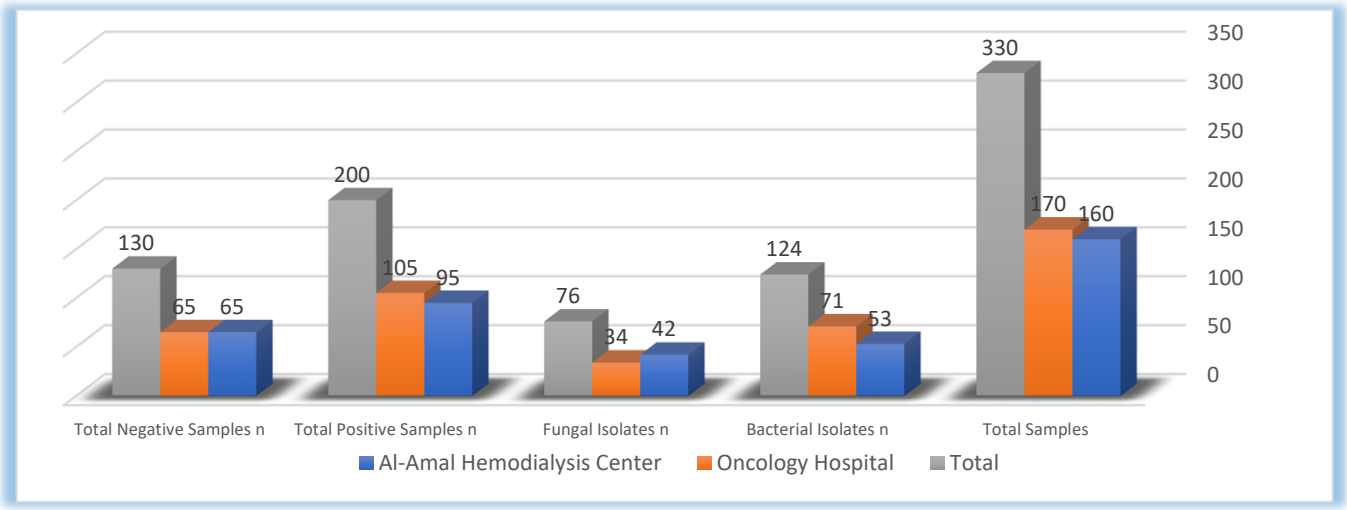

Figure 2. Distribution of microbial isolates recovered from blood cultures of immunocompromised patients (total = 200; fungal = 76, 38.0%; bacterial = 124, 62.0%).

The overall positivity rate paralleled Mahmood (36), who reported 44% catheter-related bloodstream infection among hemodialysis patients at Erbil Teaching Hospital, attributed to diabetes, hypertension, and severe anemia alongside repeated central-line exposure. Salim (37), studying immunocompromised cancer and hemodialysis patients in Iraq, reported a fungal-positivity rate of 32.5%, closely matching the 38.0% recorded here and supporting the interpretation that immunocompromised patients locally constitute a high-risk group for opportunistic fungemia. By contrast, Ali and Hamad (38) reported a markedly lower positivity rate (12.4%) among cancer patients at Nanakali Hospital, Erbil, attributable to their exclusive focus on Gram-positive bacteria and broader specimen base (urine, sputum, and wound cultures alongside blood).

Variation in positivity rates across studies reflects differences in the underlying immunosuppressive condition, blood volume drawn, timing of sample collection, and culture media employed (14). The dual composition of the present cohort—hemodialysis patients exposed to recurrent central-line contamination and oncology patients undergoing myelosuppressive chemotherapy—creates an environment particularly conducive to opportunistic BSI, plausibly explaining the comparatively high positivity observed here.

The bacterial predominance recorded (62.0% vs. 38.0%) is consistent with Karajacob *et al*. (39), who analyzed 23 studies and 14,512 patients across Southeast Asia and found bacteria accounting for over 86% of bloodstream isolates versus 4.1% for fungi. The elevated fungal proportion observed here nonetheless exceeds general BSI surveillance figures, plausibly reflecting the specific vulnerability of hemodialysis and oncology patients to opportunistic fungemia through sustained central-line access, broad-spectrum antibiotic pressure, and depth of immunosuppression.

Vonineng *et al*. (7) similarly underscored the clinical significance of fungemia among critically ill immunocompromised patients. The higher fungal proportion among hemodialysis patients (44.2% vs. 32.4%) and the converse bacterial pattern (67.6% vs. 55.8% for oncology) likely reflect divergent immunosuppressive mechanisms: hemodialysis patients experience chronic, cumulative immune dysfunction compounded by recurrent catheter exposure providing yeasts direct bloodstream access, whereas oncology patients undergo acute, cyclical chemotherapy-induced neutropenia favoring opportunistic bacteria and *Candida*, consistent with the Gram-negative predominance Vonineng et al. (7) reported among chemotherapy recipients. Lin et al. (40) similarly identified malignancy as a major driver of immunosuppression among BSI patients, reinforcing its central role in shaping bloodstream infection risk.

3.2. Identification of Candida Species

3.2.1. Morphological, Microscopic, and Biochemical Identification

Candida colonies on SDA were white to cream-coloured, convex, smooth, and characteristically odorous; microscopically, cells appeared spherical to oval or elongated, single or budding, consistent with Bhavan et al. (41) and Ellis et al. (16) (Figure 3).

CHROMagar *Candida* produced species-specific colours: *C. albicans* light green (reflecting N-acetyl-β-D-galactosaminidase activity on the chromogenic substrate) (43), *C. tropicalis* blue to blue-green, *C. krusei* dark pink with a rough, filamentous texture, *C. glabrata* smooth cream-white colonies reflecting its inability to form hyphae, and *C. parapsilosis* smaller, more dispersed white-to-cream colonies (42) (Figure 4); CHROMagar achieved approximately 96.2% diagnostic accuracy for direct identification from blood culture (44).

Biochemically, only *C. albicans* produced a germ tube in human serum, formed chlamydospores on Corn Meal Agar with Tween 80, and grew at 45 °C, reflecting its comparative thermotolerance (45, 46, 20); all isolates were urease-negative, a feature differentiating *Candida* from other yeast genera such as *Cryptococcus* (16) (Table 3; Figure 5).

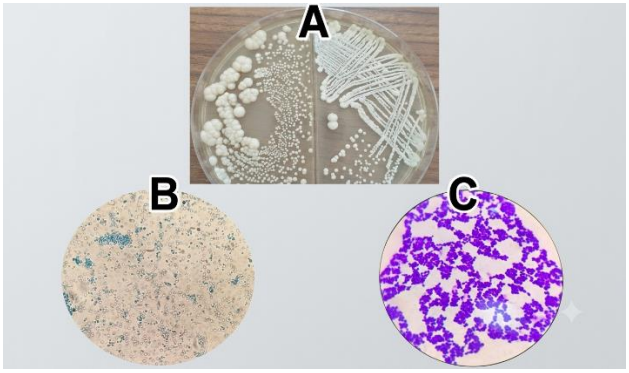

Figure 3. Morphological and microscopic characteristics of *Candida* spp. (A) Colonies on Sabouraud Dextrose Agar after 48 h incubation at 37 °C; (B) *Candida* spp. stained with Lactophenol Cotton Blue (400×); (C) *Candida* spp. stained with Gram stain (400×).

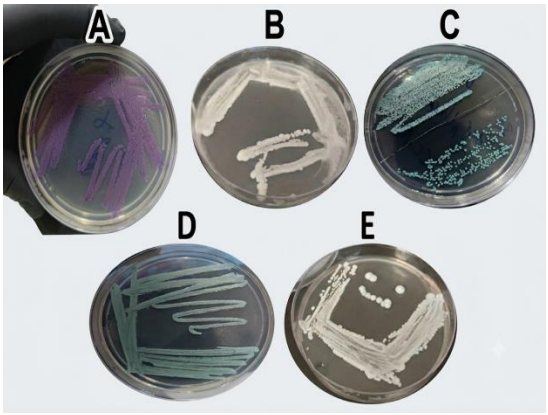

Figure 4. Colonial morphology of *Candida* spp. on HiChrome™ *Candida* Differential Agar after 24–48 h incubation at 37 °C. (A) *C. krusei* (*Pichia kudriavzevii*): dark pink, rough, crumpled colonies reflecting characteristic filamentous growth; (B) *C. glabrata*: smooth, cream-white, regular colonies, reflecting inability to form hyphae; (C) *C. albicans*: light green colonies resulting from N-acetyl-β-D-galactosaminidase activity on the chromogenic substrate; (D) *C. tropicalis*: blue to blue-green colonies resulting from hexosaminidase activity on the chromogenic substrate; (E) *C. parapsilosis*: white-to-cream, smooth colonies, more dispersed and smaller than *C. glabrata*.

Table 3. Biochemical and phenotypic characteristics of *Candida* spp. isolates.

Candida spp.	CHROMagar colour	Urease	Germ tube	Growth at 37 °C	Growth 45 °C	Chlamydospore
C. parapsilosis	Cream-white	–	–	+	–	–
C. krusei	Dark pink	–	–	+	–	–
C. glabrata	Light pink	–	–	+	–	–
C. albicans	Light green	–	+	+	+	+
C. tropicalis	Blue	–	–	+	–	–

Figure 5. Biochemical confirmatory tests for *C. albicans*. (A) Germ tube formation in human serum (400×); (B) Chlamydospore formation on Corn Meal Agar (400×); (C) Growth on SDA at 45 °C.

3.2.2. Species Distribution

Among the 76 *Candida* isolates recovered, five species were identified: *C. albicans* was most prevalent (30 isolates, 39.47%), followed by *C. krusei* (29 isolates, 38.16%), *C. glabrata* and *C. tropicalis* (7 isolates each, 9.21%), and *C. parapsilosis* (3 isolates, 3.95%) (Figure 6). Non-albicans *Candida* (NAC) species collectively accounted for 60.53% of isolates, reflecting the well-documented global epidemiological shift toward NAC dominance in candidemia among immunocompromised patients.

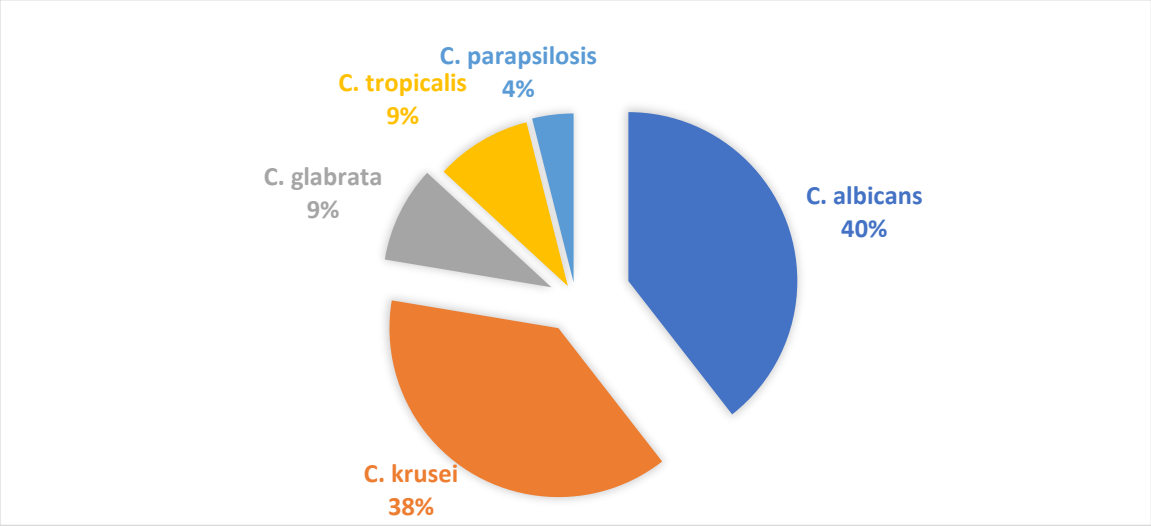

Figure 6. Species distribution of *Candida* spp. isolates recovered from blood cultures of immunocompromised patients.

The proportion of *C. albicans* recorded here is consistent with international blood-culture-based studies: Meneghello et al. (47) reported 44.32% in Italy, Kitaya et al. (48) 45% in Japan, and Pongrácz et al. (49) 54% in Hungary. The large meta-analysis by Ghojoghi et al. (50), covering 69 studies and over 9,700 cancer patients, found *C. albicans* accounted for 39.2% of candidemia isolates globally, closely matching the present finding. This is somewhat lower than the 57.7% reported by Ferngren et al. (51) in Sweden, a discrepancy attributable to well-documented geographic variation in *Candida* species distribution and to the dual composition of the present cohort (hemodialysis plus oncology patients), which may favor selection of non-albicans species.

The most distinctive finding of the present study is the unusually high proportion of *C. krusei* (38.16%). Blood-culture-based surveillance elsewhere has consistently reported this species as rare: 1.38% in Italy (47), 2.24% in Sweden (51), and 3.1% in Hungary (49). Hachem et al. (52) documented that *C. krusei* and *C. glabrata* have become increasingly common causes of candidemia in hematologic malignancy patients, driven by azole selection pressure—consistent with the heavy reliance on prophylactic fluconazole among the oncology patients in this cohort. Ghojoghi et al. (50) similarly reported that NAC prevalence in hematologic malignancies (68%) exceeds that in solid tumors (52%), partly attributable to intensive prophylactic azole use favoring selection of *C. krusei* specifically. Notably, Pongrácz et al. (49) documented that *C. krusei* carried the lowest mortality (15.4%) among *Candida* species in their cohort, versus 59.4% for *C. tropicalis* and 51.85% for *C. glabrata*, suggesting that the high proportion of *C. krusei* observed here, while a therapeutic challenge given its intrinsic fluconazole resistance, does not necessarily correspond to proportionally elevated mortality.

C. glabrata and *C. tropicalis* each accounted for 9.21% of isolates. The proportion of *C. glabrata* was

lower than reported internationally: 14.68% in Italy (47), 16% in Hungary (49), 15.2% in Japan (48), and 19.14% in Sweden (51). Ghojoghi et al. (50) noted that this species predominates in North America and Northern Europe but is comparatively less common in the Middle East and Asia. Pongrácz et al. (49) further observed that *C. glabrata* and *C. krusei* share overlapping risk factors, such that intense azole selection pressure may favor dominance of one species over the other—plausibly explaining the relative suppression of *C. glabrata* alongside the marked rise of *C. krusei* in the local Kirkuk epidemiological landscape.

C. tropicalis, at 9.21%, aligned with Kitaya et al. (48) in Japan (9.1%) and Pongrácz et al. (49) in Hungary (8%), though it exceeded the proportions reported by Meneghello et al. (47) in Italy (4.29%) and Ferngren et al. (51) in Sweden (2.80%). Ghojoghi et al. (50) identified *C. tropicalis* as the second most common NAC species in cancer-patient blood isolates globally (14.9%), predominating epidemiologically in Asia, Africa, and Latin America relative to Europe and North America—consistent with the Middle Eastern setting of the present study. Pongrácz et al. (49) linked *C. tropicalis* to the highest mortality among *Candida* species studied (59.4%), a finding corroborated by the meta-analysis of Ghojoghi et al. (50), which associated this species with hematologic malignancy and intensive care settings. Al-Ameri and Al-Musawi (53), studying Iraqi patients in Baghdad, confirmed strong biofilm-forming capacity in *C. tropicalis*, reinforcing its local clinical relevance.

C. parapsilosis recorded the lowest proportion among blood isolates (3.95%), markedly lower than reported internationally: 25.90% in Italy (47), 11.30% in Sweden (51), and 11% in Hungary (49). Ghojoghi et al. (50) reported this species accounts for 15.9% of blood isolates in cancer patients globally, predominating epidemiologically in Europe and Latin America but declining in the Middle East and Asia. This pattern is consistent with the species' well-documented association with central-line-related infection transmitted via healthcare worker hands in intensive-care settings, distinct from the endogenous translocation route characteristic of *C. albicans* and *C. krusei* in immunocompromised hosts. Kitaya et al. (48) reported that all cases of persistent catheter-related infection due to *C. parapsilosis* in their cohort were azole-resistant or dose-dependent, suggesting azole selection pressure reduces this species' survival where fluconazole prophylaxis is widely used. A Baghdad-based local study found *C. parapsilosis* to be the leading species (27.7%) among Iraqi patients (53), a distribution markedly divergent from the present findings and reflecting intra-national epidemiological heterogeneity. Salim (37), studying immunocompromised cancer and renal patients in Iraq, reported *C. albicans* as most prevalent (25.71%), followed by *C. tropicalis* (20.0%), *C. glabrata* and *C. parapsilosis* (17.14% each), and *C. krusei* (11.43%); the present study concurs with the dominance of *C. albicans* but diverges sharply in the ranking of *C. krusei* (38.16% here vs. 11.43%), underscoring marked site-to-site heterogeneity even within local Iraqi healthcare settings and reinforcing the need for periodic center-specific epidemiological surveillance.

3.2.3. Antifungal Susceptibility (Kirby-Bauer Disk Diffusion)

Amphotericin B and micafungin were the most effective agents (85.5% susceptibility each), followed by voriconazole (81.6%), caspofungin and flucytosine (71.1% each), while fluconazole recorded the highest resistance (26.3%)—attributable to the intrinsic fluconazole resistance of *C. krusei*, whose elevated proportion in this cohort drove the overall resistance rate (Figure 7). Fifteen isolates (19.7%) were classified as multidrug-resistant (MDR) and selected for confirmatory VITEK 2 and molecular identification.

These findings partially concur with Salim (37), who reported comparably high amphotericin B and

echinocandin susceptibility, though with lower echinocandin resistance than observed here (11.8% micafungin, 22.4% caspofungin), plausibly reflecting differences in isolate resistance background between settings. The markedly higher fluconazole resistance recorded here, versus only intermediate susceptibility reported by Salim (37), likely reflects the disproportionate contribution of *C. krusei* combined with extensive prophylactic fluconazole use among oncology patients, imposing sustained selection pressure (54). Azoles inhibit lanosterol 14-α-demethylase, blocking ergosterol biosynthesis (55); resistance emergence under intensive azole use is therefore an expected finding (54).

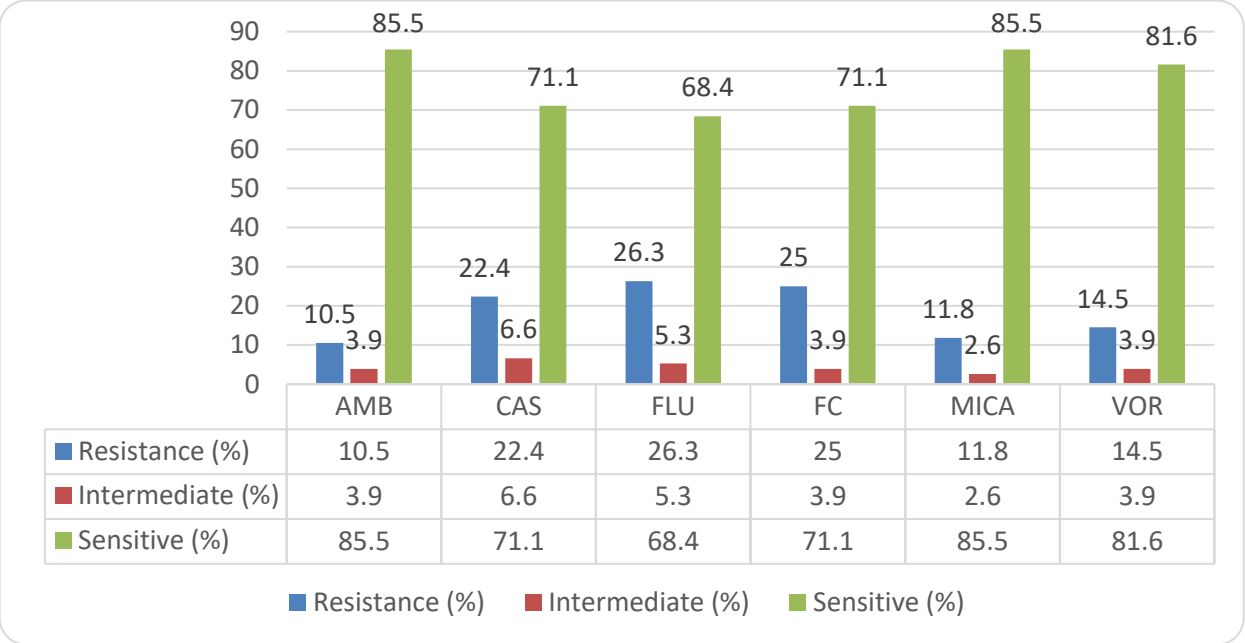

Figure 7. Susceptibility and resistance patterns of *Candida* spp. isolates to the antifungal agents tested by disk diffusion (Kirby-Bauer method).

3.2.4. VITEK 2 and Molecular Identification

VITEK 2 YST confirmed the identity of all 15 MDR isolates with >96% accuracy. ITS-region PCR yielded a consistent ~480 bp amplicon across all tested isolates (Figure 8), and Sanger sequencing of eight representative isolates deposited in GenBank confirmed species identity—reclassified under current taxonomic nomenclature as *Nakaseomyces glabratus*, *Pichia kudriavzevii*, and *Lodderomyces parapsilosis*—with sequence identities of 90.20–98.63% (*C. albicans*), 85.62% (*C. glabrata*), 94.52–96.06% (*C. krusei*), 96.67% (*C. tropicalis*), and 97.30% (*C. parapsilosis*).

Phylogenetic analysis of the eight sequenced isolates (Neighbor-Joining, MEGA12, 1000 bootstrap replicates) resolved two genetically distinct clusters separating non-albicans species from *C. albicans*, with strong nodal support (93–100%), consistent with established species boundaries (Figure 9). A separate tree restricted to the seven *C. albicans* isolates showed a generally close genetic relationship among most isolates, with limited fine-scale divergence between individual strains (Figure 10). These findings align with Ceballos-Garzón et al. (56), who reported ~99% concordance between ITS sequencing and MALDI-TOF MS for *Candida* identification.

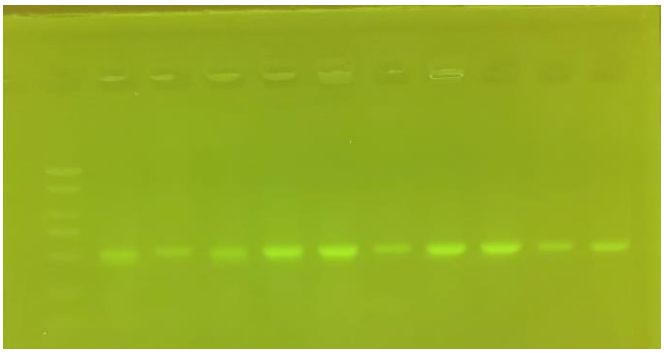

Figure 8. Agarose gel electrophoresis of ITS-region PCR products (~480 bp) for *Candida* spp. isolates.

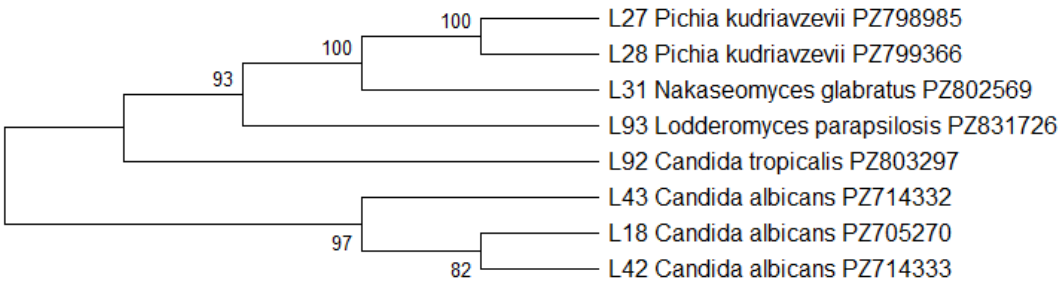

Figure 9. Phylogenetic tree of the eight sequenced *Candida* spp. isolates deposited in GenBank, based on ITS region sequences (Neighbor-Joining, MEGA12, 1000 bootstrap replicates).

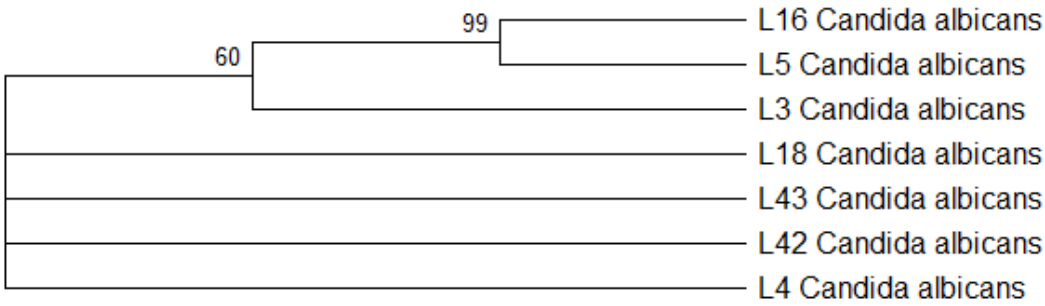

Figure 10. Phylogenetic tree of the seven *C. albicans* isolates based on ITS region sequences (Neighbor-Joining, MEGA12, 1000 bootstrap replicates).

3.3. Isolation and Identification of Bacteria

Bacterial isolates were identified through a three-tiered approach: presumptive phenotypic and biochemical characterization on selective/differential media (MacConkey Agar, Blood Agar, Mannitol Salt Agar) followed by Gram staining and standard biochemical testing (57), Kirby-Bauer susceptibility testing to select multidrug-resistant (MDR) isolates, confirmatory identification of MDR isolates by VITEK 2 Compact System (GN/GP cards; 96.8% and 96.5% reported accuracy, respectively) (27), and molecular confirmation by 16S rRNA gene amplification and Sanger sequencing.

3.3.1. Gram-Negative Bacteria

Gram-negative isolates were identified based on colonial morphology on MacConkey and Blood Agar, Gram staining, and a standard biochemical panel (Table 4; Figures 11, 12). *E. coli* produced pink, lactose-fermenting colonies on MacConkey

Agar and smooth grey-white colonies on Blood Agar, appearing as short Gram-negative rods; it was indole- and methyl red-positive, citrate-negative, consistent with Koneman et al. (57), who identified these features as key differentiators from other Enterobacteriaceae. *P. mirabilis* exhibited characteristic swarming motility and pale, lactose-negative colonies with a fish-like odour, and was urease-, methyl-red-, and citrate-positive with H2S production, consistent with Afifi et al. (58). *K. pneumoniae* produced large, mucoid, dark-pink colonies on MacConkey Agar reflecting capsular polysaccharide production, and was Voges-Proskauer-, citrate-, and urease-positive but non-motile, consistent with Akgül et al. (59) and Kumar et al. (60). *S. marcescens* produced small, pale-pink colonies attributable to variable prodigiosin production and was catalase-, Voges-Proskauer-, and citrate-positive, consistent with Drummond et al. (61). *P. aeruginosa* produced beta-haemolytic, non-lactose-fermenting colonies with a characteristic grape-like odour and was oxidase-positive, a key differential feature distinguishing it from Enterobacteriaceae (62). *M. morganii* was distinguished from the phenotypically similar *P. mirabilis* by a distinct biochemical profile (indole-, methyl-red-, and urease-positive; citrate- and oxidase-negative) (57).

3.3.1.2. Gram-Positive Bacteria

Gram-positive isolates were characterized similarly. *S. aureus*, the most numerous Gram-positive isolate, produced cream-to-yellow, beta-haemolytic colonies and Gram-positive cocci in grape-like clusters, and was catalase- and coagulase-positive with mannitol fermentation. Kateete et al. (63) emphasized that optimal *S. aureus* identification requires a combined test panel rather than a single assay, with Mannitol Salt Agar, DNase, and coagulase testing together achieving up to 100% specificity; Gillespie and Hawkey (64) confirmed coagulase positivity as the principal differential feature distinguishing *S. aureus* from coagulase-negative staphylococci.

3.3.2. Species Distribution — Gram-Negative Isolates

Gram-negative bacteria accounted for 108/124 isolates (87.10%), spanning nine species: *E. coli* was most prevalent (29, 26.85%), followed by *P. mirabilis* (22, 20.37%), *K. pneumoniae* (17, 15.74%), *S. marcescens* (14, 12.96%), *P. aeruginosa* and *M. morganii* (11 each, 10.19%), and four rare species—*B. bullata*, *E. cloacae complex*, *E. hormaechei*, and *A. lwoffii*—each contributing a single isolate (0.93%) (Figure 13).

Table 4. Phenotypic, microscopic, and biochemical characteristics of Gram-negative bacterial isolates.

Species	Gram	Shape	Catalase	Oxidase	Indole	MR	VP	Citrate	Urease	H2S	Motility	Kligler
E. coli	–	Rod	+	–	+	+	–	–	–	–	+	A/A
K. pneumoniae	–	Rod	+	–	–	–	+	+	+	–	–	A/A
P. mirabilis	–	Rod	+	–	–	–	–	–	+	+	+	K/A
S. marcescens	–	Rod	+	–	–	–	+	–	–	–	+	A/A
P. aeruginosa	–	Rod	+	+	–	–	–	+	+*	–	+	K/K
M. morganii	–	Rod	+	–	+	+	–	–	+	–	+	K/A
B. bullata	–	Rod	+	+	–	–	–	–	–	–	–	K/K
E. cloacae complex	–	Rod	+	–	–	–	+	+	–	–	+	A/A
E. hormaechei	–	Rod	+	–	–	–	+	+	–**	–	+	A/A
A. lwoffii	–	Rod	+	–	–	–	–	–	–	–	–	K/K

**P. aeruginosa isolates were urease-positive in this study (a variable trait for the species). **E. hormaechei showed negative or weak delayed-positive urease.*

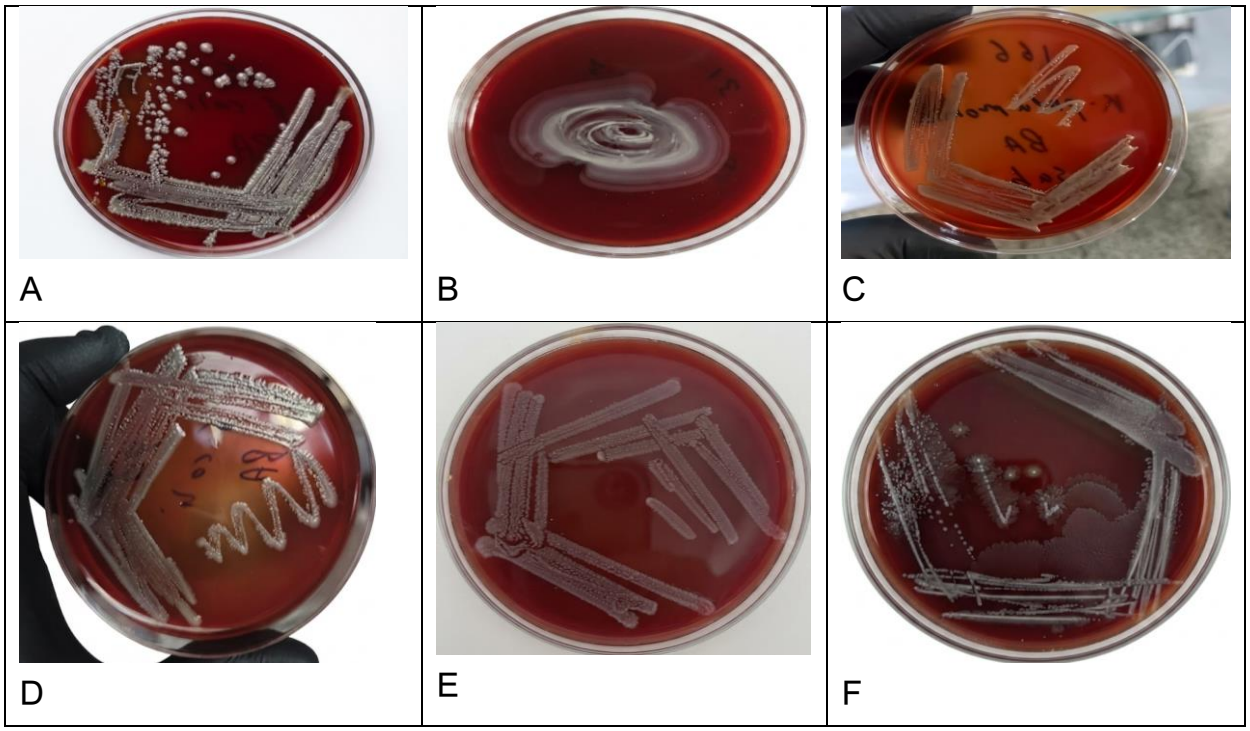

Figure 11. Colonial morphology of Gram-negative bacterial isolates on Blood Agar. (A) *E. coli*; (B) *P. mirabilis*; (C) *K. pneumoniae*; (D) *S. marcescens*; (E) *P. aeruginosa*; (F) *M. morganii*.

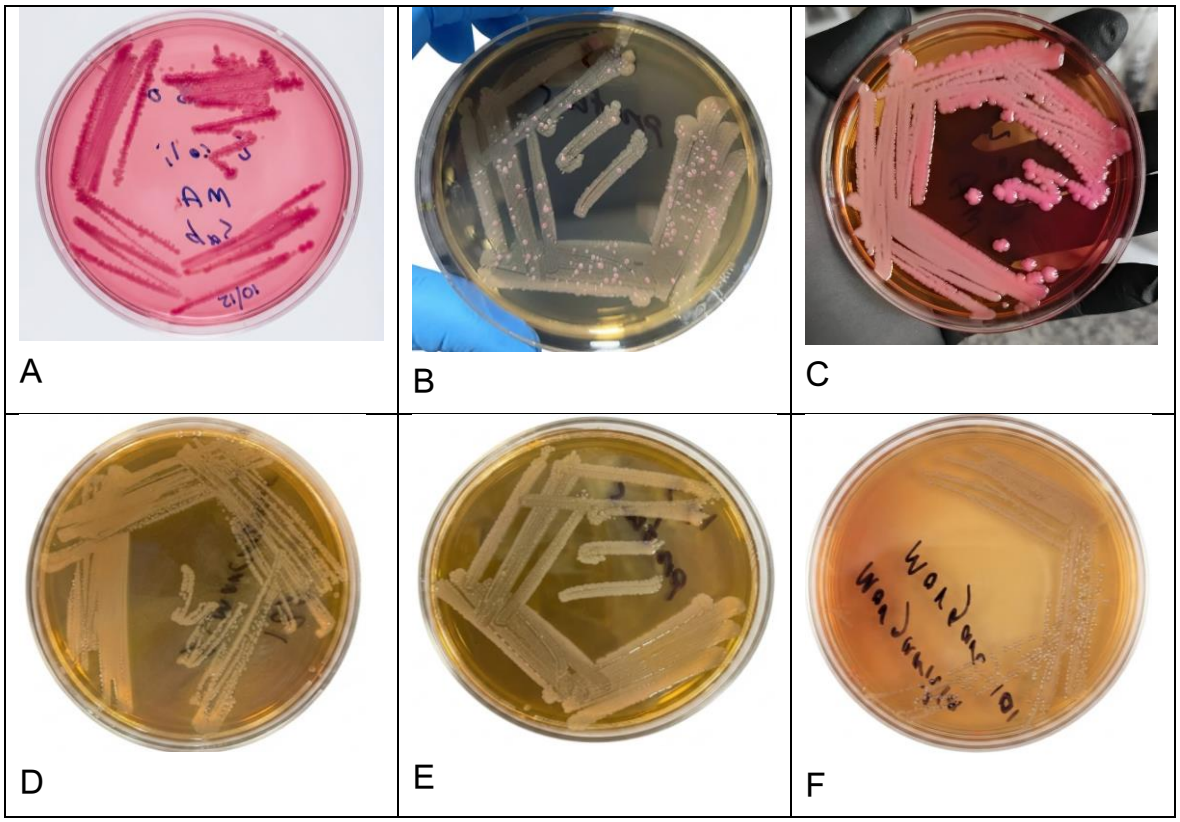

Figure 12. Colonial morphology of Gram-negative bacterial isolates on MacConkey Agar. (A) *E. coli*; (B) *P. mirabilis*; (C) *K. pneumoniae*; (D) *S. marcescens*; (E) *P. aeruginosa*; (F) *M. morganii*.

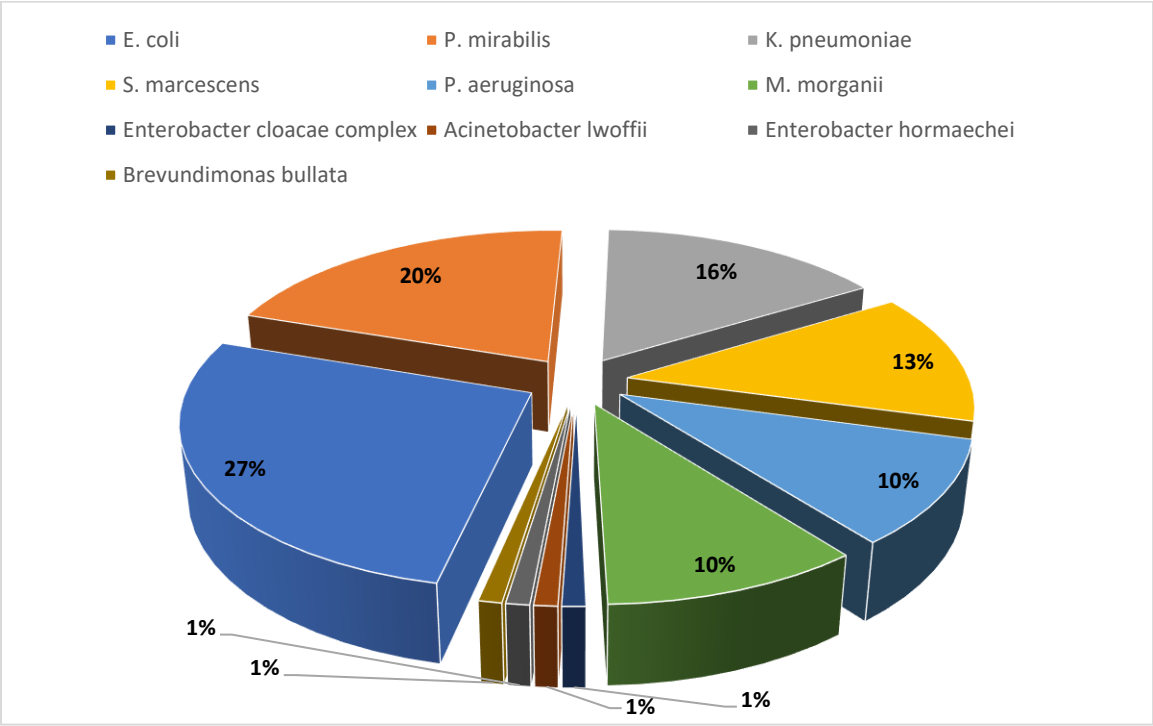

Figure 13. Species distribution of Gram-negative bacterial isolates recovered from blood cultures of immunocompromised patients.

The elevated *E. coli* proportion exceeded that reported by Thanoon and Mohammed (65) in Basra (16.6%, third-ranked), Lin et al. (40) in a Chinese immunosuppressed cohort (17.05%), and Vonineng et al. (7) in Thailand (17%), consistent with Nahar et al. (66) confirming *E. coli* as a major pathogen among immunocompromised cancer patients. This likely reflects the dual hemodialysis-oncology composition of the cohort, predisposing to enteric bacterial translocation, alongside potential strain-level geographic variation.

K. pneumoniae ranked third (13.70%), considerably lower than Ruaa et al. (67) in Najaf (41.7%), closely matching Vonineng et al. (7, 14%), but lower than Lin et al. (40, 20.06%)—differences attributable to variation in immunosuppression severity, prophylactic protocols, and local ESBL prevalence.

The most distinctive finding was the markedly elevated Morganellaceae proportion—*P. mirabilis* (17.74% of Gram-negative isolates) and *M. morganii* (8.90%), together 26.64% of all bacterial isolates. Both are recognized causes of catheter-associated urosepsis via ascending translocation, common among hemodialysis patients carrying urinary and central venous catheters (68); *P. mirabilis* urease production promotes urothelial injury and bloodstream invasion, while *M. morganii* carries intrinsic cephalosporin/penicillin resistance via chromosomal blaAmpC (69, 70).

S. marcescens (11.30%) exceeded the incidence reported by Pérez-Viso et al. (71) in Spain, where 61.7% of cases were hospital-acquired, with rising aminoglycoside and fluoroquinolone resistance, likely reflecting environmental persistence of this organism within local healthcare facilities (72).

Findings were consistent with Thanoon and Mohammed (65, 53.3% Gram-negative) and Ruaa et al. (67), confirming *K. pneumoniae*, *P. aeruginosa*, and *E. coli* dominance among cancer patients. *P. aeruginosa* (8.90%) was lower than Vonineng et al. (7, 17%, ranked first); Hernández-Jiménez et al. (73) demonstrated that prior MDR *P. aeruginosa* colonization increases bloodstream infection risk 42.1-fold, underscoring its clinical significance

despite modest prevalence here. The four rare species (0.80% each) reflect the characteristic microbial diversity of BSI in this population.

3.3.3. Species Distribution — Gram-Positive Isolates

Gram-positive bacteria accounted for 16/124 isolates (12.90%): *S. aureus* predominated (13, 81.25% of Gram-positive; 10.48% of total), while Micrococcus sp., *S. agalactiae*, and *E. faecium* each contributed a single isolate (6.25% of Gram-positive; 0.80% of total) (Figure 14).

The *S. aureus* proportion closely matched Lin et al. (40, 10.17%), though it remained considerably lower than Şibar and Şencan (74, 23%) specifically among hemodialysis patients, attributable to elevated catheter-related infection rates in that population. Tong et al. (75) confirmed *S. aureus* bacteremia mortality of 15–30%, underscoring its clinical importance despite limited representation here. The overall lower Gram-positive proportion relative to international benchmarks likely reflects predominant exposure to Gram-negative enteric bacteria via central venous catheters and invasive devices among hemodialysis and oncology patients—distinct from the pattern typical of solid-organ transplant or major surgical populations, where Gram-positive organisms more often predominate. The rare species (Micrococcus sp., *S. agalactiae*, *E. faecium*) add descriptive epidemiological value to the local spectrum of bloodstream pathogens in Kirkuk.

3.3.4. Antibiotic Susceptibility (Kirby-Bauer Disk Diffusion)

Among 11 antibiotics tested against all 124 bacterial isolates, imipenem was most effective (84.7% susceptibility), followed by gentamicin (66.9%), levofloxacin (60.5%), and amoxicillin-clavulanate (53.2%), while clindamycin recorded the highest resistance (77.4%), consistent with its primary Gram-positive activity in a predominantly Gram-negative collection. Resistance was also notable for cefixime (50.8%), ceftriaxone (41.9%), tetracycline (41.1%), cefotaxime (40.3%), cephalexin (37.9%), and ciprofloxacin (32.3%) (Figure 15). This pattern—elevated cephalosporin resistance against preserved carbapenem/aminoglycoside susceptibility—is consistent with global expansion of ESBL-producing Enterobacteriaceae, which predominated among isolates in this study.

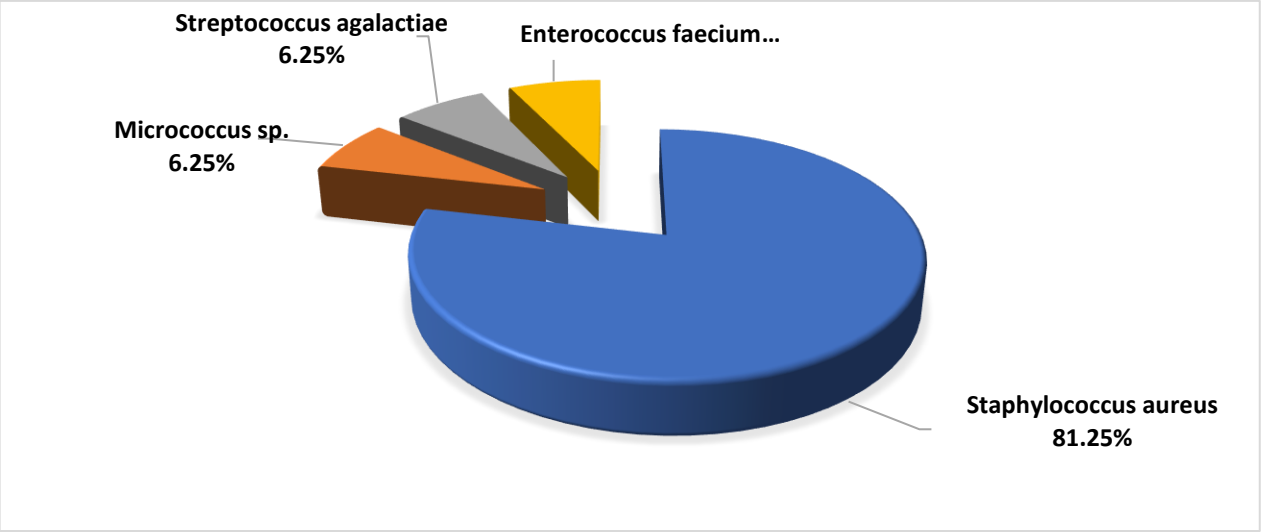

Figure 14. Species distribution of Gram-positive bacterial isolates recovered from blood cultures of immunocompromised patients.

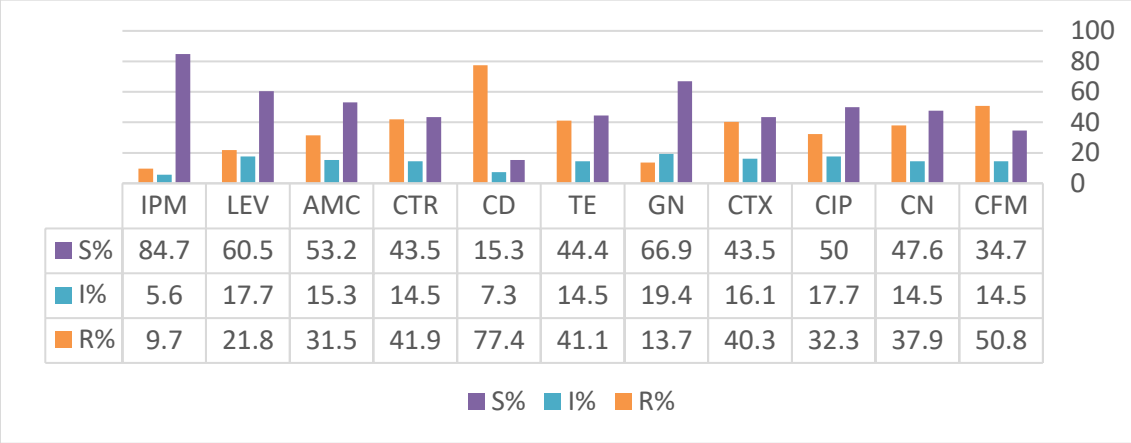

Figure 15. Susceptibility and resistance patterns of bacterial isolates to the antibiotics tested by disk diffusion (Kirby-Bauer method).

These findings parallel Han et al. (76), who reported similarly high ceftriaxone (39.9%), ceftazidime (36.7%), and aztreonam (31.2%) resistance against preserved carbapenem (<8.6%) and amikacin (<1.6%) susceptibility in a decade-long Chinese *E. cloacae complex* study, mirroring the marked imipenem superiority observed here and supporting a role for prior antibiotic exposure in driving MDR emergence.

Duhaniuc et al. (77) confirmed ESBL-producing organisms as the foremost global threat in immunocompromised patients, with production rates ranging from 34.5% (Tunisian *K. pneumoniae*) to 79.6% (Lebanese *E. coli*), reflecting regional variation in antibiotic use and prophylactic protocols that similarly explains divergence from the present findings. Wang et al. (78), studying 358 urinary tract infections among Chinese cancer patients, reported comparable susceptibility patterns—near-complete meropenem (98.75%) and imipenem (94.38%) susceptibility against near-total cephalosporin resistance and fluoroquinolone resistance exceeding 85%—supporting the interpretation that recurrent catheterization and invasive procedures among hemodialysis and oncology patients create comparable selective environments favoring ESBL-producing organisms.

3.3.5. VITEK 2 and Molecular Identification

VITEK 2 GN/GP cards confirmed 62 MDR isolates with >96% accuracy. Of the 124 isolates screened by Kirby-Bauer, 59 MDR isolates were selected for confirmatory 16S rRNA PCR and sequencing, yielding the expected ~1500 bp amplicon in all cases (Figure 16).

Sanger sequencing of 12 representative isolates deposited in GenBank confirmed species identity, with sequence similarities ranging from 95.75% (*E. cloacae complex*) to 100% (*E. coli*, *S. aureus*, Micrococcus sp.). Phylogenetic analysis of the 12 sequenced isolates (Neighbor-Joining, MEGA12) resolved two well-supported clusters (bootstrap 70–100%): one comprising all Gram-negative isolates including *P. aeruginosa*, and the second comprising Gram-positive and rare genera, consistent with established phenotypic classification and confirming 16S rRNA as a reliable tool for distinguishing major bacterial groups, notwithstanding its limited resolution between closely related species within a genus (Figure 17).

A separate tree restricted to the 17 *E. coli* isolates (Neighbor-Joining, MEGA11, 1000 bootstrap replicates) resolved two major clades at 53% nodal support, comprising several well-supported subclusters (54–100%) alongside isolates showing no clear clustering, indicating moderate genetic

heterogeneity within this species (Figure 18). This pattern is consistent with Ibrahim (79) and Suardana et al. (80), who similarly reported variable bootstrap support among Enterobacteriaceae subclusters despite common species membership—an intrinsic molecular property of 16S rRNA rather than an effect of isolate source. By contrast, Alsanie et al. (81), analysing a multi-genus isolate set, reported broadly higher bootstrap support, plausibly reflecting differences in sampling scope and sequence quality between studies. The comparatively modest support observed among *E. coli* subclusters reflects the high conservation of 16S rRNA within genus *Escherichia*, limiting variable sites available for fine-scale discrimination—particularly among single-directional reads of varying length—and likewise explains the low support (53%) at the shared root node between the two major clades.

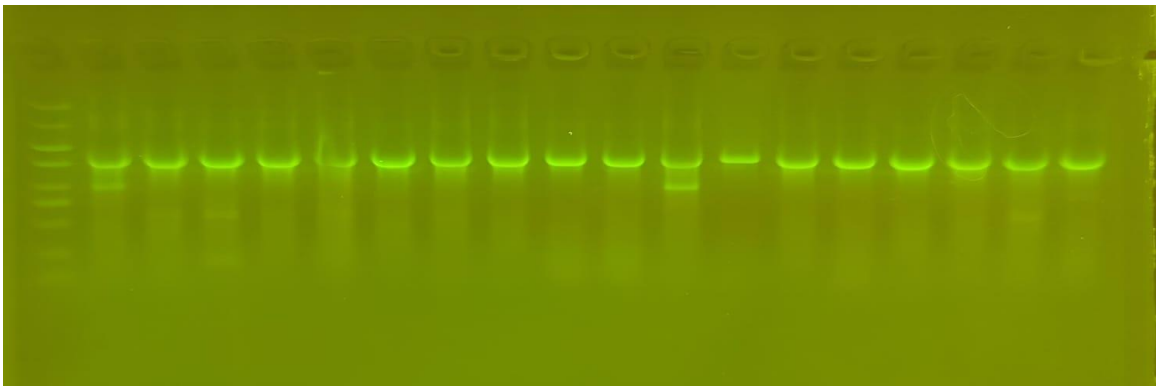

Figure 16. Agarose gel electrophoresis of 16S rRNA gene PCR products (~1500 bp) for bacterial isolates.

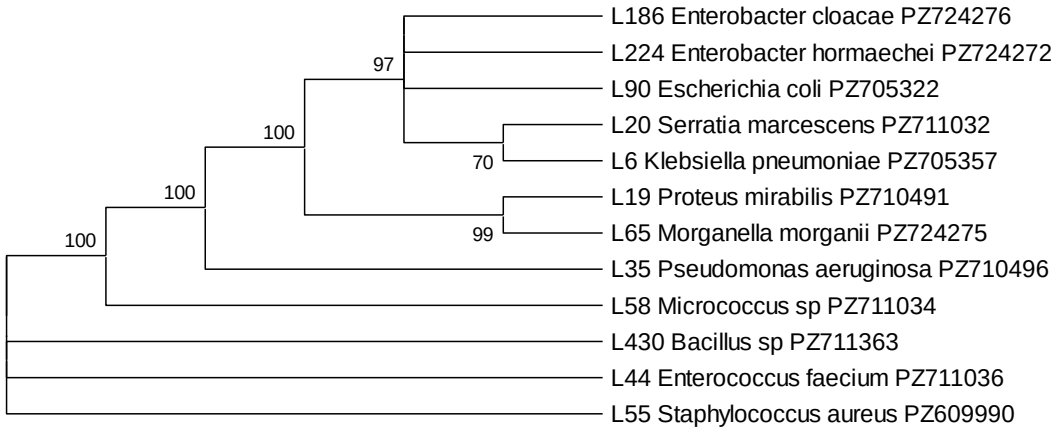

Figure 17. Phylogenetic tree of the 12 sequenced bacterial isolates deposited in GenBank, based on 16S rRNA gene sequences (Neighbor-Joining, MEGA12).

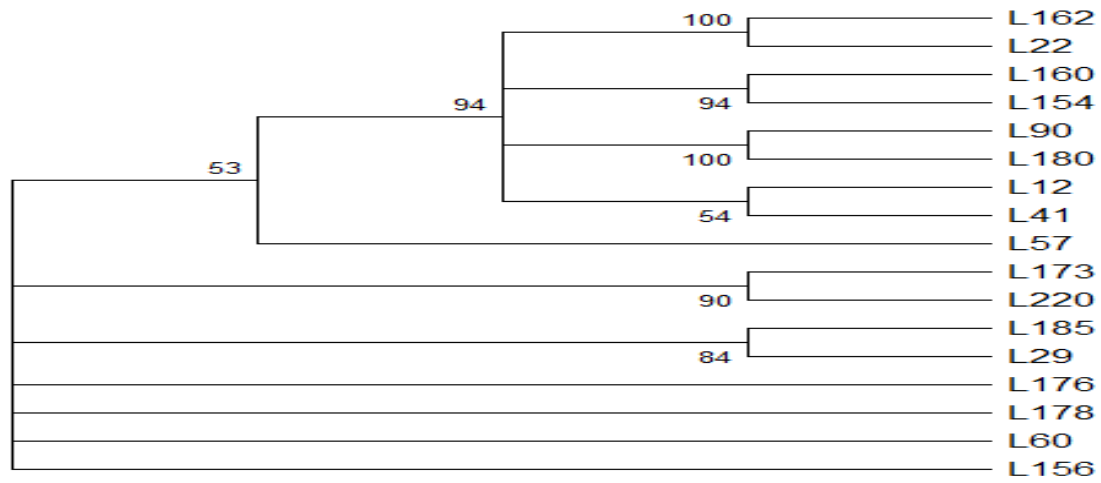

Figure 18. Phylogenetic tree of the 17 *E. coli* isolates based on 16S rRNA gene sequences (Neighbor-Joining, MEGA11, 1000 bootstrap replicates).

4. Conclusion

This study demonstrates a substantial and distinctive burden of bloodstream infection among immunocompromised patients in Kirkuk, Iraq, with Gram-negative bacteria—particularly *E. coli* and an unusually high combined proportion of Morganellaceae—and an elevated prevalence of the intrinsically fluconazole-resistant *C. krusei* among *Candida* isolates. Morphological, biochemical, VITEK 2, and molecular (ITS/16S rRNA) approaches provided concordant and reliable species-level identification, with representative isolates deposited in GenBank. The divergent bacterial and fungal profiles observed between hemodialysis and oncology patients, alongside marked resistance to fluoroquinolones, cephalosporins, and fluconazole, underscore the distinct infection risks associated with each immunosuppressive mechanism and highlight the need for continued local epidemiological surveillance to guide empirical antimicrobial therapy in this vulnerable population.

Conflict of interest: NIL

Funding: NIL

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