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# ISOLATION, IDENTIFICATION, AND ANTIMICROBIAL SUSCEPTIBILITY TESTING OF *KLEBSIELLA PNEUMONIAE* FROM PNEUMONIA PATIENTS

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**ABSTRACT**

Gram-negative bacteria, *Klebsiella pneumoniae* is an important opportunistic pathogen that causes a variety of infectious diseases in humans, including pneumonia, liver abscesses, diarrhea, and septicemia. The study's aimed to isolate and identify *Klebsiella pneumoniae* from pneumonia patients in Al-Diwanyiah teaching hospital and Al-Diwanyiah maternity and children teaching hospital, testing the sensitivity of isolates to antibiotics as well as to molecular detection Klebsiella pneumoniae isolates by PCR. The study extended from December 2024 and April 2025. Biochemical tests, Vitek 2 and *16S rRNA* identified 36 bacteria as *Klebsiella pneumoniae* from 200 samples. Antibiotic susceptibility of *Klebsiella pneumoniae* isolates against 8 of commonly used antibiotic was determined through disc-diffusion method. Results declared that the isolates were resistant to the used antibiotics except their sensitivity to Imipenem, Meropenem and Tigecycline.

**KEYWORDS:** Clinical specimens, *Klebsiella pneumoniae*, Antibiotic resistance.

## INTRODUCTION

*Klebsiella pneumoniae* is an opportunistic pathogen that is common in healthcare facilities and the general public. Conditions that fall under this category include pneumonia, liver abscesses, diarrhea, and septicemia. Patients with chronic illnesses and those with impaired immune systems are at a higher risk, although the severity of the condition might vary greatly (Patel et al., 2022). hospitalized patients have a much greater frequency of Klebsiella pneumoniae. Both community-acquired and hospital-acquired pneumonia are caused by this bacterium. While Klebsiella pneumoniae accounts for just about 3% to 5% of pneumonia infections in developed nations, that number jumps to about 15% in underdeveloped areas, which includes a large number of African nations (Hasan et al., 2021).

As *K. pneumoniae* develops resistance to several antibiotic classes, it poses a significant clinical concern. There has been a global increase in the reported prevalence of bacteria resistant to several antibiotic classes, making treatment protocols more complex(Chen and Zhang, 2021). This multidrug resistance arises from the acquisition of genes that encode enzymes like extended-spectrum beta-lactamases (ESBLs) and carbapenemases, which enzymatically deactivate numerous beta-lactam antibiotics(Singh et al., 2023). The emergence of extensively drug-resistant (XDR) *K. pneumoniae* strains, impervious to nearly all existing antibiotics, constitutes a significant public health hazard. These strains possess movable genetic elements like as plasmids that facilitate the horizontal transfer of resistance determinants, hence expediting the dissemination of resistance among bacterial populations (López-Camacho et al., 2022).

The pathogenicity of Klebsiella pneumoniae is greatly influenced by its virulence factors. In order to avoid being killed by phagocytosis and complement, its polysaccharide capsule helps it dodge the host immune system. Also, fimbriae make it easier for bacteria to stick to epithelial surfaces, which means they can colonize and build biofilms on medical equipment. This can lead to antibiotic resistance and persistent infection(Gao et al., 2023). This combination of diverse virulence mechanisms and widespread antibiotic resistance makes Klebsiella pneumoniae an extremely dangerous healthcare-associated illness (HAI) pathogen that calls for constant vigilance and the discovery of new treatment approaches (Martínez et al., 2022).

## METHODS

### Isolation and Biochemical Identification of *Klebsiella pneumoniae*

36 *Klebsiella pneumoniae* obtained from 200 clinical samples collected from patients in Al-Diwanyiah teaching hospital and Al-Diwanyiah maternity and children teaching hospital within five months (December 2024 to April 2025.), by sputum from pneumonia patients as shown in TABLE 1.

All samples transferred to laboratory and culture onto blood agar and MacConky agar medium incubated at 37° C for 24 hr. Isolates purified several times until pure isolates were obtained, exposed to microscopic and special biochemical tests then transferred to identification via VITEK 2. Also they subjected to molecular detection method using specific primer based on *16S rRNA* gene as a genetic marker for confirmed identification of *Klebsiella pneumoniae* by PCR.

**Table 1: *Klebsiella pneumoniae* isolated from patient pneumonia sources.**

| Source | Total No. | Positive samples | % |
|---|---|---|---|
| Sputum | 200 | 36 | 18 |

### Molecular Detection of *Klebsiella pneumoniae* by *16S rRNA* Gene

### DNA Extraction of Klebsiella pneumoniae

The DNA was extracted from fresh growth of *Klebsiella pneumoniae* using a DNA extraction kit (Geneaid, Taiwan). The procedure was created in accordance with the manufacturer's protocol. A NanoDrop was used to measure the concentration of DNA for both quality and quantity (Thermo Scientific NanoDrop, USA).

### Polymerase Chain Reaction Amplification of *16S rRNA Gene*

The primer 3 plus program was used to design the primers used to amplify regions of the *16S rRNA* gene. Preparation of the PCR reaction included Mastermix (Promega, Korea) with total volume (25uL) of the reaction mixture, which included 5uL of DNA template, 2uL(10pmol) for each of the primers, 12.5 µl GoTaq ®Green PCR master, and 3.5µl PCR water. The thermocycler conditions were 95 Celsius degree for two minutes of the first denaturation, 30 cycles including denaturing at 95 Celsius degree for 30 seconds, annealing at 55 Celsius degree for 30 seconds, and extension at 72 C for 1 minute, and the ultimate extension was at 72 Celsius degree for five minutes. Ethidium bromide stain was added to 1.5 percent agarose gels to separate DNA bands by electrophoresis, and UV Transilluminators were used to see DNA fragments in the PCR products (Bioneer/ Korea).

### Antibiotic resistant of *Klebsiella pneumoniae*

The modified Kirby-Power disc diffusion method was used to assess antibiotic resistance in Klebsiella pneumoniae Selective antibiotics such as meropenem, imipenem, amikacin, tigecycline, levofloxacin, ceftaxime, ceftriaxone, piperacillin/ tazobactam are used to demonstrate their effect on *Klebsiella pneumoniae* and use mueller-hinton agar medium. the results were compared using Clinical Laboratory Standards Institute (CLSI, 2024) criteria.

### Molecular detection of *Klebsiella pneumoniae*

*Klebsiella pneumoniae* isolates were activated by culturing them in brain heart infusion broth for twenty-four hours at 37 degrees Celsius. DNA was extracted using a particular extraction kit (Geneaid -Taiwan) in accordance with the manufacturer's directive *16S rRNA* gene primer were performed for detection of *Klebsiella pneumoniae* isolates as mentioned in previous studies (Yan et al., 2024).

Table 2 describe the primer sequences. PCR product was analyzed in a 1.5 percent agarose gel with 3µl of ethidium bromide stain in TBE buffer, and a UV Transilluminator was used to visualize the PCR products.

**TABLE 2: Polymerase Chain Reaction (PCR) Primers That were Used in This Study.**

| Target Genes | Primer Sequence(5′-3′) | Product Size (bp) | References |
|---|---|---|---|
| ***16S rRNA*** | F : 5′-TTAACCCGCAGAAGAAGCACC-3′ R : 5′-TTGACGTCATCCCCACCTTC-3′. | 713 | (Yan et al., 2024). |

## RESULTS

### Identification of *Klebsiella pneumoniae*

36 isolates were diagnosed as *Klebsiella pneumoniae* from (200) clinical specimens that collected from sputum of pnumonia patients in Al-Diwanyiah teaching hospital and Al-Diwanyiah maternity and children teaching hospital, through the use of conventional biochemical methods and the Vitek 2 system;. In addition, The *16S rRNA* gene was used to confirm the identity of 36 isolates.

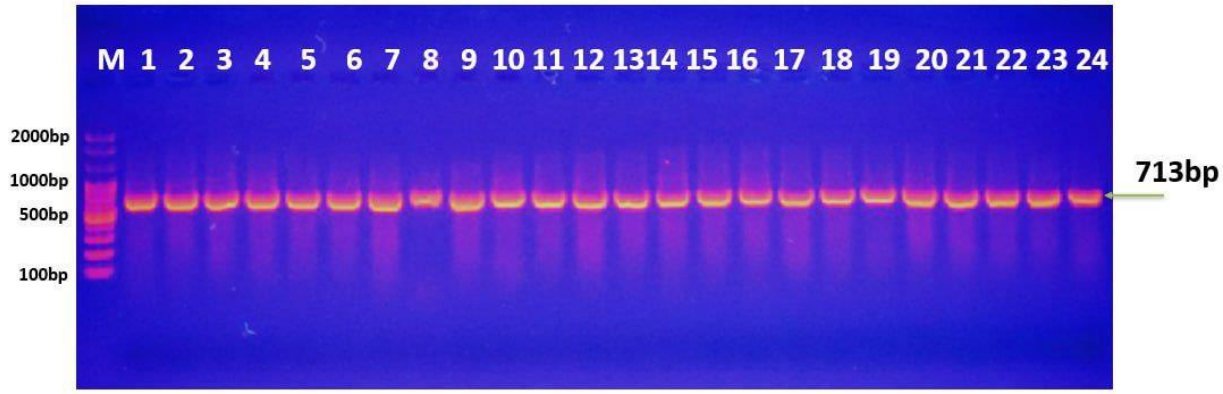
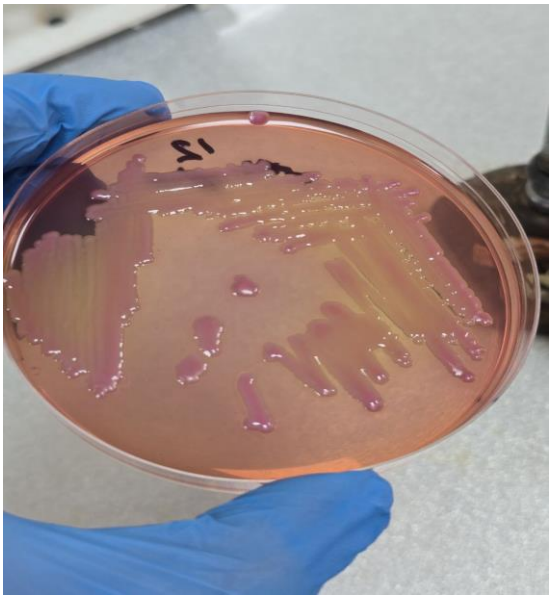
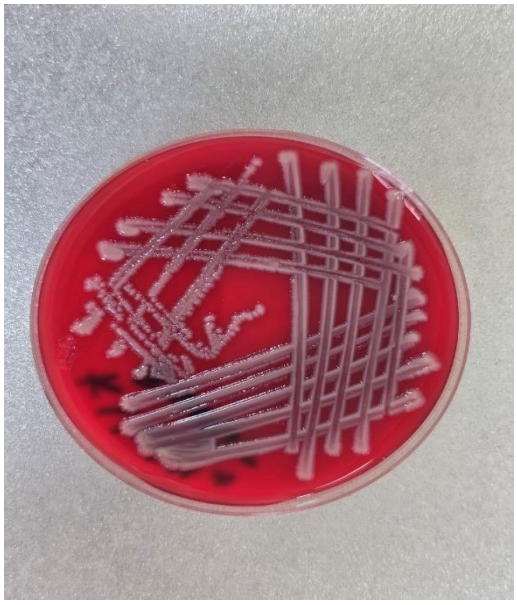


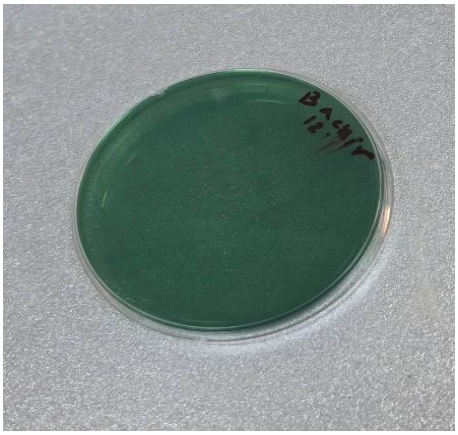

**FIGURE1: Image of agarose gel electrophoresis that describe PCR product analysis of *16S rRNA* gene in *Klebsiella pneumoniae* isolates with (713 bp) PCR product size, where ladder (100-2000bp), the number of isolates is represented by the lane (1-24).**



***Klebsiella pneumoniae* on MacConky agar**



***Klebsiella pneumoniae* ON blood agar**



***k. pneumonia* on Simon citrate agar**

***k. pneumonia* on Simon citrate agar**

**Image (1): growth of Klebsiella pneumonia on the MacConkey agar and the Blood agar medium.**

**Antibiotic resistant**

*Klebsiella pneumoniae* isolates from pneumonia patients were tested for antibiotic resistance and sensitivity, and the results are shown in the table(3). imipenem (IPM) showed 34 (94.44 %) sensitivity rate and 2 (5.55 %) intermediate rate. meropenem (MEM) showed 31 (86.11 %) sensitivity rate, 5 (13.88%) intermediate rate, and 1 (2.77%) resistance rate. Tigecycline (TGC) showed 33

(91.66 %) sensitivity rate rate and 3 (8.33 %) intermediate rate. amikacin (AK) showed 24 (63.88%) resistanc rate and 12 (33.33%) sensitivity rate. Cefataxime (CTX) showed 32 (88.88 %) resistance rate and 4 (11.11 %) intermediate rate. Ceftriaxone (CRO) showed 25 (69.44%) resistance rate and sensitivity rate 9 (25%). Levofloxacin (LEV) showed 8 (22.22) sensitivity rate and 27 (75 %) resistance rate and 1 (2.77 %) intermediate rate. piperacillin / tazobactam (TPZ) showed 24 (66.66%) resistance rate and sensitivity rate 10 (27.77). It was found that the best antibiotics in terms of sensitivity are meropenem, imipenem and Tigecycline (TGC).

**TABLE 3: the ratio of resistance, intermediate and sensitivity to antibiotic.**

| Type of antibiotic | Sensitive (S) | Intermediate (I) | Resistance ( R) |
|---|---|---|---|
| imipenem (IPM) | 34(94.44 %) | 2 (5.55 %) | 0 (0%) |
| meropenem (MEM ) | 31 (86.11 %) | 5 (13.88%) | 0 (0%) |
| Tigecycline (TGC) | 33 (91.66 %) | 3 (8.33 %) | 0 (0%) |
| amikacin (AK) | 12(33.33%) | 1 (2.77%) | 23 (63.88 %) |
| Cefataxime (CTX ) | 0 (0%) | 4 (11.11%) | 32 (88.88) |
| Ceftriaxone (CRO) | 9 (25%) | 2 (5.55%) | 25 (69.44%) |
| Levofloxacin (LEV) | 8 (22.22) | 1 (2.77 %) | 27 (75%) |
| piperacillin / tazobactam (TPZ) | 10 (27.77%) | 2 (5.55%) | 24 (66.66%) |
| $X^2$, P value | $X^2 = 160.23$, $P < 0.001$* | | |

*** Highly significant difference at P<0.05**

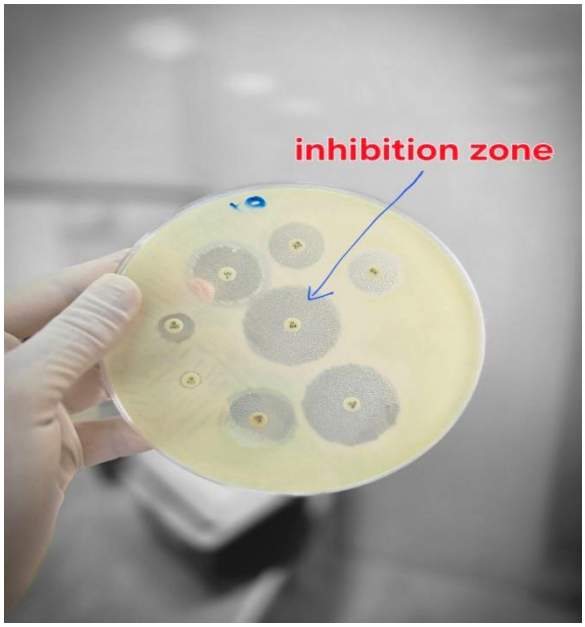



**Image (2): Antibiotic sensitivity test for *Klebsiella pneumoniae* isolates on Muller Hinton agar.**

## DISCUSSION

In the present study, *Klebsiella pneumoniae* isolated and identified from pneumonia patients samples depending on standard biochemical tests and molecular identification by *16S rRNA* gene. Because the *16S rRNA* gene is present in all bacteria, it is used as a diagnostic tool for bacteria. Furthermore, the function of the *16S rRNA* gene has remained constant over time, implying that random sequence changes are a precise measure of development (Patel, 2001). The *16S rRNA* gene has been proven to be one of the most effective tools for identification microorganisms (Janda & Abbott, 2007).

Antibiotics in the carbapenem family. In this study, the results revealed that 34 (94.44 %) isolates were sensitive to imipenem, The present result is almost similar to what was reached by (Raouf et al., 2022), who found that isolates of *Klebsiella pneumoniae* isolated from Community-acquired pneumonia patients are sensitive to imipenem by( 100 %). There is also a study conducted by ( Naqid et al., 2020), in which the sensitivity rate was (82.3%). The results of our study differ with the result obtained by (AL-Deen et al., 2023), where 40 % of *Klebsiella pneumoniae* isolates were sensitive to imipenem.

The results of our study showed that 31 isolates (86.11%) were sensitive to meropenem, and it is almost similar to the results obtained by the researcher (Hamed, 2022) and (Patilaya et al., 2019) who found that all isolates of *Klebsiella pneumoniae* isolated sensitive to meropenem at a rate of (80 %), in another study it was reported that respiratory tract isolates were sensitive to meropenem >80 (Sinanjung et al., 2020). While in a second study, it

was found that the sensitivity of meropenem in Klebsiella isolates was ~77–79% (Moustafa et al., 2023). But in another research discovered that *Klebsiella pneumoniae* isolated was resistant to meropenem at rate (45% ) and sensitive (55%). (AL-Deen et al., 2023).

The results of this study showed that isolates of *Klebsiella pneumoniae* are sensitive to Tigecycline 33 (91.66 %). This result is consistent with a study by (Bhaumik et al., 2022), in which the sensitivity rate to tigecycline was (94.7%). There is also a study consistent with this study's findings in which Tigecycline is sensitive against *Klebsiella pneumoniae*, which is (94%) (Giammanco et al., 2017).

the results revealed that 23 ( 63.88 % ) isolates were resistance to amikacin and 12 (33.33%) sensitive to amikacin That were agreement with results obtained by (AL-Deen et al., 2023) who found that, *Klebsiella pneumoniae* isolates were resistance to amikacin at rate (60 %) and sensitive for it (40%).

The resistance of Klebsiella, especially *Klebsiella pneumoniae*, to Cephalosporins (Third-generation cephalosporins) has been increasing recently because they possess enzymes such as ESBL (Extended Spectrum β-Lactamases) and AmpC β-lactamase, which play an important role in the resistance of the isolates to Cefotaxime and Ceftriaxone (Boattini et al., 2024).

In this study, the results showed that 32 (88.88%) are resistant to Cefotaxime. The results of current study are almost in agreement with the results obtained by (Kot et al., 2023), wherein the rate of Cefotaxime resistance in *Klebsiella pneumoniae* isolates was (84.4%). This study also agrees with research conducted by (Raouf et al., 2022) in which the rate of resistance to Cefotaxime (97.6 %).

In this study, Among *Klebsiella pneumoniae* isolates tested, 25 (69.44%) were Ceftriaxone -resistant, 9 (25%) were sensitive to Ceftriaxone. This study is not agreement with (Naqid et al., 2020) that showed, antibiotic susceptibility testing of isolates of *Klebsiella pneumoniae* from clinical source, 65.8% were resistant to Ceftriaxone. these results were not agreement with results obtained by (Awoke et al., 2021) who found that of *Klebsiella pneumoniae* were resistant to Ceftriaxone in rate (97 %).

Levofloxacin belongs to the Fluoroquinolones family. The results of this study showed that the percentage of *Klebsiella pneumoniae* resistance to Levofloxacin for pneumonia patients reached 27 (75 %), sensitive 8 (22.22%), and intermediate 1 (2.77 %).

This study agreement with (Ramadan et al., 2022) showed that, antibiotic susceptibility testing of isolates of *Klebsiella pneumoniae* from pneumonia patient, 85 % were resistant to levofloxacin. these results were not agreement with results obtained by (Sarkar et al., 2015), who found that Klebsiella pneumoniae were sensitive to levofloxacin in rate (83%). It also does not agree with the study conducted by (More et al., 2020), were sensitive to Levofloxacin (70%).

In the current stud 10 (27.77%) isolates of *Klebsiella pneumoniae* were sensitive to piperacillin / tazobactam, 24 (66.66%) were resistance to piperacillin / tazobactam. This result does not correspond to the results of the study obtained by (Krishna et al., 2022), in which the rate of resistance of *Klebsiella pneumoniae* to the piperacillin / tazobactam was 25 %. This study is less resistant than the study conducted by (Al-Qaysi et al., 2024) and (Hassan et al., 2023), where the resistance to piperacillin / tazobactam was (95.5%) and (100%) successively.

## CONCLUSION

The current study showed that *Klebsiella pneumoniae* isolated from hospitalized pneumonia patients from in hospitals were sensitive to Imipenem, Meropenem, and Tigecycline. The polymerase chain reaction was also effective in detecting *16S rRNA* gene

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