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Study the Molecular Identification of *Klebsiella Pneumonia* Isolated From Different Patients in AL Diwaniya City

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Annotation: This study was completed during a period of (15/11/2021) to (20/2/2022) in the bacteriology laboratories of the Gynecology Hospital in Qadisiyah Governorate. The aim of the study was to identify one of the types of Klebsiella bacteria, which is more susceptible to infection, men or women.

Besides to knowing the severity of the infection. In addition to knowing pathogenicity and the gravity of its impact on the organs of the human body. From 30 samples isolated just seven samples were (23. 3%) diagnosed as klebsiella species, about four samples (57. 2%) has been diagnosed as klebsiella pneumonia that isolated from (urine (50%), blood (25%), sputum (25%)).

Introduction

Klebsiella belongs to the tribe *Klebsiellae*, a member of the family Enterobacteriaceae, The organisms are named after Edwin *Klebs*, a 19th century German microbiologist (5). *Klebsiella* is a type of bacteria commonly found in nature, In humans, the bacteria are often present in parts of the digestive tract where they do not generally cause problems, In the United States, Klebsiella pneumoniae and Klebsiella oxytoca are the two strains responsible for most human illnesses, Many *Klebsiella* infections are acquired in the hospital setting or in long- term care facilities, In fact, *Klebsiellae* account for up to 8% of all hospital- acquired infections, People with a compromised immune system and/or people who have an implanted medical device (such as a urinary catheter or airway tube) are more at risk for *Klebsiella* infections, extensive use of antibiotics has resulted in the development of antibiotic-resistant strains of *Klebsiella*. (1, 2, 3, 4) .

Klebsiellae are nonmotile, rod-shaped, gram-negative bacteria with a prominent polysaccharide capsule, This capsule encases the entire cell surface, accounts for the large appearance of the

organism on gram stain, and provides resistance against many host defense mechanism, members of the *Klebsiella* genus typically express 2 types of antigens on their cell surface, The first is a lipopolysaccharide (O antigen); the other is a capsular polysaccharide (K antigen), Both of these antigens contribute to pathogenicity(6, 7). *Klebsiella* bacteria tend to be more round and thicker than other members of the Enterobacter family, They can be found singly in pairs or in short chains, They do not have specific growth requirements and grow well on standard laboratory media, but they grow best between 35 and 37 °C and pH 7. 2, And most breeds can live with citrate and glucose(8).

Klebsiella characteristically grow as large mucoid colonies on MacConkey agar, Citrate-containing media can be used to facilitate isolation of *Klebsiella* strains because these organisms can use citrate as a sole carbon source, rarely, blood cultures have required longer than 72 hours of incubation for radiometric detection of *Klebsiella*(9).

- **Virulence factors**

K. pneumoniae possess a number of virulence factors which share with pathogen and include capsule antigens, adhesion factors, enterotoxin produce like lipopolysaccharide as well as resistance killer effect for serum and system the gain on iron (Siderophore) and multi resistance for antibiotics which considered the main reason in spread acquired infections in hospitals, as the percentage infections 80% which led to find alternative treatments and we will mention some Iraqi research on these factors.

- **The capsule**

The capsule is considered fundamental to the virulence of *Klebsiella*, as it protects the bacterium from phagocytosis and prevents the bacteria by bactericidal serum factors [3]. Some serotypes or capsular types of *K. pneumoniae*, e. g. (K1, K2, K5, K54 and K57), have been correlating with invasive human infection illness.

- **Lipopolysaccharide**

Lipopolysaccharide represents an important and essential factor in bacterial pathogenicity, especially *K. pneumoniae*, as it is one of the superficial compositions of bacteria that help it to resist phagocytosis, and it is characterized by its ability to activate the complement factor (3). It participates in protecting bacteria against the host's Complement System. LPS consists of three parts: Lipid A, Core polysaccharide, and O antigen, which consists of a side chain of the polysaccharide, and the antigen O is responsible for the bacteria's resistance to killing (13).

- **Outer membrane proteins**

Is one of the important proteins of the gram negative bacteria are present in the outer membrane OmpA, which is characterized by most of the Enterobacteracea. OmpA is independent of the core polysaccharide in *K. pneumoniae*, which has an important role in preventing the activation of epithelial cells in the airway as it acts on NF-kB-p38- and p44 / 42- dependent pathways and thus particibate to the attenuation these cells through the inflammatory response (18).

- ***K. pneumoniae***

Klebsiella pneumoniae was first described by Carl Friedlander in 1882 as a bacterium isolated from the lungs of patients who had died from pneumonia (10)

Klebsiella pneumoniae is a Gram negative nonmotile and encapsulated bacterium found in environmental conditions as diverse as soil, plant leaves, mammalian intestines, and waste waters (11, 12). It is an opportunistic pathogen that is able to colonize the mucosal epithelium of the gut and nasopharynx and to disseminate into the deep tissues and bloodstreams of susceptible patients, causing severe infections such as pneumonia, meningitis, endophthalmitis, pyogenic liver abscesses, and bacteremia(13, 14, 15, 16). The ability of this bacterium to form a biofilm on

invasive medical devices leads to subsequent health care associated infections, particularly in the urinary and pulmonary tracts(17).

K. pneumoniae infections are difficult to treat, particularly because of the pathogen's high endogenous antibiotic resistance, for example, *K. pneumoniae* is intrinsically resistant to ampicillin, owing to the presence of β-lactamase (SHV-1) encoding genes in its chromosomal genome(18). In addition it was incriminated for the appearance of multidrug resistant (MDR) strains against third generation cephalosporins, fluoroquinolones, carbapenem and aminoglycosides(19, 20). The correlation between its wide ecological range and its ability to carry multidrug resistance genes makes of *K. pneumoniae* a good candidate for dissemination and horizontal gene transfer among the Gram negative species, This pathogen contributes to a large diffusion of widespread antibiotic resistance genes especially in its diverse niches(21, 22).

Clinical strains of *K. pneumoniae* can be divided into two main categories: the classical group (cKp) comprising of MDR strains, and the hypervirulent (hvKp) group of strains(23). HvKp strains are emerging variants of cKp that, unlike cKp strains, cause organ and life threatening infections even in healthy immunocompetent individuals, hvKp strains are considered as strict pathogens that cause infections at multiple sites including pyogenic liver abscesses, *pneumonia*, endophthalmitis, meningitis, and necrotizing fasciitis followed by metastatic spreading(24, 25). Indeed, whole genome sequencing of *K. pneumoniae* strains isolated from rectal swabs and clinical samples from the same patients showed that ~50% of *K. pneumoniae* infections result from the patients' microbiota(26).

Pathogenicity

K. pneumoniae can infect your : lungs , bladder , liver , eyes , brain, wound and blood. Healthcare places are most vulnerable to *Klebsiella* infections due to the nature of procedures that allow easy access of bacteria into the body (27).

InPulmonary Infections

K. pneumoniae has been historically recognized as a common respiratory pathogen since its discovery in 1882 and is involved in both community-acquired pneumonia (CAP) and hospital-acquired *pneumonia* (HAP)(28, 29). CAPs caused by *K. pneumoniae* are rare in Europe and North America but account for 15% of the total cases of CAP in Asia and South Africa, mainly due to the increasing prevalence of hvKp strains, medical ventilators are a major risk factor in HAPs caused by *K. pneumoniae* because they provide the pathogen with a surface on which to colonize and form biofilms, the Symptoms of lung injury include: fever, chills, coughing , yellow or blood mucus and chest pain (30, 31).

Urinary Tract Infection

K. pneumonia enters the urinary tract, it can lead to a UTI. A UTI can affect any part of the urinary system, including the urethra, kidneys, bladder, and ureters. . Classical *K. pneumoniae* strains also lead to severe disseminated infections that are difficult to treat owing to their intrinsic antibiotic resistancec . Women are at a greater risk of getting a UTI than men. *K. pneumoniae* cause UTIs in older women, UTIs don't always cause symptoms, But sometimes it causes frequent to urinate or pain and burning when urinating bloody or cloudy urine(33).

blood infection

K. Pneumoniae that enters the bloodstream can cause bacteremia, or an infection of the blood, Bacteremia needs to be treated right away, as these infections can progress to sepsis and septic shock, which can turn deadly(34).

- **Treatment**

strains of *K. pneumoniae* are resistant to most antibiotics, including carbapenems, which are considered last-resort drugs.

These bacteria produce enzymes called *Klebsiella pneumoniae* carbapenemases (KPC), which render the . antibiotics ineffective.

These hardy, high-threat-level microbes are part of a group called carbapenem- resistant *Enterobacteriaceae*, or CRE(35*). K. Pneumoniae* is generally resistant to all b-lactams, including carbapenems (36, 37, 38). Often the resistance is maintained even when associated with b-lactamase inhibitors such as clavulanic acid and tazobactam (39, 40, 41). However, depending on the phenotypic characteristics, some case reports have shown that the association with b-lactamase inhibitors slightly improves the action of some b-lactam antibiotics(42). Resistance to quinolone antibiotics such as ciprofloxacin and levofloxacin was also observed(43).

- **Antibiotics**

typically use antibiotics to treat *K. pneumoniae* infections. But the rise of antibiotic-resistant strains of the bacteria has complicated matters.

Some "superbug" strains of *K. pneumoniae* are resistant to most antibiotics, including carbapenems, which are considered last-resort drugs.

These bacteria produce enzymes called *Klebsiella pneumoniae* carbapenemase (KPC), which render the antibiotics ineffective.

These hardy, high-threat-level microbes are part of a group called carbapenem- resistant Enterobacteriaceae, or CRE.

To treat CRE, doctors rely on several powerful antibiotics that still have some effectiveness against the bacteria, particularly when used in combination, according to a 2015 report published in Open Forum Infectious Diseases.

Materials and Methods

Materials

Equipments and instruments:

Table (1): Molecular study equipments and instruments with their company and country of origin:

No.	Equipments and Instruments	Company/ Country
1	PCR thermocycler T100	BioRad/USA
2	Vortex mixer	Talboys/USA
3	Cell Disruptor Genie vortex	Scientific Industries/USA
4	High Speed Cold centrifuge	/Germany
5	High Speed centrifuge	Hettich /Germany
6	Sensitive Balance	Ohaus /USA
7	Water Bath	Polyscience/ USA
8	Micropipettes (1-10 µl, 1-20 µl, 10-100 µl, 100-1000µl)	Gibson/ France
9	Eppendorf tubes	BioBasic/ Canada
10	Incubator	BINDER
11	Laminor Air Flow (hood)	Prutscher
12	Electrophoresis	Bioneer/ Korea
13	PowerPac HC Electrophoresis Power Supply	BioRad/USA

14	U. V transilluminator	Wised/Korea
15	Tube Rack Double Panel Microcentrifuge PCR Centrifuge Tube Holder	P-ABC/ USA

Molecular study Kits

PCR detection Kits

Table (2): The PCR detection Kits used in this study with their companies and countries of origin:

No.	Kit	Company	Country
1	**Presto™ Mini gDNA Bacteria Kit**	**Geneaid**	**Taiwan**
	Gram+ Buffer		
	GT buffer lysis buffer		
	GB buffer binding buffer		
	Proteinase K		
	W1 buffer		
	Wash buffer		
	Elution buffer		
	GD column		
	Collection tube 2ml		
2	**GoTaq® Green PCR master Mix**	**Promega**	**USA**
	Taq DNA polymerase		
	dNTPs (dATP, dCTP, dGTP, dTTP)		
	Tris-HCl pH 9. 0, KCl, & MgCl2		
	Stabilizer and loading dye		

Chemicals materials and solutions:

Molecular study chemicals and solutions:

Table (3): Molecular study chemicals and solutions with their company and country of origin:

No.	Chemicals	Company/ Country
1	Absolute ethanol	CHEM-LAB/ Belgium
2	DNA Marker ladder 2000-100bp	Bioneer/ Korea
3	Ethidium bromide	BioBasic/ Canada
4	TBE buffer	iNtRON / Korea
5	Agarose	iNtRON / Korea
6	Free nuclease water	BioLabs/ UK

Primers:

The PCR primers for 16S ribosomal RNA gene were designed in This study using NCBI Genbank database and primer 3 plus. These primers were provided by ScientificReserchер. Co. Ltd in Iraq as following table (3-5):

Table (4): *The 16S ribosomal RNA* gene PCR primers with their nucleotide sequence and product size.

Primer	Sequence (5'-3')		Produc t Size	Genbank
16S rRNA gene Klebsiella pneumoniae	F	CCTGGACAAAGACTGACGCT	584bp	LC557135.3
	R	AGTTGCAGACTCCAATCCGG		

➢ Methods

This study was conducted from December (2021) to April (2022) in the Maternity and children hospital of AL- Diwaniyah Town, including blood, sputum and burns, numerous medical specimens are collected from different clinical samples. The selection process was carried out according to the (Friedrich et al. 2005)

Distinguishing of Bacterial Isolat

All isolates are described by traditional microscopic examination (Gram's stain), morphological colony characteristics on macConkey agar , blood agar and normal bacteriological tests (Podschun and Ullmann 1998).

Specimens' collection:

Different clinical samples such as urine , blood , sputum which was taken from in patients and outpatients in Maternal, and children hospital of AL Diwaniyah city during the period from 15/11/2021 till 20/2/2022. The collection process has been conducted according to (18).

Identification of Bacterial Isolates:

The isolates were identified according to (19) by using traditional microscopic examination (Gram's stain), colony morphological features on MacConkey agar and blood agar, and standard biochemical tests.

➢ DNA extraction:

The total genomic DNA of the *K. pneumoniae* was isolated using the DNA extraction and purification kit (Geneaid, USA) according to the manufacturer instructions. DNA preparations were then analyzed by electrophoresis in 1. 5% agarose gel.

➢ Polymerase chain reaction:

Polymerase chain reaction was used to amplify the entire sequences ofthe genes studied in this research. The specific primers (Bioneer, Korea)used for the amplification of these genes (20) were shown in (table 4). The PCR mixtures contained: Top DNA polymerase 1U, dNTP (dATP, dCTP, dGTP, dTTP) each: 250µM, Tris-HCl (pH 9. 0) 10mM, KCl 30mM, MgCl2 1. 5mM, Stabilizer and tracking dye . The cycling parameters of amplification were: Initial denaturation 95°C for 5min, Denaturation 95°C for 30 sec. , Annealing (58°C) for 30 sec. , Extension 72°C for 1min. , Final extension 72°C for 5min.

➢ Agarose gel electrophoresis:

The products were separated in 1. 5% agarose gel in TBE buffer (pH 8), stained with ethidium bromide, and photographed in ultraviolet light electrophoresis result noticed by using gel documentation system (21).

RESULTS AND DISCUSSION:

Klebsiella is a type of Gram-negative bacteria that can cause different types of healthcare-associated infections, including *pneumonia*, bloodstream infections, wound or surgical site infections, and meningitis. Increasingly, *Klebsiella* bacteria have developed antimicrobial resistance.

Total thirty specimens were collected from different clinical sources…(Sputum, blood and Urine) , the results showed that among the thirty isolates seven samples as *klebsiella* species (23. 3%) it was four samples from the *K. pneumonia* diagnosed in the laboratory by biochemical tests , genotype through the ***16SrRNA*** gene **(figure. 1)** and showed this evidence of the pathogenicity of these bacteria and their resistance to antibiotics .

From four specimens (57. 2%) it has been diagnosed as *k. pneumoniae* , it was 1 (25%) Isolates from sputum , 2 (50%) isolate from urine and 1

(25%) from blood

(Tabel 5).

Table 1: Numbers and percentage of *K. pneumoniae* by total isolates of bacteria and infection sites…

Site of infection	*K. pneumoniae*	Percentage (%)
Urine	2	50%
Sputum	1	25%
Blood	1	25%
Total	4	57. 2%

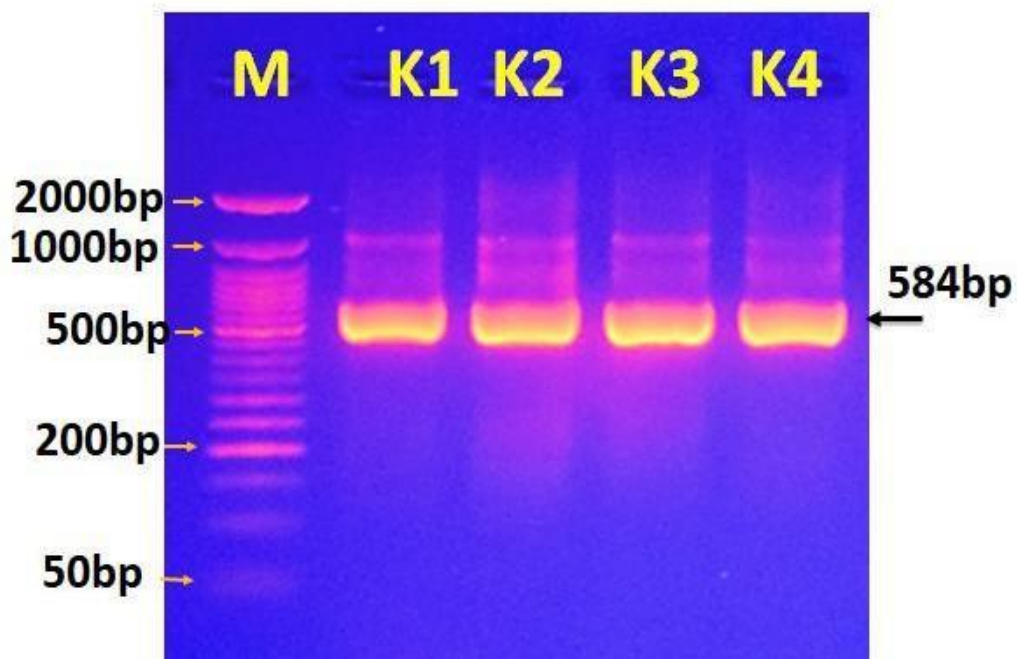

Figure (1): Agarose gel electrophoresis image that showed PCR product analysis of 16S ribosomal RNA gene for detection *Klebsiella pneumoniae* isolates. M (Marker ladder 2000-50bp). Lane (K1-K4) Showed positive Klebsiella pneumoniae isolates 16S ribosomal RNA gene at 584bp product size.

DISCUSSION

By comparing the percentage of results, it was found that the percentage of samples that were isolated from the sputum (25%) showed an agreement with the percentage of the researcher's sample (25. 4%). . .

While the percentage of other samples isolated from blood and urine showed a clear difference when compared with the researcher (44) , the increased infection rates may be due to excessive and wrong use of the drug as well as Infections can also occur through the use of contaminated medical equipment. For example, people on ventilators can contract *Klebsiella pneumonia* if

breathing tubes are contaminated with the bacteria. Long courses of antibiotics can also increase a person's risk of getting a *klebsiella* infection.

Of 4 samples, 1 (25%) were related to men and 3 (75%) were for women. In general, women are more likely than men to develop a urinary tract infection because of the difference in the anatomy on a woman’s body. The entrance to the urinary tract, bladder, and urethra is only a short distance from the colon, digestive system and anus, since bacteria from the intestine They can easily pass into the urinary system, they can increase the chance of getting an infection.

CONCLUSION

Through a review of many research studies of the Iraqi isolates of K. pneumoniae within 5 years a go, it became apparent that over time, the ability of these bacteria to resist antibiotics increases through the development of the virulence factors they possess or possess by acquiring new characteristic. This indicator shows the difficulty of discovering new antibiotics that work to kill these bacteria therefore, plant extracts have been used recently to inhibit them.

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