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Molecular study of Klebsiella spp. isolated from patients with different clinical isolate.

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Abstract

Background: Klebsiella pneumonia is one of the most important pathogen that is responsible for burn infection. The aim of this study was to investigate the prevalence rate of Klebsiella spp. among isolates from inpatients at Al-hosien hospital. Materials and Methods: A total of 100 clinical isolates were collected sequentially in different wards at the Al-Hosien, hospital, Karbala, Iraq, from December 2021 to May 2022. All isolates were diagnosed by biochemical tests and VITEK 2 system, and Klebsiella pneumonia isolates were selected for further research and 16srRNA amplification was done by PCR, and phylogenetic tree was drowned. Results: A total of 32 Klebsiella pneumonia isolates were collected from patients, the antibiogram results showed that, the highest rate of resistance was related to ampicillin (87%), and the lowest rate of resistance was related to amikacin (5%), the analysis of phylogenetic tree showed that, 8 isolates have had same genotype while another isolates have differ genotypes. Conclusion: in summary we showed that K. pneumonia have high prevalence rate among clinical isolates.

Keywords
K. pneumonia, Clinical isolates, Antibiogram, DNA sequencing.

Klebsiella species are ubiquitously found in nature, soil and animals, and they can colonise medical devices and the healthcare environment. Klebsiella species are considered opportunistic pathogens colonising mucosal surfaces without causing pathology; however, from mucosae Klebsiella may disseminate to other tissues causing life-threatening infections including pneumonia, UTIs, bloodstream infections and sepsis. K. pneumoniae infections are particularly a problem among neonates, elderly and immunocompromised individuals within the healthcare. This organism is also responsible for a significant number of community-acquired infections worldwide. Defining features of these infections are the ability to metastatically spread and their significant morbidity and mortality. Klebsiella strains associated with these infections are regarded as hyper virulent, and recent epidemiological studies indicate that these strains share specific genetic characteristics (1, 2).

Molecular typing and virulence analysis of clinical isolates are powerful tools that can shed light on multidrug resistant (MDR) Klebsiella pneumonia infections. We also used two Polymerase Chain Reaction (PCR) genotyping analyses: Enterobacterial Repetitive Intergenic Consensus (ERIC) and Random Amplified Polymorphic DNA (RAPD) to assess correlations of each with resistance patterns,

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virulence determinants, or capsule types of K. pneumoniae isolates (3, 4)

The current study was conducted to determine the extent of the spread of bacteria as a pathogen and to identify genetic variations in 16s rRNA gene for these isolates.

Materials and Methods

PCR Procedures

Extracted DNA samples, Mastermix solutions, primers, and deionized distilled water were taken from the freezer and put on ice. Each reaction used the identical PCR ingredients except for the DNA sample, for which we built a generic master mix to decrease sampling error by the needed number of samples. This combination comprises the elements specified in Table 3-3, therefore we added 15 l of Mastermix, 1 l of each primer, and 12 l of deionized distilled water to each sample. 30 microliters were included in the vial. Each vial was prepared with 29 microliters of Mastermix and 1 microliter of DNA sample until the total volume reached 30 microliters. We spun the samples for 5 seconds and then the samples. Additionally, the primer's temperature was regulated to a certain value (using the temperature gradient program of the device) (5). Tables 1 detail the temperature conditions that apply to this primer.

Table 1, PCR Cycles at Various Temperatures

Cycles	Temperature	Time	Steps
1	95	3m	Denaturation
32	95	30sec	Denaturation
	58	"45	Annealing
	72	"35	Elongation
1	90	10m	Tremination

Table 2, The specific primer 16s rRNA of gene

Primer	Sequence	Tm (°C)	GC (%)	Product size
Forward	AGAGTTTGATCCTGGCTCAG	54.3	50.0	1250
Reverse	5'GGTTACCTTGTTACGACTT-	49.4	42.1	base pair

Table 3, The Components of the Maxime PCR PreMix kit (i-Taq)

Material	Volume
i-Taq DNA Polymerase	5U/µl
DNTPs	2.5mM
Reaction buffer (10X)	1X
Gel loading buffer	1X

Electrophoresis

Electrophoresis has been done to determine DNA pieces after the process of extraction or to detect the result of the interaction of PCR during the presence of the standard DNA to distinguish the bundle size of the outcome of the interaction of PCR on the Agarose gel.

Prepare of the Agarose gel

According to Sambrook et al (1989), the agarose gel has been made in 1.5% condensation by melting 1.5 g of agarose in 100 ml of previously made TBE buffer. Agarose has been heated to boil then left to cool down at (45-50°C). The gel has been poured in the pour plate in which the plate of agarose support has been prepared after fixing the comb to make holes that would hold the samples. The gel has been poured gently not to make air bubbles and left 30 minutes to cool down. The comb has been removed gently of the solid agarose. The plate has been fixed to its stand in the Electrophoresis horizontal unit represented by the tank used in the Electrophoresis. The tank has been filled with TBE buffer in which it covers the gel surface.

Preparation of sample

3 µl of the processor loading buffer (Intron / Korea) has been mixed with 5 µl of the supposed DNA to be electrophoresis (loading dye), after the mixing process, the process of loading is now to the holes of the gel. An Electric current of 7 v\c2 has been exposed for 1-2 h till the tincture has reached to the other side of the gel. The gel has been tested by a source of the UV with 336 nm after put the gel in pool contain on 30µl Red safe Nucleic acid staining solution and 500 ml from

distilled water. Working the electrophoresis system

Red safe Nucleic acid staining solution

Red Safe Nucleic Acid Staining Solution (20,000x) is anew and safe nucleic acid stain, an alternative to the traditional Ethidium bromide (EtBr) stain for detecting nucleic acid in agarose gels. It emits green fluorescence when bound to DNA or RNA. This new stain has two fluorescence excitation maxima when bound to nucleic acid, one centered at 309nm and another at 419nm. In addition, it has one visible excitation at 514nm. The fluorescence emission of Red Safe bound to DNA is centered at 537nm. Red Safe Nucleic Acid Staining Solution (20,000x) is as sensitive as EtBr. The staining protocol for Red Safe Nucleic Acid Staining Solution (20,000x) is similar to that for EtBr. Compared to EtBr, known as a strong mutagen, Red Safe Nucleic Acid Staining Solution (20,000x) causes much fewer mutations in the Ames test. In addition, Res Safe Nucleic Acid Staining Solution (20,000x) has a negative result in mouse marrow chromophilous erythrocyte micronucleus test and mouses permary spermatocyte chromosomal aberration test. So it is wise to choose RedSafe Nucleic acid Stainig Solution (20,000x) instead of EtBr for detecting nucleic acid in agarose gels. (Cat. No. 21141), (6-8).

Statistical Analyses

The information gleaned from the outcomes of this study is given statistically and in the form of connected tables. When doing data analysis, the data are first checked for normality using the Kolmogorov-Smirnov test. If the level is more than 0.05, the data have a normal distribution; otherwise, the distribution is abnormal. After confirming the normality of the data distribution, the t-test is used for binary comparisons; in the event of an abnormal distribution, the Mann-Whitney non-parametric test is performed, which has a substantial level of significance. $P<0.05$. When comparing more than two (many) variables, the ANOVA parametric test is used for normally distributed data while the Kruskal-Wallis non-parametric test is used for normally distributed data(9)

Clinicopathological properties of patients in this study

Among 100 isolates a total of 32 Klebsiella pneumonia isolates were collected from patients in different wards of Alhosein Teaching hospital, Karbala during time period of December 2021 to May of 2022. The age of patients was from 1 to 43 years old. The patients of this study was consisted of 15 male and 17 female. For confirmation of isolates, the DNA was extracted from all isolates. In order to evaluate the quality of the extracted DNA, including the absence of smears and multiple bands, the extracted DNA was loaded on a 1% agarose gel. For this method, about 5 microliters of DNA extracted with loading buffer was placed in the wells of 1% agarose gel and electrophoresed for 45 minutes and then the gel was placed in the gel dock machine to observe the bands.

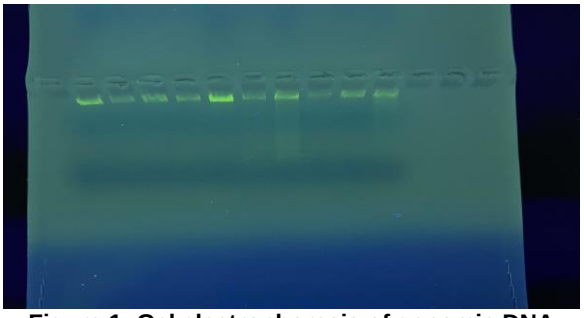


Figure 1: Gel electrophoresis of genomic DNA extraction from samples, 1% agarose gel at 5vol /cm for 30 min.

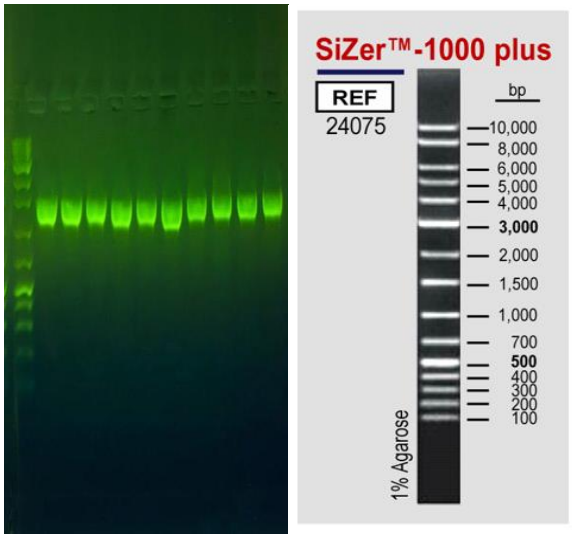


Figure 2, PCR product the band size 1250bp. The product was electrophoresis on 1.5% agarose at 5 volt/cm2. 1x TBE buffer for 1 hour. N: DNA ladder (1000 plus).

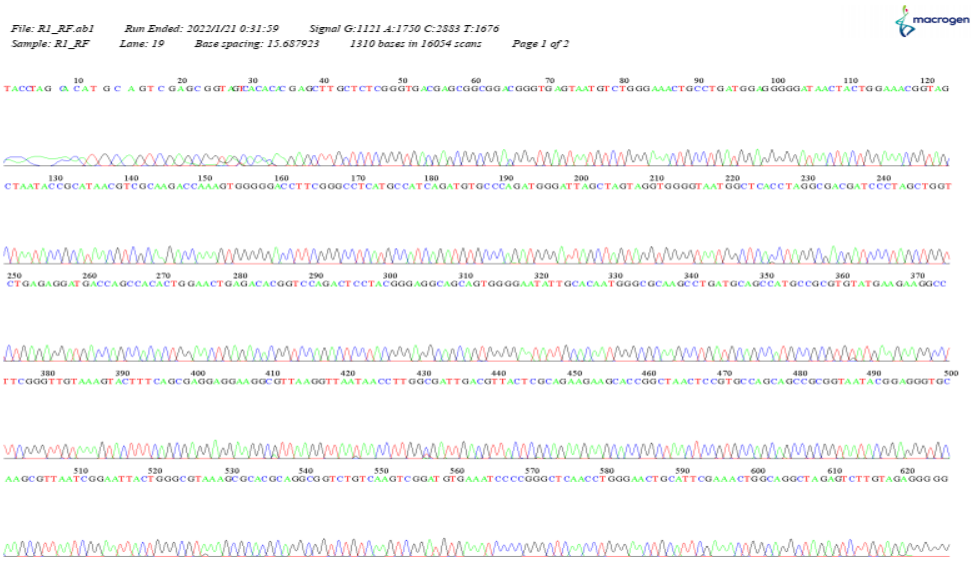


Figure 3, A representative image of sequence data from amplified part of gene.

PCR results

To identification of gene from all clinical isolated extracted DNA were subjected to PCR to amplification of 16s rRNA of gene (Figure 2). The PCR product was 1250bp. After PCR the products were subjected to sequence analysis (Figure 3).

Diagnosis and site of infection in all patients

As presented in table 4, 32 positive isolates were used for this study. This table explains the number of bactria isolates and their site of infection. 37.5% of isolates were Pneumonia, 31.25% were wound and blisters, and 31.25% of isolates were from vagina. In addition, 28.12% of pneumonia isolates were K. pneumonia while just 9.37% of patients K.aeruginosa.

Table 4, total isolate of 32 from different clinical patients. This table explains the number of Bactria isolates and their site of infection.

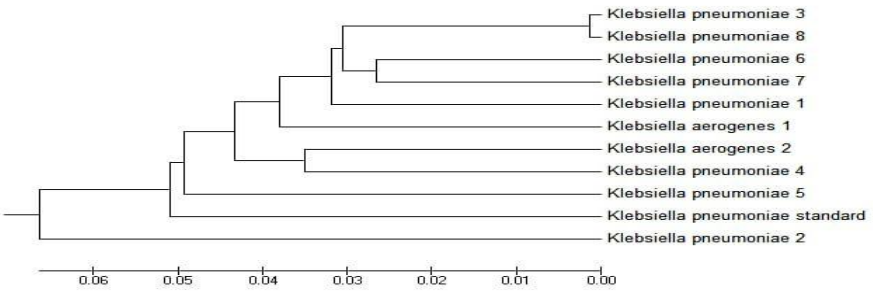
Site of infection	K. pneumonia		K. aeroginosa		Total	
	No	%	No	%	No	%
Pneumonia	9	28.12	3	9.37	12	37.5
Wound and Blisters	8	25	2	6.25	10	31.25
Vagina	9	28.12	1	3.12	10	31.25
Total	26	81.25	6	18.75	32	100

Phylogenic three of all isolates of this study

Bacterial isolation and identification were performed by sequencing. The phylogenetic tree was drowned. As presented in Figure 4 eight of clinical were Klebsiella pneumoniae in a same genotype, however just one clinical isolates was in another genotype.

Figure 4, Phylogenic three of all isolates of this study

Discussion



Klebsiella is a rod-shaped, non-motile gram-negative bacterium with a polysaccharide capsule, which covers the entire surface of the cell and creates resistance against many host defense mechanisms. Members of the Klebsiella genus express two types of antigens on the surface of their cells. Lipopolysaccharide (o antigen) and it is capsular polysaccharide (antigen k). Intestinal bacteria are different in terms of biochemical properties. Klebsiella ferments glucose and lactose. In McConkey's medium, they produce prominent pink mucoid agar colonies that are drawn when removed with an ans. Also, Klebsiella may lose its capsule due to repeated cultures. The normal place of colonization of this bacterium in a healthy person is the gastrointestinal tract, eyes, respiratory tract, urinary tract, and genital tract. K. pneumoniae exists as a saprophytic microorganism in the human nasopharynx and digestive tract. Its amount is 5-38% in stool samples and 1-6% in nasopharynx. Since there are no suitable conditions for the growth of Gram-negative bacteria on the human skin, Klebsiella is rarely observed in that area and is considered as a temporary flora. Klebsiella are considered among intestinal bacilli. In general, when these bacteria live in the intestine, they live together. Therefore, their settling in this place is considered a reservoir or a source for contamination and production of disease in other places, including the lungs and urinary tract. Klebsiella species has some virulence factors. The capsule in Klebsiella is a polysaccharide (CPS) that covers the entire surface of the cell and provides resistance to the bacteria against many host defense mechanisms. Extracellular capsule is necessary for pathogenicity. Currently, about 80 different capsular antigens are known in this bacterium. Although the polysaccharide capsule (CPS) of Klebsiella is effective in its pathogenicity, it has recently been shown that the amount of mannose in CPS reduces the virulence of the strain. In addition to k antigen, o antigen is also considered as a pathogenic factor in LPS.

Ahmed Abduljabbar Jaloob Aljanaby and colleagues reported that burns infections and urinary tract infections are the most important prevalent diseases in Asian countries, such as Iraq. Klebsiella pneumoniae is one of the most important bacteria cause this type of infections especially in hospitals. Therefore, they aimed to investigate the prevalence of multi-drug resistance K. pneumoniae and extended-spectrum beta-lactamases producing K. pneumoniae isolates from inpatients with urinary tract infection and burns infections in Al-Kufa hospital in Al-Najaf province, Iraq. They used a total of 285 clinical samples that were collected from in-patients infected with urinary tract infection (141 urine samples) and burns infections (144 burns swabs). Fourteen different antibiotics were used by disc diffusion method and 13 antimicrobials resistance genes were used by PCR technique. Their results showed that the highest resistance rate was observed for amoxicillin 25 μg and amoxicillin + clavulanic acid 20+10μg (97.67%) while the lowest resistance rate was observed for imipenem 10μg (9.30%). The most common resistance associated-genes were blaSHV (86.04%) and at lower prevalence were IMP (9.30%). They concluded that Klebsiella pneumoniae strains isolated from burns infections were more virulent than those isolated from urinary tract infections. K. pneumoniae is the cause of pneumonia, sepsis, hospital infections and urinary tract infection (UTI). One of the important organisms producing K. pneumoniae, ESBLs. Is. Pneumonia is one of the most important cases of hospital-acquired infections, especially in infants in the ICU (10).

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