Genetic diversity and antimicrobial resistance of *Escherichia coli* as microbial source tracking tools of Karaj River, Iran

Roohollah Kheiri and Leili Akhtari

ABSTRACT

The aim of this study was to analyze the enterobacterial repetitive intergenic consensus (ERIC)-types, phylo-groups and antimicrobial resistance (AMR) patterns of *Escherichia coli* and to investigate if these approaches are suitable for microbial source tracking (MST). *E. coli* strains were isolated from cattle faeces and Karaj River. For genetic diversity, AMR profile, and phylo-grouping, we applied ERIC-PCR, disk diffusion, and multiplex-PCR, respectively. Fifty isolates from each sample group were used in the study. ERIC fingerprinting produced ten different bands, demonstrating 64 unique and 36 repetitive profiles. Six isolates from the river showed the same ERIC pattern of the cattle, of which four expressed the same AMR profile. *E. coli* isolates from water were represented in A, B1, C, and D phylo-groups. Phylo-groups A, B1, and E were more prevalent in the cattle isolates and B2 was absent in both sources. Three of the water isolates with the same ERIC-type and AMR to cattle isolates showed the same phylo-groups. Genetic characteristics, AMR, and phylo-groups of the isolates from the river are diverse and complex. For accurate MST, complementary approaches should be applied together and a comprehensive library should be provided.

Key words | antimicrobial resistance, ERIC-PCR, *Escherichia coli*, microbial source tracking, phylo-groups, water

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INTRODUCTION

Escherichia coli is a normal inhabitant of the lower intestinal tract of warm-blooded animals and humans, therefore the presence of *E. coli* in water is an implicit indicator of recent faecal contamination and the risk of enteric pathogens. Although the majority of *E. coli* strains are commensals, some are known to be pathogenic, causing intestinal and extra-intestinal diseases, such as diarrhea and urinary tract infections (Lyautey *et al.* 2010). The possible reservoir of faecal contamination includes surface runoff from manure-treated agricultural land or farm animal feedlots, failing or inadequate septic systems, sewer overflow, and wildlife, along with domestic sewage disposal and animal husbandries near the bank (Kon *et al.* 2009). Water pollution raises considerable public health concern as it can transport pathogenic parasites, bacteria and viruses Borges *et al.* (2003). Hence, understanding the origin of faecal pollution is paramount in assessing associated health risks as well as the actions necessary to solve the problem (Dombek *et al.* 2000). Tracing the origin of faecal pollution by using microbiological, genotypic, phenotypic, and chemical methods is termed microbial source tracking (MST). When applying MST, it is critical to introduce applicable methods by which the faecal contamination sources can be identified (Lu *et al.* 2004). Different approaches have been proposed for tracking the contamination sources. These approaches can be broadly divided into library-dependent and library-independent techniques. Antimicrobial resistance (AMR) profiling and DNA fingerprinting-based

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methods (such as ribotyping, pulse field gel electrophoresis, REP-PCR), and phylo-typing have been reported as candidate library-dependent methods (Foley *et al.* 2009). A range of DNA fingerprinting techniques exists, among which repetitive extra-genic palindromic elements–polymerase chain reaction (REP-PCR) provides high taxonomic resolution and may act as a rapid detector of diversity and evolution of the microbial genomes (Dombek *et al.* 2000). Among REP fingerprinting methods (REP/ERIC/BOX), enterobacterial repetitive intergenic consensus (ERIC) PCR is more preferred because of the simple protocol and discriminatory power similar to PFGE (Lin *et al.* 2008). In addition to REP typing, phylo-typing is also an applicable grouping technique in which combinations of specific genes can be used to cluster *E. coli* strains into phylo-groups. Multilocus sequence typing data improves the understanding of *E. coli* phylogenetic structure and allows strains to be classified in one of the seven phylo-groups A, B_1, B_2, C, D, E, and F (Clermont *et al.* 2013).

Although *E. coli* has diverse genotypic and phylotypic characteristics, some characteristics are shared among strains exposed to similar environments due to selection pressure. The level of selective pressure exerted in a mixed area may be a useful criterion for identifying the host sources of *E. coli* in the watershed. One such tool for examining the selection pressure of *E. coli* is assessing their antimicrobial sensitivities (Ishii & Sadowsky 2008). AMR in *E. coli* has been globally identified in isolates from environmental, animal and human sources. This is a consequence of the use of antimicrobials in medicine and their application in animal husbandries, which have brought about phenotypic changes (Cantas *et al.* 2013).

This aim of this study was to determine the genetic diversity and AMR patterns of *E. coli* isolated from Khozankala cattle husbandry and Karaj River and to evaluate the applicability of these typing methods for MST.

METHODS

Study area and sample collection

This descriptive cross-sectional study was performed from August 2015 to October 2015. The authors collected 106 water samples (one sample per day) from the Karaj River, which is located in the Central Alborz Mountains with an area of 850 km^2 and length of 75 km. Water samples were collected from a given sampling site in Karaj River (with geographic coordinates of 35.9404423, 51.0742861) according to standard microbiological sampling protocols (APHA 2012). They were immediately placed in a lightproof insulated box containing ice-packs to ensure rapid cooling and shipped to the quality control office (central laboratory) of Alborz Province Water and Wastewater Company.

For enteric sampling, following autoclaving of swabs in the capped tubes, they were inserted into the rectum of cattle to insure the collection of faecal material. The swabs and adhering faecal material were then placed in the screw-capped tube, stored on ice in a cool box and shipped to the laboratory.

Bacterial isolates

To isolate *E. coli*, water samples were inoculated into 15 tubes of lauryl tryptose (LST) broth (Merck KGaA) followed by *E. coli* (EC) broth (Merck KGaA) at 44.5°C and streaked onto eosin methylene blue agar (Merck KGaA) (APHA 2012). Colonies showing metal sheen were considered as presumptive *E. coli* isolates and were subjected to IMViC (Merck KGaA), glucuronidase, and tryptophanase (Merck KGaA) tests for final confirmation (APHA 2012).

To isolate *E. coli* from faeces, following the breaking off of the top portion of the swab which was in contact with the hand, the swabs were inserted into one tube of LST broth and followed the abovementioned procedure for isolation.

Confirmed isolates were inoculated into sterile cryotube vials containing nutrient broth and were incubated overnight at 37 °C. Sterile glycerol (Merck KGaA) was then added to each vial at a final concentration of 15% (vol/vol), and the vials were stored at −70 °C. The bacterial stocks were revived in Brain Heart Infusion broth under optimal growth conditions and genomic DNA was extracted from the bacterial pellet applying the *AccuPrep*® Genomic DNA Extraction Kit (Bioneer, South Korea).

ERIC typing

The strains were fingerprinted by ERIC-PCR as described by Versalovic *et al.* (1991); however, to decrease the unspecified

bands we modified the method by using HotStart *Taq* DNA polymerase. Amplification was performed with Veriti® 96-Well Thermal Cycler (Applied Biosystems) and PCR products were evaluated by horizontal electrophoresis in 1% agarose gel (Merck KGaA) containing SYBR green (Thermo Scientific). Finally the loaded gels were visualized by Gel DOC™ XR+ (BIORAD) and analyzed by Image Lab™ 4.0 software.

Computer-assisted image analysis and cluster assignment

The positions of fingerprints on gels were normalized using GeneRuler 100 bp plus DNA Ladder (Thermo Scientific) as the external standard. Using Mesquite version 2.75 (Maddison & Maddison 2012) and PAUP 4.0 beta for Windows software (Swofford 2000), a dendrogram was generated using the unweighted-pair group method (UPGMA). Clusters were initially assigned on the basis of 90% similarity.

Diversity index

For diversity of ERIC profiles and phylo-groups in isolates from water and cattle, Shannon–Weaver, which is one of the most used diversity measures, was chosen because it accounts for both abundance and evenness of the samples and assumes that individuals are randomly sampled from an infinitely large community and all species are represented in the sample (Pielou 1975).

Phylo-typing

As previously described by Clermont *et al.* (2013), *E. coli* strains were tested for *chuA*, *yjaA*, *TSPE4.C2*, *arpA*, and *trpA* genes by PCR and the results characterized the phylo-groups including A, B1, B2, C, D, E, and F.

AMR profiling

AMR profiling was performed applying the Kirby-Bauer disk diffusion method on Mueller-Hinton agar (Merck KGaA) plates according to the Performance Standards for Antimicrobial Susceptibility Testing of the Clinical and Laboratory Standards Institute (CLSI 2012). Since we found no clear data for antibiotics prescription in villages around the Chalous River, we chose a complete panel (seven categories) of antibiotics to be used including cefotaxime (30 μg), cefalotin (30 μg), trimethoprim/sulfamethoxazole (25 μg), amoxicillin/clavulanic (20/10 μg), ampicillin (10 μg), streptomycin (10 μg), gentamycin (10 μg), nalidixic acid (30 μg), chloramphenicol (30 μg), and tetracycline (30 μg) were used.

Statistical analysis

The chi-squared test was used to find any association between the phylo-groups, ERIC clusters (E1–E15) and AMR profiles. Analysis was performed using IBM SPSS statistics software version 19.0 (USA).

MST library

To build the MST library, *E. coli* isolated from cattle was subjected to ERIC fingerprinting, phylo-grouping and AMR profile determination as described above (Kon *et al.* 2009).

RESULTS AND DISCUSSION

E. coli recovery

As described by Sakizadeh *et al.* (2015), during the sampling period, the water samples from Karaj River displayed intensive faecal contamination ranging from 70 to $1{,}600 \times 10^2$ MPN/100 ml for *E. coli*. From 50 water and 50 faecal samples, a total of 100 *E. coli* isolates (one isolate per water sample) were isolated and included in present study.

Genetic diversity of *E. coli* isolates

ERIC types

ERIC iPCR fingerprints showed ten different bands ranging from 542 bp to 3,300 bp (Figure 1), while the most common band of about 1,200 bp could be observed in 47 *E. coli* isolates from cattle and the most prevalent band of about 2,850 bp could be observed in 32 isolates from water. Fifty isolates from water produced 39 unique profiles, while 50

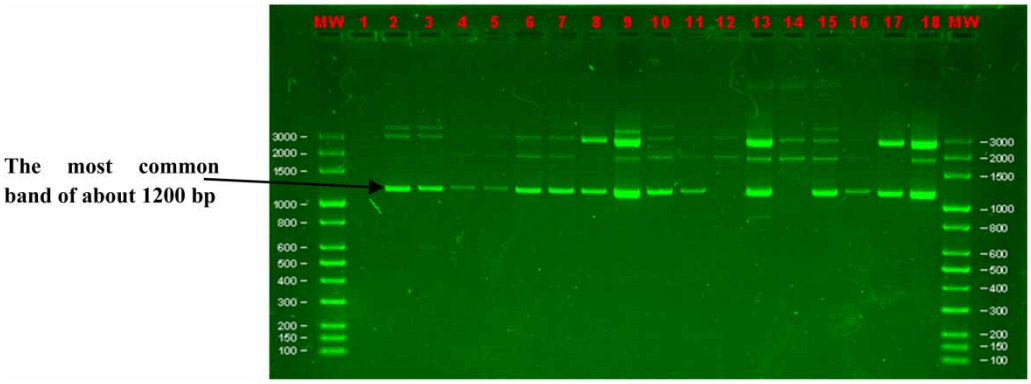

Figure 1 | ERIC-PCR fingerprinting patterns of the isolates. Lane MW: GeneRuler 100 bp plus DNA Ladder (Thermo Scientific); Lane 1–18: ERIC fingerprints of *E. coli* isolates from cattle.

isolates from cattle formed 30 single profiles. Moreover, six *E. coli* isolates from water had the same profiles as the isolates from cattle (Table 1), indicating the probability of the same origin.

Cluster analysis at a coefficient of 90% similarity, grouped 100 isolates into 15 clusters, designated E1–E15 (Figure 2). The isolates of the two sources were not uniformly distributed along the dendrogram, which means most of the clusters cannot be definitely attributed to a given source (water or cattle). In other words, E1 cluster contains ten isolates, eight from water and two from cattle, E12 cluster contains 11 isolates, nine from cattle and two from water and E3 contains seven isolates from water and three from cattle. However, E2 contains eight members of water origin, and E10 contains four isolates of cattle origin.

Diversity index for ERIC profiles in water and cattle isolates

The degree of diversity calculated for the 100 isolates, using the Shannon–Weaver index, was 3.43. The diversity was also calculated for the isolates from water ($n = 50$) and cattle ($n = 50$) separately, for which the diversity indices were 3.65 and 2.39, respectively, indicating more genetic diversity for *E. coli* of water origin.

Table 1 | Isolates of water and cattle with the same ERIC profiles

Cluster	Isolates from cattle	Isolates from water
E3	C8, C24, C38	W33
E9	C19, C21, C27, C39, C47, C50	W4, W7
E12	C25, C26	W6, W17
E13	C5, C11, C16	W37

AMR profiles

AMR tests showed no extensive drug-resistant (XDR) isolate; however, 49 (98%) and 47 (94%) isolates were MDR (resistant to two or more categories) for water- and cattle-originated *E. coli*, respectively (Tables 2 and 3). The most common resistance was observed against cefalotin followed by tetracycline, while no resistant isolate was observed against trimethoprim/sulfamethoxazole (Figure 3).

Among the isolates from water and cattle with the same ERIC profiles, four isolates from water showed the same AMR as the cattle isolates, increasing the probability of the same origin (Table 4).

Phylo-groups

E. coli strains from water and cattle showed different distribution patterns of phylo-groups. *E. coli* isolates from water were mostly represented in A, B1, and E phylo-groups, with ten (20%), 19 (38%), and eight (16%) isolates, respectively, while no B2 phylo-group was found (Tables 5), as described previously by Unno *et al.* (2009). There was a greater number of isolates in phylo-groups A and D for cattle isolates with 15 (30%) and 15 (30%) isolates, respectively, while there was one isolate for C, E, and F phylo-groups.

Among the isolates from water and cattle with similar ERIC and AMR profiles, four isolates demonstrated the same phylo-group, confirming the same origin (Tables 4).

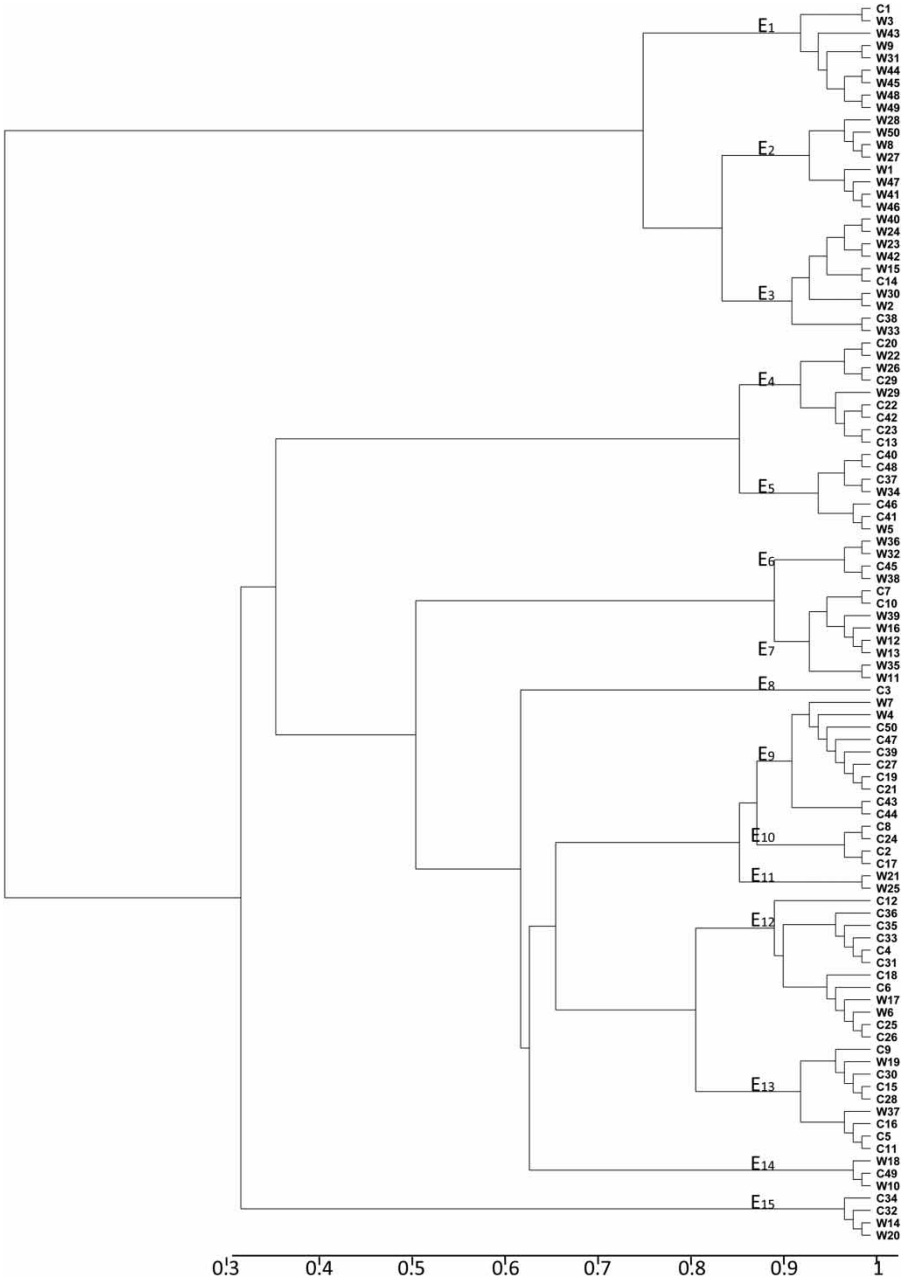

Figure 2 | Cluster analysis by ERIC-PCR fingerprint of 100 *E. coli* isolates. The bottom bar indicates the percentage of similarity. The letters on the right indicate the isolate number (C: *E. coli* isolated from cattle and W: *E. coli* isolated from water).

Diversity index of phylo-groups in water and cattle isolates

The Shannon–Weaver diversity index of phylo-groups in water and cattle isolates is shown in Table 5.

Statistical analysis

The Pearson chi-squared test did not show any significant association between ERIC types (E_1–E_{15}) and phylo-groups (p value = 0.578), ERIC types and AMR (Table 6), and

Table 2 | Phylo-groups, ERIC clusters and AMR profiles of isolates from cattle

Host	Phylo-group	Cluster	AMR profile
C1	B1	E02	CF,S
C2	D	E03	CZ,CF,SXT,C,DOX
C3	A	E01	CZ,AMP,AMC,SXT
C4	B1	E09	CT,CF,S,TE
C5	A	E05	CF,DOX
C6	C	E12	CF,S,K,N,C
C7	B1	E09	CF,S,TE
C8	A	E02	CF,DOX
C9	B1	E01	CF,SXT
C10	D	E14	CZ,CF,S,TE
C11	A	E07	CF,DOX
C12	B1	E07	CZ,AMP,AMC,S
C13	A	E07	CF,N,TE
C14	B1	E15	CZ,AMP,AMC,S,DOX
C15	D	E03	CF
C16	A	E07	CF,S
C17	B1	E12	CF,S,CF,S,K,N,C
C18	A	E14	AMP,CF,S
C19	E	E13	CZ,AMP,AMC,CF,KNCT,E
C20	A	E15	CF,SXT
C21	D	E11	CZ,AMP,AMC,CF,CRO,S,K,N
C22	B1	E04	CF,DOX
C23	B1	E03	CZ,AMP,CF
C24	A	E03	CF,S,N,C
C25	F	E11	CF,SXT,NA
C26	B1	E04	CZ,AMP,AMC,CF,S,SXT,GM,K,TE
C27	B1	E02	CZ,CF,N
C28	D	E02	CF
C29	B1	E04	CF,TE,DOX
C30	D	E03	CF,N,C
C31	A	E01	CZ,CF,S
C32	B1	E06	CF
C33	D	E03	CF,DOX
C34	B1	E05	CF,S
C35	D	E07	CZ,CF,SXT,DOX
C36	B1	E06	CF
C37	A	E13	CF
C38	B1	E06	CF,S,SXT,CT,EDOX
C39	D	E07	CF,DOX
C40	D	E03	CF,TE
C41	A	E02	CZ,CF,S
C42	D	E03	CF,S
C43	B1	E01	CF,S,CAZ,C
C44	D	E01	CZ,CF,S
C45	A	E01	CZ,CF,S
C46	D	E02	CZ,CF
C47	D	E02	CZ,AMP,CF
C48	B1	E01	CZ,AMP,AMC,CT,CF,NOR,CRO, S,TOB,SXT,CIP,N,A,LEV,C
C49	A	E01	CF,N,DOX
C50	D	E02	AMP,AMC,CF

AMC: amoxicillin/clavulanic, AMP: ampicillin C: chloramphenicol, CF: cefalotin, CTX: cefotaxime, GM: gentamycin, NA: nalidixic acid, S: streptomycin, SXT: trimethoprim/sulfamethoxazole, TE: tetracycline.

phylo-groups and AMR (Table 6), confirming the independence of these characteristics. Furthermore, Pearson chi-square calculated for the water/cattle and the phylo-groups was 0.1 (p value = 0.1) which rejects any correlation between water/cattle and phylo-groups.

DISCUSSION

Faecal contamination is considered to be one of the most difficult challenges facing environmental scientists trying to protect water for drinking, recreation or other uses. Just a few decades ago, it was impossible to identify the sources of microbial pollution. Several methods based on phenotypic and genotypic characteristics have been developed for MST; however, it is difficult to find the most reliable one (Domingo & Edge 2010; Kinzelman *et al.* 2012). This work was designed to determine the genotypic and phenotypic properties of *E. coli* to introduce the best MST to identify the sources of contamination. To do so, *E. coli* was enumerated and isolated in the Karaj River along the Chalous Road, and to build the library, *E. coli* from cattle (Khozankala husbandry) faeces was isolated. The MPN/100 ml of *E. coli* in water samples ranged from 70 to $1{,}600 \times 10^2$, which is in agreement with the study by Sakizadeh *et al.* (2015) which demonstrated faecal pollution of the river. This is not surprising, as reported by

Table 3 | Phylo-groups, ERIC clusters and AMR profiles of isolates from water

Host	Phylo-group	Cluster	AMR profile
W1	A	E01	CZ,AMP,AMC,CF,NOR,CTX,CRO,S, TOB,SXT,NA,GM,LEV,DOX
W2	F	E10	CZ,AMP,AMC,NOR,S,SXT,CIP,NA,GM, LEV,TE
W3	B1	E08	CT,CF,NOR,SXT,NA
W4	E	E12	CF,NCT,E
W5	A	E13	CF
W6	E	E12	AMP,AMC,CF,S,TOB,SXT,NA,GM,N,C, TE
W7	B1	E07	CZ,AMP,AMC,CF,NOR,S,SXT,NA,DOX
W8	A	E03	CF,DOX
W9	B1	E13	CZ,AMP,AMC,PRL,S,NA,TE
W10	B1	E07	AMC,CF,NOR,NA,C,DOX
W11	A	E13	CF
W12	B1	E12	AMP,AMC,CF,S,TOB,SXT,NA,GM,C, TE,DOX
W13	E	E04	CZ,AMP,AMC,CF,S,TOB,SXT,NA,N,C
W14	B1	E03	AMC,CF,NOR,S,CIP,NA,LEV,C,TE, DOX
W15	A	E13	CF,NOR,S,NA
W16	B1	E13	CZ,AMP,AMC,CF,NOR,S,SXT,NA,K,N, CT,E
W17	E	E10	AMC,CF,NOR,S,NA,LEV,DOX
W18	D	E12	CZ,AMP,AMC,CT,CRO,S,SXT,C,TE
W19	E	E09	CZ,AMP,AMC,CT,CF,CRO,CAZ,K,N
W20	B1	E04	CF,NOR,NA,C,TE,DOX
W21	D	E03	CF,DOX
W22	F	E04	CF,S,SXT,NA,K,N,C,TE
W23	B1	E04	CF,NA,DOX
W24	D	E03	CF,DOX
W25	C	E12	CF,S,K,N,C
W26	C	E12	CF,S,K,N,C
W27	D	E03	CF,DOX
W28	B1	E13	CZ,AMP,AMC,CF,NA
W29	B1	E04	AMP,AMC,CF,CTX,CRO,S,SXT,CAZ,C
W30	A	E13	AMP,AMC,CF,NOR,SXT,NA,C,TE
W31	B1	E12	CF,S,SXT,N,A
W32	B1	E15	AMP,AMC,CF,S,SXT,NA,C,TE,DOX
W33	C	E12	AMP,AMC,CF,NOR,S,SXT,CIP,NA,GM, LEV
W34	A	E15	AMP,AMC,CF,NOR,S,SXT,CIP,NA,LEV, C,TE
W35	C	E12	AMP,AMC,CF,NOR,S,CIP,NA,LEV,K,N, DOX
W36	B1	E12	CZ,AMP,AMC,CF,NOR,S,NA,GM,K,N
W37	E	E05	CF,NOR,S,SXT,NA,GM,LEV,DOX
W38	A	E03	CF,DOX
W39	B1	E09	CT,CF,S,TE
W40	C	E05	CF,S,C
W41	D	E05	AMP,AMC,CF,S,NA,TE,DOX
W42	A	E04	AMP,CF,S,SXT,NA,C
W43	B1	E09	AMP,AMC,CF,NOR,S,SXT,NA,TE,DOX
W44	A	E09	AMP,AMC,CF,S,SXT,C,DOX
W45	B1	E06	CZ,AMP,AMC,CF,CRO,S,SXT,N,TE, DOX
W46	C	E05	AMP,CF,NA,C
W47	B1	E09	AMP,AMC,CF,S,NA
W48	B1	E01	AMP,AMC,CF,S,SXT,TE,DOX
W49	E	E14	AMP,AMC,CF
W50	E	E09	AMP,AMC,CF,S,NA,TE,DOX

Ponce-Terashima *et al.* (2014) in some Brazilian villages surface waters, especially rivers nearby the livestock husbandries and inhabited villages, can receive contaminant from different sources.

To develop a reliable MST tool, followed by isolating *E. coli* clones, we determined the ERIC types, phylo-groups (A, B_1, B_2, C, D, E, and F) and AMR profiles.

Genetic diversity of *E. coli*

As described by Ibekwe *et al.* (2011) and Kon *et al.* (2009) the genotyping results of this study indicated considerable genetic diversity among *E. coli* isolates, particularly those isolated from water samples, although multiple isolates were obtained from the same water sampling site, by the same protocol. Our results showed a possible hypothesis for such variation is the entirely different sources of water contamination, While the genotypes inhabiting the cattle in the same environment are much more limited. A second hypothesis, described by Lu *et al.* (2004), involves genomic rearrangement during survival as the result of mutation or recombination. This result is consistent with

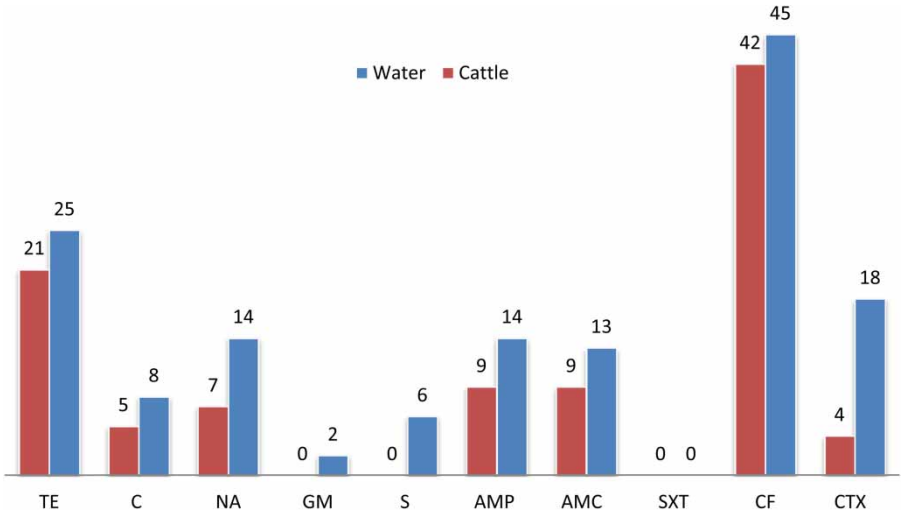

Figure 3 | Number of isolates resistant to antimicrobial agents. *E. coli* from water and cattle are shown in blue and red bars, respectively (AMC: amoxicillin/clavulanic, AMP: ampicillin, C: chloramphenicol, CF: cefalotin, CTX: cefotaxime, GM: gentamycin, NA: nalidixic acid, S: streptomycin, SXT: trimethoprim/sulfamethoxazole, TE: tetracycline). Please refer to the online version of this paper to see this figure in colour: http://dx.doi.org/10.2166/ws.2017.051.

Table 4 | Isolates of water and cattle with the same ERIC and AMR profile

Cluster	Isolates from cattle	Isolates from water
E3	C8, C24, C38	W33
E9	C39	W4
E12	C25, C26	W6
E13	C5	W37

Table 5 | Hosts and phylo-groups of the isolates

	Phylo-groups							
Host	A	B1	C	D	E	F	Total	Shannon–Weaver index
No. of isolates from cattle	14	18	1	15	1	1	50	3.305
No. of isolates from water	10	19	6	5	8	2	50	3.443
Total	24	37	7	20	9	3	100	

Table 6 | Pearson chi-square between phylo-groups and antibiotic resistance

Antibiotic resistance	ERIC types	Phylo-groups
Cefotaxime	0.6	0.518
Cefalotin	0.136	0.781
Trimethoprim/sulfamethoxazole	0.249	0.43
Amoxicillin/clavulanic	0.13	0.09
Ampicillin	0.144	0.41
Streptomycin	0.377	0.3
Gentamycin	0.824	0.1
Nalidixic acid	0.1	0.12
Chloramphenicol	0.28	0.09
Tetracycline	0.25	0.03

those reported by Campos *et al.* (2009), who observed an absence of an endemic clone and a high diversity of *E. coli* strains isolated from humans and foods.

Considering the number of bands produced for the isolates, to avoid any unspecified amplification and to increase the reproducibility of the assay, we optimized the PCR protocol by using HotStart *Taq* DNA polymerase. Based on our results, ERIC fingerprints revealed just ten different bands among 100 isolates, while Borges *et al.* (2003) reported the number of bands obtained via ERIC-PCR ranged from three to 20, with an average of 12.6 bands for each of the 98 isolates, as well as Oltramari *et al.* (2014) who generated patterns of four to 20 bands from *E. coli*. There is no doubt that the higher number of the bands will complicate interpretation and dendrogram generation, therefore, it is highly recommended by the authors to use HotStart *Taq* DNA polymerase for REP-PCR assays or more discriminating tools such as PFGE, which is based on a universal protocol for phylogenetic studies.

Our results revealed a Shannon–Weaver index of 3.65 among the 50 water isolates and 2.39 for cattle isolates confirming the higher heterogeneity of the water isolates, which is similar to those found by Borges *et al.* (2003) for *E. coli* isolates in water samples using the REP-PCR technique.

Pearson chi-square between ERIC types (E_1–E_{15}), host, AMR, and phylo-groups could not imply any significant association, suggesting no ERIC type can be attributed to a specific host, phylo-group or AMR profile, concluding that genetic diversity alone cannot be an appropriate MST tool because as shown, out of six isolates with the same ERIC fingerprints, just four isolates demonstrated a similar AMR pattern (Table 6).

AMR of *E. coli*

In this study we observed a high rate of antibiotic resistance, which is consistent with the report by Ayazi *et al.* (2015), who found an alarming increase mainly due to increases in the use of antibiotics for treatment of infections in IRI within a 5-year period. However we could not find any reliable information on the consumption of antibiotics.

Similar to our observations related to genetic diversity, we observed that the overall AMR of isolates from water is higher in comparison with isolates from cattle. The most AMR of *E. coli* isolates from both origins were against cefalotin followed by tetracycline. High AMR of the isolates from water can be attributed to the natural presence of such agents in rivers but a more possible explanation is that the isolates are excreted from animals or humans treated by these antibiotics causing overall resistance (Meireles *et al.* 2013), which may imply a high rate of tetracycline consumption in this region. Furthermore, high resistance to tetracycline is not surprising because tetracycline is often used as a growth promoter in animal food, and the corresponding resistance genes are located on mobile genetic elements that can be transmitted among bacteria (Marshal & Levy 2011). Resistance to other agents such as amoxicillin–clavulanic acid and ampicillin in *E. coli* isolates was proportionally found in both origins, possibly related to therapeutic end use for human and animal diseases (Landers *et al.* 2012). Based on our results, the high resistance of *E. coli* isolates for tetracycline and cephalothin agrees with the findings of Sayah *et al.* (2005), who found the highest levels of resistance were observed against tetracycline and cephalothin with isolates collected from domestic and wild animal faecal samples, human septage, and surface water in the Red Cedar watershed in Michigan.

In this study, *E. coli* isolates from cattle and water showed no resistance to SXT, contrary to the results reported by Schroeder *et al.* (2002), who reported a high prevalence of resistance to sulfamethoxazole among O157: H7 isolates recovered from humans and cattle (Schroeder *et al.* 2002). However, as in our study they observed a high prevalence of tetracycline resistance among the isolates.

In the current study, a statistically significant relation could not be observed between the AMR, ERIC types, host, and phylo-groups, suggesting that if AMR profile/profiling is applied alone, no accurate MST result can be gained. However, in combination with others (as a complementary tool), it can increase the possibility of correct tracking.

Phylo-grouping of *E. coli*

This report determined the prevalence of different phylo-groups of *Escherichia coli* and demonstrated that some phylo-groups may be dominant in some species. Our results confirmed the dominance of B1 and absence of B2 in cattle, which is in agreement with the studies of Unno *et al.* (2009) and Coura *et al.* (2015). Following B1 ($n = 37$), phylo-groups of A ($n = 24$), D ($n = 20$), E ($n = 9$) and C ($n = 7$) were the most prevalent, while F ($n = 3$) was rarely found in either origin. The distribution and dominance of B1 and A1 in cow, goat and sheep samples by Carlos *et al.* (2010) is consistent with our results.

As well as the ERIC diversity, a higher Shannon–Weaver index for phylo-groups of *E. coli* from water in comparison with cattle can be observed (Table 5). In other words, it can be inferred that in addition to Khozankala husbandry, *E. coli* can enter the Chalous River through various sources including wild and domestic animals, human (inhabitants and passengers), animal manure, etc., which all together necessitate the source tracking of *E. coli* origins.

Pearson chi-square did not attribute any phylo-group to any specific host (p value = 0.1) (Table 4). Taken together, as Coura *et al.* (2015) and Carlos *et al.* (2010) described, the analysis of *E. coli* population structure can be useful as a

supplementary bacterial source tracking tool, but standing alone, would not result in reliable MST results.

CONCLUSION

Overall, genotypic and phenotypic analysis revealed that *E. coli* isolates were very diverse and there was no evidence that a given group of *E. coli* isolates with distinct origin might represent a dominant population from surface water. In conclusion our results confirmed that a combination of methods including DNA-based typing and AMR profiling can be promising tools for accurate MST. However, the authors believe that despite applying all MST tools, it may be difficult to trace all bacteria in surface water, since surface water can receive various kinds of new bacterial genotypes which would not be present in our libraries and the phenotypic and genotypic characteristics of a bacterium may alter during multiplication and survival.

COMPETING INTERESTS

No conflict of interest.

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