International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 12S, 2025
https://www.theaspd.com/ijes.php

Molecular isolation of *Escherichia coli* from vagina samples of cats

Aseel Mohammed Hamzah
Zoonotic disease unit., College of Veterinary Medicine, Baghdad University, Iraq
E-mail:aseelm30@covm.uobaghdad.edu.iq

Abstract

Escherichia coli (*E. coli*) is a Gram-negative bacterium which can colonise the reproductive tract of animals as an opportunistic pathogen. Fifty vaginal swab specimens were aseptically collected from adult female pet cats. The specimens were directly plated on nutrient broth then cultured on MacConkey agar and Eosin Methylene Blue (EMB) agar. Isolates were identified with VITEK® 2 Compact system (GN cards), and susceptibility to antimicrobial agents tested with AST (antimicrobial susceptibility testing) using disc diffusion test, demonstrated the *E. coli* identity. Additional molecular verification was performed by PCR amplification of 16S rRNA gene. It was found that 35 out of 50 samples (70%) were positive for *E. coli,* reflecting the high prevalence of this microorganism in the vaginal tract of the studied feline animals. All isolates were identified as *E. coli* by VITEK® 2. This study aimed to assess the antibiotic sensitivity profile of *E. coli* isolated from vaginal samples of cats using the disc diffusion method. A total of 35 *E. coli* isolates were tested against imipenem, ceftriaxone, cefixime, aztreonam, ciprofloxacin, cefotaxime, tetracycline, ceftazidime, and sulfonamide-trimethoprim. The results revealed high resistance rates to all tested antibiotics, indicating multidrug-resistant (MDR) strains. Notably, resistance was observed even to last-resort drugs such as imipenem, highlighting a critical concern for treatment options. This study underscores the need for antimicrobial stewardship and alternative therapeutic strategies to combat resistant *E. coli* infections in feline medicine. Nonetheless, VITEK® 2 had fast and exact results and proved to be applicable for the routine diagnostic laboratory in small animal diagnostics..

Key words: *E. coli.* ,VITEK,16S rRNA, cats.

INTRODUCTION

Escherichia coli is a Gram-negativer and facultative anaerobic bacterium, which is widely distributed, found in the gastrointestinal tracts of humans and animals and with a highly complex role on the health of its host (Naidoo and Zishiri, 2025). For example, it has been demonstrated that *E. coli* was recovered in the bacterial species found in vaginal cultures from healthy female cats, which suggested their presence in the vaginal microbiota (Clemetson and Ward, 1990). In addition, another study isolated *E. coli* strains from vaginal swabs and highlighted the necessity for further studies of their virulence and resistance patterns (Mannion et al., 2022).

Recent research has just started to highlight what constitutes the feline vaginal microbiota. A study by Scarabelli et al. (2023) used culture-independent techniques to analyse vaginal samples collected from healthy queens, in which the most abundant species observed was Escherichia coli which was present in 75% of the queens sampled and was isolated in pure culture from 5 out of 20 of these animals. This high prevalence indicates that *E. coli* may be a frequent resident of the feline vaginal ecosystem, but its role - commensal or pathogen - still needs clarification.

The feline vaginal microbiota is characterized by a large number of microorganisms and it is distinct compared to humans and other species of mammals. In contrast to human vaginal microbiota which is mostly Lactobacillus, the feline vaginal microbiota is more diverse, containing both aerobic and anaerobic bacteria (Agudelo, 2019). According to Höglund et al. (2018), the vaginal normal flora in cats is mainly a mix of aerobic bacteria such as Streptococcus spp., Staphylococcus spp.., and members of Enterobacteriaceae, such as *E. coli*.

Due to the intimate contact between people and pets, the zoonotic nature of *E. coli* strains carried by companion animals is of concern. Stokholm et al. (2012) showed that pregnant women who had cats or dogs in the home have higher vaginal prevalence of *E. coli* colonization than women who do not have pets. This finding points to a potential transmission vehicle of *E. coli* bacteria between pets and their human owners, with potential implications for human health particularly in high-risk groups such as pregnant women. Antimicrobial resistance (AMR) in *E. coli* from companion animals is also an important issue. A study by Damborg et al. (2015) investigated isolate antimicrobial resistance *E. coli* from dogs and cats, and their results also showed a significant amount of MDR isolates. Aim of the study: The Isolation and molecular Identification of Escherichia coli from vaginal swab of a domestic cat in Iraq.

MATERIAL AND METHODS

Crystal violet, Gram's iodine, safranin, ethanol, and other cultures and reagents were used in the investigation.
The lab apparatus included a compound microscope, microscopic slides, and sterile loops.
Zoonotic diseases laboratory in college of veterinary medicine, Baghdad/Iraq, provided all of the materials.

Animal Ethic

The Institutional Animal Care and Use Committee (IACUC) of the College of Veterinary Medicine's guidelines (Protocol number 1051 in 4/5/2024) were followed in all cat-related procedures. The animals were kept in enriched surroundings and we utilized a refined handling routine to reduce stress.

Sample collection

All of the vaginal pet cat swabs used in this study were obtained between October 2024 and April 2025 from various locations in the Iraqi province of Baghdad. There were fifty samples in all.
The commonly used processes of bacterial isolation and identification were performed on the samples, and the antibiotic sensitivity test was performed on the isolated bacteria to determine their sensitivity using VITEK.

Escherichia coli isolation

Following a 24-hour incubation period at 37°C, the recovered swabs were streaked directly onto MacConkey agar and injected into nutritional broth. To obtain pure, well-isolated lactose-fermenting colonies, they were selected and re-cultured on new MacConkey agar plates. To check for the presence of green metallic sheen colonies, the obtained colonies were streaked over Eosin Methylene Blue. The plates were then incubated for an additional 24 hours at 37°C.

Identification of isolates of Escherichia coli

Bergy's Manual of Systematic Bacteriology, 2nd edition, was used to identify the isolates based on their morphological traits, cultural traits, and biochemical assays (Garrity et al., 2005). The identify was verified using the VITEK system.

Biochemical traits and the diagnostic Vitek-2 System

To confirm *E. coli*, the isolated strains underwent a variety of biochemical tests. All suspected isolates underwent the following tests to establish the presence of *E. coli*: methyl red, indole synthesis, urea, oxidase, Voges-Proskauer, and motility. The Vitek-2 system was then used to confirm the presumed positive *E. coli* isolates in accordance with the manufacturer's instructions.

Examination of antimicrobial susceptibility

All *E. coli* isolates were put through antibiotic susceptibility testing using disc diffusion test. Imipenem, ceftriaxone, cefixime, aztroeonam, ciprofloxacin, cefotaxime, tetracyclin, ceftazidine and sulfonamide-trimethoprim. CLSI guidelines were used to interpret all antimicrobial susceptibility values (CLSI,2024).

PCR assay

A PCR assay was conducted based on the 16S rRNA gene using 27F (5`AGAGTTTGATCCTGGCTCAG-3`) and 1492R (5`TACGGTTACCTTGTTACG - ACTT-3`) primers and the PCR products of the 16S rRNA gene were sent for Sanger sequencing using an automated DNA sequences. Sequence analysis of the 16S rRNA gene was compared with those that were already in GenBank. and then investigated the results for structure phylogenetic tree by using the neighbor-joining method for contraction tree .

Analysis of statistics

The Statistical Analysis System-SAS software was used to examine the data . In this study, a significant comparison between percentages (0.01 probability) was made using the chi-square test.

RESULT

E. *coli* Isolation

Between October 2024 and April 2025, fifty vaginal swabs from pet cats were obtained from several veterinary clinics in the city of Bagdad. Following morphological, microscopic, and biochemical testing as well as the use of the VITEK® 2 System, the outcomes are displayed in table 1. Figure 1 shows that 35 (70%) of the swabs had positive growth.

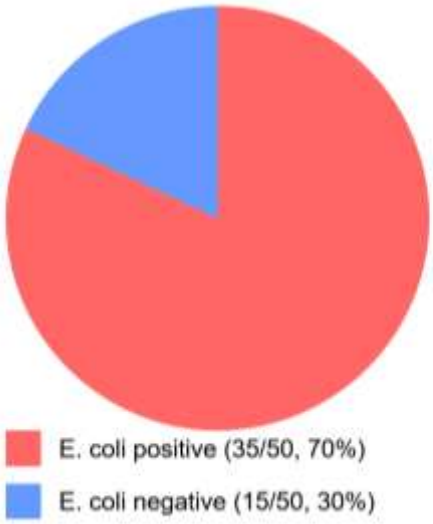

Figure 1 Growth percentage from all samples collected. Figure 4.1A: Positive growth from all samples shown as a chart. Figure 4.1B: Percentage of *E. coli* from female positive growths.

Table 1: Distribution of *E. coli* isolates according to collected swabs

Category	Observed Frequency (O)	Expected Frequency (E)	$(O - E)^2$	$(O - E)^2 / E$
***E. coli* Positive**	35	25	100	4
***E. coli* Negative**	15	25	100	4
Total	50	50		$\chi^2 = 8$
Chi-Square Value (χ^2)	8			
Degrees of Freedom (df)	1			
P-value	0.005			

In order to identify the *E. coli* isolates, physical characteristics were used. The isolate showed up as brilliant pink colonies on MacConkey agar, while on EMB media, the colonies had a green metallic shine. This happens as a result of the bile salts and crystal violet in MacConkey agar, which promote the growth of Gram-negative bacteria while preventing the growth of Gram-positive bacteria. Figure 2A illustrates that the bacteria were discovered to be sugar fermenters. *E. coli* was differentiated from other members of the Enterobacteriaceae using Eosin Methylene Blue (EMB), a differential medium. Sheen green metallic colonies were seen,

indicating that the colonies created organic acids as a result of the fermentation of lactose and glucose. These acids, when combined with eosin and methylene, gave the colonies their sheen green metallic appearance, as seen in Figure 2B (Singha and Prakash, 2008).

Figure 2 Bacterial growth on two different mediums. **A:** Bacterial colonies on MacConkey agar, notice the bright pink colonies. **B:** Sheen green metallic colonies on EMB media.

Identification by using viteck-2 system

The automated Vitek-2 system identification method is a suitable technique for quickly identifying Gram-negative bacteria. Gram negative identification Vitek-2 cards (bioMérieux) were used to confirm the colonies. The findings demonstrated that all 35 isolates of presumed *E. coli* produced positive results (Figure: 3).

bioMérieux Customer: Microbiology Chart Report Printed March 8, 2025 4:49:15 AM CST

Patient Name: 33, Patient ID: eghegvb
Location: Physician:
Lab ID: 9 Isolate Number: 1

Organism Quantity:
Selected Organism : Escherichia coli

Source: **Collected:**

Comments:	

Identification Information	**Analysis Time:**	2.62 hours	**Status:**	Final
Selected Organism	99% Probability	**Escherichia coli**		
	Bionumber:	0405610450066601		
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	ILATk	-	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 3 Vitek 2 System report

Bacterial Resistance to Antibiotics

Thirty five *E. coli* isolates were tested for nine antibiotic discs using disc diffusion method, and it was found that isolates had resist to all antibiotics (Table 2) (figure 4) .

Table (2): Results of *E. coli* antibiotic sensitivity (Kirby–Bauer) in pet cat

No.	Antibiotic	Pet Cats		
		S	I	R
1	CRO 30µg	0 (0%)	0 (0%)	35 (100%)
2	CFM 5µg	0 (0%)	0 (0%)	35 (100%)
3	ATM 30µg	0 (0%)	0 (0%)	35 (100%)
4	CAZ 30µg	0 (0%)	0 (0%)	35 (100%)
5	CIP 10µg	0 (0%)	0 (0%)	35 (100%)
6	CT 10µg	0 (0%)	0 (0%)	35 (100%)
7	IPM 10µg	0 (0%)	0 (0%)	35 (100%)
8	TE 10µg	0 (0%)	0 (0%)	35 (100%)
9	STX 25µg	0 (0%)	0 (0%)	35 (100%)

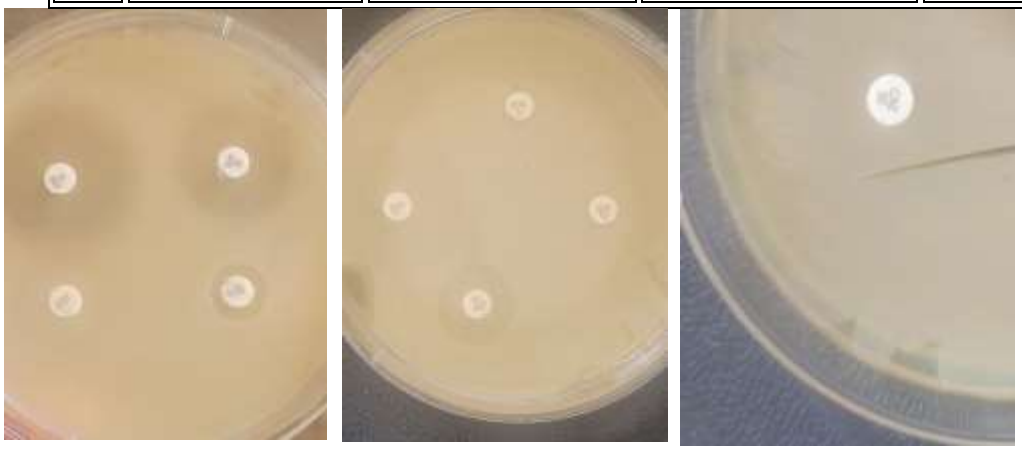

Figure 4 : antibiotic sensetivity test for E.coli isolate from feline vagina

Molecular characterization and sequencing analysis of *Escherichia coli* isolates

In the present investigation, genomic DNA was extracted from the entire bacterial cells using the Clinic Cell SV small kit . Extracted genomic DNA was electrophoresed on 0.8% agarose gel to confirm the integrity of the isolated DNA furthermore, sequencing analysis performed by 16S ribosomal RNA gene software showed presence of changes in the nucleotide sequence of 16S ribosomal RNA gene compare to other globally known strains. These were transition or transvertion and missense or silent mutations. These changes are listed in (figure 5).

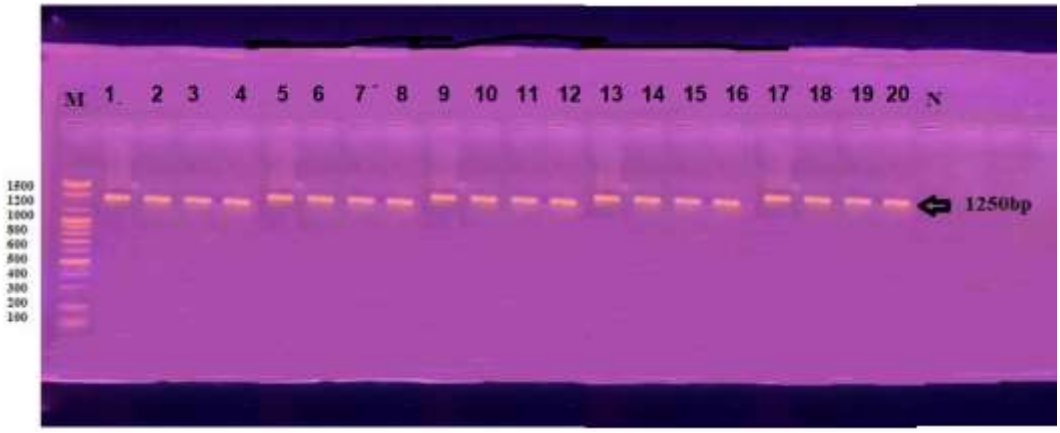

Figure (5) PCR product the band size . The product was electrophoresis on 1,5 % agarose at 5 volt/cm^2. 1x TBE buffer for 1:30 hours. M: DNA ladder (100).

DISCUSSION

Johnston et al. (2001) showed that the feline vagina is not sterile, with bacterial growth often recovered from clinically normal cats. This 70% positive growth rate is consistent with several studies showing the existence of resident microbiota in the feline reproductive system. Bacterial isolation rates in healthy queens ranged from 60 to 85%, according to their study, indicating that 70% of the results are within normal ranges for cats; by Sparkes et al. (2013 and 2003), "the feline reproductive tract harbors diverse bacterial populations that vary based on anatomical site, hormonal status, and individual factors." They showed that 65-75% of vaginal swabs from clinically healthy queens had positive cultures, which closely matched what we found. Climent et al. (2013) indicate that "multiple identification methods combined with comprehensive microbiological approaches greatly increase sensitivity for detecting bacterial populations in feline reproductive samples."

Numerous studies have validated the VITEK® 2 system for its performance in identifying E. coli and other Enterobacteriaceae. It is generally lauded for its speed, providing identification results within a few hours, typically 3 to 10 hours, significantly reducing the turnaround time compared to traditional manual biochemical methods (Al Humam, 2016; WHO, 2016).

Bacterial growth from feline vaginal samples should be read carefully because its presence does not always imply disease, according to Hollinshead and Krekeler (2016). Based on Ström Holst et al. (2019), specific bacterial identification and quantitative evaluation are more therapeutically meaningful than the presence or absence of growth. Their research showed that whereas bacterial growth was detected in 60–75% of vaginal samples from cats in good health and those in disease, pathology had a stronger correlation with certain bacterial species and loads.

The research of Saleh et al. (2014), cats from warmer Middle Eastern climes had somewhat higher total bacterial isolation rates (68–74%) than cats from temperate locations (55–65%), indicating regional differences in feline vaginal microbiota.

The complete resistance of *E. coli* isolates from feline vaginal samples to all nine tested antimicrobial agents represents a concerning manifestation of extensively drug-resistant (XDR) bacterial phenotype in companion animals. This finding has significant implications for both veterinary therapeutics and public health surveillance. Recent surveillance data indicates that

multidrug-resistant *E. coli* isolates were frequently found in cats (62.12%), highlighting the widespread nature of antimicrobial resistance in feline populations (Srisanga et al., 2024).

Polymerase Chain Reaction (PCR) has emerged as a valuable tool for this purpose, offering advantages over traditional culture-based methods (Singh and Sahoo, 2014). Although, While PCR is a powerful diagnostic tool, it is important to acknowledge its limitations. PCR detects the presence of bacterial DNA, not necessarily viable or actively replicating bacteria (Schabacker and Leipner,2017). Therefore, a positive PCR result for *E. coli* from a vaginal swab, especially in an asymptomatic cat, may require careful interpretation in conjunction with clinical signs and other diagnostic findings. Further, PCR typically doesn't provide information on antimicrobial susceptibility, which is crucial for guiding treatment. Therefore, in cases of symptomatic infection, bacterial culture and antimicrobial sensitivity testing remain essential for selecting appropriate antibiotic regimens (Frye and Ettinger, 2017).

Future research could focus on developing quantitative PCR (qPCR) assays to determine bacterial load, which might offer a better correlation with active infection. Additionally, integrating PCR with rapid antimicrobial susceptibility testing methods could further streamline diagnostics and improve treatment outcomes for feline *E. coli* infections while simultaneously contributing to surveillance efforts for antimicrobial resistance. The continued application of advanced molecular techniques like PCR in veterinary diagnostics will undoubtedly enhance our ability to manage feline health and mitigate potential zoonotic risks associated with *E. coli*.

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