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Antibiotic resistance patterns of *Escherichia coli* isolates from different aquatic environmental sources in León, Nicaragua

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Abstract

Antibiotic-resistant bacteria have emerged due to the selective pressure of antimicrobial use in humans and animals. Water plays an important role in dissemination of these organisms among humans, animals and the environment. We studied the antibiotic resistance patterns among 493 *Escherichia coli* isolates from different aquatic environmental sources collected from October 2008 to May 2009 in León, Nicaragua. High levels of antibiotic resistance were found in *E. coli* isolates in hospital sewage water and in eight of 87 well-water samples. Among the resistant isolates from the hospital sewage, ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid, trimethoprim-sulphamethoxazole was the most common multi-resistance profile. Among the resistant isolates from the wells, 19% were resistant to ampicillin, ceftazidime, ceftriaxone, cefotaxime, chloramphenicol, ciprofloxacin, gentamicin, nalidixic acid and trimethoprim-sulphamethoxazole. *E. coli* producing ESBL and harbouring $bla_{\text{CTX-M}}$ genes were detected in one of the hospital sewage samples and in 26% of the resistant isolates from the well-water samples. The $bla_{\text{CTX-M-9}}$ group was more prevalent in *E. coli* isolates from the hospital sewage samples and the $bla_{\text{CTX-M-1}}$ group was more prevalent in the well-water samples.

Keywords: Antibiotics, extended-spectrum β-lactamase, hospital, resistance, sewage, water, well
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Introduction

The emergence of antimicrobial-resistant bacteria presents a major threat to public health because it reduces the effectiveness of antimicrobial treatment, leading to increased morbidity, mortality and healthcare expenditure [1–3]. The main factor driving this process is the selective pressure of antimicrobial use in human and animal medicine, as well as in aquaculture and agriculture [1,3,4]. Consequently, this has led to the dissemination of antibiotic-resistant bacteria throughout the environment [5,6]. Residues of human and veterinary drugs are introduced into the environment via a number of pathways, but primarily from discharges of wastewater treatment plants or land application of sewage sludge and animal manure [7,8].

Due to the concern described above, some measures have been taken to address this problem; for example, the World Health Organization has ranked antimicrobials according to their importance in human medicine as a critical step in developing risk management strategies for the use of antimicrobials in animals used for food production [1]. In the European Union and some other countries such as Switzerland, the use of antibiotics as growth promoters in animal farming has been banned for the last several years. In developing countries, control of the use of antibiotics in animal farming is not being implemented and there is no information regarading the use of antibiotics for such purposes.

The importance of determining the prevalence of antibiotic-resistant bacteria in an environmental reservoir such as water is that the environmental source is not only a way of dissemination of antibiotic-resistant microorganisms among human and animal populations, but also the route by which resistance genes are introduced into natural bacterial ecosystems. Kümmerer [9], in a review about antibiotics in an aquatic environment, showed that bacteria that become resistant through the use of antibiotics during medical treatment, are an important source of resistance found in hospital effluents, municipal sewage and sewage treatment plants. Kim and Aga [7], in their review on the impact of antibiotic and antibiotic-resistant bacteria from wastewater treatment plants, showed that these sites provide favourable conditions for the proliferation of antibiotic-resistant bacteria and spread of resistance genes to non-resistant bacteria.

Thus, tracking the spread of antibiotic-resistant bacteria in water samples, such as sewage, tap and well water, is a useful source of information that can be used by policy makers in order to create risk management strategies for water environments [5]. The aim of this study was to determine the antibiotic resistance patterns of *Escherichia coli* isolates from different aquatic environmental sources in León, Nicaragua.

Materials and Methods

Sample collection and identification of *E. coli* strains

Sampling was carried out from October 2008 to May 2009 from different localities in León, Nicaragua. Samples were collected once from (i) household drinking water (n = 20); (ii) well water used for consumption (n = 87); (iii) sewage water from two municipal sedimentation treatment plants (two samples of each influent and effluent, n = 8); and (iv) sewage effluents of the main hospital in the city (n = 3).

All samples were collected in sterile 500-mL glass or polyethylene bottles without preservatives and transported at 4°C to the Department of Microbiology at National Autonomous University of Nicaragua, León, where primary isolation of *E. coli* was performed.

Samples from tap water were membrane filtered directly through 0.45-μm pore size filters (Millipore Corporation, Bedford, MA, USA), while all other water samples were subjected to serial ten-fold dilutions with phosphate-buffered saline before filtration. Membrane filters were placed on Chromogenic Selective Agar (Oxoid, Malmö, Sweden) and Difco m-FC Agar (Becton Dickinson AB, Stockholm, Sweden) plates and incubated aerobically for 24 h at 37 and 44.5°C, respectively.

The total number of coliforms was recorded, and *E. coli*-like morphology colonies (n = 1240, up to 32 bacterial colonies from each water sample were collected, when possible) were subcultured on MacConkey agar plates following an overnight incubation at 37°C and classified as *E. coli* using the PhP-RE microplates of the PhenePlate system (PhPlate, Stockholm, Sweden; http://www.phplate.se) as previously described [10,11]. Isolates with correlations higher than 0.975 to each other were assigned to the same biochemical phenotype (BPT). BPTs with identical isolates were called common (C-BPT) and those with one isolate were called single (S-BPT).

All S-BPTs and at least one isolate representing each C-BPT from each sample were subcultured onto a fresh McConkey agar plate at 37°C and stored in Brain Heart Infusion broth containing 15% (v/v) glycerol at −70°C for further characterization.

Screening for diarrhoeagenic *E. coli* (DEC) by PCR assays

After thawing, the frozen isolates were subcultured on a MacConkey agar plate and incubated at 37°C for 18 h. A smear of each bacterial culture was used as a template for PCR analyses. Screening for DEC was performed by multiplex-PCR as described [12].

Selection of the *E. coli* isolates for antibiotic susceptibility testing

For screening of antibiotic resistance, initially we selected all of the S-BPTs and at least one isolate representing each C-BPT from the results of the biochemical fingerprints of each of the 48 water samples with *E. coli* growth. Then, if the *E. coli* isolate (S-BPT or C-BPT) showed antibiotic resistance to at least one of the tested antimicrobial agents, the rest of the *E. coli* isolates from the corresponding water sample were tested for antibiotic susceptibility (when possible, up to 32 *E. coli* isolates from each water sample where a S-BPT or C-BPT was shown to be antibiotic resistant were tested) (see Table 1).

Antibiotic susceptibility testing

Minimal inhibitory concentrations (MICs) were determined by the agar dilution method for the antibiotics ampicillin (AMP; AstraZeneca, Södertälje, Sweden), amoxicillin-clavulanic acid (AMC; Sigma, Steinheim, Germany and SmithKline Beecham, Washington, DC, USA), cefotaxime (CTX; Sigma), ceftazidime (CAZ; Sigma), ceftriaxone (CRO; Sigma), ciprofloxacin (CIP; Sigma), chloramphenicol (CHL; Sigma), gentamicin (GEN; Sigma), nalidixic acid (NAL; Sigma) and trimethoprim-sulphamethoxazole (STX; Sigma). Phenotypic detection of extended-spectrum β-lactamase (ESBL), by using

TABLE 1. Total *E. coli* isolates from the water samples

Water source	Sample collected (*n*)	Samples where *E. coli* was isolated (*n*)	Samples where antibiotic-resistant *E. coli*[a] was detected	*E. coli* isolates analysed for antibiotic resistance
Tap water	20			
Well water	87	37	8	174
Sewage treatment plant	8	8	7	223
Sewage (hospital)	3	3	3	96
Total	118	48	18	493

[a]Antibiotic-resistant S-BPT or C-BPT *E. coli* isolate.

the Etest® system (Biomérieux, Solna, Sweden), was performed in those *E. coli* isolates that showed resistance to any of the third-generation cephalosporins tested. All of these analyses were carried out according to the Clinical and Laboratory Standards Institute guidelines [13]. *E. coli* ATCC 25922 and *Enterococcus faecalis* ATCC 29212 strains were used as control strains. The data from the antibiotic susceptibility testing were analysed using WHONET 5.4.

bla genes group detection and RAPD analysis

All ESBL-positive *E. coli* strains were screened for the resistance genes encoding bla_{SHV}, bla_{TEM}, $bla_{CTX\text{-}M}$ and bla_{OXA} by a multiplex PCR assay using universal primers following the procedure described by Fang *et al.* [14]. Further detection of CTX-M groups 1, 2, 9, 8 and 25 was performed using a multiplex PCR assay as described by Dallenne *et al.* [15] and a single PCR assay as described by Pitout *et al.* [16]. PCR amplification was carried out on a DNA thermal cycler GeneAmp® PCR system 9700 (Applied Biosystems Division, Foster City, CA, USA).

The epidemiological relationships between *E. coli* isolates producing ESBL from the hospital sewage water samples and from well-water samples were analysed by RAPD as described by Touati *et al.* [17] with some modifications. This analysis was carried out in 22 *E. coli* isolates from the well-water samples and 17 from the hospital sewage water samples. *E. coli* isolates showing the same antibiotic resistance and *bla* genes profile were selected. Total DNA was prepared with the QIAamp® DNA mini Kit (Qiagen, Solna, Sweden) and used for RAPD typing, which was performed using puReTaq Ready-To-Go PCR beads (GE Healthcare, Little Chalfont, Buckinghamshire, UK) together with the following primers: primer 4 (5′-AAGAGCCCGT-3′) and primer 5 (5′-AACGCGCAAC-3′) (Thermo Fisher Scientific, Limburg, Germany). PCR amplification was carried out as follows: one cycle at 94°C for 5 min, 35 (primer 4) and 31 (primer 5) cycles at 94°C for 5 s, 42°C for 30 s and 72°C for 1 min, with a final extension period at 72°C for 5 min. After amplification, the banding pattern of randomly amplified DNA was visualized and analysed on 1.5% agarose gel in Tris-acetate buffer. A negative control was included in each PCR run with no target DNA. Reproducibility of the amplification results was evaluated in parallel experiments by the repetition of the PCR reactions three times. Electrophoresed agarose gels were analysed using the BioNumerics® version 6 software (Applied Maths, Sint-Martens-Latem, Belgium). Dendrograms based on the Jaccard coefficient and unweighted pair group method using arithmetic averages (UPGMA) were generated.

Sequencing analysis

To identify the specific *bla* genes detected in the PCR assays for bla_{SHV}, bla_{TEM}, bla_{OXA} and $bla_{CTX\text{-}M}$, DNA sequence analyses of the amplicons were performed. Based on the RAPD analysis, representative *E. coli* isolates (mainly those *E. coli* isolates harbouring $bla_{CTX\text{-}M}$ plus another type of *bla* gene, i.e. bla_{SHV}) from each clonal group were selected for sequencing analysis. For bla_{SHV}, bla_{TEM} and bla_{OXA}, sequencing primers described by Fang *et al.* [14] were used. For the $bla_{CTX\text{-}M\text{-}1}$ and $bla_{CTX\text{-}M\text{-}9}$ groups sequencing primers described by Pitout *et al.*[16] were used. Amplified PCR products were purified using the QIAquick PCR Purification Kit (Qiagen) and bidirectional sequencing was performed. Each sequence was then compared with the known *bla* genes sequences (http://www.lahey.org/Studies/) by multiple-sequence alignment using the BLAST program.

Results

Screening of diarrhoeagenic *E. coli*s trains

None of the samples was positive for any of the tested diarrhoeagenic virulence markers.

Identification and selection of the *E. coli* isolates for antibiotic susceptibility testing

Antibiotic-resistant *E. coli* isolates (S-BPTs or C-BPT from the biochemical fingerprints) were detected in eight of 87 of the well-water samples, seven of eight sewage water samples from the municipal sedimentation treatment plants and in all

three hospital sewage water samples. No *E. coli* isolates were detected in any of the 20 tap water samples. A total of 493 *E. coli* isolates were included in the study (Table 1), all from the water samples with an antibiotic resistant S-BPT or C-BPT *E. coli*.

Antibiotic susceptibilities in the selected *E. coli* isolates

High levels of antibiotic resistance were mainly found in the *E. coli* isolates from the three samples of hospital sewage water (Table 2). All of the *E. coli* isolates from samples HB1 and HC1 showed resistance to ampicillin, nalidixic acid, ciprofloxacin, trimethoprim-sulphamethoxazole and chloramphenicol but were sensitive to amoxicillin-clavulanic acid, ceftazidime, ceftriaxone, cefotaxime and gentamicin. This finding indicates a very homogenous *E. coli* population in these two samples, probably one multi-resistant strain, a result that was supported by the phenotyping analyses (not shown). In contrast, *E. coli* isolates from sample HA1 showed resistance levels not only to trimethoprim-sulphamethoxazol, chloramphenicol, nalidixic acid and ciprofloxacin but also to the β-lactam drugs (except for amoxicillin-clavulanic acid) and gentamicin.

Likewise, multi-resistance patterns were mostly detected in *E. coli* isolates from the hospital sewage, with resistance to ampicillin, chloramphenicol, ciprofloxacin, nalidixic acid and trimethoprim-sulphamethoxazole as the most common multi-resistance profile in these *E. coli* isolates (67%).

Antibiotic resistance levels in the *E. coli* isolates from the remaining water samples were low or infrequent, except for the well-water sample P55, from which all of the *E. coli* isolates were resistant to all tested antimicrobial agents except for amoxicillin-clavulanic acid. ESBL-producing *E. coli* isolates were detected in 33% of the resistant isolates from the hospital sewage water and in 26% of the resistant isolates from the well-water samples.

***bla* genes group detection and RAPD analysis**

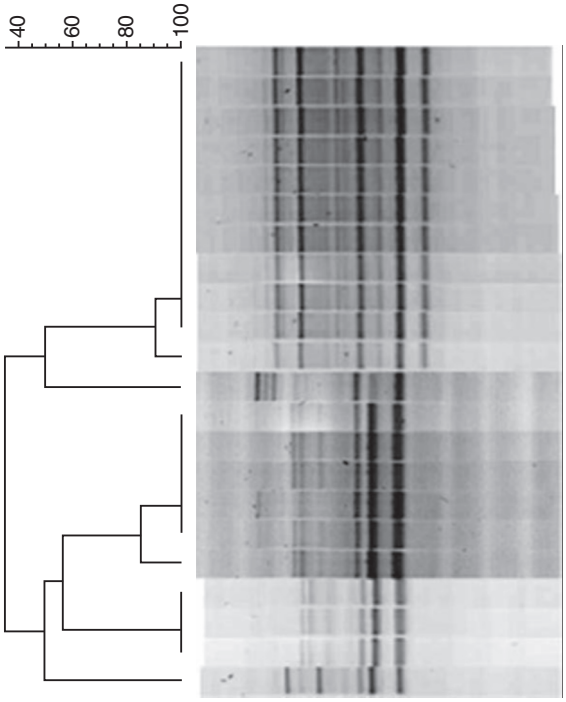
The gene encoding bla_{SHV} was more commonly detected in ESBL-producing *E. coli* isolates from the hospital sewage water (53%) than from the well-water samples (22%). The gene encoding for bla_{TEM} was only detected in ESBL-producing *E. coli* isolates from the hospital sewage water samples (14%). In contrast, the gene encoding for bla_{OXA} was only detected in ESBL-producing *E. coli* isolates from the well-water samples (57%).

PCR amplification *bla* genes showed that the $bla_{CTX\text{-}M\text{-}9}$ group was more prevalent in ESBL-producing *E. coli* isolates from the hospital sewage water samples (65%) than in well-water samples (26%). In contrast, the $bla_{CTX\text{-}M\text{-}1}$ group was more prevalent in ESBL-producing *E. coli* isolates from the

TABLE 2. Percentage of resistance of *E. coli* isolates from distinct water sources to different antibiotics

Sample code	No. *E. coli* isolates tested	Resistance (%)										
		AMP (S ≤ 8 R ≥ 32)	AMC (S ≤ 8 R ≥ 32)	CAZ (S ≤ 4 R ≥ 16)	CRO (S ≤ 1 R ≥ 4)	CTX (S ≤ 1 R ≥ 4)	GEN (S ≤ 4 R ≥ 16)	NAL (S ≤ 16 R ≥ 32)	CIP (S ≤ 1 R ≥ 4)	SXT (S ≤ 2 R ≥ 4)	CHL (S ≤ 8 R ≥ 32)
HA1	32	100		88	100	100	69	9	6	100	97
HB1	32	100						100	100	100	100
HC1	32	100						100	100	100	100
P04	24	17		17	17	17	17	17	17	17	17
P09	24	4		4	4	4		8	4	4	
P10	22	55		32	55	55	9			50	50
P11	16	6.2		13	6	6	6	6	6	6	6
P13	16	13		100	6	6	6	6	6	6	6
P17	24	13		13	13	13	13	13	13	13	13
P37	24	25		29	29	29	29			29	29
P55	24	100		100	100	100	100	100	100	100	100
SIA1[a]	32	9	3							6	
SIA2[b]	32	16								19	
SIB1[a]	31	9.7								7	
SIB2[b]	32	22						19	19		
SUA1[a]	32	69						9		6	
SUA2[b]	32	19						25		13	
SUB2[b]	32	9.4						34	22	69	

H, hospital sewage water samples; P, well-water samples; S, sewage water samples from the municipal sedimentation treatment plants: influent a and effluent b.



Strain code	ESBL type	CTX-M	RAPD-clone
P04-1	CTX-M, OXA	1	P5 ←
P04-4	CTX-M, OXA	1	P5
P04-6	CTXM	1	P5
P04-9	CTXM	1	P5
P11-02	CTXM	1	P5 ←
P13-12	CTXM	1	P5
P17-3	CTX-M, OXA	1	P5
P17-10	CTX-M, OXA	1	P5 ←
P17-24	CTX-M, OXA	1	P5
P55-07	CTXM	1	P5
P55-17	CTX-M, OXA	1	P5
P10-13	SHV, CTX-M	9	SiP4 ←
P10-08	SHV, CTX-M	9	P1
P10-14	SHV, CTX-M	9	P1
P10-16	CTXM	9	P1
P10-18	SHV, CTX-M	9	P1
P10-20	SHV, CTX-M	9	P1 ←
P10-24	SHV, CTX-M	9	P1
P10-01	CTX-M, OXA	9	P2 ←
P10-06	SHV, CTX-M	9	P2 ←
P10-07	SHV, CTX-M	9	P2
PO9-08	TEM, CTX-M, OXA	1	SiP3 ←

FIG. 1. Clonal similarity of *E. coli* from well water by RAPD-PCR. Arrows indicate *E. coli* isolates selected for sequencing analysis.

well-water samples (74%) than in hospital sewage water (34%). The genes encoding for the $bla_{\text{CTX-M-2}}$, $bla_{\text{CTX-M-8}}$ and $bla_{\text{CTX-M-25}}$ groups were not detected in any of the *E. coli* isolates studied.

Twenty-two *E. coli* isolates from the well-water samples (codes P04, P08, P09, P10, P011, P13, P17 and P55) and 17 from the hospital sewage water sample (code HA1) were selected for RAPD analysis. The analysis revealed that the isolates from the well-water samples could be separated into five clones (Fig. 1). Among these clones, P1 and P5 encompass most of the isolates (6 and 11 *E. coli* isolates, respectively). Interestingly, all of the ESBL-producing *E. coli* isolates from clone P1 harboured the gene encoding for the $bla_{\text{CTX-M-9}}$ group and most of the combination of genes encoding for bla_{SVH} and $bla_{\text{CTX-M}}$. In contrast, all of the ESBL-producing *E. coli* isolates from clone P5 harboured the gene encoding for the $bla_{\text{CTX-M-1}}$ group and most of the combination of genes encoding for bla_{TEM} or bla_{OXA} plus $bla_{\text{CTX-M}}$.

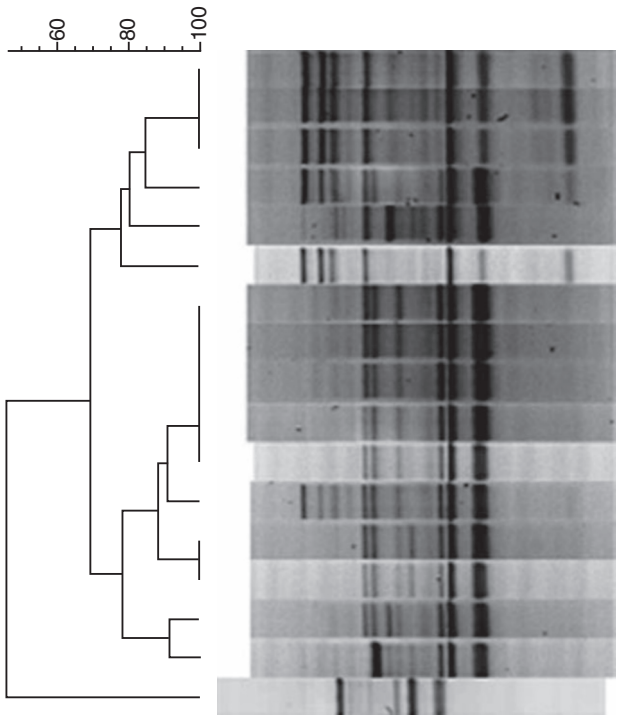
Regarding hospital sewage water samples, the RAPD analysis revealed that 17 resistant *E. coli* isolates from the hospital sewage water sample HA1 could be separated into 11 clones (Fig. 2). Among them, clone H5 encompassed the major number of *E. coli* isolates. All of them harboured the gene encoding for the $bla_{\text{CTX-M-9}}$ group and most of the combination of genes encoding for bla_{SVH} and $bla_{\text{CTX-M}}$. The $bla_{\text{CTX-M-1}}$ group was found mostly in ESBL-producing *E. coli* isolates that belonged to clone H1. The RAPD analysis did not show any clonal similarity between the ESBL-producing *E. coli* isolates from the wells and the hospital sewage water samples.

Sequencing analysis

Based on the RAPD analysis, representative isolates from each clonal group were selected for further analysis by sequencing (mainly those *E. coli* isolates harbouring $bla_{\text{CTX-M}}$ plus another type of *bla* gene, i.e. bla_{SHV}). The selected *E. coli* isolates from the well-water and the hospital sewage water samples are marked by arrows (Figs 1 and 2). After sequencing, it was found that $bla_{\text{SHV-11/-12}}$, $bla_{\text{TEM-1}}$ and $bla_{\text{OXA-1/-30}}$ were present in the *E. coli* isolates positive in the PCR assay for bla_{SHV}, bla_{TEM} or bla_{OXA}. For the $bla_{\text{CTX-M}}$ groups, it was found that the $bla_{\text{CTX-M-15}}$ gene and $bla_{\text{CTX-M-9}}$ gene were harboured in the *E. coli* isolates that were positive in the PCR assay for the $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-9}}$ groups.

Discussion

Many studies have shown the presence of antibiotic-resistant bacteria or genes conferring resistance in the aquatic environment [6,18,19]. Interestingly, this is found even in countries with a high control of the use of antibiotics (e.g. the presence of genes encoding resistance to aminoglycosides, β-lactams and tetracyclines as well as the presence of methicillin-resistant *Staphylococcus aureus* have been found in waste



Strain code	ESBL type	CTX-M	RAPD-clone
HA1-01	TEM, CTX-M	1	H1
HA1-04	TEM, CTX-M	1	H1
HA1-11	TEM, CTX-M	1	H1
HA1-12	SHV, TEM, CTX-M	9	SiH2
HA1-09	CTXM	1	SiH3
HA1-31	TEM, CTX-M	1	SiH4
HA1-03	SHV, CTX-M	9	H5
HA1-05	SHV, TEM, CTX-M	9	H5
HA1-10	SHV, TEM, CTX-M	9	H5
HA1-13	SHV, CTX-M	9	H5
HA1-28	SHV, CTX-M	9	H5
HA1-22	TEM, CTX-M	1, 9	SiH6
HA1-14	SHV, CTX-M	9	H7
HA1-25	SHV, CTX-M	9	H7
HA1-16	SHV, CTX-M	9	H8
HA1-23	CTXM	9	H8
HA1-32	TEM, CTX-M	9	SiH11

FIG. 2. Clonal similarity of *E. coli* from the hospital sewage water by RAPD-PCR. Arrows indicate *E. coli* isolates selected for sequencing analysis.

water environments from Sweden) [20]. In our study, the presence of *E. coli* resistant to at least one of the tested antibiotics was found in 18 of 118 of the environmental water samples collected in León, Nicaragua.

Although many studies have shown the relationship of DEC to diarrhoea in Nicaragua [12,21], we could not detect the presence of DEC in the environmental water samples. However, the presence of antibiotic-resistant *E. coli* strains was frequent. Perhaps a long-term environmental water study covering the diarrhoea season in Nicaragua could show the role of contaminated water in the diarrhoea disease burden in Nicaragua. Ram *et al.* [22–24], in their studies of surface water samples (drinking water, for irrigation, or other purposes) from the Indian rivers, have demonstrated the presence of antibiotic-resistant shiga toxin and enterotoxin producing *E. coli*. The authors considered that such a finding could be an important health concern due to the risk of developing waterborne outbreaks.

In many developing countries the unregulated sale and dispensing of antibiotics is very common [25,26]. Thus, it is important not only to consider the contribution of hospital effluents but also the contribution of the general community to the input of antibiotic-resistant bacteria to the aquatic environment [9]. Our results show that among all of the *E. coli* isolates included in this study, those from the hospital sewage water had higher antibiotic resistance levels to ampicillin (100%), nalidixic acid (70%), ciprofloxacin (69%), chloramphenicol (69%) and trimethoprim-sulphamethoxazole (100%) compared with *E. coli* isolates from other aquatic samples. The similarities in the frequency of antibiotic resistance found in the *E. coli* isolates from hospital sewage samples HB1 and HC1, probably as a result of a dominating strain in both samples, could be due to the fact that those sewage systems collect the water effluent from related hospital units.

Among the well-water samples that represent the contribution of the community to the input of antibiotic-resistant bacteria to the aquatic environment, *E. coli* isolates from well-water sample P55 were resistant to the tested antibiotics, which indicated a high contribution to the spread of multi-antibiotic-resistant bacteria, perhaps due to a high use of antibiotics in those settings. Kümmerer [9] showed that there is a surprisingly high incidence of antibiotic-resistant *E. coli* in rural groundwater, perhaps due to run-off from farms or leakage from septic tanks. It has been shown that improper sanitation (e.g. improper excreta management) can lead to the spread of infectious diseases such as diarrhoea. In Nicaraguan rural areas, as in many developing countries, the use of a household latrine is very common and perhaps the presence of antibiotic-resistant bacteria in well-water samples could be due to improper construction of the latrines and hence leakage to the well water.

Resistance to ampicillin, nalidixic acid, ciprofloxacin and trimethoprim-sulphamethoxazole, though lower, was also found in some of the *E. coli* isolates from the sewage water samples from the municipal sedimentation treatment plants (Table 2). Doung *et al.* [27] showed similar findings in their study of the occurrence of quinolone agents and the number of *E. coli* resistant to quinolones in hospital wastewater in Hanoi, Vietnam. They found higher levels of antibiotic-resistant bacteria in wastewater from hospitals as compared with wastewater treatment plants. It has been reported that resistant bacteria are eliminated quite well in the sewage treatment plants, which could explain the low level of resistance found in our study [9]. However, it is important to consider that there are factors that could have influenced our results, such as dilution effects and the viability of antibiotic-resistant bacteria in the environment.

In previous studies we have reported on the emergence of bacteria producing ESBL causing infections in Nicaraguan children [18,28,29]. In those isolates, the gene encoding for CTX-M was the most commonly detected. In the present study it is shown that 100% of the ESBL-producing *E. coli* isolates from both hospital sewage and the well water of the community encode the genes for $bla_{\text{CTX-M}}$. Genes encoding for bla_{TEM} and bla_{SHV} were only found in the hospital samples (44% and 53%, respectively) and the gene encoding for bla_{OXA} was only detected in the well-water samples (57%). After sequencing of selected *E. coli* isolates (Figs 1 and 2), it was found that $bla_{\text{SHV-11/-12}}$, $bla_{\text{TEM-1}}$ and $bla_{\text{OXA-1/-30}}$ were present in the *E. coli* isolates positive in the PCR assay for bla_{SHV}, bla_{TEM} or bla_{OXA}.

CTX-M has become one of the main public health concerns due to its ability to be involved in nosocomial and community-acquired infections. *E. coli* is most often responsible for producing CTX-M and seems to be a true community ESBL-producing pathogen [30,31]. In the present study, it was found that the $bla_{\text{CTX-M-15}}$ gene and $bla_{\text{CTX-M-9}}$ gene were detected in the ESBL-producing *E. coli* isolates that were positive in the PCR assay for the $bla_{\text{CTX-M-1}}$ group (more prevalent in *E. coli* isolates from the well-water samples) and $bla_{\text{CTX-M-9}}$ group (more prevalent in *E. coli* isolates from the hospital sewage water samples).

RAPD analysis did not show any clonal similarities between the ESBL-producing *E. coli* isolated from the hospital and well-water samples. However, we did find some dominant clones within samples (i.e. clone P5 encompassed most of the ESBL-producing *E. coli* from well-water samples and clone H5 most of the ESBL-producing *E. coli* in the hospital samples). In addition, the carriage of the gene encoding for the $bla_{\text{CTX-M}}$ groups was more common in some clones (i.e. all of the *E. coli* isolates from clone P5 harboured the gene encoding genes for the $bla_{\text{CTX-M-1}}$ group).

Even though we did not perform a longitudinal study of environmental water samples, our results suggest that multi-resistant ESBL-producing *E. coli* were widely spread in hospital sewage water and community water samples.

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Transparency Declaration

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