Article

Characterization of Extended-Spectrum β-Lactamase Producing- and Carbapenem–Resistant *Escherichia coli* Isolated from Diarrheic Dogs in Tunisia: First Report of *bla*$_{IMP}$ Gene in Companion Animals

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Abstract: *Escherichia coli* is an important opportunistic pathogen, causing several infections in dogs. The antimicrobial resistance of *E. coli* occurring in companion animals becomes an emerging problem. This study aimed to estimate the prevalence of ESBL-producing *E. coli* in diarrheic dogs, investigate the occurrence and molecular characterization of carbapenem-resistant isolates, and determine their virulence genes. Fecal samples were collected from 150 diarrheic dogs in Tunisia. *E. coli* isolates were screened for antimicrobial resistance against 21 antibiotics by the disk diffusion method. The characterization of β-lactamase genes, associated resistance genes, and virulence genes was studied using PCR. Among 95 *E. coli* strains, 25 were ESBL-producing, and most of them were multidrug-resistant. The most prevalent β-lactamase genes were *bla*$_{CTX-M1}$ (n = 14), *bla*$_{TEM}$ (n = 3), and *bla*$_{CMY}$ (n = 2). The *bla*$_{IMP}$ carbapenemase gene was found in two carbapenem-resistant isolates, which showed that carbapenemase-producing *E. coli* spread to companion animals in Tunisia. Different virulence genes associated with extraintestinal pathogenic *E. coli* were detected. This is the first report of the characterization of carbapenem resistance and virulence genes in dogs in North Africa. Our study showed that diarrheic dogs in Tunisia can be a potential reservoir of ESBL- or carbapenemase-producing *E. coli* with a possible risk of transmission to humans.

Keywords: antimicrobial resistance; ESBL-producing *E. coli*; carbapenemases; virulence factors; dogs; diarrhea

1. Introduction

Escherichia coli (*E. coli*), a Gram-negative bacteria belonging to the *Enterobacteriaceae* family, is frequently found in the gastrointestinal tract of both humans and companion animals [1].

Nevertheless, this opportunistic pathogen can cause intestinal and extra-intestinal diseases and may be associated with diarrheal syndrome. In dogs, acute diarrhea is one of the most common gastrointestinal problems, potentially leading to severe dehydration and death [2]. Several pathogens can be responsible for enteritis with diarrhea, including canine parvovirus type 2, protozoa such as *Giardia duodenalis* and *Cryptococcus* spp., bacteria such as *E. coli*, *Salmonella* spp., *Campylobacter* spp., *Clostridioides difficile*, *Clostridium perfringens*, and *Providencia alcalifaciens*. Some bacteria are zoonotic, particularly as in the case of *Campylobacter* spp., for which the dog is considered a reservoir with a great risk of transmission to humans, like in a study in Lebanon reporting a fecal prevalence of 17% [3,4]. In veterinary medicine, beta-lactam antibiotics are highly prescribed antibacterial agents for dogs in

order to treat infectious diseases [5]. This has led to the emergence and dissemination of resistant bacteria and allowed an increase in the Extended-Spectrum Beta-Lactamases (ESBL)-producing strains.

ESBL-producing Gram-negative bacteria are a serious threat to public health in human medicine as well as in the veterinary context. Worldwide, several studies have reported the transmission of multidrug-resistant bacteria between companion animals and their owners [6–9]. In addition, different studies all over the world have investigated ESBL-producing bacteria in companion animals [10,11]. In dogs and other companion animals, multidrug-resistant bacteria are frequently reported [12].

Recently, carbapenem resistance in companion animals has emerged, and different carbapenem resistance genes have been detected worldwide in companion animals, livestock, and sea food [13].

Antimicrobial resistance (AMR) among companion animals, particularly household pets, is a complex area that is becoming increasingly important. In this context, despite their close contact with humans, little attention has been given to the prevalence of antimicrobial resistance in companion animals. According to Dierikx et al. [14], the close contact between humans and companion animals such as dogs and cats makes the transmission of resistant organisms more likely to occur. In addition, dogs could increase the risk of transmitting zoonotic microorganisms, such as EPEC (Enteropathogenic *E. coli*) and STEC (Shiga-toxin producing *E. coli*) [15], and dogs have been suggested as a potential reservoir for ExPEC (Extra-intestinal pathogenic *E. coli*) [16]. ExPEC are facultative pathogens responsible for 80% of urinary tract infections, but also neonatal meningitis, surgical site infections, and pneumonia. ExPEC typically belongs to phylogroup B2, an *E. coli* genetic group that accumulates virulence factors, and occasionally to phylogroups D, F, or G. Food animals and pets are considered potential reservoirs of ExPEC [17]. It is noteworthy that contact with dogs or dog feces is considered a risk factor for the acquisition of resistant ExPEC [18].

The aim of this study was to determine the occurrence and molecular characterization of ESBL, carbapenemases, and virulence genes (associated with STEC and ExPEC) among ESBL-producing *E. coli* isolated from diarrheic dogs in Tunisia.

2. Materials and Methods

2.1. Sample Collection

From February 2018 to March 2019, 150 fecal samples were collected from diarrheic dogs at the National School of Veterinary Medicine of Sidi Thabet, Tunisia. All samples were obtained from the clinical department with diarrhea as the criteria of choice. The age of the dogs was between 3 months and 2 years. The number of males and females is 58 and 48, respectively.

2.2. Bacterial Isolation and Identification

MacConkey supplemented with cefotaxime (CTX, 1 µg/mL) was used to streak all samples after being enriched in water peptone buffer. Following a 24 h incubation period at 37 °C, colonies exhibiting a characteristic *E. coli* morphology and displaying a pink color were verified using chemical tests: triple sugar iron agar, Simmons' citrate agar, and urea indole medium.

2.3. Antimicrobial Susceptibility Testing

The susceptibility of strains was detected using the inhibitory zone diameter interpretation standards according to the recommendations of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2018E) [19].

The phenotypic detection of ESBL was realized by the double disk synergy test using a disk of amoxicillin and clavulanic acid placed in the center of a Mueller–Hinton agar plate surrounded by cefotaxime, ceftazidime, and cefepime disks. The enhanced inhibition zone of any of the cephalosporin disks on the side facing amoxicillin and clavulanic acid was considered an ESBL producer. Phenotypic testing for colistin was carried out using the

Colispot test$^{®}$. The isolates were tested on 21 antibiotics (Mast Ltd.Group, Merseyside, UK). The following antimicrobial disks were used (µg per disk): amoxicillin (25), piperacillin (30), cefotaxime (30), cefoxitin (30), cefepime (30), ticarcillin–clavulanic acid (75/10), amoxicillin–clavulanic acid (20/10), aztreonam (30), cephalothin (30), cefuroxime (30), ceftazidime (30), ertapenem (10), gentamicin (10), streptomycin (10), nalidixic acid (30), enrofloxacin (5), chloramphenicol (30), florfenicol (30), tetracycline (30), and trimethoprim–sulfamethoxazole (1.25/23.75).

2.4. Genomic DNA Extraction

The boiling method was used to extract the genomic DNA from each sample. In short, bacterial colonies were suspended in 1 milliliter of distilled water, and the mixture was centrifuged for five minutes at 13,200 rpm. After that, 100 µL of distilled water was added after the supernatant was removed. The samples were heated for ten minutes to 95 °C. After being boiled, the cell suspensions were diluted and kept cold (−20 °C), intended for PCR (Polymerase Chain Reaction) usage.

2.5. Identification of Antibiotic Resistance Genes

PCR amplifications were used for the searched β-lactamase-encoded genes: bla_{CTX-M}, bla_{TEM}, bla_{SHV}, bla_{CMY}, bla_{VIM}, bla_{IMP}, bla_{NDM-1}, and bla_{OXA-48} ESBL-producers' strains. The presence of genes linked to resistance to several antibiotic classes, such as quinolones, aminoglycosides, sulfonamides, and tetracyclines, was assessed. Table 1 lists the primer sequences and PCR conditions.

Table 1. Primers, sequences, and sizes of PCR products used for the detection of resistance genes.

Primers	Target	Primer Sequences (5′–3′)	Tm	PCR Product (bp)
bla_{CTX-M1}-F	bla_{CTX-M1}	ATGGTTAAAAAATCACTGCG	49 °C	876
bla_{CTX-M1}-R		TTACAAACCGTCGGTGAC		
$bla_{CTX-M-15}$-F	$Bla_{CTX-M-15}$	CACACGTGGAATTTAGGGACT	55 °C	996
$bla_{CTX-M-15}$-R		GCCGTCTAAGGCGATAAACA		
bla_{CTX-M9}-F	bla_{CTX-M9}	GTGACAAAGAGAGTGCAACGG	60 °C	856
bla_{CTX-M9}-R		ATGATTCTCGCCGCTGAAGCC		
bla_{SHV}-F	bla_{SHV}	CACTCAAGGATGTATTGTG	54 °C	885
bla_{SHV}-R		TTAGCGTTGCCAGTGCTCG		
bla_{TEM}-F	bla_{TEM}	ATTCTTGAAGACGAAAGGGC	50 °C	1150
bla_{TEM}-R		ACGCTCAGTGGAACGAAAAC		
bla_{CMY}-F	bla_{CMY}	ATGATGAAAAAATCGATATG	55 °C	1146
bla_{CMY}-R		TTATTGCAGTTTTTCAAGAATG		
OXA48-F	bla_{OXA48}	GCGTGGTTAAGGATGAACAC	56 °C	438
OXA48-R		CATCAAGTTCAACCCAACCG		
NDM-1-F	bla_{NDM-1}	GGTTTGGCGATCTGGTTTTC	52 °C	621
NDM-1-R		CGGAATGGCTCATCACGATC		
IMP-F	bla_{IMP}	GGAATAGAGTGGCTTAAYTCTC	52 °C	203
IMP-R		GGTTTAAYAAAACAACCACC		
VIM-F	bla_{VIM}	GATGGTGTTTGGTCGCATA	52 °C	390
VIM-R		CGAATGCGCAGCACCAG		

Table 1. *Cont.*

Primers	Target	Primer Sequences (5′–3′)	Tm	PCR Product (bp)
aac(3)-II-F	*aac(3)-II*	ACTGTGATGGGATACGCGTC	57 °C	200
aac(3)-II-R		CTCCGTCAGCGTTTCAGCTA		
tetA-F	*tetA*	GTAATTCTGAGCACTGTCGC	62 °C	937
tetA-R		CTGCCTGGACAACATTGCTT		
tetB-F	*tetB*	CTCAGTATTCCAAGCCTTTG	57 °C	416
tetB-R		CTAAGCACTTGTCTCCTGTT		
tetC-F	*tetC*	TCTAACAATGCGCTCATCGT	56 °C	570
tetC-R		GGTTGAAGGCTCTCAAGGGC		
sul1-F	*sul1*	TGGTGACGGTGTTCGGCATTC	62 °C	789
sul1-R		GCGAGGGTTTCCGAGAAGGTG		
sul-2F	*sul2*	CGGCATCGTCAACATAACC	50 °C	722
sul2-R		GTGTGCGGATGAAGTGAG		
sul3-F	*sul3*	CATTCTAGAAAACAGTCGTAGTTCG	51 °C	990
sul3-R		CATCTGCAGCTAACCTAGGGCTTTGGA		
Multiplex PCR qnrA-F	*qnrA*	AGAGGATTTCTCACGCCAGG		580
qnrA-R		TGCCAGGCACAGATCTTGAC		
qnrB-F	*qnrB*	GCMATHGAAATTCGCCACTG	55 °C	264
qnrB-R		TTTGCYGYYCGCCAGTCGAA		
qnrS-F	*qnrS*	GCAAGTTCATTGAACAGGGT		428
qnrS-R		TCTAAACCGTCGAGTTCGGCG		
chuA-F	*chuA*	GACGAACCAACGGTCAGGAT		279
chuA-R		TGCCGCCACTACCAAAGACA		
yji-A-F	*yjiA*	TGAAGTGTCAGGAGACGCTG	65 °C	211
yji-A-R		ATGGAGAATGCGTTCCTCAAC		
TSPE4-F	*TSPE4*	GAGTAATGTCGGGGCATTCA		154
TSPE4-R		CGCGCCAACAAAGTATTACG		

M = A or C; H = A or C or T; Y = C or T.

2.6. Phylogenetic Grouping of the Isolates

According to the previous descriptions by Clermont et al. [20], the phylogenetic grouping (A, B1, B2, and D) was examined in the isolates. The determinations of phylogenetic groups A, B1, B2, and D depending on the existence of the *chuA* and *yjaA* genes and *TSPE4.C2* determinants were detected by PCR.

2.7. Virulence Genes Identification

Each *E. coli* strain was examined for the presence of virulence genes. Multiplex PCR for Shiga-toxins (*stx1* and *stx2*), intimin (*eae*), and enterohemolysin (*ehxA*) was used to identify STEC strains. Hemolysin (*hly*), cytotoxic necrotizing factor (*cnf1*), and cytolethal distending toxin (*cdt3*) were all tested using triplex PCR. Simplex PCR was used to identify the virulence genes linked to ExPEC, which include *aer* (aerobactin system), *papA* (P fimbriae), *bfpA* (bundle forming pilus), *papG-III* (P adhesin), *fimH* (type 1 fimbriae), *traT* (serum survival gene), *ibeA* (invasion of brain endothelium), and *sfa/foc* (S and F1C fimbriae). Duplex PCR was also used to analyze the virulence genes *fyuA* (gene encoding yersiniabactin) and *iutA* (ferric aerobactin receptor). Table 2 lists the virulence gene primers and PCR conditions.

Table 2. Primers, sequences, and sizes of PCR products used for the detection of virulence genes.

Primers	Target	Primer Sequences (5′–3′)	PCR	Tm	PCR Product (bp)
Stx1-F	*stx1*	CAGTTAATGTGGTGGCGAAGG	Multiplex PCR	56 °C	348
Stx1-R		CACCAGACAATGTAACCGCTG			
Stx2-F	*stx2*	ATCCTATTCCCGGGAGTTTACG			584
Stx2-R		GCGTCATCGTATACACAGGAGC			
eae-F	*eae*	TGCGGCACAACAGGCGGCGA			629
eae-R		CGGTCGCCGCACCAGGATTC			
ehxA-F	*ehxA*	GCATCATCAAGCGTACGTTCC			534
ehxA-R		AATGAGCCAAGCTGGTTAAGCT			
fimH-F	*fimH*	TGCAGAACGGATAAGCCGTGG		56 °C	508
fimH-R		GCAGTCACCTGCCCTCCGGTA			
traT-F	*traT*	GGTGTGGTGCGATGAGCACAG		57 °C	290
traT-R		CACGGTTCAGCCATCCCTGAG			
aer-F	*aer*	TACCGGATTGTCATATGCAGACCG		56 °C	602
aer-R		AATATCTTCCTCCAGTCCGGAGAAG			
papA-F	*papA*	ATGGCAGTGGTGTCTTTTGGTG		63 °C	717
papA-R		CGTCCCACCATACGTGCTCTTC			
hly F	*hly*	GAGCGAGCTAAGCAGCTTG	Multiplex PCR	56 °C	889
hly R		CCTGCTCCAGAATAAACCACA			
cnf1-F	*cnf1*	GGGGGAAGTACAGAAGAATTA			1111
cnf1-R		TTGCCGTCCACTCTCTCACCAGT			
cdt3-F	*cdt3*	GAAAATAAATGGAATATAAATGTCCG			555
cdt3-R		TTTGTGTCGGTGCAGCAGGGAAAA			
iutA-F	*iutA*	GGCTGGACATCATGGGAACTGG	Duplex PCR	63 °C	300
iutA-R		CGTCGGGAACGGGTAGAATCG			
fyuA-F	*fyuA*	TGATTAACCCCGCGACGGGAA			880
fyuA-R		CGCAGTAGGCACGATGTTGTA			

2.8. Data Analysis and Interpretation

Using SPSS version 26 software, a Chi-square test was employed to examine any significant differences in risk factors, the associations of phylogroups, and the resistance and virulence genes (IBM Corporation, Somers, NY, USA). $p < 0.05$ was designated as the level of statistical significance.

3. Results

3.1. Bacterial Strains

Among 150 fecal samples obtained from diarrheic dogs, we isolated 95 *E. coli* isolates. A total of 25 *E. coli* isolates were ESBL-producing (26.6%), as shown in Table 3. The samples were collected from diarrheic dogs at the National School of Veterinary Medicine of Sidi Thabet, Tunisia. The age of the dogs was between three months and two years. Fifty-eight samples were from females and forty-eight were collected from males.

Table 3. Characteristics of the 25 ESBL-positive *E. coli* isolates recovered from fecal samples of diarrheic dogs.

E. coli Isolates	*β*-Lactamase Genes	Resistance to Non *β*-Lactam Antibiotics	Non *β*-Lactam Resistance Genes	Virulence Genes	Phylogroup
CM1 CTX	*bla*CTX-M15 *bla*TEM	A, PRL, CTX, CPM, TIM, ATM, KF, CAZ, GN, S, NA, FFC, C, TET		*traT, fimH, aer, fyuA, iutA*	D
CM2 CTX	*bla*CTX-M15 *bla*TEM	A, PRL, CTX, CPM, ATM, KF, NA, ENF, TET		*traT, fimH, aer, iutA*	D
CM6 CTX	*bla*CTX-M1 *bla*CTX-M9 *bla*TEM	A, PRL, CTX, ATM, KF, CAZ, GN, S, FFC, C, TET, TS	*sul1, aac(3)II*	*traT, fimH, fyuA*	A
CM8 CTX	*bla*CTX-M15 *bla*SHV	A, PRL, CTX, CPM, TIM, ATM, KF, CAZ, NA, C, ENF, TET	*tetA*	*traT, fimH, aer, iutA*	A
CM17 CTX	*bla*CTX-M15	A, PRL, CTX, CPM, ATM, CXM, KF, CAZ, S, NA, FFC, C, ENF, TET, TS	*tetA*	*traT, fimH, fyuA, iutA*	B1
CM21 CTX	*bla*CTX-M15	A, PRL, CTX, CPM, TIM, ATM, CXM, KF, S, NA, TET, TS		*traT, fimH, iutA*	A
CM22 CTX		A, PRL, CTX, CPM, TIM, KF, CXM, CAZ		*traT*	D
CM24 CTX		A, PRL, CTX, CAZ, NA, ENF		*fimH*	A
CM25		A, PRL, CTX, CPM, TIM, AUG, ATM, CAZ, S, NA, FFC, ENF, TET, C, TS		*fimH*	A
CM27 CTX	*bla*CMY	A, PRL, CTX, FOX, TIM, AUG, CAZ, TET		*traT, fimH*	A
CM28 CTX		TIM, CAZ, FFC, C, TET, TS	*sul2*	*traT, fimH, fyuA*	D
CM29 CTX	*bla*CTX-M1	A, PRL, CTX, TIM, ATM, CAZ, S		*fimH, eae*	A
CM32 CTX	*bla*CTX-M15	A, PRL, CTX, CPM, TIM, ATM, CAZ, GN, S, NA, C, ENF, TET, TS	*sul2, aac(3)II*	*traT, fimH, aer, iutA*	
CM33 CTX	*bla*CTX-M9	A, TIM, AUG, CAZ, GN, S, NA, ENF, TET, TS, NA, FFC, C, TS	*sul2*	*aer*	D
CM40 CTX	*bla*CTX-M1	A, PRL, CTX, CPM, ATM, S, ENF, TET		*fimH, aer*	A
CM45 CTX	*bla*CTX-M15	A, PRL, CTX, CPM, TIM, ATM, CAZ, S, NA, FFC, C, ENF, TET, TS		*traT, fimH, aer, bfpA, iutA*	A
CM29A		A, PRL, TIM, S, NA, FFC, ENF, TET, TS	*sul1, sul2*	*fimH*	A
CM38CTXA		A, PRL, S, TET, TS	*tetA*	*traT, fimH, aer*	D

Table 3. *Cont.*

E. coli Isolates	*β*-Lactamase Genes	Resistance to Non *β*-Lactam Antibiotics	Non *β*-Lactam Resistance Genes	Virulence Genes	Phylogroup
CM53 CTXA	$bla_{CTX\text{-}M15}$	A, PRL, CTX, S, TET, TS	*tetA*	*traT, fimH, aer*	D
CM57 CTXA	bla_{IMP}	A, PRL, CTX, CPM, TIM, AUG, ATM, CAZ, ETP, GN, S, NA, ENF, FFC, C, TET, TS	*tetA*	*traT, aer, fyuA, iutA*	A
CM58 CTXA	$bla_{CTX\text{-}M15}$	A, PRL, CTX, CPM, TIM, ATM, CAZ, ETP, GN, S, TET, TS	*sul1*	*aer*	A
CM85 CTXA	$bla_{CTX\text{-}M15}$	A, PRL, CTX, CPM, TIM, ATM, CAZ, NA, ENF, TET, TS		*traT, fimH, aer, iutA*	A
CM89 CTXA	$bla_{CTX\text{-}M1}$	A, PRL, CTX, CPM, TIM, AUG, ATM, CAZ, TET, TS	*sul2*	*traT, fimH, aer, fyuA*	A
CM91 CTXA	$bla_{CTX\text{-}M1}$ bla_{CMY}, bla_{IMP}	A, PRL, CTX, FOX, TIM, AUG, ATM, CAZ, NA, FFC, C, ENF, TET, TS		*traT, fimH, aer, iutA*	A
CM100 CTXA	$bla_{CTX\text{-}M15}$	A, PRL, CTX, CPM, TIM, ATM, KF, CXM, CAZ, ETP, NA, FFC, C, ENF, TET, TS		*traT, fimH, aer, iutA*	A

A: amoxicillin, PRL: piperacillin, CTX: cefotaxime, FOX: cefoxitin, CPM: cefepime, TIM: ticarcillin–clavulanic acid, AUG: amoxicillin–clavulanic acid, ATM: aztreonam, KF: cephalothin, CXM: cefuroxime, CAZ: ceftazidime, ETP: ertapenem, GN: gentamicin, S: streptomycin, NA: nalidixic acid, ENF: enrofloxacin, C: chloramphenicol, FFC: florfenicol, TET: tetracycline, TS: trimethoprim–sulfamethoxazole.

3.2. Antibiotic Resistance of ESBL-Producing Strains

The twenty-five ESBL-producing *E. coli* isolates were tested for their susceptibility to twenty-one antibiotic agents. We found that most of these strains were multidrug-resistant (MDR = 72%) since they were resistant to at least three families of antibiotics. Most ESBL isolates were resistant to amoxicillin (96%), tetracyclin (92%), and cefotaxime (84%). Rates of resistance for 25 ESBL-producing *E. coli* are listed in Table 4.

Table 4. Antimicrobial resistance of 25 ESBL-producing *E. coli* in diarrheic dogs.

Antibiotics	Susceptible (S)		Intermediate (I)		Resistant (R)	
	No.	%	No.	%	No.	%
Amoxicillin	0	0	1	4	24	96
Piperacillin	0	0	2	8	23	92
Cefotaxime	1	4	3	12	21	84
Cefoxitin	19	76	4	16	2	8
Cefepim	9	36	1	4	15	60
Ticarcillin/clavulanic acid	3	12	4	16	18	72
Amoxicillin/clavulanic acid	8	32	14	56	3	12
Aztreonam	1	4	7	28	17	68
Cephalothin	0	0	2	8	23	92
Cefuroxime	0	0	1	4	24	96
Ceftazidime	2	8	4	16	19	76

Table 4. *Cont.*

Antibiotics	Susceptible (S)		Intermediate (I)		Resistant (R)	
	No.	%	No.	%	No.	%
Ertapenem	18	72	4	16	3	12
Gentamicin	18	72	2	8	5	20
Streptomycin	0	0	12	48	13	52
Colistin	25	100	-	-	0	0
Nalidixic acid	5	20	5	20	15	60
Enrofloxacin	10	40	3	12	12	48
Chloramphenicol	10	40	4	16	11	44
Florfenicol	13	52	3	12	9	36
Tetracyclin	0	0	2	8	23	92
Trimethoprim/sulfomethoxazole	8	32	0	0	17	68

The isolation rate of ESBL-producing *E. coli* in this study was higher in males than females, but without statistical significance (p-value = 0.544). According to the dog's age, the ESBL frequency was lower for the category superior to 24 months than other categories without statistical significance (p-value = 0.482). The ESBL rates were higher in summer and autumn than other seasons, with this difference being statistically significant (p-value = 0.011) (Table 5).

Table 5. Prevalence of ESBL-producing *E. coli* isolated from dogs according to different risk factors.

Risk Factors	Categories	Total Tested	ESBL Producers No. (%)	*p*-Value
Gender	Male	58	15 (25.9%)	0.544
	Female	48	10 (20.8%)	
Age	<6 months	32	9 (28.1%)	0.482
	6–12 months	34	9 (26.5%)	
	12–24 months	15	4 (26.7%)	
	>24 months	25	3 (12.0%)	
Season	Autumn	10	5 (50.0%)	0.011
	Summer	4	3 (75.0%)	
	Winter	41	7 (17.1%)	
	Spring	51	10 (19.6%)	

3.3. Beta-Lactam Resistance Genes

In this study, three different β-lactamase genes belonging to Ambler molecular class A (*bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{SHV}$) were detected. The most prevalent gene was *bla*$_{CTX-M1}$ (n = 14), *bla*$_{TEM}$ (n = 3), and *bla*$_{CTX-M9}$ (n = 2), while only one isolate carried the *bla*$_{SHV}$ gene. Otherwise, two isolates carried the *bla*$_{CMY}$ gene (AmpC cephalosporinase).

3.4. Non β-Lactam Resistance Genes

Among the 25 ESBL-producing isolates, five harbored the *tetA* gene and *sul2*, three carried the *sul1* gene, and only two isolates were *aac (3)II* positive.

3.5. Phylogroups

Most of the ESBL-producing *E. coli* isolates belonged to phylogroups A (16 isolates) and D (7 isolates), and only one isolate belonged to phylogroup B1.
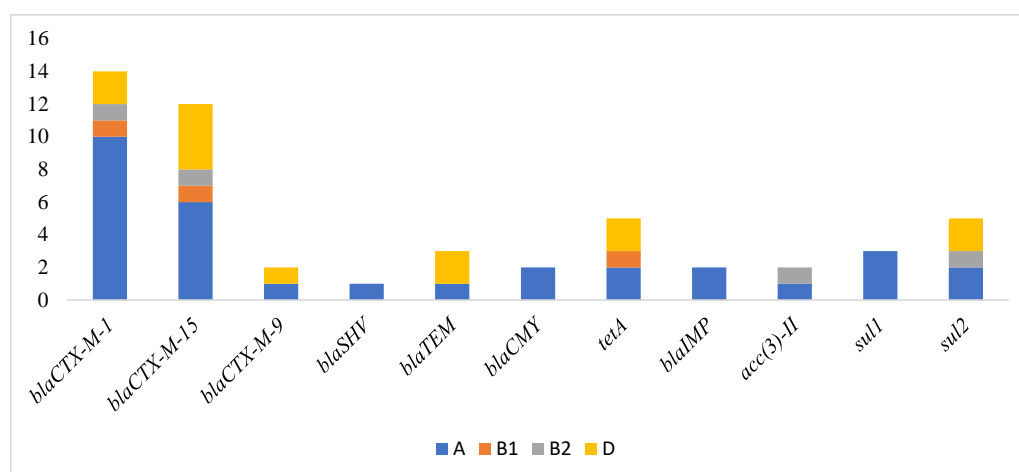
3.6. Virulence Genes

Virulence genes implicated in ExPEC related to adhesins (*fimH*, *papC*, *papG allele III*, *sfa/foc*), toxins (*hly*, *cnf1*), and invasion factors (*ibeA*, aer, *iutA*, *fyuA*) were investigated among ESBL-producing isolates.

In regard to virulence factors associated with ExPEC, a high number of strains harbored the *fimH* virulence gene (21/25, 84%), 18/25 (72%) carried the *traT* gene and 14/25 (56%) carried the *aer* gene. In addition, eight strains harbored the *iutA* gene, four harbored the *fyuA* gene, and three isolates carried both the *fyuA* and the *iutA* genes. For the *eae* and *bfpA* virulence genes, they were both found in only one ESBL each.
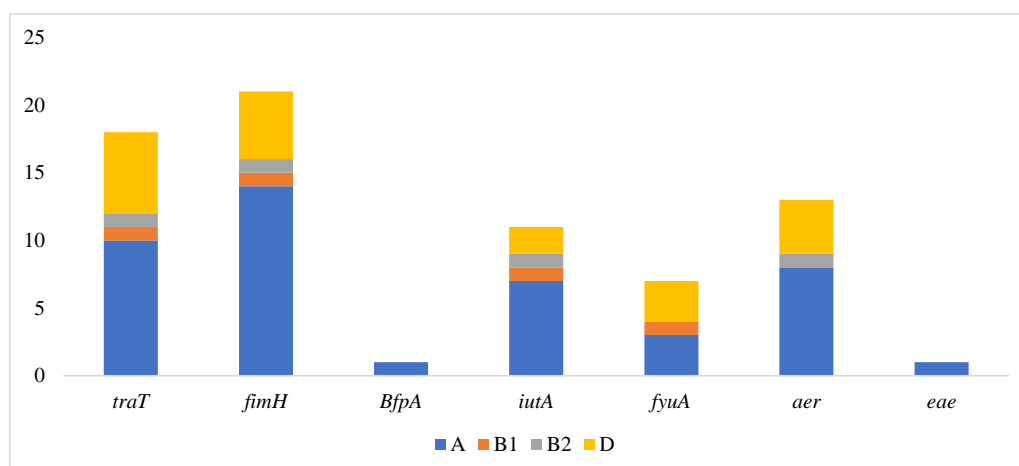
3.7. Distribution of Virulence Genes and Resistance Genes in Phylogenetic Groups

The analysis of phylogroup distribution among the resistance genes revealed that group A was the most dominant that carried virulence factors, followed by group D. β-lactamase genes $bla_{CTX-M-1}$ and $bla_{CTX-M-15}$ were distributed among phylogenetic groups, whereas $bla_{CTX-M-9}$ and bla_{TEM} were present only in groups A and D. Resistance genes bla_{SHV}, bla_{CMY}, and bla_{IMP} were associated with group A, and the difference did not reach statistical significance (Figure 1).

Figure 1. Prevalence of resistance genes and their distribution according to phylogenetic groups.

The association between virulence genes and phylogenetic groups revealed that the genes *traT*, *fimH*, and *iutA* were widely disseminated in the groups. Genes *traT*, *fimH*, *iutA*, *fyuA*, and *aer* were more strongly associated with groups A and D than other groups. Group A was the dominant group that carried the virulence genes; *BfpA* and *eae* were found only in group A (Figure 2).

Figure 2. Prevalence of the virulence genes and distribution of phylogenetic groups.

4. Discussion

In *E. coli*, ESBLs and carbapenemases are mainly responsible for the emerging resistance to the β-lactam antibiotics, especially the 3rd generation cephalosporins and carbapenems [17]. In the present study, we conducted the molecular detection and characterization of β-lactamase genes in ESBL-producing *E. coli* isolates from diarrheic dogs in Tunisia and also revealed the association between the phylogenetic groups and virulence gene profiles.

In this study, among 95 *E. coli* isolates obtained from 150 diarrheic dogs, 25 *E. coli* isolates were ESBL-producing (26.6%). The overall prevalence of *E. coli* was 62.6%, which is higher than results found in Brazil since they obtained a rate of 57.8% of *E. coli* isolated from dogs with diarrhea [21]. Our results are also higher than what was reported in Egypt [22], with a prevalence of *E. coli* of 23.7% (19/80) in the examined diseased dogs. In addition, our findings showed a rate of 26.6% of ESBL-producing *E. coli* isolated from diarrheic dogs, near the rate of 30% in India (Tudu et al. 2022), but lower than the rate of 55% reported in the Netherlands [23]. On the other hand, our results are higher than those in the United Kingdom, with only 1.9% [24].

The antimicrobial susceptibility of ESBL-producing *E. coli* isolates showed that these isolates had high rates of resistance. We found that 96% of the isolates were resistant to amoxicillin (24/25), 92% to tetracycline (23/25), and 84% to cefotaxime (21/25). These findings are higher than those found by Algammal et al., in which they reported that 100%, 84.2%, and 42.1% of the strains were resistant to tetracycline, amoxicillin, and cefotaxime, respectively, in 80 dogs suffering from bloody diarrhea [22].

In 1986, FEC-1 (Fujisawa *E. coli*-1) was the first CTX-M-type ESBL enzyme, discovered in a cefotaxime-resistant *E. coli* isolated from the feces of a laboratory dog in Japan [25]. In the year 2000, the first case of an ESBL-producing *E. coli* from animal origin was detected in Spain [26] when they reported for the first time the gene *bla*$_{SHV-12}$ from a sample of a dog with recurring chronic cystitis. A few years later, Carattoli et al. [27] detected the first cases of *bla*$_{CTX-M-1}$ producing bacteria in dogs and cats with and without pathology. Since then, different studies worldwide have reported increasing numbers of companion animals that are hosts for ESBL-producing *E. coli* [28,29].

We found three different β-lactamase genes belonging to Ambler molecular class A (*bla*$_{TEM}$, *bla*$_{CTX-M}$, and *bla*$_{SHV}$) among the strains. The most prevalent genes were *bla*$_{CTX-M1}$ (n = 14), *bla*$_{TEM}$ (n = 3), *bla*$_{CTX-M9}$ (n = 2), and only one isolate carried *bla*$_{SHV}$ (n = 1). Our results showed a total of 14 ESBL-producing *E. coli* (14/25, 56%) that harbored the *bla*$_{CTX-M-1}$ gene. In this context, many studies have reported a high prevalence of *the bla*$_{CTX-M-1}$ gene from ESBL-producing *E. coli* in companion animals [30,31]. Comparing our results to those found in the Netherlands [23], we found a higher number of ESBL harboring the *bla*$_{CTX-M-1}$ gene since they investigated 20 dogs with diarrhea and found the *bla*$_{CTX-M-1}$ gene in only two diarrheic dogs. In addition, our findings are higher than in a study in Germany [32], which showed that the *bla*$_{CTX-M-1}$ gene was less identified in dogs since they found this gene in 11 isolates among 67 dogs. For the *bla*$_{CTX-M-9}$, only two ESBL in our study carried this gene, and this is comparable to results found in Germany [32], where they found the *bla*$_{CTX-M-9}$ gene in only two isolates among 67 dogs. Our findings showed that two ESBL carried the *bla*$_{CMY}$ gene, which is similar to results found in Germany [33].

It is noteworthy that resistance phenotypes, geographical regions, and the history of antimicrobial treatments on animals can affect the prevalence of *bla*$_{CTX-M}$ genes. The high prevalence suggests a significant role for *E. coli* isolates from companion animals as ESBL gene reservoirs [31].

Given the absence of new commercialized antibiotics, numerous studies aim to develop alternatives such as the use of bacteriocins, essential oils, bacteriophages, or antimicrobials with other mechanisms of action such as metallophores. For example, the ability of metallophores to complex and transport metals into bacterial cells has also led to an alternative strategy for developing antimicrobial agents by complexing other antimicrobial metals and delivering potentially lethal concentrations of them into bacterial cells [34].

Concerning resistance to carbapenems, most reports of carbapenemase-producing *Enterobacteriaceae* have been mainly from humans [35,36], which may indicate a human-to-animal transfer through close contact [37]. In *Enterobacteriaceae*, the carbapenemases can be divided into three types: the class A carbapenemase group, which includes the KPC-type, the class B carbapenemase group (Metallo β-lactamases), and the class D carbapenemase group, which includes the OXA-48 type.

Worldwide, the most common carbapenemase type in *E. coli* is the OXA type. This type is endemic in Turkey, northern Africa, and India [38]. Worthy of note, carbapenemase genes have been identified in companion animals worldwide [33,39,40] and are associated with a high potential for dissemination [41]. In Tunisia, the first carbapenemase-producing isolate emerged from a Tunisian university hospital in 2006 [42]. Since then, different carbapenemase variants have been isolated from several origins (hospitals and wastewater effluents) in Tunisia [43,44]. Nevertheless, there has been some concern about carbapenemase-producing bacteria of animal origin all over the world [45,46], but studies on resistance to carbapenems in Tunisia are still limited [47,48]. In this context, we conducted the molecular detection and characterization of the carbapenemase genes (bla_{IMP}, bla_{OXA-48}, bla_{NDM-1}, bla_{KPC}, and bla_{VIM}) in ESBL-producing *E. coli* isolated from diarrheic dogs. It is important to highlight that our results demonstrated the presence of the bla_{IMP} gene in two isolates using PCR and sequencing and were confirmed by an E-test strip. To the best of our knowledge, this is the first report of carbapenemase-producing *E. coli* from diarrheic dogs in Tunisia. In contrast to our result, the bla_{IMP} gene was found in *E. coli* isolates from healthy rabbits in Tunisia [47] and also among *Enterobacteriaceae* isolated from wild boar (*Sus scrofa*) [49], but has never been reported before from dogs in this country.

It is noteworthy that carbapenemase genes are mainly localized on plasmids and are therefore highly transferable between different bacteria. The transfer of a plasmid carrying a carbapenemase gene suggests the possibility of dissemination in different fields (animal, human, and environmental) [50].

Carbapenemase-producing MDR bacteria were first described almost exclusively in humans, but since 2011, they have also been detected in livestock, companion animals, wildlife, and different environmental compartments, indicating their transfer to new hosts and reservoirs [51].

Worldwide, the prevalence of carbapenem-resistant *Enterobacteriaceae* among companion animals seemed to be low in Algeria, with a prevalence of 2.58% in dogs [52], as well as in France and Spain, with a prevalence of 0.6% [41,53]. This indicates that carbapenemase-producing isolates are an emerging problem and can constitute a serious threat to public health.

According to a recent study [54], there is no available data that identified the virulence genes of *E. coli* isolated from companion animals in Africa. To the best of our knowledge, this is the first report of virulence genes among *E. coli* isolated from diarrheic dogs in Tunisia. In this study, we found one ESBL-producing *E. coli* that carried the *eae* gene. In Brazil [55], the *eaeA* gene was identified in 12 (12.6%) isolates from 68 fecal samples of diarrheic dogs, which is higher than our findings. In contrast, a study in Iraq [56] showed that six (5.8%) isolates that were identified as *E. coli* O157:H7 and harbored the *eaeA* gene in 104 dogs with (11/16; 68.8%) and without diarrhea (7.9%; 7/88); this is higher than our results.

Our ESBL-producing *E. coli* with the positive *eae* gene can be classified as atypical enteropathogenic *E. coli* (EPEC) and did not harbor the *stx* and *bfpA* virulence genes of EPEC (*eae*+, *stx*–, *bfpA*–). However, in this study, we did not isolate the typical EPEC (*eae*+, *bfpA*+). Depending on their virulence genes, EPEC strains are subdivided as typical and atypical EPEC. For typical EPEC, humans are the only reservoirs, but for atypical EPEC, both animals and humans can be reservoirs, and atypical EPEC seems to be an important cause of diarrhea [57]. EPEC are characterized by the production of intimin (*eae*), and they can be classified as typical or atypical depending on the presence or absence of bundle-forming pili (*bfp*) [58]. In our data, only one isolate carried the *bfpA* gene.

Since data on virulence genes associated with dogs is not available in Africa, we compared our findings to those on other continents like Europe and America. Our findings of virulence factors associated with ExPEC showed a high rate (84%) of ESBL strains that harbored the *fimH* virulence gene (21/25), 72% (18/25) carried the *traT* gene, and 56% (14/25) carried the *aer* gene. In addition, 32% of ESBL strains harbored the *iutA* gene, 16% harbored the *fyuA* gene, and 12% carried both *fyuA* and *iutA*. These findings are higher than results found in the United States, where a rate of 69.1% for the *fimH* gene and 60.3% for the *traT* gene was reported among 68 ESBL strains isolated from dogs and cats [31]. In contrast, our rates of *iutA* and *fyuA* were 32% and 16%, respectively, lower than the findings of Liu et al. 2016.

Concerning the *stx1*, *stx2*, *eae,* and *hly* virulence genes, a recent study performed in Egypt reported that the prevalence of *stx1*, *eaeA*, and *hlyA* was 100% and 47.3% for *stx2* in fecal samples of diseased dogs suffering from hemorrhagic diarrhea [22]. These findings are higher than our results, since we found only one isolate to be *eae* positive and all isolates did not harbor the *stx1*, *stx2*, or *hly* virulence genes.

It is considered that virulent extra-intestinal *E. coli* (ExPEC) strains usually belong to phylogenetic groups B2 and D in comparison with other phylogenetic groups. In contrast, commensal strains frequently belong to phylogenetic groups A and B1. In the present study, the isolates carrying virulence genes belonged mainly to phylogenetic groups A (16 isolates) and D (7 isolates), and only one isolate belonged to B1. Our results showed that 7 isolates belonged to phylogroup D in association with virulence factors of adhesion and invasion (*fimH* and *aer*) that are linked to ExPEC infections, so 32% of the isolates may be considered ExPEC, and the remaining isolates that belonged to phylogroup A might be commensal strains or intraintestinal pathogenic (InPEC). In contrast to our findings of one isolate that belonged to phylogroup B1, Coura et al. [21] obtained eight isolates from the B1 and E phylogroups, and EPEC was the more frequent pathovar identified, known as an important diarrheagenic agent in dogs.

5. Conclusions

Our study showed that diarrheic dogs in Tunisia can be a potential reservoir of ESBL-producing *E. coli* and demonstrated that carbapenemase-producing *E. coli* in companion animals is emerging. In addition, a high occurrence of antimicrobial resistance of *E. coli* was observed in fecal samples of dogs with diarrhea, highlighting the need for prudent use of antimicrobial agents in veterinary medicine in order to decrease the selection and spread of multi-drug resistant bacteria.

Our findings strongly suggest that isolates with carbapenem resistance are currently circulating among companion animals and can act as a reservoir of resistance genes transmitted between humans and animals. Fortunately, carbapenemases are still rare among isolates of companion animals, but the number of studies on resistance to carbapenems is increasing worldwide, and this resistance must be meticulously monitored.

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References

1. Bortolami, A.; Zendri, F.; Maciuca, E.I.; Wattret, A.; Ellis, C.; Schmidt, V.; Pinchbeck, G.; Timofte, D. Diversity, virulence, and clinical significance of extended-spectrum β-lactamase-and pAmpC-producing Escherichia coli from companion animals. *Front. Microbiol.* **2019**, *10*, 1260. [CrossRef] [PubMed]

2. Duijvestijn, M.; Mughini-Gras, L.; Schuurman, N.; Schijf, W.; Wagenaar, J.A.; Egberink, H. Enteropathogen infections in canine puppies:(Co-) occurrence, clinical relevance and risk factors. *Vet. Microbiol.* **2016**, *195*, 115–122. [CrossRef] [PubMed]

3. Ghssein, G.; Barakat, R.; Nehme, N.; Awada, R.; Hassan, H.F. Fecal prevalence of Campylobacter spp. in house dogs in Lebanon: A pilot study. *Vet. World* **2023**, *16*, 2250. [CrossRef] [PubMed]

4. Campagnolo, E.; Philipp, L.; Long, J.; Hanshaw, N. Pet-associated Campylobacteriosis: A persisting public health concern. *Zoonoses Public Health* **2018**, *65*, 304–311. [CrossRef] [PubMed]

5. Dupouy, V.; Abdelli, M.; Moyano, G.; Arpaillange, N.; Bibbal, D.; Cadiergues, M.-C.; Lopez-Pulin, D.; Sayah-Jeanne, S.; De Gunzburg, J.; Saint-Lu, N. Prevalence of beta-lactam and quinolone/fluoroquinolone resistance in Enterobacteriaceae from dogs in France and Spain—Characterization of ESBL/pAmpC isolates, genes, and conjugative plasmids. *Front. Vet. Sci.* **2019**, *6*, 279. [CrossRef] [PubMed]

6. Halsby, K.D.; Walsh, A.L.; Campbell, C.; Hewitt, K.; Morgan, D. Healthy animals, healthy people: Zoonosis risk from animal contact in pet shops, a systematic review of the literature. *PLoS ONE* **2014**, *9*, e89309. [CrossRef] [PubMed]

7. Pomba, C.; Rantala, M.; Greko, C.; Baptiste, K.E.; Catry, B.; Van Duijkeren, E.; Mateus, A.; Moreno, M.A.; Pyörälä, S.; Ružauskas, M. Public health risk of antimicrobial resistance transfer from companion animals. *J. Antimicrob. Chemother.* **2017**, *72*, 957–968. [CrossRef] [PubMed]

8. Ramatla, T.; Mafokwane, T.; Lekota, K.; Monyama, M.; Khasapane, G.; Serage, N.; Nkhebenyane, J.; Bezuidenhout, C.; Thekisoe, O. "One Health" perspective on prevalence of co-existing extended-spectrum β-lactamase (ESBL)-producing Escherichia coli and Klebsiella pneumoniae: A comprehensive systematic review and meta-analysis. *Ann. Clin. Microbiol. Antimicrob.* **2023**, *22*, 88. [CrossRef] [PubMed]

9. Tseng, C.-H.; Liu, C.-W.; Liu, P.-Y. Extended-Spectrum β-Lactamases (ESBL) Producing Bacteria in Animals. *Antibiotics* **2023**, *12*, 661. [CrossRef]

10. Bogaerts, P.; Huang, T.-D.; Bouchahrouf, W.; Bauraing, C.; Berhin, C.; El Garch, F.; Glupczynski, Y.; Group, C.S. Characterization of ESBL-and AmpC-producing Enterobacteriaceae from diseased companion animals in Europe. *Microb. Drug Resist.* **2015**, *21*, 643–650. [CrossRef]

11. Rubin, J.E.; Pitout, J.D. Extended-spectrum β-lactamase, carbapenemase and AmpC producing Enterobacteriaceae in companion animals. *Vet. Microbiol.* **2014**, *170*, 10–18. [CrossRef] [PubMed]

12. Shaheen, B.; Boothe, D.; Oyarzabal, O.; Smaha, T. Antimicrobial resistance profiles and clonal relatedness of canine and feline Escherichia coli pathogens expressing multidrug resistance in the United States. *J. Vet. Intern. Med.* **2010**, *24*, 323–330. [CrossRef] [PubMed]

13. Köck, R.; Daniels-Haardt, I.; Becker, K.; Mellmann, A.; Friedrich, A.W.; Mevius, D.; Schwarz, S.; Jurke, A. Carbapenem-resistant Enterobacteriaceae in wildlife, food-producing, and companion animals: A systematic review. *Clin. Microbiol. Infect.* **2018**, *24*, 1241–1250. [CrossRef] [PubMed]

14. Dierikx, C.; van Duijkeren, E.; Schoormans, A.; van Essen-Zandbergen, A.; Veldman, K.; Kant, A.; Huijsdens, X.; van der Zwaluw, K.; Wagenaar, J.; Mevius, D. Occurrence and characteristics of extended-spectrum-β-lactamase-and AmpC-producing clinical isolates derived from companion animals and horses. *J. Antimicrob. Chemother.* **2012**, *67*, 1368–1374. [CrossRef] [PubMed]

15. Beutin, L. Escherichia coli as a pathogen in dogs and cats. *Vet. Res.* **1999**, *30*, 285–298. [PubMed]

16. 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. *J. Clin. Microbiol.* **2008**, *46*, 417–422. [CrossRef] [PubMed]

17. Pitout, J. Extraintestinal pathogenic Escherichia coli: A combination of virulence with antibiotic resistance. *Front. Microbiol.* **2012**, *3*, 9. [CrossRef] [PubMed]

18. Ukah, U.; Glass, M.; Avery, B.; Daignault, D.; Mulvey, M.; Reid-Smith, R.; Parmley, E.; Portt, A.; Boerlin, P.; Manges, A. Risk factors for acquisition of multidrug-resistant Escherichia coli and development of community-acquired urinary tract infections. *Epidemiol. Infect.* **2018**, *146*, 46–57. [CrossRef]

19. European Society of Clinical Microbiology and Infectious Diseases. *European Committee on Antimicrobial Susceptibility Testing (EU-CAST) Breakpoint Tables v 8.0 (2018) Tables for Interpretation of MICs and Zone Diameters*; European Society of Clinical Microbiology and Infectious Diseases: Basel, Switzerland, 2018.

20. Clermont, O.; Bonacorsi, S.; Bingen, E. Rapid and simple determination of theEscherichia coli phylogenetic group. *Appl. Environ. Microbiol.* **2000**, *66*, 4555–4558. [CrossRef]

21. Coura, F.M.; Diniz, A.N.; Oliveira Junior, C.A.; Lage, A.P.; Lobato, F.C.F.; Heinemann, M.B.; Silva, R.O.S. Detection of virulence genes and the phylogenetic groups of Escherichia coli isolated from dogs in Brazil. *Ciência Rural* **2018**, *48*, e20170478. [CrossRef]

22. 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*, 34. [CrossRef] [PubMed]

23. Hordijk, J.; Schoormans, A.; Kwakernaak, M.; Duim, B.; Broens, E.; Dierikx, C.; Mevius, D.; Wagenaar, J.A. High prevalence of fecal carriage of extended spectrum β-lactamase/AmpC-producing Enterobacteriaceae in cats and dogs. *Front. Microbiol.* **2013**, *4*, 242. [CrossRef] [PubMed]

24. Wedley, A.L.; Dawson, S.; Maddox, T.W.; Coyne, K.P.; Pinchbeck, G.L.; Clegg, P.; Nuttall, T.; Kirchner, M.; Williams, N.J. Carriage of antimicrobial resistant Escherichia coli in dogs: Prevalence, associated risk factors and molecular characteristics. *Vet. Microbiol.* **2017**, *199*, 23–30. [CrossRef] [PubMed]

25. Matsumoto, Y.; Ikeda, F.; Kamimura, T.; Yokota, Y.; Mine, Y. Novel plasmid-mediated beta-lactamase from Escherichia coli that inactivates oxyimino-cephalosporins. *Antimicrob. Agents Chemother.* **1988**, *32*, 1243–1246. [CrossRef] [PubMed]

26. Teshager, T.; Domínguez, L.; Moreno, M.A.; Saénz, Y.; Torres, C.; Cardeñosa, S. Isolation of an SHV-12 β-lactamase-producing Escherichia coli strain from a dog with recurrent urinary tract infections. *Antimicrob. Agents Chemother.* **2000**, *44*, 3483–3484. [CrossRef] [PubMed]

27. Carattoli, A.; Lovari, S.; Franco, A.; Cordaro, G.; Di Matteo, P.; Battisti, A. Extended-spectrum β-lactamases in Escherichia coli isolated from dogs and cats in Rome, Italy, from 2001 to 2003. *Antimicrob. Agents Chemother.* **2005**, *49*, 833–835. [CrossRef] [PubMed]

28. Ewers, C.; Grobbel, M.; Bethe, A.; Wieler, L.H.; Guenther, S. Extended-spectrum beta-lactamases-producing gram-negative bacteria in companion animals: Action is clearly warranted. *Berl. J. Soziol.* **2011**, *124*, 4–101.

29. Sun, Y.; Zeng, Z.; Chen, S.; Ma, J.; He, L.; Liu, Y.; Deng, Y.; Lei, T.; Zhao, J.; Liu, J.-H. High prevalence of blaCTX-M extended-spectrum β-lactamase genes in Escherichia coli isolates from pets and emergence of CTX-M-64 in China. *Clin. Microbiol. Infect.* **2010**, *16*, 1475–1481. [CrossRef]

30. Schaufler, K.; Bethe, A.; Lübke-Becker, A.; Ewers, C.; Kohn, B.; Wieler, L.H.; Guenther, S. Putative connection between zoonotic multiresistant extended-spectrum beta-lactamase (ESBL)-producing Escherichia coli in dog feces from a veterinary campus and clinical isolates from dogs. *Infect. Ecol. Epidemiol.* **2015**, *5*, 25334. [CrossRef]

31. 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. *Front. Microbiol.* **2016**, *7*, 1057. [CrossRef]

32. Schmiedel, J.; Falgenhauer, L.; Domann, E.; Bauerfeind, R.; Prenger-Berninghoff, E.; Imirzalioglu, C.; Chakraborty, T. Multiresistant extended-spectrum β-lactamase-producing Enterobacteriaceae from humans, companion animals and horses in central Hesse, Germany. *BMC Microbiol.* **2014**, *14*, 187. [CrossRef] [PubMed]

33. Stolle, I.; Prenger-Berninghoff, E.; Stamm, I.; Scheufen, S.; Hassdenteufel, E.; Guenther, S.; Bethe, A.; Pfeifer, Y.; Ewers, C. Emergence of OXA-48 carbapenemase-producing Escherichia coli and Klebsiella pneumoniae in dogs. *J. Antimicrob. Chemother.* **2013**, *68*, 2802–2808. [CrossRef] [PubMed]

34. Ezzeddine, Z.; Ghssein, G. Towards new antibiotics classes targeting bacterial metallophores. *Microb. Pathog.* **2023**, *182*, 106221. [CrossRef] [PubMed]

35. Raro, O.H.F.; da Silva, R.M.C.; Filho, E.M.R.; Sukiennik, T.C.T.; Stadnik, C.; Dias, C.A.G.; Oteo Iglesias, J.; Pérez-Vázquez, M. Carbapenemase-Producing Klebsiella pneumoniae From Transplanted Patients in Brazil: Phylogeny, Resistome, Virulome and Mobile Genetic Elements Harboring bla KPC–2 or bla NDM–1. *Front. Microbiol.* **2020**, *11*, 1563. [CrossRef] [PubMed]

36. Taggar, G.; Attiq Rheman, M.; Boerlin, P.; Diarra, M.S. Molecular epidemiology of carbapenemases in Enterobacteriales from humans, animals, food and the environment. *Antibiotics* **2020**, *9*, 693. [CrossRef] [PubMed]

37. Nordmann, P.; Cornaglia, G. Carbapenemase-producing Enterobacteriaceae: A call for action! *Clin. Microbiol. Infect.* **2012**, *18*, 411–412. [CrossRef]

38. Poirel, L.; Potron, A.; Nordmann, P. OXA-48-like carbapenemases: The phantom menace. *J. Antimicrob. Chemother.* **2012**, *67*, 1597–1606. [CrossRef] [PubMed]

39. Shaheen, B.W.; Nayak, R.; Boothe, D.M. Emergence of a New Delhi metallo-β-lactamase (NDM-1)-encoding gene in clinical Escherichia coli isolates recovered from companion animals in the United States. *Antimicrob. Agents Chemother.* **2013**, *57*, 2902–2903. [CrossRef] [PubMed]

40. Yousfi, M.; Mairi, A.; Bakour, S.; Touati, A.; Hassissen, L.; Hadjadj, L.; Rolain, J.-M. First report of NDM-5-producing Esche-richia coli ST1284 isolated from dog in Bejaia, Algeria. *New Microbes New Infect.* **2015**, *8*, 17. [CrossRef]

41. Melo, L.C.; Boisson, M.N.; Saras, E.; Médaille, C.; Boulouis, H.-J.; Madec, J.-Y.; Haenni, M. OXA-48-producing ST372 Escherichia coli in a French dog. *J. Antimicrob. Chemother.* **2017**, *72*, 1256–1258.

42. Ktari, S.; Arlet, G.; Mnif, B.; Gautier, V.; Mahjoubi, F.; Ben Jmeaa, M.; Bouaziz, M.; Hammami, A. Emergence of multidrug-resistant Klebsiella pneumoniae isolates producing VIM-4 metallo-β-lactamase, CTX-M-15 extended-spectrum β-lactamase, and CMY-4 AmpC β-lactamase in a Tunisian university hospital. *Antimicrob. Agents Chemother.* **2006**, *50*, 4198–4201. [CrossRef] [PubMed]

43. Nasri, E.; Subirats, J.; Sànchez-Melsió, A.; Mansour, H.B.; Borrego, C.M.; Balcázar, J.L. Abundance of carbapenemase genes (blaKPC, blaNDM and blaOXA-48) in wastewater effluents from Tunisian hospitals. *Environ. Pollut.* **2017**, *229*, 371–374. [CrossRef] [PubMed]

44. Dziri, O.; Dziri, R.; El Salabi, A.A.; Chouchani, C. Carbapenemase producing Gram-Negative bacteria in tunisia: History of thirteen years of challenge. *Infect. Drug Resist.* **2020**, *13*, 4177. [CrossRef] [PubMed]

45. Fischer, J.; Rodríguez, I.; Schmoger, S.; Friese, A.; Roesler, U.; Helmuth, R.; Guerra, B. Escherichia coli producing VIM-1 carbapenemase isolated on a pig farm. *J. Antimicrob. Chemother.* **2012**, *67*, 1793–1795. [CrossRef] [PubMed]

46. Zhang, W.-J.; Lu, Z.; Schwarz, S.; Zhang, R.-M.; Wang, X.-M.; Si, W.; Yu, S.; Chen, L.; Liu, S. Complete sequence of the bla NDM-1-carrying plasmid pNDM-AB from Acinetobacter baumannii of food animal origin. *J. Antimicrob. Chemother.* **2013**, *68*, 1681–1682. [CrossRef] [PubMed]

47. Lengliz, S.; Benlabidi, S.; Raddaoui, A.; Cheriet, S.; Ben Chehida, N.; Najar, T.; Abbassi, M. High occurrence of carbapenem-resistant Escherichia coli isolates from healthy rabbits (Oryctolagus cuniculus): First report of bla IMI and bla VIM type genes from livestock in Tunisia. *Lett. Appl. Microbiol.* **2021**, *73*, 708–717. [CrossRef] [PubMed]

48. Mani, Y.; Mansour, W.; Mammeri, H.; Denamur, E.; Saras, E.; Boujâafar, N.; Bouallègue, O.; Madec, J.-Y.; Haenni, M. KPC-3-producing ST167 Escherichia coli from mussels bought at a retail market in Tunisia. *J. Antimicrob. Chemother.* **2017**, *72*, 2403–2404. [CrossRef] [PubMed]

49. Selmi, R.; Tayh, G.; Srairi, S.; Mamlouk, A.; Chehida, F.B.; Lahmar, S.; Bouslama, M.; Daaloul-Jedidi, M.; Messadi, L. Prevalence, risk factors and emergence of extended-spectrum β-lactamase producing-, carbapenem-and colistin-resistant Enterobacterales isolated from wild boar (Sus scrofa) in Tunisia. *Microb. Pathog.* **2022**, *163*, 105385. [CrossRef]

50. Guerra, B.; Fischer, J.; Helmuth, R. An emerging public health problem: Acquired carbapenemase-producing microorganisms are present in food-producing animals, their environment, companion animals and wild birds. *Vet. Microbiol.* **2014**, *171*, 290–297. [CrossRef]

51. Grönthal, T.; Österblad, M.; Eklund, M.; Jalava, J.; Nykäsenoja, S.; Pekkanen, K.; Rantala, M. Sharing more than friendship–transmission of NDM-5 ST167 and CTX-M-9 ST69 Escherichia coli between dogs and humans in a family, Finland, 2015. *Euro Surveill.* **2018**, *23*, 1700497. [CrossRef]

52. Yousfi, M.; Touati, A.; Mairi, A.; Brasme, L.; Gharout-Sait, A.; Guillard, T.; De Champs, C. Emergence of carbapenemase-producing Escherichia coli isolated from companion animals in Algeria. *Microb. Drug Resist.* **2016**, *22*, 342–346. [CrossRef] [PubMed]

53. González-Torralba, A.; Oteo, J.; Asenjo, A.; Bautista, V.; Fuentes, E.; Alós, J.-I. Survey of carbapenemase-producing Enterobacteriaceae in companion dogs in Madrid, Spain. *Antimicrob. Agents Chemother.* **2016**, *60*, 2499–2501. [CrossRef] [PubMed]

54. Salgado-Caxito, M.; Benavides, J.A.; Adell, A.D.; Paes, A.C.; Moreno-Switt, A.I. Global prevalence and molecular characterization of extended-spectrum β-lactamase producing-Escherichia coli in dogs and cats–a scoping review and meta-analysis. *Int. J. One Health* **2021**, *12*, 100236. [CrossRef] [PubMed]

55. Puño-Sarmiento, J.; Medeiros, L.; Chiconi, C.; Martins, F.; Pelayo, J.; Rocha, S.; Blanco, J.; Blanco, M.; Zanutto, M.; Kobayashi, R. Detection of diarrheagenic Escherichia coli strains isolated from dogs and cats in Brazil. *Vet. Microbiol.* **2013**, *166*, 676–680. [CrossRef] [PubMed]

56. Hasan, M.; Yousif, A.; Alwan, M. Detection of virulent genes in *E. coli* O157: H7 isolated from puppies and adult dogs by polymerase chain reaction. *Res. J. Vet. Pract* **2016**, *4*, 1–6. [CrossRef]

57. Trabulsi, L.R.; Keller, R.; Gomes, T.A.T. Typical and Atypical Enteropathogenic Escherichia coli. *Emerging Infect. Dis.* **2002**, *8*, 508. [CrossRef]

58. Mainil, J. Escherichia coli virulence factors. *Vet. Immunol. Immunopathol.* **2013**, *152*, 2–12. [CrossRef]