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Original Research Article

Phenotypic Detection of *Escherichia coli* Isolated from Urinary Tract Infections for Cats in Babylon Provence

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Abstract: Urinary tract infection (UTI) is a very common Health problem in cats, The present study aimed to isolate and diagnose Escherichia coli causing UTIs cats in Babylon province. A total of 100 urine samples suspected of having UTIs from the age group of (2 months to 3 years) were collected from different veterinary clinics in Babylon province. 50 isolated to *Escherichia coli* were obtained in Babylon during the period from September 2023 to April 2024. The initial diagnosis of *E. coli* isolates was made using culture media (MacConkey agar, methylene blue eosin and blood agar). Biochemical tests and Vitek 2 device were used to confirm the final diagnosis of the isolates. The highest number of bacterial isolates was found in female cats, more infected than male cats, The sensitivity test was performed using the Vitek device, and the resistance was as follows: ampicillin (100%), cefazolin, trimethoprim-sulfamethoxazole (70%), piperacillin, cefoxitin, ceftriaxone, levofloxacin, ciprofloxacin (30%). The results of the study also showed absolute resistance to E. coli (ampicillin (100%) was the most resistant in cats as well as non-isolated resistance to piperacillin, ceftazidime, cefepime, amikacin, gentamicin, nitrofurantoin, and tigecycline. The ability to produce biofilm was revealed by two methods, the first is the standard plate method (MTB) and the second is the Congo red medium. the first method shown the Cat isolates (5 isolates were medium, 3 isolates were weak, and 2 isolates had no growth), As for the second method, all isolates appeared positive.

Keywords: *E. coli*, UTI, Biofilm, Antibiotic.

INTRODUCTION

Cats that have chronic diseases are more likely to develop UTIs (Freitag *et al*., 2006). UTIs are also connected to abnormally frequent urination, structural abnormalities, urothelial alterations, antibacterial features of urine, waning immunity, length of inpatient stay, and usage of antibiotics (Lekcharoensuk, *et al*., 2001; Ataya, *et al*., 2023). *E. coli* grows as normal flora in the digestive tract and is considered a pathogenic opportunistic as it can cause diarrhea, in which case it'd be called diarrheagenic E. coli (DEC) and urinary tract infections which are caused by the type called uropathogenic E. coli (UPEC) (Levinson *et al*., 2018; Riedel *et al*., 2019). Antimicrobial resistance (AMR) is a Complex problem with many Contributing Factors. Bacteria have developed numerous methods to Resist Antibiotic Action, such as Activation of Drug Efflux pumps, mutation of Antibiotic function sites by passing the target site of the antibiotics, and enzyme-mediated drug degradation (Zhang and Yang; 2022) The most important factor is Antimicrobial usage (AMU), which facilitates the selection of bacteria with acquired resistance in animals and humans (Fonseca *et al*., 2021, Garces, 2022). Antimicrobial resistance is defined as the ineffectiveness of drugs to treat health conditions caused by microorganisms, and it has led to the spread of many infections like UTIs, respiratory tract infections, and many other health conditions that are difficult to treat due to the progress these causative microorganisms have made in their antimicrobial resistance ability (Adhikary, 2020). *E. coli* form a biofilm when the environmental is unfavorable, lack of nutrient, or a high density of cells in a specific location. The biofilm is made up of polysaccharides in addition to compounds from the bacterium's environment like nutrient, minerals, amino acids, cell wall components, etc. (Billings *et al*., 2015).

First noted in 1933 by Henrici who found thick communities of bacteria grow on submerged slides in a number of different water sources (O'Toole and Wong, 2016). Many strains of E. coli elaborate a hemolysin which is responsible for the zone of beta-hemolysis surrounding bacterial colonies on blood agar. The significance of this cytolysin as a determinant of bacterial pathogenicity has been established in animal models with the use of genetically engineered, isogenic bacterial strains (Palela *et al*., 2022).

MATERIAL AND METHODS

Sampling:

The samples were obtained from Al-Qasim University Faculty of Veterinary Medicine Clinics and private pet hospitals between September (2023) to April (2024), which had been sent to Al-Qasim University Faculty of Veterinary Medicine Department of Microbiology for diagnosis. Urine samples were collected aseptically Clinically suspected specimens of pyometra and prostate infection were collected by steril containers or transport swabs. Swabs samples for bacterial examination were taken from by vaginal way. A total of 100 samples of obtained from cats were examined.

Isolation and Identification:

Upon arrival to the laboratory, samples were centrifuged at 3000 rpm for 10 minutes and the supernatant fluid was discarded (Reine and Langston, 2005). A loop from the sediment was then cultivated into nourishment broth (specify) and incubated at 37°C for 24 hours. After that, a loopful of broth was streaked onto blood agar, MacConkey agar, nutrient agar, Eosin Methylene Blue agar (EMB) and Mannitol Salt Agar (MSA) plates (Eggertsdottir, *et al*., 2007) and incubated under aerobic conditions (Luts, *et al*., 2019).

Antibiotic Sensitivity Test (AST):

Antibiotic susceptibility profile of E. coli isolated from urine sample of cat were determined by using Vitek2 system. Ten E. coli isolated from domestic cats suffering from UTIs were subjected to 14 antibiotic representing different classes of antibiotics. The results indicated diverse resistance patterns.

Biofilm Formation:

1. Congo Red Agar

The formation of biofilm was studied on Congo red agar, which consisted of 15 g of brain-heart infusion broth powder, 20 g of glucose, 50 g of sucrose, 0.8 Congo red dye, 15 g of NaCl, and 20 g of agar. E. coli was streaked on the Congo red agar and incubated at 37 °C for 7 days. The colors of the former colonies were observed; red color was a negative result, while the black color was a positive result (Gazi, 2021).

2. MTP (Micro Titer Plate)

All isolates were screened for their ability to form biofilm by the MTP method in duration of incubation, which was 24 hours, the addition of glucose Few colonies suspended in physiological saline to 0.5 McFarland and vortexed for 1 min. 96 wells flat-bottomed microtiter plates were filled with 180µl Trypticase soy broth (TSB) (Oxoid, UK) + 0.5% glucose and 20µl of bacteria suspension added to each well. Three wells per strain were incubated and their mean considered as final absorbance (Jebril, 2020).

Hemolysis Production:

Isolates were cultured by streaking on the surface of blood agar plates then incubated for 24 hours at 37 °C. Hemolysis types was observed then and as follows (Palela *et al*., 2022): α-hemolysis: Colonies will be surrounded by a green hemolysis area, β-hemolysis: Area surrounding colonies are clear and ɣ-hemolysis: No lyse is observed around colonies.

Statistical Analysis

In order to determine the statistical differences between the variables, the statistical program SPSS version 14. was used in the statistical analysis of the samples. The test of homogeneity of the sample is (chi-Sequare) and one-way a nova $p < (0.05)$.

RESULTS

Culture Characteristic of *E. coli*

E. coli isolates were identified depending on their physical characteristics. The isolate appeared as bright pink colonies when cultured on MacConkey agar and the colonies appeared green metallic sheen on EMB media (Figure 1).

Microscopic Examination:

A smear was made from a colony grown on MacConkey agar and it was stained with Gram's stain. The bacteria appeared short rods, negative to Gram's stain, and not spore forming (Figure 2).

Prevalence of E. Coli According to Collected Samples in Cats

The results indicated that the prevalence of E. coli in cats aged (less than1 year) was (58.18%) of the total 55 samples examined, while the cat aged (1-3 year) the infection rate was (40%) of the total 45 samples examined, there was no significant difference in infection rates among different age groups, Table 1. In a correlation of infection rates with gender of cat, the results showed that there was a significant increase at $P<0.05$ in the prevalence of E. coli in female cats which was 35/60(58.33%) in comparing with those in male 15/40 (37.5%) and as listed in (Table 1 and Table 2).

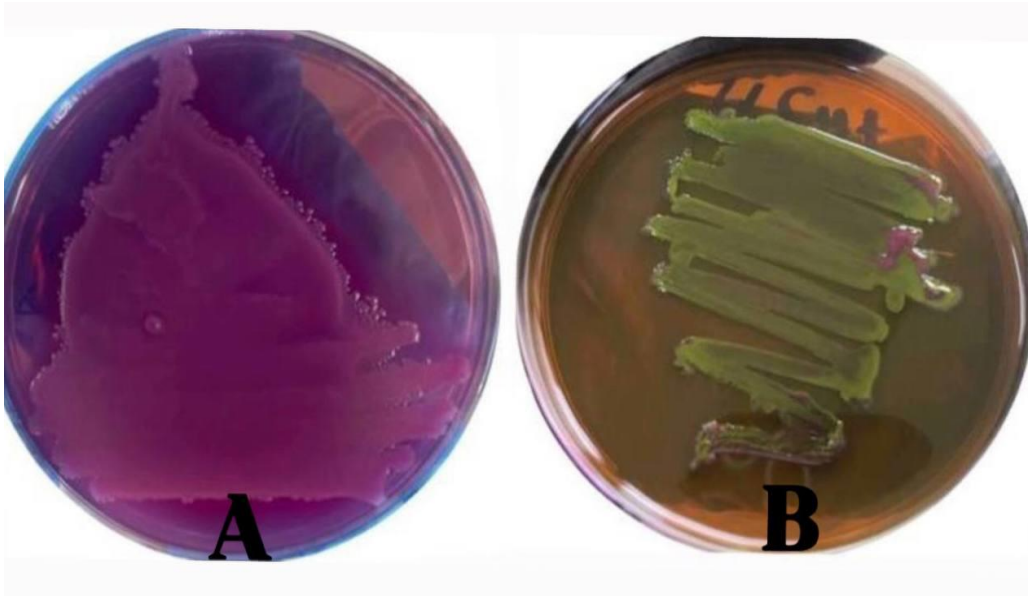

Figure 1: *E. coli* growth on 2 different mediums. A: Bacterial colonies on MacConkey agar, bright pink colonies. B: Eosin methylene blue (EMB) media, green metallic sheen colonies

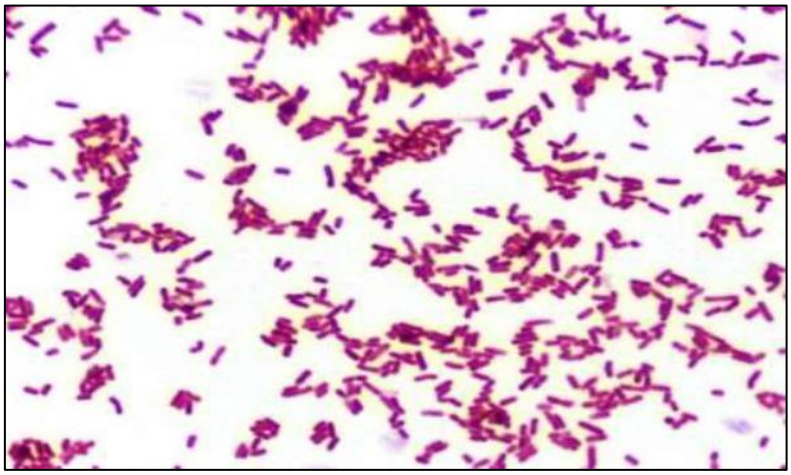

Figure 2: Gram stain of isolated E. coli (100X)

Table 1: Distribution of E. coli isolates according to age interval of cat

Age group interval	Total examined samples	Positive samples to E. coli	%
Less than 1 year	55	32	58.18
1-3 years	45	18	40
Total	100	50	50
X^2		3.27	
P value		0.07(NS)	

NS: No significant difference at P<0.05

Table 2: Distribution of E. coli isolates according to gender of cat

Gender	Total examined samples	Positive samples to E. coli	%
Male	40	15	37.5
Female	60	35	58.33
Total	100	50	50
X^2		4.16	
P value		0.041(S)	

Resistance Rates of *E. coli* in Cats:

Antibiotic susceptibility profile of E. coli isolated from urine sample of cat were determined by using Vitek2 system. The results indicated diverse resistance patterns, with the highest resistance observed against Ampicillin, cefazolin, trimethoprim-

sulfamethoxazole at percentages of (100%, 70%, 70% respectively) and piperacillin, cefoxitin, ceftriaxone, levofloxacin and ciprofloxacin (30% each one). The statistical analysis revealed that the highly significant differences among the resistance and sensitive antibiotics as listed in (Table 3).

Table 3: Antibiotic Susceptibility of E. Coli Isolates for Cats

Members	Sensitivity rate%	Resistance rate%
Ampicillin	0 (0%)	10 (100%)
Piperacillin	7 (70%)	3 (30%)
Cefazolin	3 (30%)	7 (70%)
Cefoxitin	7 (70%)	3 (30%)
Ceftazidime	10 (100%)	0 (0%)
Ceftriaxone	7(70%)	3(30%)
Cefepime	10 (100%)	0 (0%)
Amikacin	10 (100%)	0 (0%)
Gentamicin	10 (100%)	0 (0%)
Levofloxacin	3(30%)	7(70%)
Ciprofloxacin	7(70%)	3(30%)
Trimethoprim- sulfamethoxazole	3 (30%)	7 (70%)
Nitrofurantoin	10 (100%)	0(0%)
Tigecycline	10 (100%)	0 (0%)
X2	70.92	
P value	<0.0001(HS)	

Phenotypic Detection of Virulence Factors of Escherichia coli

Several virulence factors harbored by E. coli were investigated and the following sections detail all aspects of the phenotypic investigation done on the isolates.

Biofilm Detection

1. Congo Red Agar Method (CRA)

For all E. coli isolates, biofilm formation was detected by Congo red agar method (CRA) as described by Freeman *et al.*, (1989). Of the 10 *E. coli* isolates for cat. showed a biofilm-positive phenotype under the optimized conditions. CRA assay showed black colonies with dry crystalline morphology after 24-48 hours (Figure 3). Though the CRA assay can well detect biofilm-producing strains, weak producers were difficult to discriminate from biofilm negative isolates.

2- Micro Titer Plate (MTP):

Biofilm was detected in *E. coli* isolates from cats by using MTP as depicted in (Figure 4).

Figure 3: *E. coli* growth on Congo red

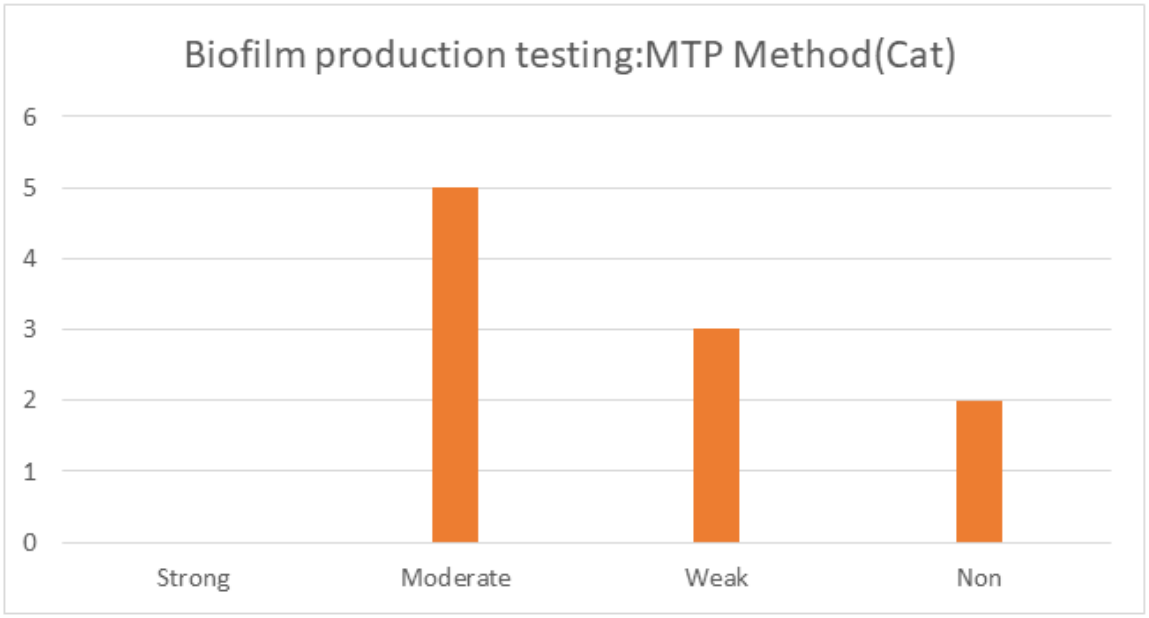

Figure 4: Biofilm formation of E. coli isolates for Cats

Hemolysin Production:

Sheep blood agar was used to test the ability of isolates to analyze red blood cells. Hemolysin production. It was found that out of 10 isolates, 8 samples (40%) were capable of degrading red blood cells to varying degrees. degrees while the remaining 2 were unable (ɣ-hemoysis) in (Figure 5).

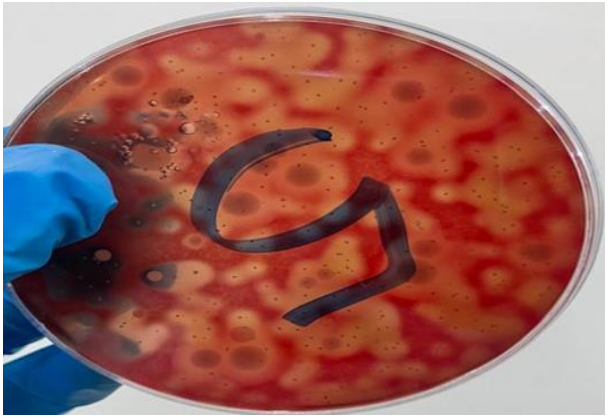

Figure 5: Hemolysin Production on Blood agar

DISCUSSION

The results of the present study showed that the prevalence of E. coli was higher in kitten (less than one year) (58.18%) in comparing with adult cat (1-3 years) (40%) (Table 1) and (Table 2). These findings in agreements with the prevalence rates reported in previous studies with values between 40% and 67% in felines (Marques *et al*., 2016; Teichmann *et al*., 2018; Hernando *et al*., 2021). In general, that mainly female cats with systemic risk factors for bacterial UTIs, such as diabetes mellitus, chronic kidney disease and hyperthyroidism, and cats with occult UTIs were included (Dorsch *et al*., 2015). In the same line, about one-third of the adult female cats undergone prior transurethral procedures, such as urethral catheterization or urogenital surgery. In the present study, female cats were significantly older than male cats (mean age 11.7-8.2 years) (Aurich *et al*., 2022). Thus, it is likely that in elderly cats with predisposing systemic diseases, female sex increases the risk for bacterial UTI. while a higher proportion of middle-aged male cats with signs of lower urinary tract and urethral obstruction need transurethral procedures or urogenital surgery, which predisposes for bacterial UTI (Mahmoud *et al*., 2024). Antibiotic resistance can arise for a number of reasons, including incorrect dosing, overuse, and failure to finish the full course of treatment for a variety of infections, as well as the acquisition of resistance in low- susceptibility bacteria through selection / spontaneous mutation, the development of resistance in enteric bacteria via R plasmids responsible for multiple drug resistance, and the transmission of resistant strains between humans and animals (Setu *et al*., 2016; Mustapha *et al*., 2019). Biofilm forming bacteria are a common cause of recurrent, and complicated UTIs in both human and animals which are normally associated with MDR bacteria (Flores-Mireles *et al*., 2015). Understanding the pathogenesis and factors associated with biofilm formation is key to the development of new therapies (Römling and Balsalobre, 2012). Among 10 E. coli isolates subjected to biofilm production, most of the isolates, 10 were biofilm formers on Congo Red Agar (CRA). This finding was like many previous studies (Subramanian *et al*., 2012; Sudheendra and Basavaraj, 2018; Katongole *et al*., 2020). The present results suggest that hemolysin is produced early in the growth cycle, and production of hemolysin is mostly associated with pathogenic bacteria therefore considered important virulence factor (Valeva *et al*., 2005). The hemolytic assay performed as an indicator of the pore forming ability of the toxins by measuring blood cells and lyses

them by oligomerizing and forming pores (Vagrali, 2009; Aziz, 2023).

CONCLUSIONS

The current study provides a basic database for the spread of inflammation, virulence factors, and genes in Babil Governorate. It is necessary to monitor the spread of the epidemic and the virulence genes of the disease in the local area. E. coli bacteria are more common among cats in rural areas. It is more common among older female cats and its prevalence is greater than in males. Escherichia coli isolates in this study revealed many virulence factors that may lead to chronic urinary tract infections cats. Escherichia coli isolates showed a high rate of resistance to various antibiotics (ciprofloxacin, Trimethoprim-sulfamethoxazole), Ampicillin was one of the most resistant antibiotics.

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