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Original Research Article

Isolation and Characterization of *Klebsiella pneumoniae* from Urinary Tract Infections: A Comparative Study of Diagnostic Methods

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Abstract: ***Background***: Urinary tract infections (UTIs) are a significant public health concern, and Klebsiella pneumoniae is a common cause of these infections. The rise of antibiotic resistance among K. pneumoniae strains necessitates accurate and timely identification for effective treatment. ***Aim***: This study aimed to isolate and identify K. pneumoniae from urine samples of patients with UTIs. It also sought to evaluate the effectiveness of different diagnostic methods, including phenotypic, biochemical, Vitek 2, and molecular techniques. ***Method***: The study was conducted between August 2023 and November 2024 in Babylon province, Iraq, encompassing both public and private healthcare facilities. A total of 100 urine samples were collected from patients with UTIs, covering a diverse range of age groups and both sexes. The study employed a combination of diagnostic approaches. Phenotypic and biochemical tests were performed for initial identification. The Vitek 2 automated system provided rapid and accurate identification. Molecular identification was carried out using PCR amplification of the 16S rRNA gene. ***Results***: *K. pneumoniae* was isolated from 12% of the urine samples. The highest prevalence was observed in the 10-30-year age group, and females had a higher incidence compared to males. All isolates were successfully identified using all diagnostic methods employed in the study. ***Conclusion*:** The study demonstrated the significant role of K. pneumoniae in causing UTIs in the studied population. The findings highlight the importance of utilizing a combination of diagnostic approaches for accurate and timely identification of this pathogen, which is crucial for effective treatment and infection control.

Keywords: *Klebsiella pneumonia*, Urinary Tract Infections (UTIs), Phenotypic Identification, Biochemical Tests, Vitek 2 System, *16S rRNA* gene.

INTRODUCTION

Urinary tract infections (UTIs) are among the most common and serious bacterial infections worldwide. Although they are treatable, controlling UTIs is becoming increasingly difficult due to the widespread antimicrobial resistance observed in uropathogens, particularly those from the *Enterobacteriaceae* family (Polse, Qarani, Assafi, Sabaly, & Ali, 2020). The length of hospital stay and the performance of invasive procedures are risk factors for acquiring *Klebsiella pneumonia*, *K. pneumonia* a gram-negative, non-motile, rod-shaped bacterium with a prominent polysaccharide-based capsule (Alattar, Emran, & Oleiwi, 2024). Klebsiella organisms are resistant to multiple antibiotics, which is believed to be a plasmid-mediated property (Karampatakis, Tsergouli, & Behzadi, 2023). In healthy people, *K. pneumoniae* is frequently found in the respiratory, gastrointestinal, and urinary systems. Hospitals are linked to the majority of *K. pneumonia* infections, which can be lethal if improperly treated (Abbas *et al*., 2024). At least 60% of women will at some point in their lives suffer the symptoms of a urinary tract infection (UTI). In the United States, 10% of women experience one or more symptomatic UTIs year. Young women between the ages of 18 and 24 who engage in sexual activity had the greatest prevalence of UTIs (Mohammed, Mahdi, & Akbar, 2024).

K. pneumoniae can leading threat in modern healthcare, recognized as a primary cause of both hospital- and community-acquired infections. Its role in

the dissemination of antibiotic resistance genes from environmental bacteria to pathogenic strains cannot be overstated. This dangerous pathogen is responsible for a range of serious infections, including hospital-acquired pneumonia, urinary tract infections, bacteremia, surgical site infections, ventilator-associated pneumonia, and septicemia. Moreover, it poses a grave risk to immunocompromised patients, leading to opportunistic infections that can be life-threatening. Combating *K. pneumoniae* is essential in our fight against antibiotic resistance and ensuring patient safety (Ahmed Hasan, T Raheem, & Mohammed Abdulla, 2021).

The identification of Klebsiella pneumoniae is traditionally based on the morphological and physiological properties of the bacteria. Typically, morphological identification involves examining both typical and atypical colonies on selective culture, which is then followed by biochemical assays. In recent years, several reports have demonstrated the feasibility of using the VITEK 2 Advanced Expert System (AES) for this purpose (Karagöz, Acar, & Körkoca, 2015). VITEK systems (bioMerieux, Marcy l'Etoile, France) is a fully automated instrument that provides species identification (ID) and antimicrobial susceptibility testing (AST) for various clinical isolates, and is currently used in many clinical microbiology laboratories worldwide (Nakasone, Kinjo, Yamane, Kisanuki, & Shiohira, 2007). Comparative analyses of phenotypic methods and the sequencing of *16S rRNA*, *khe*, and *rpoB* genes for identifying clinical isolates of Klebsiella pneumoniae often result in incorrect identifications. Consequently, biochemical profile-based identification systems are commonly used for rapid bacterial identification and susceptibility testing. In the past three decades, molecular approaches, particularly 16S *rRNA* gene, have become valuable for accurate species identification, including Klebsiella species (He *et al*., 2016). Therefore this study was aimed to isolation of *K. pneumoniae* from the urine samples of patients with urinary tract infections (UTIs). The diagnosis was performed using various routine and advanced methods. Where began with phenotypic identification and biochemical techniques, followed by VITEK-2 system, and concluded with molecular diagnosis using the housekeeping gene (*16S rRNA*). Additionally, the study classified the infected patients by age group and gender.

MATERIALS AND METHODS

Bacterial Isolates:

In this study, a total of 100 urine specimens from patients with urinary tract infections, from both sexes (male and female) and from different age groups that admitted to the AL-Hashemiya General Hospital and other private laboratories in Babylon province and its environs, Iraq, were used. This study was conducted between August 2023 to November 2024. All urine samples were positioned in clean sterile containers. Urine samples were firstly cultured on blood agar, and MacConkey agar media, and then incubated for 24 h at 37 °C.

Phenotypic identification

The bacterial colonies obtained from urine sample cultures were analyzed using various diagnostic methods. For phenotypic identification, *K . pneumoniae* produces lactose-fermenting pink colonies on MacConkey agar, while mucoid colonies are observed on blood agar. Following this, several biochemical tests were performed, including the oxidase test, Triple Sugar Iron (TSI) test, Simmon's Citrate Agar test, indole test, urease test, and motility test according to (Al-Mousawi & Al-Daraghi, 2022).

Identification by VITEK-2 system

The isolates were identified using the Vitek 2 automated system (Vitek 2 GN-card), which employs fluorescence-based technology. This system includes IDGNB cards, Vitek 2 software, and Advanced Expert System (AES) software from bioMérieux, used per the manufacturer's instructions. Bacterial strains were sub-cultured onto MacConkey agar plates to ensure purity. Suspensions were adjusted to a McFarland standard of 0.5 in sterile sodium chloride solution, with preparation and card filling completed in under 30 minutes. The VITEK 2 IDGNB card, containing 64 wells and 41 fluorescent biochemical tests, was used for identifying gram-negative bacilli. After sealing and incubating the cards for 3 hours, they were monitored every 15 minutes using kinetic fluorescence measurements. The VITEK 2 software analyzed the data and automatically reported the results t. This procedure was done according to (Khafagy *et al*., 2023).

Molecular Identification of *K. pneumoniae*

DNA extraction

Genomic DNA of bacterial isolates with 24h of age in nutrient broth was extracted using the HiMedia kit\ Indian, according to the manufacturer's instructions.

Polymerase Chain Reaction (PCR)

Specific primer were delivered by Macrogen (Seoul company, Korea). The reaction was done by using a PCR master mix (AccuPower® PCR PreMix kit. Bioneer, Korea). Where the PCR reaction mixture was achieved according to information of (Accupower ®PCR-Pre Mix-Kit). Primers, and resulting product sizes used for the identification of *K. pneumoniae*. The table specifically lists the *16S rRNA* gene, targeted using a forward primer named *16S rRNA* -F with the sequence CTACAATGGCATATACAA, and a reverse primer named *16S rRNA* -R with the sequence TTCTGATCTACGATTACT. These primers amplify a 130 base pair (bp) product. The information regarding these primers and their application is referenced to a study by (Salama, El-Mahdy, Moustafa, & Emara, 2024).

PCR combination that was employed in the study. Twenty microliters was the entire reaction

volume. Five microlitres of the amplified DNA segment, known as the template DNA, were present in this combination. It also included 2 µl of the forward primer at 10 pmole/µl and 2 µl of the reverse primer at the same concentration. Short DNA sequences known as primers start the replication process. A lyophilized (freeze-dried) master mix containing the enzymes and buffers required for the PCR reaction was also added, along with 11 µl of deionized water to bring the mixture to the final volume.

PCR thermocycling settings for *16S rRNA* gene amplification was done according to(Al-Obadi, Al-Jailawi, & Jassim, 2014). There were numerous important milestones in the program. In order to separate the double-stranded DNA, it started with an initial denaturation process that lasted five minutes at 95°C. Then came 35 cycles of denaturation (20 seconds) at 95°C, annealing (20 seconds) at 55°C (which allowed primers to attach to the DNA), and extension (30 seconds) at 72°C (where DNA polymerase creates new DNA strands). To guarantee full synthesis of all DNA fragments, a final extension step was carried out for five minutes at 72°C.

Statistical Analysis:

Statistical analysis was done using SPSS version 26to determine the percentage value.

Ethical Approval

This study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki. Prior to collecting samples, verbal and analytical consent was obtained from the patients. To secure this approval, the study protocol, subject information, and consent form were reviewed and authorized by a local ethics committee, with document number 119 0\2023, on December 17, 2023.

RESULTS

Bacterial Isolates

One hundred clinical urine specimens from patients with urinary tract infections (UTIs) were cultured for microbial analysis. Klebsiella pneumoniae was isolated from 12 (12%) of these samples. This indicates that 12% of the tested urine samples were positive for *K. pneumoniae*.

This study revealed a varying distribution of *K. pneumoniae* isolates across different age groups. The highest prevalence was observed in the 10-30 year age range, with 7 isolates (15.57%). A lower proportion of isolates, 4 (11.4%), were obtained from patients aged 31-50 years, while only 1 isolate (5.0%) was recovered from individuals older than 51 years. This higher prevalence in younger age groups (10-30 and 31-50) may be attributed to increased outdoor activity and overall higher activity levels characteristic of these demographics. Furthermore, the isolates demonstrated a skewed distribution by sex, with 9 isolates (15.0%) originating from female patients and 3 (7.57%) from male patients.

Table 1: Occurrence of K. pneumoniae depending on age of patients and sex

	No. of patients (n=100)	***K. pneumoniae* (%) (n=12)**
Age Groups (yrs.)		
10-30	45	7 (15.5%)
31-50	35	4 (11.4%)
>51	20	1 (5 %)
Sex of whole patients		
Female	60	9 (15.0%)
Male	40	3 (7.5%)

Phenotypic Biochemical identification of *K. pneumoniae* isolates

The culture characteristics on MacConkey agar revealed lactose-fermenting colonies, indicated by purple coloration, while blood agar showed mucoid colonies characteristic of bacterial capsules as in Figure (1).

All 12 isolates were identified as *K. pneumoniae* based on a suite of biochemical tests (Figure 2). These tests included Kligler Iron Agar (KIA), which exhibited acid/acid reactions indicative of lactose and/or sucrose fermentation, along with glucose fermentation. Citrate utilization was confirmed by a positive citrate test, resulting in a blue color change. Conversely, the indole test yielded negative results, showing no reaction with Kovac's reagent and the absence of a purple ring. A positive urease test, demonstrated by a pink color change in the medium, confirmed the hydrolysis of urea to ammonia and carbon dioxide. Finally, all isolates tested negative for oxidase activity. These results align with established characteristics of *K. pneumoniae* as described by (Aljanaby & Alhasnawi, 2017).

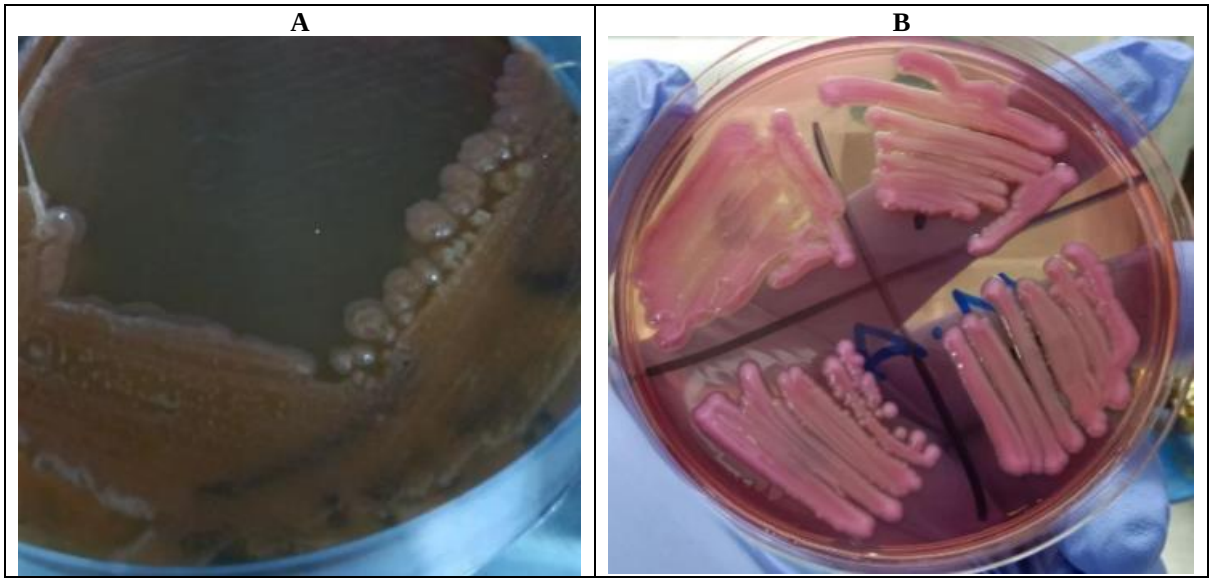


Figure 1: A) K. pneumoniae isolates mucoid appearance on Blood agar; B) K. pneumoniae isolates lactose fermenting on MacConkey's agar

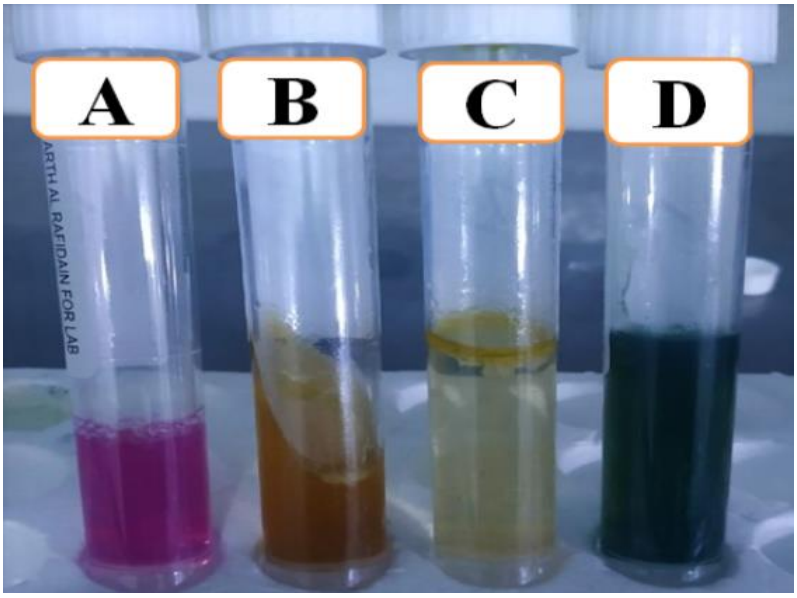


Figure 2: Refer to biochemical tests Regarding K.pneumoniae where; Tube(A): Urase positive, Tube(B): Kligler Iron Acid/ Acid, Tube(C): Indole Negative, Tube(D): Simmon Citrate positive

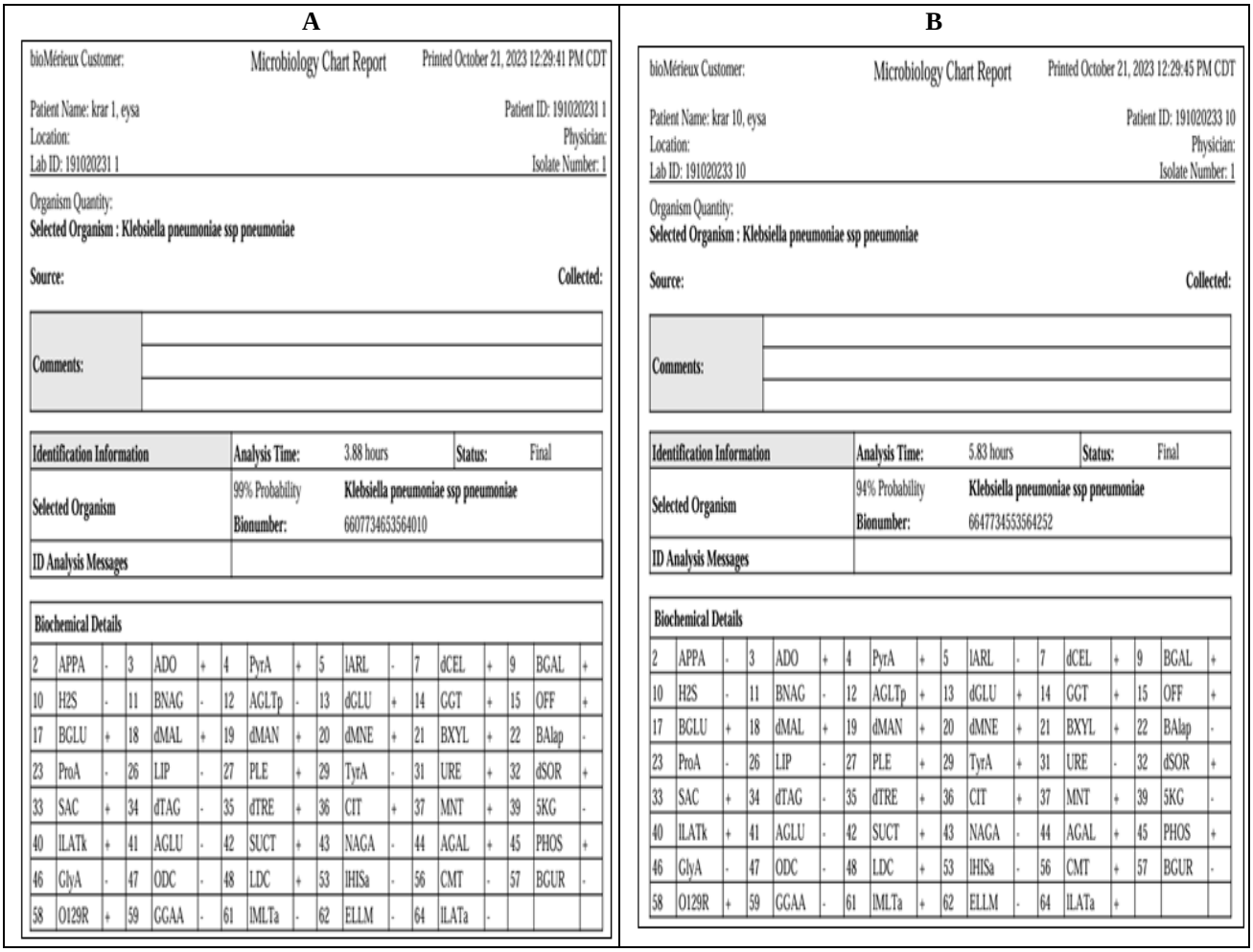
Identification of *K. pneumonia* by Vitek 2 system

The diagnosis of *K. pneumoniae* was confirmed by using Vitek2 system as shown in the figure (3). All isolates shown an excellent percentage of identification ranged from (94 - 99%).

Molecular Identification of *K. pneumoniae*

Genomic bacterial DNA Extraction

Genomic DNA was extracted from the 12 *K. pneumoniae* clinical isolates using a commercially available genomic DNA purification kit (HiMedia Bacterial DNA Extraction Kit) according to the manufacturer's instructions. Successful DNA extraction was confirmed through gel electrophoresis using a 0.7% agarose gel, run at 80 volts for 30 minutes. Following staining with 0.1% ethidium bromide, the extracted DNA was visualized as distinct, dense bands under UV transillumination (Figure 4).



A

bioMérieux Customer: Microbiology Chart Report Printed October 21, 2023 12:29:41 PM CDT

Patient Name: krar 1, eysa Patient ID: 191020231 1
Location: Physician:
Lab ID: 191020231 1 Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source: Collected:

Comments:

Identification Information	Analysis Time:	3.88 hours	Status:	Final
Selected Organism	99% Probability	Klebsiella pneumoniae ssp pneumoniae		
	Bionumber:	6607734653564010		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	-	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	-	62	ELLM	-	64	ILATa	-			

B

bioMérieux Customer: Microbiology Chart Report Printed October 21, 2023 12:29:45 PM CDT

Patient Name: krar 10, eysa Patient ID: 191020233 10
Location: Physician:
Lab ID: 191020233 10 Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source: Collected:

Comments:

Identification Information	Analysis Time:	5.83 hours	Status:	Final
Selected Organism	94% Probability	Klebsiella pneumoniae ssp pneumoniae		
	Bionumber:	6647734553564252		
ID Analysis Messages				

Biochemical Details

2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	-	12	AGLTp	+	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	-	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	-	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	ILATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	-	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	+	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	+	62	ELLM	-	64	ILATa	+			

Figure 3: A) The report of sample No.1 that it was *K. pneumoniae* Diagnosed by Vitek-2 system with probability percentage 99%; B) The report of sample No.10 that it was *K. pneumoniae* Diagnosed by Vitek-2 system with probability percentage 94%

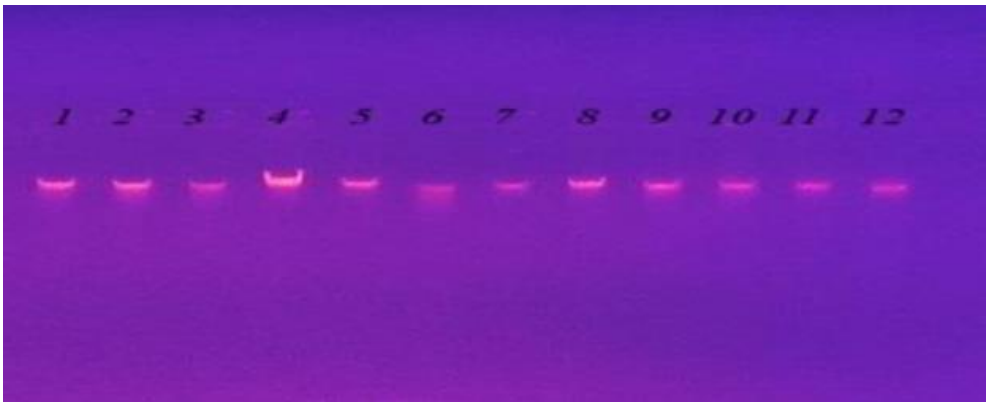


Figure 4: UV light image of agarose gel electrophoresis for bacterial genomic DNA in K. pneumoniae, where the lane (1-12) referred to isolates number. (Bacterial extraction DNA kit HiGenom, agarose size 0.7%, the volte of electrophoresis is 80 volts for 30 mints), ethidium bromide 0.1%

Molecular Identification of *K. pneumoniae* by Detection Specific *16SrRNA* gene

Molecular identification of the 12 isolates was performed via conventional PCR amplification of the *16S rRNA* gene. *K. pneumoniae*-specific primers (K 16S-F and K 16S-R) were employed, targeting a 130 base pair (bp) fragment of the *16S rRNA* gene. As shown in Figure 5, all 12 isolates yielded amplicons of approximately 130 bp, confirming their identification as *K. pneumoniae*. Therefore, PCR analysis unequivocally identified all isolates as *K. pneumoniae*.

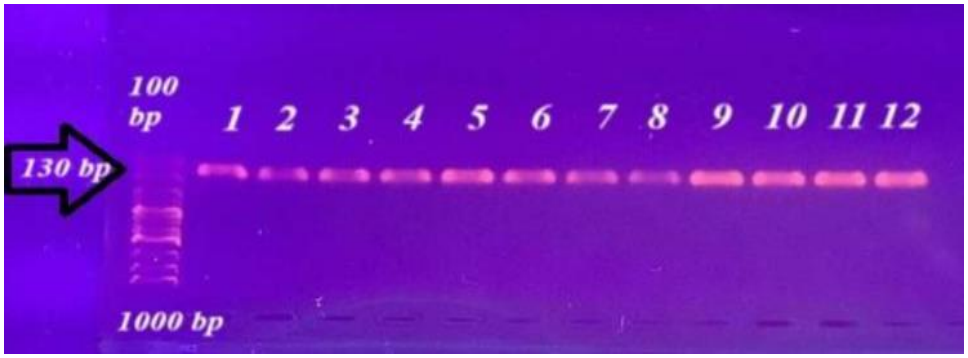


Figure 5: Agarose gel electrophoresis of PCR product of *16S rRNA* gene of *K. pneumoniae*, lane 1-12 refer to isolate`s number. (The size of ladder is 100pb (Bioneer), the volte of electrophoresis is 80V for 45 minutes, the size of agarose is 1.5%, the size of bands is 130 bp), ethidium bromide 0.1%

DISCUSSION

The results appeared as shown in Table 1, where *K. pneumoniae* was isolated from 12 (12%) urine samples from patients with urinary tract infections (UTIs). These results indicate that *K. pneumoniae* is a significant contributor to UTIs in the studied population. Perhaps the reason is that K. pneumoniae is a common bacterium found in the human gut and can easily spread to the urinary tract. A previous study by (Hafiz *et al*., 2023) aimed to assess the epidemiological and microbial-resistance characteristics of *K. pneumoniae* BSI in Saudi Arabia. The study was conducted in 2023 in Saudi Arabia and it reached that adult patients (66.4%) were at a higher risk of developing the infection than pediatric patients (33.6%). This indicates that age is a risk factor for developing *K. pneumoniae* infections. Furthermore, this study revealed a varying distribution of *K. pneumoniae* isolates across different age groups, with the highest prevalence (15.57%) observed in the 10-30 year age range. A lower proportion of isolates (11.4%) were obtained from patients aged 31-50 years, while only 1 isolate (5.0%) was recovered from individuals older than 51 years. This higher prevalence in younger age groups (10-30 and 31-50) may be attributed to increased outdoor activity and overall higher activity levels characteristic of these demographics. The isolates also demonstrated a skewed distribution by sex, with 9 isolates (15.0%) originating from female patients and 3 (7.57%) from male patients. This is consistent with other studies that have shown that women are more prone to UTIs than men due to anatomical differences. The results appeared as shown in Figure 3, where all 12 isolates of K. pneumoniae showed an excellent percentage of identification by the Vitek 2 system, ranging from 94-99%. These results indicate that the Vitek 2 system is a highly accurate method for identifying *K. pneumoniae*. Perhaps the reason is that the Vitek 2 system uses a comprehensive database of biochemical reactions to identify bacteria, which reduces the risk of misidentification. A previous study by (Aljanaby & Alhasnawi, 2017) aimed to evaluate the performance of the Vitek 2 system for identifying *K. pneumoniae* isolates. The study was conducted in 2017 in Iraq and reached a sensitivity of 97.1% and specificity of 100% for the Vitek 2 system in identifying *K. pneumoniae*. This indicates that the Vitek 2 system is a reliable method for identifying *K. pneumoniae*. The results appeared as shown in Figure 4, where the extracted DNA was visualized as distinct, dense bands under UV transillumination. These results indicate that the DNA extraction was successful. Perhaps the reason is that the HiMedia Bacterial DNA Extraction Kit used in the study is a reliable method for extracting bacterial DNA. A previous study by (Ligozzi *et al*., 2002) aimed to evaluate the performance of the Vitek 2 system for identifying *K. pneumoniae* isolates.

CONCLUSION

The accurate identification of *K. pneumoniae* is crucial for effective treatment and management of UTIs. The findings emphasize the importance of utilizing a combination of diagnostic. The study also confirmed the efficacy of various diagnostic methods for identifying *K. pneumoniae*, including traditional phenotypic and biochemical tests, the automated Vitek 2 system, and molecular identification using *16S rRNA* gene amplification.

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