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Diversity studies by using RAPD-PCR and antibiogram discrimination of *klebsiella* species isolated from pets and co-habitant human

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ABSTRACT

Klebsiella is readily transmissible among various species, encompassing both humans and animals. It plays a crucial role in the occurrence of nosocomial infections, including pneumonia, sepsis, and urinary tract infections. The aim of this research was to ascertain the genetic similarities between *Klebsiella* isolates obtained from pet animals and humans through the application of Random Amplified Polymorphic DNA (RAPD-PCR) technology. Traditional microbiological methods were employed to assess the prevalence of *Klebsiella* spp. in both pet animals and their human cohabitants. The specimens were obtained from pet animals specifically cats and dogs, through nasal swabs, fecal samples and urine samples (n = 100), while human samples were collected from patients that had infection in respiratory and urinary tracts from labs and cohabitating humans with pet animals in houses (n = 50) (sputum and urine samples) at El-Menoufia and El-Garbia Governorates. Conventional techniques identified 26 out of 150 isolates of *Klebsiella* spp. from pet animals and human, 7/50 (14%) from dogs and 11/50 (22%) from cats) and 8/50 (16%) from human samples. The incidence of *K.pneumoniae* was 16/150 (10.6%), and *K.oxytoca* was 10/150 (6.66%).

Antimicrobial susceptibility test revealed that *Klebsiella* isolates from pets and human showing 100% completely resistant to amoxicillin\calvulante and 80% sensitive to ciprofloxacin for *Klebsiella* isolates from pets.

The Polymerase Chain Reaction (PCR) and Random Amplified Polymorphic DNA (RAPD) PCR techniques were utilized to validate the *Klebsiella* isolates and ascertain the genetic fingerprints among them.

Klebsiella isolates showed degree of identity (38%) for genetic fingerprints between the sample isolated from pets and cohabitating human.

1. INTRODUCTION

Klebsiella species are recognized as rod-shaped, Gram-negative, non-motile bacteria that ferment lactose and are facultatively anaerobic, classified within the Enterobacteriaceae family. The microorganism is extensively found in various environmental settings, including soil,

manure, water, and plants, in addition to being present in the intestinal tract, skin, and oral microbiota of both animals and humans. **[1]**

Klebsiella pneumoniae is a highly contagious nosocomial agent **[2,3]**. *Klebsiella pneumoniae* ranks as the second most common species within the Enterobacteriaceae family that causes urinary tract infections (UTIs) in humans **[4]** Additionally, it has the potential to cause community-acquired infections in both humans and animals. The spread of harmful microorganisms from companion animals to people is a significant source of concern. Colony-forming bacteria are more likely to spread between species when people and pets are close, such as when animals' waste gets into people's bodies **[5-7]**.

Numerous "classic" strains of *K. pneumoniae* are present as pathobionts within the human body, alongside drug-resistant variants. A pathobiont is characterized as a symbiont that has the potential to induce disease under particular circumstances, such as compromised host immune systems and changes in microbiota composition **[8]**. *K. pneumoniae* is also capable of causing extraintestinal infections in companion animals, which may include urinary tract infections, pyometra, upper respiratory tract infections, and septicemia **[9]**. *Klebsiella pneumoniae* asymptomatically inhabits the skin, oral cavity, nasopharynx, and gastrointestinal tract **[10]**. It is a prevalent source of infections within the gastrointestinal system. Research has indicated that nearly fifty percent of infections in intensive care unit patients can be attributed to the presence of their own *K. pneumoniae* from the gastrointestinal microbiota. *Klebsiella oxytoca* is frequently found in healthcare settings. This bacterium is capable of causing severe infections, one of which presents symptoms similar to pneumonia. Additionally, *Klebsiella oxytoca* may result in urinary tract infections (UTIs), wound infections, and various other types of infections **[11]**.

Klebsiella has the potential to lead to severe bacterial infections affecting the cardiovascular, pulmonary, gastrointestinal, pancreatic, renal, and coagulation systems in both humans and animals **[12]**. The emergence of antimicrobial resistance increases the risk of treatment failure with antimicrobial therapies in both populations. Additionally, the development of antibiotic-resistant bacteria in companion animals could pose a significant threat to public health in humans . *Klebsiella* spp. represents a notable opportunistic pathogen affecting both humans and animals. However, the extensive and indiscriminate application of antibiotics has led to a rise in antimicrobial resistance. The emergence of antibiotic-resistant bacterial illnesses highlights the significance of monitoring potentially harmful germs. An investigation is being conducted on the antimicrobial resistance of strains of *Klebsiella* that exhibit high resistance to B-lactams, cephalosporins, and tetracycline **[13]**.

Klebsiella isolates with multidrug resistance (MDR), including hypervirulent *K. pneumoniae* , pose a significant public health problem worldwide **[14]**. β-lactam antibiotics represent the most frequently prescribed and commonly utilized class of antibiotics for the treatment of bacterial infections caused by Enterobacteriaceae **[13]**, which includes *Klebsiella* species. The indiscriminate use of these antibiotics can lead to the emergence of *Klebsiella* strains that produce broad-spectrum β-lactamases, such as extended-spectrum β-lactamases (ESBL) and AmpC β-lactamases, resulting in resistance. This situation poses a significant risk to the future efficacy of β-lactam medications in both human and veterinary medicine **[15]**.

A vital measure in curbing the transmission of healthcare-associated infections and enhancing infection control is the assessment of the genetic relationships among pathogenic microorganisms. The polymerase chain reaction (PCR) technique, which focuses on identifying repeating nucleotide sequences within bacterial genomes, serves as a molecular method for classifying bacterial strains **[16]**.

Random Amplified Polymorphic DNA (RAPD) is a polymerase chain reaction (PCR) technique that employs single primers with arbitrary nucleotide sequences to amplify random segments of DNA for the purpose of assessing DNA polymorphism. A single primer type binds to the genomic DNA at two distinct locations on complementary strands. The detection of RAPD polymorphism is achieved through the use of short synthetic oligonucleotides, consisting of 10 bases with random sequences, as primers in a PCR reaction. The applications of RAPD extend from investigations at the individual level to studies involving closely related species **[17]**.

In humans and pets, the level of resistance to *Klebsiella* affection is increasing, making it crucial to monitor and understand the current state of

resistance development and epidemiology for this microbe in pet animals and humans. So, the study aimed to identify genetic similarities among *Klebsiella* isolates from pet animals and humans using RAPD-PCR technology.

2. MATERIALS AND METHODS

2.1 Samples collection :

A total of 150 clinical samples were obtained in Egypt's Governorates (El-Menofia and El-Garbia). one hundred clinical samples were collected from healthy and diseased pet animals randomly (50 dogs, 50 cats) through nasal swaps (20 samples) , urine samples(20 samples) and fecal swaps (10 swaps) for each (cat and dog). In addition, 50 clinical human samples were collected at random from various private laboratories for patients suffering from urinary and respiratory illnesses, as well as those cohabiting with pets or animals in their homes (n=50). The samples were immediately transported under aseptic conditions to the Microbiology lab., Faculty of Veterinary Medicine, Menoufia University, Egypt, using transport media (peptone water) (Himedia, India) **[18]**.

2.2. Bacteriological identification of *Klebsiella* spp. :

The transferred media (peptone water) was incubated at 37°C for 24 h. before being streaked over MacConkey's agar plates (Himedia, India) and incubated for another 24 h. The probable *Klebsiella* colonies were purified by subculture onto MacConkey's agar plate, Eosin Methylene Blue (EMB) agar (Himedia, India), and sterile blood agar plates (Himedia, India). Gram's stain was used on all putative isolates, which were then inspected under a light microscope to establish morphological characteristics. Oxidase, H_2S generation, citrate utilization, and urea hydrolysis tests (Himedia, India) were used for traditional biochemical identification of the suspected *Klebsiella* isolate **[19]**

2.3. Antimicrobial susceptibility:

All confirmed *K. pneumoniae* and *K. oxytoca* isolates were tested for antimicrobial susceptibility using the Kirby-Bauer disk diffusion method. Results were interpreted based on Clinical and Laboratory Standards Institute (CLSI, 2023) criteria. The list of antimicrobial agents (Himedia, India) includes ciprofloxacin (10 µg), erythromycine (15 µg), doxycycline (30 µg), ceftriaxon (30 µg), trimethoprim-sulphamethaxazole (25 µg), amoxicillin-calvulante (20-10 µg), ampicillin-sulbactam (30 µg), imipenem (10 µg), clarithromycin (15 µg), flucloxacillin (5 µg). Approximately 100-200 µL of bacterial overnight broth was transferred into a 5 mL normal saline solution that was adjusted to match the 0.5 McFarland standard (1.5×10^8 cfu/mL). After spreading 100 µL on Mueller-Hinton agar plates (Oxoid &Biogram.) using a sterilized glass spreader, the plates were impregnated with the specified antimicrobial discs and incubated aerobically at 37°C for 24 h. Following that, the diameters of the zone of inhibition were measured and interpreted **[20]**.

2.4. Polymerase chain reaction:

2.4.1. DNA extraction

The extraction of bacterial DNA was performed utilizing the boiling method outlined in reference **[21]**. Presumptive isolates were revived and obtained from broth cultures through a technique documented in study **[22]**. The DNA templates were stored at -20°C for subsequent molecular analysis.

2.4.2. Molecular identification of presumptive isolates

Polymerase chain reaction (PCR) was employed to validate the presumptive isolates, with the results analyzed through agarose gel electrophoresis (AGE) **[23]**. This process utilized the primer sequences specific to the target species (Table,1). The data obtained from RAPD-PCR fingerprinting were transformed into binary code, reflecting the presence or absence of each band. Dendrograms were constructed using the unweighted pair group method with arithmetic average (UPGMA) and Ward's hierarchical clustering techniques. Cluster analysis and dendrogram generation were performed using SPSS version 22 (IBM 2013).

following Hunter's (1990) methodology. Similarity index (Jaccard / Tanimoto Coefficient and number of intersecting elements) between all samples was calculated using the online tool (https://planetcalc.com/1664/).

Table (1): Primer sequences of target species and their respective amplicon sizes and PCR cycling conditions.

Target Strain	Gene	Primer Sequence (5′→3′)	Size (bp)	PCR Cycling Condition	References
Klebsiella genus	*gyr*A	F: CGC GTA CTA TAC GCC ATG AAC GTA R: ACC GTT GAT CAC TTC GGT CAGG	441	Initial denaturation of 5 min at 94 °C followed by 35 cycles, denaturation at 94 °C for 30 s, annealing at 55 °C for 45 s, extension at 72 °C for 45 s and final extension at 72 °C for 10 min.	Salloum *et al*. **[42]**
K. pneumonia	*16S-23ITS*	F: ATT TGA AGA GGT TGC AAA CGAT R: TTC ACT CTG AAG TTT TCT TGT GTTC	130	Initial denaturation of 5 min at 94 °C followed by 35 cycles, denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 40 s and final extension at 72 °C for 10 min.	Salloum *et al*. **[42]**
Klebsiella oxytoca	*peh*X	F: GAT ACG GAG TAT GCC TTT ACG GTG R: TAG CCT TTA TCA AGC GGA TAC TGG	331	Initial denaturation of 5 min at 94 °C followed by 35 cycles, denaturation at 94 °C for 30 s, annealing at 55 °C for 30 s, extension at 72 °C for 40 s and final extension at 72 °C for 10 min.	Salloum *et al*. **[42]**
	RAPD 4	AAGACGCCGT	Variable	Initial denaturation of 5 min at 94 °C followed by 35 cycles, denaturation at 94 °C for 1 min, annealing at 37 °C for 1 min, extension at 72 °C for 2 min and final extension at 72 °C for 10 min.	Wasfi *et al*. **[43]**

3. RESULTS

Bacteriological examination of 150 samples from dogs, cats and human (50 samples for each) from El-Garbia and El- Menofia Governorates revealed isolation of *Klebsiella* isolates. The suspected colonies showed the characteristic properties of *Klebsiella* (Gram-negative bacilli, capsulated, non-motile, appears with turbidity of sedimentation on peptone water, Mucoid pink colony on Macconkey's agar, pink to purple without metallic green colony (Large, viscid and dome –shaped) on EMB agar).

3.1. Incidence of isolation of *Klebsiella* species:

An analysis of 150 samples obtained from dogs, cats, and humans (50 samples from each group) in El-Garbia and El-Menofia Governorates revealed that the incidence of isolation were 7/50 (14%) from Dogs and 11/50 (22%) from cats and 8/50 (16%) isolates from human samples. By application of biochemical test on pet animals' samples (TSI acid \acid, citrate positive, urea positive, indole negative for *klebsiella pneumonia* and positive for *klebsiella oxytoca*) showed *Klebsiella pneumonia* isolates n=8 (3 from dogs and 5 from cats) while *Klebsiella oxytoca* isolates n=10 (4 from dogs and 6 from cats). But in human samples, *Klebsiella pneumonia* isolates (n=8) were confirmed biochemically (Table,2).

Table (2): Incidence of isolation of *Klebsiella* from pets and human samples.

Species	**Samples**	**No .of positive samples**	**No. of *K.pneumonia***	**No. of *K.oxytoca***
Dogs	Urine	3/20	1/3	2/3

Species	Samples	No .of positive samples	No. of *K.pneumonia*	No. of *K.oxytoca*
	fecal	1/10	-------	1/1
	Nasal	3/20	2/3	1/3
	Total	7/50	3/7	4/7
cats	Urine	4/20	2/4	2/4
	Fecal	2/10	1/2	1/2
	Nasal	5/20	2/5	3/5
	Total	11/50	5/11	6/11
Human	Urine	6/25	6/6	-------
	Sputum	2/25	2/2	-------
	Total	8/50	8/8	-------

3.2. Anti-microbial sensitivity test:

The same antimicrobial agents were used to pets and human but due to the high resistance of *Klebsiella* isolates from human more antimicrobial agents were needed.

3.2.1. Anti-microbial sensitivity test for the isolated *Klebsiella* strains from pet animals

Antimicrobial susceptibility was tested by disc diffusion method for *klebsiella oxytoca* indicated that the most effective antibiotic was ciprofloxacin (80%) but completely resistant to amoxicillin\calvulante (100%), doxycyline 100% and ceftriaxone 100% (Table,3 & Fig.,1).

Antimicrobial susceptibility was tested by disc diffusion method for *klebsiella pneumonia* indicated that the most effective antibiotic was ciprofloxacin (75 %) but completely resistant to amoxicillin\calvulante (100%), doxycyline 100% and ceftriaxone 100% (Table,4 & Fig.,2).

3.2.2. Anti-microbial sensitivity test for isolated *Klebsiella* strains from human

Antimicrobial susceptibility was tested by disk diffusion method gave intermediate sensitivity result to imipenem and chloramphenicol but completely resistant to the rest of antibiotics (ciprofloxacin, doxycycline, erythromycin, clarithromycin, amoxicillin clavulanate, flucloxacillin and gentamicin) (Table,5 & Fig.,3**).**

Table (3): In-Vitro anti-microbial Sensitivity test for *Klebsiella oxytoca* isolated from dogs and cats (n=10).

Antimicrobial classes	Antimicrobial agents/conc.	R.	Int.	S.
B-lactams Penicillins	amoxicillin\calvulante AMC/20/10 µg	10(100%)	0	0
	ampicillin\sulpactam SAM/30	10 (100%)	0	0
Cephalosporins	Ceftriaxone CRO/30 µg	10 (100%)	0	0
Macrolides	Erythromycin E/15 µg	8 (80%)	2 (20%)	0
Quinolones	Ciprofloxacin CIP/10 µg	2 (20%)	0	8 (80%)
Sulfonamides	trimethoprim\sulphamethaxazole SXT/25 µg	10(100%)	0	0

Tetracycline	Doxycycline DO/30 µg	10 (100%)	0	0

R:resistant Int: intermediate S: sensitive , %: according to the no. of *K.oxytoca* (n=10)

Table (4): In-vitro anti-microbial sensitivity test for isolated *Klebsiella Pneumoniae* strains from dogs and cats (n=8).

Antimicrobial classes	**Antimicrobial agents/conc.**	**R.**	**Int.**	**S.**
B-lactams Penicillins	Amoxicillin\calvulante AMC/20/10 µg	8(100%)	0	0
	Ampicillin\sulpactam SAM/30	8(100%)	0	0
Cephalosporins	Ceftriaxone CRO/30 µg	8(100%)	0	0
Macrolides	Erythromycin E/15 µg	7(87.5%)	1(12.5%)	0
Quinolones	Ciprofloxacin CIP/10 µg	2 (25%)	0	6(75%)
Sulfonamides	trimethoprim\sulphamethaxazole SXT/25 µg	8(100%)	0	0
Tetracycline	Doxycycline DO/30 µg	8(100%)	0	0

R:resistant Int:intermediate S:sensitive , %: according to the no. of *K.pneumoniae* (n=8).

Table (5): In-Vitro anti-microbial Sensitivity test for isolated *Klebsiella* strains from human (n=8).

Antimicrobial classes	**Antimicrobial agents/conc.**	**R.**	**Int.**	**S.**
Aminoglycoside	Gentamycin GEN/10 µg	8(100%)	0	0
B-lactams Penicillins	Amoxicillin\calvulante AMC/20/10 µg	8(100%)	0	0
	Flucloxacillin FI/5 µg	8(100%)	0	0
Carbapenem	Imipenem IPM/10 µg	0	8(100%)	0
Cephalosporins	Cefadroxil CFR/30 µg	8(100%)	0	0
	Cefuroxime CXM/30 µg	8(100%)	0	0
Chloramphenicol	Chloramphenicol CLM/30 µg	0	8(100%)	0
Fluoroquinolone	Ofloxacin OFX/5 µg	8(100%)	0	0
Macrolides	Erythromycin E/15 µg	8(100%)	0	0

	Clarithromycin CLR/15 µg	8(100%)	0	0
Oxazolidinonone	Linezolid LZ/30 µg	8(100%)	0	0
Quinolones	Ciprofloxacin CIP/10 µg	8(100%)	0	0
Tetracycline	Doxycycline DO/30 µg	8(100%)	0	0

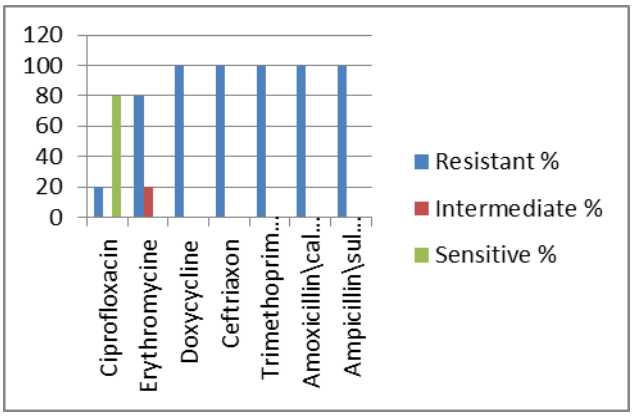


Fig. 1. Antimicrobial susceptibility patterns of *Klebsiella oxytoca* isolated from pet animals

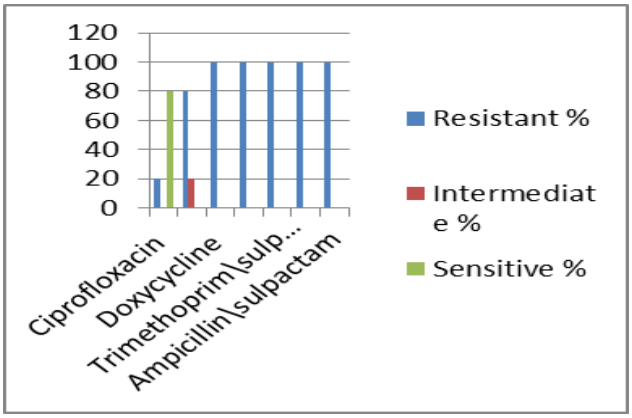


Fig. 2. Antimicrobial susceptibility patterns of *Klebsiella pneumonia* isolated from pet animals.

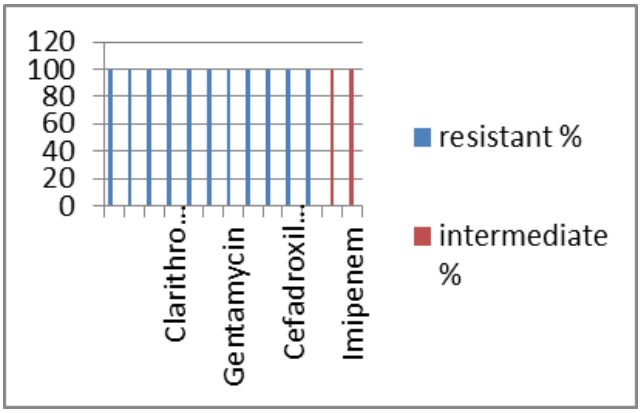


Fig 3: Antimicrobial susceptibility patterns of *Klebsiella* spp. isolated from human

3.3. Conventional PCR and RAPD PCR for identification of *Klebsiella* isolates:

3.3.1. Detection of *gyr*A gene in *Klebsiella* isolates, *K.pneumoniae 16S-23ITS* gene and *K.Oxytoca peh*X gene in *Klebsiella* isolates by PCR:

Conventional PCR was applied for *Klebsiella* isolates (n=10) that recovered from dogs, cats and human by using *gyr*A, *16S-23ITS* and *peh*X primers. The *gyr*A gene was found in all isolates and produced a specific band at 441 bp. While,The *16S-23ITS* gene was detected in three isolates with specific band at 130 bp. and also, *peh*X gene which amplified in two isolates only at 331 bp.(Table,6) The Correlation between *Klebsiella* isolated from pet animals and cohabiting human showed in table (7).

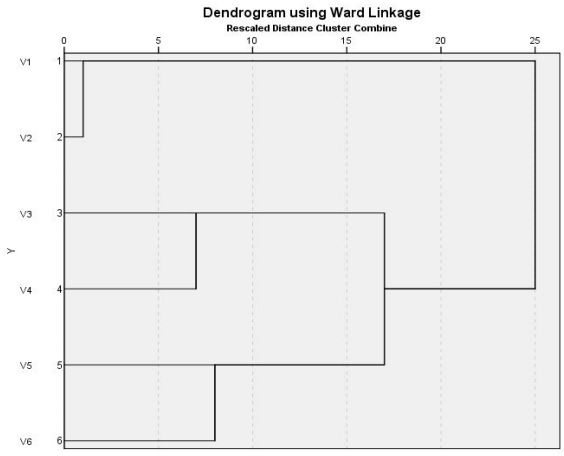
Table (6): Detection of *gyr*A gene in *Klebsiella* isolates , *K.pneumoniae 16S*-23ITS gene and *K.oxytoca peh*X gene in *Klebsiella* isolates by PCR:

No. of isolate	**Type of sample**	**Origin of isolates**	**Result of PCR for *gyr*A gene**	**Result of PCR for *K.pneumoniae 16S-23ITS***	**Result of PCR for *K.Oxytoca peh*X**
1	Nasal	Dog	+	ND	+

No. of isolate	Type of sample	Origin of isolates	Result of PCR for *gyr*A gene	Result of PCR for *K.pneumoniae 16S-23ITS*	Result of PCR for *K.Oxytoca peh*X
2	fecal	Dog	+	ND	ND
3	Urine	Dog	+	+	ND
4	Urine	Dog	+	ND	ND
5	Nasal	Cat	+	+	ND
6	Fecal	Cat	+	ND	ND
7	Urine	Cat	+	ND	+
8	Urine	Cat	+	ND	ND
9	Sputum	Human	+	ND	ND
10	Urine	Human	+	+	ND

Table (7): Correlation between of *Klebsiella* isolated from pet animals and cohabiting human.

Houses No.	Member	Type of sample		No. of positive sample		Type of *klebsiella* species	
		H	Pets	H	Pets	H	Pets
1	(2) Human & Cat	U	F	-ve	+ve	-	K.O
2	Human & Dog	U	U	+ve	-ve	K.P	-
3	Human & Cat	Sputum	N	+ve	+ve	K.P	K.P
4	Human & Cat	U	U	-ve	+ve	-	K.O
5	(2) Human & Dog	U	F	-ve	-ve	-	-
6	Human & Dog	U	N	-ve	+ve	-	K.O
7	Human & Cat	U	U	-ve	-ve	-	-
8	Human & Cat	U	N	-ve	+ve	-	K.P
9	Human & Dog	U	U	+ve	+ve	K.P	K.P
10	Human & Cat	U	N	-ve	-ve	-	-



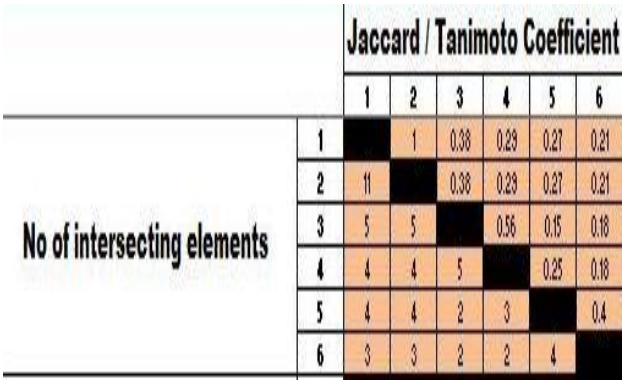


Fig. 5: Similarity index of *Klebsiella* isolates.

. **Fig.4**: Dendogram of *Klebsiella* isolates

4. DISCUSSION

Klebsiella spp., recognized as the second most common species within the Enterobacteriaceae family, is present on the mucosal surfaces of various mammals, including humans and dogs, in addition to being found in water, food and soil. In Egypt, *Klebsiella pneumonia* is notably prevalent among a range of animal species, including domestic pets, goats, sheep, and chickens. Furthermore, it is one of the most prevalent infections in humans, accounting for the third leading cause of Hospital Acquired Infections (HAI) (21.8%) **[24-26]**.

An analysis of 150 samples obtained from dogs, cats, and humans (50 samples from each group) in El-Garbia and El-Menofia governorates revealed the presence of *Klebsiella* isolates at a rate of 17.3%. The findings were consistent with the study conducted by Khalil *et al*. **[27]** which indicated a prevalence of 20.8% in Egypt. Similarly, Banerjee *et al*. **[28]** found an incidence of 22.9% in India. However, Zhang *et al*. **[9]** observed a lower incidence of 2.3% in China. Marques *et al*. **[29]** observed a higher incidence rate of 38.9% in Portugal, while Klaper *et al*. **[30]** documented an even higher incidence rate of 61.7% in Germany. The incidence varied depending on environmental conditions and the methods used for sample collection.

In this investigation, 8 (44.4%) cases of *Klebsiella pneumonia* and 10 (55.6%) cases of *Klebsiella oxytoca* were found in pets. This study aligned with the findings of Donati *et al*. **[31]**, who reported a 34.8% (31/89) incidence of *Klebsiella pneumonia* in Japan. However, it is important to note that this result is lower than their reported incidence. Zang *et al*. **[32]** found that *Klebsiella pneumoniae* was isolated in 28 out of 43 cases, representing a 65% incidence rate. The current outcome exceeds that of Ewers *et al*. **[33]** in Italy, which was 21.4% (15/70). The current investigation documented the identification of *Klebsiella oxytoca* 10 at a frequency of 55.6%, which is lower than the frequency of 25.5% reported by Zang *et al*. **[32]** for *K. oxytoca.*

Klebsiella pneumoniae was obtained from one urine sample and two nasal samples collected from dogs, as well as from two additional urine samples. one fecal sample and one nasal sample from cat. *Klebsiella oxytoca* was recovered from two urine samples, one fecal sample, and one nasal sample from dogs. Additionally, *Klebsiella oxytoca* was found in two urine samples, two fecal samples, and two nasal samples from cats. According to the findings of Lee *et al*. **[34]**, It was noted that *K. pneumoniae* and *K. oxytoca* were found in almost all lesions of companion animals. In particular, *K. pneumoniae* showed a significant prevalence in cases of gastrointestinal disorders, otitis, and respiratory illnesses. In contrast, *K. oxytoca* was prominently present in the urogenital system. The occurrence of *Klebsiella* across various lesion types was most pronounced in feces associated with diarrhea, followed by the ear canal, nasal cavity, urine, genitalia and skin.

The disc diffusion method was used to assess the antimicrobial susceptibility of *Klebsiella* bacteria isolated from pets. The results showed that ciprofloxacin was the most successful antibiotic, with a susceptibility rate of 77.7%. On the other hand, amoxicillin\calvulante, doxycycline, and ceftriaxone were found to be entirely resistant, with a resistance rate of 100% for each. A study conducted by Lee *et al*. **[34]** revealed that *Klebsiella* bacteria demonstrated considerable resistance, exceeding 40%, to β-lactam antibiotics, which encompasses third-generation cephalosporins (3GC), fluoroquinolones, and aminoglycosides. Among the three species, strains of *K. pneumoniae* that exhibit more than 50% resistance to third-generation cephalosporin medicines have been found to be strongly linked to the presence of genes that provide resistance to extended-spectrum cephalosporins (ESCs).

The antimicrobial susceptibility testing of *Klebsiella* isolated from humans revealed that imipenem and chloramphenicol exhibited intermediate sensitivity (100%), while the remaining antibiotics showed complete resistance. According to the 2017 annual report of the European Antimicrobial Resistance Surveillance Network, Portugal is one of the countries with a higher occurrence of resistance to third-generation cephalosporins, carbapenems, and fluoroquinolones in invasive *K. pneumoniae* strains. Furthermore, a significant prevalence of ESBL/AmpC-producing *K. pneumoniae* in the form of fecal colonization has been documented among inhabitants of long-term care facilities in Portugal **[35]**.

Iraz *et al*. **[36]** conducted a study in Turkey including 37 Chronic respiratory *Klebsiella pneumonia* strains. They found that all of these strains were resistant to ceftazidime, imipenem, and meropenem. Additionally, 2.7% of the bacteria were resistant to colistin, 11% to tigecycline, 19% to amikacin, and 21.6% to trimethoprim/sulfamethoxazole **[36]**. Considering all these studies, it is evident that there are variations in the antibiotic resistance profiles of *Klebsiella* strains, even across different regions. This indicated that the resistance of strains is influenced by regional treatment policies and can result in diverse resistance profiles against antibiotics. The *Klebsiella* spp. isolates were validated using molecular techniques, which are known for their rapidity and accuracy **[37]**.

Ten *Klebsiella* isolates obtained from dogs, cats, and humans were subjected to PCR testing using *gyr*A primer. All isolates had the *gyr*A gene, which resulted in the production of a distinct band measuring 441 base pairs. PCR was conducted on three *Klebsiella i*solates obtained from dogs, cats, and humans using the *K. pneumoniae 16S-23ITS* primer. The *K. pneumoniae 16S-23ITS* gene was discovered in the detected isolates, and it generated a distinct band measuring 130 base pairs. Two *Klebsiella* isolates obtained from dogs, cats, and humans were subjected to PCR analysis utilizing the *K.Oxytoca peh*X primer. The K.Oxytoca *peh*X gene was identified in the detected isolates and resulted in the amplification of a distinct 331 bp band. Several studies supported these findings, including those by Jacob *et al*. **[38]** and Trivedi *et al*. **[39]**.

This study involved 10 households that included both companion animals (dogs and cats) and humans residing in close quarters, with the aim of evaluating the prevalence of *K. pneumoniae* and the degree of transmission between animals and humans. The total number of individuals is 12 humans, 6 cats, and 4 dogs. The human participants had resided in the same household as the included companion animals for a minimum of 6 months. *K. pneumoniae* was found in 4 individuals, including 1 cat, 1 dog, and 2 humans, from a total of 10 households. Among these, 3 *Klebsiella oxytoca* strains were detected in dogs and cats. This finding is consistent with a study by Marques *et al*. **[29]**, which reported that dogs and a human living in the same household (H15) were colonized by closely related *K. pneumoniae* strains, with indistinguishable PFGE restriction patterns.

RAPD- PCR is reported to be used for serovar based typing of *Klebsiella* spp. In the present study RAPD- PCR of *Klebsiella* strains yielded different patterns consisting of 4-6 bands by which the strains were grouped into 3 clusters by the dendogram analysis (Fig.,4). Based on result of RAPD- PCR of *Klebsiella* isolates were showing highly degree of identitiy (100%) between the sample 1 & 2 (human). degree of identitiy between 1(human) and 3 (cat) showing 38% identity (human & cat in the same house). identity between two pets as 3 (cat) and 4 (dog) showing 56% identity. (Fig.,5)

In the current study, the RAPD- PCR showed its ability to detect the identity of the isolates. This result agreed with Eftekhar and Nouri **[40]** who reported that in comparison to other PCR-based methods, RAPD is a swift method for creating DNA profiles, akin to PFGE, which is regarded as the benchmark in genetic fingerprinting.

Random Amplified Polymorphic DNA (RAPD) employs a polymerase chain reaction (PCR) approach that uses arbitrary primers to attach to nonspecific regions of DNA, leading to the amplification of various fragments. These amplified

fragments are subsequently analyzed through agarose gel electrophoresis to identify variations in band patterns. This technique is both economical and relatively straightforward, making it suitable for studies on genetic diversity. Nonetheless, RAPD does have certain drawbacks, as it requires a meticulously crafted amplification protocol to ensure reproducibility of samples. Furthermore, the use of purified DNA templates is essential to avoid contamination that could compromise the PCR process **[41]**.

5. CONCLUSIONS

Consistent with previous research, the study has shown that there is a genetic similarity across clinical isolates of *Klebsiella* species which isolated from pets and cohabiting human.

We suggest that future studies should assess the genetic relatedness among bacteria isolated from pets and cohabiting human.

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