Dijlah Journal of Medical Sciences (DJMS) Vol. 3, No. 1, January, 2026, pp. 104-111
P-ISSN: 3078-3178, E-ISSN: 3078-8625, paper ID: 12

ORIGINAL ARTICLE

Molecular Profiling of *Klebsiella pneumoniae* Clinical Isolates from Dhi Qar Hospitals

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Article Info

Article history:

Received November, 05, 2025
Revised December, 01, 2025
Accepted January, 05, 2026

Keywords:

Klebsiella pneumonia,
MagA Gene,
K2A Gene

ABSTRACT

Klebsiella pneumoniae is a clinically significant pathogen responsible for a wide range of hospital-acquired infections. This study aimed to investigate its molecular characteristics and genotype distribution in patient samples from Dhi Qar, Iraq. A total of 386 clinical samples were collected from hospitals in Dhi Qar, Iraq, from patients aged 10–60 years between July and September 2022. Among these, 69 samples were identified as *Klebsiella* spp. using the API 20E and VITEK 2 systems. DNA concentrations of the isolates ranged from 47.4 to 123.8 ng/µL. All 48 suspected *K. pneumoniae* isolates underwent molecular identification by PCR amplification of the *16S rRNA* gene. The results of this study indicate that the *magA* and *k2A* genotypes may serve as reliable biomarkers for identifying the K1 and K2 serogroups of *K. pneumoniae*. For diagnosing the K1 serotype, PCR amplification of the *magA* gene using the primer sets *magA*-F and *magA*-R was sufficient. Among the 48 *K. pneumoniae* isolates, 29 (60.4%) tested positive for the *magA* gene. Overall, the findings demonstrate the effectiveness of PCR-based detection of the *magA* and *k2A* genes in identifying *K. pneumoniae* serogroups. These molecular markers can improve diagnostic accuracy and enhance understanding of infection pathways in clinical settings.

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1- INTRODUCTION

Klebsiella pneumoniae is very important pathogenic species from different infections and the isolation incidence of about 85% [1]. *Klebsiella pneumoniae* in clinical samples is very common, where pneumonia was labeled as a member of the caused by pathogenic group of organisms *(Enterococcus faecium, Staphylococcus aureus, K. pneumoniae, Acinetobacter baumannii*, *P. aeruginosa*, and *Enterobacter* spp.), which are well-known, highly virulence factor, and antimicrobials clinical pathogens [2]. *Klebsiella pneumoniae* was split into 2 distinct species. *pneumoniae*, specifically *K. pneumoniae* infection. One monomorphic clone and *rhinoscleromatis*, each associated with a distinct clinical condition (atrophic rhinitis and rhinoscleroma, respectively), have been reported [3]. Both subspecies belong to the *Klebsiella* population as a whole. *Pneumoniae* phylogenetically are thought to be hypervirulent offspring of *K. pneumonia* [4].

Kind of *Klebsiella, K. varicola*, and *K. oxytoca* have also developed and are linked to clinical infections. However, their pathogenicity profiles have not yet been extensively characterized [5, 6, 7]. *Klebsiella* is an enteric bacterium that is a part of the normal gut flora and an opportunistic pathogen. However, they can become pathogenic if they invade other tissues or sites beyond their usual residence among normal flora [8]. *Klebsiella pneumoniae*, a member of the Enterobacteriaceae family, is found in the respiratory system of about 5% of healthy individuals and is

responsible for approximately 1% of all bacterial pneumonia cases. From a medical perspective, *K. pneumoniae* considered the second most crucial species in the Enterobacteriaceae family after *E. coli* [9]. *K. pneumoniae* is a significant pathogen not only in community-acquired infectious diseases such as community-acquired pneumonia but also in hospital-acquired infections, where it frequently causes conditions like septicemia, lesions, bloodstream infections, urinary tract infections, inflammatory abdominal illnesses, and pneumonia in immunocompromised individuals [3, 10].

Strains of *Klebsiella pneumoniae* carrying the (ESBL) have been identified in various parts of the world. These genes confer resistance to a wide range of commonly used antibiotics and severely limit the treatment options used to combat the infection and this reduces the number of antibiotics used to treat *K. pneumoniae* infections [11]. Infections caused by antibiotic-resistant bacteria are clinically relevant because they are associated with relapse, prolonged hospital stays, and higher medical costs, all of which tend to occur in clusters and possibly even increase mortality [1]. This study aimed to Isolation and diagnosis of *Klebsiella pneumoniae* from patients isolates from Dhi Qar Hospitals. Also, molecular detection the *MagA* and *k-2A* genes of *K. pneumoniae.*

2- MATERIALS AND METHODS

2.1 Sample Collection

This study included 386 clinical samples were collected from hospitals in Dhi Qar, Iraq, with the ages 10-60 years old between July and September 2022. Employing regular fluid and swabs stored in sealed tubes, 386 samples were obtained aseptically from hospitals and medical individuals who go to certain clinics (Al-Hussein, Al-Habbobi, and Al-Mousawi) in the Dhi Qar government.

2.2 Isolation and identification of *Klebsiella pneumoniae*

All samples were collected from the hospitals and clinics and then cultivated on plates of MacConkey agar, EMB, and blood agar. After a full day of aerobic incubation at 37 °C, these plates were examined for the growth of bacteria. Colonies that appear pink and have a mucous consistency have been resuspended onto MacConkey agar to identify them as Lactose Fermenter colonies. On Blood agar, *Klebsiella* colonies were pale and attempted to produce gamma-hemolysis, in contrast to the pink hue seen on EMB agar [12, 13].

2.3 Morphology of colony

After being incubated at 37 C° for one day, all swabs were first classified based on the normative cultural characteristics (morphological) of the colonies formed on MacConkey, Blood and EMB media. Other traits, such as the fermentation of lactose and the lysis of blood, were also seen.

2.4 Biochemical tests

After 24 hr. incubation at 37 C°, a new bacterial culture was added to peptone broth, and 10 drops of Kovacs were introduced through every test tube. If a crimson ring forms at the broth's surface after 10 min, it's a good sign. In order to determine whether or whether bacteria are capable of producing the treptophanase enzyme, which is necessary for the hydrolysis of tryptophan into indole, pyruvic acid, and ammonia, a test was developed.

2.5 Molecular study of *Klebsiella pneumoniae* by PCR

Use of PCR for diagnostic and identifying the target genes by using suitable primers. The following primers were used in this study to identify the target genes in *Klebsiella* spp. isolates:

NO.	Primer	sequence	Size	Ref.
1	*16S rRNA*	**F ATT TGA AGA GGT TGC AAA CGA T**	1500 bp	13
		R TTC ACT CTG AAG TTT TCT TGT GTT C		
2	*magA*	**F GGT GCT CTT TAC ATC ATT GC**	1283 bp	12
		R GCA ATG GCC ATT TGC GTT TGC GTT AG		
3	*K2A*	**F CAACCATGGTGGTCGA TTAG**	543bp	14
		R TGGTAGCCATATCCCTT TGG		

3- RESULTS AND DISCUSSION

3.1 *Klebsiella pneumoniae* identification

Multiple morphological, and biochemistry tests were conducted to determine the identification of the isolated *Klebsiella pneumoniae*. From those 386 clinical samples, it was shown that *Klebsiella spp* account was 20.7% (no.= 69) of these samples, with subtypes including *Klebsiella pneumoniae* (48 swabs), 14.3%; *K. oxytoca* (6 separates), 1.8%; *K. ornitholytica* (4 separates), 1.2%; and *K. terrigena* (11 swabs), 3.3%. Culture, microscopy, and biochemistry data were used to confirm the identity of bacterial swabs. The API pattern (API 20E) and the platform VITEK 2 were also used to authenticate the bacterial swabs ' identities. Under a microscope, the *Klebsiella* suspects appeared as G (-ve), non-motile, tiny, straight rods that were either single or in pairs. When studied by oil immersion after capsule stain, all isolated had a recognizable capsule as a test area around the bacterium.

Bacterial cultures were identified by applying biochemical assays. In vitro microbial virulence factor (IMVIC) findings distinguish them from other LF genera such as *E. coli, Citrobacter*, and *Serratia*. Results for indole resistance in *Klebsiella* were (-ve) (except *K. oxytoca*). Certain intestinal bacteria that produce the enzyme tryptophanase are characterized by their capacity to hydrolyze tryptophan to indole, a property used in the indole test. *Klebsiella* and another indole (-ve) bacteria do not make tryptophanase; therefore, when Kovac's reagent is added to an indole-free broth, no red ring will appear on the surface of the soup. These results agreed with those declared by [14].

Klebsiella demonstrated favorable reactivity for citrate, making it clear that the citrate in Simmon citrate media is crucial for determining whether or not the bacteria swabs are capable of growing on it as a distinct source of carbon and energy. The pH indicator bromothymol blue is present in Simmon's medium. The carbon dioxide used in the production of Klebsiella combines with the other media elements to form an alkaline molecule, resulting in a shift in color from green to blue in a pH marker to signify a (+ve) citrate test. Kligler Iron Agar (KIA) is used to distinguish between various *Enterobacteriaceae* genera by measuring the bacteria's ability to ferment carbohydrates and produce hydrogen sulfide. A KIA slant has the same lactose and glucose as a teaspoon. When the medium was acidic, the pH indicator (phenol red) caused it to turn from orange-red to yellow. KIA also includes ferrous sulfate, which creates a black deposit to identify H2S-producing bacteria, and sodium thiosulfate, a substrate for H2S generation. Urease tests (+ve) for *Klebsiella* and (-ve) for Enterobacter may be used to distinguish between the two types of bacteria, whereas urease test differentiate Klebsiella isolates from Enterobacter isolates as it was positive for *Klebsiella* and negative for Enterobacter [15]. Urease enzyme catalyzes the breakdown of urea, and the bacteria that can produce this enzyme is able to detoxify the waste products and to drive metabolic energy from its utilization which change the medium color from yellow to purple-pink, indicating urease positive test. Klebsiella can produce urease enzyme and gives urease positive test [16].

3.2 API 20E system

Figure indicates that the API 20E system was used to verify the results conducted on *Klebsiella* spp. (69 isolates). All bacterial swabs' findings matched these norms**.**

Figure (2): The API 20E system for *Klebsiella pneumoniae* positive results

3.3 VITEK 2 system

Klebsiella pneumoniae was definitively diagnosed using this technique. This technique allowed for the rapid, effective identification of germs outside potentially contaminating environments. The tests validated the findings from the morphological, biochemical, and API 20E systems. Therefore, all (69) swabs formerly thought to be *Klebsiella spp*. may now be confidently classified as *Klebsiella*. The API 20E diagnostic system may it gave excellent results and differentiate *Klebsiella* spp. Moreover, the diagnosis of this system is high accuracy [17].

3.4 Molecular identification of *Klebsiella pneumoniae* using PCR

3.4.1 16S rRNA gene amplefication

All 48 swabs underwent molecular identification by amplifying the *16S rRNA* through a polymerase chain reaction with 16S-F and K 16S-R Primers, designed explicitly for the amplification of *Klebsiella pneumoniae16S rRNA*. The amplified fragments were approximately 1500 bp in size, consistent with the findings of, who used the same Primers. All 48 swabs yielded (+ve) results with a band size of 1500 bp, confirming their identity as *Klebsiella pneumoniae*. These results corroborate the initial findings that all swabs were *Klebsiella pneumoniae*.

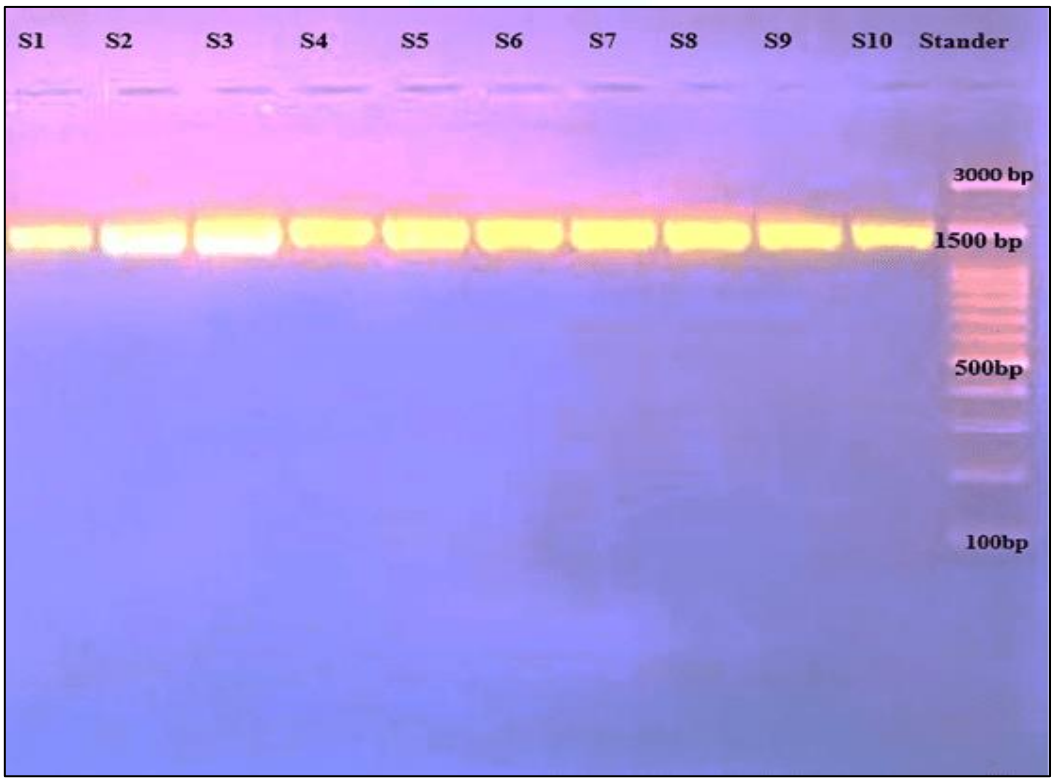

Figure (3): Agarose gel electrophoresis of amplification of PCR products for *16S rRNA* gene (1500 bp). Lane stander, 100bp DNA ladder; lanes 1-10, *Klebsiella pneumoniae* S1 – S10 isolates; (2% agarose, 80V for 2hr).

3.4.2 MagA and *k-2A* genes Amplefication

To diagnose *Klebsiella pneumoniae* serotype K-1, PCR amplification of the *magA* gene urinary tract infection a particular primed set was sufficient (*magA*-F) and (*magA*-R). After applying amplification with these primers to a total of 48 *Klebsiella pneumonias* strains, 29 (60.4%) tested (+ve) for the presence of the *magA* gene. These findings show that all 29 of these dangerous swabs belong to the K-1serotyp. Figure- 3 indicates that the approximate size of the PCR reaction was 1283 bp, a size also seen when employing the same primers according to [18]**.** *mag-A* is found inside an operon unique to serotypK-1 cps gene clusters. The studies [19, 20] analyzed 134 *Klebsiella pneumoniae* swabs from around the globe and came to the same conclusion: mag-A is exclusive to the *Klebsiella pneumoniae* capsule serotype K-1 gene cluster, whereas all the Non-K-1 strains tested (-ve) for the

molecule. Thus, *magA* PCR testing provides an efficient and reliable molecular diagnostic tool for *Klebsiella pneumoniae* serotype K-1swa.

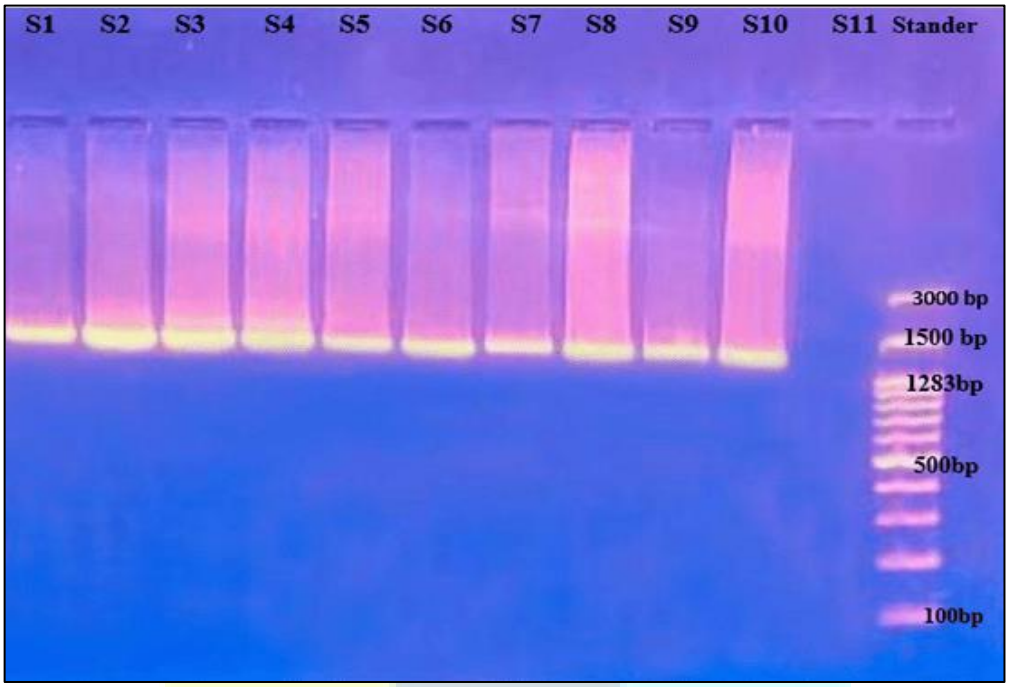

Figure (4): Agarose gel electrophoresis of amplification of PCR products for *Mag-A* gene(1283 bp). Lane stander, 100bp DNA ladder; lanes 1-11, *Klebsiella pneumoniae* S1 – S11 isolates; (2% agarose, 80V for 2hr).

PCR was used to identify *Klebsiella pneumoniae* serotyp K-2 by amplifying the k-2A gene using Primers (*k-2A*-F and *k-2A*-R). This primer was tested by amplification of 48 *Klebsiella pneumoniae* swabs. Figure 4 shows that the PCR reaction was about 543 bp, consistent with the results from [21]. *Klebsiella pneumoniae* isolates with the *k-2A* segment of 543 bp were found in 14 samples (29.2%). The findings indicated that the K-2 serotype was present in these (pathogenic) strains. *Klebsiella pneumoniae* serotype K-2 capsule identification may be possible using a highly specific k-2A ORF-9 PCR study that matches the *mag-A* area in the cps gene clusters of K-1 strains [22].

Other (5 isolates) were classified as Non-K-1/K-2 because they did not match the molecular serotypes of K-1 (29 swabs) or K-2 (14 swabs) after mag-A and k-2A amplification. However, the non-K-1/K-2 strain did not produce mag-A and k-2Aspecific amplicon while being less dangerous and able to serotype with both K-1 andK-2. This may benefit the new test over a traditional serotyping method, in which such cross-reactions occur Always by (18)**,** who stated that *Klebsiella pneumoniae* was most common among those of serotypK-1, and [23], who indicated that *Klebsiella pneumoniae* was most common among those of serotypK-2, the data showed that K. serotypK-1 of *Klebsiella pneumoniae* is the most common and causes the most severe illnesses. Corresponding with all those results, [24] indicated that K-1 and K-2 serotypes of *Klebsiella* were present.

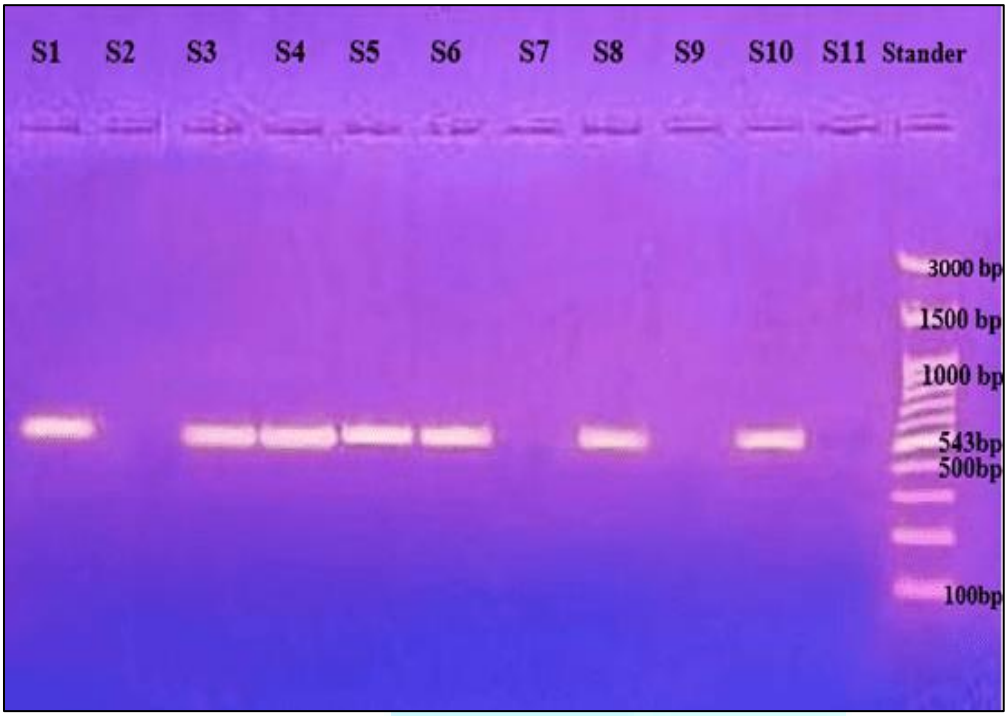

Figure (5): Agarose gel electrophoresis of amplification of PCR products for *K-2A* gene (543 bp). Lane stander, 100bp DNA ladder; lanes 1-11, *Klebsiella pneumoniae* S1 – S11 isolates; (2% agarose, 80V for 2hr).

This study's findings also contradicted those of which found that *Klebsiella pneumonia* isolates were 14.3% (7/49), 38.8% (19/49), and 46.9% (23/49) serotypK-1, K-2, and Non-K-1/K-2, respectively. According to [29], K-1 accounts for 46.6% (34/73) of all cases, followed by Non-K-1/K-2 at 32.9% (24/73) and K-2 at 20.5% (15/73). They also mentioned that K-1 and K-2swabs were far more common than non-K-1 and non-K-2 swabs (Non-K-1/K-2; 49/73 vs. 24/73) [25, 26].

4- CONCLUSION

This study confirmed the accurate identification of *Klebsiella pneumoniae* using morphological, biochemical, API 20E, VITEK 2, and PCR-based methods. Molecular analysis revealed that 60.4% of isolates carried the *magA* gene (K1 serotype) and 29.2% carried the *k2A* gene (K2 serotype), indicating the predominance of these virulent serogroups. The PCR detection of *magA* and *k2A* proved to be a reliable and specific method for serotyping *K. pneumoniae*. These findings highlight the high prevalence of pathogenic K1 and K2 strains in clinical samples from Dhi Qar. Overall, molecular markers significantly enhance diagnostic accuracy and support better understanding of infection severity and distribution.

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