

Isolation and identification of Enterobacter spp from pet animals Ear Infections in Al-Hilla Veterinary clinic, Iraq

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ABSTRACT

Objective: To isolation and identification of Enterobacter spp. from ear infection in pet animals in Babylon province by traditional method Automated Vitek 2 system as well as determined the antibiotic sensitivity test.

Methods; One hundred swab of ear infection were isolated from dogs and cats ear infections in different veterinary clinic in Babylon province, at a period between 1 January to 1 July 2022.

Results: The results of pet animals swab collection revealed that the percentage of gram negative was 71(71%) while the positive was 29 (29%) as well as the biochemical test showed that the enterococcal cloaca percent was (64%), Klebsiella (19%), positive bacteria (6%), E. coli 3% and no growth was 7%. The biochemical tests showed positive for Catalase, Citrate. They gave negative reaction for Urease, Oxidase, MR (Methyl Red), Indole. By the other hand, the result of isolated E. cloaca that was plated onto culture media. then subcultures on MacConkey agar (MAC) and XLD agar. The Antimicrobial susceptibility test of E.cloaca against 55 conventional antimicrobial revealed that E.cloaca varied to a large extent. The isolated bacteria showed sensitivity for the most commonly used antimicrobials for treatment of bacterial otitis media in dog and cat which were amoxicillin, amoxicillin+clavulanic acid, cephalospins, gentamicin and fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin) azithromycin, clarithromycin, neomycin and ploymyxin-B. In contrast, the present study showed that E.cloaca resistant for three antibiotic belongs from beta lactam group (Cefsulodin, Loracarbet and Cefpodoxime).

Conclusion: the production of inhibitor-resistant β -lactamases by E. cloacae isolates could be increasingly common in nosocomial pathogens other than E. coli or K. pneumoniae in pet animals ear infection in Al-Hilla city Babylon province, Iraq.

KEYWORDS: Enterobacter spp., ear infection, Vitek2, Cat and Dog.

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INTRODUCTION

In the underdeveloped world, ear infections are the leading cause of hearing loss, although being entirely preventable (1,2). Ear infections affect about 10-20 % of the dogs and cats, as well as it is one of the most common health problems of the pet animals (3). Ear infections are usually caused by yeast, ear mites and bacteria, with bacteria being the most common cause. Ear problem in dog and cat are usually identified by observing head shaking/ tilting, smelly ears, ear scratching, lack of balance, unusual back-and-forth eye movements, redness inside the ear, swelling of the ear and/or brown, yellow, or bloody discharge from the ear. The problem may be in one or both the ears at any age. Ear infections are usually caused by Staphylococcus,

Pseudomonas and Streptococcus species (4). The most common bacterial causes of otitis media in animals and human beings include Escherichia coli, Proteus spp., Staphylococcus aureus, Streptococcus pneumoniae, Moraxella (Branhamella) catarrhalis, and Haemophilus influenzae (5). Besides Staphylococcus intermedius, Staphylococcus hyicus, Corynebacterium spp., and Enterobacter spp., isolates, Proteus mirabilis was identified as the most frequent cause of otitis in dogs (6). Enterobacter cloacae are pathogens that are commonly isolated from human clinical specimens. They cause an extensive range of nosocomial infections involving skin and soft tissue, the lower respiratory tract, urinary tract, and the CNS infections. The antibiotic-resistance properties of these bacteria had been

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described by several authors worldwide (7). We also look into the prevalence of methicillin-resistant *S. aureus* (MRSA) among *S. aureus* isolates and the presence of the *mecA* gene in MRSA samples from Babylon, Iraq.

MATERIALS AND METHODS

Study design: One hundred ear infection swabs were recruited from the veterinary clinics in Babylon province (Hilla city), Iraq, for this study. Al-Qasim green University in Iraq was home to the study's Faculty of Microbiology.

Sample collection: One or both ears may have been swabbed aseptically to gather samples. Medical professionals collected samples, which were then transferred to the microbiological lab in an ice box within an hour after being immersed in nutrient broth (Oxoid, UK) as a transport media.

Isolation and identification: Each clinical sample was inoculated onto plates of XLD and MacConkey Agar (Oxoid, UK) and then incubated at 35-37°C for 20 hours. *E. cloaca* was identified as colonies of color.

Antibiotic susceptibility of Staphylococcus aureus isolates by VITEK2 system

Antimicrobial sensitivity testing of staph. Aureus was performed by Vitek-2 automated susceptibility system according to manufacturer's instructions. Antibiotic susceptibility of staph. Aureus against 66 antibiotics belonging to 9 classes of antimicrobial agents was tested, as shown in Table (2).

Statistical Analysis: SPSS (Armonk, New York: IBM Corp.) version 17 (Statistical Package for the Social Sciences) was used for the calculations (8).

RESULTS

Sample collection and Geographical isolation: The results of pet animals swab collection illustrated in table (1) and figure (1) revealed that the percentage of gram negative was 71(71%) while the positive was 29 (29%) as well as the biochemical test showed that the enterococcal cloaca percent was (64%), Klebsiella (19%), positive bacteria (6%), *E. coli* 3% and no growth was 7%.

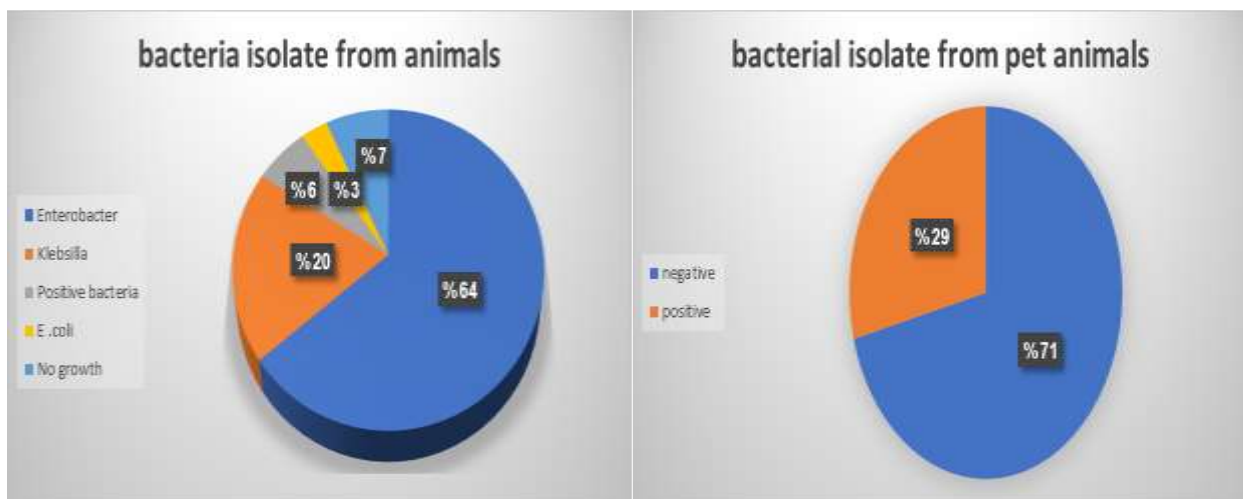


Table (1): Geographically distribution of different swab collection from ear infection in pet animals at Babylon province.

No	Location	Cat	dog	Total
1	Babylon veterinary hospital	3	1	4
2	Janet ard babil clinic	9	2	11
3	Flamango clinic	10	5	15
4	Al awaeal clinic	11	6	17
5	Safary clinic	10	3	13
6	Muayad clinic	6	3	9
7	Dr. Enas clinic	9	4	13
8	Dr Ali alsharaa clinic	8	4	12
9	Nelover clinic	3	3	6
	Total	69	31	100

Biochemical Identification of Enterobacter spp .

The biochemical tests for pet animal samples all isolates showed positive for Catalase, Citrate. They gave negative reaction for Urease, Oxidase, MR (Methyl Red), Indole. In the present study, these conventional biochemical tests that

carried out, and the results were compared with the results documented by Probert et al., (9). The result of isolated *E. cloaca* that was plated onto culture media. Fig (2, A) then subcultures on MacConkey agar (MAC) and XLD agar in Fig (2, B). The current finding strongly consistent with (9, 10 11).

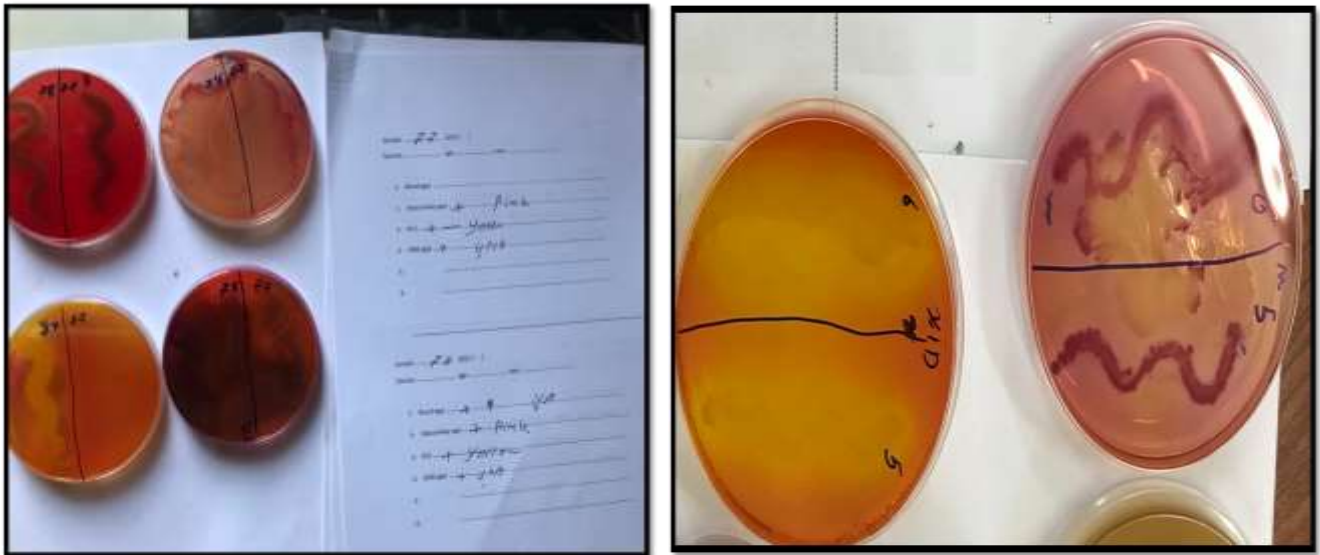


Figure (2): A- *Enterobacter* on culture media; B- *Enterobacter* on XLD and MacConkey.

Identification and Antibiotic susceptibility of *Enterobacter cloaca* isolates by VITEK2 system:

The VITEK 2 system has proven the identification of the *Enterobacter cloaca* that have been taken from cat and dog ear swabs. The main thing that sets VITEK 2 devices

apart is that they can diagnose bacterial isolates faster and more accurately. Using (64) molecular tests to make a diagnosis with special kits and away from contamination, which could make it hard to find a pathogen.

Table (2): Antibiotic sensitivity of isolated *E. cloaca* by using VITEK2 system

Antimicrobial	MIC	Interpretation	Antimicrobial	MIC	Interpretation
Ticarcillin	<=8	S	Gentamicin		S
Ticarcillin /clavulanic acid	<=8	S	Isepumicin		S
Piperacillin	<=4	S	Netilmicin		S
+ Piperacillin/sulbactam			Tobramycin		S
Piperacillin/tazobactam	<=4	S	Cinoxacin		
+cefsulodin		R	Nalidixic Acid		
+ceftobiprole		S	Ciprofloxacin		S
+loracarbet		R	Delafloxacin		
+cefpodoxime		R	Enrolloxacin		
+ceftibutin			Flerosacin		
+cefotaxime		S	Guttiloxacin		S
+cefovecin			Lev ofloxacin		S
+ceftazidime	<=1	S	Lomefloxacin		
+ceftiofur			Marbofloxacin		
Ceftizoxime		S	moxifloxacin		
Cettriaxone		S	Norfloxacin		
Cefoperazone /Sulbactam		S	ofloxacin		S
Ceftazidime:Avibactam		S	pefloxacin		
Cefepime	<=1	S	Pradofloxacin		
+Cefpirome		S	Tosufloxacin		
Aztreonam	<=1	S	Doxyeycline		

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Doripenem+		S	Minocycline	2	S
+Faropenem			Tetracycline		S
Imipenem	<=0,25	S	Colistin		
.Meropenem	<=0,25	S	Rifampicin		
Panipenem+			Trimethoprim		
+Meropenem/Vaborbactam		S	Trimethoprim/ Sulfamethosazole	<=20	S
Amikacin	<=2	S			

DISCUSSION

In this study, 64 bacterial sample were identified as *Enterobacter* spp. Using commercialized systems, such as biochemical tests and Vitek2 system. These techniques present limitations in species' identification, particularly within the *E. cloacae* complex and between *E. hormaechei*, *E. cloacae*, *E. asburiae*, *E. kobei*, and *E. ludwigii*, owing to the high similarity between them (12). For the right drug treatment, it is important to correctly identify the species, and wrong identifications could hurt the patient's health (13). Antimicrobial susceptibility test of *E. cloaca* in table (2) against 55 conventional antimicrobial revealed that *E. cloaca* varied to a large extent. The isolated bacteria showed sensitivity for the most commonly used antimicrobials for treatment of bacterial otitis media in dog and cat which were amoxicillin, amoxicillin+clavulanic acid, cephalospins (cefalexin, cefdinir, cefixime, ceftriaxone, cefuroxime), gentamicin and fluoroquinolones (ciprofloxacin, ofloxacin, levofloxacin) azithromycin, clarithromycin, neomycin and ploymyxin-B (13). These findings agreed with Singh et al., (3). In contrast, the present study showed that *E. cloaca* resistant for three antibiotic belongs from beta lactam group (Cefsulodin, Loracarbet and Cefpodoxime) .According to a recent meta-analysis report, an estimated 48.9% of Gram-negative (*Enterobacteriaceae*) clinical isolates in Ethiopia are ES β L producers, most of them are classified as MDR because they usually carry genes encoding resistance to antibiotics other than beta-lactams (14). Results of this study are in agreement with a study of Alzaidi and Mohammed in Iraq (15), who founded that all *E. cloacae* isolates recovered to be susceptible to cefoxitin disks as per CLSI guidelines (CLSI, 2019), confirming their ability to produce AmpC β -lactamases. For our knowledge, the present study is the first study that isolated *Enterobacter. cloaca* as the most prominent bacteria caused otitis in dog and cat in Iraq. It is the same as that reported in Italy by Piccolo et al., (2020) when they isolated *E. cloaca* in cat when they reported that 34.5% from all isolated bacteria was *Enterobacter* spp.

CONCLUSION

From this study, we can conclude that the production of inhibitor-resistant β -lactamases by *E. cloacae* isolates could be increasingly common in nosocomial pathogens other than

E. coli or *K. pneumoniae* in pet animals ear infection in Al-Hilla city Babylon province, Iraq.

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Conflict of interest: None.

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