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Multidrug resistance and class 1 integron presence in *Escherichia coli* isolates from a polluted drainage ditch's water

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ABSTRACT

The impact of contamination of water drainage ditches in the development of antibiotic-resistant bacteria has been scarcely studied in Mexico. In this regard, 101 isolates of *E. coli* were obtained from water samples from a ditch in Sinaloa, during one year. The antimicrobial resistant profiles, the presence of the class 1 integron and evolutionary relationship of *intI1* sequences were determined. The 47.5% of strains were resistant and 5.9% multidrug resistant (MDR) with an average multiple antibiotic resistance index value of 0.45. The highest resistance was registered with β-lactam (39.6%) and quinolone (9.9%). The *intI1* gene was detected in 11.9% of the isolates, and no association with MDR was found. Sequence were associated with human and animal host isolates. MDR *E. coli* isolates with *intI1* gene highlight the potential risk of the ditch's water to human health. An attenuation effect of MDR *E. coli* isolates in the outlet water was observed.

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Anthropogenic pollution; antibiotic resistance; aquatic environment; intI1; *E coli*

Introduction

Escherichia coli (*E. coli*) naturally resides in the gastrointestinal tract of animals and humans, is widely distributed in aquatic and terrestrial natural environments; can be used as an indicator organism to survey fecal contamination, and develop patterns of antibiotic resistance (Hutinel et al. 2019; Zhang et al. 2020).

Water is essential for life and is used for drinking, agricultural irrigation, recreational activities among others. However, freshwater sources are threatened by contamination with antibiotics and other xenobiotics; contaminants released through agricultural runoffs, sewage discharges, and leaching from nearby farms. Besides, contaminated water is recognized as one of the main sources to transmit and disseminate antibiotic resistance (Nnadozie and Odume 2019; Zhang et al. 2020). In this respect, water from agricultural drainage ditches in Sinaloa, Mexico, has several uses downstream for instance crop irrigation, water for livestock, and in aquaculture farms. Unfortunately, these drainage ditches commonly receive sewage and wastewater from nearby rural communities and crop irrigation highly contaminated (Ahumada-Santos et al. 2014; Moeder et al. 2017).

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Integrons are genetic elements associated with plasmids and transposons, and they are involved in horizontal gene transfer between pathogenic and commensal bacteria. The main structural elements of an integron are recombination site (attI), promoter (Pc), the integrase gene (*intI*) that catalyzes recombination between the attC site of circular gene cassettes, and the attendant recombination site (attI). The amino acid sequences of *intI* define the class of integrons (Gillings et al. 2015; Zhang et al. 2020). The *intI1* gene has been considered a good proxy for anthropogenic pollution (Gillings et al. 2015). The class 1 integrons are involved in the spread of antimicrobial resistance (AMR) by the acquisition and dissemination of antibiotic resistance genes (Gillings 2018), representing a risk to human health (Zhang et al. 2020).

This study aimed to investigate the antimicrobial resistance profiles and presence of class 1 integron *intI1* gene in *E. coli* isolates from water from a ditch highly polluted in Sinaloa, Mexico; in addition, it analyzed the ditch performance to decrease the resistant bacteria in the drained water.

Materials and methods

Sample collection and isolation of *Escherichia coli*

Escherichia coli strains were isolated from 57 water samples from the ditch "La Michoacana" in Sinaloa, Mexico. Samples were collected monthly during one year at five equidistant sampling sites (SS) selected as previously described by Ahumada-Santos et al. (2014) and Moeder et al. (2017). Water was sampled at a 15 cm depth from the surface, kept at 4 °C, and analyzed within 8 h. Total coliforms (TC) and fecal coliforms (FC) were recovered by the membrane filtration technique (APHA, AWWA, WEF 2005). Membrane was transferred to the culture media *m*-Endo Agar and FC Agar, and the plates were incubated at 37°C (TC) or 45°C (FC) for 24 h. Presumptive *E. coli* colonies were identified by Gram staining and biochemical tests. Subsequently, strains were stored in BHI medium with DMSO at −80°C until use.

Antimicrobial susceptibility testing

The antimicrobial susceptibility patterns of the isolates were determined using the Kirby – Bauer disk diffusion method (CLSI 2018). All assays were carried out in duplicate and the *E. coli* ATCC 25922 was used as a reference strain. Antibiotic susceptibility was interpreted according to CLSI guidelines (CLSI 2022). Fourteen antimicrobials (ACCUTRACK multidisks for Gram GN1 Negatives) of five different classes were used at concentrations listed in Table S1. The multiple antibiotic resistance (MAR) index of each strain was calculated by dividing the number of antibiotics to which the isolate was resistant by the number of antibiotics to which the isolate was exposed (Krumperman 1983).

DNA extraction

Escherichia coli were cultured overnight in agar MacConkey plates and DNA extraction was carried out according to Velarde Félix et al. (2015) with some modifications. The cell culture was suspended in 1 mL of extraction buffer (TRIS base 30 mM, EDTA 15 mM, pH 8.5) and mixed with 350 µL of CTAB 10%. The bacterial suspension was lysed (100 °C/20 min), added with 250 µL of NaCl 5 M, incubated (−20 °C/15 min), and centrifuged (12,000×g/10 min). The DNA was washed with 600 µL chloroform and precipitated with 900 µL isopropanol. The concentration, purity, and integrity of the DNA were verified by using a UV spectroscopic analysis (NanoDrop) and 2% agarose gel electrophoresis. DNA samples were preserved at −20°C until use.

Detection of class 1 integron intI1 gene

The class 1 integron in the *E. coli* isolates was identified by PCR using primers for the integrase (*intI1*) gene according to Ahumada-Santos et al. (2020). The amplicons were analyzed in 2% agarose gel electrophoresis, purified using the Wizard®SV Gel and PCR Clean-Up System (Promega), and sequenced in both directions (Macrogen Inc.). DNA sequence data were analyzed using CLUSTALW software, the GenBank database (Table S2), and the BLAST algorithm on the NCBI website.

Evolutionary relationship analysis of intI1 sequences

A total of 12 nucleotide sequences obtained from partial sequences of the *intI1* gene of the study strains (Table S3) were aligned with 22 sequences of *E. coli* of a different geographical/biological origin available in GenBank (Table S2). In addition, the reference sequences from *Mus musculus*, *Enterococcus faecalis*, and *Staphylococcus aureus* were used as external controls. The alignments were performed by using the CLUSTAL W and MUSCLE algorithms with MEGA7 (64-bit) MacOS and Graphical software.

Phylogenetic reconstruction

The evolutionary history was inferred using the Unweighted Pair Group Method with Arithmetic Mean (UPGMA). The evolutionary distances were computed using the Maximum Composite Likelihood method and expressed in the units of the number of base substitutions per site. The analysis involved 37 nucleotide sequences. All positions containing gaps and missing data were eliminated, and the final dataset comprised 651 positions. Evolutionary analyses were conducted in MEGA7.

Results

A total of 101 *E. coli* isolates were obtained from five ditch points. The months with the highest number of isolates were January (27.7%), July (12.9%), and December (11.9%), while no strains were isolated in March. The highest number of isolates considering the SS in descending order was SS4 (27.7%), SS5 (22.8%), SS2 (20.8%), SS3 (17.8%), and SS1 (10.9%).

Antimicrobial susceptibility

The antimicrobial susceptibility profile analysis displayed that 47.5% of strains were resistant to at least one out of the 14 tested antibiotics. The 18.8% of strains were susceptible to all antibiotics. The 70.3% of strains presented intermedia resistance at least to one of the antibiotics evaluated. The most outstanding resistance by drug was for CD (26.7%) and CXM (18.8%), and all strains were susceptible to AK (100%) (Table 1). Analysing the bacterial resistance associated with the antibiotic classes, the β-lactam (39.6%) and quinolones (9.9%) presented the highest resistance, while the aminoglycosides (100%) and fluoroquinolones (97%) exhibited the highest susceptibility (Table S4).

The highest percentage of resistant strains was recovered in January (12.9%) and July (8.9%), while the lowest percentage (1%) was in April, May, and November. Analyzing resistant isolates recovery by SS, SS2 showed the highest value (13.9%), and resistant percentages decreased downstream the ditch. Besides, the recovery percentages of sensitive isolates were maximum in January (27.7%) and minimum in April (3%) (Figure 1).

The β-lactam-resistant *E. coli* strains were recovered in all months of the study, being the only antibiotic family with such characteristics. Specifically, isolates with CD resistance indicated the highest frequency in six of the months and four of the SS studied; resistance to CXM was registered

Table 1. Antibiotic susceptibility of the 101 *Escherichia coli* isolates.

Antibiotic (Class[1])	% Resistance	% Intermediate resistance	% Susceptible	%R + %IR
Cefdinir (B)	**26.7**	**18.8**	54.5	**45.5**
Cefuroxime (B)	**18.8**	**31.7**	49.5	**50.5**
Nalidixic acid (Q)	**9.9**	**30.7**	59.4	**40.6**
Ceftazidime (B)	7.9	8.9	83.2	16.8
Cefotaxime (B)	7.9	3.0	89.1	10.9
Cefixime (B)	5.9	1.0	93.1	6.9
Ceftriaxone (B)	5.0	1.0	94.1	5.9
Nitrofurantoin (N)	5.0	5.9	89.1	10.9
Norfloxacin (F)	5.0	1.0	94.1	5.9
Gentamicin (A)	5.0	0.0	**95.0**	5.0
Aztreonam (B)	4.0	2.0	94.1	5.9
Ofloxacine(F)	3.0	0.0	**97.0**	3.0
Ciprofloxacin (F)	3.0	0.0	**97.0**	3.0
Amikacin (A)	0.0	0.0	**100.0**	0.0

[1]Class: A: Aminoglycosides, B: β-lactams, F: Fluoroquinolones, Q: Quinolones, N: Nitrofurans. Numbers in bold type indicates the three highest value of resistance (R), intermediate resistance (IR), susceptibility, and %R+%IR.

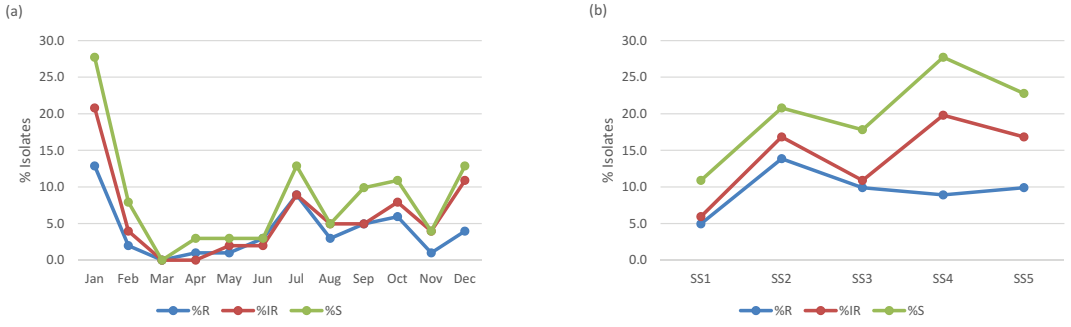

Figure 1. Antibiotic susceptibility of the 101 *Escherichia coli* isolates distributed by (a) month and (b) sample site (SS). %R: resistance, %IR: intermediate resistance, %S: susceptible.

only in April, May, November, and December; while resistance to the five antibiotic classes studied was detected in October and in SS1, SS2, and SS4. In addition, isolates of SS1 and SS4 were resistant to more antibiotics, followed by SS2, SS5, and SS3 (Figure 2).

Multidrug Resistance (MDR)

The 5.9% of the *E. coli* isolates were resistant to three or more classes of antimicrobials and classified as multidrug-resistant (MDR) (Magiorakos et al. 2012). In 83.3% of the MDR isolates, aminoglycosides (GM), β-lactams (CD), and quinolones (NA) were the most represented antibiotic-class resistance; these strains were isolated more frequently in September and October and SS1. The MDR isolates presented a MAR index value >0.2 with an average of 0.45, and 50% of them had the *intI1* gene. The 167 isolate was the only one resistant to the five classes of antibiotics evaluated, displayed the highest MAR index (0.93), and had the *intI1* gene (Table 2).

Detection of intI1 gene

The *intI1* gene was detected in 11.9% of the *E. coli* isolates, occurring more frequently in January, July, August, and October and the SS3. Among the isolates with the *intl1* gene, 41.7% were resistant to CXM, 100% were sensitive to AK, 50% were resistant to β-lactams, and 33.3% did not present resistance to any antibiotic. These isolates presented a MAR index value >0.07 with an average of 0.29, and 25% were classified as MDR (Table 3).

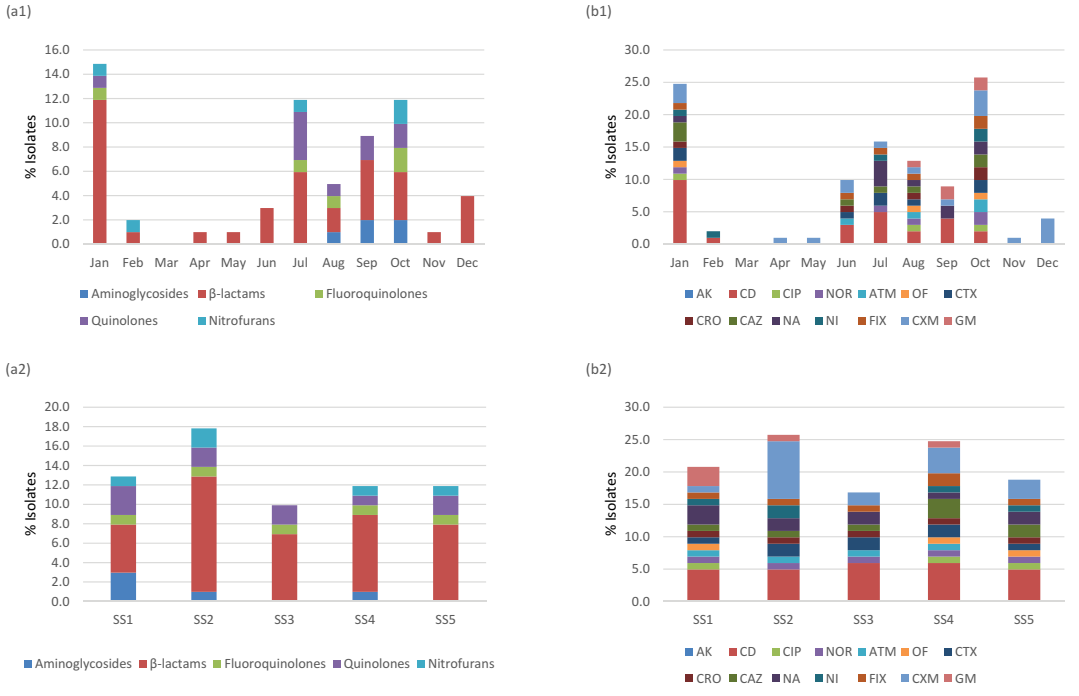

Figure 2. Accumulated percentages of resistant *Escherichia coli* isolates by class of antibiotics (a) and individual antibiotics (b), distributed by month (a1; b1) and sampling site (a2; b2).

Table 2. Characteristics of multidrug resistant (MDR) *Escherichia coli* isolates.

Isolate	Month	Sampling Site	Resistance by antibiotic[1] (numbers of antibiotics)	Resistance by class[2] (numbers of class)	MAR[3]Index	*intl1*
34	Jan	5	CD,CIP,NOR,OF,CTX,CRO,CAZ,NA,FIX,CXM (10)	B,F,Q (3)	0.71	-
147	Aug	4	CIP,NOR,OF,NA,GM (5)	A,F,Q (3)	0.36	Yes
151	Sep	1	CD,NA,GM (3)	A,B,Q (3)	0.21	-
152	Sep	1	CD,NA,GM (3)	A,B,Q (3)	0.21	-
167	Oct	1	CD,CIP,NOR,ATM,OF,CTX,CRO,CAZ,NA,NI,FIX,CXM,GM (13)	A,B,F,Q,N (4)	0.93	Yes
169	Oct	2	CD,NI,CXM,GM (4)	A,B,N (3)	0.29	Yes

[1]Antibiotics: CD: Cefdinir, CIP: Ciprofloxacin, NOR: Norfloxacin, ATM: Aztreonam, OF: Ofloxacin, CTX: Cefotaxime, CRO: Ceftriaxone, CAZ: Ceftazidime, NA: Nalidixic acid, NI: Nitrofurantoin, FIX: Cefixime, CXM: Cefuroxime, GM: Gentamicin.
[2]Class: A: Aminoglycosides, B: β-lactams, F: Fluoroquinolones, Q: Quinolones, N: Nitrofurans.
[3]MAR: Multiple Antibiotic Resistance.

Sequence analysis of intl1 gene

Analyzing the 12 *intl1* sequences of the *E. coli* isolates, they exhibited >99.9% identity to those reported in GenBank. Nine of them had a single-nucleotide change, six at position 60 (C-T) and three at position 65 (G-C). Evolutionary relationship analysis allowed their classification into four groups observed from left to right and up and down (Figure 3). Sequence from isolate 206 is in the first group and is associated with ten isolates from human and chicken hosts. In the same group also are present APEC pathogenic strains and phylogenetic group A, B1, B2, D, and F. In the second phylogenetic group, the sequence 18 is related to four sequences isolated in Mexico from human host classified in the B2 and A phylogenetic groups. Sequences from isolates 24, 159, 167, and 189 are in the branch with sequences of strains obtained in Mexico, India, and Switzerland from human and chicken hosts; some of these isolates are of the phylogenetic groups A and B1. Finally,

Table 3. Characteristics of *Escherichia coli* isolates with class 1 integron gene *intI1*.

Isolate	Month	Sampling Site	Resistance by antibiotic[1] (number of antibiotics)	Resistance by class[2] (number of classes)	MAR[3] Index	MDR[4]
18	Jan	5	CD (1)	B (1)	0.07	-
24	Jan	3	-		-	-
75	Apr	3	-		-	-
122	Jul	3	NA (1)	Q (1)	0.07	-
134	Jul	4	-		-	-
145	Aug	3	CD,ATM,CTX,CRO,CAZ,FIX,CXM (7)	B (1)	**0.50**	-
147	Aug	4	CIP,NOR,OF,NA,GM (5)	A,F,Q (3)	**0.36**	Yes
159	Sep	4	-		-	-
167	Oct	1	CD,CIP,NOR,ATM,OF,CTX,CRO,CAZ,NA,NI,FIX,CXM,GM (13)	A,B,F,Q,N (5)	**0.93**	Yes
169	Oct	2	CD,NI,CXM,GM (4)	A,B,N (3)	**0.29**	Yes
189	Nov	2	CXM (1)	B (1)	0.07	-
206	Dic	2	CXM (1)	B (1)	0.07	-

[1]Antibiotics: CD: Cefdinir, CIP: Ciprofloxacin, NOR: Norfloxacin, ATM: Aztreonam, OF: Ofloxacin, CTX: Cefotaxime, CRO: Ceftriaxone, CAZ: Ceftazidime, NA: Nalidixic acid, NI: Nitrofurantoin, FIX: Cefixime, CXM: Cefuroxime, GM: Gentamicin.
[2]Class: A: Aminoglycosides, B: β-lactams, F: Fluoroquinolones, Q: Quinolones, N: Nitrofurans.
[3]MAR: Multiple Antibiotic Resistance. Numbers in bold type indicates MAR index >0.2.
[4]MDR: Multi Drug Resistance.

sequences from isolates 75, 147, and 169 share the branch with five sequences isolated in Mexico and Spain from human and pig, respectively. The reference sequences of external controls are grouped in separated branches. The sequences of *intI1* genes were deposited in the GenBank (Table S3).

Discussion

Water environments influenced by anthropogenic activities have been widely related to the presence of antibiotic-resistant bacteria (ARB), antibiotic resistance genes (ARG), and the acquisition and dissemination of antibiotic resistance (Nnadozie and Odume 2019; Amarasiri et al. 2020). MDR *E. coli* isolates with *intI1* gene, obtained previously from ditches water (Ahumada-Santos et al. 2014; Moeder et al. 2017), were analyzed in the present work. It was observed an increased resistance to β-lactams (2nd and 3rd generation cephalosporins) and quinolones (1st generation) and decreased resistance to aminoglycosides and fluoroquinolones (3rd generation quinolones). Similar results were reported in isolates from surface water used for crop irrigation in the studied region, differing only with a lower percentage of resistance for NA (3.5%) (Chaidez-Quiroz et al. 2009; Canizalez-Roman et al. 2019). In India (Kaushik et al. 2019; Singh et al. 2021), Brazil (Canal et al. 2016; Tolentino et al. 2021), and the Philippines (Paraoan et al. 2017), similar resistance profiles for isolates from surface waters disturbed by anthropogenic activities were similar to those from the ditch studied , although with higher percentages in some cases. In Mexico, the presence of antibiotics in aquatic contaminated environments is considered a determinant factor for antimicrobial resistance development; however, reports of resistance profiles of *E. coli* isolates in these locations are scarce (Valdez-Carrillo et al. 2020). As in this research, 5% or less of the *E. coli* isolates obtained from waters of the San Pedro River in Aguascalientes and Xochimilco´s Lake in Mexico City were resistant to GM, AK, CRO, and CIP; reporting complete susceptibility to CTX and higher resistance to NI (Ramirez Castillo et al. 2013; Rosas et al. 2015). The differences in *E. coli* antibiotic resistance may be due to different factors: *e.g.*, the physicochemical characteristics of the water bodies that allow greater diffusion of pollutants; the regulation and management of antibiotics in hospital environments, aquaculture and livestock; the presence and dissemination of ARG; and the nature of polluting effluents (*e.g.*, wastewater, metals, and organic contaminants). Such factors can

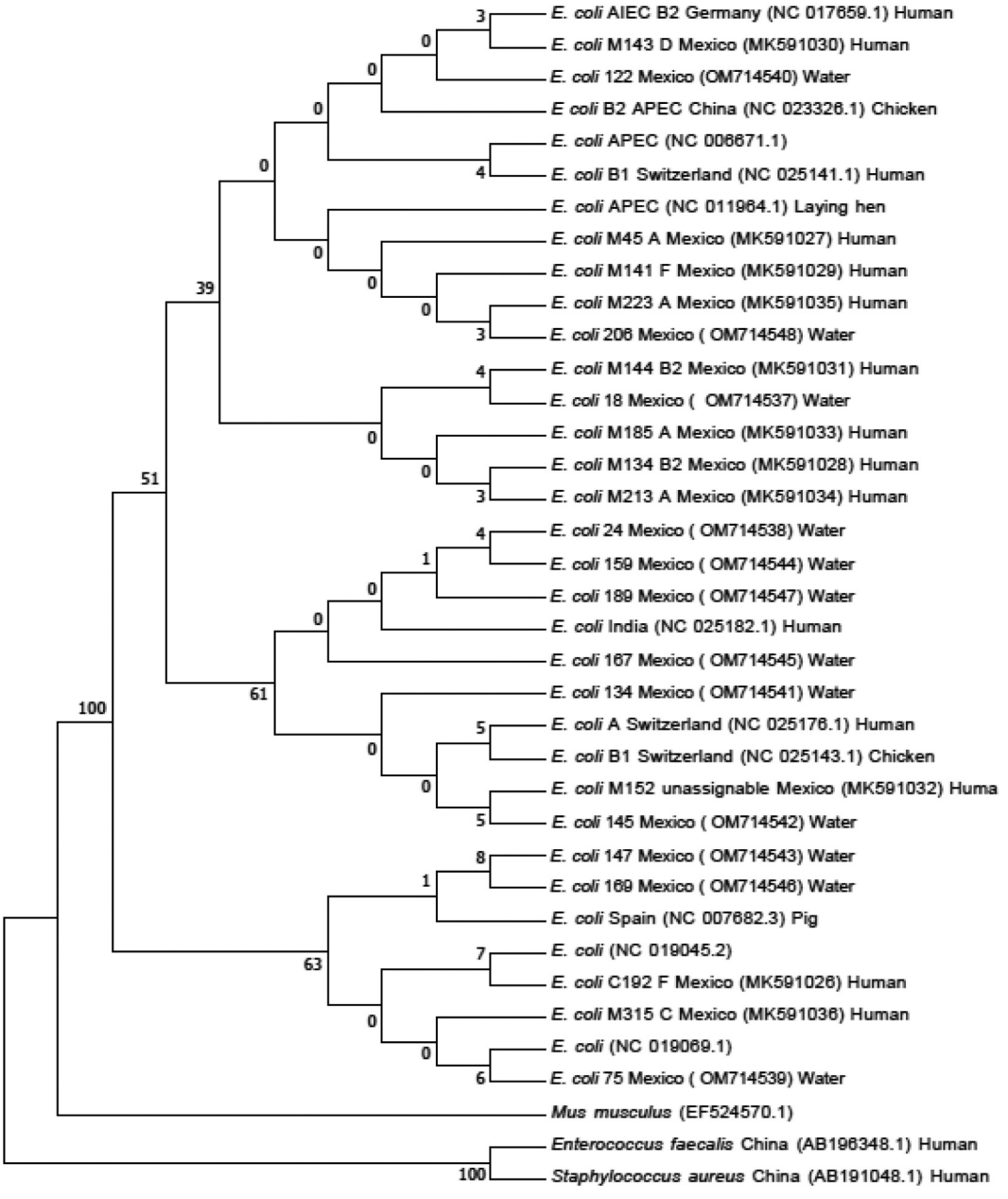

Figure 3. Evolutionary relationship of *Escherichia coli* integron class 1 *intI1* gene. The optimal tree with the sum of branch length = 3. 49,640,052 is shown. The percentage of replicate trees in which the associated taxa clustered together in the bootstrap test (1000 replicates) is shown next to the branches.

lead to selective pressure and increase the horizontal gene transfer among the bacterial communities (Nnadozie and Odume 2019; Lin et al. 2021).

The 5.9% of the *E. coli* isolates in this study were MDR, and higher percentages have been reported for surface water isolates from other regions (Paraoan et al. 2017; Kaushik et al. 2019; Singh et al. 2021). In Sinaloa, Canizalez-Roman et al. (2019) reported an MDR value of 17%; authors defined MDR as the resistance to three or more antibiotics; with this criterion, 22.9% of our isolates must be MDR. The average MAR index values of our isolates were 0.16 for the resistant and 0.45 for the MDR ones; similar values have been reported for isolates from equally disturbed water samples (Kaushik et al. 2019; Singh et al. 2021). A MAR index value >0.2 can be considered an environmental indicator of high-risk contamination and exposure to antibiotics (Krumperman 1983; Singh et al. 2021). Thus, the analyzed ditch water can be described as highly contaminated with antibiotic residues. This ditch receives water discharges from a rural community and cultivation area, being contaminated with pesticides and pharmaceuticals, among other pollutants (Moeder et al. 2017).

The susceptibility profiles of the *E. coli* water isolates for each family of antibiotics corresponded to those reported for clinical *E. coli* isolates, as well as with the prescription and consumption of antibiotics in Mexico, although compared with some particular antibiotics, higher resistance is reported (Ponce de Leon 2018; Sánchez-Huesca et al. 2020; Vázquez-Zamora et al. 2020). In Sinaloa, higher resistance to β-lactams and quinolones is reported for isolates from diarrheal cases and stool samples. These isolates had higher susceptibility to CAZ than CIP and NA (Canizalez-Roman et al. 2016) and greater susceptibility to cephalosporins than to the aminoglycoside GM (Uribe-Beltrán et al. 2017). There is a probable relationship between the consumption and misuse of these antibiotics with the presence of ARB in aquatic environments, presenting a continuous entry of ARB and even residues of antibiotics and ARG in bodies of water in the region (Hutinel et al. 2019).

It is worrying that nine of 14 evaluated antibiotics among those with the highest resistance are within the Watch group of the WHO AWaRe (World Health Organization Access, Watch, Reserve) categorization. Furthermore, it is more alarming the presence of isolates resistant to antimicrobials (ATM) classified into the Reserve group antibiotics (WHO 2021). The presence of these resistant bacteria in the studied ditch water reflects the improper medical prescription and misuse of antibiotics and the significant risk to human health; thus, the population near the ditch is at higher risk. The population is exposed to infectious agents that cannot be treated successfully. Interestingly, isolate named 167 presented resistance to the five classes of antibiotics evaluated and the *intI1* gene, evidencing that water environments influenced by anthropogenic activities act as sources of MDR bacteria (Kaushik et al. 2019; Valdez-Carrillo et al. 2020). Thus, the potential risk to public health of the bacteria circulating in the ditch water is evident.

A remedial effect of organic contaminants and coliform bacteria in the ditch water studied was reported (Ahumada-Santos et al. 2014; Moeder et al. 2017). The same effect was found for the isolated ARB; they were distributed in the ditch water varying in space and temporality, reaching lower ARB in SS5 (Figure S1). This effect is related to reducing antibiotics and ARG in natural or constructed wetlands, lessening the selective pressure exposure by sub-inhibitory concentrations of antibiotics and the transfer of ARG between resistant and sensitive bacteria that co-inhabit the same environment (Li et al. 2019).

The analysis of space and temporality of antibiotic resistance presented a higher presence of ARB in SS2 during summer (Figure S1). This higher ARB was related to the close punctual input of domestic wastewater discharges and the high population density. In contrast, SS4 had the lowest isolated ARB and did not receive domestic wastewater inputs, only an inlet for rainwater and irrigation water for nearby crops. Wastewater inflows into aquatic environments have been reported as contaminating hotspots, where the levels of ARB and ARG increased (Li et al. 2019), the same behavior was evidenced in the studied ditch. In SS5 (exit point of the flow in the ditch), ARB were only isolated from September to February. This point is close to a fertilizer manufacturing company and receives mainly agricultural runoff. In September, a hurricane hit and flooded the studied region; besides, pesticides were applied from November to February. These situations could indicate that

ARB can reach the ditch due to agriculture runoff, where pesticides in aquatic environments can induce resistance in bacteria (Ramakrishnan et al. 2019). Resistance development is also promoted due to increased fecal contamination and ARG in surface water after storms (Ahmed et al. 2021). During the summer, the high temperatures and the lack of rain allow the development and concentration of bacteria in water environments (Ahumada-Santos et al. 2014), which increases the possibility of transfer ARG (Li et al. 2019). Also, diarrheal diseases increase, and since domestic discharges are the only source of water entering the ditch, the presence of residues of antibiotics used in diarrhea treatment and ARB can increase, as presented in June and July (Figure S1).

The *intI1* gene was detected in 11.9% of the *E. coli* isolates, resulting in similar values to that reported in Brazil (14%) (Canal et al. 2016), and lower than those in India (52%-75%) (Kaushik et al. 2019; Singh et al. 2021) and the Philippines (67%) (Paraoan et al. 2017). The *intI1* gene has been considered a good anthropogenic pollution indicator (Gillings et al. 2015), consistent with the high presence of organic and bacterial contaminants in the ditch water, derived mainly from anthropogenic activity (Ahumada-Santos et al. 2014; Moeder et al. 2017). These isolates represent potential disseminators and reservoirs of AGR among other bacteria and environments, as well as a risk to human health (Canal et al. 2016; Zhang et al. 2020).

The highest number of isolates with *intI1* gene was observed in SS3. However, it was the only SS without MDR isolates (Figure S2) and resistance to a lower diversity of antibiotics. This indicates that the resistance presented by the isolates of this SS may be mediated by molecular mechanisms, such as plasmids or transposons (Gillings et al. 2015; Zhang et al. 2020). Although the MDR profile is widely associated with integrons (Kaushik et al. 2019), the lack of correlation in *E. coli* isolates in water environments has been reported (Canal et al. 2016; Singh et al. 2021). SS3, followed by SS2 and SS4, has a higher population density close to and a higher negative impact of untreated wastewater. These sampling sites were the source of the highest number of *E. coli* isolates with *intI1* gene detected. Comparing SS1 and SS5, mainly influenced by pluvial water, agricultural runoff, and industrial wastewater, it is suggested that the presence of *intI1* gene in the isolates of the studied ditch is mainly affected by domestic wastewater.

Escherichia coli isolates with *intI1* gene appeared mainly in January, July, August, and October, months in which resistance to a greater diversity of antibiotics was detected. Analyzing by temporality, the registered resistance could be related to the presence of the *intI1*; however, only 50% of the isolates were classified as MDR. This pattern must be further analyzed by characterizing the *intI1* variable region to identify the ARG presence and associate them with the MDR profiles found. Despite not having this information, 50% and 25% of the isolates with *intI1* gene were resistant to the class of β-lactams and aminoglycosides, and it is precisely the gene cassettes carrying ARG to these classes of antibiotics that have been mostly associated with the integron class 1 (Zhang et al. 2018).

The presence of *intI1* has been cataloged as a type of environmental contaminant DNA, with greater abundance and prevalence in agricultural animals, humans, and waste streams derived from human activity (Gillings 2018). This relationship was found in the sequence analysis, and it indicated the negative impact of human activities on the quality of the ditch´s water and the spread of antibiotic resistance. The *intI1* gene sequences of the *E. coli* isolates had >99.9% identity to those reported in GenBank, agreed with the conserved phylogenetic distribution reported for the *intI1* gene in the *Enterobacteriaceae* family and *E. coli* bacteria (Zhang et al. 2018).

Conclusion

The 47.5% of *E. coli* isolates were resistant and 5.9% resulted MDR. The *intI1* gene was detected in 11.9% of the isolates, but no MDR association was found. MDR *E. coli* isolates with *intI1* gene highlight the potential risk to human health in the water from the "La Michoacana" ditch. However, the ditch has an attenuation effect of MDR *E. coli* isolates in the outlet water. The information generated is helpful for the creation of laws, regulations, and environmental monitoring programs

that consider multiresistance as an integral problem between humans, animals, and the environment, indicated by the WHO in its "One Health" initiative.

Disclosure statement

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