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Molecular and Phenotypic Characterization of ESKAPE Pathogens from Cancer Patients

Zahraa Farazdaq Hasan*, Akhter Ahmed Ahmed

Department of Biology, College of Science, Salahaddin University- Erbil, Erbil, Iraq.
*Corresponding author : zahraa.hasan@su.edu.krd

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Abstract

Cancer patients are particularly susceptible to antimicrobial-resistant ESKAPE (*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae,* Acinetobacter baumannii*, Pseudomonas aeruginosa, and Enterobacter cloacae*) pathogens due to their immunocompromised status and frequent exposure to antibiotics. The ultimate objective of this study was to investigate at the molecular features, trends of antimicrobial resistance, and biofilm-forming abilities of ESKAPE bacteria that were taken from cancer patients. Samples were collected from July to October 2024 in the Bacteriology Laboratory at Nanakali Hospital, Erbil, Kurdistan Region, Iraq. As a result, 80 analyzed isolates from different clinical specimens were obtained from various cancer patients. Conventional microbiological methods were used for initial identification, and the VITEK 2 automated system was used for confirmation. Biofilm formation was evaluated through a microtiter plate assay, and antimicrobial susceptibility was determined using an automated system. Molecular validation of six chosen ESKAPE isolates was achieved using 16S rRNA gene sequencing. After that, a phylogenetic analysis was done. The findings indicated that ESKAPE pathogens comprised 26 of 80 isolates (32.5%) obtained from cancer patients, with multi-drug resistance phenotypes being particularly prevalent in Klebsiella pneumoniae. The production of biofilms differed among species; yet, no significant correlation was seen between MDR status and biofilm strength. These results highlight the clinical significance of ESKAPE pathogens in infections impacting immunocompromised cancer patients.

1. Introduction:

Antibiotics are widely used medications to treat and prevent potentially fatal bacterial infections in the medical field [1]. The ability of microorganism to survive being exposed to drugs that would typically kill them or stop them from growing is known as antibiotic resistance [2]. However, the so-called ”antibiotic resistance phenomenon” resulted from the widespread use of antibiotics, the lack of new, effective medications, and ineffective infection control measures [3]. Antimicrobial resistance is one of the greatest risks to human health, according to the World Health Organization (WHO), and it poses considerable challenges regarding managing infections in cancer patients with impaired immune systems [4]. Unfortunately, if nothing changes, the impact of a ”post-antibiotic era” may arise, which would lead to higher global healthcare expenses and lower clinical results [5]. The rapid spread of multidrug resistant (MDR) bacteria has surpassed the researchers' ability to develop new antibiotics, and even with modern technology advances, no significant new antibacterial class has been created over the past quarter century [1].

It is difficult to discuss MDR without mentioning the role of the six nosocomial pathogens that make up the ESKAPE pathogens, *Klebsiella pneumoniae (K. pneumoniae)*, *Pseudomonas aeruginosa (P. aeruginosa)*, *Staphylococcus aureus (S. aureus)*, *Acinetobacter baumannii (A. baumannii)*, *Enterococcus faecium (E. faecium)*, and *Enterobacter cloacae (E. cloacae)*, which together constitute the most important antibiotic-resistant pathogens [5, 6]. Their capacity to "escape" antimicrobial action through multiple mechanisms renders them highly resistant, and their capacity to establish biofilms on hospital surfaces and healthcare equipment enhances their persistence in clinical settings [7].

Immunocompromised populations, including cancer patients, have a higher prevalence of drug-resistant infections associated with ESKAPE groups. These patients are extremely vulnerable to bacterial infections caused by surgical complications, chemotherapy- or radiation-induced white blood cell shortages, and the use of immunosuppressive medicines during treatment [8]. Also, antibiotic medication and chemotherapy can cause dysbiosis in the microbiota [2], which makes resistance even worse by spreading opportunistic bacteria that have antibiotic resistance genes. Antibiotic resistance in cancer patients is associated with an elevated risk of infection and a reduced likelihood of survival [8].

Moreover, total cancer treatment outcomes are still impacted by infection-related death [4], and notably, ESKAPE infections are frequently linked to prolonged infections, longer hospital stays, and increased mortality rates in this population [8]. The purpose of this study was to collect pathogenic bacteria from cancer patients and examine their antibiotic resistance profiles, biofilm-forming capabilities, and molecular attributes, including 16S rRNA gene sequencing and phylogenetic analysis.

2. Material and Methods:

2.1 Sample Collection:

An overall of 80 clinical isolates, with ages spanning from 9 months to 83 years, were obtained from urine, blood, sputum, stool, and various swabs from both cancer inpatients and outpatients from July to October, 2024, in the Bacteriology Laboratory at Nanakali Hospital, Erbil, Kurdistan Region, Iraq.

2.2 Bacterial Identification and Antimicrobial Resistance Assessment:

Initial identification and characterization of all samples were performed using standard biochemical assays according to [9], followed by species confirmation with the Vitek-2 automated system (BioMérieux, France). Pure cultures were kept on Mueller–Hinton agar (MHA; Scharlau, Spain) slants under refrigeration, while Aliquots were preserved in 30% glycerol at 20 °C for long-term storage.

Antimicrobial susceptibility testing was conducted via the VITEK 2 system against a range of antibiotics, including (amikacin, amoxicillin/clavulanate, ampicillin, ampicillin /sulbactam, benzylpenicillin, cefazolin, cefepime, cefotaxime, ceftazidime, ceftazidime/avibactam, ceftolozane/tazobactam, ceftriaxone, cefuroxime, cefuroxime axetil, ciprofloxacin, clindamycin, colistin, doripenem, doxycycline, ertapenem, erythromycin, fosfomycin, fusidic acid, gentamicin, imipenem, levofloxacin, linezolid, meropenem, minocycline, moxifloxacin, nitrofurantoin, oxacillin, piperacillin/tazobactam, rifampicin, teicoplanin, tetracycline, tigecycline, tobramycin, trimethoprim/sulfamethoxazole, vancomycin).

The antimicrobial susceptibility results from the VITEK 2 system were utilized to categorize ESKAPE isolates as MDR or non-MDR bacteria according to their susceptibility profiles.

2.3 Static Biofilm Assay:

The microplate technique described by Limban et al. [10] was used with minor modification to assess the capacity of ESKAPE isolates to produce biofilms. In summary, 10μl of an overnight culture was pipetted into each well of a sterile flat bottom microtiter plate (MTP; Citotest Labware, China) with 200 μl of sterile Nutrient Broth (NB) containing 2% glucose. The wells with sterile medium were used as a control. Plates were incubated under static conditions at 37°C for a total of 24 hours. After incubation, the supernatant fluid from each well was removed, and the wells were gently flushed with sterile Phosphate Buffer Solution (PBS) thrice to remove non-adherent cells. Plates were dried in an oven (Memmert, Germany) at 55°C. Each well received 200 μl of 1% Crystal Violet solution, which was let to stain for 10 minutes at room temperature.

Plates were rinsed three times with sterile PBS. Crystal Violet, which adhered to bacteria in wells, was solubilized with 95% ethanol. The optical density (OD) of the supernatant fluid containing solubilized Crystal Violet in each well was quantified using an ELISA reader (BioTek Instruments, USA) at a wavelength of 630 nm to assess its direct association with biofilm biomass. Biofilm formation was quantitatively classified based on the measured OD values following the criteria proposed by Mathur et al. [11], where OD $<$0.120 indicated weak biofilm production, OD 0.120–0.240 indicated moderate production, and OD $>$ 0.240 indicated strong biofilm production.

2.4 CGenomic DNA Extraction and Molecular Identification of Bacterial Isolates:

A subset of representative strains (one isolate per species) was selected from the identified ESKAPE isolates for molecular study, prioritizing isolates with high multidrug resistance and strong biofilm-forming capacity. A Bacteria DNA Preparation – Solution Kit (Jena Bioscience, Germany) was used to get the genomic DNA of these six ESKAPE isolates. Before carrying out this extraction, each isolate had to be sub-cultured

in a NB and incubated at 37°C for a total of 48 hours in order to have a fresh biomass to work with.

The 16S rRNA was amplified by PCR [12] with universal primers 27F (5-AGAGTTTGATCMTGGCTCAG-3) and 1492R (5-CGGTTACCTTGTTACGACTT-3). A PCR master mix with a total reaction volume of 25 μl was produced, including 3 μl of genomic DNA, 12.5 μl of 2X GoTaqGreen Master Mix (Promega, USA), 1 μl each of forward and reverse primers, and 7.5 μl of nuclease-free water. The program cycle was conducted under the following parameters: initial denaturation at 95ºC for 5 minutes. The amplification comprises 35 cycles: denaturation at 94ºC for 30 seconds, annealing at 57ºC for 30 seconds, and extension at 72ºC for 1 minute, concluding with a final extension at 72ºC for 10 minutes.

The target sequence of 1400 bp from the isolates in the 16S ribosomal RNA variants was successfully amplified. The size of PCR products was confirmed using 2% agarose gel electrophoresis in 1XTBE buffer, employing Safe dye (Bioland, USA) for staining. The amplified gene has been identified by comparing fragment size to the 100bp DNA marker (Froggabio/Canada). PCR products from isolates, utilizing both forward and reverse primers, were sent to Macrogen in South Korea for sequencing. The quality of the DNA that was extracted was evaluated using a Nano-Drop UV spectrophotometer. Phylogenetic trees have been established. All sequence files were analyzed using MEGA11 and subsequently aligned with NCBI-BLAST (http://www.ncbi.nlm.nih.gov/BLAST/).

2.5 Statistical Analysis:

Statistical analysis was conducted with GraphPad Prism version 9.0, and relationships among categorical variables were evaluated using Fisher's exact test.

3. Results:

Of all the 80 clinical samples obtained from cancer patients, 26 isolates (32.5%) were identified as ESKAPE pathogens, while 54 isolates (67.5%) were categorized as non-ESKAPE bacteria Figure 1. *K. pneumoniae* was the predominant ESKAPE pathogen at 12.5%, followed by *P. aeruginosa* at 7.5%, *S. aureus* at 5.0%, *A. baumannii* at 3.75%, *E. faecium* at 2.5%, and *E. cloacae* at 1.25%. These were isolated from various specimen categories. Maximum represented by urine 31.25% (n = 25), followed by blood 26% (n = 21), stool 20% (n = 16), and swab samples 13.75% (n = 11) and sputum 8.75% (n = 7). In Table 1, the frequency of bacterial isolates is presented by specimen category.

3.1 Demographic and Clinical Characteristics of the Samples:

The clinical presentations of the cancer patient population included a wide range of hematological and solid malignancies. Leukemia was identified as the leading condition, contributing to 50 of the total 80 cases, with a nearly balanced

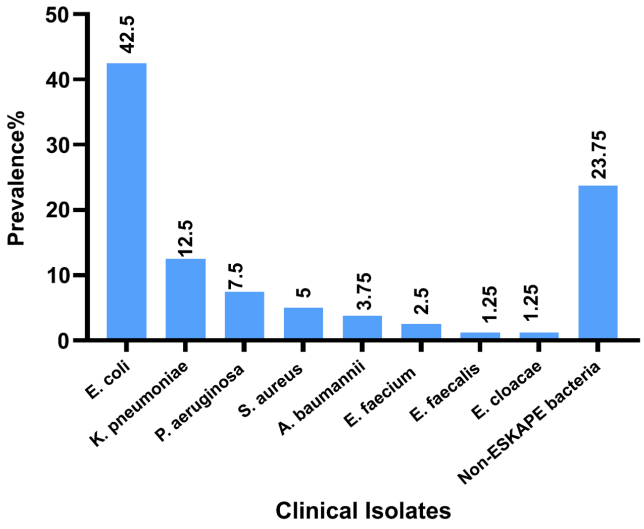

Figure 1. Prevalence of ESKAPE isolates in cancer patients.

distribution between males (27) and females (23). Multiple myeloma was identified in 9 cases, consisting of 7 males and 2 females, while a total of 8 cases had been diagnosed with lymphoma, consisting of 7 males and 1 female. Additionally, sarcoma was identified in 3 cases, consisting of 1 male and 2 females, and solid tumors contributed to a total of 10 cases, consisting of 4 males and 6 females. Overall, 46 of the 80 patients were males and 34 females. These clinical characteristics are summarized in Table 2. Table 3 presents the distribution of the bacterial isolates based on the underlying cancer types, including both ESKAPE and non-ESKAPE bacteria, thereby indicating the unique roles played by the various malignancies in the isolation of the bacterial species.

3.2 Antimicrobial Susceptibility Testing (AST):

Antimicrobial susceptibility testing was carried out with the VITEK 2 system. The *K. pneumoniae* isolates (K1–K10) displayed different susceptibility patterns Figure 2. Responses to third- and fourth-generation cephalosporins, such as ceftriaxone, ceftazidime, and cefepime, exhibited variability, with around fifty percent of the isolates demonstrating non-susceptibility.

The effectiveness of β-lactam / β -lactamase inhibitor combinations varied across isolates. arbapenems, such as meropenem, imipenem, and ertapenem, exhibited diverse responses; some isolates maintained susceptibility, whilst others displayed intermediate or resistant phenotypes.

Additionally, *P. aeruginosa* isolates showed considerable variation in antibiotic susceptibility. Antipseudomonal β-lactams, such as ceftazidime, cefepime, and piperacillin /tazobactam, have inconsistent susceptibility profiles. Fluoroquinolones (ciprofloxacin and levofloxacin) had variable efficacy, with resistance observed in some strains. Aminoglycosides,

Table 1. Prevalence of bacterial isolates in clinical specimens in cancer patients.

Bacterial Isolate	No. of Isolates	Blood	Urine	Sputum	Swab	Stool
K. pneumoniae	10	2	6	0	1	1
P. aeruginosa	6	3	1	1	1	0
S. aureus	4	2	0	1	1	0
A. baumannii	3	0	0	3	0	0
E. cloacae	1	1	0	0	0	0
Non-ESKAPE bacteria	54	13	16	2	8	15
Total	80	21(26.25%)	25(31.25%)	7(8.75%)	11(13.75%)	16(20%)

Table 2. Gender -based Distribution of cancer types among patients.

Types of cancer	No. cases	Male	Female
Leukemia	50	27	23
Myeloma	9	7	2
Lymphoma	8	7	1
Sarcoma	3	1	2
Solid Tumors	10	4	6
Total	80	46	34

including tobramycin, gentamicin, and amikacin, have shown inconsistent effectiveness against *P. aeruginosa*, remaining effective in some isolates, therefore suggesting their potential as isolate-specific treatment alternatives.

Acinetobacter baumannii isolates demonstrated substantial resistance to the majority of tested β-lactams and carbapenems, including imipenem and meropenem, with the majority of isolates categorized as resistant. On the other hand, colistin remained effective against most isolates, whereas tigecycline showed restricted yet discernible susceptibility, highlighting the need for last-resort treatments.The *E. cloacae* isolate showed less sensitivity to cephalosporins such as ceftriaxone and ceftazidime, but carbapenems like meropenem and imipenem still worked. Among Gram-positive organisms, *E. faecium* isolates exhibited consistent susceptibility to glycopeptides (vancomycin and teicoplanin) and linezolid. *S. aureus* isolates consistently demonstrated resistance to oxacillin, whereas sensitivity to vancomycin and linezolid was primarily preserved, exhibiting varied responses to erythromycin and clindamycin. The susceptibility profiles that were acquired from the testing were used to categorize the isolates as either MDR or non-MDR bacteria. The distribution of MDR phenotypes showed that MDR isolates were much more common among the ESKAPE bacteria that were being evaluated. *K. pneumoniae* exhibited a complete MDR profile, with all 10 isolates categorized as MDR. Likewise, all isolates of *S. aureus, E. faecium*, and *E. cloacae* had an MDR phenotype. *Pseudomonas aeruginosa* exhibited both MDR and non-MDR profiles among non-fermenting Gram-negative bacteria, with three isolates designated as MDR and three as non-MDR, indicating the greatest proportion of non-MDR isolates in the collection. *Acinetobacter baumannii* exhibited heterogeneous resistance patterns, which included two MDR isolates and one non-MDR strain. Table 4 shows that most of the isolates (84.6%) were MDR phenotypes, whereas just a small number (15.4%) were not MDR.

3.3 Biofilm Formation:

The capacity of the isolated ESKAPE species to produce biofilms showed variability across the biofilm assay. *K. pneumoniae* exhibited an even distribution of biofilm formation strength, with four weak, three moderate, and three strong biofilm-forming isolates. *P. aeruginosa* demonstrated a strong tendency toward biofilm formation, as no weak biofilm-forming isolates were observed; four isolates were classified as strong biofilm formers, while two showed moderate biofilm-forming ability. All *S. aureus* isolates exhibited strong biofilm formation. *Acinetobacter baumannii* displayed a balanced biofilm profile, with one weak, one moderate, and one strong biofilm-forming isolate. Among *Enterococcus* species, *E. faecium* showed only weak biofilm-forming ability, represented by two isolates. In addition, *E. cloacae* were represented by a single isolate exhibiting weak biofilm-forming ability. The distribution of weak, moderate, and strong biofilm-forming capacities among the analyzed ESKAPE species is summarized in Table 5.

Table 3. Prevalence of bacterial isolates among various cancer types.

Bacteria	Type of cancer				
	Leukemia	Sarcoma	Lymphoma	Myeloma	Solid Tumors
K. pneumoniae	7	0	1	1	1
P. aeruginosa	5	0	0	0	1
S. aureus	0	0	1	2	1
A. baumannii	0	0	2	1	0
E. faecium	2	0	0	0	0
E. cloacae	1	0	0	0	0
Non-ESKAPE bacteria	35	3	4	5	7
Total (%)	50(62.5)	3(3.75)	8(20)	9(11.25)	10(12.5)

Table 4. Prevalence of bacterial isolates among various cancer types.

Bacterial species	MDR (n)	Non-MDR (n)
K. pneumoniae	10	0
P. aeruginosa	3	3
S. aureus	4	0
A. baumannii	2	1
E. faecium	2	0
E. cloacae	1	0
Total (%)	22(84.6)	4(15.4)

3.4 Biofilm Strength in Relation to MDR Status among ESKAPE Isolates:

The correlation between the strength of biofilm formation and multidrug resistance was assessed among the ESKAPE isolates Table 6. MDR isolates were found more frequently in weak, moderate, and strong biofilm-forming groups than in non-MDR isolates. Fisher's exact test revealed no statistically significant association between biofilm strength and multidrug-resistant status ($p = 0.3689$).

3.5 Molecular Identification of Selected ESKAPE Isolates:

DNA was successfully extracted from seven selected isolates from the ESKAPE group. The quality and quantity of the DNA were verified using a NanoDrop. The DNA purity values ranged between 1.63 and 2.02. These numbers show that the chemicals and proteins employed during extraction were eliminated in a way that was acceptable. Also, the DNA concentrations ranged from 117 to 466 ng μL^{-1}, which showed that there was adequate template volume for Sanger sequencing to follow.

The 16S rRNA gene was amplified using PCR for selected ESKAPE isolates, and each sample produced a clear DNA band of approximately 1400 bp. Figure 3 shows the electrophoretic profile results. Each sample showed a distinct band at the 1.4 kb region, confirming successful amplification of the universal 16S rRNA gene. The PCR results were sequenced to validate each bacterium's species identity. The sequencing of the amplified 16S rRNA gene fragments yielded high-quality readings for all selected isolates. BLAST analysis demonstrated substantial sequence similarity with pertinent reference sequences in the NCBI GenBank database, hence validating species-level identification and supporting the phenotypic results obtained from the VITEK® 2 system. Figure 4 displays typical Sanger sequencing chromatograms of partial 16S rRNA gene regions for each species.

The BLAST outcomes revealed that the sequences were quite close to the reference sequences, which confirmed the first identifications made with Vitek-2. The NCBI GenBank database received all of the sequences. The accession codes are *K. pneumoniae* (PV762025), *P. aeruginosa* (PV762021), *S. aureus* (PV762022), *A. baumannii* (PV762024), *E. faecium* (PV762023), and *E. cloacae* (PV762020). Phylogenetic analysis utilizing partial 16S rRNA gene sequences indicated that the research isolates clustered into their corresponding species-specific clades when compared with reference sequences obtained from the NCBI database. The study consistently identifies clusters that are closely related to known reference strains of *K. pneumoniae, P. aeruginosa, S. aureus, A. baumannii, E. faecium, and E. cloacae*, with strong bootstrap support. Figure 5 shows the intricate phylogenetic relationships.

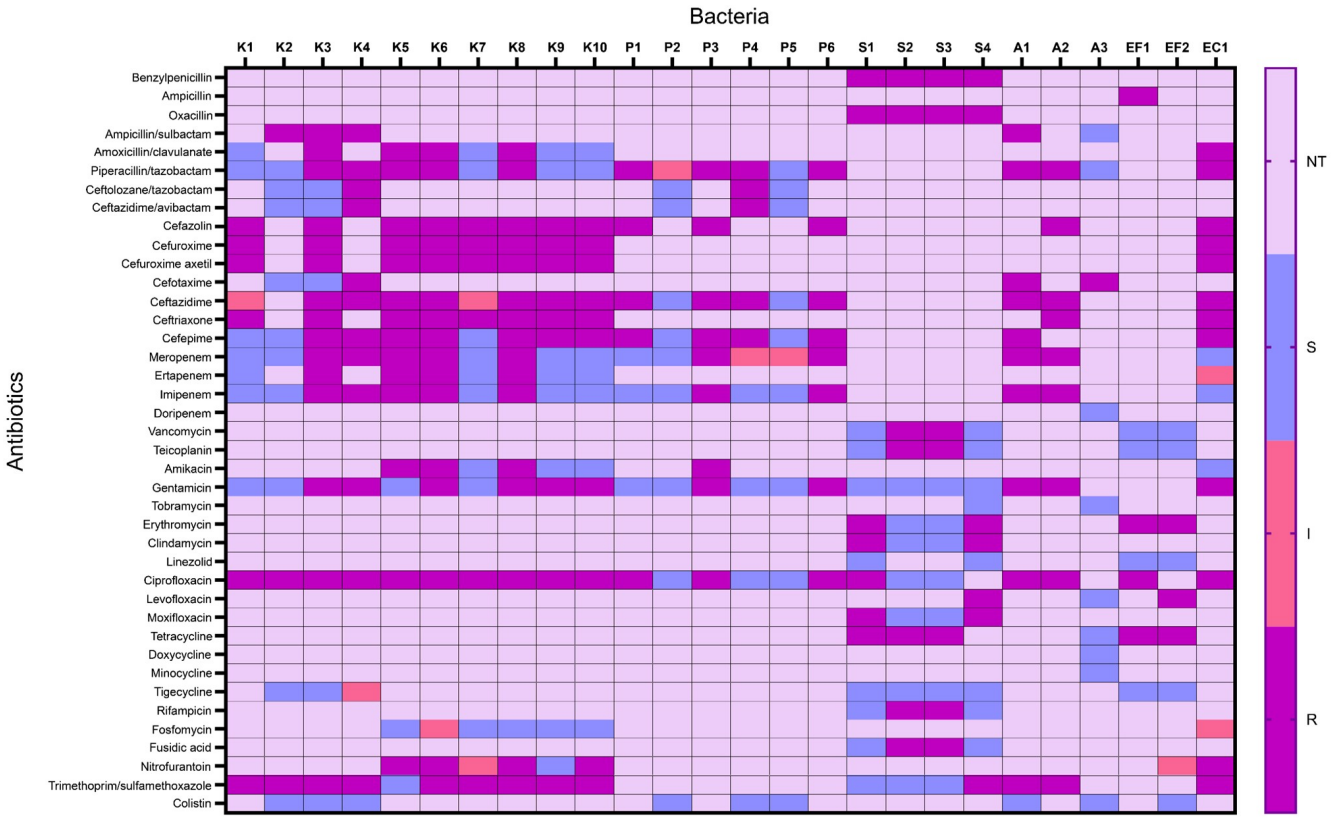

Figure 2. map showing the antimicrobial susceptibility profiles of ESKAPE bacterial isolates determined using the VITEK® 2 automated system. Columns represent bacterial isolates arranged in the following order: *K. pneumoniae* (K1–K10), *P. aeruginosa* (P1–P6), *S. aureus* (S1–S4), *A. baumannii* (A1–A3), *E. faecium* (EF1–EF2), and *E. cloacae* (EC1). Rows represent individual antibiotic agents. Color coding indicates susceptibility interpretation as resistant (R), intermediate (I), susceptible (S), or not tested (NT).

Table 5. Quantitative Assessment of Biofilm-Forming Capacity in ESKAPE isolates from cancer Patients.

Bacterial Isolates	No. of Isolates	Weak No. (%)	Moderate No. (%)	Strong No. (%)
K. pneumoniae	10	4(40)	3(30)	3(30)
P. aeruginosa	6	0	2(33.4)	4(66.6)
S. aureus	4	0	0	4(100)
A. baumannii	3	1(33.33)	1(33.33)	1(33.33)
E. faecium	2	2(100)	0	0
E. cloacae	1	1(100)	0	0

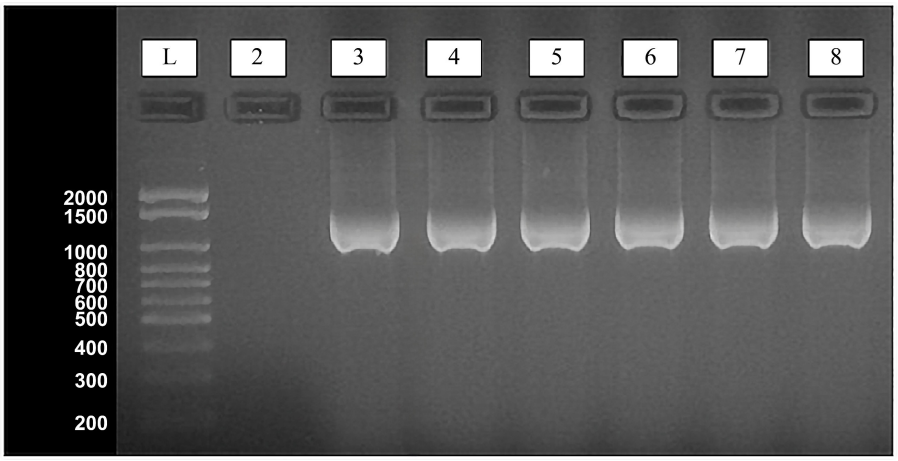

Figure 3. Agarose gel electrophoresis of 16S rRNA gene amplification from different bacterial isolates from cancer patients. Lane 1: Ladder of 100bp, Lane 2: Negative control, Lane 3: *E. cloacae*, Lane 4: *P. aeruginosa*, Lane 5: *S. aureus*, Lane 6: *E. faecium*, Lane 7: *A. baumannii*, and Lane 8: *K. pneumoniae*, showing clear single bands of approximately 1400 bp, corresponding to the expected size of the amplified 16S rRNA fragment.

Table 6. Distribution of biofilm formation strength according to MDR status among ESKAPE isolates (n = 26).

Biofilm strength	MDR (n)	Non-MDR (n)	Total
Weak	7	1	8
Moderate	4	2	6
Strong	11	1	12
Total	22	4	26

Figure 4. Representative partial 16S rRNA gene Sanger sequencing chromatograms of selected ESKAPE isolates. Panels correspond to (A) *K. pneumoniae*, (B) *P. aeruginosa*, (C) *S. aureus*, (D) *A. baumannii*, (E) *E. faecium*, and (F) *E. cloacae*. The displayed sequence regions represent high-quality reads used for downstream BLAST analysis and phylogenetic reconstruction.

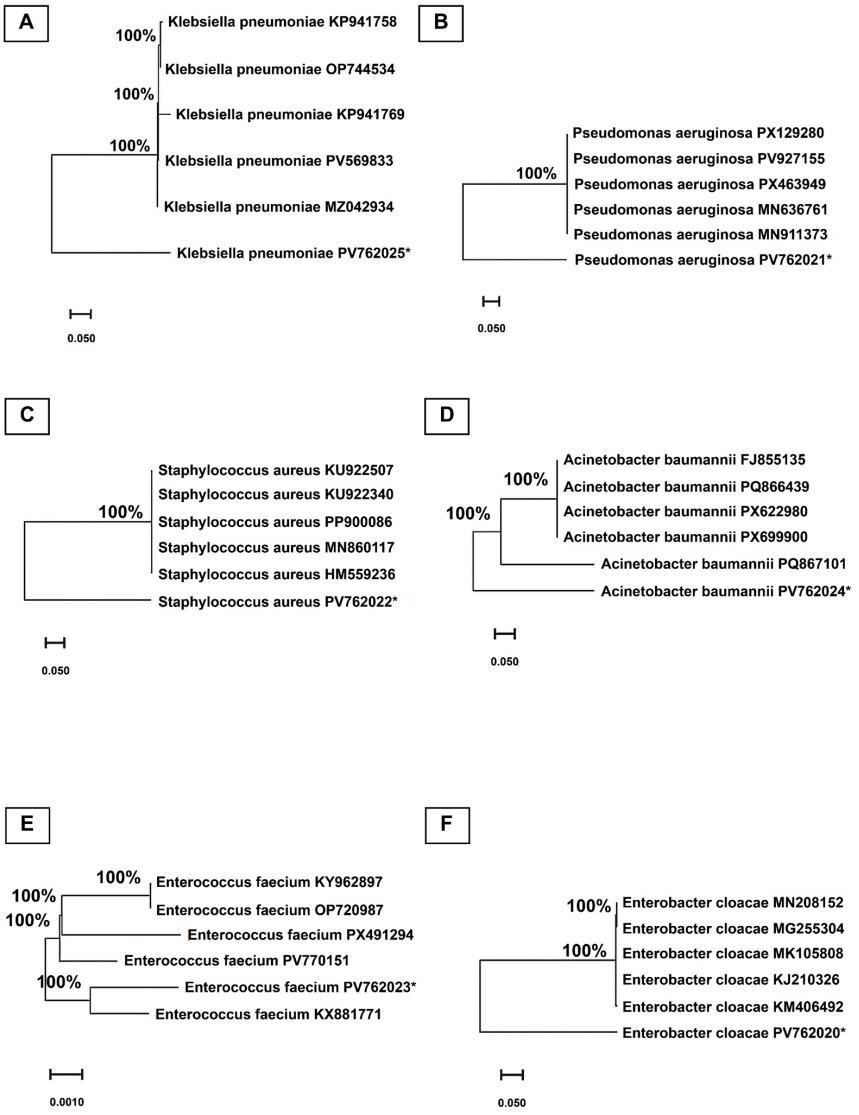

Figure 5. Maximum Likelihood phylogenetic- trees based on partial 16S rRNA gene sequences of study isolates and related reference sequences retrieved from the NCBI database. Panels represent phylogenies of (A) *K. pneumoniae* (GenBank accession no. PV762025), (B) *P. aeruginosa* (PV762021), (C) *S. aureus* (PV762022), (D) *A. baumannii* (PV762024), (E) *E. faecium* (PV762023), and (F) *E. cloacae* (PV762020). Study isolates analyzed in the present work are indicated by an asterisk (*). Bootstrap support values (1,000 replicates) are shown at branch nodes. Scale bars represent nucleotide substitutions per site.

4. Discussions:

Infectious diseases represent a major risk for cancer patients, ranking as the second-leading cause of cancer deaths [2]. Chemotherapy and other treatments often weaken the immune systems of cancer patients, leaving them susceptible to opportunistic infections. Consequently, the compromised immune systems of these individuals necessitate the simultaneous use of antibiotics to eliminate bacterial cells in infections. Regrettably, antibiotic resistance may arise as a result of this repeated exposure to antibiotics [13, 14]. The emergence of antimicrobial-resistant infections presents an urgent concern for successful cancer treatment. Among these drug-resistant nosocomial infectious isolates are ESKAPE pathogens, which are currently the forefront of cancer infections and antimicrobial-resistant problems [8]. The higher recovery of isolates from urine and blood specimens reflects the prominence of urinary tract and bloodstream infections in cancer patients, particularly those requiring prolonged catheterization, intensive care support, or invasive diagnostic and therapeutic interventions [2]. Unlike community-acquired infections, urinary infections in hospitalized cancer patients are increasingly associated with healthcare-associated Gram-negative pathogens, including *K. pneumoniae*, Enterobacter species, *P. aeruginosa*, and *A. baumannii*, which are widely acknowledged for their capacity to endure in hospital settings and colonize indwelling medical equipment. The frequent involvement of these organisms in oncology settings may be attributed to their intrinsic and acquired resistance mechanisms, biofilm-forming capacity, and selective pressure imposed by prolonged antibiotic exposure, all of which complicate infection control and therapeutic management in cancer care [15]. In the current study, the hematological malignancies, especially leukemia, contributed to the majority of cancer cases. Prior study corroborates this, demonstrating that individuals with hematological malignancies have a significant risk of infection development because of the drug-intensive treatments they go through. Leukemia patients have a high risk of developing bacterial infection because of neutropenia [16]. Furthermore, the research sample had a greater number of men than women, aligning with previous findings by Khan, Papier [17] demonstrating that cancer incidence and infection susceptibility may vary by sex. Different forms of cancer may have different bacterial isolates because of differences in the patients' immune systems, how strong the therapy is, and how long they stay in the hospital. A broad range of approaches is employed to identify the ESKAPE group, including automated and conventional microbiological procedures that reliably identify and authenticate bacterial isolates from clinical samples. All bacterial isolates were first identified by standard procedures, including cultural characteristics on various media and biochemical tests. After that, the Vitek-2 automated system, which is a quick and accurate way to identify bacteria, was used to confirm the results [18]. This comprehensive technique improves the reliability of detecting bacteria and offers a good basis for further laboratory tests, such as antibiotic susceptibility testing [19]. Examination of isolates indicated that members of the Enterobacterales group, particularly K. pneumoniae, exhibited multidrug resistance. This result aligns with the systematic review performed by Ntim et al. [13], which included 132 studies published between 2000 and 2024 that showed that isolates taken from cancer patients often have MDR characteristics. On the other hand, non-fermenting Gram-negative bacteria like *P. aeruginosa* and *A. baumannii* showed both MDR and non-MDR phenotypes, which shows that resistance patterns are not the same across the board. For Gram-positive bacteria, *S. aureus* and *E. faecium* were mostly categorized as MDR. In general, MDR phenotypes were the most common among most isolates. This shows how serious antibiotic resistance is in infections that impact cancer patients with weak immune systems. These results highlight the necessity of ongoing monitoring and effective antimicrobial stewardship efforts in oncology environments.

The heat-map-based antimicrobial susceptibility study revealed distinct species-specific resistance patterns among ESKAPE infections. *K. pneumoniae* and *A. baumannii* exhibited considerable resistance to -lactams and carbapenems, thus confirming their designation as high-priority nosocomial pathogens. Recent cancer investigations have recorded similar resistance patterns, demonstrating that prolonged antibiotic exposure and hospital-associated selective pressure facilitate multidrug resistance in Enterobacterales and non-fermenting Gram-negative bacteria [13].

The variable susceptibility observed among *P. aeruginosa* isolates underscores intra-species heterogeneity, emphasizing the necessity for isolate-specific antimicrobial therapy instead of empirical escalation [20].

The ESKAPE pathogens exhibited clear species-dependent differences in biofilm formation. *K. pneumoniae* had a significant ability to form biofilms; according to Li and Ni [21], 54% of clinical K. pneumoniae isolates as strong biofilm producers (29% moderate, 14% weak). Additionally, *P. aeruginosa* is a potent biofilm-former and a model organism for biofilm research. In the present study, all *P. aeruginosa* isolates formed moderate to strong biofilm with no weak biofilm producers detected, consistent with its well-known role in device-associated infections [22]. Enterococcus faecium and *E. cloacae* isolates were weak biofilm producers. A. baumannii showed a more even distribution of weak, moderate, and strong biofilm formers. These patterns probably show that the species have different adhesion factors and virulence features [23, 24]. Isolates were grouped by resistance class (MDR and non-MDR), and there was no significant association between antibiotic resistance and biofilm strength (Fisher's $p = 0.3689$). For example, similar proportions of MDR isolates exhibited weak (7 of 22) and strong (11 of 22) biofilm forma-

tion, indicating that high biofilm production was not confined to more-resistant strains. This result is consistent with Cepas et al.[25], who analyzed two species of microorganisms included among the ESKAPE pathogens (*K. pneumoniae* and *P. aeruginosa*), and Son et al. [26], who analyzed two species: *E. faecium* and *E. faecalis*. Both studies reported no clear link between antibiotic resistance and biofilm-forming ability. However, according to Anik et al. [27]. No overall association was found between antimicrobial resistance phenotype and biofilm formation.

Molecular identification of the selected ESKAPE isolates was performed using 16s rRNA gene sequencing. 16S rRNA is widely used in clinical practice, where it has served as a powerful tool for bacterial identification and diagnostics, and is described as one of the most studied sequences ever [28]. One significant thing that makes the 16S rRNA gene useful is that it has many conserved and hypervariable sections. This means that there are many ways to construct PCR primers [29]. Phylogenetic analysis utilizing partial 16S rRNA gene sequences validated the taxonomic classification of the study isolates, as all sequences consistently grouped within their respective species-specific clades alongside reference sequences obtained from the NCBI GenBank database [30]. This clustering corroborates the reliability of the molecular identification method utilized in this study and illustrates the appropriateness of partial 16S rRNA gene sequencing for species-level identification of clinically significant ESKAPE pathogens. These findings substantiate the efficacy of 16S rRNA gene sequencing as a reliable method for verifying bacterial identity in clinical microbiology contexts.

5. Conclusions:

This study thoroughly examined antibiotic resistance patterns, biofilm formation abilities, and genotypic characteristics of ESKAPE bacteria isolated from cancer patients in Erbil, Kurdistan, Iraq. A wide variety of methods are used for identification of ESKAPE group, using conventional microbiological techniques, including culture characteristics on different media and biochemical tests, and automated (Vitek-2 automated system) to accurately identify and validate bacterial isolates. *K. pneumoniae* was the most frequently isolated organism, followed by *P. aeruginosa* and *S. aureus*, and the bacterial isolates were more frequently found in urine samples compared to other clinical specimens. Leukemia, was the majority of cancer cases, and the study population included a higher proportion of men than women.

Furthermore, the findings revealed a high prevalence of MDR isolates, especially in Enterobacterales, alongside species-specific differences in biofilm-forming ability. Additionally, there was no significant difference between antibiotic resistance and biofilm formation. Molecular identification using 16S rRNA sequencing and phylogenetic analysis confirmed the accurate species-level identification of the selected isolates. Collectively, these results highlight the clinical importance of ESKAPE pathogens in immunocompromised patients and emphasize the need for appropriate diagnostic approaches and effective antimicrobial management strategies in oncology settings.

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Data Availability Statement: All of the data supporting the findings of the presented study are available from corresponding author on request.

Declarations:

Conflict of interest: The authors declare no conflicts of Interest.

Ethical approval: This study was reviewed and approved by the Research Ethics Committee Office (RECO) of the Department of Biology, College of Science, Salahaddin University-Erbil. The ethical approval was issued on 8 September 2024 under the reference number 4S/316. All samples were handled according to ethical standards, and patient information was kept confidential.

Author contributions: Akhter A. Ahmed designed the study and supervised the research work. Zahraa F. Hasan collected the samples, performed the data analysis, and wrote the manuscript. Akhter A. Ahmed reviewed and edited the manuscript. All authors read and approved the final manuscript.

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References

[1] Mirabela Romanescu, Camelia Oprean, Adelina Lombrea, Bianca Badescu, Ana Teodor, George D Constantin, Minodora Andor, Roxana Folescu, Delia Muntean, Corina Danciu, et al. Current state of knowledge regarding who high priority pathogens—resistance mechanisms and proposed solutions through candidates such as essential oils: A systematic review. *International journal of molecular sciences*, 24(11):9727, 2023, doi:10.3390/ijms24119727.

[2] Amila K Nanayakkara, Helen W Boucher, Vance G Fowler Jr, Amanda Jezek, Kevin Outterson, and David E Greenberg. Antibiotic resistance in the patient with cancer: Escalating challenges and paths forward. *CA:*

a cancer journal for clinicians, 71(6):488–504, 2021, doi:10.3322/caac.21697.

[3] Talar Ibrahim Hasan and Akhter Ahmed Ahmed. Characterization, antibacterial and antibiofilm evaluation of biosynthesized silver nanoparticles from pseudomonas aeruginosa against drug resistant acinetobacter baumannii. *Al Mustansiriyah Journal of Pharmaceutical Sciences*, 23(3):307–321, 2023, doi:10.32947/ajps.v23i3.1049.

[4] Minichil Worku, Gizeaddis Belay, and Abiye Tigabu. Bacterial profile and antimicrobial susceptibility patterns in cancer patients. *PLoS One*, 17(4):e0266919, 2022, doi:10.1371/journal.pone.0266919.

[5] Jessica B Kelly, Aaron C Nolan, and Merve S Zeden. How can we escape the eskapes: Antimicrobial resistance mechanisms and what lies ahead? *PLoS pathogens*, 20(6):e1012270, 2024, doi:10.1371/journal.ppat.1012270.

[6] Lejla Daruka, Márton Simon Czikkely, Petra Szili, Zoltán Farkas, Dávid Balogh, Gábor Grézal, Elvin Maharramov, Thu-Hien Vu, Levente Sipos, Szilvia Juhász, et al. Eskape pathogens rapidly develop resistance against antibiotics in development in vitro. *Nature Microbiology*, 10(2):313–331, 2025, doi:10.1038/s41564-024-01891-8.

[7] Peter Vella, Reshma Srilakshmi Rudraraju, Thomas Lundbäck, Hanna Axelsson, Helena Almqvist, Michaela Vallin, Gunter Schneider, and Robert Schnell. A fabg inhibitor targeting an allosteric binding site inhibits several orthologs from gram-negative eskape pathogens. *Bioorganic & Medicinal Chemistry*, 30:115898, 2021, doi:10.1016/j.bmc.2020.115898 .

[8] Kafayat Yusuf, Venkatesh Sampath, and Shahid Umar. Bacterial infections and cancer: exploring this association and its implications for cancer patients. *International Journal of Molecular Sciences*, 24(4):3110, 2023, doi:10.3390/ijms24043110.

[9] Patricia M. Tille. *Bailey Scott's Diagnostic Microbiology*. Elsevier, 2025.

[10] Carmen Limban, Alexandru V Missir, Ileana C Chirita, George M Nitulescu, Miron T Caproiu, Mariana C Chifiriuc, and Anca M Israil. Synthesis and antimicrobial properties of new 2-((4-ethylphenoxy) methyl) benzoylthioureas. *Chemical Papers*, 65(1):60–69, 2011, doi:10.2478/s11696-010-0092-9.

[11] T Mathur, S Singhal, S Khan, DJ Upadhyay, T Fatma, and A Rattan. Detection of biofilm formation among the clinical isolates of staphylococci: an evaluation of three different screening methods. *Indian journal of medical microbiology*, 24(1):25–29, 2006, doi:10.1016/S0255-0857(21)02466-X.

[12] Pragya Lakshmi, Alok Bharadwaj, and Ranjan Kumar Srivastava. Molecular detection and identification of bacteria in urine samples of asymptomatic and symptomatic pregnant women by 16s rrna gene sequencing. *Archives of Clinical Infectious Diseases*, 15(15):e101136, 2020, doi:10.5812/archcid.101136.

[13] Onyansaniba K Ntim, Aaron Awere-Duodu, Abdul-Halim Osman, and Eric S Donkor. Antimicrobial resistance of bacterial pathogens isolated from cancer patients: a systematic review and meta-analysis. *BMC Infectious Diseases*, 25(1):296, 2025.

[14] A Abd-Al-Ridha Al-Abdullah et al. Phenotyping and genotyping evaluation of e. coli produces carbapenemase isolated from cancer patients in al-basrah, iraq. *Archives of Razi Institute*, 78(3):823, 2023, doi:10.22092/ari.2022.359869.2493.

[15] Li Yajun, Zhou Xuan, Tian Juan, Tu Rui, Xiao Zuyan, Zhang Bingbing, Zhou Ruiqi, Du Guiqin, and Zhao Tao. Risk factors for catheter-associated urinary tract infection in an intensive care unit: a matched case-control study. *BMC Infectious Diseases*, 25(1):617, 2025, doi:10.1186/s12879-025-10839-0.

[16] Aiah M Khateb, Ahmed S Barefah, Zinab O Doha, Marwah M Albakri, Osman Radhwi, Ali Algiraigri, and Esam I Azhar. Microbial infections in lymphocytic leukemia: A 12 year analysis in the saudi population. *Journal of Infection and Public Health*, page 103005, 2025, doi:10.1016/j.jiph.2025.103005.

[17] Maira Khan, Keren Papier, Kirstin Pirie, Joshua Atkins, Tim Key, and Ruth Travis. Sex differences in cancer incidence: Prospective analyses in the uk biobank. *Cancer Research*, 84(6_Supplement):4850–4850, 2024, doi:10.1038/s41416-025-03028-y.

[18] Parviz Mohajeri, Hossein Faridafshar, and Sara Kooti. Identification and antimicrobial susceptibility testing of streptococcus agalactiae associated urinary tract infections using vitek 2 system. *Iranian journal of microbiology*, 17(2):239, 2025, doi:10.18502/ijm.v17i2.18383.

[19] Richard Bigge, Boyke Bunk, Wolfram W Rudolph, Florian Gunzer, Sina M Coldewey, Thomas Riedel, and Percy Schröttner. Comparative study of different diagnostic routine methods for the identification of acinetobacter radioresistens. *Microorganisms*, 10(9):1767, 2022, doi:10.3390/microorganisms10091767.

[20] Aiman M Momenah, Rafat Ahmed Bakri, Naif A Jalal, Sami S Ashgar, Rakan Fahad Felemban, Farkad Bantun, Sumyya H Hariri, Abeer A Barhameen, Hani Faidah, and Hamdi M Al-Said. Antimicrobial resistance pattern of pseudomonas aeruginosa: An 11-year experience in a tertiary care hospital in makkah, saudi arabia. *In-*

fection and Drug Resistance, pages 4113–4122, 2023, doi:10.2147/idr.S409726.

[21] Yin Li and Ming Ni. Regulation of biofilm formation in klebsiella pneumoniae. *Frontiers in microbiology*, 14:1238482, 2023, doi:10.3389/fmicb.2023.1238482.

[22] Felipe Francisco Tuon, Leticia Ramos Dantas, Paula Hansen Suss, and Victoria Stadler Tasca Ribeiro. Pathogenesis of the pseudomonas aeruginosa biofilm: a review. *Pathogens*, 11(3):300, 2022, 10.3390/pathogens11030300.

[23] Aisha M Alamri, Afnan A Alsultan, Mohammad A Ansari, and Amani M Alnimr. Biofilm-formation in clonally unrelated multidrug-resistant acinetobacter baumannii isolates. *Pathogens*, 9(8):630, 2020, doi:10.3390/pathogens9080630.

[24] Vanessa Silva, Catarina Freitas, Jessica Ribeiro, Gilberto Igrejas, and Patricia Poeta. Comparative analysis of antibiotic resistance and biofilm formation in enterococcus spp. across one health domains. *FEMS microbes*, 6:xtaf005, 2025, 10.1093/femsmc/xtaf005.

[25] Virginio Cepas, Yuly López, Estela Muñoz, Dora Rolo, Carmen Ardanuy, Sara Martí, Mariona Xercavins, Juan Pablo Horcajada, Jordi Bosch, and Sara M Soto. Relationship between biofilm formation and antimicrobial resistance in gram-negative bacteria. *Microbial Drug Resistance*, 25(1):72–79, 2019, doi:10.1089/mdr.2018.0027.

[26] Yongwoo Son, Yeung Bae Jin, Eun-Jeong Cho, Ae Ra Park, Rochelle A Flores, Binh T Nguyen, Seung Yun Lee, Bujinlkham Altanzul, Kwang Il Park, Wongi Min, et al. Comparative analysis of antibiotic resistance and biofilm characteristics of two major enterococcus species from poultry slaughterhouses in south korea. *Veterinary Sciences*, 11(4):180, 2024, doi:10.3390/vetsci11040180.

[27] Tasnimul Arabi Anik, Rahat Uzzaman, Khandaker Toyabur Rahman, Abir Hossain, Faruk Islam, Mosammod Nowshin Tasnim, Shahin Ara Begum, Humaira Akhter, and Anowara Begum. Comparative analysis of biofilm formation and antibiotic resistance in five eskape pathogen species from a tertiary hospital in bangladesh. *Antibiotics*, 14(8):842, 2025, doi:10.3390/antibiotics14080842.

[28] Oldřich Bartoš, Martin Chmel, and Iva Swierczková. The overlooked evolutionary dynamics of 16s rrna revises its role as the "gold standard" for bacterial species identification. *Scientific reports*, 14(1):9067, 2024, doi:10.1038/s41598-024-59667-3.

[29] Hayley B Hassler, Brett Probert, Carson Moore, Elizabeth Lawson, Richard W Jackson, Brook T Russell, and Vincent P Richards. Phylogenies of the 16s rrna gene and its hypervariable regions lack concordance with core genome phylogenies. *Microbiome*, 10(1):104, 2022, doi:10.1186/s40168-022-01295-y.

[30] Sana MH Al-Shimmary, Nadira S Mohamed, Safaa AS Al-Qaysi, and Asmaa M Salih Almohaidi. Phylogeny analysis of gyrb gene and 16s rrna genes of pseudomonas aeruginosa isolated from iraqi patients. *Research Journal of Pharmacy and Technology*, 14(5):2517–2521, 2021, doi:10.52711/0974-360X.2021.00443.

التوصيف الجزيئي والظاهري لممرضات (*ESKAPE*) المعزولة من مرضى السرطان

* زهراء فرزدق حسن، أختر أحمد أحمد

قسم علوم الحياة، كلية العلوم، جامعة صلاح الدينأربيل، أربيل، العراق

* الباحث المسؤول: *zahraa.hasan@su.edu.krd*

الخلاصة

يُعد مرضى السرطان أكثر عرضة للإصابة بممرضات (*ESKAPE*) المقاومة للمضادات الحيوية، وهي (*Enterococcus faecium* و *Staphylococcus aureus* و *Klebsiella pneumoniae* و *Acinetobacter baumannii* و *Pseudomonas aeruginosa* و *Enterobacter cloacae*) ، وذلك بسبب ضعف المناعة لديهم والتعرض المتكرر للمضادات الحيوية. هدفت هذه الدراسة إلى التحري عن الخصائص الجزيئية وأنماط المقاومة للمضادات الحيوية وقابلية تكوين الأغشية الحيوية لبكتيريا (*ESKAPE*) المعزولة من مرضى السرطان. جُمعت العينات خلال المدة من تموز إلى تشرين الأول 2024 في مختبر البكتريولوجي بمستشفى نانكلي، أربيل، إقليم كردستان، العراق. وقد تم تحليل 80 عزلة بكتيرية تم الحصول عليها من عينات سريرية مختلفة لمرضى السرطان. استُخدمت الطرق الميكروبيولوجية التقليدية للتشخيص الأولي، بينما استُخدم نظام (*VITEK 2*) الآلي للتأكيد. كما تم تقييم تكوين الغشاء الحيوي باستخدام اختبار أطباق المايكروتايتر، وتم تحديد الحساسية للمضادات الحيوية باستخدام النظام الآلي. وأُجري التحقق الجزيئي لست عزلات مختارة من (*ESKAPE*) باستخدام تسلسل جين (*16SrRNA*) ، تلاه تحليل النشوء والتطور. وأظهرت النتائج أن ممرضات (*ESKAPE*) شكّلت 26 عزلة من أصل 80 عزلة (32.5%) تم الحصول عليها من مرضى السرطان، وكانت أنماط المقاومة المتعددة للأدوية أكثر شيوعًا بشكل خاص في *Klebsiella pneumoniae* . كما اختلفت القدرة على تكوين الأغشية الحيوية بين الأنواع البكتيرية، إلا أنه لم تُلاحظ علاقة معنوية بين حالة المقاومة المتعددة للأدوية وشدة تكوين الغشاء الحيوي. وتبرز هذه النتائج الأهمية السريرية لممرضات (*ESKAPE*) في العدوى التي تصيب مرضى السرطان ذوي المناعة الضعيفة.

الكلمات الدالة: المقاومة المتعددة للأدوية؛ تكوين الغشاء الحيوي؛ مرضى السرطان؛ ممرضات (*ESKAPE*) .

التمويل: تم تمويل هذا البحث من قبل جامعة صلاح الدين ـ أربيل، أربيل، العراق.

بيان توفر البيانات: جميع البيانات التي تدعم نتائج الدراسة المقدمة متاحة من المؤلف المسؤول عند الطلب.

اقرارات:

تضارب المصالح: يقر المؤلف انه ليس لديهم تضارب في المصالح.

الموافقة الأخلاقية: تمت مراجعة هذه الدراسة والموافقة عليها من قبل مكتب لجنة أخلاقيات البحث العلمي (*RECO*) التابع لقسم الأحياء، كلية العلوم، جامعة صلاح الدين ـ أربيل. وقد صدرت الموافقة الأخلاقية بتاريخ 8 سبتمبر 2024 تحت الرقم المرجعي 4 *S*/316 . وتم التعامل مع جميع العينات وفقًا للمعايير الأخلاقية، مع الحفاظ على سرية معلومات المرضى.

مساهمات المؤلفين: قامت اختر احمد احمد بتصميم الدراسة والاشراف على العمل البحثيكما قامت زهراء فرزدق حسن بجمع العينات، واجراء تحليل البانات، وكتابة المخطوطة. قام جميع الؤلفين بقراءة النسخة النهائية والموافقة عليها.