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# Emergence of Carbapenem Resistant *Escherichia coli* Isolated from Human and Companion Animals Associated with Urinary Tract Infection

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**Abstract** | One of the most substantial emerging concerns facing the One Health concept is the growing role of animals in social contact with human as pets in the global increasing of resistance to antibiotics used in human infections therapy as carbapenems which is considered one of the human use last resorts. Extraintestinal *Escherichia coli* constitute the main etiologic factor implemented in urinary tract infections (UTIs) in humans and animals. The hypothesized risky role of companion animals in the load and dissemination of multidrug resistant uropathogenic *E. coli* (UPEC) which causes community-acquired UTI in pets and human community has been monitored. The current study aimed to investigate the prevalence of shared uropathogenic *E. coli* serotypes isolated from companion animals and human as well as assessed their virulence determinants which implemented in pathogenesis and severity of infection via phenotypic and molecular methods. Besides that, the study determined their multidrug resistance patterns, particularly the existence of the gene responsible for carbapenem resistance. A cross-sectional study performed on one hundred and ninety two urine samples owned to urinary infected humans, dogs and cats were obtained from Egyptian laboratories in Great Cairo governorates. The samples were cultured onto the suitable media; the presumptive *E. coli* colonies were identified phenotypically, biochemically via Vitek2® and serotyping. The virulence of *E. coli* isolates were characterized phenotypically via hemolysis, Congo red binding, Vero cell cytotoxicity. The antibiotics resistance pattern was assessed by disk diffusion test. Also, the *E. coli* isolates were molecularly confirmed and screened for presence of 3 virulence genes (*fim*H, *iss* and *iut*A) as well as *bla*NDM-1gene which responsible for carbapenem resistance. The data were statistically analyzed using Chi-square ($\chi 2$) via (SPSS) version 20.0 software. Our results revealed sixty three *E. coli* isolates were obtained (32.81%); dogs (34), cats (8) and humans (21). The highest prevalent serotypes were O25; 13 (20.63%) followed by O78; 10 (15.87%) with 13 untypable isolates (20.63%). The *E. coli* isolates showed positivity for hemolysis production (68.25%), Congo red binding (73.01%), Vero cell cytotoxicity (41.27%). The phenotypic antibiotic resistance pattern showed presence of average unusual high imipenem resistance (30.33%), and clindamycin (84.3%). On the other side, gene identification via polymerase chain reaction revealed existence of species specific '*yai*O' (100%), virulence; '*fim*H' (71.43%), '*iut*A' (26.98 %) and '*iss*' (20.63%). The harboring of *bla*NDM-1gene was presented among 15 *E. coli* isolates as (23.81%); dog (11), human (3) and one in cat. The obtained data of our study asserted the potential role of companion animals in transmission of zoonotic *E. coli* serotypes especially those of exposing resistance to several antibiotic groups. The significant existence of *bla*NDM-1gene among *E. coli* isolates which responsible for resistance against carbapenems as a last antibiotic resort may constitute a public health concern and highlights the need of more regulation and monitoring of antimicrobial use in pet clinics.

**Keywords**: Carbapeneme, Egypt, Dog, Cat, Patient, UPEC, Virulence

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## INTRODUCTION

Urinary tract infections (UTIs) are considered one of substantial causes of morbidity in dogs and cats. The main illnesses represented by ascending / upper UTI (pyelonephritis), recurrent and cystitis with association of varying conditions and clinical signs (Weese *et al*., 2019). The most significant one is the recurrent type which could be not only hard to treat but also high cost for pet owners as well as clinicians because of diverse rounds of therapy (Amphaiphan *et al*., 2021).

*Escherichia coli* is assumed as a global threat because of the lowering options for antimicrobial therapy. Companion animals as dog and cat could be a reservoir of multidrug *E. coli*, and the numbers of pets and households owning pets are considerably increased (Teng *et al*., 2023).

Among the numerous causative agents of UTI; extraintestinal pathogenic *E. coli* (ExPEC) are the most strains frequent in pets and humans as they are isolated in nearly half of total urine cultures from dogs and cats (Aurich *et al*., 2022). They constitute pathotypes frequently characterized by the existence of specific virulence determinants such as adhesins, toxins, and lipopolysaccharide that increase pathogenicity outside the intestinal tract (Johnson and Russo, 2002). Furthermore, *E. coli* is the most prevalent pathogen in other extra-intestinal infections such as pyometra and assumed to be a risk factor for development of resistant urogenital infection in women (Flament-Simon *et al*., 2020).

The elevation in antimicrobial resistance induced by the dissemination of multidrug-resistant (MDR) bacteria, such as cephalosporinase (AmpC), extended-spectrum beta-lactamase (ESBL), and carbapenemase-producing *E. coli*, is an ongoing global concern responsible for thousands of deaths each year (Ruiz, 2021). These *E. coli* strains are leading to treatment failure due to their capacity to hydrolyze carbapenems as well as third- and fourth-generation cephalosporins which have been assumed critically substantial antimicrobials to human and veterinary medicine (WHO, 2019).

The One Health concept, as purposed by Zinnstag *et al*. (2012), may aid to fulfill progresses to maintain health globally. This approach would target to initiate an extra value to what human, animal, and environmental health working alone can accomplish. Today, companion animal caretakers address physical, social, and psychological health benefits from incorporating companion animals into their lives. Human-pets bonds are growing as they live together, share same spaces and have a mutual effect on each other's health as well as the surrounding environment (Kamal *et al*., 2023).

However, the role of pets, especially dogs and cats, is often under respected in One Health communications. In near decades, dogs and cats more often spend their life indoors in contact with their owners. For the human, there may be an increasing risk of the transfer of zoonotic diseases due to habit trends such as sleeping with pets, permitting pets to lick the face or wounds, bite accidents, the importation of rescue dogs, and soil contact (Overgaauw *et al*., 2020). There are a number of zoonotic infectious illnesses, in addition to resistant bacteria, that may be transferred directly or indirectly from companion animals, raising public-health concerns (Baede *et al*., 2017).

The emerging antimicrobial resistance is prevalent among *E. coli* isolated from the urine of dogs and cats associated with UTI, which is not only further frustrating therapy but also constitute one health concern; the risky development of zoonotic and community acquired UTI when pet owners are in contact (Damborg *et al*., 2018). Because of the emerging expansion of extensively drug-resistant (XDR) and pan drug-resistant (PDR) Gram-negative bacteria, which are resistant to last-resort drug groups as carbapenems constitute a grave burden in human medicine (Endimiani *et al*., 2020).

Mobilizable carbapenemase-encoding determinants such as $bla_{NDM}$ (New Dehli metallo-β-lactamase) in *E. coli* have been recognized and increasingly reported globally among veterinary sources including pets (Alba *et al*., 2021). Interestingly, pets and people in contact having similar ESBL-producing *E. coli* strains uphold substantiation of inter-

species dissemination of resistant bacteria between humans and animals (Hong *et al*., 2019).

The target of the current study was to investigate the prevalence and serotyping of antibiotic-resistant *Escherichia coli* among UT infected pets and humans. Also, the study aimed to screen the presence of gene responsible for carbapenem resistance in addition to some virulence determinants.

## MATERIALS AND METHODS

### Ethical Approval

The study was performed according to National Research Centre Ethics Committee, Egypt guidelines and approved under the ethical number 13050415-1.

**Study design:** The present cross-sectional study was performed during October 2022 to September 2023. The study was designed based on the answer trials of the following questions;

- What is the incidence percentage of *E. coli* isolation from urine samples among the examined species?
- How the obtained *E. coli* isolates behave versus the tested antibiotics?
- Are the obtained *E. coli* isolates carry virulence and carbapenem resistance genes?
- Whether multidrug-resistant *E. coli* in companion animals poses a risk to human health?

**Setting:** the study was carried out on laboratory samples collected from different human and pet laboratories in Great Cairo governorates, Egypt.

**Participants:** laboratory urine samples of human, dog, and cat. The case history of cases revealed suffering from different urinary infection signs and not receiving antimicrobial therapy.

**Variables:** prevalence of carbapenems resistant *E. coli*, existence of 3 virulence genes were considered as the independent variables while multidrug resistance rate was a dependent variable.

**Limitations:** the samples were restricted to;

- The geographical area: laboratories located in the 3 governorates constitute Great Cairo region.
- The targeted species; human, dogs, and cats.
- The targeted disease; urinary tract infections.
- The cases have not received antibiotic therapy.

**Data source/measurements:** The data were obtained by analyzing urine samples belonged to human, dog, and cat. The prevalence of UPE, antimicrobial resistance and existence of carbapenems resistance gene as well as other 3 virulence genes was determined by conducting culture, biochemical, serological identification, antibiotics sensitivity testing and PCR assays on the isolates.

**Sample size:** by screening of the cases presented in the laboratories located within the three studied governorates (Cairo, Giza, and Qalyubia) during the study period, the urine samples obtained from urinary tract infected cases were chosen, and their total number was 192 samples, distributed as follows: (dog ≈ 81, cat≈ 38 and human ≈73).

***E. coli* Isolation and Identification:** The gathered urine samples were cultured onto MacConkey agar, and aerobically incubated overnight at 37°C. The pink lactose-fermenting colonies were picked up and sub-cultured on Eosin methylene Blue (EMB) agar to gain pure green metallic sheen colored colonies. Relied on the morpho-biochemical features, the selected pure colonies were initially identified (Quinn *et al*., 2002). The complete identification was achieved via Vitek2® compact system (Biomérieux, Marcy l'Etoile, France).

**Serotyping identification:** The determination of O and K antigens was performed using the method formerly described by (Guinée *et al*., 1981) with available O and K antisera (Polyspecific test reagents Anti-Coli, Sifin diagnostics gmbh, Germany). *E. coli* isolates that did not react with any antisera were categorized as non-typeable.

### Virulence Assays

**Hemolysis assay:** *E. coli* isolates were propagated on blood agar base supplemented with 5% washed sheep erythrocytes. The plates were incubated at 37°C for 24 hours and hemolytic activity of the isolates was recorded.

**Congo red (CR) binding test:** *E. coli* isolates were tested for their growth status on Congo red medium. The reaction was best seen after 18, 24, 48 and 72 hours of incubation at 37°C and then left at room temperature for an additional 2 days (not to exceed 4 days). Orange colonies were considered positive and different intensities in the dye uptake were expressed as +, ++ and +++ (Berkhoff and Vinal, 1986).

**Vero cell cytotoxicity activity:** The test was carried out in 96 well tissue culture plates comprising 24 hours monolayer sheet of Vero cells. Fifty microliters of each *E. coli* culture extracts were added to duplicate wells, sealed with plastic film, and incubated at 37°C in 5% $CO_2$ incubator. After 24 hours, the plates were examined under inverted microscope after fixation with 10% formalin and crystal violet stained (Giugliano *et al*., 1982).

**Table 1:** Primers sequences, temperatures and cycling conditions used for amplification of the tested genes.

| Gene | Sequence (5′-3′) | Denaturation | Annealing | Extension |
|---|---|---|---|---|
| *yaiO* | TGATTTCCGTGCGTCTGAATG<br>ATGCTGCCGTAGCGTGTTTC | 95°C 30sec | 58°C 30sec | 72°C 60sec |
| *fimH* | GTGCCAATTCCTCTTACCGTT TGGAATAATCGTACCGTTGCG | 96°C 30sec | 64°C 1min | 72°C 60sec |
| *iss* | ATG TTA TTT TCT GCC GCT CTG<br>CTA TTG TGA GCA ATA TAC CC | 94°C 30sec | 63°C 30sec | 72°C 30sec |
| *iutA* | GGCTGGACATGGGAACTGG<br>CGTCGGGAACGGGTAGAATCG | 94°C 30sec | 53°C 30sec | 72°C 30sec |
| $bla_{NDM-1}$ | GGTTTGGCGATCTGGTTTTC<br>CGGAATGGCTCATCACGATC | 94°C 20sec | 52°C 30sec | 72°C 45sec |

**Antimicrobial Susceptibility Test:** *E. coli* isolates were tested for their antibiotic susceptibility using disk diffusion technique onto Muller–Hinton agar and incubated aerobically overnight at 37 °C. Nineteen antibiotics supplied by (TM Media, Titan Biotech Ltd, India); Amikacin (AK;30µg), Amoxicillin/Clavulanic acid (AMC; 30µg), Ampicillin/Sulbactam (SAM;20µg), Aztreonam (AZM;15µg), Cefixime (CFM;5µg), Cefotaxime (CTX;30µg), Cefoxitin (FOX;30µg), Ceftazidime (CAZ;30µg), Ceftriaxone (CRO;30µg), Cefuroxime (CXM30µg) 25 µg), Ciprofloxacin (CIP; 5µg), Clindamycin (DA;2µg), Erythromycin (E;15µg), Fosfomycin (FF; 30µg), Imipenem (IPM;10µg), Levofloxacin (LEV;5µg), and Norfloxacin (NOR;10µg), Ofloxacin (OFX;5µg). The diameter of the inhibition zone around antibiotic disks was measured according to (CLSI, 2020).

***E. coli* DNA Extraction:** Bacterial DNA was extracted from the *E. coli* isolates using GF-1 DNA Extraction Kit (Vivantis Technologies, Malaysia), persuading the manufacturer's guidelines.

### Primers and Testing Conditions of Polymerase Chain Reaction (PCR)

Monoplex PCR assays were applied for the amplification of species specific *yaiO* gene (115bp) (Molina *et al*., 2015), carbapenemase gene (*bla*NDM-1) (621bp) (Poirel *et al*., 2011). Also, some virulence genes; (adherence) *fimH* (164bp) (Hojati *et al*., 2015), (serum resistance) *iss* gene (266bp) (Yaguchi *et al*., 2007) and (iron acquisition) *iutA* (300bp) (Ananias and Yano, 2008).

PCR reaction was carried out using SimpliAmp™ Thermal Cycler (USA) in a final volume of 25 µl reaction involving 12.5 µl of 2x Red Mix Master Mix (Meridian Bioscience, UK), one µl (10 µM) of each primer and one µl of target DNA and 9.5 µl of doubled distilled water. The PCR products were analyzed by InGenius3 gel documentation system (Syngene, UK). The used primers and cycling conditions were mentioned in Table 1.

### Statistical Analysis

Statistical analysis was conducted using (SPSS) version 20.0 software (SPSS Inc., Chicago, IL). Chi-square ($\chi^2$) was used to compare the frequencies variations obtained.

P values < 0.05 were deemed statistically significant.
To do the statistical evaluation we used the following model

$$Yij = \mu + Si + eij$$

Where:
Yij: the value of the trait (observation); µ: general mean; Si: the fixed appearance of the *E. coli* isolate on studied trait; eij: experimental error.

**Quality control:** To ensure validity and reliability of the results, quality control measures were taken by using validated laboratory protocol for sample analysis. All laboratory procedures were done in accordance with standard operating procedures and CLSI guidelines. The reference strain of *E. coli* (ATCC 26122) was used in quality control for culture and susceptibility tests as well as for the detection of ESBL.

## RESULTS AND DISCUSSION

### *E. coli* Isolation and Identification

Relied on morphological culture appearance, Gram-staining, classical biochemical testing, and Vitek-2; a total of 63 *E. coli* isolates were obtained (32.81%). In detailed; 34 out of 81(41.97%) and 8 out of 38 (21.05%) isolates were recovered from dog and cat samples respectively. On the other hand, the human samples revealed 21 isolates (31.82%). The growth of lactose fermenter pink colonies and metallic green ones on MacConckey and EMB agar plates respectively assumed a successful culture of *E. coli*. The development of positive indole, methyl red with negative Voges–Proskauer and citrate tests as well as via Vitek-2 compact system gave further identification and confirmation.

### *E. coli* Serotyping

Out of total 63 isolates of *E. coli* in the current study, 50 could be serotyped to 11 different serotypes and 13 were

found non-typeable (Table 2). There were non-significant impacts of distribution of *E. coli* among dog, cat and human at P≥0.05 (0.669, 0.317 and 0.663 respectively). The majority was belonged to serotypes O25 (13; 26%), and O78 (10; 20%). Serotypes O26 K60, O78 K80 and O126 K71 were isolated from the three species.

**Table 2:** Distribution of obtained *E. coli* serotypes among examined urine samples belonged to the three species.

| Serotype | Dog | Cat | Human | Total |
|---|---|---|---|---|
| O6 K- | 1 | - | 3 | 4 |
| O8 K- | - | - | 2 | 2 |
| O25 K11 | 9 | - | 4 | 13 |
| O26 K60 | 2 | 1 | 1 | 4 |
| O44 K74 | 4 | - | - | 4 |
| O78 K80 | 5 | 3 | 2 | 10 |
| O118 K- | 2 | - | - | 2 |
| O119 K69 | 3 | - | - | 3 |
| O126 K71 | 2 | 1 | 1 | 4 |
| O142 K86 | 1 | 1 | - | 2 |
| O145 K- | 1 | - | 1 | 2 |
| | 30 | 6 | 14 | 50 |
| Untypable | 4 | 2 | 7 | 13 |
| Total | 34 | 8 | 21 | 63 |

**Figure 1:** Colonial appearance of *E. coli* isolates on Congo red medium.

## Hemolytic Activity of *E. coli* Isolates

The sixty-three *E. coli* strains were tested for their hemolytic activities, 28 serotypes exposed clear zone of α- hemolysis with a percentage 44.44%, while 15 strains displayed incomplete greenish zone of β-hemolysis (23.81%). On the other side, the rest 20 strains showed no hemolysis with an incidence 31.75%.

## Congo Red Binding Activity of *E. coli* Isolates

Congo red uptake assay was used to distinguish between the potential invasive and non–invasive *E. coli*. Forty-six isolates (73.01%) were positive in variable degrees indicated by the growth of orange red colonies (Figure 1).

## Vero Cell Cytotoxicity

Only 26 out of tested 63 *E. coli* isolates (41.27%) were able to produce cytopathic effect on the Vero cells. Among examined species; 12 human isolates (19.05%), 11 canine (17.46%) then 3 feline isolates (4.76%). The higher one was O78 (5 strains) followed by serotype O25 (4 strains). The overall results of phenotypic virulence characteristics among the three species and isolated *E. coli* serotypes were shown in Figure (2) and Table (3). The results revealed that there were non-significant impacts of phenotypic virulence characteristics among the obtained *E. coli* serotypes.

**Table 3:** Display of tested phenotypic virulence characteristics among the obtained *E. coli* serotypes.

| | α- hemolysis | β- hemolysis | no hemolysis (γ) | Congo red binding activity | Vero cell cytotoxicity |
|---|---|---|---|---|---|
| O6 | 2 | 1 | - | 3 | 2 |
| O8 | 1 | - | 1 | 1 | 2 |
| O25 | 7 | 2 | 4 | 10 | 4 |
| O26 | 2 | - | 2 | 2 | 1 |
| O44 | 3 | 1 | - | 4 | 3 |
| O78 | 6 | 1 | 3 | 8 | 5 |
| O118 | - | - | 2 | 1 | - |
| O119 | 3 | - | - | 2 | 1 |
| O126 | - | 1 | 3 | 2 | 1 |
| O142 | 1 | 2 | - | 2 | - |
| O145 | - | - | 2 | - | - |
| Untypable | 3 | 7 | 3 | 11 | 7 |
| Total | 28 | 15 | 20 | 46 | 26 |

## Antimicrobial Susceptibility

Results of antimicrobial sensitivity assessment of *E. coli* isolates from samples recovered from human, dogs, and cats are presented in Figure 3. The canine strains exhibited high-

er resistance to most examined antibiotics as, clindamycin, norfloxacin, ofloxacin (100%), levofloxacin and cefuroxime (91.2%) as well as notable unusual resistance against imipenem (52.94%). The canine isolates were susceptible to amikacin (82.4%), erythromycin (79.4%) and aztreonam (76.5%). Among feline isolates, the high resistance was exposed to clindamycin (87.5%), amoxicillin/ clavulanic acid and aztreonam (62.5%) while sensitive to most cephalosporins. Contrarily, the isolates derived from human origin revealed high resistance to cephalosporins but high sensitivity to imipenem (81%).

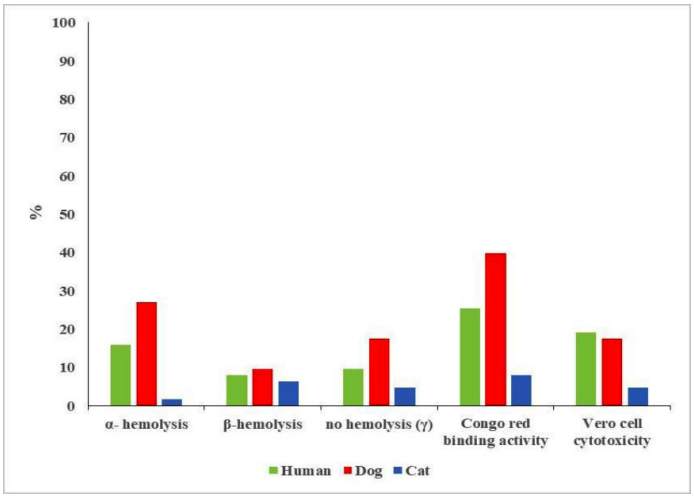


**Figure 2:** Display of tested phenotypic virulence characteristics among the sampled species *E. coli* isolates.

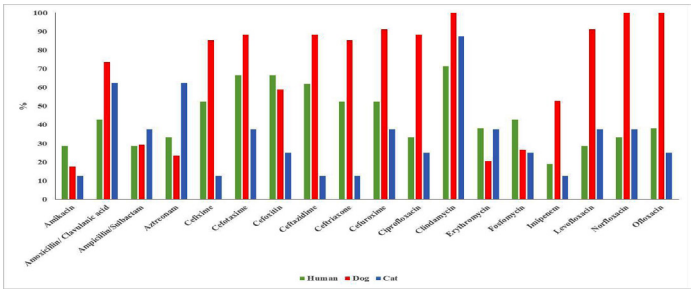

**Figure 3:** Resistance of *Escherichia coli* isolates to the antibacterial agents.

## Molecular Identification and Characterization of *E. coli* Isolates

All *E. coli* isolates amplified the species specific *yaiO* gene at 115 bp using PCR method. After that, all 63 *E. coli* isolates were characterized with regard to presence of the carbapeneme resistance gene as well as three virulence genes. The present study revealed that a number of 15 *E. coli* isolates harbored *bla*NDM-1gene (23.81%) Figure 4.

On the other hand, 52 (82.54%) of the *E. coli* isolates were positive for at least one of the examined 3 virulence genes. The highest prevalence was shown in feline isolates 7/8 (87.5%), followed by human isolates 18/21 (85.71%) then canine isolates as 27/34 (79.41%). The detailed molecular profile was shown in Table 4. The results exposed significant impacts of *E. coli* isolates' molecular profile and distribution of tested genes among examined species; (0.012, 0.033, 0.018) for *iut* A, *fim*H and *bla*-NDM respectively), but non-significant for *iss* gene (P = 0.097).

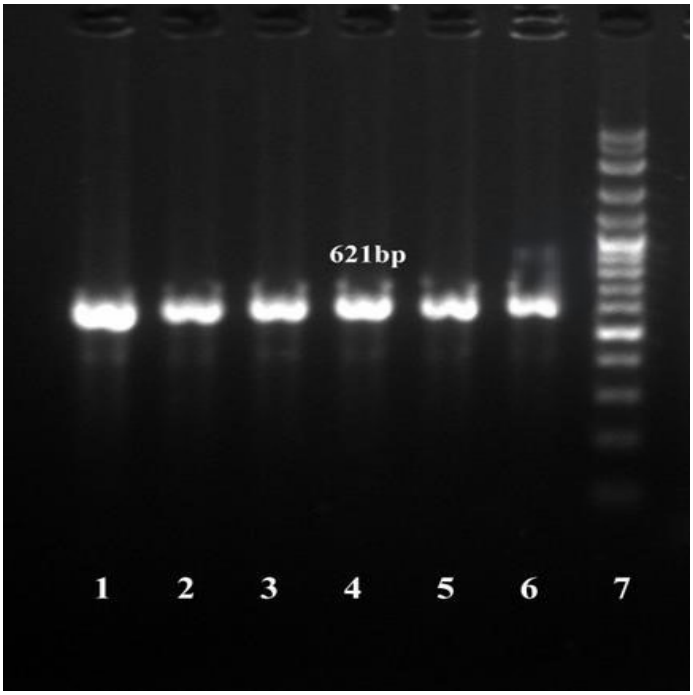


**Figure 4:** PCR amplification of carbapeneme resistance gene (*bla*NDM-1) gene in *E. coli* isolates. Lane 1: control positive. Lanes 2–6: positive amplification of PCR product (621bp). Lane 7: 100 bp DNA (1500) ladder.

Drug-resistant bacteria can constitute an important One Health concern which can be transmitted via either close contact between humans and pets or via the home environment (Bhat, 2021).

*Escherichia coli* are not only a commensal organism in humans and animals, but also a causative agent of extraintestinal illnesses as urinary tract infections (Aurich *et al*., 2022).

In our study, the cultural and phenotypic as well as biochemical identification results exposed that out of 192 examined urine samples, a total of 63 *E. coli* isolates were obtained (32.81%). In detailed; 34 out of 81(41.97%) and 8 out of 38 (21.05%) isolates were recovered from dog and cat samples respectively. On the other hand, the human samples revealed 21 isolates (31.82%).

Our results were greatly agreed with Iranian study in which *E. coli* was found in 44.4% of examined canine urine samples (Yousefi and Torkan, 2017). Also, our results coincided with a Spanish survey that revealed a parallel incidence of (44.6%) among analyzed 3270 dogs and 1673 cats of suspected UTI (Darwich *et al*., 2021). While, very high incidence of *E. coli* (86.64%) was obtained in a Chinese study

(Zhou *et al*., 2022). Contrarily, low prevalence was determined in urine samples examined in Korean study; (9.20%) dogs and (2.84%) cats (Choi *et al*., 2023).

**Table 4:** detailed molecular profile of *E. coli* isolates and distribution of tested genes among examined species.

| | Species | Number | iss | iut A | fimH | Bla-NDM |
|---|---|---|---|---|---|---|
| *E coli* O6 K- | Dog | 1 | - | - | - | - |
| | Human | 3 | - | 2 | 2 | 1 |
| *E coli* O8 K - | Human | 2 | - | - | 1 | - |
| *E coli* O25 K 11 | Dog | 9 | 3 | 2 | 7 | 3 |
| | Human | 4 | 1 | 1 | 3 | 1 |
| *E coli* O26 K 60 | Dog | 2 | 1 | 1 | 2 | 2 |
| | Cat | 1 | - | - | 1 | - |
| | Human | 1 | - | - | 1 | - |
| *E coli* O44 K 74 | Dog | 4 | 2 | 1 | 3 | 1 |
| *E coli* O78 K 80 | Dog | 5 | 2 | 1 | 4 | 2 |
| | Cat | 3 | - | 1 | 2 | - |
| | Human | 2 | - | 1 | 2 | - |
| *E coli* O118 K - | Dog | 2 | 1 | 1 | 1 | 1 |
| *E coli* O119 K 69 | Dog | 3 | - | - | - | - |
| *E coli* O126 K 71 | Dog | 2 | - | 1 | 2 | 1 |
| | Cat | 1 | 1 | 1 | 1 | 1 |
| | Human | 1 | - | - | 1 | 1 |
| *E coli* O142 K 86 | Dog | 1 | - | - | 1 | - |
| | Cat | 1 | - | - | 1 | - |
| *E coli* O145 K - | Dog | 1 | - | - | - | 1 |
| | Human | 1 | - | 1 | - | - |
| Untypable | Dog | 4 | 1 | 1 | 3 | - |
| | Cat | 2 | - | - | 2 | - |
| | Human | 7 | 1 | 2 | 5 | - |
| Total | Dog | 34 | 10 | 8 | 23 | 11 |
| | Cat | 8 | 1 | 2 | 7 | 1 |
| | Human | 21 | 2 | 7 | 15 | 3 |

Our *E. coli* incidence result regarded human samples was harmonized with another Egyptian study in Kafr Elsheikh University Hospital; (36.4%) among 100 UTI neonates (Baz *et al*., 2021). While, in El-Minia governorate; 134 *E. coli* strains were isolated from 583 collected urine samples of outpatients with community-acquired–UTIs (22.98%), (Farahat *et al*., 2021). Higher prevalence of *E. coli* (64%) in UTI human samples was reported in Dhaka, Bangladesh, (Islam *et al*., 2022).

Our results revealed that 43 out of 63 *E. coli* strains showed hemolytic activity (68.25%). Nearly parallel result was achieved by demonstration of hemolytic activity in 17 out of 24 *E. coli* strains (70.83%) (Prada *et al*., 1991).

Congo red uptake assay was used to distinguish between the invasive and non–invasive *E. coli* (Sharma *et al*., 2006). Our result of Congo red uptake assay revealed positivity of 73.01% of *E. coli* isolates. Algammal *et al*. (2022) found that 16/19 of the tested isolates (84.2%) were positive for Congo-red uptake from which 3 isolates belonged to O26.

The result of Vero cell cytotoxicity revealed 26 out of 63 *E. coli* isolates (41.27%) were able to produce CPE on the Vero cells. The result was agreed with a study of Maldonado *et al*. (2005) who mentioned that 42% of 93 isolates exposed positive cytotoxicity. At the point of view, Starcic *et al*. (2002) proposed that uropathogenic *E. coli* strains frequently have hemolytic and cyto-necrotic activities as the production of hemolysin was usually linked with the output of cytotoxic necrotizing factor.

Generally, transmission of ExPEC between pets and humans was recorded and noticed to be highly genetic related but differed in their adhesins which define host specificity (Beutin, 1991). Our results of typable *E. coli* isolates serotyping exposed a total of 11 serotypes (Table 2); (dogs≈10), (cats≈4) (human≈7). It was proposed that the high similarity in the genomic backbone between certain human- and animal-origin *E. coli* strains within serogroup O6 asserts the hypothesis of zoonotic potential (Johnson *et al*., 2008). Yuri *et al*. (1999) addressed O4, O6, O25 and many untypable *E. coli* serotypes obtained from 80 dogs and 35 cats with UTI. Tanabe *et al*. (2022) determined O6:H1 and O25:H4 then O8 and O126 as the most prevalent in UTI outpatient's urine samples in Brazil. The zoonotic importance of serotype O78:H10 was reported in outbreak documented in Copenhagen in 1991(Olesen *et al*., 1994). Furthermore, O126:K71 was detected in 44/110 (40%) of *E. coli* strains analyzed from the human urine in an Egyptian study (Osman *et al*., 2012).

The growing emergence of MDR bacteria, inducing UTI in dogs and cats, which can be transmitted via either close contact between humans and pets or via the home environment constitutes an important One Health concern and represents a prominent therapeutic challenge (Endimiani *et al*., 2020). Results of antimicrobial sensitivity assessment of *E. coli* isolates from samples recovered from human, dogs, and cats are presented in Figure 3, in which the high resistances to many antibiotics were observed. The canine strains exhibited higher resistance to most examined antibiotics; clindamycin, norfloxacin, ofloxacin (100%), levofloxacin and cefuroxime (91.2%) as well as notable unusual resistance against imipenem (52.94%). The canine isolates were susceptible to amikacin (82.4%), and erythromycin (79.4%). Among feline isolates, the high resistance was ex-

posed to clindamycin (87.5%) while sensitive to most cephalosporins. Contrarily, the isolates derived from human origin revealed high resistance to cephalosporins but highly sensitive to imipenem (81%). The antibiogram pattern of uropathogenic *E. coli* was determined in different previous reports. *E. coli* noted to be resistant to most of cephalosporins and quinolones (Islam *et al*., 2022). The higher incidence of antibiotic resistance of *E. coli* strains isolated from dogs suffered from UTIs was reported in study performed by Yousefi and Torkan (2017). In a Swiss study, all *E. coli* strains that obtained from UI dogs and cats' cases were found to be resistant to cephalosporins and fluoroquinolones but sensitive for amikacin (Huber *et al*., 2013). On the other direction, in a Chinese study, it was noteworthy that 25% of isolates were resistance to imipenem and meropenem (Liu *et al*., 2016). An Egyptian study addressed 93 UPEC isolates obtained from premenopausal and postmenopausal women suffering from uncomplicated UTI. The identified isolates exposed resistance to cefotaxime and ceftazidime (Ali and Yakout, 2021). Another very recent study conducted on 128 hospital acquired uropathogenic *E. coli* isolates in Zagazig, Egypt that revealed the highest resistance to cefoxitin (93%), cefepime (92%) (El Maghraby *et al*., 2024). The extended use of quinolones, particularly ciprofloxacin, in the outpatients is the reason of elevation in resistance to these drugs; remarkably higher in developing countries (55.5–85.5%) (Kot, 2019). Cek *et al*. (2014) pointed to the correlation between the growing prophylactic use of broad-spectrum antibiotics in the urological conditions and elevated antimicrobial multi-resistance of bacteria; 85% in Africa and makes therapy more difficult. Carbapenems as imipenem constitute the best choice for UTI therapy caused by ESBL-producing strains (Idil *et al*., 2016). Carbapenem resistance (CR) is deemed a marker for XDR and PDR Gram-ve bacteria because it is accompanied with a broad scope of co-resistance to other antimicrobial agents (Tacconelli *et al*., 2018). A high existence of carbapenemase enzymes has been recorded in companion animals, assuming their role in the cross-species transfer of carbapenem-resistant determinants (Ramírez-Castillo *et al*., 2023).

Molecularly, all 63 *E coli* isolates were confirmed via amplification of highly species specific *yaiO* gene which is unique to *E. coli* and encodes a protein found to be expressed and existed in the outer membrane (Molina *et al*., 2015).

Concerning the emergent carbapenem resistance gene which was achieved in fifteen isolates (23.81%); the canine isolates exposed considerable high existence 11/34 (32.35%), then human isolates 3/21(14.28%) while only one feline isolate harbored the gene (12.5%). In Egypt, Ramadan *et al*. (2020) described 2 different NDM alleles; *bla*NDM-5 obtained from healthy person's urine and seven environmental dogs' samples as well as *bla*NDM-1 obtained from infected patient's urine. Cui *et al*. (2018) isolated an *E. coli* ST167 strain from companion dog as a first case for *bla*NDM-1 gene positivity in Beijing, China. A very recent study identified the first case of carbapenem resistance gene; *bla*NDM in *E. coli* strain isolated from female dog suffered from pyometra in Japan (Harada *et al*., 2024). Five *E. coli* strains isolated from 129 healthy or ill dogs and cats (3.88%) in veterinary clinics located in 6 Chinese cities were found to carry *bla*NDM (Kuang *et al*., 2022). In Italy, an *E. coli* ST167 recovered from a hospitalized dog and found to harbor carbapenem-resistance gene *bla*NDM, the novel molecular approach demonstrated that the strain was related to other strains isolated from a Swiss dog and Italian human clinical cases (Alba *et al*., 2021). Hong *et al*. (2019) identified two *E. coli* isolates that carried *bla*NDM gene one from cat and the other one was from a dog in South Korea. A study from Saudi Arabia concerned the existence of carbapenem-resistant UPEC clones in CA-UTIs reported the presence of carbapenemases genes; NDM-1 and 5 in examined *E. coli* strains (Abd El Ghany *et al*., 2018).

In addition to the link with diverse antimicrobial resistance determinants, the categorization as an international MDR high-risk strain is also associated with the bacterial pathogenicity, capacity to colonize and survive in the hosts for more than 6 months, ability for transmission between hosts, global distribution and the ability to induce recurrent infections (Mathers *et al*., 2018).

On the other side, UPEC strains have a definitive genetic diversity comprising significant adhesins as different types of fimbriae and iron acquisition systems. Adhesions permit bacteria to evade expulsion via urination, so give the chance to bacterial proliferation within the uroepithelium followed by infection of nearby cells, and finally persist in urinary tract (Sharma *et al*., 2021).

Forty-five isolates succeed to amplify the fimbrial gene '*fimH*' at 164 bp (71.43%). *fimH* is a prime determinant, responsible for synthesis of the adhesive portion of type 1 fimbriae which has high binding affinity to urinary receptors that permit bacteria to adhere, proliferate, infect uroepithelial cells, and finally persist in urinary tract (Sharma *et al*., 2021; Sokurenko *et al*., 2004). Mahmoud *et al*. (2022) detected *fimH* gene in 87.5% of *E. coli* isolates obtained from Tomcats suffering from urine retention in Ismailia, Egypt. Another Egyptian study addressed 22 *E. coli* isolated from 80 urine samples of UTI patients in Minia university hospital and exposed *fimH* gene in 20 (90.9%), (Abd El-Baky *et al*. 2020). Decano *et al*. (2021) detected *fimH* gene in all *E. coli* ≈55 (100%) obtained from clinically UTI in- and out-patients in East Africa and serotype O25

was the most one identified. In North Carolina University, a study detected *fimH* gene in 91% of 69 UPEC isolates obtained from dog urine suffered from UTIs (Gilbertie *et al*. 2020). A descriptive study performed in Lima, Peru on 75 uropathogenic *E. coli* isolates with detection of *fim*H in 74 isolates (98.7%), (Matta-Chuquisapon *et al*. 2020).

Regarding the other two tested virulence genes; our results demonstrated the existence of *iut*A and *iss* genes in 17 (26.98%) and 13 (20.63%) in *E. coli* isolates respectively. The *iut*A gene is responsible for encoding aerobactin which considered one of the ferric protein siderophore that aids iron acquisition after making free iron availability (Robinson *et al*. 2018). It is assumed that the *iut*A expression in extraintestinal pathogenic *E. coli* isolates give a mirror to their virulence (Chouikha *et al*. 2008). While, *iss* gene is a substantial factor for increasing serum survival via complement resistance resulted in ongoing of septicemia and lethality (Biran *et al*., 2021). The detailed prevalence of the *iut*A and *iss* genes was demonstrated in Table 4.

Aurich *et al*. (2023) detected *iut*A gene among uropathogenic *E. coli* ST372 isolated from dogs (14.1%) as well as human-related ST73 (17.1%) in infected cats by a total of (15.2%). While, Valat *et al*. (2020) found *iut*A and *iss* genes by (16.8%) and (15.5%) respectively among UPEC canine isolates in the Parisian suburbs laboratories. Among 59 *E. coli* strains isolated from urinary samples of dogs presented to Zurich hospital, Switzerland, *iss* gene was detected in 4 strains of ST533 (7.55%), (Huber *et al*., 2013). Momtaz *et al*. (2013) surveyed 123 *E. coli* isolates obtained from symptomatic UTIs patients in Tehran, Iran and detected 10 isolates harbored *iss* gene (8.13%).

Among surveyed 165 *E. coli* strains obtained from UTI dogs in Shaanxi, China; 16 (9.7%) isolates were positive for *iut*A gene (Liu *et al*., 2016). Another survey was conducted on 2443 *E. coli* isolates recovered from infected dogs and cats in 6 regions of USA. The results revealed the existence of *iut*A gene in non-ESBL producers (72.2%), ESBL producers (38.2%), CTX-M producers (44%), and finally in non-CTX-M producers (22.2%), (Liu *et al*., 2016). In an Australian study among 26 isolates belonged to O75 (24 from human, and 2 from dogs); *iut*A gene was detected in all isolates (100%), (Platell *et al*., 2012).

## CONCLUSIONS AND RECOMMENDATIONS

*Escherichia coli* are implemented in a high proportion of UTIs associated with both humans and companion animals. The ExPEC have considerable virulence genes that promote the adherence, biofilm formation, iron utilization and resistance to host immune response. Furthermore, this study highlights the emergence seriousness of resistance versus last-resort antibiotics as carbapeneme in pets which increase the treatment challenges. Provide spotlighting on MDR bacteria that may be bi-directionally transferred between humans and companion animals. Further molecular epidemiological studies are needed to understand the transmission dynamics of multidrug especially carbapeneme resistant bacteria which constitute a potential threat to public health. Antibiotic misuse has implemented in the elevating emergence of multidrug-resistant and pan-resistant strains in humans, companion animals, rather than water and food animals. Our data also assume that the existence of carbapeneme resistant *E. coli* among companion animals requires further continued surveillance.

The need for a global action plan to address the antimicrobial resistance crises requires a One Health approach supported by scientific data that can be used to raise awareness of decision-makers and the general population. Therefore, it is necessary to establish national standards for the rational use of antibiotics in companion animals.

## NOVELTY STATEMENT

The study exposed the existence of carbapenem resistance genes in Egyptian UPE isolates and subsequent developing of resistance against carbapenem and other antibiotics classes.

Demonstrating the role of companion animals in distribution of the multidrug resistant E. coli among human contacts community in Egypt.

## AUTHORS' CONTRIBUTIONS

Ashraf Hakim conceptualized, designed the research work, acquired data, and drafted as well as formatted manuscript. Doaa Diab Khalaf and Engy Farahat conducted microbiological and biochemical identification as well as antibiotic sensitivity. Hussein Abuelhag, Mohammed Darwish Mohammed and Wahid El-Dabae carried out serotyping and virulence assays besides statistics analysis. Amany Nabil Dapgh performed Vitek identification. Ehab Ali Fouad extracted DNA and performed PCR assays and supervised by Khaled Abd El-Razik. All the authors listed read and approved the final manuscript.

### Conflict of Interest

The authors have declared no conflict of interest.

## REFERENCES

Abd El-Baky, R.M., Ibrahim, R.A., Mohamed, D.S., Ahmed, E.F., Hashem, Z.S. Prevalence of virulence genes and their

association with antimicrobial resistance among pathogenic *E. coli* isolated from Egyptian patients with different clinical infections. Infection and Drug Resistance, 2020; 13: 1221-1236. https://doi.org/10.2147/IDR.S241073

Abd El Ghany, M., Sharaf, H., Al-agamy, M.H., Shibl, A., Hill-Cawthorne, G.A., Hong, P.Y. Genomic characterization of NDM-1 and 5, and OXA-181 carbapenemases in uropathogenic *Escherichia coli* isolates from Riyadh, Saudi Arabia. PLoS One, 2018;13(8): e0201613. https://doi.org/10.1371/journal.pone.0201613

Alba, P., Taddei, R., Cordaro, G., Fontana, M.C., Toschi, E., Gaibani, P., Marani, I., Giacomi, A., Diaconu, E.L., Iurescia, M., Carfora, V., Franco, A. Carbapenemase IncF-borne blaNDM-5 gene in the *E. coli* ST167 high-risk clone from canine clinical infection, Italy. Veterinary Microbiology, 2021; 256:109045. https://doi.org/10.1016/j.vetmic.2021.109045

Algammal, A.M., El-Tarabili, R.M., Alfifi, K.J., Al-Otaibi, A.S., Hashem, M.E.A., El-Maghraby, M.M., Mahmoud, A.E. Virulence determinant and antimicrobial resistance traits of emerging MDR Shiga toxigenic *E. coli* in diarrheic dogs. AMB Express, 2022; 12(1):34. https://doi.org/10.1186/s13568-022-01371-4

Ali, G.H., Yakout, M.A. Comparative study of ESBL production among uropathogenic *Escherichia coli* clinical isolates from pre- and post-menopausal women in Egypt. Current Microbiology, 2021; 78(9):3516-3525. https://doi.org/10.1007/s00284-021-02599-2

Amphaiphan, C., Yano, T., Som-In, M., Kungwong, P., Wongsawan, K., Pusoonthornthum, R., Salman, M.D., Tangtrongsup, S. Antimicrobial drug resistance profile of isolated bacteria in dogs and cats with urologic problems at Chiang Mai University Veterinary Teaching Hospital, Thailand (2012-2016). Zoonoses Public Health, 2021; 68(5):452-463. https://doi.org/10.1111/zph.12832

Ananias, M., Yano, T. Serogroups and virulence genotypes of *Escherichia coli* isolated from patients with sepsis. Braz J Med Biol Res, 2008; 41(10):877–83. https://doi.org/10.1590/S0100-879X2008001000008

Aurich, S., Prenger-Berninghoff, E., Ewers, C. Prevalence and antimicrobial resistance of bacterial uropathogens isolated from dogs and cats. Antibiotics (Basel), 2022; 11(12):1730. https://doi.org/10.3390/antibiotics11121730

Aurich, S., Wolf, S.A., Prenger-Berninghoff, E., Thrukonda, L., Semmler, T., Ewers, C. Genotypic characterization of uropathogenic *Escherichia coli* from companion animals: predominance of ST372 in dogs and human-related ST73 in cats. Antibiotics (Basel), 2023; 13(1):38. https://doi.org/10.3390/antibiotics13010038

Baede V.O., Broens E.M., Spaninks M.P., Timmerman A.J., Graveland H., Wagenaar J.A., Duim B., Hordijk J. Raw pet food as a risk factor for shedding of extended-spectrum beta-lactamase-producing *Enterobacteriaceae* in household cats. PLoS ONE, 2017; 12: e0187239. https://doi.org/10.1371/journal.pone.0187239

Baz, A.M.K., El-Agamy, O.A.E., Ibrahim, A.M. Incidence of urinary tract infection in neonates with significant indirect Hyperbilirubinemia of unknown etiology: case-control study. Italian Journal Pediatrics, 2021; 47(1):35. https://doi.org/10.1186/s13052-021-00982-0

Berkhoff, H.A., Vinal, A.C. Congo red medium to distinguish between invasive and non-invasive Escherichia coli pathogenic for poultry. Avian Diseases, 1986; 30(1):117-21. https://doi.org/10.2307/1590621

Beutin, L. *Escherichia coli* as a pathogen in dogs and cats. Veterinary Research, 1999; 30(2-3), 285–298.

Bhat A.H. Bacterial zoonoses transmitted by household pets and as reservoirs of antimicrobial resistant bacteria. Microbial Pathogen 2021; 155:104891. https://doi.org/10.1016/j.micpath.2021.104891

Biran, D., Sura, T., Otto, A., Yair, Y., Becher, D., Ron, E.Z. Surviving serum: the *Escherichia coli iss* gene of extraintestinal pathogenic *E. coli* Is required for the synthesis of group 4 capsule. Infection Immunity, 2021; 89(10):e0031621. https://doi.org/10.1128/IAI.00316-21

Cek, M., Tandoğdu, Z., Wagenlehner, F., Tenke, P., Naber, K., Bjerklund-Johansen, T.E. Healthcare-associated urinary tract infections in hospitalized urological patients – a global perspective: results from the GPIU studies 2003–2010. World J Urology, 2014; 32(6):1587–1594. https://doi.org/10.1007/s00345-013-1218-9

Choi, J.H., Ali, M.S., Moon, B.Y., Kang, H.Y., Kim, S.J., Song, H.J., Mechesso, A.F., Moon, D.C., Lim, S.K. Prevalence and characterization of extended-spectrum β-lactamase-producing *Escherichia coli* isolated from dogs and cats in South Korea. Antibiotics (Basel), 2023; 12(4):745. https://doi.org/10.3390/antibiotics12040745

Chouikha, I., Bree, A., Moulin-Schouleur, M., Gilot, P., Germon, P. Differential expression of *iut*A and *ibe*A in the early stages of infection by extra-intestinal pathogenic *E. coli*. Microbes and Infection, 2008; 10(4), 432–8. https://doi.org/10.1016/j.micinf.2008.01.002

Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. Supplement M100≤ S30. Wayne, Pennsylvania, 2020.

Cui, L., Lei, L., Lv, Y., Zhang, R., Liu, X., Li, M., Zhang, F., Wang, Y. blaNDM-1-producing multidrug-resistant *Escherichia coli* isolated from a companion dog in China. Journal of Global Antimicrobial Resistance, 2018; 13:24-27 https://doi.org/10.1016/j.jgar.2017.10.021

Damborg, P., Gumpert, H., Johansson, L., Jana, B., Frimodt-Møller, N., Guardabassi, L. Dogs as reservoirs of *Escherichia coli* strains causing urinary tract infection in their owners. bioRxiv, 2018; 302885. https://doi.org/10.1101/302885

Darwich, L., Seminati, C., Burballa, A., Nieto, A., Durán, I., Tarradas, N., Molina-López, R.A. Antimicrobial susceptibility of bacterial isolates from urinary tract infections in companion animals in Spain. Veterinary Record, 2021; 188(9): e60. https://doi.org/10.1002/vetr.60

Decano, A.G., Pettigrew, K., Sabiiti, W., Sloan, D.J., Neema, S., Bazira, J., Kiiru, J., Onyango, H., Asiimwe, B., Holden, M.T.G. Pan-resistome characterization of uropathogenic *Escherichia coli* and *Klebsiella pneumoniae* strains circulating in Uganda and Kenya, isolated from 2017-2018. Antibiotics (Basel), 2021; 10(12):1547. https://doi.org/10.3390/antibiotics10121547

El Maghraby, H.M., El-Sayed, H.A., Hussein, S., El Azawy, D.S., Attia, O., Orabi, E.E., Fahmy, Y.A. Detection of phylogrouping, adhesin, and extended spectrum β-lactamases genes in hospital acquired uropathogenic *Escherichia coli* isolates. Mol Biol Rep., 2024; 51(1):143. https://doi.org/10.1007/s11033-023-08983-4

Endimiani, A., Brilhante, M., Bernasconi, O.J., Perreten, V., Schmidt, J.S., Dazio, V., Nigg, A., Gobeli Brawand, S., Kuster, S.P., Schuller, S. Employees of Swiss veterinary clinics colonized with epidemic clones of carbapenemase-

producing *Escherichia coli*. Journal of Antimicrobial and Chemotherapy, 2020; 75:766–768. https://doi.org/10.1093/jac/dkz470

Farahat, E.M., Hassuna, N.A., Hammad, A.M., Fattah, M.A., Khairalla, A.S. Distribution of integrons and phylogenetic groups among *Escherichia coli* causing community-acquired urinary tract infection in Upper Egypt. Canadian Journal of Microbiology, 2021; 67(6):451-463. https://doi.org/10.1139/cjm-2020-0292

Flament-Simon, S.C., de Toro, M., García, V., Blanco, J.E., Blanco, M., Alonso, M.P., Goicoa, A., Díaz-González, J., Nicolas-Chanoine, M.-H., Blanco, J. Molecular characteristics of extraintestinal pathogenic *E. coli* (ExPEC), uropathogenic *E. coli* (UPEC), and multidrug resistant *E. coli* isolated from healthy dogs in Spain. Whole genome sequencing of canine ST372 isolates and comparison with human isolates causing extraintestinal infections. Microorganisms, 2020; 8:1712. https://doi.org/10.3390/microorganisms8111712

Gilbertie, J.M., Levent, G., Norman, K.N., Vinasco, J., Scott, H.M., Jacob, M.E. Comprehensive phenotypic and genotypic characterization and comparison of virulence, biofilm, and antimicrobial resistance in urinary *Escherichia coli* isolated from canines. Veterinary Microbiology, 2020; 249:108822. https://doi.org/10.1016/j.vetmic.2020.108822

Giugliano, L.G., Mann, G.F., Drasar, B.S. Response of mammalian cell lines to the toxins of *Escherichia coli*. Journal of Medical Microbiology, 1982; 15: 531-539. https://doi.org/10.1099/00222615-15-4-531

Guinée, P.A.M., Jansen, W.H., Wadström, T., Sellwood, R. *Escherichia coli* associated with neonatal diarrhoea in piglets and calves. Curr. Top. Vet. Anim. Sciences, 1981; 13:126-162. https://doi.org/10.1007/978-94-009-8328-1_18

Harada, K., Miyamoto, T., Sugiyama, M., Asai, T. First report of a blaNDM-5-carrying *Escherichia coli* sequence type 12 isolated from a dog with pyometra in Japan. Journal of Infection and Chemotherapy, 2024: S1341-321X (24)00048-5. https://doi.org/10.1016/j.jiac.2024.02.013

Hojati, Z., Zamanzad, B., Hashemzadeh, M., Molaie, R., Gholipour, A. The *FimH* Gene in uropathogenic *Escherichia coli* strains isolated from patients with urinary tract infection. Jundishapur J Microbiology, 2015; 8(2): e17520. https://doi.org/10.5812/jjm.17520

Hong, J.S., Song, W., Park, H.M., Oh, J.Y., Chae, J.C., Shin, S., Jeong, S.H. Clonal spread of extended-spectrum cephalosporin-resistant Enterobacteriaceae between companion animals and humans in South Korea. Front. Microbiology, 2019; 10:1371. https://doi.org/10.3389/fmicb.2019.01371

Huber, H., Zweifel, C., Wittenbrink, M.M., Stephan R. ESBL-producing uropathogenic *Escherichia coli* isolated from dogs and cats in Switzerland. Veterinary Microbiology, 2013; 162(2-4):992-996. https://doi.org/10.1016/j.vetmic.2012.10.029

Idil, N., Candan, E.D., Rad, A.Y., Aksoz, N. High trimethoprim-sulfamethoxazole resistance in ciprofloxacin-resistant *Escherichia coli* strains isolated from urinary tract infection. Minerva Biotecnology, 2016; 28(3):159–163.

Islam, M.R., Hoque, M.J., Uddin, M.N., Dewan, A., Haque, N.B., Islam, M.T., Islam, M.H., Hasan, M.A. Antimicrobial resistance of *E. coli* causing urinary tract infection in Bangladesh. Mymensingh Medical Journal, 2022; 31(1):180-185.

Johnson, J.R., Johnston, B., Clabots, C.R., Kuskowski, M.A., Roberts, E., DebRoy, C. Virulence genotypes and phylogenetic background of *Escherichia coli* serogroup O6 isolates from humans, dogs, and cats. Journal of Clinical Microbiology, 2008; 46(2):417-22. https://doi.org/10.1128/JCM.00674-07

Johnson, J.R., Russo, T.A. Extraintestinal pathogenic *Escherichia coli*: "The other bad *E. coli*" Journal of Laboratory and Clinical Medicine, 2002; 139:155–162. https://doi.org/10.1067/mlc.2002.121550

Kamal, A.M., Dell, C.A., Kang, T. Recognizing zooeyia to promote companion animal welfare in urban Bangladesh. Animals, 2023; 13(9): 1523. https://doi.org/10.3390/ani13091523

Kot, B. Antibiotic Resistance among uropathogenic *Escherichia coli*. Polish Journal of Microbiology, 2019; 68(4):403-415. https://doi.org/10.33073/pjm-2019-048

Kuang, X., Yang, R., Ye, X., Sun, J., Liao, X., Liu, Y., Yu, Y. NDM-5-producing *Escherichia coli* co-harboring mcr-1 gene in companion animals in China. Animals (Basel), 2022; 12(10): 1310. https://doi.org/10.3390/ani12101310

Liu, X., Liu, H., Li, Y., Hao, C. High prevalence of β-lactamase and plasmid-mediated quinolone resistance genes in extended-spectrum cephalosporin-resistant *Escherichia coli* from dogs in Shaanxi, China. Frontier Microbiology, 2016; 7:1843. https://doi.org/10.3389/fmicb.2016.01843

Liu, X., Thungrat, K., Boothe, D.M. Occurrence of OXA-48 carbapenemase and other β-lactamase genes in ESBL-producing multidrug resistant *Escherichia coli* from dogs and cats in the United States, 2009-2013. Frontier Microbiology, 2016; 7: 1057. https://doi.org/10.3389/fmicb.2016.01057

Mahmoud, A.E., El-Maghraby, M.M., Eltarabili, R.M., Soliman, E.S. Epidemiological investigations on microbial infection and crystals causing feline lower urinary tract disease in tomcats in Ismailia, Egypt. Open Veterinary Journal, 2022; 12(2):290-302. https://doi.org/10.5455/OVJ.2022.v12.i2.18

Maldonado, Y., Fiser, J.C., Nakatsu, C.H., Bhunia, A.K. Cytotoxicity potential and genotypic characterization of *Escherichia coli* isolates from environmental and food sources. Applied Environmental Microbiology, 2005; 71(4):1890-8. https://doi.org/10.1128/AEM.71.4.1890-1898.2005

Mathers, A.J., Peirano, G., Pitout J.D. The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant *Enterobacteriaceae*. Clinical Microbiology Review, 2015; 28:565–591. https://doi.org/10.1128/CMR.00116-14

Matta-Chuquisapon, J., Valencia-Bazalar, E., Marocho-Chahuayo, L., Gonzales-Escalante, E., Sevilla-Andrade, C.R., 2020. Presence of *fimH* and *afa* genes in urinary isolates of extended-spectrum beta-lactamases producing *Escherichia coli* in Lima, Peru. Rev Peru Med Exp Salud Publica. 37(2), 282-286. https://doi.org/10.17843/rpmesp.2020.372.4829

Molina, F., López-Acedo, E., Tabla, R., Roa, I., Gómez, A., Rebollo, J.E. Improved detection of *Escherichia coli* and coliform bacteria by multiplex PCR. BMC Biotechnology, 2015; 15:48. https://doi.org/10.1186/s12896-015-0168-2

Momtaz, H., Karimian, A., Madani, M., Safarpoor Dehkordi, F., Ranjbar, R., Sarshar, M., Souod, N. Uropathogenic *Escherichia coli* in Iran: serogroup distributions, virulence factors and antimicrobial resistance properties. Annual Clinical Microbiology and Antimicrobiolgy, 2013; 12:8. https://doi.org/10.1186/1476-0711-12-8

Olesen, B., Kolmos, H.J., Orskov, F., Orskov, I. Cluster of multiresistant *Escherichia coli* O78:H10 in greater Copenhagen.

Scandinavian Journal of Infectious Diseases, 1994; 26: 406-10. https://doi.org/10.3109/00365549409008613

Osman, K.M., Mustafa, A.M., Elhariri, M., AbdElhamed, G.S. Identification of serotypes and virulence markers of *Escherichia coli* isolated from human stool and urine samples in Egypt. Indian Journal of Medical Microbiology, 2012; 30(3): 308-13. https://doi.org/10.4103/0255-0857.99492

Overgaauw, P. A. M., Vinke, C. M., Hagen, M. A. E. V., Lipman, L. J. A. (). A One Health Perspective on the human-companion animal relationship with emphasis on zoonotic aspects. International journal of environmental research and public health, 2020; 17(11): 3789. https://doi.org/10.3390/ijerph17113789

Platell, J.L., Cobbold, R.N., Johnson, J.R., Clabots, C.R., Trott, D.J. Fluoroquinolone-resistant extraintestinal *Escherichia coli* clinical isolates representing the O15:K52:H1 clonal group from humans and dogs in Australia. Comp. Immunology, Microbiology and Infectious Diseases, 2012 35: 319–324. https://doi.org/10.1016/j.cimid.2012.02.002

Poirel, L., Walsh, T.R., Cuvillier, V., Nordmann, P. Multiplex PCR for detection of acquired carbapenemase genes. Diagnostic Microbiology Infectious Diseases, 2011; 70(1):119-23. https://doi.org/10.1016/j.diagmicrobio.2010.12.002

Prada, J., Baljer, G., De Rycke, J., Steinrück, H., Zimmermann, S., Stephan, R., Beutin, L. Characteristics of alpha-hemolytic strains of *Escherichia coli* isolated from dogs with gastroenteritis. Veterinary Microbiology, 1991; 29(1):59-73. https://doi.org/10.1016/0378-1135(91)90110-2

Quinn, P.J., Markey, B.K., Carter, M.E., Donnelly, W.J.C., Leonard, F.C. Veterinary Microbiology and Microbial Disease. 2002.

Ramadan, H., Gupta, S.K., Sharma, P., Ahmed, M., Hiott, L.M., Barrett, J.B., Woodley, T.A., Frye, J.G., Jackson, C.R. Circulation of emerging NDM-5-producing *Escherichia coli* among humans and dogs in Egypt. Zoonoses Public Health, 2020; 67(3):324-329. https://doi.org/10.1111/zph.12676

Ramírez-Castillo, F.Y., Guerrero-Barrera, A.L., Avelar-González, F.J. An overview of carbapenem-resistant organisms from food-producing animals, seafood, aquaculture, companion animals, and wildlife. Frontier Veterinary Sciences, 2023; 10:1158588. https://doi.org/10.3389/fvets.2023.1158588

Robinson, A.E., Heffernan, J.R., Henderson, J.P. The iron hand of uropathogenic *Escherichia coli*: the role of transition metal control in virulence. Future Microbiology, 2018; 13:745–56. https://doi.org/10.2217/fmb-2017-0295

Ruiz, J. Antimicrobial Resistance, from Bench-to-Publicside. Microbes Infection and Chemotherapy, 2021; 1: e1182. https://doi.org/10.54034/mic.e1182

Sharma, K., Soni, S., Meharchandani S. Congo red dye agar test as an indicator test for detection of invasive bovine *Escherichia coli*. Veterinary Arhiv, 2006; 76: 363- 366.

Sharma, K., Thacker, V.V., Dhar, N., Clapés Cabrer, M., Dubois, A., Signorino-Gelo, F., Mullenders, J., Knott, G.W., Clevers, H., McKinney, J.D. Early invasion of the bladder wall by solitary bacteria protects UPEC from antibiotics and neutrophil swarms in an organoid model. Cell Rep, 2021; 36(3):109351. https://doi.org/10.1016/j.celrep.2021.109351

Sokurenko, E.V., Feldgarden, M., Trintchina, E., Weissman, S.J., Avagyan, S., Chattopadhyay, S. Selection footprint in the *FimH* adhesin shows pathoadaptive niche differentiation in *Escherichia coli*. Molecular Biological Evolution, 2004; 21(7):1373–83. https://doi.org/10.1093/molbev/msh136

Starcic, M., Johnson, J.R., Stell, A.L., van der Goot, J., Hendriks, H.G., van Vorstenbosch, C., van Dijk, L., Gaastra, W. Hemolytic *Escherichia coli* isolated from dogs with diarrhea have characteristics of both uropathogenic and necrotoxigenic strains. Veterinary Microbiology, 2002; 85(4):361-77. https://doi.org/10.1016/S0378-1135(02)00003-2

Tacconelli, E., Carrara, E., Savoldi, A. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infectious Diseases, 2018; 18:318–27. https://doi.org/10.1016/S1473-3099(17)30753-3

Tanabe, R.H.S., Dias, R.C.B., Orsi, H., de Lira, D.R.P., Vieira, M.A., Dos Santos, L.F., Ferreira, A.M., Rall, V.L.M., Mondelli, A.L., Gomes, T.A.T., Camargo, C.H., Hernandes, R.T. Characterization of uropathogenic *Escherichia coli* reveals hybrid isolates of uropathogenic and diarrheagenic (UPEC/DEC) *E. coli*. Microorganisms, 2022; 10(3):645. https://doi.org/10.3390/microorganisms10030645

Teng, L., Feng, M., Liao, S., Zheng, Z., Jia, C., Zhou, X., Nambiar, R. B., Ma, Z., Yue, M. (). A Cross-sectional study of companion animal-derived multidrug-resistant *Escherichia coli* in Hangzhou, China. Microbiology spectrum, 2023; 11(2), e0211322. https://doi.org/10.1128/spectrum.02113-22

Valat, C., Drapeau, A., Beurlet, S., Bachy, V., Boulouis, H.J., Pin, R., Cazeau, G., Madec, J.Y., Haenni, M. Pathogenic *Escherichia coli* in dogs reveals the predominance of ST372 and the human-associated ST73 extra-intestinal lineages. Frontier Microbiology, 2020; 11:580. https://doi.org/10.3389/fmicb.2020.00580

Weese, J.S., Blondeau, J., Boothe, D. International Society for Companion Animal Infectious Diseases (ISCAID) guidelines for the diagnosis and management of bacterial urinary tract infections in dogs and cats. 2019; 247:8-25. https://doi.org/10.1016/j.tvjl.2019.02.008

World Health Organization (WHO). Critically important antimicrobials for human medicine. 6th revision WHO advisory group on integrated surveillance of antimicrobial resistance (AGISAR) 2019. [(accessed on 15 January 2022)].

Yaguchi, K., Ogitani, T., Osawa, R., Kawano, M., Kokumai, N., Kaneshige, T. Virulence factors of avian pathogenic *Escherichia coli* strains isolated from chickens with colisepticemia in Japan. Avian Diseases, 2007; 51(3):656–62. https://doi.org/10.1637/0005-2086(2007)51[656:VFOAPE]2.0.CO;2

Yousefi, A., Torkan, S. Uropathogenic *Escherichia coli* in the urine samples of Iranian dogs: Antimicrobial resistance pattern and distribution of antibiotic resistance genes. Biomedical Research International, 2017; 2017:4180490. https://doi.org/10.1155/2017/4180490

Yuri, K., Nakata, K., Katae, H., Tsukamoto, T., Hasegawa, A. Serotypes and virulence factors of *Escherichia coli* strains isolated from dogs and cats. Journal of Veterinary Medicine Science, 1999; 61(1):37-40. https://doi.org/10.1292/jvms.61.37

Zhou, Y., Ji, X., Liang, B., Jiang, B., Li, Y., Yuan, T., Zhu, L., Liu, J., Guo, X., Sun, Y. Antimicrobial resistance and prevalence of extended spectrum β-lactamase-producing *Escherichia coli* from dogs and cats in Northeastern China from 2012 to 2021. Antibiotics (Basel), 2022; 11(11):1506. https://doi.org/10.3390/antibiotics11111506

Zinsstag J., Meisser A., Schelling E., Bonfoh B., Tanner M. From 'two medicines' to 'One Health' and beyond. Onderstepoort Journal of Veterinary Researches, 2012; 79(2): 5. https://doi.org/10.4102/ojvr.v79i2.492