International Journal of Food Microbiology 304 (2019) 1–10

Contents lists available at ScienceDirect

International Journal of Food Microbiology

journal homepage: www.elsevier.com/locate/ijfoodmicro

Detection of antimicrobial-resistance diarrheagenic *Escherichia coli* strains in surface water used to irrigate food products in the northwest of Mexico

Adrian Canizalez-Roman[a,b,*], Jorge Velazquez-Roman[a], Marco A. Valdez-Flores[a], Héctor Flores-Villaseñor[a,c], Jorge E. Vidal[d,e], Secundino Muro-Amador[c], Alma Marlene Guadrón-Llanos[a], Edgar Gonzalez-Nuñez[c], Julio Medina-Serrano[a,f], Gabriela Tapia-Pastrana[g], Nidia León-Sicairos[a,h]

[a] CIASaP, School of Medicine, Autonomous University of Sinaloa, 80246 Culiacan, Sinaloa, Mexico
[b] The Women's Hospital, Secretariat of Health, 80127 Culiacan, Mexico
[c] The Sinaloa State Public Health Laboratory, Secretariat of Health, 80020 Culiacan, Sinaloa, Mexico
[d] Hubert Department of Global Health, Rollins School of Public Health, Emory University, Atlanta, GA 30322, USA
[e] Antibiotic Resistance Center, School of Medicine, Emory University, Atlanta, GA, USA
[f] Coordinación de Investigación en Salud, Delegacion IMSS, 80220 Culiacan, Sinaloa, Mexico
[g] Laboratorio de Investigación Biomédica, Hospital Regional de Alta Especialidad de Oaxaca, Oaxaca 71256, Mexico
[h] Pediatric Hospital of Sinaloa, 80200 Culiacan, Sinaloa, Mexico

ARTICLE INFO

Keywords:
E. coli
Diarrheagenic
Antibiotic resistance
Water sources

ABSTRACT

Water contamination by pathogenic bacteria is a global public health problem. Contamination of surface water utilized to irrigate food products, or for human consumption, causes outbreaks of foodborne and waterborne disease. Of these, those caused by diarrheagenic *Escherichia coli* (DEC) strains present substantial morbidity and mortality. The aim of this study was, therefore, to investigate the microbiological quality of surface water and the presence of DEC strains in different water bodies. A total of 472 water samples were collected from irrigation canal, dam, river, and dike water bodies from January through December 2015 in Sinaloa, a State located in Northwestern Mexico. Our studies demonstrated that 47.0% (222/472) of samples contained thermotolerant coliforms above permissive levels whereas *E. coli* strains were isolated from 43.6% (206/472). Among these *E. coli* isolates, DEC strains were identified in 14% (29/206) of samples including in irrigation canal (26/29) and river water (3/29) collected from the northern (83%) and central area (17%). Isolated DEC strains were classified as enteroaggregative *E. coli* (EAEC) 34.4% (10/29), enteropathogenic *E. coli* (EPEC) 31.0% (9/29), diffuse adherent *E. coli* (DAEC) 27.5% (8/29), and enterotoxigenic *E. coli* (ETEC) 6.8% (2/29). Moreover, 90% of isolated DEC strains exhibited resistance to at least one commonly prescribed antibiotic in Mexico whereas 17% were multi-drug resistant. In conclusion, the presence of DEC strains in surface water represents a potential source for human infection, and thus routine monitoring of DEC in surface water and other indirect affected areas should be considered at northwestern Mexico.

1. Introduction

Water is the most important natural source for life, being essential for humans and indispensable for all ecosystems (WHO, 2011). However, there are several factors that have affected the quality of fresh water, including the influence of organic materials that are incessantly discharged into the environment, and the fecal pollution derived mainly from human settlements and cattle, pigs, and poultry rearing facilities that can carry a myriad of pathogens. According to the 2017 report of the joint monitoring program, there are 2.3 billion people worldwide who still do not have basic sanitation facilities, and at least 1.8 billion people are estimated to drink water contaminated with feces (WHO, 2017). Thus, an important aspect to consider is to analyze for microorganisms present in water, in which the most common infectious agents include *Salmonella sp*, *Shigella sp*, *Giardia lamblia*, *Vibrio cholerae*, *Cryptosporidium parvum*, pathogenic *Escherichia coli* strains and enteric viruses (Leclerc et al., 2002).

To date, there is no single approach to encompass the analysis of a water sample for all pathogenic agents of interest. According to the World Health Organization (WHO), thermotolerant coliforms including

* Corresponding author.
E-mail address: canizalez@uas.edu.mx (A. Canizalez-Roman).

https://doi.org/10.1016/j.ijfoodmicro.2019.05.017
Received 19 October 2018; Received in revised form 16 May 2019; Accepted 20 May 2019
Available online 22 May 2019

Escherichia coli strains, can be used as indicator of water sanitary quality (WHO, 2001). Despite *E. coli* is considered a commensal bacterium that it is normally found in the gut of humans and warm-blooded animals, it can become an important pathogen causing disease in intestinal and extra-intestinal tissues of humans and other mammals (Robins-Browne et al., 2016).

The pathotypes of *E. coli* that are associated with intestinal disease are known as diarrheagenic *E. coli* (DEC) (Robins-Browne et al., 2016). Based on a number of virulence factors (e.g., adhesins, capsule, and invasiveness), and thus pathogenic mechanisms, clinical presentation, and epidemiology, DEC strains are classified into six different categories. *i)* Enteropathogenic *E. coli* (EPEC) strains are characterized by the presence of a pathogenicity island known as the "locus of enterocyte effacement" (LEE). The LEE island carry the *eae* gene that encodes an outer membrane adhesive protein known as intimin. EPEC strains also carry a plasmid called EPEC adherence factor (EAF), because it encodes genes of the bundle forming pili (BFP), including *bfpA*. Intimin and BFP are responsible for the EPEC's ability to produce attaching and effacing (A/E) lesions, and their genes (i.e., *eae*, and *bfpA*) have been used for the molecular identification of EPEC strains. EPEC strains can be subtyped in typical and atypical strains. Atypical EPEC carries LEE and therefore presents the A/E phenotype but does not possess the EAF plasmid [*bfpA*$^-$ (Trabulsi et al., 2002)]. *ii)* Enterotoxigenic *E. coli* (ETEC) strains are defined by the production of enterotoxins including the heat labile and heat-s toxins (LT and ST) (Nataro and Kaper, 1998; Robins-Browne et al., 2016). *iii)* Enteroinvasive *E. coli* (EIEC) strains invade the epithelium of the colon using the transcription activator v*irF*, and the type three secretion system (T3SS). The T3SS is encoded in the *p*Inv plasmid, which also carry the invasion associated pathogen antigen (*ipaH*) (Adler et al., 1989). *iv)* Enteroaggregative *E. coli* (EAEC) strains present a characteristic aggregative adherence (AA) pattern on diverse cell lines that is associated to a 60-MDa plasmid. The pAA plasmid carries virulence genes including the anti-aggregation protein transporter (*CVD432*), aggregative adherence fimbria I and II (*aggA/aggAII*), dispersin secretory protein (*aap*), and the transcriptional activator gene (*aggR*) which is a central regulator of EAEC virulence. EAEC strains carrying the AggR regulon are classified as typical EAEC (Aslani et al., 2011; Jenkins et al., 2006). *v)* Diffuse adherent *E. coli* (DAEC) strains are characterized by the presence of genes encoding afimbrial (Afa) or fimbrial (Dr) adhesins, which are responsible for their diffusely adherent phenotype on HEp2 cells (Clements et al., 2012). *vi)* Vero toxin-producing/Shiga toxin-producing *E. coli* (VTEC/STEC) are terms used in the developed world for pathotypes that cause enteric *E. coli* infections associated to production of toxins. Enterohemorrhagic *E. coli* (EHEC) is the most prevalent subgroup, which carries in addition to *stx1 and*/or *stx2* gene encoding for shiga toxins the *eaeA* gene encoding intimin responsible for attaching and effacing lesions (Clements et al., 2012; Nataro and Kaper, 1998). Most of these categories (EPEC, EIEC, EHEC, ETEC and EAEC serotypes) are important waterborne pathogens; these strains are commonly transmitted through contaminated water and have been isolated from gastroenteritis patients (Ram et al., 2009; Teng et al., 2004; Yang et al., 2007).

Despite advances in food and water management and sanitation, food and waterborne diseases continue to occur in developed countries. The 2011 German *E. coli* food-borne outbreak was due to a new Shiga toxin (Stx) type 2a-producing enteroaggregative *E. coli* (EAEC), which was extended to 15 countries around the world (Greece, United Kingdom, Austria, Spain, Czech Republic, Netherlands, Norway, Denmark, France, Luxembourg, Poland, Sweden, Switzerland, Canada, and United States) (Boisen et al., 2015; Canizalez-Roman et al., 2013; Rasko et al., 2011). Mexico, like many developing countries, experiences a high incidence of foodborne and waterborne diseases (Aijuka et al., 2018; Delgado-Gardea et al., 2016). In 2013, our group demonstrated DEC contamination of different food products in Sinaloa, Mexico (Canizalez-Roman et al., 2013). We then showed that multidrug resistant DEC strains were isolated from nearly one out of four cases of acute diarrhea diagnosed in Sinaloa (Canizalez-Roman et al., 2016). Sinaloa is a State located in the northwest of Mexico that presents large irrigation sources, which has contributed to position it as one of the main agronomic States of Mexico. Sinaloa distributes annually large amount of vegetables and fruits within the country and exports to different international destinations (INEGI, 2016).

Since many DEC can be transported by river channels, drains, streams, and dike, and deposited into bodies of water that could later be used for irrigation, recreation or even drinking, it is of vital importance to monitor the quality of fresh water sources in order to decrease waterborne infections and thereby controlling the morbidity and mortality caused by contaminated water (Leclerc et al., 2002). Moreover, the occurrence of antibiotic-resistant bacteria is very common worldwide, due in part to the widespread use of antimicrobial compounds in human therapy, and feed additives used to promote growth in both animals and aquaculture (Giowanella et al., 2015; Poma et al., 2016).

Thus, the aim of this study was to evaluate the microbiological quality of irrigation canal, river, dike and dam water in the State of Sinaloa, Mexico. To accomplish this following using WHO recommendations, we analyzed thermotolerant coliforms and *E. coli* bacteria contamination. We additionally investigated the prevalence and distribution of different DEC strains using a recently-developed scheme of PCR reactions. Finally, the antibiotic resistance profiles of DEC strains were determined.

2. Materials and methods

2.1. Bacterial strains

DEC reference strains utilized in this study belong to our laboratory collection and include ETEC H10407 (*lt* + and *st*+) (Fleckenstein et al., 2000), EPEC E2348/69 (*eae* + and *bfpA*+), EIEC (*ipaH*+ and *virF*-), EHEC O157:H7 EDL933 (*eae*+, *hlyA*+, *stx*1+, and *stx*2+), DAEC (*daaE*+), EAEC O42 (*aggR*+, *aap*+, *pCVD*432+, and *aafII*+) and *E. coli* DH5α (Canizalez-Roman et al., 2013). Bacteria were routinely grown overnight in Luria-Bertani (LB) broth (0.5% yeast extract, 1% tryptone and 0.5% NaCl) and incubated at 37 °C in a shaker incubator (Thermo Scientific, Waltham, Massachusetts USA).

2.2. Sample collection

Water samples were collected from different water bodies situated in 12 out of 18 municipalities of Sinaloa, from January to December 2015. A total of 472 samples were collected from: irrigation canal (n = 429), river water (n = 29), dike water (n = 9) and dam water (n = 5) (Fig. 1). Approximately, 300 mL of water samples were collected in 500 mL sterile, wide mouth, plastic bottles, according to the American Public Health Association, and placed in 4 °C cooling boxes for processing within 6 h of collection (APHA, 2012).

2.3. Bacteriological analyses

Samples were first screened for thermotolerant coliforms, as fecal origin bacteria, which include *Escherichia spp., Klebsiella spp., and Enterobacter spp.,* and the subgroup *E. coli* that produce β-galactosidase but not urease, as described by the Standard and Bacteriological Analytical Manual of the Food and Drug Administration (Feng et al., 2002) and Mexican Official Norms (NOM-127-SSA1–1994; NOM-003-ECOL-1997) reported in the Official Journal of the Federation (NOM, 1994, 1998).

The most probable number (MPN) of fecal coliforms was determined according to the FDA's Bacteriological Analytical Manual. Serial dilutions of water samples (1 mL, 0.1 mL, and 0.01 mL) were inoculated in lauryl sulfate tryptose broth media and incubated at 35 °C for 48 h. Positive tubes, which presented turbidity and formed gas, were seeded into brilliant green broth with 2% lactose and further incubated

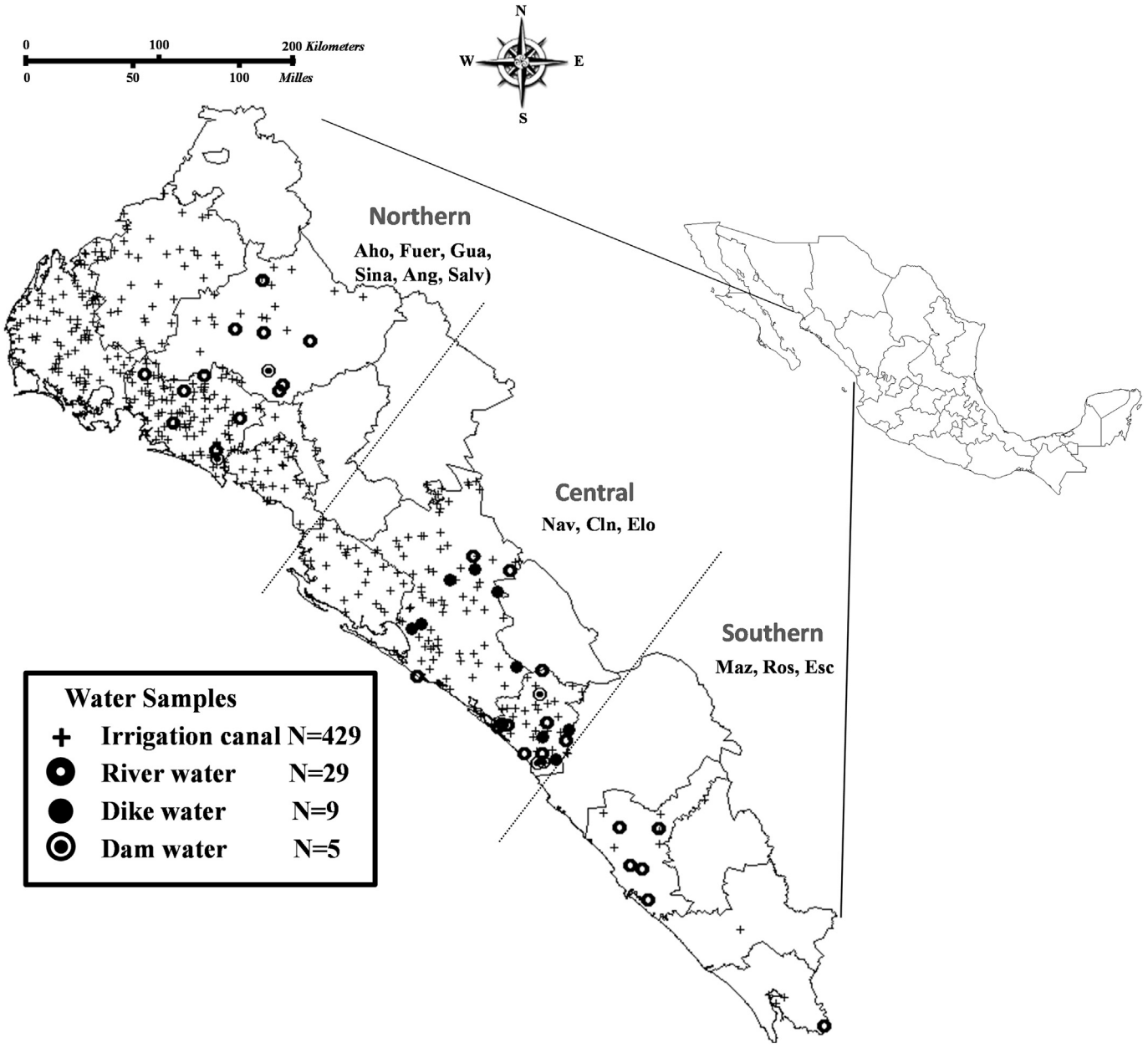

Fig. 1. Map showing the geographical locations of sampling sites. A total of 472 water samples (irrigation canal, $n = 429$; dike water, $n = 9$; dam water $n = 5$; and river water, $n = 29$) were collected from 222 different sites during 2015 distributed in regions from northern (120 sites: Aho = Ahome, Fuer = El Fuerte, Gua = Guasave, Sina = Sinaloa de Leyva, Ang = Angostura, Salv = Salvador Alvarado), central (93 sites: Nav = Navolato, Cln = Culiacán, Elo = Elota) and southern (9 sites: Maz = Mazatlán, Ros = Rosario, Esc = Escuinapa) Sinaloa.

at 35 °C for 48 h and inoculated into *E. coli* (EC) broth (45 °C -24 h). Samples yielding ≥1104 coliforms cfu/g, or cfu/mL, were considered contaminated with coliforms according to Mexican norms (NOM-003-ECOL-1997 and NOM-001-ECOL-1996) (NOM, 1994, 1998).

Positive tubes for EC broth were streaked on McConkey agar. Pink-colored colonies were presumptive identified as *E. coli*, and transferred to both eosin methylene blue (EMB) and violet red bile agar. These colonies were further phenotypically (Biochemical, API 20E; bioMerieux) and genotypically (PCR, as described below) identified.

2.4. Sequential multiplex PCR, scheme to identify DEC strains

2.4.1. Preparation of template DNA

Five colonies of presumptive *E. coli* per sample were grown in 3 mL of LB broth for 18 h to reach stationary phase. Cells were centrifuged at 10,000 ×*g* for 10 min, the pellet was resuspended in 0.3 mL of distilled water, and heated at 100 °C for 10 min, vortexed for 10 s, and centrifuged at 12000 ×*g* for 3 min. DNA-containing supernatants were transferred into 0.5 mL microfuge tubes and stored at −20 °C until used.

2.5. Sequential multiplex PCR

E. coli strain were confirmed molecularly by PCR according to the protocol described by Tsen et al. (1998). DEC strains among *E. coli* isolates were further identified using a scheme of sequential multiplex, duplex, and singleplex PCR reactions, as previously reported (Canizalez-Roman et al., 2013). These sequential multiplex PCR reactions identify the most prevalent DEC groups in decreasing order; reaction #1 identified EPEC strains and negative samples were further

Table 1
Detection of Coliforms and *Escherichia coli* in water samples from Sinaloa Mexico, during 2015.

Water sample collected from:	N (%)	Coliforms N (%)	*E. coli* N (%)
Irrigation canal	429 (90.8)	206 (48.0)	190 (44.3)
Dike	9 (1.9)	3 (33.3)	3 (33.3)
Dam	5 (1.05)	2 (40.0)	2 (40.0)
River	29 (6.14)	11 (37.9)	11 (37.9)
Total	472	222 (46.6)	206 (43.6)

used as template for reactions #2 and #3 to identify EAEC strains, and so on. Multiplex PCR reaction #1 contained primers to amplify the intimin gene (*eae*) and the structural subunit of the bundle-forming pilus (Bfp) gene (*bfp*A) and detected both typical (*eae* + and *bfp*A +) and atypical (*eae* + and *bfp*A-) EPEC strains. Multiplex PCR reaction #2 contained primers detecting a gene encoding a regulator protein (*aggR*) and a fimbriae gene (*aafII)*. Multiplex PCR reaction #3 contained primers to amplify the gene coding a protein dispersin (*aap*) and the AA pattern associated plasmid (*pCVD432*) to identify typical (*pCVD432* + and *aggR* + and/or *aafII* + *aap* +) and atypical (*pCVD432* + and *aggR*- and/or *aafII* + *aap* +) EAEC strains. Duplex PCR reaction #4 amplified both the gene encoding the heat labile toxin (*Lt*) and that encoding the heat stable toxin (*stII*) present in ETEC strains. PCR reaction #5 contained primers that amplified the structural subunit gene of the F1845 fimbria (*daaE*) to detect DAEC strains. Detection of EIEC strains was performed with reaction #6, this reaction contained primers to amplify the invasion genes (*virF*) and (*ipaH)*. Negative strains and DEC *eae* + strains (from reaction #1) were screened in multiplex PCR reaction #7 to detect possible EHEC strains. This reaction amplified genes *stx1* and/or *stx2,* EHEC strains encode Shiga toxin genes and the *eae* gene whereas STEC strains were *eae*-. Finally, multiplex PCR reaction #8 contained primers that amplified the hemolysin gene (*hlyA*), the *rfbE* gene coding for production of the lipopolysaccharide O of *E. coli* O157 and the *fliC* gene which encodes the *E. coli* flagellum H7 serotype and so detecting EHEC O157:H7 strains. Primer sequences, PCR conditions, and product sizes were reported by Canizalez-Roman et al. (Canizalez-Roman et al., 2013).

2.6. Antimicrobial agent susceptibility testing

Antibiotic susceptibility testing of pathogenic isolates was performed by the Kirby-Bauer disk diffusion method (Bauer et al., 1966) following the guidelines developed by the Clinical Laboratory Standard Institute (CLSI) (Cockerill, 2011). Mueller-Hinton agar plates were swabbed with inoculum prepared as indicated by the CLSI protocol and the following antibiotic disks (BD BBL, Franklin Lakes, NJ) were added to the plates under a sterile environment: gentamicin, ciprofloxacin, nalidixic acid, sulfamethoxazole-trimethoprim, tetracycline, ampicillin, ceftazidime, cefotaxime and chloramphenicol. The plates were incubated at 37 °C for 18 to 20 h. The diameters (in millimeters) of clear zones of growth inhibition around the antimicrobial agent disks were measured using a precision digital caliper (Absolute, Mitutoyo, Japan). *E. coli* ATCC 25922 and *E. coli* ATCC 35218 obtained from the American Type Culture Collection (ATCC) were used as control. Recommendations by the National Antimicrobial Resistance Monitoring System for *E. coli* were utilized to define breakpoints of antibiotics and thus categorize the isolates as resistant, intermediate, or sensitive. Multidrug resistance (MDR) was defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories. Antibiotics were selected based on their importance in treating human (Amabile-Cuevas, 2010), or animal *E. coli* infections and their used as feed additives to promote growth in animals. They represent different classes of antimicrobial agents that are available to treat Gram negative infections in Mexico.

2.7. Comparison of antibiotic resistance of DEC strains and statistical analysis

Statistical analysis of antibiotic resistance of DEC strains isolated from food items (Canizalez-Roman et al., 2013), clinical isolates (Canizalez-Roman et al., 2016), investigated in previous studies, and surface water (the current study) was performed. In these three studies, strains were isolated from the same geographical regions of Sinaloa Mexico, and antibiotic resistance was investigated using the same CLSI-recommended method, as detailed above. Data were analyzed using Sigma Plot software (version 11.0; Systat Software, Inc., USA) and Epi Info TM version 7. Percentages were compared using a Pearson chi-square test to establish mutual relatedness among the DEC resistant to antibiotics. *p*-values of 0.05 were considered statistically significant.

3. Results

3.1. Fecal coliform and E. coli in different water bodies

A total of 472 water samples including 429 from irrigation canal, 9 from dike water, 5 from dam water, and 29 river water samples were collected from different sites distributed in northern, central, and southern of Sinaloa (Fig. 1). Selected samples were first analyzed for fecal contamination indicators such as thermotolerant coliforms and *E. coli* bacteria.

Results of water fecal contamination analysis revealed that 47% (222/472) of the studied samples were contaminated above the permissible level with fecal coliforms ($\geq$ 1104 cfu), according to both Mexican guidelines and WHO guidelines. Of these samples, 43.6% (206/472) mainly contained *E. coli*, as detected by conventional bacteriological cultures and PCR analysis. The prevalence of the isolated *E. coli* is shown in Table 1. *E. coli* strains were isolated from all studied water sources. In irrigation canals *E. coli* strains were detected in 44.3% (190/429) of samples, in dam water in 40.0% (2/5) of samples, in river water in 37.9% (11/29), and dike water 33.3% (3/9).

3.2. Detection of diarrheagenic E. coli pathotypes from water samples

All 206 *E. coli* strains were further examined with a multiplex PCR approach to identify DEC strains. Our studies revealed that DEC strains were detected in 14% (29/206) of samples containing *E. coli*, which represented a prevalence of 6.14% (29/472) in all screened water samples. Twenty-six DEC strains were isolated from irrigation canal and 3 in river water samples. DEC strains were not detected in dam and dike water (Table 2). The most frequent DEC pathotype identified was enteroaggregative *E. coli* (EAEC) accounting for 34.5% (10/29), with seven typical and three atypical strains. Enteropathogenic *E. coli* (EPEC) was isolated from 31.0% (9/29) of water samples with eight typical and only one atypical strain. Diffuse adherent *E. coli* (DAEC) comprised 27.6% (8/29); and enterotoxigenic *E. coli* (ETEC) 6.89% (2/29) of strains. Enteroinvasive *E. coli* (EIEC) and enterohemorrhagic *E. coli* (EHEC) were not isolated from these water samples (Table 3).

3.3. Geographical locations of diarrheagenic E. coli pathotypes

For this study, Sinaloa was divided into three regions as follows, norther, central, and southern regions (Fig. 2). Most DEC strains were detected in irrigation canal and river samples from the northern region 24/29 (83%) with DAEC, EAEC, typical, and atypical EPEC and ETEC categories. The remaining DEC were isolated from samples collected at the central region 5/29 (17%) with DAEC, EAEC, typical EPEC, and ETEC categories. It is noteworthy that in the southern region DEC strains were not isolated. This is probably because the water sample size was much smaller in the southern region (16) than at the central (159) or the northern region (297).

Antimicrobial resistance of diarrheagenic *E.coli* strains isolated from

Table 2
Detection of diarrheagenic *Escherichia coli* pathotypes from water samples.

Water samples	No.	Diarrheagenic *Escherichia coli* (DEC)					
		EAEC (%)		EPEC (%)		DAEC (%)	ETEC (%)
		Typical	Atypical	Typical	Atypical		
Irrigation canal	26	7 (26.9)	3 (11.5)	8 (30.76)	1 (3.84)	6 (23.1)	1 (3.8)
River	3	*		*		2 (66.7)	1 (33.3)
Total	29	10 (34.5)		9 (31.0)		8 (27.6)	2 (6.9)

DEC pathotypes were not detected in dike and dam water samples. EIEC and EHEC were not detected in analyzed samples. *Denotes absence of DEC pathotypes.

water sources.

The antibiotic resistance of isolated DEC strains is shown in Table 3. Ninety percent (26/29) of DEC strains were resistant to at least one antimicrobial agent; 51% (15/29) were resistant to at least two; and 17% (5/29) were multi-drug resistant. Of note, nearly half of the strains were resistant to cefotaxime (48%), followed by ampicillin (44%), tetracycline (38%), sulfamethoxazole-trimethoprim (14%), ceftazidime (10%), and gentamicin (7%). A very low resistance rate was observed for chloramphenicol, nalidixic acid, and ciprofloxacin (4%). Among analyzed DEC, EPEC strains presented resistance to tetracycline and cefotaxime (44%), sulfamethoxazole-trimethoprim, ampicillin, and gentamicin (22%), and ciprofloxacin and nalidixic acid (11%). EAEC strains showed resistance to ampicillin and cefotaxime (40%), and tetracycline and ceftazidime (20%), while DAEC isolates presented resistance to cefotaxime (75%), ampicillin (63%), tetracycline (38%), and trimethoprim-sulfamethoxazole and chloramphenicol (13%). Finally, ETEC strains presented resistance to tetracycline and ampicillin (7%), and trimethoprim-sulfamethoxazole (4%) (Table 3).

3.4. Multiple antimicrobial resistance phenotype of E. coli strains

Overall 17% of DEC strains were multi-drug resistant and ~70% were resistant to only one or two antibiotics. EPEC strains, for example, were 55.5% (5/9) resistant to one drug, 33% (3/9) resistant to two, and 11% (1/9) multi-drug resistant. DAEC isolates were 38% (3/8) resistant to one and two drugs and 25% (2/8) multi-drug resistant, while EAEC strains were found as the less resistant DEC pathotype with 30% (3/10) of strains resistant to one and two antibiotics and 10% (1/10) multi-drug resistant (Table 3). We isolated only two ETEC strains, one strain was resistant to two drugs and, despite the low isolation rate, the other strain was multi-drug resistant.

An analysis of co-resistance to antibiotics was made and is shown in Table 4. The more prevalent co-resistance observed for DEC strains were tetracycline + ampicillin (n = 3, 30%) and ampicillin + cefotaxime (n = 3, 30%), followed by tetracycline + cefotaxime (n = 1, 10%), tetracycline + Sulfamethoxazole-trimethoprim (n = 1, 10%), ampicillin + ceftazidime (n = 1, 10%) and cefotaxime + gentamicin (n = 1, 10%). More prevalent co-resistance combinations to three antibiotics was tetracycline + ampicillin + cefotaxime (n = 2, 66.6%) followed by tetracycline + ampicillin + Sulfamethoxazole-trimethoprim (n = 1, 33.3%). Co-resistance to four antibiotics only was to tetracycline + Sulfamethoxazole-trimethoprim + gentamicin + ciprofloxacin and to five antibiotics only was to tetracycline + Sulfamethoxazole-trimethoprim + ampicillin + cefotaxime + chloramphenicol (Table 4).

3.5. Comparison of antibiotic resistant DECs obtained from different sources

The susceptibility to antibiotics obtained in the current study for

Table 3
Antibiotic resistance among *diarrheagenic Escherichia coli* strains isolated from water samples.

Class and antimicrobial	Total (n = 29)	Pathotypes and percentage of resistance			
		EPEC	EAEC	DAEC	ETEC
		(n = 9)	(n = 10)	(n = 8)	(n = 2)
Aminoglycoside					
Gentamicin	2/29 (6.9%)	2/9 (22.2%)	0	0	0
Quinolones and Fluoroquinolones					
Ciprofloxacin	1/29 (3.5%)	1/9 (11.1%)	0	0	0
Nalidixic acid	1/29 (3.5%)	1/9 (11.1%)	0	0	0
Sulfonamides and potentiated sulfonamides					
Sulfamethoxazole-trimethoprim	4/29 (13.8%)	2/9 (22.2%)	0	1/8 (12.5%)	1/2 (50%)
Tetracyclines					
Tetracycline	11/29 (37.9%)	4/9 (44.4%)	2/10 (20%)	3/8 (37.5%)	2/2 (100%)
Beta lactams					
Ampicillin	13/29 (44.8%)	2/9 (22.2%)	4/10 (40%)	5/8 (62.5%)	2/2 (100%)
Cephalosporins					
Ceftazidime	3/29 (10.4%)	0	2/10 (20%)	1/8 (12.5%)	0
Cefotaxime	14/29 (48.3%)	4/9 (44.4%)	4/10 (40%)	6/8 (75%)	0
Phenicols					
Chloramphenicol	1/29 (3.5%)	0	0	1/8 (12.5%)	0
Antibiotics resistance to:					
0 antibiotics	3 (10.3%)	0	3 (30%)	0	0
1 antibiotic	11 (37.9%)	5 (55.5%)	3 (30%)	3 (37.5%)	0
2 antibiotics	10 (34.5%)	3 (33.3%)	3 (30%)	3 (37.5%)	1 (3.5%)
3 antibiotics	3 (10.3%)	0	1 (10%)	1 (12.5%)	1 (3.5%)
4 antibiotics	1 (3.5%)	1 (11.1%)	0	0	0
5 antibiotics	1 (3.5%)	0	0	1 (12.5%)	0

Strains with resistance to 6, 7, 8 and 9 drugs were not detected.

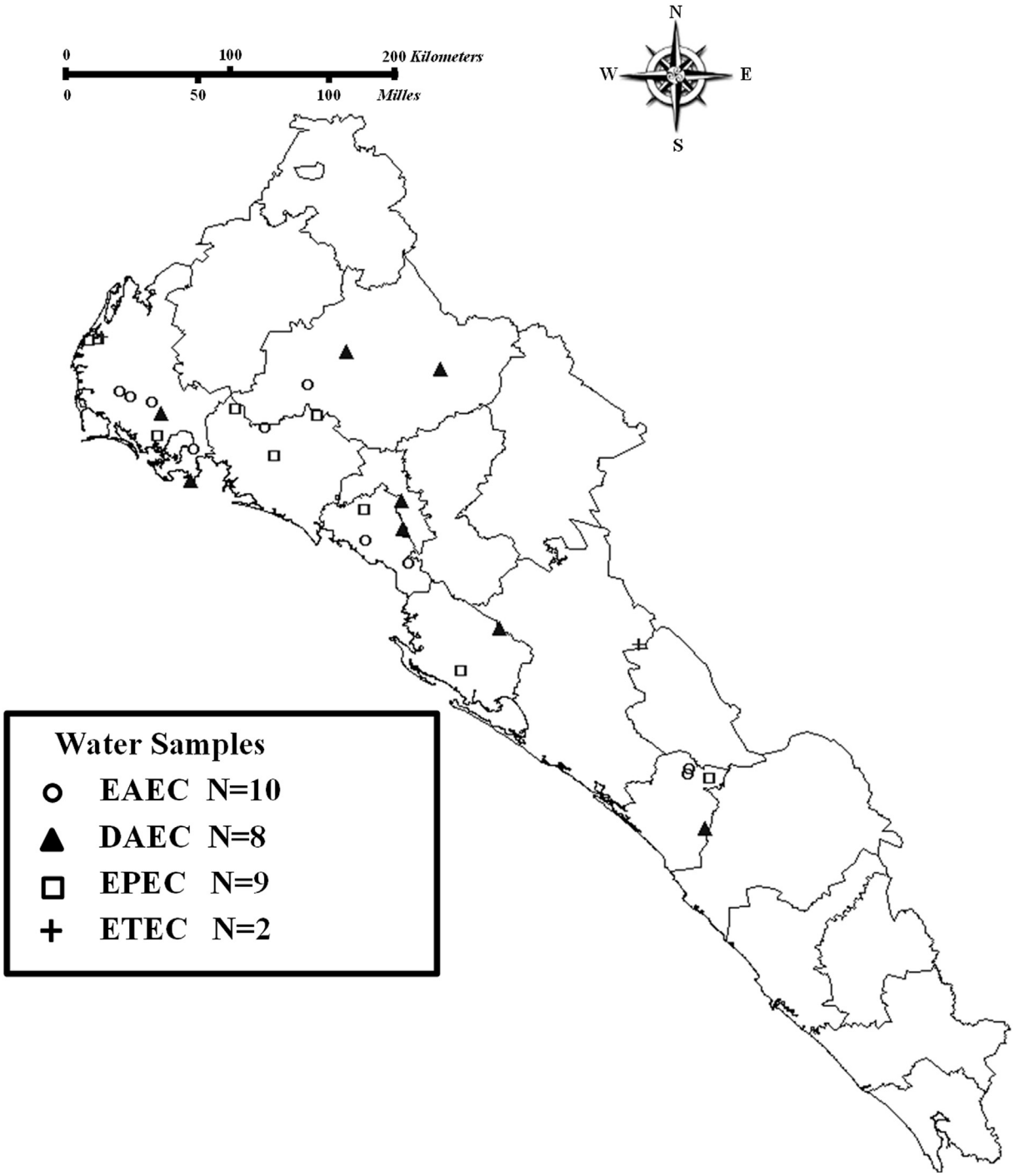

Fig. 2. Geographical locations of DEC isolates from water samples. A total of 29 DEC were detected in 472 water samples (irrigation canal $n = 26$ and river water $n = 3$) during 2015 in regions of northern (Aho = Ahome, Fuer = El Fuerte, Gua = Guasave, Sina = Sinaloa de Leyva, Ang = Angostura, Salv = Salvador Alvarado), central (Nav = Navolato, Cln = Culiacán, Elo = Elota) and southern (Maz = Mazatlán, Ros = Rosario, Esc = Escuinapa) Sinaloa.

DEC strains isolated from water samples ($n = 29$) was compared with that obtained from previous CLSI-recommended antibiotic resistance studies, performed by our group, on DEC strains isolated from humans ($n = 242$) (Canizalez-Roman et al., 2016) and Food samples ($n = 56$) (Canizalez-Roman et al., 2013). Except for resistance to ceftazidime and cefotaxime, the proportion of DEC strains with resistance to all other antibiotics was statistically significant higher in strains isolated from humans than that from DEC strains isolated from food or water samples (Fig. 3). In contrast, a similar proportion of DEC strains with resistance to all antibiotics was observed when strains isolated from food and water sources were statistically analyzed ($p > 0.10$). Interestingly, the prevalence of strains with resistance to the cephalosporin cefotaxime was statistically significant increase in DEC strains isolated from water (~50%) than in those DEC isolated from human sources [~25%, ($p = 0.03$)].

4. Discussion

This study describes the presence of DEC strains in several water sources of Sinaloa, one of the main regions of Mexico where food products are produced and distributed nationally, and exported to USA and other international destinations. Different surface waters including

Table 4
Multiple resistance to antibiotics of DEC strains isolated in water samples from Sinaloa.

Number of antibiotics resistant to	Strains (N)	Co-Resistant to Antibiotic evaluated	DEC Strains			
			EPEC	EAEC	DAEC	ETEC
2		Tetracycline and Ampicillin		1	1	1
		Tetracycline and Cefotaxime	1			
	10	Tetracyclineand Sulfamethoxazole-trimethoprim	1			
		Ampicillin and Cefotaxime		1	2	
		Ampicillin and Ceftazidime		1		
		Cefotaxime and Gentamicin	1			
3		Tetracycline and Ampicillin and Cefotaxime		1	1	
	3	Tetracycline and Ampicillin and Sulfamethoxazole-trimethoprim				1
4	1	Tetracycline and Sulfamethoxazole-trimethoprim and Gentamicin and Ciprofloxacin	1			
5	1	Tetracycline and Sulfamethoxazole-trimethoprim and Ampicillin and Cefotaxime and Chloramphenicol			1	

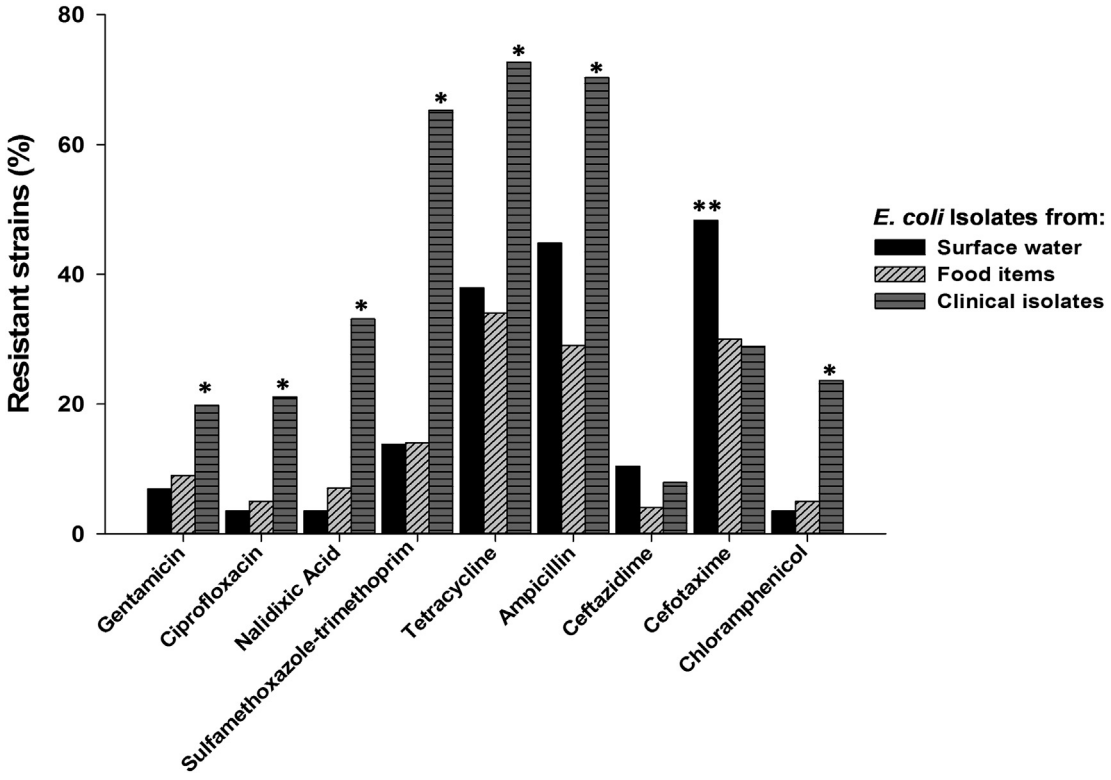

Fig. 3. Comparison of antibiotic resistant DECs obtained from different sources. Antimicrobial resistance (%) of diarrheagenic *E. coli* strains isolated from surface waters (this study, $n = 29$), food items ($n = 56$) (Canizalez-Roman et al., 2013), and clinical isolates ($n = 242$) .(Canizalez-Roman et al., 2016). The proportion of DEC strains resistant to antibiotics from clinical isolates was statistically significant high than those isolated from surface water and food items p value ≤ 0.05 (*). The proportion of DEC strains with resistance to antibiotics isolated from surface water were statistically significant high than those DEC from clinical specimens and food items p value ≤ 0.05 (**).

irrigation canal, dike water, dam water, and river water, were represented by 472 water samples. Nearly half of the samples analyzed (43.6%, 206/472) contained high, non-permissible, levels of *E. coli* contamination. Molecular analysis of *E. coli* isolates demonstrated that 14% (29/206) of contaminated water contained DEC strains. The most frequently isolated DEC category was EAEC, followed by EPEC, DAEC, and ETEC. Isolated DEC strains were present only in irrigation canal and river water, and mostly in the northern and central region of Sinaloa. Whereas all DEC strains bear pathogenic potential, we identified a subset of DEC strains (17%) that had a multi-drug resistance phenotype and therefore represent a threat to those in risk of infection. To our knowledge this is the first study that assessed the bacteriological quality of water sources of Sinaloa.

Contamination of surface waters, particularly in developing countries, is a big concern. Besides studies describing outbreaks of waterborne bacterial infections in Mexico, the prevalence and categorization of enteropathogenic bacteria in water samples has been poorly studied. There are only a few reports in which the microbiological quality of water has been evaluated (Gortares-Moroyoqui et al., 2011; Ramirez Castillo et al., 2013; Requena-Castro et al., 2018). In this study, all four selected water bodies exhibited an overall poor bacteriological quality with 46.6% of analyzed samples surpassing Mexican and WHO irrigation water guidelines for fecal coliforms (NOM, 1994, 1998; WHO, 2001, 2006), and in 43.6% of samples were isolated *E. coli*. Irrigation canal was found as the most affected with 44.3% of *E. coli* contamination. Fecal coliforms levels were found similar to that reported by Ramirez-Castillo et al. for San Pedro River, Aguascalientes, Mexico (Ramirez Castillo et al., 2013); while, *E. coli* contamination was found lower to that reported by Giowanella et al. whose study found 69% of *E. coli* contamination in rivers of Caritibia,

Paraná, Brazil (Giowanella et al., 2015). The high prevalence of water contamination found in Sinaloa could be mainly explained due to the use of rivers for recreational activities, the intense agricultural activities, industry, and the poor sanitation facilities (Poma et al., 2016).

DEC strains have been isolated from diverse ecological niches ranging from mammalians intestines to various aquatic environments including surface waters, and different food products. In a previous study conducted by our group, DEC strains were isolated from several food items including meat products, dairy products, beverages and ice, seafood and fish, and prepared foods in Sinaloa (Canizalez-Roman et al., 2013). We also demonstrated that DEC strains were the main cause of gastrointestinal disease in 25% of patients presenting acute diarrhea during a 4-year period of surveillance in Sinaloa (Canizalez-Roman et al., 2016). We have further identified in this study a potential source of DEC strains that contaminates food products and perhaps cause a significant burden of human gastrointestinal disease. Of the 206 isolated *E. coli,* 14% of them were molecularly characterized as DEC, from which four different categories were detected based on the analysis of genes encoding for specific virulence factors. The prevalence of DEC strains was lower in comparison to the reported by Ramirez-Castillo et al. (57%) (Ramirez Castillo et al., 2013) and Requena-Castro et al. (42.5%) (Requena-Castro et al., 2018) in studies analyzing water samples from the San Pedro river, Aguascalientes, Mexico and the Bravo river, Tamaulipas, Mexico, respectively. The lower prevalence of DEC strains found in the present study could be explained due to the fact that both reports were focused on river waters, and their sampling sites were selected based on the presence of important discharges of treated and untreated wastewater into the rivers. Meanwhile, in this study, four different water bodies were analyzed and the sampling sites were randomly selected.

This study also shows that out of 29 detected DEC, 26 corresponded to irrigation canal, and 3 to river water samples, with no DEC isolated from dams and dikes water samples. The most frequently isolated category from irrigation water was EAEC, followed by EPEC, DAEC, and ETEC. The increased prevalence of EAEC found in this study is in line with the reported by Aijuka et al. for irrigation water in South Africa (Aijuka et al., 2018). None of the EAEC strains isolated in this study carried the *stx*2 gene. For river water, the only DEC strains isolated were DAEC and ETEC although other categories such as EPEC, ETEC and STEC have been reported to be present in other rivers of Mexico (Ramirez Castillo et al., 2013; Requena-Castro et al., 2018). It is noteworthy that the DEC frequency pattern found (EAEC, EPEC, DAEC and ETEC) for irrigation canal was similar to that reported previously for DEC strains isolated in cases of human infections (Canizalez-Roman et al., 2016). This may suggest that in Sinaloa, humans have been acquiring infection by a direct contact with irrigation water or by consuming food contaminated with irrigation water such as vegetable and fruits. The absence of EIEC and EHEC strains implies that selected water bodies may not be major reservoirs of these categories, and thus indicates a low risk for EIEC and EHEC strains associated diseases, as reported in our previous study (Canizalez-Roman et al., 2016).

Different DEC strains have been associated with sporadic cases of diarrheal diseases in human and a number of waterborne gastroenteritis outbreaks. EHEC was the first strain recognized as pathogen during an outbreak of hemorrhagic colitis in 1982 (Riley et al., 1983) and, up to now, represent the most often isolated strain from outbreaks (Gobin et al., 2018; Lienemann et al., 2011). The major outbreak caused by EAEC strains was in Germany 2011, which was due to a Shiga toxin-producing EAEC strain infecting over 4000 individuals (Bielaszewska et al., 2011; Rasko et al., 2011); while, the pathogenicity of atypical EAEC has not been completely described (Huang et al., 2006). Typical EPEC strains are highly prevalent in developing community settings, and were initially associated with gastroenteritis outbreaks in infants and, in recent studies, it has been also isolated from waterborne outbreaks in adults (Nataro and Kaper, 1998; Wedley et al., 2013). Very little is known about the pathogenicity of atypical EPEC with only a few studies indicating an association with endemic diarrhea outbreaks (Alikhani et al., 2006; Wedley et al., 2013), and there are no reports of DAEC causing outbreaks with only a few studies showing its virulence (Giron et al., 1991; Vial et al., 1988). Waterborne outbreaks due to ETEC have been extensively reported in developing countries (Ram et al., 2009), and it represents the most common cause of “travelers' diarrhea” affecting individuals from industrialized countries travelling to the developing world (Poma et al., 2016).

The occurrence of antibiotic-resistant bacteria, particularly pathogenic *E. coli*, has become a serious public health threat. In this study, DEC isolates from both irrigation canal and river water were resistant to most of the traditionally used antimicrobial agents in Mexico. The overall high antimicrobial resistance found could be explained due to the indiscriminate use of antibiotics and discharge of industries, particularly antibiotic production plants and urban wastewater treatment plants, as suggested by several authors (Kruse and Sorum, 1994; Sidrach-Cardona et al., 2014). However, while comparing the antimicrobial resistant DEC found in the present study with the antimicrobial resistant DEC previously reported in Sinaloa for food items (Canizalez-Roman et al., 2013) and clinical isolates (Canizalez-Roman et al., 2016), we concluded that clinical isolates exhibited higher resistance for most of the analyzed antibiotics (Fig. 3). We hypothesized that, in the northwest of Mexico, the increase of DEC-induced cases of human gastroenteritis is caused by the spread of antibiotic resistance clones.

The prevalence of multi-drug resistance phenotypes of *E. coli* described here are in line with previous reports, particularly those performed in developing countries (Aslani et al., 2011; Poma et al., 2016; Ram et al., 2009). Xu et al. (2018) stated that many bacteria mutate to acquire their multi-antimicrobial resistance by the effect of the environment pressure. Several bacterial antibiotic-resistant genes, such as those towards ß-lactams, amoxicillin/ampicillin (bla_{TEM}), tetracycline (*tet*), chloramphenicol (*cmlA*), vancomycin (*van*), and streptomycin/spectinomycin (*aadA*) have been reported in bacteria from various aquatic ecosystems (Thevenon et al., 2012). These genes are often inserted in integrons, transposons, and plasmids, from where they can be exchanged to other bacteria by horizontal transfer (Giowanella et al., 2015; Ochman et al., 2000). Thus, waters containing antibiotics and antibiotic-resistant bacteria may play an instrumental role in the spread of genes encoding resistance factors.

5. Conclusions

This study demonstrates a moderate prevalence of diarrheagenic *E. coli* strains in various water sources of Sinaloa, Mexico. However, a subset of these DEC strains have a high pathogenic potential as they exhibited a multi-drug resistant phenotype. If these multi-drug resistant, water-borne pathogens, infect humans and cause gastrointestinal disease, the physician will have limited treatment options. The presence of diarrheagenic *E. coli* in waters warrants the implementation of environmental safety strategies in order to avoid the dissemination of clones to people leaving in the area but particularly those more vulnerable communities who utilize these waters for domestic and industrial purposes. In Sinaloa, as one of the main agro-industrial regions of Mexico that produce food for exportation, there is a necessity for better management practices, remediation strategies, and overall increased awareness of the global issues associated with water pollution.

Declaration of Competing Interest

No conflict of interest to declare.

Acknowledgments

This work was supported by grants from PROFAPI 2015/057, to

A.C.R. We thank the Department of Microbiology and Epidemiology, the Sinaloa State Public Health Laboratory, and Elizandra Quiñonez for their technical help.

References

Adler, B., Sasakawa, C., Tobe, T., Makino, S., Komatsu, K., Yoshikawa, M., 1989. A dual transcriptional activation system for the 230 kb plasmid genes coding for virulence-associated antigens of Shigella flexneri. Mol. Microbiol. 3, 627–635.

Aijuka, M., Santiago, A.E., Giron, J.A., Nataro, J.P., Buys, E.M., 2018. Enteroaggregative Escherichia coli is the predominant diarrheagenic E. coli pathotype among irrigation water and food sources in South Africa. Int. J. Food Microbiol. 278, 44–51.

Alikhani, M.Y., Mirsalehian, A., Aslani, M.M., 2006. Detection of typical and atypical enteropathogenic Escherichia coli (EPEC) in Iranian children with and without diarrhoea. J. Med. Microbiol. 55, 1159–1163.

Amabile-Cuevas, C., 2010. Antibiotic resistance in Mexico: a brief overview of the current status and its causes. J. Infect. Dev. Ctries. 4, 126–131.

APHA, A., 2012. WEF. Standard Methods for examination of water and waste water; 1360 pp. ISBN 978–087553–013-0, Washington D. DC.

Aslani, M.M., Alikhani, M.Y., Zavari, A., Yousefi, R., Zamani, A.R., 2011. Characterization of enteroaggregative Escherichia coli (EAEC) clinical isolates and their antibiotic resistance pattern. Int. J. Infect. Dis. 15, e136–e139.

Bauer, A.W., Kirby, W.M., Sherris, J.C., Turck, M., 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. 45, 493–496.

Bielaszewska, M., Mellmann, A., Zhang, W., Kock, R., Fruth, A., Bauwens, A., Peters, G., Karch, H., 2011. Characterisation of the Escherichia coli strain associated with an outbreak of haemolytic uraemic syndrome in Germany, 2011: a microbiological study. Lancet Infect. Dis. 11, 671–676.

Boisen, N., Melton-Celsa, A.R., Scheutz, F., O'Brien, A.D., Nataro, J.P., 2015. Shiga toxin 2a and Enteroaggregative Escherichia coli–a deadly combination. Gut Microbes 6, 272–278.

Canizalez-Roman, A., Gonzalez-Nunez, E., Vidal, J.E., Flores-Villasenor, H., Leon-Sicairos, N., 2013. Prevalence and antibiotic resistance profiles of diarrheagenic Escherichia coli strains isolated from food items in northwestern Mexico. Int. J. Food Microbiol. 164, 36–45.

Canizalez-Roman, A., Flores-Villasenor, H.M., Gonzalez-Nunez, E., Velazquez-Roman, J., Vidal, J.E., Muro-Amador, S., Alapizco-Castro, G., Diaz-Quinonez, J.A., Leon-Sicairos, N., 2016. Surveillance of Diarrheagenic Escherichia coli strains isolated from diarrhea cases from children, adults and elderly at northwest of Mexico. Front. Microbiol. 7, 1924.

Clements, A., Young, J.C., Constantinou, N., Frankel, G., 2012. Infection strategies of enteric pathogenic Escherichia coli. Gut Microbes 3, 71–87.

Cockerill, F.R., 2011. Performance standards for antimicrobial susceptibility testing: twenty-first informational supplement M02-A10 and M07-A08. Clinical and Laboratory Standards Institute (CLSI).

Delgado-Gardea, M.C., Tamez-Guerra, P., Gomez-Flores, R., Zavala-Diaz de la Serna, F.J., Eroza-de la Vega, G., Nevarez-Moorillon, G.V., Perez-Recoder, M.C., Sanchez-Ramirez, B., Gonzalez-Horta Mdel, C., Infante-Ramirez, R., 2016. Multidrug-resistant bacteria isolated from surface Water in Bassaseachic falls National Park, Mexico. Int. J. Environ. Res. Public Health 13.

Feng, P., Weagant, S.D., Grant, M.A., Burkhardt, W., Shellfish, M., Water, B., 2002. BAM: Enumeration of Escherichia coli and the Coliform Bacteria. Bacteriological analytical manual, 13–19. Available online at: https://www.fda.gov/food/foodscienceresearch/laboratorymethods/ucm064948.htm (Accessed August 27, 2018).

Fleckenstein, J.M., Lindler, L.E., Elsinghorst, E.A., Dale, J.B., 2000. Identification of a gene within a pathogenicity island of enterotoxigenic Escherichia coli H10407 required for maximal secretion of the heat-labile enterotoxin. Infect. Immun. 68, 2766–2774.

Giowanella, M., Bozza, A., do Rocio Dalzoto, P., Dionisio, J.A., Andraus, S., Guimaraes, E.L., Pimentel, I.C., 2015. Microbiological quality of water from the rivers of Curitiba, Parana state, Brazil, and the susceptibility to antimicrobial drugs and pathogenicity of Escherichia coli. Environ. Monit. Assess. 187, 673.

Giron, J.A., Jones, T., Millan-Velasco, F., Castro-Munoz, E., Zarate, L., Fry, J., Frankel, G., Moseley, S.L., Baudry, B., Kaper, J.B., et al., 1991. Diffuse-adhering Escherichia coli (DAEC) as a putative cause of diarrhea in Mayan children in Mexico. J. Infect. Dis. 163, 507–513.

Gobin, M., Hawker, J., Cleary, P., Inns, T., Gardiner, D., Mikhail, A., McCormick, J., Elson, R., Ready, D., Dallman, T., Roddick, I., Hall, I., Willis, C., Crook, P., Godbole, G., Tubin-Delic, D., Oliver, I., 2018. National outbreak of Shiga toxin-producing Escherichia coli O157:H7 linked to mixed salad leaves, United Kingdom, 2016. Euro Surveill. 23.

Gortares-Moroyoqui, P., Castro-Espinoza, L., Naranjo, J.E., Karpiscak, M.M., Freitas, R.J., Gerba, C.P., 2011. Microbiological water quality in a large irrigation system: El Valle del Yaqui, Sonora Mexico. J. Environ. Sci. Health A Tox. Hazard. Subst. Environ. Eng. 46, 1708–1712.

Huang, D.B., Nataro, J.P., DuPont, H.L., Kamat, P.P., Mhatre, A.D., Okhuysen, P.C., Chiang, T., 2006. Enteroaggregative Escherichia coli is a cause of acute diarrheal illness: a meta-analysis. Clin. Infect. Dis. 43, 556–563.

INEGI, 2016. Instituto Nacional de Estadística y Geografía, Anuario estadístico y geográfico de Sinaloa 2016. INEGI, pp. Available online at: http://internet.contenidos.inegi.org.mx/contenidos/Productos/prod_serv/contenidos/espanol/bvinegi/productos/nueva_estruc/anuarios_2016/702825083687.pdf (Accessed August 27, 2018).

Jenkins, C., Chart, H., Willshaw, G.A., Cheasty, T., Smith, H.R., 2006. Genotyping of enteroaggregative Escherichia coli and identification of target genes for the detection of both typical and atypical strains. Diagn. Microbiol. Infect. Dis. 55, 13–19.

Kruse, H., Sorum, H., 1994. Transfer of multiple drug resistance plasmids between bacteria of diverse origins in natural microenvironments. Appl. Environ. Microbiol. 60, 4015–4021.

Leclerc, H., Schwartzbrod, L., Dei-Cas, E., 2002. Microbial agents associated with waterborne diseases. Crit. Rev. Microbiol. 28, 371–409.

Lienemann, T., Pitkanen, T., Antikainen, J., Molsa, E., Miettinen, I., Haukka, K., Vaara, M., Siitonen, A., 2011. Shiga toxin-producing Escherichia coli O100:H(−): stx2e in drinking water contaminated by waste water in Finland. Curr. Microbiol. 62, 1239–1244.

Nataro, J.P., Kaper, J.B., 1998. Diarrheagenic Escherichia coli. Clin. Microbiol. Rev. 11, 142–201.

NOM, 1994. Norma Oficial Mexicana, NOM-127-SSA1, Salud ambiental. Agua para uso y consumo humano. Límites permisibles de calidad y tratamientos a que debe someterse el agua para su potabilización. Diario Oficial de la Federación, Available online at: http://www.dof.gob.mx/nota_detalle.php?codigo=2063863&fecha=2063831/2063812/2061969 (Accessed August 27, 2018).

NOM, 1998. Norma Oficial Mexicana, NOM-003-ECOL-1997, Que establece los lımites máximos permisibles de contaminantes para las aguas residuales tratadas que se reúsen en servicios al público. Diario Oficial de la Federación 21, Available online at: http://dof.gob.mx/nota_detalle.php?codigo=4893449&fecha=4893421/4893409/4891998 (Accessed August 27, 2018).

Ochman, H., Lawrence, J.G., Groisman, E.A., 2000. Lateral gene transfer and the nature of bacterial innovation. Nature 405, 299–304.

Poma, V., Mamani, N., Iniguez, V., 2016. Impact of urban contamination of the La Paz River basin on thermotolerant coliform density and occurrence of multiple antibiotic resistant enteric pathogens in river water, irrigated soil and fresh vegetables. Springerplus 5, 499.

Ram, S., Vajpayee, P., Singh, R.L., Shanker, R., 2009. Surface water of a perennial river exhibits multi-antimicrobial resistant Shiga toxin and enterotoxin producing Escherichia coli. Ecotoxicol. Environ. Saf. 72, 490–495.

Ramirez Castillo, F.Y., Avelar Gonzalez, F.J., Garneau, P., Marquez Diaz, F., Guerrero Barrera, A.L., Harel, J., 2013. Presence of multi-drug resistant pathogenic Escherichia coli in the San Pedro River located in the state of Aguascalientes, Mexico. Front. Microbiol. 4, 147.

Rasko, D.A., Webster, D.R., Sahl, J.W., Bashir, A., Boisen, N., Scheutz, F., Paxinos, E.E., Sebra, R., Chin, C.S., Iliopoulos, D., Klammer, A., Peluso, P., Lee, L., Kislyuk, A.O., Bullard, J., Kasarskis, A., Wang, S., Eid, J., Rank, D., Redman, J.C., Steyert, S.R., Frimodt-Moller, J., Struve, C., Petersen, A.M., Krogfelt, K.A., Nataro, J.P., Schadt, E.E., Waldor, M.K., 2011. Origins of the E. coli strain causing an outbreak of hemolytic-uremic syndrome in Germany. N. Engl. J. Med. 365, 709–717.

Requena-Castro, R., Aguilera-Arreola, M.G., Martinez-Vazquez, V., Bocanegra-Garcia, V., 2018. Prevalence of virulence genes of Escherichia coli in surface waters of the Rio Bravo in Reynosa city, Tamaulipas. Mex. J. Biotechnol. 3, 87–93.

Riley, L.W., Remis, R.S., Helgerson, S.D., McGee, H.B., Wells, J.G., Davis, B.R., Hebert, R.J., Olcott, E.S., Johnson, L.M., Hargrett, N.T., Blake, P.A., Cohen, M.L., 1983. Hemorrhagic colitis associated with a rare Escherichia coli serotype. N. Engl. J. Med. 308, 681–685.

Robins-Browne, R.M., Holt, K.E., Ingle, D.J., Hocking, D.M., Yang, J., Tauschek, M., 2016. Are Escherichia coli Pathotypes still relevant in the era of whole-genome sequencing? Front. Cell. Infect. Microbiol. 6, 141.

Sidrach-Cardona, R., Hijosa-Valsero, M., Marti, E., Balcazar, J.L., Becares, E., 2014. Prevalence of antibiotic-resistant fecal bacteria in a river impacted by both an antibiotic production plant and urban treated discharges. Sci. Total Environ. 488-489, 220–227.

Teng, L.J., Hsueh, P.R., Liaw, S.J., Ho, S.W., Tsai, J.C., 2004. Genetic detection of diarrheagenic Escherichia coli isolated from children with sporadic diarrhea. J. Microbiol. Immunol. Infect. 37, 327–334.

Thevenon, F., Adatte, T., Wildi, W., Pote, J., 2012. Antibiotic resistant bacteria/genes dissemination in lacustrine sediments highly increased following cultural eutrophication of Lake Geneva (Switzerland). Chemosphere 86, 468–476.

Trabulsi, L.R., Keller, R., Tardelli Gomes, T.A., 2002. Typical and atypical enteropathogenic Escherichia coli. Emerg. Infect. Dis. 8, 508–513.

Tsen, H.Y., Lin, C.K., Chi, W.R., 1998. Development and use of 16S rRNA gene targeted PCR primers for the identification of Escherichia coli cells in water. J. Appl. Microbiol. 85, 554–560.

Vial, P.A., Robins-Browne, R., Lior, H., Prado, V., Kaper, J.B., Nataro, J.P., Maneval, D., Elsayed, A., Levine, M.M., 1988. Characterization of enteroadherent-aggregative Escherichia coli, a putative agent of diarrheal disease. J. Infect. Dis. 158, 70–79.

Wedley, A.L., Elajnef, H.M., Fletcher, J.N., 2013. Characterization of a novel EAST-negative enteropathogenic E. coli strain implicated in a food-borne outbreak of diarrhoea in adults. APMIS 121, 494–502.

WHO, 2001. Guidelines, standards and health: Assessment of risk and risk management for water-related infectious disease. World Health Organization, Geneva, Switzerland, Available online at: http://www.who.int/water_sanitation_health/dwq/iwaforeword.pdf (Accessed August 27, 2018).

WHO, 2006. Guidelines for the Safe Use of Wastewater, Excreta and Greywater, Third, edition ed. World Health Organization. Available online at. http://www.who.int/water_sanitation_health/sanitationwaste/wastewater/wastewater-guidelines/en/, Accessed date: 27 August 2018.

WHO, 2011. Outbreaks of *E.* In: *coli* O104:H4 Infection: Update 30. World Health Organization, Available Online at, . http://www.euro.who.int/en/health-topics/disease-prevention/food-safety/news/news/2011/07/outbreaks-of-e.-coli-o104h4-infection-update-30 (Accessed August 27, 2018).

WHO, 2017. WHO/UNICEF Joint Monitoring Program for Water Supply, Sanitation and Hygiene (JMP) – 2017 Update and SDG Baselines. World Health Organization, Available online at: http://www.who.int/mediacentre/news/releases/2017/launch-version-report-jmp-water-sanitation-hygiene.pdf (Accessed August 27, 2018).

Xu, X., Xu, L., Yuan, G., Wang, Y., Qu, Y., Zhou, M., 2018. Synergistic combination of two antimicrobial agents closing each other's mutant selection windows to prevent antimicrobial resistance. Sci. Rep. 8, 7237.

Yang, J.R., Wu, F.T., Tsai, J.L., Mu, J.J., Lin, L.F., Chen, K.L., Kuo, S.H., Chiang, C.S., Wu, H.S., 2007. Comparison between O serotyping method and multiplex real-time PCR to identify diarrheagenic Escherichia coli in Taiwan. J. Clin. Microbiol. 45, 3620–3625.