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Antimicrobial Resistance Characteristics and Phylogenetic Groups of *Escherichia coli* Isolated From Diarrheic Calves in Southeast of Iran

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Abstract

Background: Antimicrobial resistance (AR) is one of the main challenges in diarrheal diseases in human and animals. Regardless of the main reason of the disease, approximately all antimicrobial actions including treatment, control, and prevention are mostly centralized against *Escherichia coli* strains.

Objectives: This work was focused on AR and determination of phylogenetic groups in *E. coli* isolates (n = 170) obtained from calves with diarrhea.

Materials and Methods: Isolates were molecularly characterized for 17 AR genes and 3 phylogenetic sequences. AR phenotyping was performed on all strains for 12 antimicrobial agents by using disc diffusion method.

Results: All AR genes but *qnrS* were identified with different prevalence in *E. coli* isolates; the most common genes were *aadA* (20%), bla_{TEM} (11.7%), and *sulII* (11.2%) belonging to aminoglycoside, β-lactamase, and sulphonamide families, respectively. Resistance to the penicillin and sulphamethoxazole drugs was found in 100% of isolates and was followed by resistance to tetracycline (73.5%), streptomycin (60%), trimethoprim sulphamethoxazole (56.5%), and kanamycin (53.5%). The phylogenetic groups A and B1 with the frequency of 65.8% and 30.6% considerably surrounded the majority of isolates, respectively.

Conclusions: In Iran, diarrheic calves have an important role as the reservoir of resistant *E. coli* strains to some drugs which are registered for treatment of calf diarrhea.

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Background

Antimicrobial resistance (AR) in *Escherichia coli* is a significant issue in the treatment and control of diarrheal diseases[1] and exposure to antimicrobial agents amplifies the resistance in *E. coli* population inhabited in animal and human intestinal tracts. Besides, the transmission of resistance genes is probable and also helps to spread out AR traits to other pathogenic and non-pathogenic organisms.[2] Treatment with antibiotics such as amoxicillin, sulfonamides etc. is common in diarrheic calves.[3] Consequently, calves are suggested as one of the major reservoirs for antimicrobial drug resistance amongst food-producing animals.[4]

Escherichia coli can be categorized into four recognized phylogenetic groups A, B1, B2, and D; this classification have been performed using a triplex polymerase chain reaction (PCR) based method defined by Clermont et al based on the detection of three genetic sequences called *chuA*, *yjaA*, and TSPE4.C2.[5] It is now well accepted that achievement of evolutionary data through phylogenetic analysis of *E. coli* can help to determine the pathogenic and non-pathogenic nature of the bacterium.[6]

Objectives

The objectives of present study were to isolate *E. coli* from calf diarrhea and determinate their phenotypic and genotypic AR in order to show the importance of diarrheic calves as reservoirs of antimicrobial resistant *E. coli* in Iran.

Materials and Methods

Sampling, Culture and *Escherichia coli* Confirmation

A collection of 170 fecal samples were obtained from diarrheic calves via rectal swabs from different dairies in southeast of Iran (Kerman province). Each sample belonged to one animal between 1 to 8 weeks of age. The

swabs were placed in Amies medium (BBL, USA) and transferred to laboratory within about 6 hours and immediately cultured onto MacConkey agar (Merck, Germany) and held in aerobic incubation condition at 37°C for 18 to 24 hours. Biochemical confirmations were performed on suspected colonies using IMViC (indole, methyl-red-Voges-Proskauer, citrate) tests[7] and finally confirmed *E. coli* isolates were subjected to next steps.

PCR for Antimicrobial Resistance Genes and Phylogenetic Grouping

DNA extraction was carried out by NaOH method as described previously.[8] Simplex and multiplex PCR methods were employed to detect the AR genes and three sequences for phylogenetic grouping. In this work, 17 AR genes including *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-15}$, *bla*$_{OXA}$, *sulI*, *sulII*, *dhfrV*, *dhfrI*, *aadA*, *aac(3)-I*, *floR*, *cat1*, *qnrA*, *qnrB*, *qnrS*, *tetA*, and *tetB* were screened in each strain.[9-14] Determination of phylogenetic groups was done based on the presence or absence of *chuA*, *yjaA*, and TSPE4.C2 sequences in each *E. coli* isolate using a triplex PCR.[5] Positive and negative controls were used in each reaction; clinical *E. coli* strains 17DN for *qnrS*, *sulI*, and *sulII;* 21DN for *qnrB*; 25DN for *tetA*, *tetB*, *aadA*, *aac(3)-I*, *floR*, and *cat1;* 170DN for *dhfrI* and *dhfrV* all were kindly provided by Dr Reza Ghanbarpour from Faculty of Veterinary Medicine, Shahid Bahonar University of Kerman. Distilled water was used as negative control.

For PCR, reaction mixtures were mostly prepared in a total volume of 25 µL containing 12.5 µL Hot Start 2x Master Mix (Ampliqon, Denmark), 0.5µM of each primer (Table 1), 3 µL of centrifuged (13 000 rpm for 1 min-

Table 1. Primers Used for Detection of Antimicrobial Resistance Genes and Phylogenetic Sequences

Target	Sequence (5'-3')	Size (bp)		Reference
Antimicrobial Resistance Genes				
bla$_{CTX-15}$	CGC TTT GCG ATG TGC AG	550	60°C	12
	ACC GCG ATA TCG TTG GT			
bla$_{TEM}$	AAA ATT CTT GAA GAC G	1080	50°C	13
	TTA CCA ATG CTT AAT CA			
bla$_{SHV}$	TTA ACT CCC TGT TAG CCA	768	50°C	13
	GAT TTG CTG ATT TCG CCC			
bla$_{OXA}$	TCA ACT TTC AAG ATC GCA	591	48°C	10
	GTG TGT TTA GAA TGG TGA			
aadA	TGA TTT GCT GGT TAC GGT GAC	284	58°C	14
	CGC TAT GTT CTC TTG CTT TTG			
sulI	TTC GGC ATT CTG AAT CTC AC	822	58°C	14
	ATG ATC TAA CCC TCG GTC TC			
dhfrV	CTG CAA AAG CGA AAA ACG G	432	58°C	14
	AGC AAT AGT TAA TGT TTG AGC TAA AG			
aac(3)-I	ACC TAC TCC CAA CAT CAG CC	157	55°	14
	ATA TAG ATC TCA CTA CGC GC			
dhfrI	AAG AAT GGA GTT ATC GGG AAT G	391	55°	14
	GGG TAA AAA CTG GCC TAA AAT TG			
tetA	GTG AAA CCC AAC ATA CCC C	887	50°C	14
	GAA GGC AAG CAG GAT GTA G			
tetB	CCT TAT CAT GCC AGT CTT GC	773	50°C	14
	ACT GCC GTT TTT TCG CC			
sulII	GCG CTC AAG GCA GAT GGC ATT	293	69°C	11
	GCG TTT GAT ACC GGC ACC CGT			
floR	TAT CTC CCT GTC GTT CCA G	399	56°C	14
	AGA ACT CGC CGA TCA ATG			
cat1	AGT TGC TCA ATG TAC CTA TAA CC	547	56°C	14
	TTG TAA TTC ATT AAG CAT TCT GCC			
qnrA	AGA GGA TTT CTC ACG CCA GG	580	54°C	9
	TGC CAG GCA CAG ATC TTG AC			
qnrB	GGM ATH GAA ATT CGC CAC TG	264	54°C	9
	TTT GCY GYY CGC CAG TCG AA			
qnrS	TTT GCY GYY CGC CAG TCG AA	428	54°C	9
	GCA AGT TCA TTG AAC AGG GT			
Phylogenetic Grouping				
chuA	GAC GAA CCA ACG GTC AGG AT	279	55°C	5
	TCG CCA GTA CCA AAG ACA			
yajA	TGA AGT GTC AGG AGA CGC TG	211	55°C	5
	ATG GAG AAT GCG TTC CTC AAC			
TSPE4.C2	GAG TAA TGT CGG GGC ATT CA	152	55°C	5
	CGC GCC AAC AAA GTA TTA CG			

ute) DNA template, and PCR grade water. The majority of thermal cycling programs were: 95°C for 15 minutes (initial denaturation and activation of DNA polymerase), 95°C for 1 minute (denaturation), 54-65°C for 1 minute (annealing), 72°C for 1 minute (extension), and finally 72°C for 10 minutes (final extension). The stages 2, 3 and 4 were repeated for at least 30 cycles in each program and annealing temperatures were optimized as mentioned in Table 1. The PCR products were checked on the agarose gel (1.5%), after electrophoresis at 85 V for 45 minutes, staining with ethidium bromide, and imaging by a Gel Doc 1000 (Vilber Lourmat, France).

Antimicrobial Resistance Phenotyping

Twelve antimicrobial agents including penicillin (P; 10 µg), cefotaxim (CTX; 30 µg), ceftazidime-clavulanic acid (CZA; 30/10 µg), streptomycin (S; 10 µg), kanamycin (K; 30 µg), spectinomycin (SPT; 100 µg), gentamicin (GM; 10 µg), tetracycline (TE; 30 µg), florfenicol (FF; 30 µg), enrofloxacin (NFX; 10 µg), sulphamethoxazole (SMZ; 250 µg), and trimethoprim sulphamethoxazole (SXT; 1.25/23.75 µg) were used for AR phenotyping of all *E. coli* isolates.

A suspension of each organism with turbidity similar to a 0.5 McFarland standard was prepared in approximately 2 mL of sterile saline. A sterile swab was dipped into the inoculum tube and inoculation was performed on a Mueller-Hinton (MH) agar plate by streaking the swab 3 times over the entire agar surface. Antimicrobial disks were placed on the surface of the agar and plates were incubated at 37°C for 24 hours.

Finally, susceptibility to the antimicrobials was measured using Kirby–Bauer disc diffusion method according to the Clinical and Laboratory Standards Institute (CLSI).[15] *E. coli* strain ATCC 25922 was considered as a quality control. Isolates were classified as susceptible (S) and resistant (R) based on zone diameters of bacterial growth inhibition in MH agar presented in CLSI 2013: P (R≤13 mm and 17 mm≤S), CTX (R≤22 mm and 26 mm ≤S), CZA (R≤16 mm and 22 mm≤S), S (R≤11 mm and 15 mm≤S), K (R≤13 mm and 18 mm≤S), SPT (R≤18 mm and 18 mm≤S), GM (R≤12 mm and 15 mm≤S), TE (R≤11 mm and 15 mm≤S), FF (R≤14 mm and 19 mm≤S), NFX (R≤16 mm and 23 mm≤S), SMZ (R≤12 mm and 17 mm≤S), and SXT (R≤10 mm and 16 mm≤S).

Statistically Analysis

Proportions were compared through "N-1" chi-square test using MedCalc software (https://www.medcalc.org/). Calculations were performed in 95% confidence level and *P* value ≤ .05 was considered as marginal significance. For comparison of AR genotype with their phenotype in each antimicrobial group, the isolates were classified into 4 categories including phenotype$^+$/genotype$^+$, phenotype$^+$/genotype$^-$, phenotype$^-$/genotype$^+$, and phenotype$^-$/genotype$^-$. Then, proportions were compared by chi-square test.

Results

Antimicrobial Resistance Genes

Totally, 65 (38.2%; 95% CI: 30.9-45.5%) isolates were determined as AR gene-positive strains which had at least one of the 17 examined genes (Figure 1). Among the 65 isolates, the most frequent AR sequences were related to aminoglycoside (58.5%; 95% CI: 46.5-70.5%), sulphonamide (52.3%; 95% CI: 40.1-64.4%), and β-lactamase (47.7%; 95% CI: 35.5-59.8%) and in each family, the most common genes were *aadA* (52.3%), bla_{TEM} (30.8%), and *sulII* (29.2%), respectively. These amounts in association with tetracycline, amphenicols, quinolones, and trimethoprim families were 41.5%, 35.3%, 26.1%, and 24.6%, respectively. Forty-two different genetic AR profiles were found and 38/65 (58.5%; 95% CI: 46.5-70.5%) of strains carried 2 or more AR genes. Among 18 positives for VG sequences, the genes *aadA*, bla_{TEM}, bla_{SHV}, bla_{CTX-15}, *qnrS*, and *dhfrV* were amplified from 8 (44.4%; 95% CI: 21.4-67.3%) strains, with *aadA* as the most prevalent (Table 2).

Antimicrobial Resistance Phenotypes

All (100%) isolates showed resistance to the drugs P and SMZ, and resistance to TE (73.5%), S (60%), SXT

Table 2. Prevalence of Each Antimicrobial Resistance Gene and Phenotype Among *E. coli* Isolates

Genotype	Prevalence No. (%)	Phenotype	Prevalence No. (%)
β-Lactam[a]			
bla_{CTX-15}[b]	3 (1.7)	P[c]	170 (100)
bla_{TEM}	20 (11.7)	CTX	38 (22.3)
bla_{SHV}	7 (4.1)	CZA	23 (13.5)
bla_{OXA}	1 (0.6)		
Aminoglycoside			
aadA	34 (20)	S	102 (60)
aac(3)-I	4 (2.3)	K	91 (53.5)
		SPT	61 (35.8)
		GM	39 (22.9)
Tetracycline			
tetA	15(8.8)	TE	125 (73.5)
tetB	12 (7)		
Amphenicol			
cat1	14 (8.2)	FF	28 (16.4)
floR	9 (5.2)		
Quinolones			
qnrA	-	NFX	56 (32.9)
qnrB	2 (1,1)		
qnrS	15 (8.8)		
Sulphonamide			
sulI	15 (8.8)	SMZ	170 (100)
sulII	19 (11.1)		
Trimethoprim			
dhfrI	3 (1.7)	SXT	96 (56.4)
dhfrV	13 (7.6)		

[a]Name of antimicrobial family/group.
[b]Name of antimicrobial resistance genes in abbreviated and italic form.
[c]Name of antibiotics in abbreviated form.

(56.5%), and K (53.5%) was considerable, too. The fewer frequencies of AR were related to NFX (32.9%), SPT (22.9%), GM (22.9%), CTX (22.3%), and FF (16.5%), respectively. Surprisingly, seventy diverse patterns of AR panels were observed in phenotyping stage; the two profiles P/SMZ (n=27) and P/SMZ/SXT/S/K/SPT/GM/TE/NFX/FF (n=11) were the most frequent profiles. Totally, 143 (84.11%; 95% CI: 78.1-90.1%) strains had multi (3≤)-drug resistance to the tested antibiotics. Among 18 VG-positive isolates, resistance was registered as follows: P (18/18), SMZ (18/18), TE (12/18), S (10/18), K (8/18), SXT (7/18), SPT (7/18), CTX (7/18), GM (5/18), CZA (3/18), NFX (1/18), and FF (none of isolates) (Table 2).

Phenotypic/Genotypic Antimicrobial Resistance

In this study, all (n = 170) isolates showed the phenotypic resistance to at least one of the β-lactam antimicrobials. Among them, 26 (15.2%) isolates carried one or more of related genes but remaining 144 isolates (84.8%) lacked these genes. In aminoglycoside family, 122 isolates demonstrated resistance to one or more of S, K, SPT, and GM antibiotics from which only 31 (25.4%) isolates had *aadA* and/or *aac(3)-I*; whereas three strains possessing the aminoglycoside genes did not show the correlated resistance phenotype. On the basis of phenotypic method, 125 isolates displayed to be resistant to tetracycline from which 19 (15.2%) isolates had each or both of the related genes. Two isolates were positive for tetracycline genes, but had not resistance phenotype to TE. In this research, phenotypic resistance to florfenicol was detected in low prevalence (n = 28/170). Although the *catI* and/or *floR* genes were amplified from 6 (21.4%) FF-resistant isolates, 9 positives for florfenicol genes did not show the resistance to FF in disk diffusion method. Totally, 56 enrofloxacin-resistant isolates were observed during antibiogram stage from which 7 (12.5%) strains carried at least one of the three associated AR genes, while 9 isolates having the enrofloxacin genes did not represent any resistance to

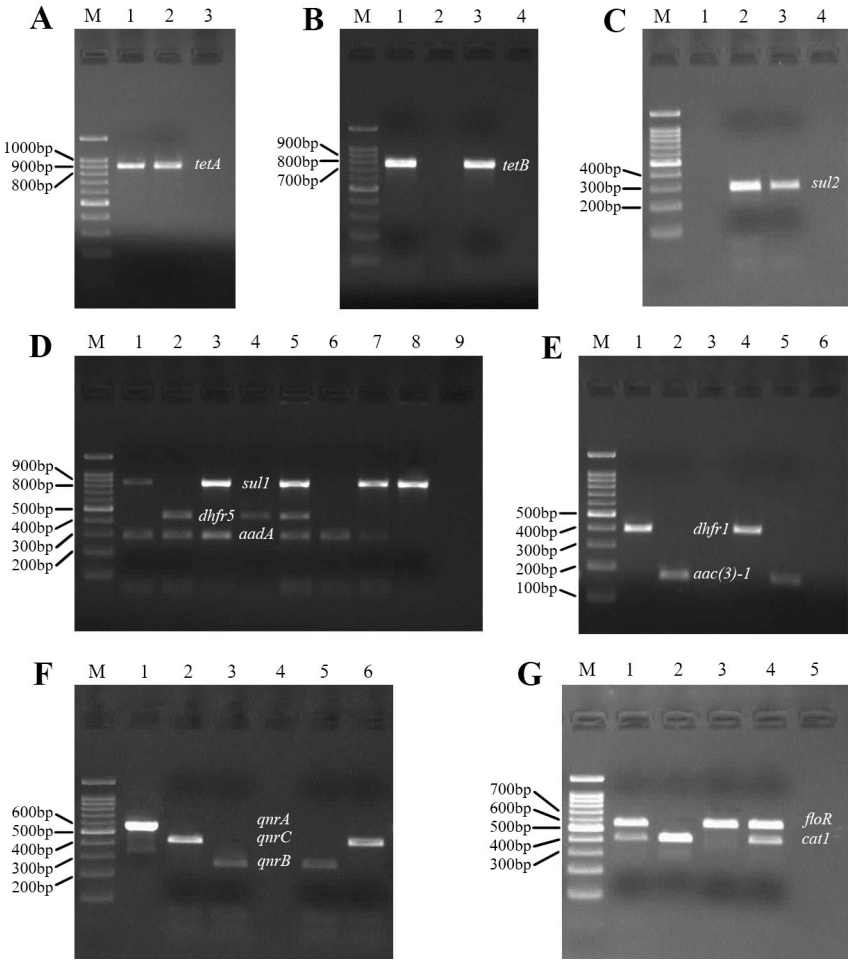

Figure 1. Agarose Gel Electrophoresis of Antimicrobial Resistance Genes Among *Escherichia coli* Isolates From Fecal Samples.
A, M, marker (100 bp); lane 1, positive-control; lane 2, positive-sample; lane 3, negative-control. B, M, marker (100 bp); lane 1, positive-control; lane 2, negative-control; lane 3, positive-sample, lane 4, negative-sample. C, M, marker (100 bp); lane 1, negative-control; lane 2, negative-control; lane 3, positive-sample, lane 4, negative-sample. D, M, marker (100 bp); lane 1-2, positive-control; lane 3-8, positive-samples; lane 9, negative-control. E, M, marker (100 bp); lane 1-2, positive-control; lane 3, negative-control; lane 4-5, positive-sample, lane 6, negative-sample. F, M, marker (100 bp); lane 1-3, positive-control; lane 4, negative-control; lane 5-6, positive-sample. G, M, marker (100 bp); lane 1, positive-control; lane 2-4, positive-sample, lane 5, negative-sample.

NFX in plate. Results of sulfamethoxazole antibiotic were exactly similar to β-lactam family; 170 isolates were resistant phenotypically and among them, 26 (15.2%) isolates were *sul1* and/or *sul2*-positve. These amounts were different for trimethoprim sulfamethoxazole-resistant isolates including 96 SXT-resistant strains among which 14 (14.6%) isolates were positive for *dhfrI* and/or *dhfrV* genes.

Relationships between AR genes and corresponding phenotypes were analyzed in each antimicrobial family. The statistical comparison of phenotype$^-$/genotype$^-$ and phenotype$^+$/genotype$^+$ isolates showed that there were significant differences among all antibiotic families except for aminoglycoside. The difference between phenotype$^-$/genotype$^+$ and phenotype$^+$/genotype$^+$ isolates was considered not to be quite statistically significant in florfenicol and enrofloxacin, unlike other groups. And finally the phenotype$^+$/genotype$^-$ isolates formed a notable proportion of strains ($P < .0001$; $df = 1$).

Phylogenetic Groups

Although four major *E. coli* phylo-groups (A, B1, B2, and D) and five sub phylo-groups (A_0, A_1, B1, $B2_3$, and D_1) were recognized in this study, majority of strains were within A (65.8%; 95% CI: 58.7-73%) and B1 (30.6%; 95% CI: 23.6-37.5%) phylo-groups. Only 1 and 5 samples were detected as members of B2 and D, respectively, and none of the strains were identified for $B2_2$ and D_2 phylogenetic groups. A_1 and B1 were the predominant sub phylo-groups among AR-gene positive strains, but VG-positive isolates were mostly allocated to A_0 and B1 (Table 3).

Discussion

One of the critical issues and concerns in public health is increasing AR among bacterial populations. AR decreases therapeutic impact of antibiotics and may be transmitted to other microorganisms by related genes. In this study, prevalence of genetic and phenotypic AR was evaluated among the *E. coli* isolates to 6 antibiotic groups. The most prevalent AR genes and phenotypes were correlated to β-lactam, sulphonamide, aminoglycoside, and tetracycline families. Moreover, the specific AR genes of *aadA* (streptomycin and spectinomycin), bla_{TEM} (penicillins and first generation of cephalosporins), and *sulII* (sulphonamides) were determined as the most common genes, which is in agreement with a study on ruminant *E. coli* strains in Spain in that predominant resistance genes were bla_{TEM} in β-lactam, *tetA* in tetracycline, *aadA* in aminoglycosides, *catI* in chloramphenicol, and *sulI* and *sulII* in sulfamethoxazole groups.[16] In the Spanish study, *dhfrI* was predominant gene in trimethoprim group, unlike to our results. Furthermore, in agreement with previous studies on diarrheic neonatal calves, prevalence of genetic AR compared to ceftiofur (bla_{CTX-15}), apramycin (*aac3-I*), and florfenicol (*floR*) were found in low rate.[17] Frequency of AR gene was different in diverse researches which may be similar or dissimilar to our results.

In phenotypic level, isolates had resistance to two (penicillin and sulphonamide) or more of tested antibiotics; 84.11% of isolates were defined as multi ($3 \leq$)-drug resistant. Regardless of P and SMZ, resistance to TE, S, SXT, and K were observed in high frequency, while the isolates represented a considerable susceptibility to five antibiotics including CTX, CZA, GM, FF and NFX, respectively. Our results was comparable with a study in Iran that showed the extensive resistance to penicillin and sulphonamide. Our study was in agreement with the Iranian study in prevalence of SXT and NFX, but there were significant differences about S, TE, and GM. Although there is agreement between our results and previous studies in family level, there are some differences in frequency of specific antibiotics.[18]

Our isolates were significantly resistant to the antibiotics streptomycin, tetracycline, and sulphonamide, having prophylactic and therapeutic usages in calf diarrhea. Other choices for above-mentioned applications are amoxicillin, neomycin, ampicillin, and chloramphenicol,[2] and evaluation of phenotypic resistance to them should not be neglected. Irregular consumption of antibiotics and nourishment of calves with AR contaminated milk are the important risk factors that amplify the selection of resistant strains in gut.[19] Horizontal transferring of resistant bacteria and genes to environment, foods, and other hosts is completely probable.[20] Therefore, tracing AR in commensal microorganisms can help us to adopt strategies for controlling resistance.

In this research, *E. coli* isolates possessing β-lactamase and sulphonamide AR genes showed the phenotypic resistance to corresponding antibiotics, but approximately 60% of gene-positives for amphenicol and quinolone and 10% of gene-positive strains for aminoglycoside and tetracycline isolates did not show the related AR phenotype. Additionally, only 12%-25% of isolates displaying AR phenotype had the correlated genes. Based on AR phenotype/genotype analysis, all AR genes but *floR* and *qnr* had been expressed. Attendance of isolates with AR pheno-

Table 3. Prevalence of Phylogenetic Subgroups Among Isolates Based on Presence or Absence of AR Genes (%)

Phylo-group	A		B1	B2	D	
Phylo-subgroup	A_0	A_1	B1	$B2_3$	D1	Total
AR gene-positive isolates	14 (21.5)	29 (44.6)	20 (30.8)		2 (3.1)	65 (100)
AR gene-negative isolates	50 (47.6)	19 (18.1)	32 (30.5)	1 (0.9)	3 (2.9)	105 (100)
No. of isolates which belong to each phylo-subgroup	64 (37.6)	48 (28.2)	52 (30.6)	1 (0.6)	5 (3)	170 (100)

type without genotype may also be due to resistance genes that have not been studied during research. Nevertheless, some studies have pointed to association between prevalence of AR genes and phenotypes, while there are some reports about isolates without the phenotypic expression of AR genes.[21] Various forms of resistance to antimicrobial drugs can be correlated to some factors such as oxidative stress, iron depletion, and retained motility which change expression of resistant genes.[22]

In phylogenetic analysis, all isolates were classified into phylo-groups and their subsets; majority of our positives for AR and multi-drug resistance were allocated to A, followed by B1 phylogenetic group. Totally, the three groups A, B1, and D contain the major phylogenetic groups of antibiotic-resistant *E. coli*.[23] In a report, B1 was the most virulent phylo-group, but A and D were the predominant groups in non-virulent isolates.[23] It seems that phylo-groups A and B1 are more common in animals than in humans and B1 is predominant in herbivorous animals.[24] Relatedness of pathogenic *E. coli* with B2 and D phylo-groups is documented in some researches [24] and A and B1 phylogenetic groups are predominantly distributed in commensal *E. coli* populations.[25]

This work shows that diarrheic calves are reservoirs for non-pathogenic phylogenetic groups (A and B1) and important AR. Resistance to β-lactam, streptomycin, tetracycline, and sulphonamide groups is significantly common. Because of transmission probability of resistance traits to other microorganisms and hosts, these forms of resistance may lead to a series of consequences such as complications in antimicrobial treatment process in animals and human. Thus, for management of resistant strains, screening of AR among gut commensals should be continued and also evaluation of further different AR genes is required for each antimicrobial category.

Authors' Contributions

ZN conducted literature searches, provided summaries of previous research studies, and performed sampling and laboratory tests. RG designed the study and wrote the protocol. MS evaluated the statistical analysis and final manuscript.

Conflict of Interest Disclosures

The authors declare that they have no conflict of interests.

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References

1. World Health Organization. Antimicrobial Resistance Global Report on Surveillance. Geneva: WHO; 2014:1-34.
2. Dzidic S, Bedeković V. Horizontal gene transfer-emerging multidrug resistance in hospital bacteria. Acta Pharmacol Sin. 2003;24(6):519-526.
3. Constable PD. Antimicrobial use in the treatment of calf diarrhea. J Vet Intern Med. 2004;18(1):8-17.
4. Khachatryan AR, Hancock DD, Besser TE, Call DR. Role of calf-adapted *Escherichia coli* in maintenance of antimicrobial drug resistance in dairy calves. Appl Environ Microbiol. 2004;70(2):752-757.
5. Clermont O, Bonacorsi S, Bingen E. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol. 2000;66(10):4555-4558.
6. Chaudhuri RR, Henderson IR. The evolution of the *Escherichia coli* phylogeny. Infect Genet Evol. 2012;12(2):214-226. doi:10.1016/j.meegid.2012.01.005.
7. Markey B, Leonard F, Archambault M, Cullinane A, Maguire D. Clinical Veterinary Microbiology. Elsevier Health Sciences; 2013.
8. Jajarmi M, Ghanbarpour R, Sharifi H, Golchin M. Distribution pattern of EcoR phylogenetic groups among shiga toxin-producing and enteropathogenic *Escherichia coli* isolated from healthy goats. Int J Enteric Pathog. 2015;3(3):e27971.
9. Cattoir V, Poirel L, Rotimi V, Soussy CJ, Nordmann P. Multiplex PCR for detection of plasmid-mediated quinolone resistance qnr genes in ESBL-producing enterobacterial isolates. J Antimicrob Chemother. 2007;60(2):394-397.
10. Colom K, Pérez J, Alonso R, Fernández-Aranguiz A, Lariño E, Cisterna R. Simple and reliable multiplex PCR assay for detection of *blaTEM*, *blaSHV* and *blaOXA–1* genes in Enterobacteriaceae. FEMS Microbiol Lett. 2003;223(2):147-151.
11. Kerrn M, Klemmensen T, Frimodt-Møller N, Espersen F. Susceptibility of Danish *Escherichia coli* strains isolated from urinary tract infections and bacteraemia, and distribution of sul genes conferring sulphonamide resistance. J Antimicrob Chemother. 2002;50(4):513-516.
12. Messai Y, Benhassine T, Naim M, Paul G, Bakour R. Prevalence of β-lactams resistance among *Escherichia coli* clinical isolates from a hospital in Algiers. Rev Esp Quimioter. 2006;19(2):144-151.
13. Sharma J, Sharma M, Ray P. Detection of TEM & SHV genes in Escherichia coli & Klebsiella pneumoniae isolates in a tertiary care hospital from India. Indian J Med Res. 2010;132:332-336.
14. Van TTH, Chin J, Chapman T, Tran LT, Coloe PJ. Safety of raw meat and shellfish in Vietnam: an analysis of Escherichia coli isolations for antibiotic resistance and virulence genes. Int J Food Microbiol. 2008;124(3):217-223. doi:10.1016/j.ijfoodmicro.2008.03.029.
15. Cockerill F, Patel J, Alder J, Bradford P, Dudley M, Eliopoulos G. Performance standards for antimicrobial susceptibility testing: twenty-third informational supplement; M100-S23. Wayne, PA: CLSI; 2013.
16. Medina A, Horcajo P, Jurado S, et al. Phenotypic and genotypic characterization of antimicrobial resistance in enterohemorrhagic *Escherichia coli* and atypical enteropathogenic *E. coli* strains from ruminants. J Vet Diagn Invest. 2011;23(1):91-95.
17. Ahmed AM, Younis EE, Osman SA, Ishida Y, El-khodery SA, Shimamoto T. Genetic analysis of antimicrobial resistance in *Escherichia coli* isolated from diarrheic neonatal calves. Vet Microbiol. 2009;136(3):397-402. doi:10.1016/j.vetmic.2008.11.021.
18. Pereira R, Santos T, Bicalho M, Caixeta L, Machado V, Bicalho R. Antimicrobial resistance and prevalence of virulence factor genes in fecal Escherichia coli of Holstein calves fed milk with and without antimicrobials. J Dairy Sci. 2011;94(9):4556-4565.
19. Duse A, Waller KP, Emanuelson U, Unnerstad HE, Persson Y, Bengtsson B. Risk factors for antimicrobial resistance in fecal Escherichia coli from preweaned dairy calves. J Dairy Sci. 2015;98(1):500-516. doi:10.3168/jds.2014-8432.

20. Yamamoto S, Iwabuchi E, Hasegawa M, et al. Prevalence and molecular epidemiological characterization of antimicrobial-resistant *Escherichia coli* isolates from Japanese black beef cattle. J Food Prot. 2013;76(3):394-404. doi:10.4315/0362-028X.JFP-12-273.
21. Sheikh AA, Checkley S, Avery B, et al. Antimicrobial resistance and resistance genes in *Escherichia coli* isolated from retail meat purchased in Alberta, Canada. Foodborne Pathog Dis. 2012;9(7):625-631. doi:10.1089/fpd.2011.
22. Suzuki S, Horinouchi T, Furusawa C. Prediction of antibiotic resistance by gene expression profiles. Nat Commun. 2014;5:5792. doi:10.1038/ncomms6792.
23. Valat C, Auvray F, Forest K, et al. Phylogenetic grouping and virulence potential of Extended-Spectrum β-Lactamase-producing *Escherichia coli* in cattle. Appl Environ Microbiol. 2012;78(13):4677-82. doi:10.1128/AEM.00351-12.
24. Carlos C, Pires MM, Stoppe NC, et al. *Escherichia coli* phylogenetic group determination and its application in the identification of the major animal source of fecal contamination. BMC Microbiol. 2010;10:161. doi:10.1186/1471-2180-10-161.
25. Ishii S, Meyer KP, Sadowsky MJ. Relationship between phylogenetic groups, genotypic clusters, and virulence gene profiles of *Escherichia coli* strains from diverse human and animal sources. Appl Environ Microbiol. 2007;73(18):5703-5710.