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Molecular Characterization, Serotyping, and Antimicrobial Resistance Profiles of *Escherichia coli* Isolates from Companion Animals in El-Menoufia Governorate, Egypt

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ABSTRACT

They have been further divided into seven *E. coli* pathotypes based on the various virulence characteristics All domestic animals and the environment contain *Escherichia coli*, which is easily dispersed throughout various compartments. *E.coli* is also a "highly relevant and representative indicator of the global antimicrobial resistance (AMR) problem," according to the World Health Organization (WHO). It is categorized into multiple pathotypes that cause intestinal and extraintestinal diseases, including skin and soft tissue infections, gastroenteritis, urinary tract infections (UTI), and septicemia. A total no. of 100 (urine ,nasal and stool) samples were collected from the El-Menoufia governorate (40 dogs, 50 cats, and 10 Egyptian nisnas), In addition, further identification of *E.coli* species was performed using PCR targeting for the *16srRNA* gene. The results showed that *E.coli* was isolated with incidence of 19 (47.5%) , 21 (42%) and 2 (20%) from dogs ,cats and Egyptian nisnas respectively. All detected *E. coli* isolates harbored the *16srRNA* gene.

PCR technique were applied to detect the beta lactamases resistance genes (*bla*SHV, *bla*TEM, *bla*CTXM, and *bla*OXA). Each isolate has the gene *bla*TEM, one isolate has the genes *bla*SHV and *bla*TEM, and four isolates have the genes *bla*TEM and *bla*CTXM. With the Incidence of (100%) *bla*TEM, (40%) *bla*CTXM and (10%) *bla*SHV .

and clinical changes brought about by pathogenic *E. coli* strains: Shiga-like toxin producing (STEC), enterotoxigenic/heat-labile/heat-stable enterotoxins generating (ETEC), diffusely adherent (DAEC), enteroinvasive (EIEC), enterohemorrhagic (EHEC), enteropathogenic (EPEC), and enteroaggregative (EAEC). From pets, phylogroup B2 was found to be quite prevalent. All isolates had the phylogenetic genes *chuA*, *yjaA*, and *tspE4.C2* detected by PCR, except for two isolates which lacked *tspE4.C2*.

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1. INTRODUCTION

2. One of the earliest microorganisms to enter the human intestine after birth is *Escherichia coli*, a Gram-negative bacterium. On the other hand, *E. coli* frequently causes infections in the gastrointestinal system and other areas of both human and animal bodies. More specifically, appendicitis, pneumonia, meningitis, endocarditis, and gastrointestinal infections are among the several dangerous illnesses and diseases that *E. coli* can cause, but it usually causes urine infections [1, 2, 3].
3. Because it may cause severe infections in both humans and animals, *E. coli* is a unique bacterium in the world of microbiology. But it also has a significant impact on the autonomic microbiota of various hosts. The main mechanism of *E. coli* pathogenicity is the transfer of resistant *E. coli* between humans and animals through a variety of pathways. *E. coli* is a major source of resistance genes in veterinary and human medicine that may be the cause of treatment failures [4].
4. *Escherichia coli* (*E. coli*) is a common cause of gastrointestinal illnesses and a factor in the development of antibiotic resistance in both people and animals [5]. Shigatoxigenic *E. coli* (STEC), attaching and effacing shigatoxigenic *E. coli* (AE-STEC), enterotoxigenic *E. coli* (ETEC), and enteropathogenic *E. coli* (EPEC) are some of the pathotypes of pathogenic *E. coli* that were grouped based on their virulence determinants [6].
5. The presence of AMR genes, particularly those associated with ESBL, in *E. coli* isolated from veterinarians and healthy pets suggests that these *E. coli* sources may act as reservoirs for antibiotic resistance, increasing the possibility of negative effects on humans and animals. This is because *E. coli* is considered a great indicator of antimicrobial resistance (AMR) for a variety of species [4]. These results emphasize how crucial it is to apply efficient AMR management strategies in veterinary clinics because both humans and animals might harbor bacteria resistant to widely used antibiotics [7]. When treating gastrointestinal disorders in dogs and cats, beta-lactam antibiotics are the most commonly prescribed antimicrobial medicines. However, a considerable rise in resistant *E. coli* isolates was seen following oral administration of amoxicillin-clavulanic acid [8].
6. *Escherichia coli* typically exhibited 100% ampicillin and 100% amoxicillin–clavulanic acid resistance. Extended-spectrum β-lactamases (ESBLs) genotypes, particularly *bla*CTX-M, *bla*TEM, and *bla*SHV, were shown to be quite prevalent, may

have important consequences for the health of pets, veterinary professionals, and pet owners as well as the environment [9].

7. Using molecular phylotyping on multiple strains of *E. coli* may help identify the links between them. Three genetic markers (*chuA*, *yjaA*, and *tspE4.C2*) were used to categorize *E. coli* isolates into four phylogenetic groupings (A, B1, B2, and D) [10].
8. While phylogroups A and B1 are closely connected to commensal or enteropathogenic *E.coli* associated with animal infection, the majority of virulent human extraintestinal strains are made up of B2 and, to a lesser degree, D phylogroups [11].
9. There is little information available regarding the relationships between harmful *Escherichia coli* strains that are Multi Drug Resistant (MDR). Finding the phylogenetic groups for pet strains of *E.coli* was also necessary to validate the connection between these species. So, this study aimed to examine the phylogenetic groups and AMR genes in *E.coli* species isolated from pets to investigate potential connections between pathogenic MDR strains**.**

10. MATERIALS AND METHODS

11. 2.1. Sampling:

12. Throughout the months of June through July and September of 2023, a total no. of 100 clinical samples were collected from the El-Menoufia governorate. Nasal, urine, and stool samples were obtained from 100 clinical samples from companion animals (40 dogs, 50 cats, and 10 Egyptian nisnas), In order to isolate *E. coli* bacteria, the samples were sent in a sterile plastic bag that had preserved in an ice box to Microbiology lab, Faculty of Veterinary Medicine's, Shebin El-Kom University /Egypt. Transport media (peptone water) (Himedia, India) (Oxoid, UK) were used to incubate them for 12 to 18 hours at 37 °C [12].

13. 2.2. Bacteriological and biochemical identification of *E. coli* isolates:

14. A loopful of infected peptone broth was streaked individually on blood agar plates (Himedia, India), Macconkey's agar medium, and (Oxoid, UK) provided the Eosin Methylene Blue (EMB) agar medium, which was incubated for 24 to 48 hours at 37 °C. [13]. Every probable isolate that was seen under a light microscope had Gram's stain applied in order to identify the morphological characteristics that made *E. coli* appear red and rod-shaped. By employing conventional biochemical techniques, the likely *E. coli* isolates were identified, including the urea hydrolysis test, citrate utilization test, oxidase test, catalase test, indole test

and H2S production test (Himedia, India).

15. **2.3. Serological Identification of *E. coli* isolates**:

16. In order to determine Enteropathogenic types, Quick diagnostic *E. coli* antisera sets (DENKA SEIKEN Co., Japan) were used to serologically identify the isolates [14].

17. **2.4. Antimicrobial Susceptibility Test:**

18. Examination of Microbiological Susceptibility All confirmed *E. coli* isolates were screened using the Kirby-Bauer disk diffusion method, and the data were interpreted according to the guidelines set forth by the CLSI. [15]. The antibiotics that were employed were ampicillin\sulbactam (SAM,30), gentamycin (GEN,10) µg, ciprofloxacin (Cip, 10 µg), erythromycin (E15 µg), doxycycline (Do,30 µg), ceftriaxone (CRO, 30 µg), trimethoprim\ sulphamethaxazole (SXT, 25 µg), amoxicillin\clavulanate (AMC,20\10 µg) (Oxoid, Biogram) as antibiotics.

19. Briefly, 5 mL of regular saline solution was mixed with 100–200 µL of the bacterial overnight broth, which had been adjusted to meet the 0.5 McFarland standard (0.5 x10^8 cfu/mL). Then, using a sterile glass spreader, 100 µL was applied to Mueller-Hinton agar plates (Himedia, India). The plates were then impregnated with the previously indicated antimicrobial discs and incubated aerobically for 24 hours at 37 °C. Following that, the zone of inhibition diameters was measured and interpreted using the standards suggested by the CLSI. [15].

20. **2.5. Molecular identification:**

21. **2.5.1. DNA Extraction:**

22. The boiling procedure, as outlined by Jackson. [16], was used to extract bacterial DNA. In short, presumptive isolates were resuscitated and extracted from the broth cultures. An aliquot of 1000µL of cell suspension containing 10^7 cells/mL from each of *Escherichia coli* was transferred to microtubes and incubated. Cell suspensions were centrifuged at 4,500 rpm for 5 min at 4°C, and the pellets obtained were used for DNA extraction by boiling method with a modification. The collected material was placed into a tube containing 50 µL nuclease-free water, then subjected to boiling at 100^{o}C for five minutes. The mixture was centrifuged at 3000g for 10 minutes. The DNA-containing upper aqueous phase was transferred into a separate 2 ml Eppendorf tube and 0.7 volumes of cold absolute ethanol was added. The aqueous phase was recovered by centrifugation for 20

min, and genomic DNA was precipitated by ethanol. The pellet was washed in cold 70% ethanol then after a further centrifugation step the ethanol was removed, and the nucleic acid pellet was allowed to dry before being resuspended in aqueous TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0). The process requires three times centrifugation to collect the cells, to eliminate the cell debris after the boiling procedure to pellet the total precipitated DNA [17], and 1 milliliter of isolates on nutritional broth was created. The DNA templates were kept for subsequent molecular analysis at -20°C

23. **2.5.2. Presumptive Isolate Molecular Identification**

24. Polymerase chain reaction (PCR) methods were used to confirm the presumed isolates, and agarose gel electrophoresis (AGE) was used to evaluate the PCR results [18]. Table 1 lists the oligonucleotide primers that were employed as well as the cycling conditions.

25. **2.5.2.1. Molecular identification of *E. coli* isolates**:

26. *16SrRNA* primer were used for detetion of *16Sr*RNA gene to confirm the isolated *E. coli* strains.

27. **2.5.2.2. Phylogrouping of the *E. coli* isolates:**

28. The Clermont method was used to phylogroup various *E. coli* strains according to the presence or lack of three particular genes (*chu*A, *yja*A, and *tspE4.C2*) in order to ascertain the relationships between *E. coli* pathotypes, phylogroups, and antibiotic resistance (Table 1). According to Clermont. [10], Using the phylogenetic grouping approach, *E. coli* strains can be divided into four phylogroups: A (*chu*A-/*TspE4.C2*-), B1 (*chu*A-/*TspE4.C2* +), B2 (*chu*A + /*yja*A +), and D (*chu*A + /*yja*A-).

29. **2.5.2.3. Molecular identification of ESBL *E. coli* isolates:**

30. Despite their phonotypical resistance, the antibiotic resistance genes of every isolated strain of *E. coli* were described at the molecular level. According to Fang. [19], Using the multiplex PCR assay was optimized to detect all target genes in a single reaction, ESBLs encoding the genes *bla*TEM, *bla*SHV, *bla*CTXM, and *bla*OXA were found.

Table (1): Primer sequences for oligonucleotides Source: Germany's Metabion.

Bacteria	Gene	Sequence 5`------3`	Amplified product	Cycling conditions of the different primers during cPCR	Reference
E. coli	*16sr*RNA	F:CCC CCT GGA CGA AGA CTG AC	401 bp	1 cycle (95 °C, 8 min) 30 cycles (95 °C, 30 s/58 °C, 30 s/72 °C, 30 s) 1 cycle (72 °C, 7 min	Wang *et al*., 2002
		R:ACC GCT GGC AAC AAA GGA TA			
Antibiotic resistance gene (ESBLs)	***blaSHV***	F:CTT TAT CGG CCC TCA CTC AA	237 bp	1 cycle (95 °C, 5 min) 30 cycle (94 °C, 30 s/62 °C, 90 s/ 72 °C, 1 min) 1 cycle (72 °C, 10 min	Fang *et al*., 2004
		R:AGG TGC TCA TCA TGG GAA AG			
	blaTEM	F:CGC CGC ATA CAC TAT TCT CAG AAT GA	445 bp		Monstein *et al.* 2007
		R:ACG CTC ACC GGC TCC AGA TTT AT			
	***bla*CTXM**	F:TATCAGAGGTAGTTGGCGTCAT	593 bp		Boyd *et al.* 2004
		R:GTTCCATAGCGTTAAGGTTTCAT T			
	***bla*OXA**	F:ACA CAA TAC ATA TCA ACT TCG C	813bp		Ouellette et al., 1987
		R:AGT GTG TTT AGA ATG GTG ATC			
Phylogroup encoding genes	***ChuA***	F:GAC GAA CCA ACG GTC AGG AT	279 bp	1 cycle (94 °C, 5 min) 30 cycle (94 °C, 30 s/55 °C, 30 s/72 °C, 30 s) 1 cycle (72 °C, 7 min)	Clermont *et al*., 2000
		R:TGC CGC CAG TAC CAA AGA CA			
	YjaA	F:TGA AGT GTC AGG AGA CGC TG	211 bp		*Clermont et al.* 2000
		R:ATG GAG AAT GCG TTC CTC AAC			
	*Tsp**E4.C2***	F:GAG TAA TGT CGG GGC ATT CA	152 bp		Boyd *et al.*2004
		R:CGC GCC AAC AAA GTA TTA CG			

31. 3.RESULTS:

32. 3.1. Incidence of *E. coli*:

33. Bacteriological analysis of 100 pet animal (urine, nasal ,fecal) samples from the El-Menoufia governorate (dogs (n = 40), cats (n = 50), and Egyptian nisnas (n = 10)) revealed isolation of *E-coli* strains. *E-coli* isolates are Gram-negative and has a round, red rod-shaped bacillus that grows on EMB agar media with a characteristic metallic

green sheen and on the MacConkey agar plates They are pink to dark pink, dry, donut-shaped, and have a dark pink area where bile salts have precipitated. With biochemical findings, indole, MR. and citrate test are positive, but VP, urea test and H2S are negative.

Table 2 and Figure 1 showing that the incidence of *E. coli* isolates were (6/15) 40%. (6/20)30 %, cat, and (1/4) 25% in urine samples of dogs, cats, and Egyptian nisnas, respectively. In stool samples (9/15) 60, (10/20) 25%, and (1/4) 25% of dogs, cats, and Egyptian nisnas, respectively. In nasal samples (4/10) 40%, (5/10) 50%, and (0/2) 0% of dogs, cats, and Egyptian nisnas, respectively.

Table (2): the prevalence of *E. coli* isolation by sample type:

Species \Samples	Types	Total no.	No. of positive samples	Incidence %
Dogs	Urine	15	6	40
	Stool	15	9	60
	Nasal	10	4	40
	Total	40	19	47.5
Cats	Urine	20	6	30
	Stool	20	10	50
	Nasal	10	5	50
	Total	50	21	42
Egyption nisnas	Urine	4	1	25
	Stool	4	1	25
	Nasal	2	-	0
	Total	10	2	20

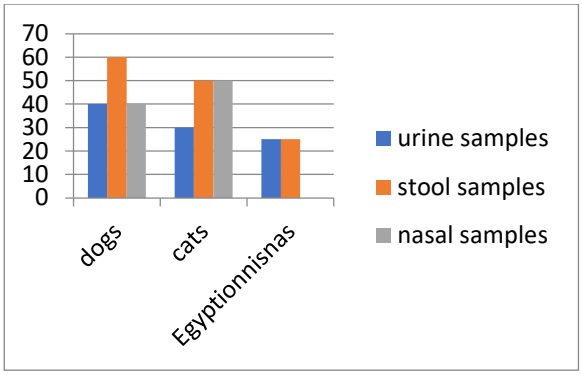

Fig. (1): the prevalence of *E. coli* isolation by sample type

3.2. Results of *E. coli* Isolate Serotyping:

E. coli isolates from Egyptian nisnas (O119:K69 (EPEC/EHEC), auto agglutination) (urine, stool) were identified serologically (Table, 3). Dog *E. coli* isolates were identified by serology (O44:K74 (EPEC/EAggEC), O119:K69 (EPEC/EHEC), O164: K- (EIEC), O25:K11 (EPEC/ETEC), unidentified sample) (stool, nasal, urine, stool, stool), respectively. But isolates from cats were (O44:K74 (EPEC/EAggEC), O44:K74 (EPEC/EAggEC), O119:K69 (EPEC/EHEC), O25:K11 (EPEC/ETEC), O125:K70 (EPEC/EHEC) (stool, nasal, urine, stool, stool), respectively.

Table (3): Serotyping of *E.coli* isolated from dogs, cats and Egyptian nisnas as pets (n=12):

Animal	Numbe	Type	Polyvalent	Monovalen

	r	of sample		t
Egyptian nisnas	2	Urine Stool	II auto agglutination	O119:K69 auto agglutination
Dogs	5	Stool Nasal Urine Stool Stool	I II III III unidentified	O44:K74 O119:K69 O164:K- O25:K11 Unidentified
Cats	5	Stool nasal Urine Stool Stool	I I II III I	O44:K74 O44:K74 O119:K69 O25:K11 O125:K70
Total	12	12	10	10

3.3. Antimicrobial susceptibility patterns of *E. coli:*

The strains were highly susceptible to ciprofloxacin (73.8%), completely resistant to amoxicillin/calvulante (100%) and followed by ampicillin/sulpactam, trimethoprim/sulphamethaxazole, erythromycin, doxycycline, and ceftriaxone (97.6%, 95.3%, 88.1%, 85.7%, and 80.9%), according to the results of the antimicrobial susceptibility patterns of *E. coli* is displayed in fig. (2) and table (4).

Table (4): Patterns of antibiotic resistance in *E. coli* species isolated from household pets (dogs, cats, and Egyptian nisnas) (n= 42)

Antimicrobial Class	Antimicrobial Agents \conc.	R	%	Int.	%	S	%
Quinolones	Ciprofloxacin (CIP 10)	4	9.5	7	16.7	31	73.8
Tetracycline	Doxycycline (DO 30)	36	85.7	6	14.3	0	0
Cephalosporins	Ceftriaxon (CRO 30)	34	80.9	1	2.4	7	16.7
Macrolides	Erythromycin e (E 15)	37	88.1	5	11.9	0	0
Sulfonamides	Trimethoprim \sulphamethaxazole (SXT 25)	40	95.3	0	0	2	2.7
Aminoglycoside	Gentamicin (GEN 10)	16	38.1	26	61.9	0	0
B-lactams Penicillins	Amoxicillin\ Calvulante (AMC 20\10)	42	100	0	0	0	0
	Ampicillin\ Sulpactam (SAM 30)	41	97.6	1	2.4	0	0

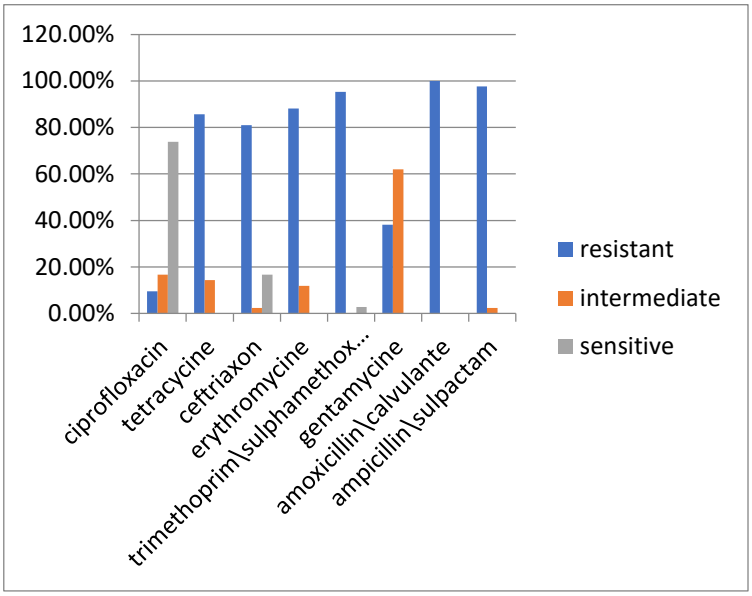

Fig. (2): Patterns of antibiotic resistance in *E. coli* species isolated from household pets (dogs, cats, and Egyptian nisnas) (n= 42)

3.4. Result of *16Sr*RNAgene detection in *E-coli* isolates by PCR:

Using the 16S rRNA primer, ten *E. coli* isolates were isolated from different samples of dogs (n = 4),

cats (n = 4), and Egyptian nisnas (n = 2). All isolates have the *16S r*RNA gene at 401 bp , as indicated in Fig(10).

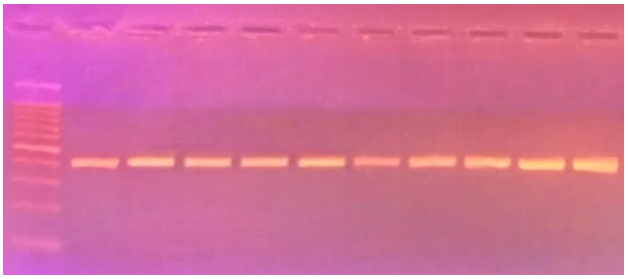

Fig 10: PCR for detecting *16srRNA* gene in *E. coli* strains.

Lane M: 100-1000 bp DNA marker; Lanes 1-10: Positive strains for *16srRNA* gene at 401 bp.

3.5. Multiplex PCR for detecting *bla*SHV, *bla*TEM, *bla*CTXM and *bla*OXA genes in *E. coli* strains.

E. coli isolates can be tested using multiplex PCR to find the beta lactam resistance genes (*bla*SHV, *bla*TEM, *bla*CTXM, and *bla*OXA). Every isolate has the gene *bla*TEM, while isolate number seven has the genes *bla*SHV and *bla*TEM, and isolates 1, 3, 4, and 6 have the genes *bla*TEM and *bla*CTXM, as indicated in table (5) and Fig(11).

Table (5): Multiplex PCR results for B lactam resistance genes

No. of isolate	Type and origin of sample	***bla*SHV gene**	***bla*TEM *gene***	***bla*CTXM *gene***	***bla*OXA *gene***
1	Urine (cat)	-	+	+	-
2	Urine (nisnas)	-	+	-	-
3	Nasal (dog)	-	+	+	-
4	Nasal (cat)	-	+	+	-
5	Stool (dog)	-	+	-	-
6	Urine (dog)	-	+	+	-
7	Stool (cat)	+	+	-	-
8	Stool (nisnas)	-	+	-	-
9	Stool (cat)	-	+	-	-
10	Stool (dog)	-	+	-	-

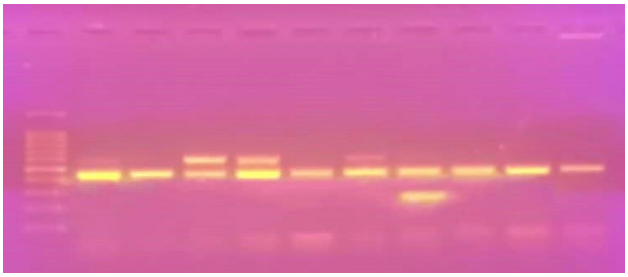

Fig 11: Multiplex PCR for detecting *bla*SHV, *bla*TEM , *bla*CTXM and *bla*OXA genes in *E. coli* strains.

Lane M: 100-1000 bp DNA marker.

Lanes 2, 5, and 8, 9 ,10: Positive strains for *bla*TEM gene at 445bp.

Lanes 1, 3,4, and 6: Positive strains for *bla*TEM and *bla*CTXM *g*enes at 445 and 593bp, respectively.

Lane 7: Positive strains for *bla*SHV and *bla*TEM genes at 237and 445 bp, respectively.

3.6. Phylogenetic grouping of *E. coli*

By using PCR, phylogenetic genes (*chuA*, *yjaA*, and *tspE4.C2*) were found. All isolates have (*chuA, yjaA, TspE4.C2) gene*s except isolates number (1,9) don't have (*tspE4.C2*) gene and all isolates from (B2) phylogenetic group as shown in table (6) and fig(12).

Table (6): PCR-based Phylogentic grouping of *E. coli*

No. of isolate	Type & origin of samples	***chuA Gene***	***YjaA Gene***	***tspE4.C2 gene***	**Phylogroup**	**Pathogenesis**
1	Urine(cat)	+	+	-	B2	EHEC/EPEC
2	Urine(nisnas)	+	+	+	B2	EPEC
3	Nasal (dog)	+	+	+	B2	EHEC/EPEC
4	Nasal (cat)	+	+	+	B2	EAggEC/EPEC
5	Stool (dog)	+	+	+	B2	EaggEC/EPEC
6	Urine(dog)	+	+	+	B2	EIEC
7	Stool (cat)	+	+	+	B2	EaggEC/EPEC
8	Stool(nisnas)	+	+	+	B2	auto agglutination
9	Stool (cat)	+	+	-	B2	EHEC/EPEC
10	Stool (dog)	+	+	+	B2	ETEC/EPEC

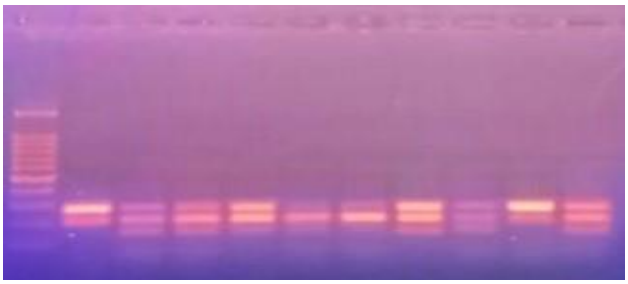

Fig 12: Triplex PCR for detecting *chuA, yjaA* and *TspE4*.C2 genes in *E. coli* isolates.

Lane M: 100-1000 bp DNA marker.

Lanes 1 and 9: Positive strains for *yja*A and *chu*A genes at 211 and 279 bp, respectively.

Lanes 2,3,4,5,6,7,8 and 10: Positive strains for *TspE*4.C2, *yjaA* and *chuA* genes at 152, 211 and 279 bp, respectively.

DISCUSSION

One of the most important pathogens and the most prevalent commensal habitant of the gastrointestinal tracts of warm-blooded animals is *E. coli*. It is a member of the Enterobacteriaceae family of bacteria. *Escherichia coli* continues to be one of the most frequent causes of several common bacterial infections in pets. *E. coli* is the most frequent cause of newborn meningitis, septicemia, enteritis, urinary tract infections, and other clinical illnesses.Diarrhea in pets is also frequently linked to *E. coli* [20]. Pathogens known as Enteropathogenic *Escherichia coli* (EPEC) are linked to gastrointestinal disorders. Antimicrobial resistance may hinder required therapies, and EPEC can be present in dogs and cats [21].

The current study's bacteriological analysis of 100 pet animal samples (40 dogs, 50 cats, and 10 Egyptian Nisnas) collected from the El-Menoufia governorate showing that *E. coli* were isolated with an incidence of 19 (47.5%) in dogs, 21(42%) in cats, and 2(20%) in Egyptian Nisnas. These findings were consistent with kukanih.et al.[22], who indicated that *E. coli* isolated in pets had an incidence of 43% in dogs and cats.

Using the 16SrRNA primer in this study, ten *E. coli* isolates were examined by PCR. All isolates had the *16SrRNA* gene at 401 pb. This outcome was consistent with Handl et al. [23], who found that *16S r*RNA gene was present in most *E. coli* strains.

A varied set of bacteria commonly linked to gastrointestinal diseases are known as diarrheagenic *Escherichia coli* (DEC) strains. According to their virulence factors, some strains of *E. coli* can be categorized as pathotypes, including Shiga toxin-producing *E. coli* (STEC), enteropathogenic *E. coli* (EPEC), enterotoxigenic *E. coli* (ETEC), enteroaggregative *E. coli* (EAEC), enteroinvasive *E. coli* (EIEC), and enterohaemorrhagic *E. coli* (EHEC) [24]. Prior to the discovery of specific virulence components in dangerous strains, *E. coli* was mostly classified by the serologic identification of the O (lipopolysaccharide, LPS) and H (flagellar) antigens [20]. O44:K74 (EPEC/EAggEC), O119:K69 (EPEC/EHEC), O25:K11 (EPEC/ETEC), and O164: K- (EIEC) were the serologically identified dog isolates of *E. coli* in this study. This result was consistent with Ali et al. [25] who said that the serotype O164:K-(EIEC) of *E. coli* was also recovered from dogs.

The cat’s *E. coli* strains which detected in this study were identified serologically was O44:K74 (EPEC/EAggEC), O119:K69 (EPEC/EHEC), O25:K11 (EPEC/ETEC), and O125:K70 (EPEC/EHEC). This results agreeded with Krause et al. [26] who stated that pets and domestic animals are a substantial natural reservoir of AEEC strains, some of which are known to be human pathogens (O145:[H28],O177:[H11], O26:[H11],O128:H2, O103:H2).

To the best of our knowledge, the serological identification of *E. coli* from Egyptian nisnas, which was (O119:K69) (EPEC/EHEC), may have been the first documented in Egypt.

The pathogenic types of *E. coli* strains are determined by the host's clinical signs and the kind of virulence factor that is present. A collection of strains from the same species that produce a common disease is called

a pathotype. Three pathotypes are extra intestinal (ExPEC), and there are at least seven primary pathotypes of enteric *E. coli*. When contaminated food or water is ingested, intestinal diseases spread through the fecal-oral pathway. The majority of EPEC strains cause diarrhea in children and animals, particularly in cases when cleanliness is poor. Hemorrhagic colitis, or HUS, is frequently caused by the food-borne bacteria EHEC. Because they produce Shiga-like toxins (also called Shiga toxin producing *E. coli*, or STEC) that are similar to those produced by Shigella dysenteriae, typical EHEC strains are the most virulent diarrhoeagenic *E. coli* that have been identified to date [20,27].

According to the current investigation, *E. coli*'s antimicrobial susceptibility patterns were 100% resistant to amoxicillin and calvulanate, extremely sensitive to ciprofloxacin (73.8%), and very resistant to trimethoprim and sulphamethaxazole (95.3%).These findings concurred with Rybarikova et al. [28], who found that ampicillin, ciprofloxacin, and amoxicillin/clavulanic acid had lesser resistance at 35%, 2.0%, and 1.0%, respectively, and higher resistance to sulfamethoxazole/trimethoprim and nalidixic acid at 81% and 50%. Almeida et al. [29] who found reduced resistance to gentamicin and ciprofloxacin, disagreed with the same findings. Furthermore, Sobur et al. [30] found reduced gentamicin and ciprofloxacin resistance at 13.2% and 16.98%, respectively. He also showed how extended use of antibiotics contributes to the development of multidrug-resistant strains, hence monitoring antibiotic use is essential to lowering the risk of multidrug resistance.

In present study, the antimicrobial susceptibility patterns of *E. coli* in Egyption nisnas were 100% susceptible to ciprofloxacin and totally resistant to amoxicillin and calvulante. The resistant patterns of bacteria have been varied by geographical location and by time so periodically testing of antibiotic resistant is really important. *E. coli* strains are the leading causes of serious bacterial infections in health society .Mobile genetic elements including transposons, plasmids and integrons contribute to lateral transfer of resistance genes in bacteria. *E. coli* can be intrinsically resistant to some special antibiotics and have gens which are responsible for resistance to some of antibiotics such as aminoglycosides, flouroquinolones and β-lactamas[31 –32].

Additionally, it was noted that dogs and cats may host multidrug-resistant (MDR) bacteria, and that MDR and Extended-spectrum beta-lactamases (ESBLs) are produced by *E. coli*. may spread zoonotically. Similarly, people can affected by their pets resistant bacteria [33],[34].

Numerous studies review and assess *E. coli* and other Enterobacteriaceae that produce ESBL/AmpC, with an emphasis on the molecular epidemiological and phylogenetic data now accessible for the chromosomal background and the acquired episomal β-lactamase types [35].

The PCR used in this research to determine the beta lactamases resistance genes (*bla*SHV, *bla*TEM, *bla*CTXM, and *bla*OXA) in isolates of *E. coli* showed that every isolate had the gene *blaTEM*, isolate number seven had the genes *bla*SHV and *bla*TEM, and isolates 1, 3, 4, and 6 had the genes

*bla*TEM and *bla*CTXM, as indicated in table (5). These findings concurred with Ewers et al. [35] who found that the most significant intermediary of resistance to a variety of β-lactam antibiotics in *E. coli* is the synthesis of β-lactamase. The most common producers of β-lactamases are encoded on plasmids in *E. coli*. Gram-negative bacteria are increasingly developing multidrug resistance due to β-lactamases, which also give resistance to cephalosporins and penicillins.

There are numerous β-lactamase types that have been identified. Moreover, Awosile et al. [36] found that the *bla*SHV gene was present in a low percentage (1.1%), whereas the percentages of *bla*TEM, *bla*CMYII, and *bla*CTXM were 84.1%, 52.2%, and 30.7%, respectively.

Moreover, in the present study multiplex PCR for identifying beta lactamase resistance genes (*bla*SHV, *bla*TEM, *bla*CTXM, and *bla*OXA) in Egyption nisnas isolates of *E. coli* showed that every isolate possessed the *bla*TEM gene.

BlaTEM-1 genes were found in ampicillin and/or amoxicillin resistant *E. coli* [37]. The *E. coli* isolates may be grouped into four phylogenetic groups (A, B1, B2, and D) according to the results of amplification for the C2 non-coding region and the *chuA*, *yjaA*, and *tspE4* genes [38]. *E. coli* strains were classified into phylogroups A, B1, B2, C, D, E, and F according to the Clermont procedure [39], which is based on the presence or lack of the genes *arpA*, *chuA*, *yjaA*, *trpA*, and *TspE4* [21].

Conditional extraintestinal pathogens B2 and D are members of a potentially pathogenic group that have virulence-related genes, while A and B1 groups are frequently identified in symbiotic groups [40].

Phylogenetic genes (*chuA*, *yjaA*, and *tspE4.C2*) were studied in the current investigation using multiplex PCR. It was shown that all isolates had these genes, with the exception of isolates 1 and 9, which lacked the *tspE4.C2* gene. Table (5) lists every isolate from the (B2) phylogenetic group. Using PCR, phylogenetic genes (*chuA*, *yjaA*, and *tspE4.C2*) may be found in Egyptian Nisnas isolates.

In the present study, the detected isolates were belonged to B2 which is the more virulent strains. This results in agreement with et al. litster et al.[41] who reported that the more virulent strains of ExPEC recovered from UTIs usually belong to the B2 and D phylogenetic groups of *E. coli* and *E. coli* phylogenetic group B2 was the most prevalent strains [42]. One potential zoonotic agent that could cause UTIs in humans is the *E. coli* phylogroup B2 [43]. Numerous studies have consistently indicated that the B2 phylogroup is substantially related with UTI in people [44] and [45]. Lazarus et al. [46] stated that the strains' molecular phylotyping revealed the existence of the B2 phylogroup, which accounts for a percentage of extraintestinal infections in humans. Conversely, Staji et al. [47] found that phylogroup B1 accounted for the majority of *E. coli* strains recovered from pets.

There is an anthropozoonotic relationship, which supports the theory that B2 UPEC strains are more commonly derived from "animals that live with humans." Phylogroup B2 has been shown to include extraintestinal virulent strains (extraintestinal pathogenic *E. coli* [ExPEC]), which express numerous virulence factors, However, more recently, an increase in B2 phylogroup strains was observed in human [43].

1. **CONCLUSIONS**
2. MDR *E. coli* strains are becoming a significant problem that spreads widely among pets and reduces the effectiveness of therapeutic medications. The main cause of diarrhea in pets may be pathogenic strains of *Escherichia coli*. Resistance to β-lactam antibiotics can be transmitted through phenotypic antimicrobial resistance, especially in isolates that are resistant to extended-spectrum cephalosporin (ESC. one or more resistance genes in combination. Controlling the use of antibiotics in pets and maintaining good cleanliness helps stop multidrug resistance in animals from spreading.so the extensive using of antibiotics in treatment of pet animals should be controlled.
3. In the end, it helps to stop the using of ineffective antibiotics by facilitating the creation of biosecurity protocols and antimicrobial usage standards. Future research should broaden the sampling locations and incorporate additional samples. Furthermore, deeper insights on MDR *E. coli* bacteria from diarrheal pets may be obtained by the use of whole genome or gene sequencing.

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Authors' declarations

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A.R.S: Data collection, Formal Analysis, Project administration, Resources, Writing – review and editing.

M.M.Z: Conceptualization, Data curation, Formal Analysis, Resources, Supervision, , Writing – review and editing.

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