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A New Blend of Phenotypic and Genotypic Application as a Zoonosis *Escherichia coli* Transmission Detector

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ABSTRACT

Aims *Escherichia coli* is a Gram-negative bacteria classified into non-pathogenic and pathogenic serotypes. This study aimed to blend phenotypic and genotypic techniques to identify *Escherichia coli* isolated from patients and dogs.

Materials & Methods 80 samples were collected from humans and dogs with UTI or diarrhea cultured on MacConkey, EMB, and nutrient agars. The suspected *E. coli* bacteria was confirmed using a PCR-based molecular method. Two bacterial identification techniques were applied to detect the zoonotic transmission possibility; a phenotypic analyzer using Antibiotic Susceptibility test Patterns and molecular genotypic technique depending on the presence of four exact genes in each isolate.

Findings All the isolated *E. coli* were multi-drug and strongly resistant (100%) to Amoxicillin+clavulanic acid, Oxacillin, and Vancomycin. Meanwhile, they were highly susceptible to imipenem (100%). The ASP5 (i) was the dominant pattern among human and animal isolates and the most common shared pattern among the four groups of samples. The results showed that 31 of 50 (62%) isolates have similar ASPs; however, only 16 (53.3%) shared the same phylogenetic groups. Furthermore, the molecular genetic group C is highly prevalent in dog isolates, whereas group E was the commonest among human isolates.

Conclusion Both methods would be more accurate and better explain bacterial transmission and acquiring new antibiotic-resistance genes.

Keywords Public Health; Zoonotic Diseases; Clermont Phylotyping; *Escherichia coli*; Antibiotic Sensitivity Profile

CITATION LINKS

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Introduction

Escherichia coli is a Gram-negative, facultatively anaerobic, rod-shaped, coliform bacterium, which is an important part of the healthy intestinal tract of humans and animals [1, 2]. *Escherichia coli* is a bacterium belonging to the Enterobacterales order and the Enterobacteriaceae family, a group of bacteria commonly found in the intestines of warm-blooded organisms, including humans and animals. As a species, *E. coli* is one of the most versatile and widely studied microorganisms due to its wide range of pathogenic and non-pathogenic strains, making it an essential component of human and animal gut microbiota. The bacterium's ability to thrive in diverse environments has contributed to its presence in healthy individuals and those suffering from infections [3]. In addition to being a part of the normal gut flora, some strains of *E. coli* have evolved into significant pathogens capable of causing a range of diseases, including urinary tract infections, gastrointestinal illnesses, and even systemic infections [4].

More than 160 serotypes of *E. coli* have been identified, each categorized based on their surface antigens. These antigens include the H antigen, which represents the components of flagella responsible for motility; the O antigen, which refers to the oligosaccharide polymer found on the outer membrane of the bacterium; and the K antigen, which is associated with the polysaccharides of the capsule that help the bacteria evade host immune responses. These surface antigens are crucial in identifying, classifying, and pathogenesis of various *E. coli* strains, making them an important part of microbial taxonomy and immunology [3].

E. coli is not a uniform species but a complex and genetically diverse organism. Based on genetic substructures and phylogenetic analysis, *E. coli* can be divided into several groups. These groups include A, B1, B2, C, D, E, F, and clade. Each group is associated with a distinct set of genetic markers contributing to the bacterium's survival ability in different environments [5]. Pathogenic *E. coli* strains, which cause infections outside the intestine, are most commonly found in groups B2 and D. These groups have been linked to extra-intestinal infections such as urinary tract infections (UTIs), bloodstream infections, and meningitis. On the other hand, non-pathogenic *E. coli* strains, which are typically harmless and found as part of the normal gut flora, are most often categorized into groups A or B1 [4, 5]. Groups E and F are considered to be associated with the primary groups B2 and D, providing further genetic insight into the bacterium's phylogenetic relationships.

An interesting aspect of *E. coli* classification is that some strains exhibit similar phenotypic characteristics but possess different genetic structures. These strains are classified under a hidden clade called clade I [6]. This phenomenon highlights the complexity and adaptability of *E. coli*, as well as the challenges in distinguishing strains based solely on outward appearance or behavior. Despite these variations, all strains of *E. coli*, pathogenic or not, share the ability to colonize the human gastrointestinal tract shortly after birth, which underscores the bacterium's important role in the gut microbiota [7].

E. coli is a commensal bacterium, which means it exists in a symbiotic relationship with its host, providing certain benefits while generally not causing harm. As a facultative anaerobe, *E. coli* thrives in environments where oxygen levels vary, such as in the human gut. Its ability to adapt to anaerobic conditions allows it to colonize various parts of the intestines, competing with other microorganisms for nutrients and space. This competition is a significant part of how *E. coli* maintains its presence in the gut. *E. coli* is the most prevalent facultative anaerobe in the human intestinal microbiota, making it a dominant player in maintaining a balanced microbial ecosystem [8].

However, despite its commensal nature, certain strains of *E. coli* have acquired pathogenic potential, turning it into a major cause of infections in humans and animals. These pathogenic strains often harbor virulence factors that enhance their ability to adhere to and invade host tissues, evade immune responses, and produce toxins that can cause severe diseases. Pathogenic strains, such as uropathogenic *E. coli* (UPEC), are responsible for urinary tract infections, while enterohemorrhagic *E. coli* (EHEC) can cause foodborne illnesses and hemolytic uremic syndrome. The genetic diversity of *E. coli* and its ability to evolve rapidly through horizontal gene transfer makes it an ever-present challenge for healthcare systems worldwide [7-9].

Generally, *E. coli* is considered a non-pathogenic bacteria, but some serotypes can cause diseases inside and outside of the intestinal tract. Two of the most common diseases these bacteria contribute to are various cases of diarrhea and urinary tract infections, which are also common between animals and humans [10]. However, due to the abundance and incorrect use of antibiotics, *E. coli* has become capable of developing resistance to many of the available antibiotics prescribed by many doctors and veterinarians [11]. One of the methods used to determine the optimal choice of antibiotics to eliminate these bacteria and monitor the extent of their development and sensitivity to specific antibiotics is to perform an antibiotics sensitivity test, which is a laboratory procedure performed to determine the extent of the effect of antibiotics on various types of bacteria and fungi [12].

Approximately 80% of urinary tract infections (UTIs) are caused by extraintestinal pathogenic *Escherichia coli* (ExPEC) isolates [8]. These ExPEC isolates have

become increasingly resistant to first-line antibiotics such as ciprofloxacin and trimethoprim, with resistance to these drugs frequently reported in Europe, America, and many parts of Asia. The rising prevalence of extended-spectrum beta-lactamases (ESBLs) in ExPEC, particularly non-TEM/SHV ESBLs such as CTX-M enzymes, has become a serious clinical issue globally, especially in the past decade [4]. The emergence of antibiotic-resistant superbugs is thus a global problem [4].

In 2000, Clermont and colleagues described a molecular genetic-based technique (triplex PCR) that can divide *E. coli* isolates into four phylogroups [13]. But with a growing body of multi-locus sequence data and genome data of *E. coli*, a new PCR-based method (over 95% validation) is developed so that an *E. coli* isolate can be assigned to one of eight genetic phylogroups (A, B1, B2, C, D, E, F) and cryptic clades (II to V) for identification [14].

So, this study aimed to blend two different classification methods: Phenotypic (antibiotic sensitivity profile) and genotypic (Clermont genetic phylotyping) techniques to identify *Escherichia coli* isolated from patients and dogs suffering from urinary tract infection or diarrhea to determine the zoonotic transmission possibility of these bacteria, which will offer a good tool for controlling and curing the effect of these causative agents on animal and human public health.

Materials and Methods

Sample collection

Eighty samples were collected from two groups; 40 from humans and 40 from dogs. Each group is evenly divided into urine and stool samples. Human samples were obtained from Al-Sadr Teaching Hospital and Basra Teaching Hospital patients. As for the dog samples, they were collected from various veterinary clinics and hospitals in Basra Governorate. Samples collection was done using swabs and collection cups, then transported directly to the central laboratory at the College of Veterinary Medicine, University of Basrah, within a period not exceeding two hours to perform bacterial culture.

Isolation and identification of *E. coli*

Samples were directly swabbed on MacConkey and nutrient agar (as a control) and incubated at 37°C for 24 hours. After the first incubation, well-defined single pink colonies were picked up and streaked on Eosin-methylene blue (EMB) agar using a sterile loop. Plates were then incubated at 37°C for 24 hours. Typical single metallic green sheen color colonies were noticed.

DNA extraction

All bacterial isolates' genomic DNA (gDNA) was extracted using boiling [15]. Bacteria were cultured in 5ml nutrient broth containing falcon tubes and incubated in a shaking incubator at 37°C, 180rpm for 24 hours. The bacterial pellet was harvested by centrifugation at 5000rpm for 5 minutes, then resuspended with 100µL molecular grade water and transported into a clean 1.5ml Eppendorf tube. Subsequently, the Eppendorf tube was incubated in a water bath at 100°C for 10min. Then 450µL of molecular grade water was added to the components and mixed well, followed by a 10-minute centrifugation at 12,000rpm. The supernatant (containing the gDNA) was collected in a new Eppendorf tube and stored at -20°C until used.

Molecular detection of *E. coli*

A species-specific pair of primers confirmed the isolated *E. coli* identity by partially amplifying the 16S rRNA gene. A 20µL PCR reaction mix was prepared by adding 10µL GoTaq green master mix, 1µL of 10pmol/µL forward primer and reverse primer, 3µL template DNA, and 5µL nuclease-free water. The mixture was subjected to a PCR amplification program started with an initial denaturation step at 95°C for 5min, followed by 35 cycles for each of the following steps: Denaturation 94°C/30sec, annealing 62°C/30sec, extension 72°C/30sec, ending with a final extension step at 72°C/3min and held at 4°C. The PCR products were analyzed and size-fractionated on 1.5% agarose gel. The amplicon's sizes were measured by loading a 100bp standard DNA ladder (Promega; USA) alongside the samples. The gel was run in a TBE buffer at 100V (constant voltage) for 30 minutes. The DNA bands (replicons) were displayed on the UV transilluminator safety imaging system. The Band's size was estimated by comparison to the DNA ladder bands.

Table 1. Primers used in this study to detect and classify *Escherichia coli*

Genes	Primer sequence (5´ to 3´)	Amplicon size	References
16S rRNA	F: GACCTCGGTTTAGTTCACAGA R: CACACGTGACGCTGACCA	585 bp	Wang *et al.* 1996 [20]
arpA	F: AACGCTATTCGCCAGCTTGC R: TCTCCCCATACCGTACGCTA	400 bp	Clermont *et al.* 2013 [21]
chuA	F: ATGGTACCGGACGAACCAAC R: TGCCGCCAGTACCAAAGACA	288 bp	
yjaA	F: CAAACGTGAAGTGTCAGGAG R: AATGCGTTCCTCAACCTGTG	211 bp	
tspE4.C4	F: CACTATTCGTAAGGTCATCC R: AGTTTATCGCTGCGGGTCGC	152 bp	
trpAgpC.1	F: AGTTTTATGCCCAGTGCGAG R: TCTGCGCCGGTCACGCCC	220 bp	
ArpAgpE.f	F: GATTCCATCTTGTCAAAATATGCC R: GAAAAGAAAAAGAATTCCCAAGAG	301 bp	

F: Forward primer; R: Reverse primer

Antibiotic susceptibility test of *E. coli*

Preparation of McFarland

A Barium chloride solution of 1% concentration was prepared by dissolving 1gm of anhydrous barium chloride in 99ml distilled water; also, 1% of H_2SO_4 was prepared by mixing 1 ml of (H_2SO_4) with 99ml distilled water. Then, McFarland (0.5) is achieved by properly mixing 50µl of 1% Bacl2 with 9.95 ml of 1% H_2SO_4. To adjust the bacterial solution to McFarland standard, both tubes (bacterial suspension, McFarland) were held together side by side in front of a good light source to compare and match the turbidity of one another properly.

Preparation of bacterial inoculum

The bacterial isolates were streaked on nutrient agar and incubated for 24 hours. A few colonies were picked up and put inside a 4ml normal saline-containing tube. Vortex was applied, and the bacterial suspensions were adjusted to the 0.5 McFarland standard [16]. Subsequently, bacterial colonies were added until they reached the right concentration. The resulting bacterial suspension was used to inoculate Molar Hinton agar for the antibiotic susceptibility test.

Applying antibiotic disks

Several types of commercially available antibiotic disks were used. The disks were evenly placed and lightly pressed on the surface of a cultured Mueller Hinton Agar plate (5 disks/plate) as the antibiotic of interest diffused into the agar. A gradient concentration was created; the highest concentration was the nearest to the applied disk, and it gets lower when far from it [17]. After 24 hours of incubation at 37°C, the formed inhibition zones around the antibiotic disks were measured using a centimeter scale. Then, the obtained inhibition zone diameters were analyzed by comparing them to the standard measurements [18].

Antibiotic Susceptibility Patterns (ASP)

Specific bacterial categories were made by observing the resistance ability of the tested bacterial isolates against several antibiotics [19]. Each category includes the same antibiotics susceptibility-behaved bacteria. The final resulting behavior was the antibiotic susceptibility patterns. Each ASP (present in this study) was labeled with a unique number starting from 1 to 9, regarding the total number of antibiotic resistances. Number 1 is the highest. Furthermore, sub-patterns were recorded in each ASP regarding resistance to the type of antibiotics.

***Escherichia coli* molecular genetic phylotyping classification (Clermont method)**

A method described by Clermont and colleagues was applied [14]. They proved that *E. coli* strains can be assigned to one of eight molecular phylogroups (A, B1, B2, C, D, E, F) and cryptic clades (II to V) regarding the presence of particular genes (arpA, chuA, yjaA and tspE4.C4) in their genome. Which made a novel identification method for *E. coli* isolates. A PCR-based technique was used to identify these genotypes. Two types of PCR approaches (multiplex and uniplex) were applied. The multiplex PCR was used to identify types A, B1, B2, C, D, E, and F. Whereas uniplex PCR was used to differentiate between types A and C, E and D, E, and clade I by amplifying trpAgpC.1 and ArpAgpE.f genes, respectively. A 25µL multiplex PCR reaction was carried out using four specific pairs of primers to amplify arpA, chuA, yjaA, and tspE4.C4 (Table 1). The PCR reaction tube contains 12.5µL GoTaq green master mix, 1µL of each of the forward and reverse primers, 3µL gDNA template, and 2µL nuclease-free water. The PCR program started with the initial denaturation step 94°C/4 min followed by 30 cycles of the following steps (denaturation 94°C/5sec, annealing 59°C/20sec, extension 72°C/20sec), ending with final extension step 72°C/5min. The PCR products were run on 1.5% agarose gel and visualized under UV.

A blend of phenotypic and genotypic identification techniques

A newly suggested dual-detecting system (a blend of ASP and Clermont) was used to obtain a pheno-geno-bacterial identity. Which relies on *E. coli* phenotype and genotype characteristics obtained from the ASP sub-groups and Clermont phylotypes, respectively. The resulting identity technique is called the blend.

Findings

The findings showed that 60 (75%) isolates had pink colonies when grown on MacConkey agar. However, only 50 (62.5%) isolates appeared with a metallic-green sheen when grown on Eosin-methylene blue (Figure 1).

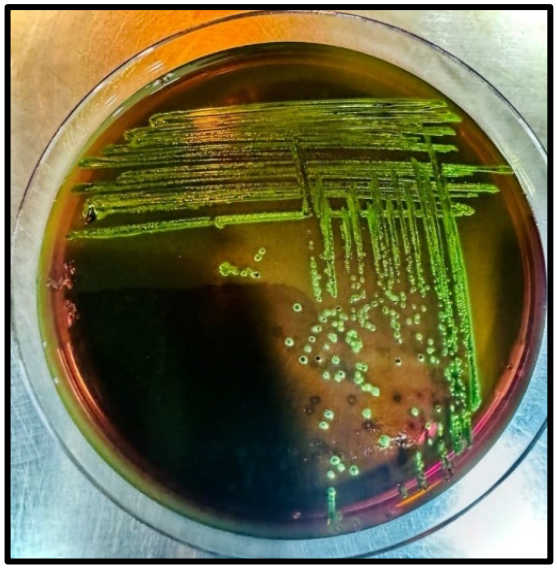

Figure 1. *Escherichia coli* colonies on EMB agar. Small, rounded, distinctive metallic green sheen colonies can be clearly notified

All 50 isolates were confirmed as *E. coli* using a molecular genetic detection technique that partially amplifies the 16SrRNA gene utilizing species-specific primers in the polymerase chain reaction technique (Figure 2).

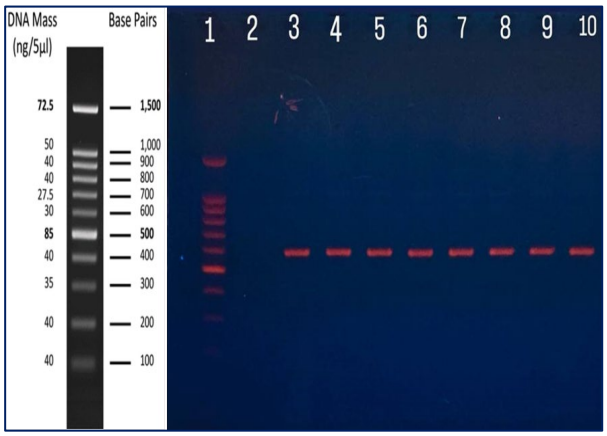

Figure 2. An agarose gel image displays the partially amplified 16S rRNA gene of the tested *E. coli* using a species-specific pair of primers. One clear band was reported at approximately 585 bp for each bacterial sample. Lane 1: DNA ladder 100 bp (Promega; USA), lane 2: Negative control, lanes 3-10: Single amplicon approximately 585bp

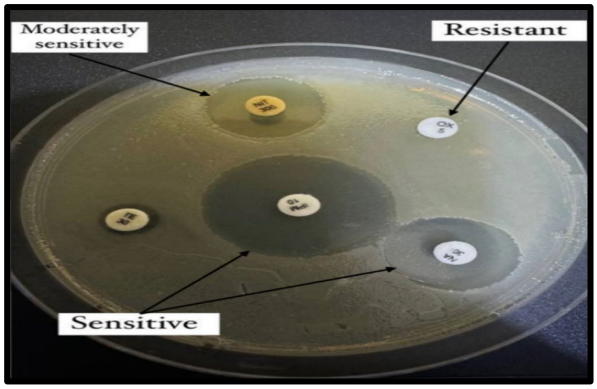

Figure 3. Antibiotic sensitivity test results. All the tested isolates were categorized into sensitive, resistant or intermediate, after being grown on Muller Hinton agar against all the utilized antibiotics by comparing the produced inhibition zones with the standard measurements

Table 2. Shows the number of resistant/isolates according to the used antibiotics

Antibiotics class	Antibiotic	Dogs		Human		Total
		Urine	Fecal	Urine	Fecal	
Aminopenicillins	Amoxicillin+clavulanic acid	3/3	17/17	10/10	20/20	50/50
Penicinillase	Oxacillin	3/3	17/17	10/10	20/20	50/50
Glycopeptides	Vancomycin	3/3	17/17	10/10	20/20	50/50
Carbapenems	Imipenem	0/3	0/17	0/10	0/20	0/50
Macrolides	Erythromycin	3/3	13/17	10/10	20/20	46/50
Aminoglycosides	High-level gentamycin	0/3	2/17	2/10	2/20	6/50
Lincosamides	Clindamycin	3/3	14/17	10/10	19/20	46/50
Aminoglycosides	Amikacin	0/3	1/17	2/10	5/20	8/50
	Kanamycin	0/3	0/17	5/10	6/20	11/50
Fluorquinolones	Ciprofloxacin	0/3	3/17	5/10	7/20	15/50
	Levofloxacin	0/3	1/17	3/10	5/20	9/50
Macrobid	Nitrofurantoin	2/3	8/17	2/10	0/20	12/50
Tetracyclines	Tetracycline	2/3	9/17	9/10	16/20	36/50
Cephalosporins 3rd generation	Ceftazidime	2/3	16/17	7/10	12/20	37/50

The prevalence rate of *E. coli* observed during the current study was 3 (15%) and 10 (50%) isolates in urine and 17 (85%) and 20 (100%) isolates in fecal samples from humans and dogs, respectively. Most isolated *E. coli* were multidrug-resistant (MDR; Figure 3).

Table 3. The antibiotic susceptibility profiles (phenotypic) and the Clermont genotypes (genotypic) distribution and repetition of *E. coli* isolates identified in human and dogs

Classification type	Human	Dogs
Antibiotic susceptibility profile/repetition		
ASP1	2	0
ASP2	6	1
ASP3	5	2
ASP4	3	5
ASP5	5	5
ASP6	6	4
ASP7	3	1
ASP8	0	1
ASP9	0	1
Clermont genotypes/repetition		
A	3	5
B1	5	3
B2	5	-
C	2	10
D	-	-
E	9	-
F	2	1
Clades	-	-

Antibiotic susceptibility testing was performed on all isolates using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (MHA). Fourteen antibiotics were used (Table 2), as the diversity in their choice depended on the different families of antibiotics available. Almost all isolates (100%) were resistant to Amoxicillin/clavulanic acid (AMC) and oxacillin but were sensitive to imipenem and high-level gentamycin.

The resulting Clermont genotypes were compared across the tested *E. coli* isolates. Six (A, B1, B2, C, E, and F) and four (A, B1, C, and F) genotypes were identified in human and dog isolates, respectively (Table 3; Figure 4).

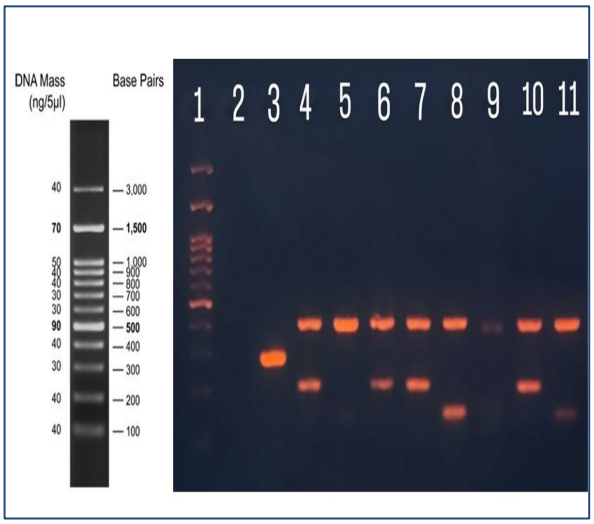

Figure 4. Agarose gel electrophoresis image of multiplex PCR for *E. coli* molecular genetic phylotyping classification. Lane 1: DNA ladder 100bp (Promega, USA); Lane 2: Negative control; Lane 3: Approximately 288bp of chuA gene (F phylogroup); Lane 4, 6, 7 and 10: Two bands approximately 400bp and 211bp of arpA gene and yjaA gene, respectively (A or C phylogroups); Lane 5: Single band 400bp arpA gene (A phylogroup); Lane 8 and 11: Two bands approximately 400bp and 152 bp of arpA gene and TspE4.C2, respectively (B1 phylogroup)

The major type reported in human isolates was E, followed by B1, B2, and A, whereas type C was the dominant type among dog isolates, followed by A and B1. However, type E did not appear in dog samples.

The current data clarified the presence of identical genotypes of different isolates from different sources. Approximately 53.3% of the same ASP sub-types shared the exact Clermont genotype. For example, almost all ASP2 (ii), ASP1 (i), and ASP7 (iv) isolates harbored Clermont type B1, B2, and E, respectively (Table 4).

The newly suggested blend detecting system (dual of ASP and Clermont) gave fewer shared types but is more specific and showed that the ASP5 (i) C and ASP2 (ii) B1 are the most possible zoonotic *E. coli* isolates (Figure 5).

Table 4. Antibiotic susceptibility pattern of the isolated *E. coli*

ASP	No. of isolates	Sub pattern	Resistant antibiotics (number of isolates)		No resistant antibiotics
ASP1	2	(i)	AMC, OX, VA, E, CD, AK, K, CIP, LEV, TE, CAZ	(2)	11
ASP2	7	(i)	AMC, OX, VA, E, CD, CIP, TE, CAZ, NIT, HLG		10
		(ii)	AMC, OX, VA, E, CD, AK, K, CIP, TE, CAZ	(3)	
		(iii)	AMC, OX, VA, E, CD, K, CIP, LEV, TE, HLG		
		(iv)	AMC, OX, VA, E, CD, CIP, LEV, TE, CAZ, NIT	(2)	
ASP3	7	(i)	AMC, OX, VA, E, CD, CIP, NIT, TE, CAZ		9
		(ii)	AMC, OX, VA, E, CD, LEV, NIT, TE, CAZ		
		(iii)	AMC, OX, VA, E, CD, AK, K, TE, CAZ		
		(iv)	AMC, OX, VA, E, CD, CIP, LEV, TE, CAZ	(2)	
		(v)	AMC, OX, VA, E, CD, K, NIT, TE, CAZ		
		(vi)	AMC, OX, VA, E, HLG, CD, K, CIP, TE		
ASP4	8	(i)	AMC, OX, VA, E, CD, NIT, TE, CAZ	(4)	8
		(ii)	AMC, OX, VA, E, CD, CIP, NIT, CAZ		
		(iii)	AMC, OX, VA, E, HLG, CD, TE, CAZ		
		(vi)	AMC, OX, VA, E, CD, AK, TE, CAZ		
		(v)	AMC, OX, VA, E, CD, K, CIP, TE		
ASP5	10	(i)	AMC, OX, VA, E, CD, TE, CAZ	(8)	7
		(ii)	AMC, OX, VA, E, CD, NIT, CAZ		
		(iii)	AMC, OX, VA, E, CD, LEV, TE		
ASP6	10	(i)	AMC, OX, VA, CD, NIT, CAZ		6
		(ii)	AMC, OX, VA, AK, NIT, CAZ		
		(iii)	AMC, OX, VA, E, CD, CAZ	(3)	
		(vi)	AMC, OX, VA, E, CD, NIT		
		(v)	AMC, OX, VA, E, CD, TE	(3)	
		(vi)	AMC, OX, VA, E, K, TE		
ASP7	4	(i)	AMC, OX, VA, CD, CAZ		5
		(ii)	AMC, OX, VA, E, CD	(3)	
ASP8	1	(i)	AMC, OX, VA, E		4
ASP9	1	(i)	AMC, OX, CAZ		3
Total	50	29		(50)	-

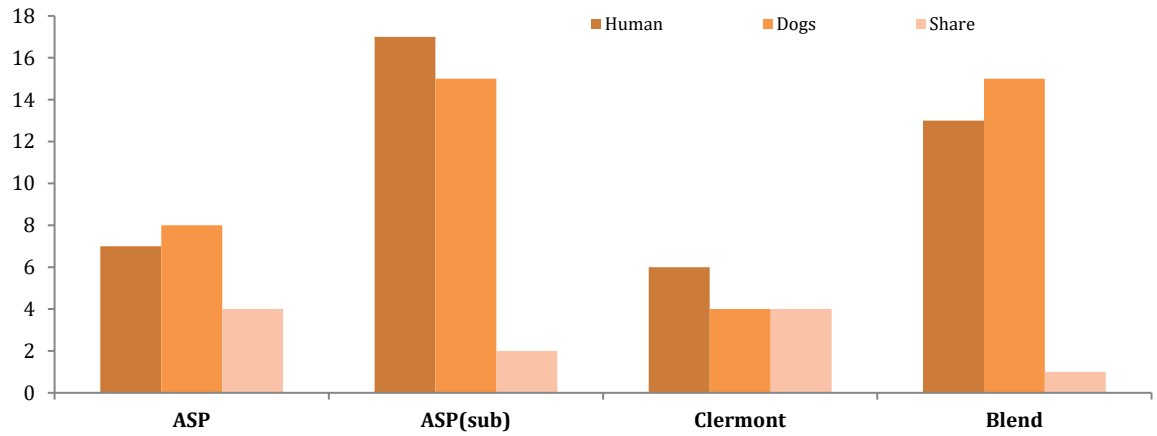

Figure 5. A schematic diagram of phenotypic (antibiotic susceptibility profile), genotypic (Clermont molecular genotypes), and the blend (dual detection) categories distribution and relationship of *Escherichia coli* strains isolated from humans and dogs. The blue columns represent the proportions of human isolates compared to dog isolates (orange columns) and the shared profiles (between human and dog isolates) in the grey columns.

Discussion

Most *E. coli* strains are commensals inhabiting the intestinal tract of humans and warm-blooded animals [1]. However, some strains are pathogenic and can cause various diseases such as diarrhea, pneumonia, bacteremia, urinary tract infection (UTI), neonatal meningitis, and uncomplicated cholecystitis [21]. Since diarrhea and urinary tract infections are the

most common zoonotic diseases caused by *Escherichia coli* [22], the current study focused on samples collected from patients and dogs suffering from either diarrhea or urinary tract infections.

Detection of this kind of *E. coli* isolates from dog fecal samples is one health concern. The presence of this type of bacteria in pets could be a serious threat to human health [23]. Furthermore, it would reduce the efficient treatment of bacterial diseases [24]. The prevalence rate of MDR *E. coli* observed during the current study in diarrheic dogs was 90%, which was higher than that recorded in research conducted in China [25], which detected 54.81% (n=74 of 135) MDR *E. coli* from diarrheal samples of dogs. This finding could be related to the daily feeding style and food type, particularly raw feed, as several studies reported that feeding dogs raw meat can increase the percentage of multi-drug resistance *E. coli* in their feces [26-29], as the majority of the studied samples came from, at least one uncooked meal per day feeding dogs. This would allow this bacteria to be a causative agent of intestinal infection with clinical diarrhea.

To our knowledge, this is the first study conducted on the prevalence of MDR *E. coli* in UTI cases of dogs in Iraq. On the contrary, many other countries have conducted reports of MDR bacteria in their animals [22, 30-32]. In the field of veterinary medicine, poor empirical decisions, the use of antibiotics for nonbacterial illnesses, owners' mistakes in administration, and prolonged treatment duration are the primary causes of poor patient outcomes and main factors in developing multi-drug resistance bacteria [33].

The current study reported that half of the tested dog's urine samples were positive for bacterial growth tests, all containing MDR *E. coli*. This finding aligns with Garces *et al*. [32], who reported that approximately 44.5% of dog UTI cases were related to *E. coli*. Moreover, the results were slightly lower than Aurich *et al.* [22] and Smoglica *et al*. [30] reported in Germany and Italy, respectively. This might be related to the difficulty in UTI diagnosis and treatment.

E. coli was found in just 15% of human urine samples associated with UTIs, and every isolate demonstrated multidrug resistance. This finding was slightly lower than other studies conducted in Iraq, such as Mohammed *et al*. [34], who reported that approximately 23.5% of the human UTI-causing *E. coli* were multidrug resistant. A possible explanation of these variances might be related to the time of sample collection during the year and the presence of other UTI-causing pathogens. The current study showed that almost all human fecal samples (96%) were positive for MDR *E. coli* tests. A possible reason to explain this observation is the presence of *E. coli* bacteria naturally in some parts of the digestive tract (such as the intestine) as a normal flora, making it easily reported in fecal samples [35]. Furthermore, how antibiotics are misused and administered would activate the bacterial defense tools, therefore developing MDR bacteria [36-39].

Nearly all the isolates (100%) resisted Amoxicillin/clavulanic acid (AMC) and oxacillin, yet they showed susceptibility to imipenem and high-dose gentamycin. These results reflect those of Naqid *et al*. [40], who also found that the studied *E. coli* have a high resistance rate against AMC and are susceptible to imipenem. Surprisingly, the majority of the isolates were resistant to vancomycin. This finding could be related to the accumulation of phosphatidic acid inside the bacterial cells because of a genetic disorder that alters the outer membrane's physical properties, making it very difficult for vancomycin to enter the periplasm and reach its target [41]. Remarkably, all human samples were resistant to Erythromycin; meanwhile, dog samples were resistant to ceftazidime. A possible explanation for these results may be the difference in type, reason, and rate of drugs used in the human and veterinary fields.

The bacterial isolates' antibiotic susceptibility was recorded as Antibiotic Susceptibility Patterns (ASP). Human samples exhibited 7 main patterns with 17 sub-patterns across 30 isolates, while dogs showed eight main patterns with 15 sub-patterns. The presence of this diversity in antibiotic resistance ability within the same type of bacteria (*E. coli*) reflects the extent of the risk of the response to curing and disease control, which will be more dangerous if these bacteria were transmitted to another organism, such as humans or vice versa. Interestingly, the results demonstrate that there are some isolates harbored the same ASP sub-pattern even though they were obtained from different sources (human and dogs), such as ASP2 (ii), ASP2 (iv), ASP4 (i), ASP6 (iii), ASP5 (i) and ASP7 (ii), making this technique an initial bacterial zoonotic indicator tool. Furthermore, it would almost certainly raise one health issue.

The transmission ability of E. coli was analyzed using the Clermont genotyping method. Six genotypes (A, B1, B2, C, E, F) were found in human isolates, while four (A, B1, C, F) were identified in dog isolates. Type E was predominant in humans, while type C was most common in dogs, with type E absent in dog samples. This is also in accordance with other earlier observations [43].

The genetic classification tool (Clermont) indicates a lower matching percentage of the isolates among similar phenotypic isolates. There are three likely causes for these differences. A possible explanation for this finding may be the transfer of a special antibiotic resistance gene between different types of bacteria of the same genus [29, 43], which could be related to any mobile genetic element such as plasmids or genomic cassettes [44]. Another possible explanation is the co-occurrence activity of antibiotic resistance genes [45-47] and the possibility of more than one gene coding for the same resistance trait.

It could be concluded that, although the phenotypic test gave a relatively higher pattern rate, genetic tests remain the most accurate way for bacterial confirmation and identification. Alongside the idea of the transfer of the special antibiotic resistance gene between different types of bacteria of the same genus could produce a new generation of bacteria regarding the ASP typing technique even though these bacteria came from the same ancestors (mother cells) and vice versa. Using the blend (both methods) gives a better idea about bacterial transmission and the acquisition of new antibiotic resistance genes, therefore, a better tool to understand the bacterial identity and zoonotic possible activity, which will provide more information to decrease and control the effect of these causative agents on the public health.

Conclusion

Using a dual-method approach, combining antibiotic susceptibility patterns and genetic classification techniques would significantly enhance the accuracy of the findings. This combined approach offers a more detailed picture of how bacterial strains spread across different populations and environments, and it can also help identify the mechanisms behind the acquisition of new antibiotic-resistance genes.

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