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Original Article

Characteristics of ciprofloxacin-resistant Enterobacteriaceae isolates recovered from wastewater of an Algerian hospital

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Abstract

Introduction: Hospital effluents are a source of environmental pollution by drugs, antibiotic-resistant bacteria, and resistance genes. Quinolones, particularly ciprofloxacin, are commonly detected in these effluents, contributing to the emergence of antimicrobial resistance. The objective of this study was to characterize ciprofloxacin-resistant Enterobacteriaceae in hospital effluents.

Methodology: Isolates were selected on Tergitol-7 agar supplemented with ciprofloxacin and genotyped by ERIC-PCR. Antibiotic susceptibility testing was done using the disk diffusion method, and minimum inhibitory concentrations were determined using the agar dilution method. Resistance genes, integrons, phylogenetic groups, and sequence types were identified by PCR and sequencing.

Results: A total of 17 ciprofloxacin-resistant isolates were characterized: *Escherichia coli*, *Escherichia vulneris*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Citrobacter freundii*, and *Citrobacter koseri/farmeri*. Isolates presented concomitant resistance to nalidixic acid, ciprofloxacin, ofloxacin, and pefloxacin. A diversity in mutation patterns in *gyrA* and *parC* genes and new amino-acid substitutions in GyrA subunit were observed. Quinolone plasmidic resistance genes *qnrB1*, *qnrB2*, *qnrB5/19*, *qnrS1*, and *aac(6')-Ib-cr* were detected. Resistance to other antibiotic classes was observed. Class 1 integrons and resistance genes $bla_{\text{CTX-M-15}}$, $bla_{\text{OXA-1}}$, *sul1*, *sul2*, *sul3*, *tetA*, *tetB*, *aadA1/2*, *aadA5*, *aph(3')-Ia*, *aac(3)II*, *dfrA1*, *dfrA5*, *dfrA7*, and *dfrA12* were detected. Bacterial tolerance to cadmium, zinc, and mercury was observed with the presence of the *merA* gene. *E. coli* isolates belonged to phylogenetic groups A, B1, and D and to sequence types ST405, ST443, ST101, ST10, and ST347.

Conclusions: This study highlighted bacterial multidrug resistance linked to ciprofloxacin and, consequently, the risk of bacterial exposure to this antibiotic.

Key words: quinolones; ciprofloxacin; heavy metals; resistance; hospital effluents; Enterobacteriaceae.

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Introduction

The rapid development of antibiotic resistance is worrying as it affects broad-spectrum molecules; we are facing an absence of new effective molecules. Quinolones are a major class of antibiotics commonly used in a wide range of infections in both communities and hospitals. Quinolone bacterial resistance occurs mainly by successive mutations in chromosomal genes of quinolone targets that are DNA gyrase and topoisomerase IV, mainly in their subunits GyrA and ParC, respectively. In addition, three plasmidic resistance gene classes have been described, which encode proteins of protection of the target (*qnr*), acetylation of antibiotic (*aac(6')Ib-cr*), and specific efflux (*qepA* and *oqxAB*). A worldwide constant increase in bacterial resistance to quinolones was observed during the last decade, inherent to the massive use of antibiotics in human therapy and in intensive livestock, particularly poultry, in some countries [1,2]. Hospital effluents discharged into the sewer system, usually without pre-treatment, have been reported to be an influent source of antimicrobial residues and antibiotic-resistant bacteria to the wastewater treatment plant (WWTP) [3]. Furthermore, resistant bacteria and resistance genes are often not completely removed in WWTPs; therefore, they can spread and reach humans through natural surface waters and the food chain [4,5]. Quinolones are excreted in their biologically active form and released in aquatic environments, especially hospital effluents, where they are stable and may impact bacterial antibiotic resistance [3,4]. Quinolones, particularly ciprofloxacin, are distinguished by the cross-resistance between them and with non-quinolone antimicrobials [6,7], hence the interest of the characterization of quinolone resistance selected by the complex environment of hospital effluents, more so as

very few data on antibiotic resistance in non-clinical environments are available in Algeria. For this purpose, we proposed, in this study, to characterize the ciprofloxacin resistance in Enterobacteriaceae isolates collected from the wastewater of a hospital in Algiers. Ciprofloxacin is the only quinolone prescribed in Algeria, the one prescribed most widely in the world, and also the one most frequently detected in surface waters, especially in hospital effluents [1,3,4].

Methodology

Isolation, identification, and genotyping of bacterial isolates

Wastewater samples were collected three times on the same day in March 2010 from the final collector of effluents of Zemirli hospital located in El Harrach, a district east of Algiers. The hospital has a capacity of 281 beds and includes the following medical units: internal medicine, general surgery, orthopedic surgery, neurosurgery, intensive care, medical and surgical emergencies, forensic medicine, laboratories (microbiology, biochemistry, and anatomo-cytopathology), blood transfusion, and medical imaging. Support services include kitchens, laundry, and a maintenance workshop. Samples were pooled and 100 mL volumes of the homogenate and serial tenfold dilutions were filtered on cellulose nitrate membranes of 0.45 µm-pore-size (Millipore, Molsheim, France), and filters were placed onto Tergitol-7 agar (Merck, Darmstadt, Germany) supplemented with ciprofloxacin (1 µg/mL). After incubation at 37°C for 24 hours, ciprofloxacin-resistant isolates were identified by the API 20E system (bioMérieux, Marcy l'Étoile, France) and genotyped by enterobacterial repetitive intergenic consensus polymerase chain reaction (ERIC-PCR) using primer ERIC2 [8]. Fingerprints were visually compared; strains dissimilar by one band or more were considered to be different.

Antibiotic and heavy metal resistance testing

Antibiotic resistance profiles of ciprofloxacin-resistant isolates were determined using the disk diffusion method on Mueller-Hinton agar according to the recommendations of the Antibiogram Committee of the French Society for Microbiology (CA-SFM) (www.sfmmicrobiologie.org). Antibiotics disks were purchased from Bio-Rad, Marnes la Coquette, France. Cefotaxime-resistant isolates were screened for extended-spectrum beta-lactamase (ESBL) production by the double-disk synergy test (DDST) [9]. The minimal inhibitory concentrations (MICs) of ciprofloxacin were determined using the agar-dilution method and interpreted according to the guidelines of CA-SFM (www.sfmmicrobiologie.org). *Escherichia coli* ATCC 25922 was used as a control strain for antimicrobial susceptibility testing. MICs of five heavy metals were determined using the agar dilution method. The experimental plates were prepared by supplementing Muller-Hinton medium with following metal concentrations: 100, 200, 400, 800, 1,600, and 3,200 µg/mL for Cu^{2+} ($CuCl_2$) and Zn^{2+} ($ZnCl_2$); 400, 800, 1,600, 2,400 and 3,200 µg/mL for Pb^{2+} ($Pb(NO3)_2$); 12.5, 25, 50, 100, 200, 400, and 800 µg/mL for Cd^{2+} ($Cd(NO3)_2$.4H2O); 2.7, 13.57, 27.15, and 54.3 µg/mL for Hg^{2+} ($HgCl_2$). MIC values indicative of metal tolerance were 200 µg/mL for Cd^{2+}, 1,600 µg/mL for Zn^{2+}, 3,200 µg/mL for Cu^{2+} and Pb^{2+} [10], and 54.3 µg/mL for Hg^{2+} [11].

Detection of antibiotic resistance genes and integrons

Amplification of quinolone resistance-determining regions (QRDRs) of *gyrA* and *parC* genes was done by PCR as previously described [12,13]. Simplex and multiplex PCR were used to screen for the following resistance genes: plasmid-mediated quinolone resistance (PMQR) genes *qnrA*, *qnrB*, *qnrS*, *qnrC*, *qnrD*, *aac-(6')-Ib*, and *qepA* [14]; beta-lactamases bla_{OXA-1}, bla_{TEM}, and bla_{CTX-M} [8,15]; tetracycline efflux pumps *tetA*, *tetB*, *tetC*, *tetD*, and *tetE* [15]; dihydropteroate synthases *sul1*, *sul2*, and *sul3* [15]; dihydofolate reductase gene clusters *dfrA1*, *dfrA5*, *dfrA7*, *dfrA8*, and *dfrA12* [16]; aminoglycosides adenyltransferases, acetyltransferases and phosphotransferases *aadA1/2*, *aadA5*, *aac(3)II*, *aph(3')-Ia*, *aph(3')-IIa* [15]; and mercuric reductase *merA* [17]. The screening for class 1 integrons was done by PCR targeting the *int1* gene [8]. PCR-obtained products of *gyrA*, *parC*, PMQR, and bla_{CTX-M} genes were sequenced and analyzed with the BLAST and FASTA programs of the National Center for Biotechnology Information (www.ncbi.nlm.nhi.gov).

Phylogenetic grouping and multilocus sequence typing of E. coli isolates

Phylogenetic group determination was based on PCR detection of *chuA* and *yjaA* genes, and anonymous DNA fragment TspE4.C2, as previously described [18]. Multilocus sequence typing (MLST) was performed according to Mark Achtman's MLST scheme based on PCR amplification and sequencing of set of seven housekeeping genes (*adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, and *recA*); sequence types (ST) were identified using the *E. coli* MLST database website (http://mlst.ucc.ie/mlst/dbs/Ecoli).

Table 1. Antimicrobial resistance profiles, resistance genes, phylogenetic groups, and sequence types of ciprofloxacin-resistant *Enterobacteriaceae* isolates from hospital effluents.

Isolates	ERIC profile	MICs of CIP (µg/mL)	Antimicrobial resistance profiles	QRDR mutations		Antimicrobial resistance genes	Class 1 integrons	Phylogenetic group	ST
				GyrA	ParC				
Ec 13	Pc1	8	CIP^R PEF^R OFX^R NA^R TE^R SSS^R SXT^R Hg^R Cd^R Zn^R	S83L	S80I	*qnrB 5/19*, *aadA1/2*, *dfrA1*, *merA*	*int 1*	A	ST347
Ec 70	Pc2	32	CIP^R PEF^R OFX^R NA^R TE^R Cd^R Zn^R	S83L, D87N	S80I	*tetA*	-	A	ND
Ec 108	Pc3	32	CIP^R PEF^R OFX^R NA^R TE^R SSS^R SXT^R Cd^R Zn^R	S83L, D87N	S80I	*aadA1/2*, *tetA*, *sul1*, *sul2*, *dfrA12*	-	A	ND
Ec 3	Pc4	32	CIP^R PEF^R OFX^R NA^R K^R TE^R SXT^R SSS^R Hg^R Cd^R Zn^R	S83L, D87N	S80I, E84G	*aadA1/2*, *aph(3')Ia*, *tetA*, *tetB*, *sul2*, *sul3*, *dfrA5*, *merA*	*int 1*	A	ND
Ec 12	Pc5	8	CIP^R PEF^R OFX^R NA^R TE^R SSS^R SXT^R Hg^R Cd^R Zn^R	S83L, D87N	S80I	*sul2*, *dfrA5*, *merA*	*int 1*	B1	ST101
Ec 4	Pc6	64	CIP^R PEF^R OFX^R NA^R CTX^R CAZ^R CRO^R CPO^R ATM^R K^R TE^R SSS^R SXT^R Hg^R Cd^R Zn^R	S83L, D87N	S80I, L99M	*CTX-M-15*, *aph(3')Ia*, *tetA*, *tetB*, *sul2*, *dfrA5*, *merA*	-	D	ST405
Ec 65	Pc7	32	CIP^R PEF^R OFX^R NA^R TE^R SSS^R SXT^R Hg^R Cd^R Zn^R	S83L, D87N	S80I	*tetB*, *dfrA5*, *merA*	-	B1	ST443
Ec 42	Pc8	8	CIP^R PEF^R OFX^R NA^R TE^R SSS^R SXT^R Hg^R Cd^R Zn^R	S83L, D87N	S80I	*tetA*, *sul2*, *dfrA1*, *dfrA5*, *merA*	*int 1*	B1	ST101
Ec 68	Pc9	32	CIP^R PEF^R OFX^R NA^R Cd^R Zn^R	S83L, D87N	S80I	-	*int 1*	A	ND
Ec 107	Pc10	32	CIP^R PEF^R OFX^R NA^R TE^R SSS^R SXT^R Hg^R Cd^R Zn^R	S83L	S80R	*qnrS1*, *aadA1/2*, *tetA*, *sul1*, *sul2*, *dfrA5*, *dfrA12*, *merA*	*int 1*	A	ST10
Ec 24	Pc11	128	CIP^R PEF^R OFX^R NA^R K^R TE^R SSS^R SXT^R Hg^R Cd^R Zn^R	S83L, D87N	S80I	*aph(3')Ia*, *aadA5*, *tetB*, *sul2*, *dfrA7*, *merA*	*int 1*	A	ST10
Ec 72	Pc12	128	CIP^R PEF^R OFX^R NA^R CTX^R CAZ^R CRO^R K^R TE^R SSS^R SXT^R Hg^R Cd^R Zn^R	S83L, D87N	S80I	*CTXM-15*, *aph(3')Ia*, *tetB*, *sul1*, *sul2*, *dfrA5*, *merA*	*int 1*	D	ST405
Ev 1	Pv1	16	CIP^R PEF^R OFX^R NA^R K^R TE^R SSS^R Hg^R Cd^R Zn^R	S83L, D87N	S80I	*aph(3')Ia*, *tetB*, *sul2*, *merA*	-		ND
Kp 2	Pk 1	32	CIP^R PEF^R OFX^R NA^R CTX^R CAZ^R CRO^R CPO^R FEP^R ATM^R GM^R TE^R SSS^R SXT^R Hg^R Cd^R Zn^R	S83F, D87A	S80I	*aac(6')Ib-cr*, *CTXM-15*, *oxa-1*, *aac(3)II*, *tetA*, *sul2*, *dfrA5*, *merA*	*int 1*		ND
Ko 50	Po 1	32	CIP^R PEF^R OFX^R NA^R CTX^R CRO^R GM^R TE^R SSS^R SXT^R Hg^R Cd^R Zn^R	T83I	S80I, M157L	*qnrB1*, *aac(6')Ib-cr*, *CTXM-15*, *oxa-1*, *aac(3)II*, *tetA*, *sul2*, *dfrA5*, *merA*	*int 1*		ND
Cf 20	Pf1	16	CIP^R PEF^R OFX^R NA^R CTX^R CAZ^R CRO^R TE^R SXT^R SSS^R Hg^R Cd^R	T83I	S80I, N111D	*qnrB2*, *aadA1/2*, *sul1*, *sul2*, *dfrA1*, *merA*	-		ND
Cfk 44	Pfk1	8	CIP^R PEF^R OFX^R NA^R TE^R SSS^R SXT^R Hg^R Cd^R Zn^R	T83L, D87N	S80I, N111D	*tetA*, *sul2*, *dfrA5*, *merA*	*int 1*		ND

QRDR: quinolone resistance-determining region; Ec: *E. coli*; Ev: *E. vulneris*; Kp: *K. pneumoniae*; Ko: *K. oxytoca*; Cf: *C. freundii*; Cfk: *C. farmeri/Koseri* ; ERIC: enterobacterial repetitive intergenic consensus; MIC: minimal inhibitory concentration; CIP: ciprofloxacin; PEF: pefloxacin; OFX: ofloxacin; NA: nalidixic acid; CTX: cefotaxime ; CRO: ceftriaxone; CAZ: ceftazidime; CPO: cefpirome; FEP: cefepime; ATM: aztreonam; GM: gentamicin; K: kanamycin; TE: tetracycline; SSS: sulfonamides; SXT: trimethoprim/sulfamethoxazole; *int1*: integrase; - : absence; ST: sequence type; ND: not determined.

Results

A total of 60 ciprofloxacin-resistant Enterobacteriaceae isolates was recovered and identified; they were divided into 51 isolates of *Escherichia coli*, 4 *Klebsiella pneumoniae*, 2 *Citrobacter freundii*, 1 *Klebsiella oxytoca*, 1 *Escherichia vulneris*, and 1 *Citrobacter koseri/farmeri*. Molecular typing of the 60 isolates by ERIC-PCR showed high clonality; 12 DNA profiles were observed for the 51 *E. coli* isolates, and those of *Klebsiella pneumoniae* and *Citrobacter freundii* isolates were similar. A sample of 17 strains representative of the different genetic profiles was retained for further study: *E. coli* (n = 12), *E. vulneris* (n = 1), *K. pneumoniae* (n = 1), *K. oxytoca* (n = 1), *C. freundii* (n = 1), and *C. koseri/farmeri* (n = 1). All results are presented in Table 1. Quinolone susceptibility testing of these isolates showed MICs of ciprofloxacin from 8 µg/mL to 128 µg/mL and concomitant resistance to nalidixic acid, ciprofloxacin, ofloxacin, and pefloxacin. Sequence analysis of the QRDR of the *gyrA* and *parC* genes revealed mutations in all strains. Among *E. coli* isolates, 8 harbored the amino acid substitution pattern GyrA: S83L + D87N, ParC: S80I, and each of the 4 remaining isolates carried the pattern GyrA: S83L + D87N, ParC: S80I + E84G; GyrA: S83L + D87N, ParC: S80I + L99M; GyrA: S83L, ParC: S80I; and GyrA: S83L, ParC: S80R.

Concerning strains of other species, the mutation pattern GyrA: S83L + D87N, ParC: S80I was observed in *E. vulneris*, GyrA: S83F + D87A, ParC: S80I in *K. pneumoniae*, GyrA: T83L + D87N, ParC: S80I in *C. koseri/farmeri*, and GyrA: T83I, ParC: S80I in *K. oxytoca* and *C. freundii*.

Besides ParC substitutions within QRDR, additional substitutions were detected at position M157L in *K. oxytoca* isolate and at N111D in the 2 *Citrobacter* isolates. The screening for PMQR determinants by multiplex PCR and sequencing was positive for 6 isolates, *qnrB1* allele in *K. oxytoca*, *qnrB2* in *C. freundii*, *qnrB5/19* and *qnrS1* in *E. coli*, and the variant *aac(6')-Ib-cr* in *K. pneumoniae* and *K. oxytoca*. No *qnrA*, *qnrD*, *qnrC*, and *qepA* genes were detected.

Regarding the non-quinolone antibiotics, resistance was observed for beta-lactams cefotaxime (5/17), ceftriaxone (5/17), ceftazidime (4/17), cefpirome (2/17), cefepime (1/17), and aztreonam (2/17); aminoglycosides kanamicin (5/17) and gentamicin (2/17); tetracycline (16/17); trimethoprim-sulfamethoxazole (14/17), and sulfonamides (15/17). Fifteen isolates had a multidrug-resistance phenotype, they were resistant to at least three antibiotic classes.

The resistance genes detected were $bla_{\text{CTX-M-15}}$ (n = 4), $bla_{\text{OXA-1}}$ (n = 2), *sul2* (n = 13), *sul1* (n = 4), *sul3* (n = 1), *tetA* (n = 9), *tetB* (n = 6), *aadA1/2* (n = 5), *aadA5* (n = 1), *aph(3')-Ia* (n = 5), *aac(3)II* (n = 2), *dfrA5* (n = 10), *dfrA1* (n = 3), *dfrA12* (n = 2), and *dfrA7* (n = 1). Class 1 integrons were detected in 11/17 strains.

Assessment of heavy metal tolerance showed tolerance of all the tested strains to cadmium, 16/17 to zinc, and 14/17 to mercury. The search for the mercuric reductase gene *merA* showed its presence in the 14 mercury-tolerant strains and in 10/11 strains harboring a class 1 integron.

The phylogenetic grouping of the 12 *E. coli* isolates allowed for the isolates to be assigned them to phylogroups A (n = 7), B1 (n = 3) and D (n = 2). MLST performed on 8 isolates enabled the definition the following STs (and associated phylogroups): D / ST405 (n = 2), B1 / ST443 (n = 1), B1 / ST101 (n = 2), A / ST10 (n = 2), and A/ ST347 (n = 1).

Discussion

The detection of ciprofloxacin resistance in Enterobacteriaceae isolates, mainly *E. coli*, is in accordance with hospital effluents being the source of quinolone-resistant fecal bacteria [19], and the concomitant resistance to multiple quinolones is consistent with cross-resistance between ciprofloxacin and other quinolones [6]. There was a diversity of mutation patterns among the tested *E. coli* isolates. The S83L, D87N + S80I mutation profile has been reported to be the most prevalent in clinical settings and in the environment [13,20-23], while the other profiles were found at low prevalence [21,23,24]; our results are in agreement with these reports. Among ParC substitutions, S80I was the most common, S80R and E84G were less frequent as previously reported [23], while L99M was described for the first time in this study. MICs of ciprofloxacin appear not to be dependent on the mutation patterns, contrary to what was reported in aquatic isolates [22]. Although very prevalent among Enterobacteriaceae, the GyrA: S83L + D87N, ParC: S80I pattern was, however, not described in *E. vulneris*; GyrA: S83F + D87A, ParC: S80I; and GyrA: T83I, ParC: S80I were reported, respectively, in clinical *K. pneumoniae* [13] and in *C. freundii* [25], but only T83I substitution was described in *K. oxytoca* [26]. The pattern GyrA: T83L + D87N, ParC: S80I in *C. koseri/farmeri* and the substitution T83L were described for the first time in this study. Only the M157L substitution had already been described as a single mutation in ParC in an isolate of *K. oxytoca* resistant to quinolones [13]. Few studies have been

devoted to the effect of mutations located outside the QRDR; however, the findings of Ruiz *et al.* [13] and Zurfluh *et al.* [23] suggest a role of these additional substitutions in quinolone resistance. Further investigations should be continued.

PMQR genes detected in this study are among the most frequently described in clinical, animal, food, and environmental Enterobacteriaceae [13,27-29]. Although our strains were resistant to quinolones, the prevalence of PMQR genes was relatively low, consistent with the fact that the contribution of these genes in the level of quinolone resistance is negligible. The genes have, in fact, also been found in both quinolone-resistant and -susceptible isolates; their role is to facilitate the emergence of resistance and to strengthen it [23,30,31].

The resistance to beta-lactams, aminoglycosides, tetracyclines, sulfonamides, and trimethoprim/sulfamethoxazole, resulting in multidrug-resistance phenotype, supports the association between ciprofloxacin resistance and resistance to other antibiotics as previously reported [6,7,32]. Resistance to ciprofloxacin can even be used as a multidrug resistance marker [7]. This cross-resistance is related to shared mechanisms or genetic supports of resistance to ciprofloxacin and unrelated drugs. Multidrug resistance is also linked to the ability of ciprofloxacin to induce mutational changes in efflux regulatory genes [33] or mobilization of multiresistance elements through the SOS response [32].

Various resistance genes were detected. $bla_{\text{CTX-M-15}}$ was found in two *E. coli* isolates and in *K. pneumoniae* and *K. oxytoca* isolates harboring *aac(6')-Ib-cr* and *aac(6')-Ib-cr* + *qnrB1*, respectively; it is the most widespread ESBL gene in the world [34]. The association of CTX-M-15 with ciprofloxacin resistance is commonly reported in human, animal, and aquatic environmental isolates [23,35,36] by its location on the plasmids carrying PMQR genes, particularly *aac(6')-Ib-cr*, or chromosomal integration which might facilitate co-selection processes [23,34,35].

Resistance genes *sul1*, *sul2*, *sul3*, *tetA*, *tetB*, *aadA*, *aph(3')-Ia*, *aac(3)II*, *aac(6')-Ib-cr*, *dfrA1*, *dfrA5*, *dfrA7*, and *dfrA12*, common in clinical settings, were also reported in food, animal, and aquatic environments, and are often associated with resistance to fluoroquinolones. Combinations of these genes in the same strain are frequently found because they are commonly carried as gene cassettes by class 1 integrons [15,37-40]. Class 1 integrons were detected in 11/17 strains, which is consistent with their role in the emergence and spread of antibiotic resistance in aquatic environments [41] and their high prevalence among ciprofloxacin-resistant isolates [42,43].

The co-selection of heavy metal resistance by antibiotics and vice versa is a widely reported phenomenon in complex environments such as hospital effluents, which may contain antibiotic residues and heavy metals simultaneously [3]. Almost all of the strains were resistant to three heavy metals of the five tested; this reflects the ability of these bacteria to persist in the environment. This antibiotic and heavy metal multiresistance of strains can be linked to cross-resistance through the same mechanism and/or co-resistance due to a common mobile genetic support [44]. The detection of mercuric reductase gene *merA*, a part of operon *merTCFPAD*, in most isolates resistant to mercury and harboring a class 1 integron augurs the presence of integron-containing Tn21-related transposons which usually carry this operon. This mobile genetic structure allows combination of resistance to antibiotics and to mercury [45].

Phylogenetic groups A, B1, and D have been found to be prevalent in human and animal wastewater [46]; our results are consistent with these findings. Phylogroups A and B1 are considered less virulent or even commensal compared to the B2 phylogroup and, to a lesser extent, the D phylogroup [47]. Moreover, A and B1 were described as the most frequently resistant to antibiotics, as shown in our results; indeed, it seems that there is a trade-off between antibiotic resistance, particularly to quinolones (ciprofloxacin), and virulence in *E. coli* [32].

Sequence types 405, 101, and 10, already reported from human and animal sources, are internationally successful sequence types. They are linked to phylogroups D, B1, and A, respectively [48], and are associated with ciprofloxacin resistance [49,50]. ST405 was associated with the spread of CTX-M-15 ESBL [48,50].

Conclusions

This study highlighted bacterial multiple-antibiotic and heavy metal resistance in hospital effluents, which is linked to ciprofloxacin resistance through selective pressure, co-resistance, and cross-resistance. This should draw attention to the consequences of exposure of bacteria to fluoroquinolones in general and ciprofloxacin in particular through their heavy and/or inappropriate use and their release in hospital effluents.

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Authors' contributions

LA: experimental work and manuscript drafting; YM: acquisition of data, interpretation of results and manuscript revision; VE: participation to experimental work; CT: analysis of results and manuscript revision; RB: conception and design of the study, analysis and interpretation of results and manuscript drafting.

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