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Prevalence of pathogenic and antibiotics resistant *Escherichia coli* from effluents of a slaughterhouse and a municipal wastewater treatment plant in Dakar

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The aim of this study was to detect and characterize pathogenic and antibiotic resistant *Escherichia coli* isolated from slaughterhouse wastewater and the effluents of a municipal wastewater treatment plant (WWTP) in Dakar, Senegal. Genetic markers associated with enterohemorrhagic *E. coli* (EHEC) and extraintestinal pathogenic *E. coli* (ExPEC) were screened in 268 *E. coli* isolates from slaughterhouse and 272 *E. coli* isolates from WWTP. Concerning antimicrobial resistance, 40 *E. coli* isolates were studied for each of the two sampling sites. None of the *E. coli* isolates harboring the genes associated with EHEC was detected in WWTP. Of the 13 tested virulence genes, *fuyA* and *f17* were the most prevalent. The pathogenic and resistant *E. coli* was found in the raw wastewater and the influent of slaughterhouse. The final rejection used for urban gardening, including the watering of vegetables does not contain *E. coli*. These results showed that, after treatment, the sanitation risks associated with the reuse water from WWTP were low.

Key words: *Escherichia coli*, wastewater, enterohemorrhagic *E. coli* (EHEC), extraintestinal pathogenic *E. coli* (ExPEC), antimicrobial resistance, slaughterhouse.

INTRODUCTION

Waterborne pathogens are frequently associated with fecal pollution from diverse sources such as wastewater, agricultural fecal wastes and wildlife fecal droppings (Leclerc et al., 2002). Domestic and slaughterhouse wastewater contains important microbiological contaminations. Wastewater treatment has an impact on the microbial quality of surface waters. Most wastewater treatment plants (WWTP) are designed to eliminate organic matter, which allows elimination of pathogens at the same time (Curtis, 2003). *Escherichia coli* is the most used indicator to monitor the microbial quality of water (Wu et al., 2011).

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In Senegal, the use of wastewater for agriculture is becoming more and more widespread, especially in the areas around Dakar, where many programs designed to stimulate and make horticultural activities viable alternative were put in place. It is generally near the wastewater sources, where they are more or less stagnant, and plots are gardened, mainly by either poor inhabitants from peri-urban zones, or rural migrants. Even if they fill the water deficit and inputs (fertilizers), at present, their most common use mode (by spray irrigation) are associated with major health risks.

The main risk associated with reusing this water is one of sanitation, due to the survival of the pathogenic germs which it contains. Since 1982, the *Escherichia coli* strains in particular, the enterohemorrhagic *E. coli* (EHEC) have often been the cause of epidemics diarrhea, cases of hemorrhagic colitis, and of hemolytic uremic syndrome (HUS) (Caprioli *e*t al., 2005). The most recent example is the outbreak of hemolytic uremic syndrome and bloody diarrhea caused by *E. coli* O104:H4 in June 2011 in Germany (Frank et al., 2011). The intestinal pathogenic *E. coli* (InPEC) is a large group of *E. coli* that cause diarrhea, which is sometimes very severe due to the release of toxins. Otherwise, the extraintestinal pathogenic *E. coli* (ExPEC) is harmless when they remain in the gut, but they cause a range of infections elsewhere in the body. They are the biggest single cause of blood poisoning, urinary-tract infections and infectious disease in humans which can lead to serious complications and death (Bonacorsi and Bingen, 2005; Ron, 2006; Wiles et al., 2008). Trying to establish what is causing these rapid increases of pathogenic and antibiotic resistance *E. coli* is now the subject of a major worldwide research effort. The use of livestock waste, sewage sludge and animal processing waste can facilitate the spread of resistant *E. coli* isolates and/or pathogenic bacteria on a large scale in soils and shallow water sources Kabiru et al. (2015). This practice can therefore represent a threat to public health. Currently, knowledge on the behavior and characteristics of pathogenic *E. coli* in the environment, in particular, during the wastewater purification process, is incomplete, and no plan for the surveillance of pathogenic and/or resistant *E. coli* in the environment has been put in place in Senegal.

The objective of the present study was to evaluate the prevalence of EHEC, ExPEC and the antimicrobial resistance of *E. coli* in effluents of a WWTP and slaughterhouse. Pathogenic *E. coli* were further characterized, in order to specify their ability to cause illness in humans, and to allow comparisons between isolates from the different origins.

MATERIALS AND METHODS

Study sites and sampling

Samples were taken from a municipal wastewater treatment plant (WWTP) located in Dakar. The WWTP serviced population equivalent to 2,000,000 and wastewater from a hospital and the raining wastewater were included. In the WWTP, samples were taken from three treatment step: (1) raw wastewater, (2) clarified effluent and (3) the treated effluent. The decontamination of effluent is done by addition of chlorine. Thus, treated water is used in the irrigation of the horticultural productions, the watering of technopole golf course and to provide water to building firms. Ten liters of effluent were collected separately at the three different levels of treatment process.

Slaughterhouse is medium capacity (10 tons daily). It deals mainly on cattle (74% of the annual tonnage), sheep and goat (19% of the annual tonnage) and smaller quantities of pigs (1% of the annual tonnage) with a slaughter room for cattle, a room for sheep and goats and a room for pigs. The average water consumption of the site is approximately 150 m^3 per day. Slaughterhouses are not equipped with a pre-treatment system or wastewater treatment. All slaughter waste (physiological liquid and water for cleaning tasks) are discharged directly into the piping system which opens into the sea through the Bay of Hann. Only the solid portion of the gastric contents is recovered to protect downstream of the items arriving the wholesale elements likely to cause clogging of the pipe system. This part that corresponds to the manure is used for application in vegetable growing.

The effluent samples were taken from the cattle slaughter room. And for better targeting of the period of strong activity of the slaughterhouse, the sample was taken in the morning between 8:00 and 10:00 and a volume of approximately 10 L was collected directly in sterile bottles. All samples were transported to the laboratory in isothermal containers with eutectic plates, and processed upon arrival at the laboratory.

Enumeration and isolation of *E. coli*

Ten liters of the four samples were concentrated stepwise to a final volume of 5 ml by centrifugation. Decimal dilutions of the concentrated samples were then plated onto selective medium for *E. coli* (Petrifilm™ Select *E. coli* count plate, Grosseron, Saint Herblain, France). The plates were incubated at 42°C for 24 h; and bacterial enumeration was performed. For each sample, about 20 Petrifilm™ Select *E. coli* count plates were inoculated with the dilution giving about 20 well isolated colonies. After incubation, isolates were picked for each sample and were grown separately with agitation at 37°C in Luria Bertani broth. They were then stored at -80°C in Luria Bertani broth containing 30% glycerol in 96-well µl plates for further analysis. In total, 540 isolates were saved.

Screening for potentially pathogenic *E. coli*

The collection of 540 isolates was screened for the presence of 3 virulence genes associated with EHEC (*eae, stx*$_1$ and *stx*$_2$), and 10 virulence genes associated with ExPEC (*sfa/focDE, kpsMT* K1*, hlyA, hlyF, fuyA, papEF, afa*/*draBC*, *clbN*, *f17A* and *cnf*) as previously described by Diallo et al. (2013) (Table 1).

Phylogenetic group classification

E. coli isolates positive for at least one virulence gene were tested for phylogenetic groups using multiplex PCR with the *chuA* and *yjaA* genes and the DNA fragment TSPE4. C2 according to the method described by Clermont et al. (2000). Representative *E. coli* Reference Collection strains were used as template control.

Characterization of *stx*- and *eae*- positive *E. coli* isolates

The *eae* - positive *E. coli* were PCR-tested for the presence of *bfpA*

Table 1. Primers used for the detection of virulence genes associated with ExPECs and EHECs.

Pathotype	Target gene	Seauences forward and reverse	Control strains	Size (Pb)	References
ExPEC	*f17A*	GCA GAA AAT TCA ATT TAT CCT TGG CTG ATA AGC GAT GGT GTA ATT AAC	*25KH9*	537	Bertin, 1996
	cnf	CAA TGG CAA CAA AAA TAC CTT GAA CGA CGT TCT TCA TAA GTA TC	*28C*	1147	Yamamoto et al., 1995
	clbN	GTT TTG CTC GCC AGA TAG TCA TTC CAG TTC GGG TAT GTG TGG AAG G	*IHE3034*	733	Johnson et al., 2008a
	papEF	GCA ACA GCA ACG CTG GTT GCA TCA T AGA GAG AGC CAC TCT TAT ACG GAC A	*J96*	336	Yamamoto et al., 1995
	afa/draBC	GGC AGA GGG CCG GCA ACA GGC CCC GTA ACG CGC CAG CAT CTC	*A30*	559	Johnson and Stell, 2000
	kpsMT (K1)	TAG CAA ACG TTC TAT TGG TGC CAT CCA GAC GAT AAG CAT GAG CA	*SP15*	153	Johnson and Stell, 2000
	hlyA	AAC AAG GAT AAG CAC TGT TCT GGC T ACC ATA TAA GCG GTC ATT CCC GTC A	*J96*	1177	Johnson and Stell, 2000
	sfa/focDE	CTC CGG AGA ACT GGG TGC ATC TTA C CGG AGG AGT AAT TAC AAA CCT GGC A	*J96*	410	Yamamoto et al., 1995
	FyuA[a]	TGA TTA ACC CCG CGA CGG GAA CGC AGT AGG CAC GAT GTT GTA	*J96*	880	Johnson and Stell, 2000
	HlyF[a]	TCG TTT AGG GTG CTT ACC TTC AAC TTT GGC GGT TTA GGC ATT CC	*SP15*	444	Moulin-Schouleur et al., 2007
EHEC	eae	AGGCTTCGTCACAGTTG CCATCGTCACCAGAGGA	*Sakaï* (RIMD 0509952)	570	China et al., 1996
	SLT1	AGAGCGATGTTACGGTTTG TTGCCCCCAGAGTGGATG		388	
	SLTII	TGGGTTTTTCTTCGGTATC GACATTCTGGTTGACTATCTT		807	

and EPEC adherence factor (EAF) plasmid as already described (Franke et al., 1994; Gunzburg et al., 1995). E2348/69 was used as control strain (Levine et al., 1985). Based on PCR results, *E. coli* isolates were classified as Shiga toxin-producing *E. coli* (STEC) or atypical EPEC. *E. coli* isolates positive for *stx1* and/or *stx2* genes were classified as STEC, isolates positive for *eae* gene, and negative for stx1, stx2, bfpA and EPEC adherence factor (EAF) plasmid were classified as atypical EPEC (Trabulsi et al., 2002). The five major serotypes associated with EHEC (O157:H7, O26:H11, O145:H28, O111:H8 and O103:H2) were investigated in STEC and potential EPEC isolates, by real-time PCR with conditions and control strains described elsewhere (Madic et al., 2011; Perelle et al., 2004, 2005).

Antimicrobial susceptibility testing

A subcollection of 80 isolates of *E. coli* including 40 isolates from slaughterhouse wastewater and 40 isolates from city wastewater and WWTP were chosen ramdomly and subjected to antimicrobial susceptibility testing. *E. coli* isolates positive to one EHEC- or EXPEC-associated genetic marker were also tested. Antimicrobial susceptibility tests were performed by using a disk diffusion method according to the CLSI standards (CLSI, 2009a) on Mueller-Hinton agar (Bio-Rad Laboratories). *E. coli* ATCC 25922 was used as the control strain. The 16 antibiotic disks (Bio-Rad Laboratories) used in this study were ampicillin (10 µg), amoxicillin (20 µg) with clavulanic acid (10 µg), cephalothin (30 µg), ceftazidime (30 µg), cefotaxime (30 µg), cefepime (30 µg), cefuroxime (30 µg), streptomycin (10µg), gentamicin (10 µg), kanamycin (30 µg), nalidixic acid (30 µg), ciprofloxacin (5 µg), tetracycline (30 µg), chloramphenicol (30 µg), trimethoprim (5 µg), sulfonamides (300 µg). The susceptibility breakpoints for all antimicrobials were those recommended by CLSI (CLSI, 2008; 2009b). Isolates were classified as multiresistant when they exhibited resistance to three or more classes of antimicrobial agents (Schwarz et al., 2010).

Statistical analysis

A Pearson's Chi-squared test was used to test if antimicrobial resistant were evenly distributed among sampling points. The statistical significance was set at a P value <0.05. These analyses were performed using the statistical R.2.13.0.

RESULTS

E. coli counts

Regarding urban WWTP, raw wastewater and clarified effluent contained 1.5×10^6 CFU/mL, and 1.1×10^2 CFU/mL *E. coli*, respectively. After treatment, the final rejection used for urban gardening, including the watering of vegetables contained no *E. coli*. Slaughterhouse

Table 2. Prevalence of virulence genes in city wastewater and slaughterhouse wastewater.

Virulence genes	Slaughterhouse wastewater (n= 268)	WWTP		Total (n=540)
		Raw (n= 183)	clarified (n=89)	
ExPEC-associated genetic markers				
papEF	0% (0)	0.5% (1)	0% (0)	0.1% (1)
clbN	0.37% (1)	1.0% (2)	0% (0)	0.5% (3)
kpsMTK1	0% (0)	0% (0)	0% (0)	0% (0)
sfa/focDE	0% (0)	0% (0)	0% (0)	0% (0)
hlyF	0.37% (1)	0.5% (1)	0% (0)	0.3% (2)
f17A	0% (0)	0% (0)	0% (0)	0% (0)
f17a	0% (0)	0% (0)	0% (0)	0% (0)
f17c-A	0% (0)	0.5% (1)	0% (0)	0.1% (1)
f111	0% (0)	2.1% (4)	0% (0)	0.7% (4)
afa/draBC	0% (0)	0% (0)	1.1% (1)	0.1% (1)
cnfx	1.1% (3)	0% (0)	0% (0)	0.5% (3)
fuyA	1.4% (4)	0.5% (1)	1.1% (1)	1.1% (6)
EHEC-associated genetic markers				
eae	0.7% (2)	0% (0)	0% (0)	0.3% (2)
stx1	0.7% (2)	0% (0)	0% (0)	0.3% (2)
stx2	0.7% (2)	0% (0)	0% (0)	0.3% (2)

wastewater contained 3×10^6 CFU/mL *E. coli* that were discharged into the environment without treatment.

Prevalence and characteristics of ExPEC in WWTP

The collection of isolates was screened for ExPEC associated genetic markers (*papEF, sfa*/*focDE afa/draBC*, *fuyA*, *cnfx*, *f17A, hlyA, hlyF, clbN* and *kpsMT* K1). One of these genes was detected in 13 of the 540 tested isolates: 9 in city wastewater and 4 in slaughterhouse (Table 4). The most prevalent gene was *fuyA* (1.1%) followed by *f17* (0.9%), *clbN* (0.5%), *cnfx* (0.5%), *hlyF* 0.3%), *papEF* (0.1%) and *afa/dra* (0.1%) (Table 2). The *f17* gene was only detected in city wastewater strains, whereas *cnfx* was only detected in strains isolated from the effluent from the slaughterhouse. The genes *kpsMT K1* and *sfa/focDE* were absent on the entire collection. A weak association of genes was noted. The majority of these strains was assigned to group B1 (9 strains) following by B2 (2 strains), D (1 strain) and A1 (1 strain). It is interesting to note that the strains belonging to group B2 and D were found in the WWTP. Except one strain which was susceptible to the antibiotics, the strains from WWTP carrying gene associated with ExPEC were resistant at least to one antibiotic.

Prevalence and characteristics of STEC and atypical EPEC

EHEC associated genetic markers (*stx1, stx2* and *eae* genes) were screened in the collection of the 540 isolates. Four isolates including two *eae*-positive *E. coli* isolates, and two *stx*-positive isolates (STEC) were detected in the slaughterhouse wastewater. The two STEC from slaughterhouse carried both *stx1* and *stx2* genes (Table 4). Any isolate harboring both *eae* and *stx* gene was isolated. The two *eae*-positive were negative for *bfpA* and EPEC adherence factor (EAF) plasmid, which justifies their classification as atypical EPEC (Kaper et al., 2004). In order to specify the ability to cause illness in humans, these 4 isolates were better characterized. The specific detection, by real time PCR, showed none of the 4 isolates was assigned to the five major serotypes frequently associated with EHEC (O157, O26, O145, O103 and O111). In additional, the strains harboring gene associated with EHEC were allocated in groups B1. The STEC and atypical EPEC strain detected from the slaughterhouse were susceptible to all tested antibiotics.

Prevalence of antimicrobial resistant *E. coli*

Antimicrobial resistance to all the antimicrobials tested and multiresistance were detected more frequently in isolates from WWTP. Ampicillin, amoxicillin + clavulanic acid, cephalotin, streptomycin, tetracycline and trimethoprim resistances were observed in isolates from all origins (Table 3). Acid nalidixic, chloramphenicol, ciprofloxacin, sulfonamide and cefepime resistances were only observed in WWTP. Gentamicin, cefotaxime, ceftazidime and cefuroxime resistances were not

Table 3. Prevalence (%) of resistant strains by antibiotics observed within each sampling points.

Antibiotics family	Antibiotics[a]	Location		p-value[b]
		Slaughterhouse (n=40)	WWTP (n=40)	
	CTX	0 (0)	0 (0)	NS
	AMC	2 (5)	5 (12.5)	NS
	CAZ	0 (0)	0 (0)	NS
β-Lactam	AM	6 (15)	17 (42.5)	< 0.05
	CEF	2 (5)	5 (12.5)	NS
	CXM	0 (0)	0 (0)	NS
	FEP	0 (0)	1 (2.5)	NS
	GEN	0 (0)	0 (0)	NS
Aminosides	KAN	1 (2.5)	0 (0)	NS
	STR	1 (2.5)	9 (22.5)	< 0.05
Tétracycline	TE	1 (2.5)	17 (42.5)	< 0.005
Phenicol	C	0 (0)	1 (2.5)	NS
Sulfonamide	SSS	0 (0)	14 (35)	< 0.005
	TMP	1 (2.5)	13 (32.5)	< 0.005
Fluoroquinolones	CIP	0 (0)	1 (2.5)	NS
Quinolones	NA	0 (0)	5 (12.5)	< 0.05

[a]AM, ampicillin; AMC, amoxicillin clavinic acid ; CEF, cephalothin; CXM, cefuroxime; CTX, cefotaxime; CAZ, ceftazidime ;FEP, cefepime ; KAN, kanamycin ; STR, streptomycin; C, chloramphenicol; NA, nalidixic acid; CIP, ciprofloxacin; SSS, sulfonamide, TMP, trimethoprim; TET, tetracycline; CIP, ciprofloxacin. [b] NS no significant.

Table 4. Characterization of *E. coli* isolates harboring gene associated with ExPEC and EHEC.

Location	Strain code	API 20E code	Combination of virulence genes	Resistance phenotype[a]	Phylogenetic groups
	SN01	5144572	stx1/stx2	S	B1
	SN02	5144572	tx1/stx2	S	B1
Slaughterhouse	SN03	5044573	eae	S	B1
	SN04	5144572	eae	S	B1
	SN05	5144572	Cnfx/fuyA	S	B1
	SN06	5144572	Cnfx/fuyA	S	B1
	SN07	5144572	clbN/hlyF/fuyA	S	B1
	SN08	5144172	Cnfx/fuyA	S	B1
	SN09	5144572	f111	TE	B1
	SN10	5144572	f111	TE	B1
WWTP	SN11	5144572	f111	TE	B1
	SN12	5144572	f111	TE	B1
	SN13	5144532	clbN/papEF/fuyA	TE/C/SSS/TMP	B2
	SN14	5144532	clbN	TE/C/SSS/TMP	B2
	SN15	7044572	hlyf	SSS/TMP	D
	SN16	5144572	f17c/f111	S	B1
	SN17	5144532	afa/dra/fuyA	AM/TE/TMP	A

[a]AM, ampicillin; C, chloramphenicol; NA, nalidixic acid; SSS, sulfonamides, TMP, trimethoprim; TE, tetracycline; S, susceptible to all tested antibiotics.

detected from all collection. The resistance frequencies towards the tested antimicrobials varied among the different origins (0 to 50%). By comparing the sampling sites, the largest number of resistant strains was found in

isolates from raw wastewater and clarified effluent. Among isolates from WWTP, antimicrobial resistance frequencies were highest for tetracycline, ampicillin, sulfonamide, trimethoprim and streptomycin.

The multi-resistant strains were observed mostly at the urban sewage treatment plant with 32.6% of strains resistant to at least four antibiotics. Whereas, concerning the number of strains resistant to two and three antibiotics, no significant difference was noted between slaughterhouse and WWTP (Table 3).

DISCUSSION

The first objective of this study was to evaluate the proportion of *E. coli* contamination of raw wastewater, effluents slaughterhouse and the final rejection. The results did not show a significant difference between the load of *E. coli* in slaughterhouse effluents and human sewage. Moreover, the same results were found in several studies conducted both on the effluent from slaughterhouses and the effluent of human origin (Czajkowska et al., 2008) and also on hospital effluents (Tsai et al., 1998). In contrast, the *E. coli* strains were absent after treatment of effluent at the WWTP. These results are opposed to those obtained in several studies conducted in France and elsewhere (Vernozy-Rozand et al., 2002; Loukiadis et al., 2006). This difference could be justified by the various methods of wastewater treatment used in a wastewater treatment plant and the final destination of the treated water. The wastewater treatment is done by chlorine at the WWTP. This water is reused for irrigation of vegetable crops in the peri-urban area of Dakar and also by building construction companies.

The same result were obtained with sludge dehydrated and stabilized with lime (Reinthaler et al., 2010). The treatment had eliminated the *E. coli* strains in the effluent, meaning that raw sewage and slaughterhouse effluents characteristics that are without treatment may play an important role in the dissemination of *E. coli* in the environment (Kabiru et al., 2015). The Dakar slaughterhouse effluents are discharged directly without treatment in the sea at the bay of Hann which is a swimming area. The authors detected pathogenic and resistant *E. coli* in the raw wastewater before treatment and the effluent of slaughterhouse, indicating a potential risk for the microbiological pollution of water. The absence of eae-positive and stx-positive strains in human effluent is not surprising. Domestic ruminants (especially cattle) are considered to be the main reservoir of EHEC strains for human infection (Hancock et al., 2001; Mainil and Daube, 2005). The two *eae*-positive isolates identified in the present study are ‘atypical EPEC’ or a-EPEC, because they do not harbour any *bfpA* gene. This is in full agreement with the observation that *Bfp* is only produced in human strains, with the exception of a few strains from dogs and cats (Goffaux et al., 2000; Chen and Frankel, 2005). Other studies showed the presence of *eae*-positive *E. coli* isolates in effluents from human and animal origin (Holler et al., 1999; Vernozy-Rozand et al., 2004; Garcia-Aljaro, 2005; Awais et al., 2007; Ayaz et al., 2014). Loukiadis et al. (2006) detected 54 *eae*-positive *E. coli* isolates among 5,001 isolates from various effluents from 12 slaughterhouses in France. Here, it was confirmed that the prevalence of *eae*-positive *E. coli* isolates in effluents was very low.

In order to investigate the prevalence of ExPEC, ten genetic markers associated with ExPEC was screened in the collection of 540 isolates. The results showed that ExPEC associated genetic markers were detected in the human and animal effluents. This is in agreement with other studies showing that *E. coli* strains with uropathogenic virulence characteristics were present in sewage of human (Muhldorfer et al., 1996; Boczek et al., 2007; Anastasi et al., 2010; Diallo et al., 2013) and animal origin (Sabate et al., 2008). Besides *fuyA* gene which encodes the iron uptake system, *f17* related gene was the most prevalent. All strains carrying *f17* genes were detected in human effluent. This finding is surprising. The F17 – Fimbriated *E. coli* are very common in cattle populations. This family of the fimbriae related F17 is frequently isolated from clinical cases of mastitis in cattle. For instance, in the Netherlands, 55% of isolates from mastitic milk expressed FI7-related fimbriae (Nemeth et al., 1994; Lipman et al., 1995). The *E. coli* strains harboring F17 gene were isolated from raw wastewater and had the same profile. Further characterization of the isolates carrying virulence genes associated with ExPEC majority were classified as Group B1. This could be explained by a low rate of association of genes observed with the strains. Two strains were grouped as B2, one expressed the genes *clbN* / *papEF*/*fuya* and the second one expressed the *clbN*. The *clbN* gene is a marker for the 3’ region of the *pks* island, encoding colibactin that induces DNA double-strand breaks in eukaryotic cells, and *pks* island was shown to be significantly associated with bacteremia (Nougayrede et al., 2006; Johnson et al., 2008b). Previous epidemiological studies have shown that *E. coli* isolated from extra intestinal infections harbored many virulence genes and belong mostly to phylogenetic group B2 and, to a lesser extent, group D (Bingen et al., 1998; Boyd and Hartl, 1998; Picard et al., 1999; Johnson and Russo, 2005). Strains carrying virulence genes associated with ExPEC detected in this study cannot be considered as potential ExPEC strains. However, these strains remain a danger to public health due to their rapid growth and plasticity of their genomes.

One the other hand, the study shows that human effluents had the higher number of resistant strains than slaughterhouse (23/40 versus 7/40). This result shows that the use of antibiotics in animals is not yet widespread. Most of the slaughtered cattle come from

traditional livestock farming characterized by low utilization of veterinary inputs, which is not the case in the human sector. These results contrast with those obtained by Martel et al. (1981), in France who observed very high resistance levels that exceeded 50% for all strains of *E. coli* bovine studied towards the ampicillin, streptomycin, kanamycin, chloramphenicol, tetracycline and sulfonamides (Martel et al., 1981). The 15 strains of *E. coli* (37.5%) of the urban sewage were resistant to more than 2 antibiotics and resistance profile of the most common resistance profile was the association among ampicillin, streptomycin and tetracycline. The results confirm those obtained by Holzel et al. (2010) where was *E. coli* from sewage sludge, had the most frequent resistance to streptomycin (56.5%), followed by doxycycline (54.7%) and ampicillin (19.8%). In addition, the results are consistent with those obtained by Schroeder et al. (2002) who showed that 75% of *E. coli* strains resistant to ampicillin were also resistant to streptomycin and tetracycline.

The present study shows the importance of monitoring samples collected in the environment for the presence of virulence and resistant microorganisms. It was shown that the raw wastewater is a reservoir for a variety of virulence factors associated with ExPEC and EHEC, and that the treatment of effluents is very important. For preventative reasons, treatment of effluent before use by gardener should be given preference. Raw wastewater without effective treatment to eliminate pathogenic *E. coli* should no longer to be used in irrigating vegetables.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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