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Isolation and Phenotyping Study of *Klebsiella pneumoniae* Isolated from Otitis in Cats and Screening their Antibiotic Sensitivity

Article Info.

Author

Majida. A. Jasim[1] ،Aamer.Y.Alchalaby[1] ،Omar.K.Alhankawe[2].

1.Department of Microbiology, College of Veterinary Medicine, University of Mosul.

2.Department of Internal and Preventive Medicine, College of Veterinary Medicine, University of Mosul, Mosul, Iraq.

Corresponding Author Email Address: draameryeh@uomosul.edu.iq

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Abstract

Klebsiella spp. are becoming more common in companion animals, leading to a serious risk to their health and welfare and frequently having deadly consequences. The current study aims to isolate *Klebsiella pneumoniae* bacteria from feline otitis media and to study antibiotic sensitivity and identify antibiotic-resistant bacteria. Fifty ear swabs from otitis (ear swabs) in cats suffering from ear infections from different private clinics in the Nineveh Governorate were collected between August and November of 2025. All samples were promptly transferred to the Department of Microbiology, College of Veterinary Medicine, University of Mosul, Iraq, for microbiological processing after being placed in a sterile container. Enrichment media (blood agar and brain heart infusion agar) and selective media (MacConkey agar) were used for isolation. The suspected colony was diagnosed using Gram stain and biochemical tests and confirmed by Vitek and Render (kits that contain a group of biochemical tests). Result 28% (14/50) of the cat samples contained *K. pneumoniae* isolates that were positive. Additionally, the antibiotic susceptibility tests reveal complete resistance to metronidazole and amoxicillin. In contrast, it has shown complete sensitivity to ofloxacin. In conclusion, show the significance of *K. pneumoniae* in the causes of otitis in cats, and also the resistance to many antibiotics that were used poses a threat to public health and society; therefore, the indiscriminate use of antibiotics should be avoided.

Keywords: *Klebsiella pneumoniae,* otitis, cats, antibiotic sensitivity**.**

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Introduction

Otitis is an inflammation of the ear or pinna. Otitis externa is the term used when the condition affects only the external canal, which is outside the tympanic membrane. When both the tympanum and the tympanic bulla are impacted, the condition is referred to as "otitis media." Otitis interna implies damage to the hearing system; neurologic symptoms and deafness are usually present (1). Some veterinary practices fail to identify otitis media, an inflammatory disease of the middle ear cavity (2). Because the widely used clinical approach for diagnosing and treating canine otitis rarely produces satisfactory results when applied to cats, otitis in cats can also be a difficult clinical problem (1). One of the most common Gram-negative bacteria, *Klebsiella pneumoniae*, is closely connected to the *Enterobacteriaceae* family, which can also include other well-known pathogens, including *Escherichia coli* and *Salmonella spp*. (3-4). Despite being a normal microbiota*, K.pneumoniae* is a significant nosocomial opportunistic bacterium because of several virulence factors, such as a capsule, fimbriae adhesion, an iron acquisition system, and the formation of a biofilm that enables it to elude a host's innate immunity (5-7). Lipopolysaccharide (O) and capsular (K) antigens are often the primary ingredients on the cell surface that influence the pathogenicity and different serotypes of the bacteria (8). Due to biofilm formation, efflux pumps, β-lactamase generation, and further amplification from enzyme alteration and porin loss, multidrug resistance has dramatically increased over the past ten years (9). *Klebsiella pneumoniae* is a gram-negative, rod-shaped, facultatively anaerobic, lactose-fermenting bacillus that is not motile. It is a member of the Enterobacteriaceae family, measures 2 µm by 0.5 µm, and appears on MacConkey agar as mucoid lactose (10).

The prevalence of resistance increased mortality and morbidity rates and lowered the efficacy of conventional medications used frequently to treat *K. pneumoniae*. (11). Antibiotics are utilized extensively to stop infection from being worse, but the preferred medication is still required, especially in severe systemic diseases with a worse prognosis (12-13). Among the bacteria that are aware of drug resistance is the *Klebsiella* species. Gram-negative *Klebsiella* bacteria can sometimes cause harm to both humans and animals, despite being frequently found in oral, cutaneous, and intestinal flora, which cause bacterial illnesses in large animals and poultry. Antibiotic resistance in *Klebsiella* species has been noted in a number of studies (14). Due to the severity of *K.pneumoniae* infection in both humans and animals and the limited studies on otitis in cats in Iraq, we investigate *K.pneumoniae* from otitis-diseased pet cats and estimate antibiotic sensitivity.

Materials and Methods

Samples: Between August and November of 2025, fifty ear swabs from cats with otitis were taken from different clinics in the Nineveh Governorate. After being placed in a sterile container,

all samples were transferred to the microbiological lab in the Department of Microbiology, College of Veterinary Medicine, University of Mosul, Iraq.

Bacteriological examination

All ear swab samples were cultured in the brain heart infusion broth (Scharlau/Spain) (for primary enrichment steps); after that, an inoculum of pure colonies from enriched media was subcultured on both MacConkey agar and blood agar (Scharlau/Spain). All cultures were incubated at 37°C aerobically for 18 hours. The suspected colonies that grow in blood and MacConkey agar are similar in phenotype to *K.pneumoniae*. All samples identified on culture media underwent Gram staining and biochemical testing. Biochemical diagnostic tests included indole, methyl red, Voges-Proskauer, Simon citrate, oxidase, and catalase tests for all isolates, and the VITEK2® system (French/ BioMérieux) is a new automated system for identifying bacteria and testing their sensitivity using a fluorescence-based technology and Render MA120 ID&AST System operation. (Shenzhen Render Bio-tech Co., Ltd).

A suspension of pure culture of Gram-negative cocci is inoculated into the Enterobacteriaceae ID&AST plate (MA120-SL). This ID/AST combination panel includes susceptibility wells with varying concentrations of different antimicrobial agents. The panel also contains a growth reference well at appropriate well locations.

Bacterial identification: The identification panel utilizes a series of chromogenic biochemical tests to identify the organisms. Utilization and degradation of specific substrates are detected by various indicator systems. The colour change observed due to bacterial metabolites or after the addition of a chromogenic agent is detected using the colorimetric principle.

Antimicrobial susceptibility test

The test was conducted using the Bauer-Kirby method. A standard bacterial inoculation of 1.5×10^8 CFU/ml was used to perform the test (15). Eleven of the popular antibiotic discs were used in this study, which are listed in Table 1. The World Health Organization states that the results were categorized into three intervals: resistance, intermediate, and sensitive. The inhibition zone was measured in millimeters. (16).

Results

Bacteriological isolation

The results showed isolation of 14 positive samples for *K. pneumoniae* isolates from the otitis case with 28% (14/50) isolate rate. On MacConkey and blood agar, every colony resembled the typical

morphological appearance that is colonies appear mucoid, round and large pink on MacConkey agar, indicating fermentation of lactose and acid production of *K. pneumoniae* (Figure 1). Moreover, organisms appear gram-negative rod-shaped and yet it appeared distinct clear halo surrounding the bacterial cell, indicating the presence of a thick polysaccharide capsule (Indian ink stain) is shown in Figure 2&3. Based on biochemical tests, 28% tested isolates are positive for catalase, urea, and citrate and negative for indole and methyl red, as shown in Figures 4 and 5. The VITEK2® Compact system confirmed that all isolates belong to *K.pneumoniae* with a purity of up to 94%, as shown in Figure 5. And the Render MA120 ID&AST, as shown in Figure 6

Table 1: Antibiotics used for the antibiotic sensitivity test in the current study

Antimicrobial class	Antibiotic	Concentration (µg/disc)	Disc Code
Glycyline	Tetracycline	30µg	TE
Aminoglycoside	Tobramycin	10 µg	TOB
Aminoglycoside	Neomycin	10µg	CIP
Aminoglycoside	Gentamicin	10 µg	NIT
Sulphonamide	Sulfamethoxazole/Trimethoprim	25 µg	SXT
Nitrofurans	Nitrofurantoin	30µg	MET
Fluoroquinolone group	Ciprofloxacin	5µg	GEN
Fluoroquinolone group	Ofloxacin	5µg	OF
Nitrimidazole	Metronidazole	30µg	N
Amphenicols	Chloramphenicol	10µg	C
β-lactam	Amoxillin	10µg	AX

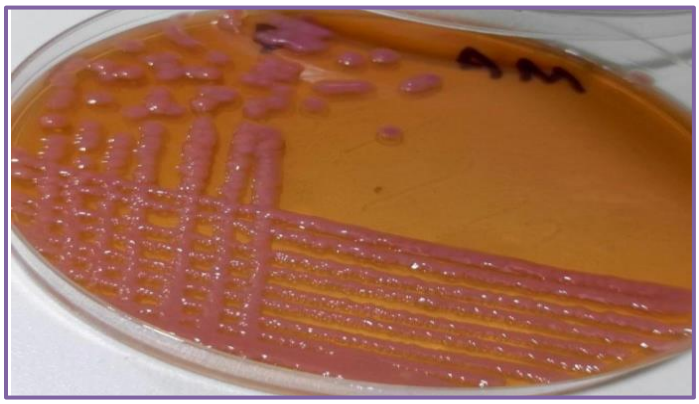


Figure 1: *K.pneumoniae* isolates with mucoid consistency on MacConkey agar

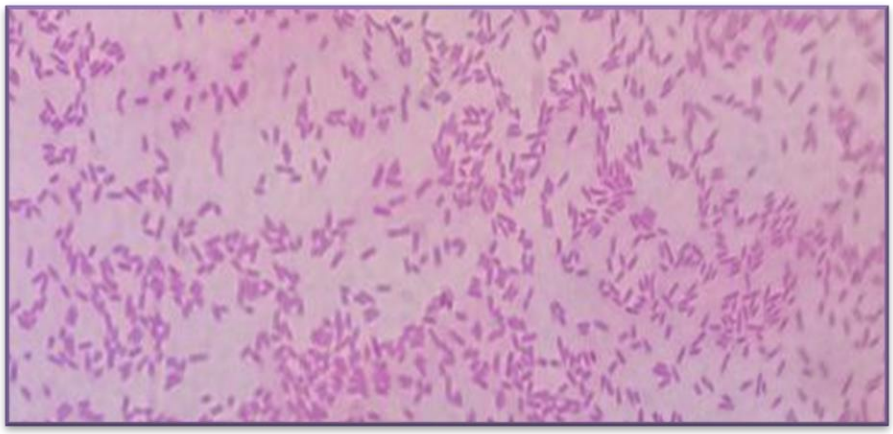


Figure 2: staining of *K. pneumoniae* by Gram stain (Gram-negative red coccobacilli)

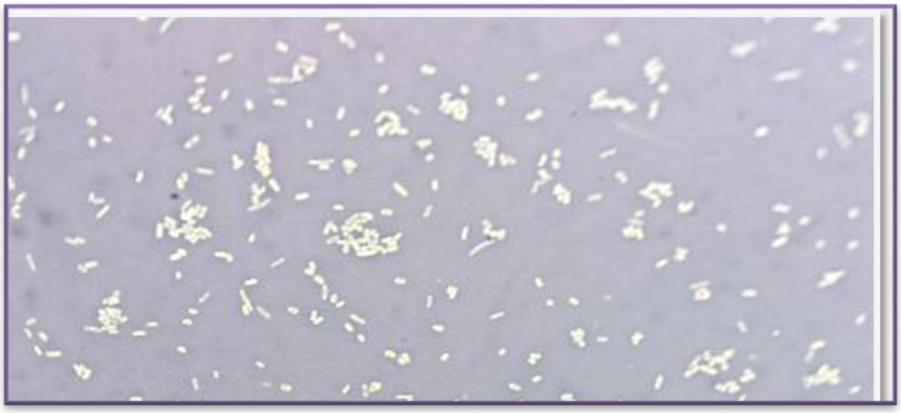


Figure 3: staining of *K .pneumoniae* by Indian ink stain (rod, clear halo surrounding the bacterial cell

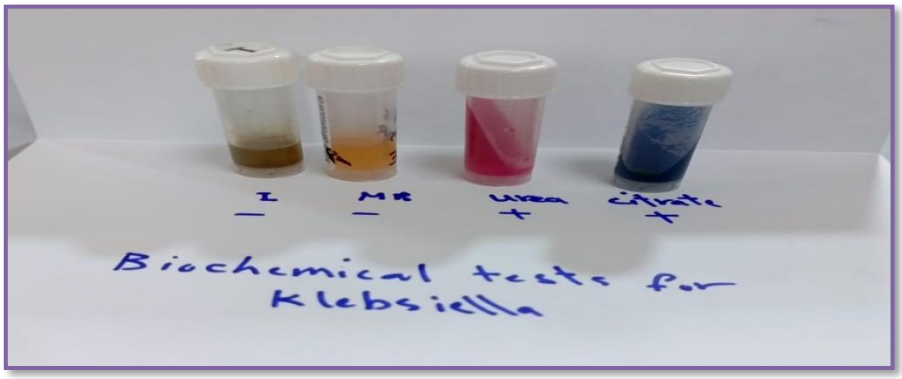


Figure 4: Basic biochemical tests for the diagnosis of *Klebsiella pneumonia*, right to left showed negative results for indole and methyl red, and positive results for urea and citrate.

Figure 5: Positive result of the Catales for *K.pneumoniae* test (immediate formation of a bubble after adding H2O2 to the bacterial colony).

bioMérieux Customer:

Microbiology Chart Report

Printed October 9, 2025 3:55:03 PM AST

Patient Name: ., Majida 25
Location:
Lab ID: 1388

Patient ID: 1388
Physician:
Isolate Number: 1

Organism Quantity:
Selected Organism : Klebsiella pneumoniae ssp pneumoniae

Source: Research

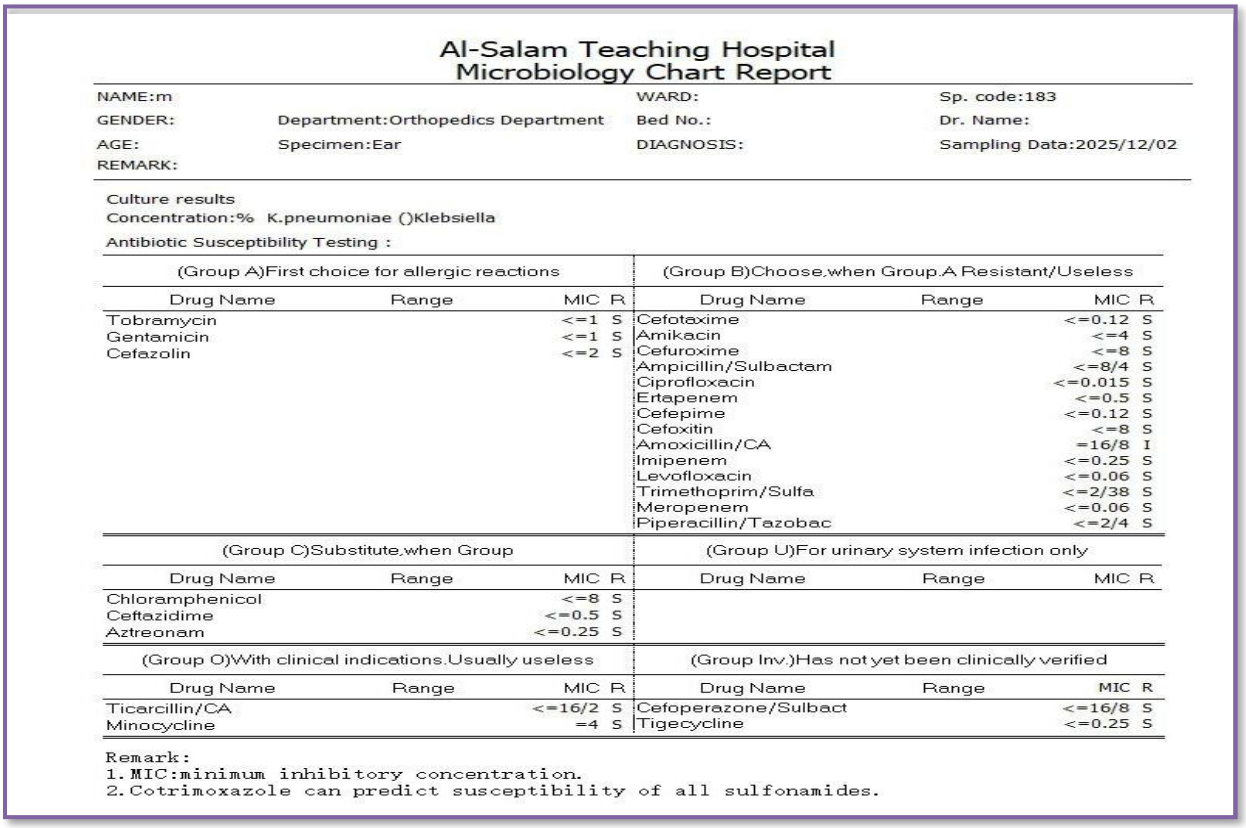
Collected:

Comments:	

Identification Information	**Analysis Time:**	7.73 hours	**Status:**	Final
Selected Organism	94% Probability **Bionumber:**	**Klebsiella pneumoniae ssp pneumoniae** 6627735753565053		
ID Analysis Messages				

Biochemical Details																
2	APPA	-	3	ADO	+	4	PyrA	+	5	lARL	-	7	dCEL	+	9	BGAL	+
10	H2S	-	11	BNAG	+	12	AGLTp	-	13	dGLU	+	14	GGT	+	15	OFF	+
17	BGLU	+	18	dMAL	+	19	dMAN	+	20	dMNE	+	21	BXYL	+	22	BAlap	-
23	ProA	+	26	LIP	-	27	PLE	+	29	TyrA	+	31	URE	+	32	dSOR	+
33	SAC	+	34	dTAG	-	35	dTRE	+	36	CIT	+	37	MNT	+	39	5KG	-
40	lLATk	+	41	AGLU	-	42	SUCT	+	43	NAGA	-	44	AGAL	+	45	PHOS	+
46	GlyA	+	47	ODC	-	48	LDC	+	53	lHISa	-	56	CMT	-	57	BGUR	-
58	O129R	+	59	GGAA	-	61	lMLTa	+	62	ELLM	+	64	lLATa	+			

Figure 6: Identification of *k. pneumonia,* purity of up to 94% using the VITEK2® system.



Al-Salam Teaching Hospital
Microbiology Chart Report

NAME:m | WARD: | Sp. code:183
GENDER: | Department:Orthopedics Department | Bed No.: | Dr. Name:
AGE: | Specimen:Ear | DIAGNOSIS: | Sampling Data:2025/12/02
REMARK:

Culture results
Concentration:% K.pneumoniae ()Klebsiella
Antibiotic Susceptibility Testing :

(Group A)First choice for allergic reactions				(Group B)Choose,when Group.A Resistant/Useless			
Drug Name	Range	MIC	R	Drug Name	Range	MIC	R
Tobramycin		<=1	S	Cefotaxime		<=0.12	S
Gentamicin		<=1	S	Amikacin		<=4	S
Cefazolin		<=2	S	Cefuroxime		<=8	S
				Ampicillin/Sulbactam		<=8/4	S
				Ciprofloxacin		<=0.015	S
				Ertapenem		<=0.5	S
				Cefepime		<=0.12	S
				Cefoxitin		<=8	S
				Amoxicillin/CA		=16/8	I
				Imipenem		<=0.25	S
				Levofloxacin		<=0.06	S
				Trimethoprim/Sulfa		<=2/38	S
				Meropenem		<=0.06	S
				Piperacillin/Tazobac		<=2/4	S

(Group C)Substitute,when Group				(Group U)For urinary system infection only			
Drug Name	Range	MIC	R	Drug Name	Range	MIC	R
Chloramphenicol		<=8	S				
Ceftazidime		<=0.5	S				
Aztreonam		<=0.25	S				

(Group O)With clinical indications.Usually useless				(Group Inv.)Has not yet been clinically verified			
Drug Name	Range	MIC	R	Drug Name	Range	MIC	R
Ticarcillin/CA		<=16/2	S	Cefoperazone/Sulbact		<=16/8	S
Minocycline		=4	S	Tigecycline		<=0.25	S

Remark:
1. MIC:minimum inhibitory concentration.
2. Cotrimoxazole can predict susceptibility of all sulfonamides.

Figure 7: Identification of *K.pneumoniae* by using Render MA120 ID reading

Antimicrobial susceptibility testing (AST)

Klebsiella pneumoniae has shown complete resistance to metronidazole and amoxicillin. And (50%) to the tetracycline, (33.3%) to the nitrofurantoin, (25%) to the tobramycin, gentamycin, and (16.7%) to the ciprofloxacin, sulfamethoxazole/trimethoprim, chloramphenicol and (8.3%) to the neomycin. In contrast, it has shown complete sensitivity to ofloxacin, and (75%) to ciprofloxacin, sulfamethoxazole/trimethoprim, and (66.7%) to tobramycin, gentamycin, and (58.3%) to the chloramphenicol, and (25%) to the neomycin, nitrofurantoin and (8.3%) to the tetracycline. table (2) and figure 7,8.

Table 2. Antibiotic Sensitivity tests of*: K.pneumoniae*

Antibiotic	Disc concentration	Sensitive (%)	Intermediate (%)	Resistance (%)
Tetracycline	TE-30µg	1 (8.3)	5 (41.7)	6 (50)
Tobramycin	TOB-10 µg	8 (66.7)	1 (8.3)	3(25)
Neomycin	N-10µg	3 (25)	8 (66.7)	1 (8.3)
Gentamicin	GEN-10µg	8 (66.7)	1 (8.3)	3 (25)
Ciprofloxacin	CIP-5µg	9 (75)	1 (8.3)	2 (16.7)
Ofloxacin	OF-5µg	12 (100)	0	0
Sulfamethoxazole/ Trimethoprim	SXT-25 µg	9 (75)	1 (8.3)	2 (16.7)
Metronidazole	MET-30 µg	0	0	12 (100)
Chloramphenicol	C-10µg	7 (58.3)	3 (25)	2 (16.7)
Amoxicillin	AX-10µg	0	0	12(100)
Nitrofronatoin	NIT-30 µg	3(25)	5(41.7)	4(33.3)

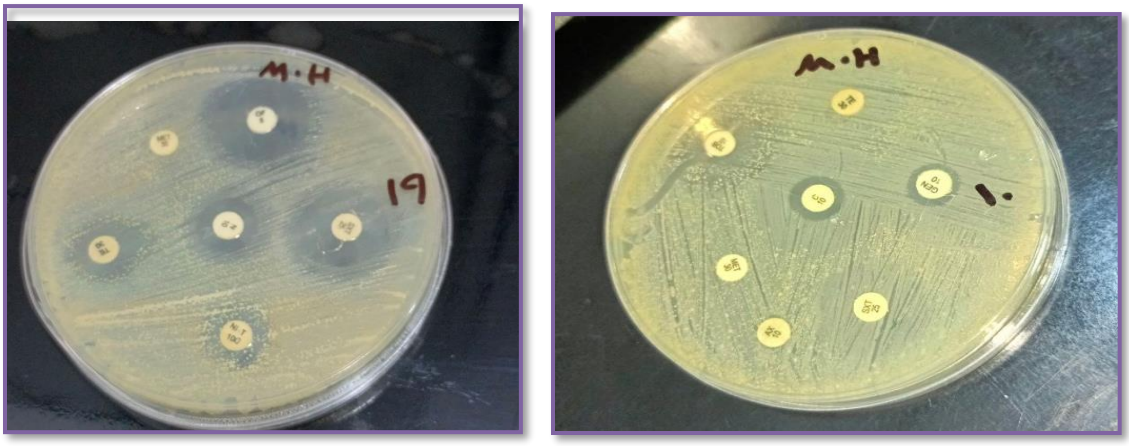


Figure 7: *K.pneumoniae* is resistant to most antibiotics used in the study

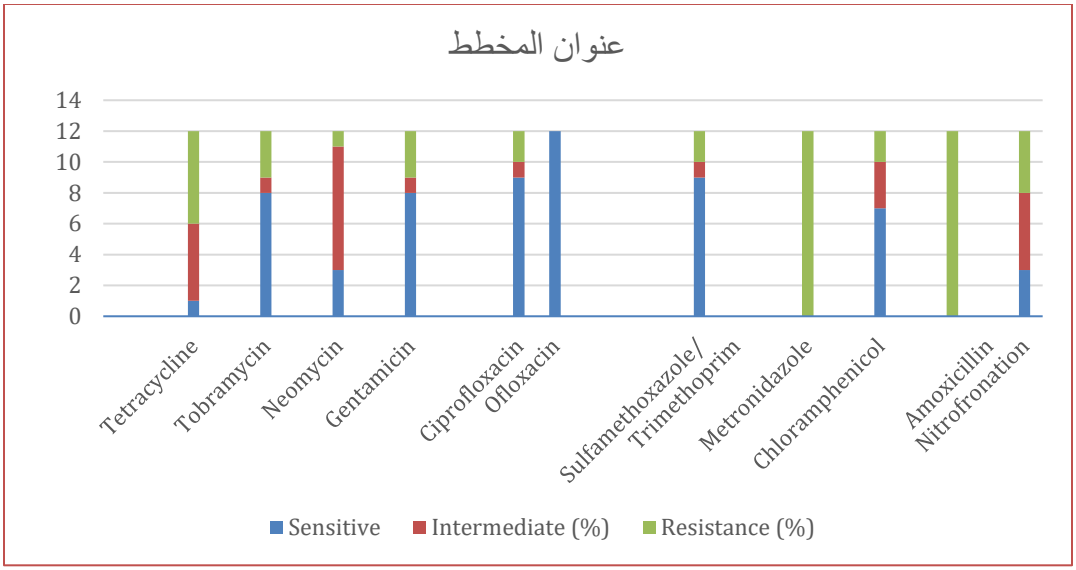


Figure 8: The antibiotic sensitivity test of *K. pneumoniae*

Discussion

These bacteria belong to the Enterobacteriaceae family and are facultatively anaerobic. There are 77 capsular antigens (K antigens) in this genus, which lead to different serogroups (17–18-19). Using the culture method, the study's findings revealed that 14 isolates, or 28% of the 50 samples, had *Klebsiella spp*. characteristics. Colonies that grow on MacConkey agar and ferment lactose sugar to form mucoid, bright pink colonies—the hallmark of *Klebsiella spp*. This is the distinguishing feature of *Klebsiella* species. Additionally, the biochemical (VITEK2® Compact system) and microscopic (Gram and Indian ink stain) tests reveal that these bacteria are *Klebsiella spp*., and these findings are consistent with numerous studies that generally employ conventional techniques (20-21). Due to its correlation with severe infections and multidrug-resistant patterns, *K.pneumoniae* is considered an emerging concern in veterinary dermatology (22). Due to variables like climate, population density, antimicrobial use patterns, and hygiene practices, the geographic distribution of bacterial pathogens in otitis externa varies significantly.

(23) It depends on who is aware of the local bacterial pathogens and the risk factors for bacterial otitis externa (24). Anatomical anomalies (such as stenotic ear canals or excessive hair inside the ear canal) are predisposing factors. Environmental factors (such as high humidity, excessive moisture, or poor ventilation in the ear canal) are conducive to microbial growth). (25). The low *K.pneumoniae* rate (28% of isolates) is in contrast to reports from other nations that found much

higher rates; however, this discrepancy may be explained by differences in environmental exposures and antimicrobial use patterns (26). Over time, bacteria have become resistant to medicines, and several multidrug-resistant microorganisms have been created to help with more appropriate medication selection. (27-28) Because resistant *K.pneumoniae* strains reduce the effectiveness of several medications, they are extremely important for human health. (29). According to our research, the susceptibility of *K.pneumoniae* isolates to particular medications varies. The remarkable capacity to get foreign genetic elements that encode resistance and hyper-virulence, alter or mutate protein-coding genes, and generate β-lactamase enzymes are potential origins of resistance (30- 31). In the study conducted to isolate *Klebsiella pneumoniae*, resistance was recorded (50%) for tetracycline (25%) for gentamicin, and research conducted in Bogor and Indonesia confirmed that there was a similar percentage of results between them. In one study, isolates of *K.pneumoniae* obtained from clinical cats in Bogor, Indonesia, were resistant to tetracycline and gentamicin due to the presence of different genes such as *tetA* and *qnrS* at different rates of prevalence: 57.2% and 28.5%, respectively (29).

Because of its superior effectiveness against anaerobic bacteria and protozoa, metronidazole—one of the primary antimicrobials used in regular veterinary treatment, particularly in companion animals—showed the highest resistance rate. Resistance mechanisms to this medication may have developed as a result of its frequent use. (32). In this study, it was observed that all isolates presented multidrug resistance, being classified as MRD. There are other reports in the literature where this multidrug resistance to this pathogen has been observed (33-34). In both veterinary and human medicine, the emergence of multidrug-resistant strains of *K.pneumoniae* has been a significant issue. Because it generates an enzyme that confers resistance to multiple types of antibiotics, this bacterium can cause resistance in up to 95% of the antimicrobials currently on the pharmaceutical market, severely limiting treatment options. It is evident from this study that certain bacteria exhibited resistance to every type of antibiotic examined. 97% of the isolates in the IRMA study were categorized as multidrug-resistant bacteria, and the patterns of resistance varied greatly, mostly affecting the bacteria that are most frequently utilized in veterinary care. Because infections brought on by multidrug-resistant germs frequently do not respond to traditional treatments, this outcome is seen as a public health concern (35). A study conducted on *Klebsiella pneumonia* demonstrated complete resistance to amoxicillin in otitis in cats. This is consistent as a percentage with Zhang et al., who showed that *K.pneumoniae* isolates have greater resistance to amoxicillin. who explained in zhang that the rate of resistance of clinical isolates of *Klebsiella pneumoniae* bacteria is 82.9% to amoxicillin in cats. (36) Research in Zhang confirms a similar percentage of amoxicillin resistance between the two studies.

Conclusion These results show that *K.pneumoniae* in the one of the causes of otitis in cats and also resistance to many antibiotics that were used poses a threat to public health and society; therefore, the indiscriminate use of antibiotics should be avoided.

Acknowledgments

Thanks to the staff of the Microbiology Department of the College of Veterinary Medicine for supporting the completion of this study.

Conflict of interest

A conflict of interest is absent.

Ethical approval

The certificate with the number UM.VET 2025.034 on 7/8/2025, which was given by the Commission of Scientific Morals, was used to collect data and provided the moral cover to carry out the research in the College of Veterinary Medicine.

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دراسة عزل وتحديد النمط الظاهري لجراثيم كليبسيلا الرئوية المعزولة من التهاب الأذن لدى القطط، وفحص حساسيتها للمضادات الحيوية.

ماجدة احمد جاسم[1] ,عامر يحيى الجلبي[1] ,عمر خزعل الحنكاوي[2].

1-فرع الاحياء المجهرية، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

2- فرع الطب الباطني والوقائي، كلية الطب البيطري، جامعة الموصل، الموصل، العراق

الخلاصة

تتزايد أعداد جراثيم الكلبسيلا في الحيوانات الأليفة، مما يُشكل خطرًا جسيمًا على صحتها وسلامتها، وغالبًا ما تكون عواقبه وخيمة. تهدف هذه الدراسة إلى عزل جراثيم الكلبسيلا الرئوية من التهاب الأذن لدى القطط، ودراسة حساسيتها للمضادات الحيوية، وتحديد الجراثيم المقاومة لها. جُمعت خمسون عينة من مسحات الأذن لقطط مصابة بالتهابات الأذن من عيادات بيطرية خاصة مختلفة في محافظة نينوى، وذلك خلال الفترة من آب إلى تشرين الثاني 2025. نُقلت جميع العينات فورًا إلى قسم الأحياء المجهرية، كلية الطب البيطري، جامعة الموصل، العراق، لإجراء التحاليل الكيموحيوية بعد وضعها في عبوات معقمة. استُخدمت أوساط إثراء (أجار الدم وأجار مستخلص القلب والدماغ) وأوساط انتقائية (أجار ماكونكي) للعزل. شُخصت المستعمرة المشتبه بها باستخدام صبغة غرام والاختبارات الكيميائية الحيوية، وتأكد التشخيص باستخدام مجموعتي Vitek وRender (مجموعة من الاختبارات الكيميائية الحيوية). أظهرت النتائج أن 28% (50/14) من عينات القطط احتوت على عزلات إيجابية من جراثيم كليبسيلا الرئوية. بالإضافة إلى ذلك، كشفت اختبارات حساسية المضادات الحيوية عن مقاومة كاملة للميترونيدازول والأموكسيسيلين، في حين أظهرت حساسية كاملة للأوفلوكساسين. ، تُبين هذه النتائج أهمية جراثيم كليبسيلا الرئوية في التسبب بالتهاب الأذن لدى القطط، كما أن مقاومتها للعديد من المضادات الحيوية المستخدمة تُشكل خطرًا على الصحة العامة والمجتمع؛ لذا، ينبغي تجنب الاستخدام العشوائي للمضادات الحيوية.

الكلمات المفتاحية: كليبسيلا الرئوية، التهاب الأذن، القطط، حساسية المضادات الحيوية.