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Original Article

Phenotypic, Antibiotyping, and Molecular Detection of *Klebsiella Pneumoniae* Isolates from Clinical Specimens in Kirkuk, Iraq

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Abstract

Klebsiella pneumoniae is globally responsible for hospital- and community-acquired infections. This study aimed to determine the prevalence of *K. pneumoniae* and investigate the antibiotic resistance profile among clinical specimens at Azadi Teaching Hospital in Kirkuk, Iraq, and detect the *rpoB* gene for molecular identification of *K. pneumoniae* in comparison with phenotypic and biochemical methods. In total, 250 clinical specimens were collected from patients in Azadi Teaching Hospital in Kirkuk, Iraq, between January 2018 and May 2018. The isolates were identified by morphologic and biochemical testing. Kirby-Bauer disk diffusion method was used in the antibiotics susceptibility test. Following that, 19 (7.6%) *K. pneumoniae* isolates were isolated from 250 clinical specimens (5 [5.61%] and 14 [8.69%] from males and females, respectively), and most of them (n=12; 11.76%) were isolated from the age group of 10-35 years old. The isolates were reported high resistance towards various types of antibiotics, especially penicillins and cephalosporins. In contrast, *K. pneumoniae* showed very low resistance to imipenem and amikacin (5.26% and 10.52%, respectively). The range of multidrug-resistant *K. pneumoniae* isolates in this study was estimated at 100%. In gene detection, all isolates in this study showed PCR product with 108 bp by *K. pneumonia* specific primer (*rpoB*). Developed antibiotic policies and regular surveillance of antibiotic susceptibility patterns may help to overcome the indiscriminate use of antibiotics that is a major cause of the emergence of drug resistance among pathogens.

Keywords: *K. pneumoniae*, Multidrug Resistance, *rpoB* Gene

1. Introduction

Klebsiella pneumoniae is an important opportunistic pathogen that causes a variety of infectious diseases in humans, including septicaemia, liver abscesses, diarrhea, and pneumonia. The development of antibacterial resistance is considered a serious challenge in hospitals and health care centers over the world. *K. pneumoniae* strains that are rapidly developing multidrug-resistant (MDR) are considered a critical threat to the patients causing a high fatality rate due to low therapy options. The World Health Organization announced antimicrobial resistance (AMR) as one of the main global problems (1). *K. pneumoniae* is globally known as one of the major causes of hospital- and community-acquired infections and plays a significant role in the propagation of antibacterial resistance genes from environmental bacteria to pathogenic bacteria (2).

This bacterium has developed resistance to antibacterial agents more readily than most bacteria by the production of Carbapenemase and Extended-Spectrum β- Lactamase enzymes (1, 3). The most significant risk factor of AMR is exposure to antibiotics, and the main cause which contributes to

expanding the spreading of resistant bacteria strains is the prolonged and intensive use of antimicrobial agents in health care settings (4). The pathogenic bacteria cause many infections, such as hospital-acquired pneumonia, urinary tract infection, bacteremia, surgical site infection, ventilator-associated pneumonia, and septicemia, in addition to the opportunistic infections that occur among immunocompromised patients (5, 6).

β-subunit of RNA polymerase is encoded by the *rpoB* gene which is considered a core gene candidate for the identification of bacteria and phylogenetic analyses, particularly when studying closely related isolates (7, 8).

The emergence of AMR in *Klebsiella* spp. isolates is of great concern worldwide in human medicine. Therefore, this study aimed to determine the prevalence of *K. pneumoniae* and investigate the antibiotic resistance profile among clinical specimens at Azadi Teaching Hospital in Kirkuk, Iraq, and detect the *rpoB* gene for the molecular identification of *K. pneumoniae* in comparison with phenotypic and biochemical methods.

2. Material and Methods

2.1. Samples

In total, 250 clinical specimens were collected from patients (89 and 161 males and females, respectively; age range: 10-70 years), including urine (n=100), vaginal swabs (n=50), wound (n=50), and throat (n=50) from Azadi Teaching Hospital in Kirkuk, Iraq, between January 2018 and May 2018.

2.2. Culturing and Identification

The samples were streaked on blood and MacConkey agar and then incubated at 37°C for 24 h. Gram stain, capsule stain, and many biochemical tests, such as oxidase, IMViC, urea hydrolysis, H2S production, lactose fermentation, lysine decarboxylase, coagulase, gas production, and catalyzes were used for *K. pneumoniae* identification (1, 9).

2.3. Antibiotic Sensitivity Test

Kirby-Bauer disc diffusion method was utilized to detect the sensitivity of isolates to Gentamicin (10 µg), Cefotaxime (30 µg), Meropenem (10 µg), Ciprofloxacin(5µg), Tobramycin (10 µg), Amikacin (30 µg), Ampicillin (10 µg), Ceftazidime (30 µg), Nitrofurination (30 µg), Rifampin (5 µg), Amoxicillin (25 µg), Cefixime (5 µg), Doxycycline (10 µg), Imipenem (10 µg), Aztreonam (30 µg), Nalidixic (30 µg), Chloramphenicol (30 µg), Cephalothin (30 µg), and Trimethoprim/Sulphamthoxazol (1.25/23.75µg). *K. pneumoniae* ATCC 1290 was employed as control strains (10).

Resistance to three or more various classes of antibiotics was considered MDR *K. pneumoniae* (11).

2.4. Polymerase Chain Reaction

DNA of bacterial isolates was extracted by using the Bioneer kit. Polymerase chain reaction (PCR) test was performed with species-specific primers forward primer 5'-CAACGGTGTGGTTACTGACG-3', and reverse primer 5'-TCTACGAAGTGGCCGTTTTC-3' was used for the amplification of the *K. pneumoniae* target genes (*rpoB*). The reaction was performed in a 20 µl volume, 3 µl of a ready Master Mix, 2 µL of each primer, and 5 µL of DNA, while nuclease-free water was used to complete the volume. The PCR program included initial denaturation in one cycle for 5 min at 95°C, amplification in 35 cycles each of 30 sec. at 94°C, 30 sec. at 55°C, and 30 sec. at 72°C, followed by a final extension cycle for 7 min. at 72°C (1, 4).

2.5. Data Analysis

The data were analyzed in SPSS software (version 16.0), and a p-value less than 0.05 was considered statistically significant.

3. Results

The prevalence of *K. pneumoniae* among clinical specimens was 19 (7.6%) isolates, and the highest percentage of the isolates from throat swabs was obtained at 12% (n=6) (Table 1).

The isolates were distributed among 5 male (5.61%) and 14 female (8.69%) patients, and most of them (n=12; 11.76%) were isolated from patients within the age range of 10-35 years old (Table 2).

Table 1. Prevalence of *K. pneumoniae* depending on the source of specimens

Source of specimens	No. of samples	No .of *K. pneumoniae* (%)
Urine	100	7(7%)
Vagina	50	4 (8%)
Wound	50	2 (4%)
Throat	50	6 (12%)
Total	250	19 (7.6%)

Table 2. Prevalence of *K. pneumoniae* depending on patients' gender and age

Age Groups (yrs.)	No. of patients (n=250)	*K. pneumoniae* (%) (n=19)
10-35 (11.76%)	102	12
36-55	98	4 (4.08%)
<56	50	3 (6%)
Gender of total patients		
Female	161	14 (8.69%)
Male	89	5 (5.61%)

3.1. Antibiotic Susceptibility Pattern

K. pneumoniae isolates were reported high resistance towards various types of antibiotics, especially penicillins and cephalosporins. In contrast, *K. pneumoniae* showed very low resistance to imipenem and amikacin (5.26% and 10.52%, respectively) (Figure 1).

Table 3 tabulates the antibiotyping of the *K. pneumoniae* isolates that are classified under different groups (Types 1-16) relying on antimicrobial-resistant patterns. Antibiotyping of Type 1 shows resistance towards 20 antibiotics representing 100% of antibiotics used in this study, while the last type (Type 16) shows resistance toward 4 antibiotics.

3.2. DNA Extraction and Identification of *K. pneumoniae* by PCR

The DNA extraction from 19 *K. pneumoniae* was made by the Bioneer kit. The purity and the concentration of DNA specimens were ranged from (1.6- 2) to (60-130) ng/ul, respectively. All isolates in this study showed PCR product with 108 bp by *K. pneumonia* specific primer (*rpoB*) that performed *K. pneumoniae* (Figure 2).

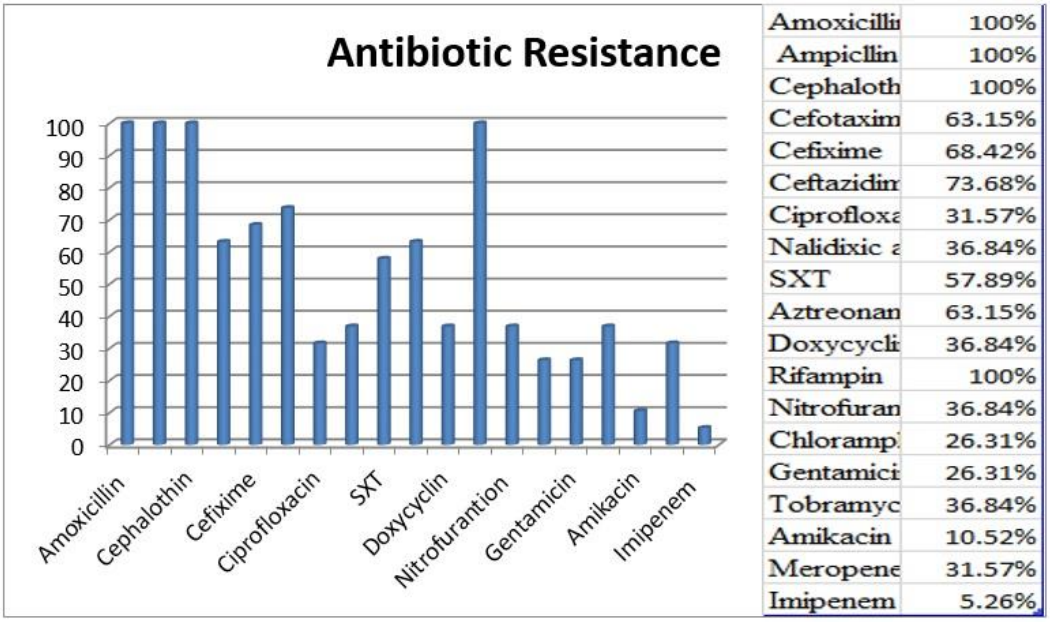

Figure 1. Resistance pattern of isolates

Table 3. Antibiotyping of *K. pneumoniae* isolates

Type	No. of resistant antibiotic	No. of isolates (%)	Resistance of antibiotics
1	20	1 5.26%	AM,RA,AX,CEP,CTX,CAZ,CFM,ATM,SXT,C,DO,MEM,NA,TOB,CIP,NT,AK, IMP.
2	19	1 5.26%	AM,RA,AX,CEP,CTX,CAZ,CFM,ATM,SXT,C,DO,MEM,NA,TOB,CIP,NT,AK.
3	16	1 5.26%	AM,RA,AX,CEP,CTX,CAZ,CFM,ATM,SXT,C,DO,MEM,NA,TOB,CIP,NT.
4	15	1 5.26%	AM,RA,AX,CEP,CTX,CAZ,CFM,ATM,SXT,NT,NA,TOB,CIP,MEM,DO.
5	15	1 5.26%	AM,RA,AX,CEP,CTX,CAZ,CFM,ATM,SXT,GN,NA,NT,CIP,TOB.DO.
6	14	1 5.26%	AM,RA,AX,CEP,CTX,CAZ,CFM,ATM,SXT,GN,NA,NT,CIP,TOB.
7	13	1 5.26%	AM,RA,AX,CEP,CTX,CAZ,CFM,ATM,SXT,C,DO,MEM,GN.
8	11	1 5.26%	AM,RA,AX,CEP,CTX,CAZ,CFM,ATM, SXT ,NA,NT.
9	11	1 5.26%	AM,RA,AX,CEP,CTX,CAZ,CFM,ATM, SXT ,C,GN.
10	11	1 5.26%	AM,RA,AX,CEP,CTX,CAZ,CFM,ATM, SXT,GN,TOB.
11	9	2 10.52%	AM,RA,AX,CEP,CTX,CAZ,CFM,ATM,SXT.
12	8	1 5.26%	AM,RA,AX,CEP,CTX,CAZ,CFM,ATM.
13	6	1 5.26%	AM,RA,AX,CEP,CAZ,CFM.
14	6	1 5.26%	AM,RA,AX,CEP,CAZ,MEM.
15	5	1 5.26%	AM,RA,AX,CEP,DO.
16	4	3 15.8%	AM,RA,AX,CEP.

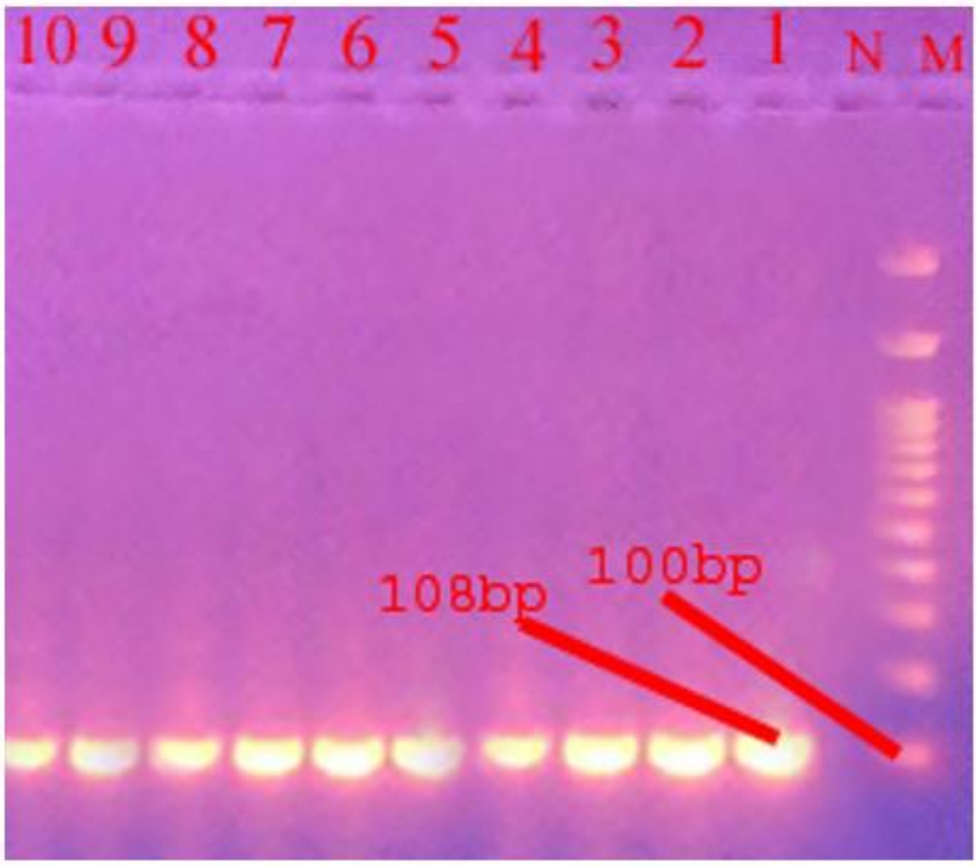

Figure 2. PCR product of (*rpoB*) gene for *K. pneumonia* by gel electrophoresis. Lane L: 100bp DNA ladder; Lane N: negative; Lane Lanes 1-10: Clinical isolates

4. Discussion

The prevalence of *K. pneumoniae* was 7.6% in this study, while other studies reported such percentages as 4.03%, 17.36%, and 32.48% (12-14). These differences in the mean prevalence rates among various studies could be related to differences in geographical location and hygienic practices of the population (15, 16). The highest percentage of *K. pneumoniae* isolates were reported among female patients since the highest number of collected samples in this study were from females; moreover, the age group of 10-35 years represented the highest percentage of *K. pneumoniae* (Table 2) because the young age group participated in outdoor activities, and they are the most activated group. This result is in line with the findings of the studies conducted by Al-Rubaye, Hamza (17), as well as Kadum (12) in Iraq. Ampicillin, amoxicillin, and cephalothin showed the lowest effect towards *K. pneumoniae* isolates, while amikacin and imipenem revealed the highest effect (Figure 1). These results were supported by studies performed by Namratha, Sreeshma (13), as well as Nirwati, Sinanjung (14).

The range of population exposure to antimicrobial agents with the hygienic culture of them and the type of clinical samples that examined were considered major reasons in the variation of prevalence rate of bacterial resistance among many studies (15, 16). Several factors act in the growth of antibiotic resistance, such as the use of antibiotics in the community, hospital, environment, agriculture, and animal production. Furthermore, since there is a possibility to buy antimicrobial agents without prescription, this made the

antimicrobial agents be used extremely. Prolonged and intensive use of antibiotics in health care settings are considered major factors in the wide spreading of severe dangerous nosocomial infections (4). The rate of MDR *K. pneumoniae* isolates in this study was estimated at 100% (Table 3), and the results of a study conducted by Manjula, CM (18) supported this finding. They showed that out of 41 isolates, 37 (90.2%) of them were MDR. Many studies have used a combination of antibiotics in their treatments to avoid emerging new resistant strains.

The MDR bacterial isolates are causing a global challenge in curing infections; as a result, the use of antibiotic stewardship programs is of critical importance in the optimization and monitoring of antibiotics use. Moreover, the Rational Use of Medicine Program is urged on the importance of the collaboration between microbiologists and clinicians to get effective management of infections (19). *K. pneumoniae* is the most prevalent cause of nosocomial infections and is considered an opportunistic pathogen due to the difficulty and misclassification in the detection of this bacterium in the laboratory (20). Therefore, molecular identification is highly necessary for accurate detection. All isolates in this study demonstrated PCR product with 108 bp by *K. pneumonia* specific primer (*rpoB*) that performed *K. pneumoniae* (Figure 2).

This finding is consistent with the results of a study performed by Hadi (21) in Kufa University that showed 100% PCR product representing *K. pneumonia*; however, it was not in line with the results of a study conducted by Al- Rubaye, who showed 87.93% PCR product that represented *K. pneumonia* (17). This could have been related to the type of clinical specimens and the type of laboratory identification methods used in various studies.

5. Conclusion

Careful selection of antimicrobial agents is suggested in this study for proper and accurate management. Due to the high prevalence of MDR *K. pneumoniae* infections, this will aid in decreasing the rate of mortality and morbidity. The development of antibiotic policies and regular surveillance of antimicrobial sensitivity patterns may aid to overcome the overuse of antibiotics that is the main cause of drug resistance development among pathogens.

Authors' Contribution

Study concept and design: S. A. H.
Acquisition of data: T. F. R.
Analysis and interpretation of data: H. M. A.
Drafting of the manuscript: H. M. A.
Critical revision of the manuscript for important intellectual content: S. A. H.
Statistical analysis: T. F. R.
Administrative, technical, and material support: S. A. H.

Ethics

All procedures performed in this study involving human participants were in accordance with the ethical standards of the Kirkuk University, Iraq.

Conflict of Interest

The authors declare that they have no conflict of interest.

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