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Isolation and molecular identification of Klebsiella pneumonia from different human samples in Anbar province

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Abstract

This study aimed to isolate and molecular identifying of Klebsiella pneumonia from different human samples in Anbar province. Patients sent to clinics in the province of Anbar with burns, and wounds of all ages and gender s provided a total of 92 samples. The samples were collected using sterile, single-use cotton swabs, while urine samples were taken by tube and centrifuged and take from sediment, then all stored in BHI broth, and then cultured for 24 hours at 37°C on MacConkey agar. The suspected isolates were diagnosed according to microbiological reference. The DNA of suspected bacteria were extracted by using of kit purchased from Geneaid company, the extraction process was carried out in accordance with the manufacturer's instructions. Two genes were examined among the suspected isolates, these were 16S rRNA and rmpA. Two hours of electrophoresis were carried out using a 70 volt/35 mAmp current. With the use of a UV transilluminator, the DNA bands were seen and captured on camera. The results demonstrate that 12 (13%) K. pneumoniae isolates were found from 92 clinical samples, with 8 (66.7%) coming from urine, 3 (25%) from burns, and 1 (8.3%) from a wound. The antibiotic sensitivity test showed that the highest sensitivity rate of K. pneumonia was recorded to imipenem (70%), gentamycin (65%), ciprofloxacin (60%) and streptomycin (50%), while the lowest sensitivity rate was recorded to tetracycline (20%) and trimethoprim (30%). The 16S rRNA gene was used for the molecular identification of all isolates (12). When the rmpA primer was used to amplify 12 K. pneumoniae isolates, 3 isolates (25%) tested positive for the rmpA gene. In conclusion, Klebsiella pneumonia was isolated at significant point especially from patient with UTI and these isolates were confirmed molecularly by using 16S rRNA gene.

Keywords
Klebsiella pneumonia, Urine, Burns, Wound

One of the most prevalent pathogens in hospitals with high death rates is Klebsiella pneumoniae, which also causes diarrhoea, endophthalmitis, septicemia, meningitis, liver abscesses, urinary tract infections, pneumonia, as well as bacteremia [1, 2]. About one-third of all Gram-negative infections, including burns, UTIs, surgical wound infections, etc., are caused by this bacteria.

One of the major issues with antibiotic therapy and a major source of concern globally is the quick establishment of drug resistance to *K. pneumoniae* isolates [3]. We may be able to treat *K. pneumoniae*

infections more successfully if new knowledge on the patterns of antibiotic resistance in bacterial agents becomes available.

Due to their positioning on various integrons, the *qnr* genes are one of the plasmid-mediated quinolone resistance agents that produce highly fast resistance to Enterobacteriaceae [4-6]. Nalidixic acid and uoroquinolones, among other quinolones, bind to topoisomerase IV and DNA gyrazes and prevent DNA replication. But these antibiotics are now resistant to usage because of their excessive and improper use [7]. The management of these organisms has become increasingly challenging in recent years due to high levels of qnr-mediated resistance among uoroquinolones and quinolones-resistant Enterobacteriaceae isolates [8].

An essential infection control method for assessing the connections between various bacterial isolates is molecular typing. It aids in identifying the origins of contamination, examining the pathogen dispersion status, understanding how many infections changed over time, deciding on the most effective course of treatment for illnesses, and lowering the dangers of antibiotic resistance [9].

This study aimed to isolate and molecular identifying of *Klebsiella pneumonia* from different human samples in Anbar province.

Materials and Methods

Sampling and bacterial isolation

Patients sent to clinics in the province of Anbar with burns, and wounds of all ages and genders provided a total of 92 samples. The samples were collected using sterile, single-use cotton swabs, while urine samples were taken by tube and centrifuged and take from sediment, then all stored in BHI broth, and then cultured for 24 hours at 37°C on MacConkey agar.

The form, size, colour, texture, and other morphological characteristics of colonies from bacterial cultures were characterised [10]. Then, using the Gram technique, a touch from a colony was spread and stained. Cells were then examined under a microscope to determine their Gram response and form and organization [11].

Antibiotic Susceptibility test

Utilizing the Kirby-Bauer disk diffusion method, isolates of *K. pneumoniae* were tested for their resistance to several antibiotics. This was accomplished using the Mueller-Hinton agar (Merck, Germany) medium. Using the principles of the Clinical and Laboratory Standards Institute (CLSI), ciprofloxacin (5 µg) trimethoprim (5 µg), gentamicin (10 µg) , cefoxitin (30 µg), ceftriaxone (30 µg), streptomycin (10 µg), tetracycline (30 µg), and imipenem (10 µg) were used to assess antibiotic resistance. [12]. All of the inoculation plates were incubated aerobically for 18–24 hours at 37 °C.

Molecular identification of K. pneumonia

The DNA of suspected bacteria were extracted by using of kit purchased from Geneaid company, the extraction process was carried out in accordance with the manufacturer's guidelines .

Table 1: Description of the primer sequence.

Gene name	Genes sequence	bp	Reference
16S rRNA	F 5'- AGAGTTTGATCCTGGCTCAG3'* R 5'- GGTTACCTTGTTACGACTT- 3'*	130	[11]
rmpA	F ACT GGG CTA CCT CTG CTT CA R CTT GCA TGA GCC ATC TTT CA	536	

The PCR conditions was described in table 2.

Steps	Time (min.)	Temperature (℃)	Cycle number
Initial denaturation	5	94	1
Denaturation	0.5	94	35
Annealing	1	55	

Extension	1	72	
Hold	10	4	1

Two hours of electrophoresis were carried out using a 70 volt/35 mAmp current. With the use of a UV transilluminator, the DNA bands were seen and captured on camera.

Statistical analysis

Chi-Square x^2 was used for comparison between different percentages in this study by using Statistical analysis system (SAS), 2012 [13].

Results and Discussion

Results of this study illustrated that the highest sensitivity rate was recorded to

Table (3) findings demonstrate that 12 (13%) *K. pneumoniae* isolates were found from 92 clinical samples, with 8 (66.7%) coming from urine, 3 (25%) from burns, and 1 (8.3%) from a wound.

Table 3. Distribution of K. pneumonia isolates from different clinical samples by using bacteriological methods

Samples	Number	Percentage
Burns (23)	3	25%
Wounds (28)	1	8.3%
Urine samples (41)	8	66.7%
Total (92)	12	13%

This outcome was consistent with Hansen's [14] findings, which revealed that this species was the most prevalent among others and accounted for 86% of the clinically isolated Klebsiella species.

According to Abdul Razzaq et al. [15], K. pneumoniae was found in 29.1% (46/158) of the isolates. Omar-Zahid [16] discovered that K. pneumoniae accounted for 79.12% of all Klebsiella spp. isolates from clinical specimens, constituting 54.16% of the total isolates.

These findings corroborated those of [17] and [18], who discovered that the *K. pneumoniae* accounted for 50% of the *Klebsiella spp.* isolated from urinary tract infections, indicating that *K. pneumoniae* plays a significant role in UTI development.

While a research carried out in Syria found that 53.09% of patients with UTI inflammation had Klebsiella spp. isolated from their hospital, the study by According to Al-Dhahri [19], 17.2% of *Klebsiella spp.* were isolated from UTIs. According to Al-Hilfi's [20] explanation, 2.1% of the *Klebsiella spp.* were isolated from the UTI. According to Mahesh et al. [21], wound sources were the source of the fewest instances and urine sources the most.

The antibiotic sensitivity test revealed that the highest sensitivity rate (significant at $P<0.01$) of *K. pneumonia* was recorded to imipenem (70%), gentamycin (65%), ciprofloxacin (60) and streptomycin (50%). While the lowest sensitivity rate was recorded for tetracycline (20%) and trimethoprim (30%), table (4) and figure (1). This finding is consistent with that of [22], who discovered that meropenem (20%), gentamycin (25%), and streptomycin (30%) had the highest antibiotic sensitivity in *K. pneumoniae* isolates, while trimethoprim (63%) and cefoxitin (45.45%) had the lowest.

In a study conducted in Iran, the rates of ciprofloxacin resistance were 23.8%, cefoxitin resistance was 11.5%, gentamicin resistance was 29.2%, and tetracycline resistance was 35.4%. Their findings for gentamicin and tetracycline are comparable to those of the current study; however, our study found higher incidences of ciprofloxacin resistance [23]. Another study found that ciprofloxacin (60%), trimethoprim (61%), and gentamycin (59%), respectively, had the highest rates of resistance among isolates [24].

Table 4: Antibiotic sensitivity tests to Klepsiella pneumonia.

Antibiotics	Dose	Sensitivity %	Resistance %
imipenem	(10 µg)	70	20
gentamicin	(10 µg)	65	35
ciprofloxacin	(5 µg)	60	40
Streptomycin	(10 µg)	50	50
ceftriaxone	(30 µg)	40	60
cefoxitin	(30 µg)	40	60
trimethoprim	(5 µg)	30	70
tetracycline	(30 µg)	20	80
Chi-Square x^2 Value		15.33**	
** (P<0.01).			

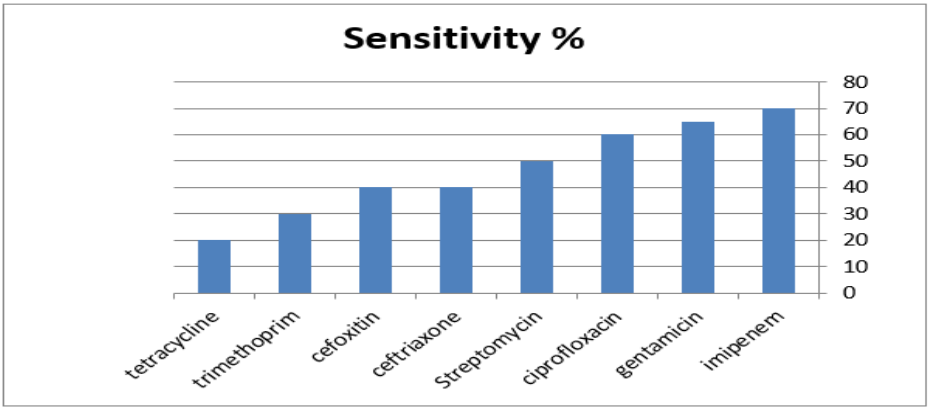

Figure 1: Antibiotic sensitivity of K. pneumonia

The PCR amplification of *K. pneumonia* is carried out using specific primers that relate to the 16S rRNA gene, was used for the molecular identification of all isolates (12) (figure 2).

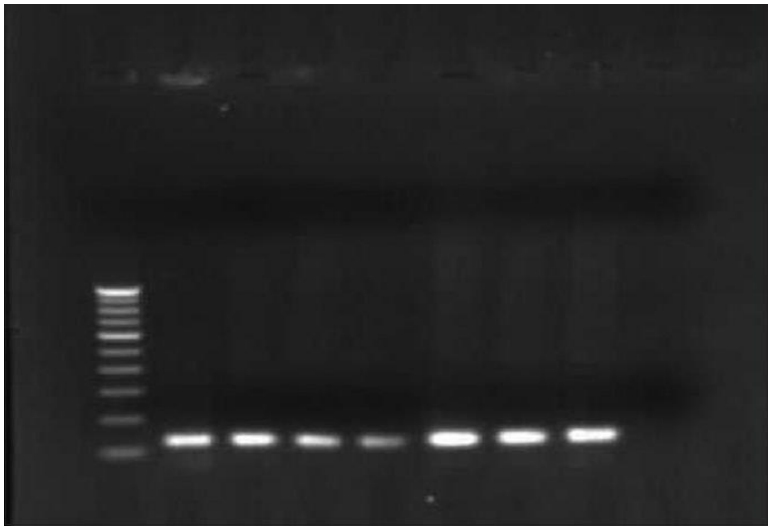

Figure 2: K. pneumoniae 16S rRNA gene amplification using gel electrophoresis. The positive bands were at 130bp

When the *rmpA* primer was used to amplify 12 *K. pneumoniae* isolates, 3 isolates (25%) tested positive for the *rmpA* gene (Figure 2).

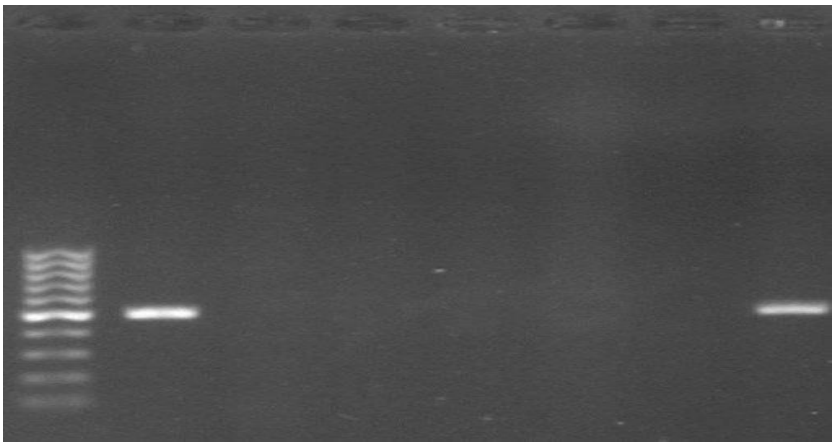

Figure 2. K. pneumoniae rmpA gene amplification using gel electrophoresis. The positive bands were at 536bp.

K. pneumoniae identification were usually done by using the 16S-23S [25]. The study of microbial evolution relies heavily on rRNA sequences, and the 16S rRNA genes in particular have emerged as the gold standard for estimating population diversity [26]. Amplification of the 16S rRNA gene offers a highly precise and adaptable method for classifying bacteria to the species level, Even if using biochemical methods it is notoriously difficult to identify the target species [27]. These results were verified by a number of clinical isolates, the former of which had already been identified by biochemical testing, according to Turton et al. [11]. He reported that 16S rRNA was employed in a multiplex PCR that was performed on isolates of the Klebsiella species, and the results indicated that every isolate produced a clear band with a molecular size of 130 bp.

Numerous studies have linked *rmpA* to virulence, according to Turton et al. [11]; *rmpA* was most often found in the serotype K2 and was similarly connected to numerous capsular types (K1 and K2). The *rmpA* gene is a plasmid-mediated promoter of the capsular polysaccharide synthesis and enhancer of a highly mucoviscous phenotype [28]. Nassif et al. provided the first description [29]. Despite the connection between *rmpA* and the clinical symptoms caused by K. pneumoniae, *rmpA* remained undiscovered for more than ten years. Yu et al. [30] showed that *rmpA*-carrying strains were connected to both the invasive clinical condition and the hypermucoviscosity phenotype. According to Nassif et al. [31], deletion of the *rmpA* gene may result in a 1000-fold reduction in virulence in mouse lethality assays.

Conclusion

Klebsiella pneumonia were isolated at significant point especially from patient with UTI and these isolates were confirmed molecularly by using 16S rRNA gene.

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