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Brief Original Article

Molecular epidemiological of extended-spectrum β-lactamase producing *Escherichia coli* isolated in Djibouti

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Abstract

Introduction: While the molecular epidemiology of extended-spectrum-β-lactamase (ESBL)-producing *E. coli* is well known in Europe due to effective surveillance networks and substantial literature, data for Africa are less available, especially in Djibouti.

Methodology: We studied 31 isolates of ESBL-producing *E. coli* from Djibouti and compared these molecular results with data available in Africa.

Results: Susceptibility rates were 3.2% for ceftazidim, 48.4% for piperacillin-tazobactam, 90.3% for amikacine and 16.1% for ofloxacin. No isolate showed resistance to carbapenems or colistin. 30 *E. coli* (96.8%) were positive to $bla_{\text{CTX-M-15}}$, 1 (3.2%) to $bla_{\text{CTX-M-14}}$ and 10 (32.3%) to narrow-broad-spectrum bla_{TEM}. No bla_{SHV} were detected. Fluoroquinolone resistance analysis showed that 30 ofloxacin-resistant *E. coli* had the mutation Ser-83->Leu on the *gyrA* gene. 24 *E. coli* (77.4%) harboured the plasmid-borne *aac(6 ')-Ib-cr* gene. No *E. coli* carried the genes *qnrA*, *qnrB* and *qepA*. 10 isolates (32.3%) belonging to the ST131 clone. The plasmid incompatibility group most widely represented in our collection was IncFIA/IB/II.

Conclusions: There is no major difference with African epidemiology. In particular, we notice the international diffusion of specific clonal group ST131.

Key words: *E. coli*; ESBL; Africa; Djibouti; ST131.

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Introduction

Escherichia coli (*E. coli*), a Gram-negative bacillus belonging to the *Enterobacterales* family, colonizes the digestive tract and can cause infection, especially of the urinary, intestinal or neonatal varieties. It is the primary agent of bacteraemia and urinary tract infections in Europe [1]. In Africa, *E. coli* is the second-most prevalent *Enterobacterales* responsible for community-acquired bacteraemia and one of the main agents of diarrhea, causing more than 10% of deaths in children under 5 years [2,3]. Antibiotic resistance in *E. coli* has steadily increased over the years, leading to the emergence and the international spread of multidrug-resistant *Enterobacterales*. This phenomenon is linked to a growing selection pressure for antibiotics, sometimes associated with misuse, interpersonal transmission, and a significant contribution of the environment and animal feed. While the molecular epidemiology of extended-spectrum-β-lactamase (ESBL)-producing *E. coli* is well known in Europe due to effective surveillance networks and substantial literature, data for Africa are less available, often being limited to the Northern part of the continent. Despite the importance of the problem and its consequences on health and economic measures, few countries in Africa have national surveillance and control programmes against resistance, as recommended by the World Health Organization [4]. During his tenure, a biologist colleague, working at the laboratory of the military hospital Bouffard, reported an increase of third-generation cephalosporin-resistant *Enterobacterales* (6% in 2009 to 24% in 2014 on isolates from clinical samples; unpublished data). Consequently, we decided to establish a genetic resistance profile of ESBL-producing *E. coli* isolates collected before the closure of the military hospital Bouffard in Djibouti and to compare these molecular results with other data available in Africa.

Methodology

Bacterial isolates and setting

Thirty-one strains of ESBL-producing *E. coli* isolated from 31 different patients were collected in 2015 at the military hospital Bouffard in Djibouti.

These isolates were obtained from clinical specimens such as urine (12 samples), blood cultures (2 samples), bronchoalveolar fluids (2 samples), pus (5 samples) and from rectal swabs (10 samples for screening purposes). Of these 31 isolates, 20 (64.5%) were isolated from men and 11 (35.5%) from women. Of the patient base, 20 (64.5%) patients were hospitalized, 4 (12.9%) were at the emergency unit and 7 (22.6%) had medical consultation in hospital. Regarding military service status, 29 (93.5%) patients were native or Djiboutian soldiers, 2 (6.4%) were French servicemen.

Isolates were sent to our laboratory in France for further phenotypic and molecular investigation: *E. coli* identification performed in Djibouti was confirmed by a matrix-assisted laser desorption ionization-time of flight mass spectrometry system in France (MALDI-TOF, Microflex®, Bruker Daltonics, GmbH, Bremen, Germany).

Susceptibility testing

Antimicrobial susceptibility was determined by the disc diffusion method on Mueller Hinton agar plates (Biorad, Marnes-la-coquette, France). Quality control strains were used in particular extended-spectrum β-lactamase producing *Klebsiella pneumoniae* ATCC 700603. The results were interpreted according to the Antibiogram Committee of the French Society of Microbiology 2017. ESBL production was confirmed by double-disc synergy testing. The minimum inhibitory concentration (MIC) of colistin was determined on each isolate in a liquid medium using the UMIC Colistine® kit (Biocentric, Bandol, France).

Primers, DNA extraction, PCR conditions and sequencing

Primers to target β-lactamases genes encoding TEM; SHV; CTX-M; quinolones resistance genes *gyrA*, *qnrA, qnrB, qnrS*, *qepA, aac*(6')-*Ib-cr* and aminoglycoside resistance genes *armA*, *rmtA*, *rmtB* were used. The *E. coli* phylogenetic groups (A, B1, B2 and D) were determined using a PCR-based method according to the presence of the two genes (*chuA* and *yjaA*) and an anonymous DNA fragment (TsPE4.C2) [5]. Primers allowing detection of the *E. coli* O25b-ST131 clone were used according to Clermont *et al*. [6]. The *E. coli* isolates were typed by multilocus sequence typing (MLST) by amplifying partial fragments of eight housekeeping genes (*dinB, icdA, pabB, polB, putB, trpA, trpB* and *uidA*) following the protocols available at the website: http://bigsdb.pasteur.fr/ecoli/ecoli.html. Plasmid incompatibility was studied according to the work of Compain *et al.* by several PCRs targeting the relaxase genes of each type of plasmids [7].

All primers used in this study are listed in Supplementary Table 1.

Lysis by boiling was used for total DNA extraction as previously described [8]. Simplex PCR was performed in a 50 µL mix with 2U of Taq DNA polymerase, 10XPCR buffer, $MgCl_2$ (Qiagen, Courtaboeuf, France), 200 µM of each deoxynucleotide triphosphate (dNTP Mix, Eurobio, Courtaboeuf, France), 0.2 pmol/µL of each primer, 40 µL of sterile water and 2 µL of total DNA extract. PCR conditions consisted of an initial activation at 95°C for 5 min, followed by 35 cycles of 95°C for 1 min, 58°C for 40 s and 72°C for 40 s with a final extension at 72°C for 7 min. PCR products were separated with QIAxcel Advanced System (Qiagen, Hilden, Germany). PCR products were purified using the QIAquick PCR purification kit (Qiagen, Hilden, Germany) and subjected to DNA sequencing using BigDye terminator v3.1 cycle sequencing kit (Applied Biosystems, Foster City, CA, USA) and an Applied Biosystems 3730 XL capillary sequencer. Sequence analysis was carried out using CEQ 8000 system analysis (Beckman Coulter, Brea, USA).

Results

Of 31 ESBL-producing *E. coli* analysed no isolate showed resistance to carbapenems (ertapenem). All isolates were susceptible to colistin, with a mean MIC at 0.27 mg/L. Susceptibility rates were 83.9% for cefoxitin, 3.2% for ceftazidim, 48.4% for piperacillin-tazobactam, 41.9% for temocillin, 90.3% for amikacin, 38.7% for netilmicin, 19.4% for tobramycin, 41.9% for gentamicin, 12.9% for ofloxacin and 19.4% for ciprofloxacin.

PCR testing for β-lactamase genes followed by sequencing revealed 30 *E. coli* (96.8%) positive to extended spectrum β-lactamases $bla_{\text{CTX-M-15}}$ and 1 (3.2%) to $bla_{\text{CTX-M-14}}$ and 10 (32.3%) to narrow-spectrum Ambler class A β-lactamases: 6 (19.4%) to $bla_{\text{TEM-6}}$, 2 (6.5%) to $bla_{\text{TEM-190,}}$ 1 (3.2%) to $bla_{\text{TEM-2,}}$ 1 (3.2%) to $bla_{\text{TEM-169}}$. No bla_{SHV} were detected. Eight *E. coli* were co-carriers of both TEM and CTX-M.

Fluoroquinolone resistance analysis showed that all the ofloxacin-resistant *E. coli* (83.9%) had the mutation known and described in the literature on the *gyrA* gene (QRDR region with mutations at positions 83 (Ser->Leu)) and/or carried a plasmid-borne resistance gene.

Table 1. Resistance genes, sequence types, other antibiotic resistance gene, plasmid replicon type of the 31 isolates studied.

Isolate	Sequence type	Phylogroup	Beta-lactam genes (*bla*$_{CTX-M}$)	Fluoroquinolone resistance					Aminoglycoside resistance				Plasmid relaxase typing
				Ofloxacin	*Ciprofloxacin*	*gyrA* mutation(s)	*qnrS*	*aac(6')-Ib-cr*	Amikacin	Gentamicin	Tobramycin	Netilmicin	
1	ST-43	B2	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	R	R	R	IncFIA, IncFIB, IncFII
2	ST-43	B2	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	S	R	S	IncFIA, IncFIB, IncFII
3	ST-741	B1	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	-	S	S	S	S	IncFIA, IncFIB, IncFII
5	ST-43	B2	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	R	R	R	IncFIA, IncFIB, IncFII
16	ST-43	B2	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	R	R	R	IncFIA, IncFIB, IncFII
19	ST-2	A	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	S	R	S	IncFIA, IncFIB, IncFII
20	ST-2	A	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	S	R	S	IncFIA, IncFIB, IncFII
24	ST-132	A	*bla*$_{CTX-M-15}$	S (acid nalidixic R)	S	-	-	-	S	R	R	R	NT
26	ST-741	A	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	R	R	S	IncFIA, IncFIB, IncFII, IncI1
27	ST-2	A	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	S	R	S	IncFIA, IncFIB, IncFII, IncI1
31	ST-8	D	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	S	R	S	IncFIA, IncFIB, IncFII
33	ST-721	D	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	R	R	R	IncFIA, IncFIB, IncFII, IncK-B/O
39	ST-2	A	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	R	R	I	IncFIA, IncFIB, IncFII, IncI1
45	ST-3	D	*bla*$_{CTX-M-15}$	S (acid nalidixic S)	S	NR	-	+	S	R	R	I	IncFIA, IncFIB, IncFII, IncI1
57	ST-53	B2	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	R	R	R	NT
59	ST-43	B2	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	R	S	R	R	IncFIA, IncFIB, IncFII
71	ST-43	B2	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	R	R	I	NT
78	ST-8	B2	*bla*$_{CTX-M-14}$	S (acid nalidixic S)	S	-	-	-	S	S	S	S	IncFIA, IncFIB, IncFII
84	ST-2	A	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	I	R	R	R	IncFIA, IncFIB, IncFII
99	ST-829	D	*bla*$_{CTX-M-15}$	S (acid nalidixic S)	S	NR	-	-	S	S	S	S	IncFIA, IncFIB, IncFII
105	ST-692	A	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	+	+	S	R	R	R	IncFIA, IncFIB, IncFII, IncI1
116	ST-740	D	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	-	S	S	S	S	IncFIA, IncFIB, IncFII
118	ST-43	B2	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	S	R	I	IncFIA, IncFIB, IncFII
120	ST-43	B2	*bla*$_{CTX-M-15}$	R	R	Ser-83->Leu	-	+	S	R	R	I	IncI1
122	ST-698	A	*bla*$_{CTX-M-15}$	R	S	-	+	-	S	S	S	S	NT

Table 1 (continued). Resistance genes, sequence types, other antibiotic resistance gene, plasmid replicon type of the 31 isolates studied.

125	ST-500	A	*bla*$_{\text{CTX-M-15}}$	R	R	Ser-83->Leu	-	+	S	R	R	R	IncFIA, IncFIB, IncFII, IncI1
126	NT	D	*bla*$_{\text{CTX-M-15}}$	R	R	Ser-83->Leu	-	+	S	R	R	R	IncFIA, IncFIB, IncFII
128	ST-44	D	*bla*$_{\text{CTX-M-15}}$	R	S	Ser-83->Leu	-	-	S	S	S	S	NT
129	ST-2	A	*bla*$_{\text{CTX-M-15}}$	R	R	Ser-83->Leu	-	+	S	R	R	R	IncFIA, IncFIB, IncFII
136	ST-43	B2	*bla*$_{\text{CTX-M-15}}$	R	R	Ser-83->Leu	-	+	I	R	R	R	IncFIA, IncFIB, IncFII
137	ST-43	B2	*bla*$_{\text{CTX-M-15}}$	R	R	Ser-83->Leu	-	+	S	R	R	R	IncFIA, IncFIB, IncFII, IncI1

NT: non typeable; NR: no result; S/I/R: susceptible/intermediate/resistant according to CASFM 2017 (derived from EUCAST 2017).

Two *E. coli* (6.5%) were positive for the plasmid-borne *qnrS* gene: the both were resistant to ofloxacin, one was resistant to ciprofloxacin, while the other was susceptible. Interestingly, 24 *E. coli* (77.4%) harboured the plasmid-borne *aac(6')-Ib-cr* gene: all these bacteria were resistant to ciprofloxacin except for one *E. coli* (isolate 45) that was susceptible to all quinolones and resistant to gentamicin. No *E. coli* carried the plasmid-mediated quinolone resistance genes *qnrA*, *qnrB* and *qepA*.

Regarding aminoglycoside resistance, *armA, rmtA* and *rmtB* PCRs performed on the three amikacin intermediate/resistant *E. coli* (9.7%) were negative.

Finally, the IncFIA/IB/II, IncI1 and IncB/O/K plasmids were detected respectively in 25 (80.6%), 8 (25.8%) and 1 (3.2%) of the 31 *E. coli* studied. Five isolates remained non-typable within the 18 major incompatibility groups [7].

Regarding molecular epidemiology, 12 *E. coli* belonged to phylogroup B2 (38.7%), 11 to group A (35.5%), 7 to group D (22.6%) and 1 to group B1 (3.2%). ST131 PCR revealed 10 isolates (32.3%) belonging to the ST131 clone. These results were confirmed by sequence analysis using the MLST website. Indeed, the 10 isolates identified ST131 by conventional PCR were typed ST43, one of the 3 major clades of ST131 clones. The other clones identified belonged to 13 different STs: ST2 (19.3%), ST741 (6.4%), ST8 (6.4%) and ST3, 44, 53, 132, 500, 692, 698, 721, 740, 829 (3.2%). One non-ST131-*E. coli* remained unclassified because of the impossibility to amplify one of its MLST genes despite several assays and conditions.

All results are summarized in Table 1.

Discussion

We have provided genetic data on molecular epidemiology in ESBL-producing *E. coli* collected in Djibouti. To our knowledge, this study is the first report of molecular epidemiology in ESBL in this country. A total of 31 isolates of ESBL-producing *E. coli* recovered from clinical samples and rectal swabs were analysed. Although limited, especially by the small number of isolates, this study allowed three major results to be highlighted.

First, the *bla*$_{\text{CTX-M-15}}$ gene was the most commonly detected ESBL-encoding gene in our collection, as reported by other works conducted in North America, Europe, and South America [9]. Data from Malawi, Nigeria, and Tanzania are very similar with respectively 95.2%, 85.7%, and 100% of ESBL-producing *E.coli* belonging to the CTX-M-15 group [3,9,10]. This further strengthens the converging data about the high diffusion of this gene, including this phenomenon as reported in the Horn of Africa.

Secondly, genetic explorations showed a high molecular diversity and frequent combinations of antibiotic-resistance plasmids, in particular those encoding beta-lactamases and resistance to fluoroquinolones. The virulent extra-intestinal phylogroup B2 was predominant in our collection, followed by the commensal phylogroup A. These data are consistent with the results of a similar study conducted in Malawi in 2017 in which 34% of *E.coli* belonged to phylogroup B2 and 23.4% to phylogroup A2 [3]. These groups were also predominant in Tanzania in the study of Mshana *et al*. In our study, *bla*$_{\text{CTX-M-15}}$ gene was detected in the 5 different *E. coli* phylogroups and in 14 different STs. One-third of the CTX-M-15-producing *E. coli* group belonged to the ST131 clone. This predominance of ST131 despite the genomic diversity of CTX-M-15 has already been

described in Tanzania, Asia, Europe, and America [3,11]. The ST131 is a pandemic clone responsible for the high incidence of extraintestinal pathogenic *E. coli* and has been known widely for its contribution to the worldwide dissemination of multidrug resistance [12]. The success of this ST has been explained by its acquisition of fluoroquinolone resistance and additional virulence factors [13]. Concerning the genetic support of resistance, the plasmid incompatibility group most widely represented in our collection was IncFIA/IB/II. It is also highlighted in studies carried out in Malawi and Tanzania with respectively 89% and 73% of the *E. coli* studied [3,11]. The IncF plasmids groups represent the most prevalent incompatibility type and have been identified worldwide in *Enterobacterales* from different sources [14]. The IncF plasmids have been reported to be characterized by a low copy number with a size >100kb and having more than one replicon to promote the initiation of replication. The multi-replicon status has been described by Villa *et al*. to be one means by which plasmids with a narrow host range can accomplish broad host range replication [15]. The IncF plasmids possess great versatility of intracellular adaptation by the rapid evolution of the regulatory sequences of the replicons which explains why they are widely diffused in *Enterobacterales* [15]. Various plasmids that differ in incompatibility groups and replicon types have been characterized in *E. coli* ST131 isolates, but the narrow-host-range IncF is the most common Inc type detected in ST131 *E. coli* with $bla_{CTX\text{-}M}$ genes [16].

Finally, in our collection, quinolone resistance was clearly correlated to mutations observed on the *gyrA* gene. Chromosomal resistance is therefore, as in Europe, still predominant. However, the plasmid-borne variant *aac(6')-Ib-cr* was also very common (77.4%). Its prevalence in our study was higher than in other reports in Africa. The variant was evidenced in Egypt, Morocco and Malawi in only 10 to 20% of ESBL-producing *E.coli* isolates [3,17,18]. The *cr* variant of *aac(6')-Ib* encodes an aminoglycoside acetyltransferase that confers reduced susceptibility to ciprofloxacin by *N*-acetylation of its piperazinyl amine. The presence of *aac(6')-Ib-cr* alone increases substantially the frequency of selection of chromosomal mutants upon exposure to ciprofloxacin [19]. Conversely, the *qnr* and *qepA* genes were poorly represented in our collection (< 10% for *qnr*, 0% for *qep*), as already reported in Algeria, Morocco, and Malawi [3,18,20], whereas their prevalences appeared to be higher in Egypt, ranging from 17 to 23% for *qnr* and 15% for *qep* in ESBL-producing *E.coli* [17]. Plasmids carrying the *aac(6')-Ib-cr* variant were associated with different incompatibility groups but mainly with the IncF type [21]. In previous reports, this determinant was carried by a variety of replicons, such as IncFII, IncN, and IncFIA [22,23].

Conclusion

In conclusion, we describe a collection of 31 ESBL-producing *E. coli* isolates in Djibouti. To our knowledge, this is the first available data in this country. There is no major difference in this data when compared with epidemiology in other African countries. In particular, we will note the international diffusion of specific clonal group ST131. The establishment of surveillance networks and the creation of standardized technical and epidemiological protocols are essential to control and prevent the circulation and dissemination of these multi-resistant *Enterobacterales*. A continuity in the study of the *E. coli* and others *Enterobacterales* is essential in order to detect any emergent phenomena (e.g. carbapenemase).

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Annex – Supplementary Items

Supplementary Table 1. Primers used in this study.

Gene/Function	Primer sequence	Amplicon size (bp)
ChuA F	5'-GACGAACCAACGGTCAGGAT-3'	279
ChuA R	5'-TGCCGCCAGTACCAAAGACA-3'	
YjaA F	5'-TGAAGTGTCAGGAGACGCTG-3'	211
YjaA R	5'-ATGGAGAATGCGTTCCTCAAC-3'	
TspE4C2 F	5'-GAGTAATGTCGGGGCATTCA-3'	152
TspE4C2 R	5'-CGCGCCAACAAAGTATTACG-3'	
ST131 F	5'-TCCAGCAGGTGCTGGATCGT-3'	347
ST131 R	5'-GCGAAATTTTTCGCCGTACTGT-3'	
CTXM1 F	5'-TGGTTTAAAAAATCACTGCG-3'	876
CTXM1 R	5'-ATTACAAACCGTCGGTGAC-3'	
CTXM2 F	5'-ACTCAGAGCATTCGCCGCTCA-3'	879
CTXM2 R	5'-TTATTGCATCAGAAACCGTG-3'	
CTXM9 F	5'-ATGGTGACAAAGAGAGTGCAACG -3'	837
CTXM9 R	5'-ACAGCCCTTCGGCGATGATTC-3'	
SHV F	5'-CGCCGGGTTATTCTTATTTGTCGC-3'	1016
SHV R	5'-TCTTTCCGATGCCGCCGCCAGTCA-3'	
TEM F	5'-ATGAGTATTCAACATTTCCG-3'	861
TEM R	5'-CCAATGCTTAATCAGTGAG-3'	
gyrA F	5'-CTGAAGCCGGTACACCGT-3'	577
gyrA R	5'-GGATATACACCTTGCCGC-3'	
qnrA F	5'-GATAAAGTTTTTCAGCAAGAGG-3'	543
qnrA R	5'-ATCCAGATCGGCAAAGGTTA-3'	
qnrB F	5'-GACAGAAACAGGTTCACCGGT-3'	469
qnrB R	5'-CAAGACGTTCCAGGAGCAACG-3'	
qnrS F	5'-GGAAACCTACAATCATACATA-3'	600
qnrS R	5'-GTCAGGATAAACAACAATACC-3'	
qep F	5'-AACTGCTTGAGCCCGTAGAT-3'	185
qep R	5'-TTGCTGGAGTTCTTCCACTG-3'	
aac(6')-1b F	5'-ATGACTGAGCATGACCTTG-3'	476
aac(6')-1b R	5'-AACCATGTACACGGCTGG-3'	
armA F	5'-AGGTTGTTTCCATTTCTGAG-3'	590
armA R	5'-TCTCTTCCATTCCCTTCTCC-3'	
rmtA F	5'-CTAGCGTCCATCCTTTCCTC-3'	635
rmtA R	5'-TTTGCTTCCATGCCCTTGCC-3'	
rmtB F	5'-ATGAACATCAACGATGCCCT-3'	769
rmtB R	5'-CCTTCTGATTGGCTTATCCA-3'	
dinB F	5'-GTTTTCCCAGTCACGACGTTGTATGAGAGGTGAGCAATGCGTA-3'	450
dinB R	5'-TTGTGAGCGGATAACAATTTCCGTAGCCCCATCGCTTCCAG-3'	
icdA F	5'-GTTTTCCCAGTCACGACGTTGTAATTCGCTTCCCGGAACATTG-3'	516
icdA R	5'-TTGTGAGCGGATAACAATTTCATGATCGCGTCACCAAAYTC-3'	
pabB F	5'-GTTTTCCCAGTCACGACGTTGTAAATCCAATATGACCCGCGAG-3'	468
pabB R	5'-TTGTGAGCGGATAACAATTTCGGTTCCAGTTCGTCGATAAT-3'	
polB F	5'-GTTTTCCCAGTCACGACGTTGTAGGCGGCTATGTGATGGATTC-3'	450
polB R	5'-TTGTGAGCGGATAACAATTTCGGTTGGCATCAGAAAACGGC-3'	
putP F	5'-GTTTTCCCAGTCACGACGTTGTACTGTTTAACCCGTGGATTGC-3'	456
putP R	5'-TTGTGAGCGGATAACAATTTCGCATCGGCCTCGGCAAAGCG-3'	
trpA F	5'-GTTTTCCCAGTCACGACGTTGTAGCTACGAATCTCTGTTTGCC-3'	561
trpA R	5'-TTGTGAGCGGATAACAATTTCGCTTTCATCGGTTGTACAAA-3'	
trpB F	5'-GTTTTCCCAGTCACGACGTTGTACACTATATGCTGGGCACCGC-3'	594
trpB R	5'-TTGTGAGCGGATAACAATTTCCCTCGTGCTTTCAAAATATC-3'	
uidA F	5'-GTTTTCCCAGTCACGACGTTGTACATTACGGCAAAGTGTGGGTCAAT-3'	600
uidA R	5'-TTGTGAGCGGATAACAATTTCCCATCAGCACGTTATCGAATCCTT-3'	

MLST sequencing	5’-GTTTTCCCAGTCACGACGTTGTA-3’	NA
MLST sequencing	5’-TTGTGAGCGGATAACAATTTC-3’	
IncFIIK-f	5’-CGATATTCTKGAACCCCGTA-3’	297
IncFIIK-r	5’-TCATTGCCCATAATYCGTCC-3’	
IncFtot-f	5’-ATCAGGAMCCACAGTTACAC-3’	753
IncFtot-r	5’-GTTTCATGATRTCRCGACTGAG-3’	
IncFV-f	5’-CATCAAAGCGAARGAGYAGTTCAC-3’	642
IncFV-r	5’-TTACCCTCWGCAATATGRCGRA-3’	
IncN-f	5’-CCAGTTTAGAAACCCGATCA-3’	332
IncN-r	5’-CGTAATCGTCGTAAATGCTG-3’	
IncL/M-f	5’-TCGCTATATCGTTGGTGATG-3’	491
IncL/M-r	5’-GGGTATGTTGGGCAATTTTC-3’	
IncA/C-f	5’-TGATTGAGAAAGTGCGGAACTC-3’	659
IncA/C-r	5’-CACACCATAGGAGAACTCGT-3’	
IncI1-f	5’-TTTAATCATGAGCAAACGCC-3’	764
IncI1-r	5’-ACAACYCTGTCACCATAATC-3’	
IncHI1-f	5’-GATGGTATGTGGATAGTCCG-3’	368
IncHI1-r	5’-GAATATGCCTCACYATCGCT-3’	
IncHI2-f	5’-AATGACGTGTGGTTGAATACGA-3’	225
IncHI2-r	5’-AGAATAGAAATCAGCGGTCC-3’	
IncX1-f	5’-GAAAATGGTCGTAAGTCAGCT-3’	845
IncX1-r	5’-TTCAGYCTGACAAGATCACCTGC-3’	
IncX2-f	5’-CGAAAATGGTCGTAAGTCTGCA-3’	560
IncX2-r	5’-CATTACAAAAGCCCGTCCTG-3’	
IncX3-f	5’-TGTTGACAAGGAATTCAGGGT-3’	736
IncX3-r	5’-ATGGTCGTAGCCAACATCAA-3’	
IncX4-f	5’-TTAAACGCGCTAAAGGGACTGG-3’	996
IncX4-r	5’-GGGCAATATTTTCAATCTGCCAG-3’	
IncQ1-f	5’-GATTTATCACCTTACGGCGAA-3’	962
IncQ1-r	5’-TACCTCCCGCAACTCTTTGG-3’	
IncU-f	5’-TATGACACTCTAGCCGGGTT-3’	743
IncU-r	5’-TGGATCTTGTCTTCCCACGGTT-3’	
IncP1-f	5’-TGAAGTACATCACCGACGAG-3’	424
IncP1-r	5’-GTTTCGTGATTGTCACGCTC-3’	
IncW-f	5’-CATCGAGTTCGATACCCTTG-3’	531
IncW-r	5’-GCCACATAGTAAACGTCCTT-3’	
IncB/O/K-f	5’-GAATGCCATYATTCCGMAMAA-3’	800
IncB/O/K-r	5’-GTGATATACAGACCATCACTGG-3’	