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Determination of pathogenic *E. coli* and antimicrobial resistance genes in dogs and human semen: Evidence of multidrug-resistance and antimicrobial resistance gene profiles

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ABSTRACT

Escherichia coli (*E. coli*) causes urethritis, epididymitis, epididymal-orchitis, and prostatitis in men. It also increases semen leukocytes (pus). This can impact the individual's reproductive capacity. This study was established for achieving the following aim: determination of resistant genes encoded in the DNA of the pathogenic *E. coli*, by isolating and identifying pathogenic *E. coli* using standard traditional methods and the Congo red test, followed by PCR targeting the phoA gene, which amplified at 720 bp, which confirmed isolated pathogenic *E. coli* strains. A total of 112 samples of semen were collected and laboratory examined microbiologically. The results showed that 3% of them were from dogs' seminal fluid, and 16.7% were from men. Antimicrobial sensitivity testing was carried out using 7 antibiotic groups, including trimethoprim/sulfamethoxazole (sulphonamides), erythromycin (macrolides), clindamycin (lincosamides), tetracycline (tetracyclines), vancomycin (glycopeptides), linezolid (oxazolidinones), and norfloxacin (fluoroquinolones). The results of this work showed that the five pathogenic *E. coli* strains isolated were resistant to four groups of antibiotics, exhibiting complete resistance (100%) to erythromycin (macrolides), clindamycin (lincosamides), vancomycin (glycopeptides), and linezolid (oxazolidinones). Because the isolated pathogenic *E. coli* strains were resistant to more than two antibiotic groups, they were recorded as multidrug-resistant strains. The PCR technique applied for the detection of resistance genes revealed that three of the four tested resistance genes: tetracycline (tetA) was positive (100%), trimethoprim (dfrA) was positive (75%), and the erythromycin (ermB) gene was positive (100%) in human samples. It was concluded that the determination of the main resistance genes of the isolated pathogenic *E. coli* was achieved.

1. INTRODUCTION

Escherichia coli (*E. coli*) is a member of the Enterobacteriaceae family, characterized by accelerated growth at 37 °C and tolerance to high temperatures up to 49 °C (Poor et al., 2024). It appears to be a Gram-negative, rod-shaped (bacillus), non-sporulated, flagellated, and usually facultative anaerobe (Jang et al., 2017). E. coli includes both pathogenic and nonpathogenic strains (Ramos et al., 2020). The pathogenic strains are considered one of the most important members of bacteria and influential microbes that infect humans and animals, leading to high economic losses (Cocco et al., 2023).

The multi-drug-resistant strains of pathogenic *E. coli* are associated with high economic losses, more than $40,000 per hospital encounter (Nelson et al., 2021). So this antimicrobial resistance (AMR) pattern represents a global danger for human and veterinary medicine, according to the World Health Organization (2021) and the World Organization for Animal Health (2024). E. coli strain represented a zoonotic disease because it could be transmitted between cats, dogs, and humans due to prolonged contact (Carvalho et al., 2016), as in the case of UTI well transmitted from dog to human or human to dog (Nielsen et al., 2022). The pathogenic *E. coli* strains can induce disease conditions in humans and animals by possessing specific virulence factors that help these bacteria to be colonized (biofilm formation) and cause infections in the host by the ability to adhere to the epithelial cell lining and produce their toxin or invade the tissues (Mueller and Tainter, 2023). Otherwise, the non-pathogenic *E. coli* strains do not possess the pathogenic virulence factors and biological phenomena (biofilm formation), but these bacteria are considered the normal micro-flora of the gut, which have benefic values as secreting vitamins (vitamin K) and prevent the colonization of pathogenic bacteria via the competition for nutrient substances and sites of attachment. Consequently, the differentiation between pathogenic and non-pathogenic *E. coli* must be done by an accurate, rapid, easy, and cheaper test, known as the Congo Red test.

Congo red test was achieved by using the Congo red dye for differentiation between pathogenic and non-pathogenic *E. coli* by coloration of the secreted amyloid (virulence factor) by the pathogenic *E. coli*. Congo red dye could be used either by dyeing (staining) the slide films or by adding it to the microorganism growth medium (Courtney et al., 2015).

Alkaline phosphatases, including three families (PhoA, PhoX, and PhoD), these families play an important role in the mineralization of organic phosphorus. PhoA is an essential component of Pho regulation for numerous microbes and has a lower distribution than PhoX and PhoD. (PhoA) can establish the di sulfide-bond via the formation of

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complexes by the peri-plasmic thiol through the di sulfide interchange protein DsbA (Elfageih et al., 2020). In the case of *E. coli*, the alkaline phosphatase PhoA induces the mineralization of phytic acids, phosphate monoesters, and phosphate diesters (Zhou et al., 2021). In addition to the precipitation of Ca phosphate (Cosmidis et al., 2015), the phoA gene was of wide use in the determination of protein localization and membrane topology due to its secretion to extracellular or peri-plasmic space via a protein channel in the *E. coli* membrane to make its product active when it was outside the plasma membrane (Zhou et al., 2021).

The phenotypic characteristics of pathogenic *E. coli* were represented as an important source of resistance genes, which play a significant role in both human and veterinary medical treatment (Joddha et al., 2023). Furthermore, PCR targeting antimicrobial resistance genes is an effective tool to identify antimicrobial resistance (Nasser et al., 2022). In this study, the studied genotypes of *E. coli* are characterized by the development of a wide range of resistance genes against a variety of antibiotics, such as tetracyclin (tetA), vancomycin (vanA), erythromycin (ermB), and trimethoprim/sulfamethoxazole (dfrA). Consequently, the aim of this work was established for the isolation and identification of pathogenic *E. coli*. Furthermore, the determination of resistance genes encoded on the DNA of the multi-drug-resistant (MDR) strains.

2. MATERIAL AND METHODS

2.1. Ethical Approval

The study was done according to an approved protocol by the Ethical Committee, Faculty of Veterinary Medicine, Benha University (BUFVTM16-08-24).

2.2. Samples

Seminal fluid samples (n = 112) from men (n = 12) from hospitals, clinics for In vitro Fertilization (IVF) and private medical analysis laboratories. The Seminal fluid samples from dogs (n = 100) were obtained from the Animal Reproduction Research Institute, Giza, veterinary clinics.

The seminal samples collected from short hair Germaine chipper breeds by disinfecting the perennial region by alcohol then gentile menstruation with discarding the pre-ejaculate watery substance following by collecting samples in sterile containers.

The seminal samples were collected after 2 to 5 days of sexual abstinence, following strict genital hygiene measures to avoid contamination of the collected samples in sterile screw-capped glass tubes (Noor et al., 2020). The seminal samples were collected from male cases with a history of not taking antibiotic treatments for about one week (WHO, 2021 and ISO, Geneva2021).

The collected samples must be freshly examined as soon as possible through 30-minute liquefaction at 37°C, with determination of volume (2.5 ml) of men samples while in dogs the volume (5-10 ml) and the pH are (Alkaline) for both men and dog samples. The optical microscope was used for tabulating the seminal enumeration and morphological characters (Scaruffi et al., 2023).

2.3. Isolation and identification of E. coli from seminal samples

The isolation and identification of *E. coli* from seminal samples were performed according to the methods described by McVey *et al.*, (2022); Basavaraju and Gunashree, (2023). Samples were inoculated into nutrient broth, and then the growing colonies were cultivated on the nutrient agar plates (McVey *et al.*, 2022). Sub-culture on the solid agar plates media such as blood agar (Oxoid CM 0271) and MacConkey agar (Oxoid CMO 0115). The isolated *E. coli* strains were purified by re-cultivation on Eosin Methylene Blue Agar (EMBA) plates (E. Merck, Darmstadt, Germany) with incubation at 37 °C for 24 h (Basavaraju and Gunashree, 2023), with incubation for 24-48 hrs at 37 °C in aerobic conditions with 5% CO2 tension (Maniarasu and Kumar, 2022). Morphological characteristics were demonstrated by gram staining of isolated strains (Basavaraju and Gunashree, 2023). Biochemical tests included IMVC (Indole production, citrate utilization test, methyl red, Voges-Proskauer test), as well as oxidase, catalase test, urease, and cultivation on the triglyceride sugar iron agar tubes (McVey *et al.*, 2022).

2.4. The differentiation between pathogenic and non-pathogenic of E. coli

The application of Congo red test for distinguishing of pathogenic *E. coli* from the non-pathogenic once, which was carried out by the addition of the Congo red dye either to the growing media in concentration of 0.5%, or to staining the glass slide film of *E. coli.* The Congo red dye neither affects the growth nor the quantitative rates and enumeration. (Courtney, 2015). The selected pathogenic *E. coli* by the Congo red test was undergoes the confirmatory test for identification.

2.5. In –vitro anti-microbial sensitivity test

Antimicrobial sensitivity tests were performed on the identified selective pathogenic *E. coli* strains in vitro using Mueller-Hinton agar (Oxoid, Hampshire, England) by the agar diffusion method. Seven antimicrobial groups of discs were used, including erythromycin (E/15 µg) (Bio analysis, Turkey), tetracycline (TE/30 µg) (Himedia, India), trimethoprim/sulfamethoxazole (SXT/25 µg) (Bio analysis, Turkey), vancomycin (VAN/30 µg) (Tm Media), clindamycin (DA/2 µg) (Bio analysis, Turkey), linezolid (LEN/30 µg) (Tm Media, India) and norfloxacin (NOR/10 µg) (Bio analysis, Turkey). The interpretation of the obtained results was determined according to the Clinical and Laboratory Standards Institute (CLSI 2021) (Table 1).

Table (1): Antimicrobial standardized discs, concentration, and interpretation of their effect (CLIS 2021) for pathogenic *E. coli*

Antimicrobial disks		Disk concentration	Zone of inhibition(mm)		
			Resistant <mm {R}	Intermediate mm range {IS}	Sensitive >mm {S}
Erythromycin	E/15	15 µg	13	14-22	23
Tetracyclin	TE/30	30 µg	14	15-18	19
Trimethoprim/sulphmethoxazole	SXT/25	25µg	10	11-15	16
Vancomycin	VAN/30	30 µg	14	15-16	17
Clindamycin	DA/2	2 µg	10	11-13	14
Linezolid	LEN/30	30 µg	15	16-20	21
Norflaxocin	NOR/10	10 µg	12	13-16	17

2.6. Molecular identification of phoA and some antibiotic resistance genes of E. coli

2.6.1. DNA extraction

The identified selected pathogenic *E. coli* strains were used for the extraction of DNA, by Qiagen Mini Kit (Hombrechtikon, Switzerland), (Am. 2016).

2.6.2. Molecular identification of phoA gene

The pathogenic *E. coli* strains were identified using PCR with specific primers. For molecular characterization, the primers set encoded for *pho*A of *E. coli* (Hu *et al.*, 2011), were used (Table 2). The PCR reaction was made by the using a total volume of 25µl of DNA template, consisted of 5pmol of each primer and 5µl of 1X PCR master (Cat. No.51304 Jena bioscience, GmbH, Germany). The thermal cycler (Perkinelmer, Walthan, USA), For the PCR mixtures, these thermal cycles were used; 50 ℃ for 2 min., (1 cycle), 40 cycles of 95 ℃ for 45 s, 50 ℃ for1 min., and 72 ℃ for 1 min., (1 cycle).

Table (2): Oligonucleotide primers sequences

Bacteria	Gene	Primer sequence (5'-3')	Length of amplified product	Reference
E. coli	*pho*A	*F* CGATTCTGGAAATGGCAAAAG *R* CGTGATCAGCGGTGACTATGAC	720bp	(Hu *et al.*, 2011)
	*tet*A	F GGTTCACTCGAACGACGTCA R CTGTCCGACAAGTTGCATGA	570 bp	Randall *et al.* 2004
	ermB	F GAAAAAGTACTCAACCAAATA R AATTTAAGTACCGTTACT	639 bp	Nguyen *et al.*, 2009
	*dfr*A	F TGGTAGCTATATCGAAGAATGGAGT R TATGTTAGAGGCGAAGTCTTGGGTA	425 bp	Grape *et al.*, 2007
	*van*A	F GGCAAGTCAGGTGAAGATG R ATCAAGCGGTCAATCAGTTC	763 bp	Maharjan *et al.*, 2021

Table (3): Thermal cycles of the primers during PCR

Bacteria	Gene	Primary denaturation	Secondary denaturation	Annealing	Extension	No. of cycles	Final extension
E. coli	*pho*A	94℃ 5min	94 ˚C 30sec.	55 ˚C 40sec.	72 ˚C 45sec.	35	72 ˚C 10min.
	*tet*A	94˚C 5 min.	94˚C 30 sec.	50˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
	*erm*B	94˚C 5 min.	94˚C 30 sec.	45˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
	*dfr*A	94˚C 5 min.	94˚C 30 sec.	60˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.
	*van*A	94˚C 5 min.	94˚C 30 sec.	54˚C 40 sec.	72˚C 45 sec.	35	72˚C 10 min.

3.6.3. Molecular identification of antibiotic-resistant gene

Genotyping detection of four antibiotics resistant genes, (*tet*A) tetracycline, trimethoprim (*dfr* A*)*, vancomycin (*van* A) and erythromycin *(erm* B) in five of pathogenic *E. coli* strains including (two from each human and three from each dogs) (Table2).

3. RESULTS

3.1. Prevalence of *E. coli* in collected seminal fluid samples:

The pathogenic *E. coli* isolates appeared as Gram-negative, medium in size, and rod-shaped bacterium. On nutrient agar and blood agar, the colonies exhibited non-pigmented bumps with beta hemolysis, while on MacConkey agar, they appeared as round brilliant pink non-mucoid bumps due to lactose fermenting action Moreover, the pathogenic *E. coli* displayed greenish metallic shine calories on EMB agar plates, indicating B-glucuronidase positivity. Furthermore, biochemical tests revealed coagulase positive with negative catalase and oxidase, as summarized in (Table4).

Table (4): Biochemical characters of isolated *E. coli*

Characteristics	*E. coli*
Gram Staining	Negative
Shape (Cocci/Diplococci/Rods)	Rods
Catalase	Negative (-ve)
Oxidase	Negative (-ve)
Coagulase	Positive(+ve)
MR	Positive (+ve)
VP	Negative (-ve)
Indole	Positive (+ve)
Citrate	Negative (-ve)
Urease	Negative (-ve)
Nitrate Reduction	Positive (+ve)
H2S	Negative (-ve)
Gas	Positive (+ve)
Lactose	Positive (+ve)
Glucose	Positive (+ve)

4.2. Differentiate between pathogenic and non-pathogenic *E. coli* isolates.

The traditional methods for identifying pathogenic *E. coli* may not provide accurate results. Therefore, it is crucial to use a special dye that can quickly differentiate between pathogenic and non-pathogenic *E. coli*. A study found five isolates of *E. coli* out of 112 seminal fluid samples, which accounted for human (n = 12) and dogs (n = 100), which isolated from human (7/12) then after subculture recorded (5/12) but about dogs isolated (25/100) after sub culture (12/100).

In this experimental work, Congo-red dye is used for staining glass films from pathogenic *E. coli* growing colonies or it is added as a 0.5% solution to the culture media. The results show completely red colonies of pathogenic *E. coli* associated with colorless colonies of non-pathogenic *E. coli*, either under a microscope or on the culture media plates, which accounted for pathogenic *E. coli* 4.5% of the samples (Table5). These isolates were present in human seminal fluid (2/12) 16.7% and dog seminal fluid (3/100) 3%, (Figure1).

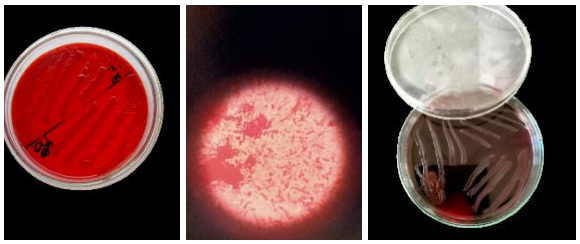

Figure (1-a): Biofilm-forming bacteria on Congo red agar plate Represented as whole red colonies of pathogenic E. coli,on Macconky agar plate. (fig1-b) Microscopic by oil emersion lenses showing ,pathogenic. *coli* staining with red color Congo red dye. (fig1-c) Dark red colonies clarified non-pathogenic E. coli, on Macconky agar plate.

Table (5): Prevalence of an incidence pathogenic *E. coli* isolation from different seminal fluid samples by using Congo red test

Seminal fluid samples	Number of samples	Negative samples		Positive samples	
		No.	%	No.	%
Human	12	10	83.3	2	16.7
Dogs	100	97	97	3	3
Total	112	107	95.5	5	4.5

*percentage in relation to total No. of each examined seminal fluid sample (12,100&112 for total)

PCR analysis had been demonstrating an amplification of the *pho*A gene of *E. coli* at 720 base pairs (Figure 2).

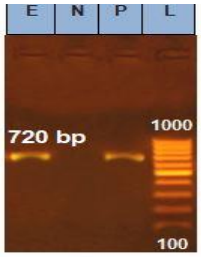

Figure 2: Agarose gel electrophoresis of phoA gene (720bp) *E. coli* L: 100bp ladder, P: Control positive phoA (720 bp) (N: Control negative, *E. coli* strain isolated from seminal fluid for human & dogs.

3.3 Antimicrobial resistance of *E. coli* isolates

Antibiogram sensitivity test recording that the pathogenic *E. coli* strains were completely sensitive to trimethoprim/sulfamethoxazole, tetracycline and norfloxacin (100%). On the other hand, they exhibited complete resistance (100%) to erythromycin, clindamycin, vancomycin and lenzolide, as indicated in the research findings presented in (Table 6).

Table (6): The effectiveness of various antimicrobial agents against pathogenic *E. coli* strains

Antimicrobial class	Antimicrobial Agent	Disc diffusion	Number of isolations Isolation %						A.A
			Sensitive		Intermediate		Resistance		
			No.	%	No.	%	No.	%	
1)Flote pathway inhibitions	Trimethoprim /Sulfamethoxazole Sxt	25mg	5	100%	0	0	0	0	S
2)Macrolides	Erythromycin E	15mg	0	0	0	0	5	100%	R
3)Lincomycins	Clindomycin Cli	2mg	0	0	0	0	5	100%	R
4) Tetracycline	Tetracycline Te	30mg	5	100%	0	0	0	0	S
5)Glycopeptid antibiotics	Vancomycin Van	30mg	0	0	0	0	5	100%	R
6)Linezolid	Lenzolid Len	30mg	0	0	0	0	5	100%	R
7)Fluor quinolones	Norfloxacin Nor	10mg	5	100%	0	0	0	0	S

A.A: Antibiogram activity %: Percent of resistant's group (7)

4.4 Molecular identification of antibiotic-resistant genes

In this research, the PCR technique was used to detect two resistance genes in pathogenic *E. coli* strains. The results showed that the gene (*tet*A) was present in (100%) of the samples, while the gene (*dfr*A) was present in (75%) of the samples. Additionally, the gene (*erm*B) was founded in (100%) of the human samples but was not detected in dog semen. The gene (*van*A) was present in (50%) of both human and dog seminal fluid samples. These findings were summarized in (Table 7 and Figure 3&4).

The genotypic analysis of the isolated pathogenic *E. coli* strains revealed the presence of several resistance genes. Specifically, the *tet*A gene associated with tetracycline resistance was detected in 100% of the isolates. Additionally, the *dfr*A gene conferring trimethoprim resistance was found in 75% of the samples, and the *erm*B gene encoding erythromycin (macrolide) resistance was present in 100% of the human samples.

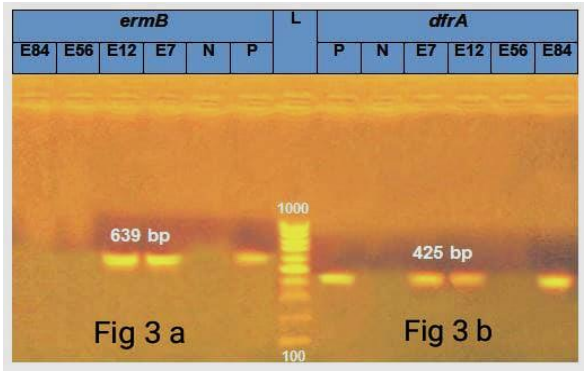

Fig (3-a): Erythromycin resistant gene (ermB). Lane L: 100-1000bp.DNA Ladder. Fig (3-b):Trimethoprim resistant gene (dfrA). Lane L: 100-1000bp.DNA Ladder

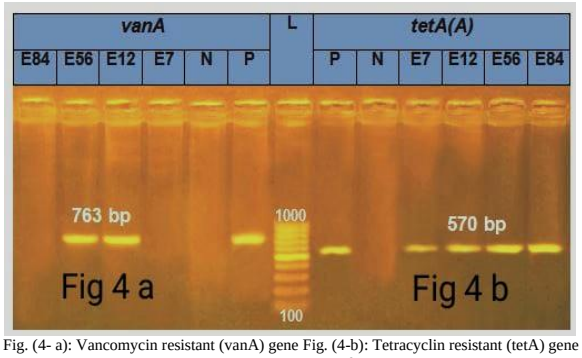

Fig. (4- a): Vancomycin resistant (vanA) gene Fig. (4-b): Tetracyclin resistant (tetA) gene Tetracycline (*tet* A) positive (100%), trimethoprim (*dfrA)* positive (75%) and ermB gene was positive (100%) in human samples.

Table (7): Screening of resistance genes for pathogenic *E. coli* strains

E. coli Sample ID	*ermB*	*VanA*	*tet A*	*dfrA*
7	+	-	+	+
12	+	+	+	+
56	-	+	+	-
84	-	-	+	+
Total NO.	2	2	4	3
%	50	50	100	75

*Human samples are (7&12) *dog samples are (56&84)

4. DISCUSSION

Pathogenic *E. coli* strains have been found in the seminal fluid of both humans and dogs, and they play an important role in sperm motility and vitality, leading to male infertility. This can affect successful breeding programs and raise health concerns for the offspring (Domrazek et al., 2024). Additionally, these strains are known to induce male accessory gland infections, especially prostatitis and epididymitis (Fijak et al., 2018).

The incidence of pathogenic *E. coli* in human seminal fluid samples in our study was 16.7%, which is lower than the rates reported by Uday et al. (2022) of 23.53% and Ďuračka et al. (2023) of 78.9%. However, our findings agree with Mu'azu et al. (2021), who reported an incidence of 14.7%. In seminal fluid samples from dogs, a recorded incidence of 3% was observed. However, noteworthy findings by Albaqly et al. (2022) and Lechner et al. (2023) reported substantially higher incidences of 14.7% and 26%, respectively.

The differentiation between pathogenic and non-pathogenic *E. coli* has historically been challenging due to the difficulty, expense, and time consumption of tests and advanced techniques required. However, the use of Congo red dye has provided a solution to these problems. Staining with Congo Red (CR) dye offers a qualitative means for detecting extracellular amyloids in both in vitro and in vivo settings (Yakupova et al., 2019). Specifically, only the pathogenic E. coli will bind this dye, resulting in a visible change in color and appearance. Interestingly, it has been observed that Congo red does not inhibit the growth of the tested microorganism, suggesting its potential for further research and application in the field (Courtney et al., 2015). Additionally, the presence of an extracellular adhesive amyloid fiber known as curli, which facilitates adhesion and accelerates biofilm formation, further underscores the importance of the Congo red staining method in differentiating between pathogenic and non-pathogenic E. coli (Figure 1). This test recorded the accurate number of pathogenic *E. coli* in both men and dogs' samples, which was two from men and three from dogs. Furthermore, PCR can detect the phoA gene at 720 bp, indicating the presence of pathogenic *E. coli* strains. Previous studies by Su et al. (2015) have extensively discussed and validated this approach (Table 2, Figure 2).

The prevalence of multidrug resistance in *E. coli* is a growing concern in both human and veterinary medicine on a global scale (El-Shazely et al., 2020). In the study of antibiotic sensitivity in our investigation, we used seven antibiotic group disks: trimethoprim/sulfamethoxazole (sulfonamide), erythromycin (macrolides), clindamycin (lincomycin), tetracycline (tetracycline), vancomycin (glycopeptide), linezolid (linezolid), and norfloxacin (fluoroquinolones). In the data tabulated in Table 6, it was found that the pathogenic *E. coli* strains exhibited complete sensitivity to three groups of drugs: sulfonamides (trimethoprim/sulfamethoxazole), tetracyclines (tetracycline) and fluoroquinolones (norfloxacin), with 100% susceptibility. Conversely, the same results showed a 100% resistance to erythromycin (macrolides), clindamycin (lincomycin), vancomycin (glycopeptide), and linezolid (linezolid), as indicated in Table 6. However, other previous research showed differences in some of the results of the current study, including that tetracycline had a high resistance percentage to E. coli (72.6%, 83.3%, 81.8%, and 73.6%), according to Mu'azu et al., 2021; Enwuru et al., 2020; and Ďuračka et al., 2023, respectively. In contrast, Faisal and Salman (2021) reported a lower resistant percentage of 40% and 47.3%, respectively.

In the use of erythromycin disks, high resistance rates were observed by Enwuru et al. (2020) and Swidan et al. (2020), who reported 95% and 87.5% resistance, respectively. However, some studies reported contrasting results. (Nasrallah et al., 2018) found a lower resistance rate of 22%, while Silago et al., 2020) observed complete sensitivity (100%), which differs from the findings of the current experiment. These varying results highlight the need for further investigations to better understand the resistance patterns of erythromycin against *E. coli* across different studies and geographical regions.

The reported results for the resistance of *E. coli* to trimethoprim/sulfamethoxazole show a wide range of findings across multiple studies: Nasrallah et al. (2018); Abbas et al. (2019), and Muhammed et al. (2022) all reported high resistance rates, with percentages of 64%, 54%, and 92.8%, respectively. (Silago et al., 2020; and Ahmed et al., 2022) both observed complete resistance (100%) to trimethoprim/sulfamethoxazole, which is the same result as the current study. In contrast, Li et al. (2014) reported a high sensitivity rate of 66%, while Olana et al. (2022–2023) found a relatively low sensitivity of 9.3%. These conflicting findings should be subjected to more research to explain the resistance patterns of trimethoprim and sulfamethoxazole across different geographic regions and study settings. This current study, as well as the study by Olana et al. (2022-2023), recorded complete resistance (100%) to vancomycin. In contrast, Nasrallah et al. (2018) detected a high sensitivity of 64% to vancomycin. (Silago et al., 2020) observed complete sensitivity (100%), which is not a sample to the current study. The complete resistance (100%) observed in the current study, as well as in the study by Olana et al. (2022-2023). The noted norfloxacin resistance rates for *E. coli* isolates across various studies demonstrate significant variations; Ahmed et al. (2022) reported an even higher norfloxacin resistance rate of 92.2%. In contrast, some studies have reported moderate to high levels of norfloxacin sensitivity: Shah et al. (2017) found that 55% of the *E. coli* isolates exhibited moderate sensitivity to norfloxacin, and Al-Jebouri and Mdish (2019) reported a moderate norfloxacin sensitivity rate of 46%.

The recorded Clindamycin susceptibility of *E. coli* isolates varies widely across different studies. Nasrallah et al. (2018) observed a moderate clindamycin sensitivity rate of 40% among the *E. coli* isolates. In contrast, Silago et al. (2020) reported complete clindamycin sensitivity with a 100% susceptibility rate. On the other hand, some studies have reported high levels of clindamycin resistance in E. coli. The current experiment conducted by the research team observed a complete clindamycin resistance rate of 100% among the *E. coli* isolates. Similarly, Swidan et al. (2020) also reported a 100% clindamycin resistance rate in their study.

The current study reported a complete linezolid resistance rate of 100% among the *E. coli* isolates. This finding is in stark contrast with the observations made by Nasrallah et al. (2018), who reported a linezolid sensitivity rate of 70% in their study.

The existing literature has well documented the concerning issue of pathogenic *E. coli* strains being represented as multidrug-resistant (MDR) agents. This worrying trend has been observed in both human and veterinary medicine settings. As highlighted by Poirel et al. (2018), the primary driver behind the emergence of MDR *E. coli* is the remarkable capacity of these strains to accumulate antibiotic resistance genes, primarily through the process of horizontal gene transfer. This ability to rapidly acquire and disseminate resistance genes across bacterial populations poses a significant challenge to effective treatment and infection control.

The PCR analysis of the *E. coli* isolates from the human samples revealed the following key findings: Tetracycline resistance gene (tetA): 100% of the isolates were positive for the tetA gene. The tetA gene was amplified at the expected

size of 570 bp (Figure 4-b), consistent with the observations reported by Li et al. (2014). Trimethoprim resistance gene (dfrA): 75% of the isolates were positive for the dfrA gene. The dfrA gene was amplified at the expected size of 425 bp (Fig. 3-b), further corroborating the findings of Li et al. (2014). Macrolide resistance gene (ermB): 100% of the isolates were positive for the ermB gene. The ermB gene was amplified at the expected size of 639 bp (Figure 3-a). Vancomycin resistance gene (vanA): The vanA gene was amplified at the expected size of 763 bp (Figure 4-a) (Table 7). The presence of a specific resistance gene in the DNA of the *E. coli* isolates does not necessarily mean that the gene is being actively expressed or functional. This important nuance requires further consideration in the interpretation of the PCR results. Let us expand on this concept: The detection of resistance genes, such as tetA, dfrA, ermB, and vanA, through PCR analysis indicates the presence of the genetic determinants for these resistance mechanisms. However, the mere presence of these genes does not automatically imply that they are being expressed or conferring functional resistance to the corresponding antibiotics. In other words, the presence of the resistance genes, as detected by PCR, does not necessarily equate to the actual expression and functionality of the resistance phenotype. The bacteria may harbor the genetic potential for resistance, but the resistance may not be actively manifested or confer a selective advantage to the bacteria under the given conditions. The discrepancy between the phenotypic resistance and genotypic resistance observed in this experiment is an important aspect to address.

In this experiment, tetracycline, when tested by the antibiotic sensitivity test, showed 100% susceptibility, while the result of PCR was estimated to be the presence of a resistance gene (tet A). Instead of the Erythromycin, in dog-tested samples it was explained as 100%) resistant, but the PCR recorded the absence of resistance gene (ermB), This finding aligns with the observations reported by Nguyen et al. (2009), where the presence of resistance genes did not necessarily correlate with the phenotypic resistance profile.

These discrepancies between genotpic and phenotypic resistance highlight the complex and multifaceted nature of antimicrobial resistance. They emphasize the importance of utilizing a combination of genotypic and phenotypic approaches to fully understand the resistance profile of bacterial isolates and inform appropriate treatment strategies.

Further investigations, such as whole-genome sequencing, gene expression analysis, and comprehensive antibiotic susceptibility testing, may help elucidate the underlying mechanisms responsible for these observed discrepancies.

The primary aim of this study, as you mentioned, was to determine the prevalence and antimicrobial susceptibility of E. coli from clinical samples. This information is crucial for understanding the epidemiology of *E. coli* infections and guiding appropriate treatment strategies. Finally, the isolation of pathogenic *E. coli* from the seminal fluid of both human and dog samples is a significant finding that highlights the importance of preventing the spread of these microorganisms.

5. CONCLUSIONS

This study emphasizes the importance of using phenotypic and genotypic approaches for fully identifying *E. coli* isolates' antibiotic resistance patterns. This data is essential for appropriate antibiotic selection and E. coli infection treatment for humans and animals.

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