Original Article

β-lactam and fluoroquinolone resistance in Enterobacteriaceae from imported and locally-produced chicken in Mozambique

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Abstract

Introduction: Plasmid-mediated resistance to β-lactam and fluoroquinolone antibiotics was investigated in Enterobacteriaceae isolated from retailed frozen chickens from Brazil, South Africa and Mozambique.

Methodology: Carcass swabs and the liquid thaw of 33 chickens from each of the three countries constituted the total sample size of 198. Isolates were identified by biochemical tests, antibiotic susceptibility was ascertained by the disc diffusion assay and β-lactamases were detected using the double-disk synergy test. PCR was used to detect the presence of blaCTX-M, blaSHV, blaTEM, blaCMY, blaKRX, blaFOX, blaOXA, qnrB, qnrD, qnrS and qepA genes. A random selection of CTX-M genes was sequenced.

Results: The 198 samples yielded 27 (13.6%) putative extended-spectrum β-lactamase (ESBL)-positive isolates, 15 from carcass swabs and 12 from the liquid thaw from 22 chickens with 19, 5 and 3 isolates from South African, Mozambican and Brazilian chicken, respectively. Isolates exhibited the following resistance: ampicillin 100%, ceftriaxone 89%, trimethoprim-sulfamethoxazole 78%, cefotaxime 74%, ciprofloxacin 70%, ceftazidime 67%, cefoxitin 22% and gentamicin 8%. The predominant putative ESBL gene was blaSHV (85%), followed by blaCTX-M (62.9%) and blaTEM (44.4%) whilst blaKRX and blaOXA were the most common pAmpC genes at 33.3%. The predominant plasmid-mediated fluoroquinolone-resistance gene was qepA (22.2%). DNA sequencing identified blaCTX-M-55/-79/-101/-164. ERIC–PCR profiles did not show strong evidence of clonality.

Conclusion: The Mozambican population is exposed to a reservoir of plasmid-mediated, and hence mobile β-lactam and quinolone resistance genes via imported, and to a lesser extent, locally produced poultry. This presents a food safety concern.

Key words: antibiotic resistance; ESBL; pAmpC; CTX-M; Enterobacteriaceae; chicken.
These resistance mechanisms to both antibiotic classes have been described in food-borne pathogens [4–6].

In Africa, the most common ESBL genes detected in bacteria among food-producing animals are bla\text{CTX-M}, followed by bla\text{TEM}-52 and bla\text{SHV}-12 genes and mostly in \textit{Escherichia coli}. Antibiotic resistance in food products in Africa have also been reported [7–10].

The poultry industry has been considered a potential reservoir of ESBL-producing Gram-negative bacteria that may be acquired by humans through handling or consumption of contaminated meat [11]. As chicken is one of the most consumed foods by the Mozambican population, we undertook a study to investigate ESBL and pAmpC-mediated \(\beta\)-lactam resistance as well as plasmid-mediated fluoroquinolone resistance in \textit{Enterobacteriaceae} isolated from imported and locally produced chicken in Maputo, Mozambique.

**Methodology**

**Study Sample**

Ninety-nine frozen chickens, consisting of 33 chickens imported from each of South Africa and Brazil, and 33 locally produced in Mozambique, were purchased from three different major supermarkets in Maputo City. Eleven chickens from each country were randomly purchased weekly during May 2015 from each of the three supermarkets selected as they carried chickens produced in all three countries. The chickens were from a single producer in each country.

Two samples were taken from each chicken, one from the liquid thaw considered a proxy for the carcass rinsate and the other from a swab of the carcass yielding a total of 198 samples from 99 chickens. One milliliter of liquid thaw was collected in a sterile tube and used for culturing procedures. The carcass sample was taken by passing the swab into different internal parts of the carcass. Samples were cultured on ESBL-selective media, i.e., MacConkey agar (Oxoid Ltd, Basingstoke, Hampshire, England) plates supplemented with 2 mg/L of ceftriaxone using the streak method [12].

**Species identification and antimicrobial susceptibility testing**

All Gram-negative, oxidase-negative bacilli were identified to species level using biochemical tests (sugars with Kliger iron agar, motility-indole-ornithine agar, citrate agar, urea agar, lysine agar) [12]. After overnight incubation at 37°C, lactose and glucose fermenters, gas producers, indole-positive, lysine and ornithine-positive and motile isolates were identified as \textit{E. coli}. \textit{Citrobacter} spp. were identified as all Gram-negative bacilli which were lactose and glucose fermenters, gas producers, citrate and urease-positive and motile. The production of sulfuric acid served as a differentiator between \textit{Citrobacter diversus} and \textit{Citrobacter freundii}. \textit{Enterobacter} spp. were identified as lactose and glucose fermenters, gas producers, citrate- and ornithine-positive and motile isolates. \textit{Enterobacter} spp. which were urease-positive were identified as \textit{Enterobacter cloacae} and those that were lysine-positive were identified as \textit{Enterobacter agglomerans} [13].

Antimicrobial susceptibility testing (AST) was undertaken using the agar disc diffusion assay according to Clinical and Laboratory Standards Institute (CLSI) guidelines [14] with the antibiotic panel consisting of ampicillin, cefotaxime, cefoxitin, ceftazidime, cefuroxime, ciprofloxacin, gentamicin, amoxicillin/clavulanic acid and trimethoprim-sulfamethoxazole. \textit{E. coli} ATCC 25922 was used as the control.

**Phenotypic detection of putative ESBLs, and pAmpCs**

ESBL production was confirmed using the double-disk synergy test (DDST) using ROSCO disks containing cephalosporins (cefepime, cefotaxime, ceftazidime,) with and without clavulanic acid [15]. \textit{E. coli} ATCC 35218 producing TEM-1 and \textit{Klebsiella pneumoniae} ATCC 700603, producing SHV-18 were used as controls. The phenotypic AmpC confirmation test was based on inhibition of AmpC by cloxacillin and boronic acid derivatives [15] with \textit{E. coli} ATCC 25922 and \textit{E. coli} ATCC 35218 as controls. Results were considered positive when the inhibition zones of the combined discs were ≥ 5 mm compared to that of the antibiotic discs alone.

**DNA extraction, PCR and electrophoresis**

Genomic DNA was extracted from overnight bacterial cultures using the GeneJET Genomic DNA Purification Kit (Thermo Scientific, Waltham, USA) as per manufacturer’s guidelines. PCR amplification of ESBL, pAmpC, \textit{qnr} and \textit{qepA} genes was performed with primers and conditions previously described [16–19], in a final volume of 25 µL, containing 12.5 µL of Phusion Flash High-Fidelity PCR Master Mix (Thermo Scientific, Waltham, USA), 7.5 µL of sterilized distilled water, 2 µL of each primer and 1 µL template DNA. PCR was undertaken in a T100™ Thermal cycler (Bio-Rad, Hercules, USA). The cycle comprised of preliminary denaturation for 10 seconds at 98°C, followed by 35 cycles of denaturation at 98°C for 1 second, annealing for 5 seconds and elongation at 72°C.
for 7 minutes. Annealing temperatures and primers are described in the Supplementary Table 1. Amplicons were visualized by electrophoresis in 1.5% agarose gels for 40 minutes at 120 V, stained with gel red and detected by ultraviolet trans-illumination.

Randomly selected CTX-M PCR amplicons were purified and sequenced by Inqaba Biotec South Africa, using Sanger dideoxy sequencing technology. Sequences were analyzed using Basic Local Alignment Search Tool, available on the website of the National Center for Biotechnology Information (http://www.ncbi.nlm.nih.gov/blast/BLAST.cgi) and BioEdit (http://www.mbio.ncsu.edu/bioedit/bioedit.html).

ERIC-PCR was undertaken in a total reaction volume of 10 µL, which contained 2 µL of template DNA and 0.1 µL of 100 µM primers ERIC 1 and ERIC 2 [20] and 5 µL of DreamTaq Green PCR Master Mix (Thermo Scientific, Waltham, USA). PCR conditions were as follows: 94°C for 3 minutes, 30 cycles of 30 seconds of denaturation at 94°C, 1 minute of annealing at 50°C, 8 minutes of extension at 65°C and a final elongation of 16 minutes at 65°C, in an Applied Biosystems 2720 Thermal Cycler. The ERIC-PCR products were loaded onto 1.0% (w/v) agarose gels and subjected to electrophoresis at 80V using 1× TAE buffer. Amplification products were visualized by UV trans-illumination (Syngene, Cambridge, UK) after staining in 0.1 mg/mL ethidium bromide for 15 minutes. Genotypic variations were analyzed using the Gel Compare II version 6.0 software package (Applied Maths) by Jacquard and Unweighted Pair Group Method with Arithmetic mean (UPGMA) cluster analysis to produce a dendrogram. Optimization and band tolerance were set at 1% and 80% similarity cut-off was used to define clusters.

Results

Isolates identified

Of the 198 samples, 27 were putatively ESBL-positive: 15 from the carcass swabs and 12 from the liquid thaw from a total of 22 chickens. Five chickens

Table 1. Antibiotic susceptibility results.

| Country   | Code | Species     | Source | AMC | CTX | CAZ | CRO | FOX | GEN | AMP | SXT | CIP |
|-----------|------|-------------|--------|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| South Africa | A3d  | E. agglomerans | Thaw   | S   | R   | R   | R   | R   | S   | R   | R   | R   |
|           | A10e | E. coli      | Carcass | R   | R   | R   | R   | S   | S   | R   | R   |
|           | A10d | E. coli      | Thaw   | S   | R   | R   | R   | S   | S   | R   | R   |
|           | A12c | E. coli      | Carcass | S   | I   | S   | R   | S   | R   | S   |
|           | A13d | E. coli      | Thaw   | S   | I   | R   | S   | S   | R   | R   |
|           | A17c | E. coli      | Carcass | I   | R   | R   | S   | S   | R   | S   |
|           | A19c | C. diversus  | Carcass | S   | R   | R   | R   | S   | R   | S   |
|           | A20c | E. coli      | Carcass | S   | R   | R   | R   | S   | R   | R   |
|           | A21c | E. coli      | Carcass | R   | R   | R   | R   | S   | R   | R   |
|           | A25c | E. coli      | Carcass | S   | I   | S   | R   | S   | R   | R   |
|           | A25d | E. coli      | Thaw   | I   | R   | R   | S   | S   | R   | R   |
|           | A26c | C. freundii  | Carcass | I   | R   | R   | S   | S   | R   | R   |
|           | A26d | C. freundii  | Carcass | S   | R   | R   | R   | I   | S   | R   | R   |
|           | A27d | E. coli      | Thaw   | S   | R   | R   | R   | S   | R   | R   |
|           | A29c | E. coli      | Carcass | S   | R   | R   | S   | S   | R   | R   |
|           | A30c | E. coli      | Carcass | S   | R   | I   | S   | S   | R   | R   |
|           | A30d | E. coli      | Thaw   | R   | R   | R   | R   | S   | R   | R   |
|           | A33d | E. coli      | Thaw   | R   | R   | R   | S   | S   | S   | R   | R   |
|           | A32d | E. coli      | Thaw   | S   | R   | R   | R   | S   | S   | R   | R   |
|           | B17c | E. coli      | Carcass | R   | S   | S   | S   | S   | S   | R   | S   |
| Brazil    | B18c | E. coli      | Carcass | R   | S   | R   | I   | R   | R   | S   | S   |
|           | B23d | C. freundii  | Thaw   | S   | S   | S   | S   | I   | S   | R   | S   |
|           | M15d | E. cloacae   | Thaw   | S   | R   | S   | R   | S   | R   | R   |
|           | M27c | E. coli      | Carcass | R   | R   | R   | R   | S   | R   | S   |
| Mozambique| M27d | E. coli      | Thaw   | R   | R   | R   | R   | S   | R   | R   |
|           | M30d | E. coli      | Thaw   | R   | R   | R   | R   | S   | R   | R   |
|           | M32c | E. coli      | Carcass | S   | S   | S   | S   | R   | S   | R   | R   |

R-resistant; S-sensitive; I-intermediate; AMC - amoxicillin/clavulanic acid (≥ 26-S, 23-25-I, ≤ 22-R); CTX - ceftaxime (≥ 18-S, 14-17-I, ≤ 13-R); CAZ - ceftazidime (≥ 21-S, 18-20-I, ≤ 17-R); CRO-ceftriaxone (≥ 23-S, 20-22-I, ≤ 19-R); FOX-cefoxitin (≥ 18-S, 15-17-I, ≤ 14-R); GEN-gentamicin (≥ 15-S, 13-14-I, ≤ 12-R), AMP-ampicillin (≥ 17-S, 13-16-I, ≤ 13-R); SXT – trimethoprim-sulphamethoxazole (≥ 16-S, 11-15-I, ≤ 10-R); CIP-ciprofloxacin (≥ 21-S, 16-20-I, ≤ 15-R).
(4 from South Africa and 1 from Mozambique) yielded positive results from both the carcass swab and the liquid thaw (12 isolates). Of the 27 ESBL-positive isolates, 19, 5 and 3 isolates were from South African, Mozambican and Brazilian chickens, respectively. The isolates were identified as *Citrobacter diversus* (n = 1), *Citrobacter freundii* (n = 3), *Enterobacter agglomerans* (n = 1), *Enterobacter cloacae* (n = 1) and *E. coli* (n = 21) (Tables 1 and 2).

**Susceptibility testing results**

Resistance to ampicillin was 100%, followed by resistance to ceftriaxone (89%), gentamicin (78%), trimethoprim-sulfamethoxazole (78%), cefotaxime (74%), ciprofloxacin (70%), ceftazidime (67%), amoxicillin/clavulanic acid (22%) and cefoxitin (2%). Multi-drug resistance (resistance to three or more antibiotic classes) was evident in 19 (70.4%) isolates, with 79/-101/-164, with *bla*<sub>CTX-M</sub> being most common (Table 2). *bla*<sub>MOX</sub> (33.3%) and *bla*<sub>TEM</sub> (33.3%) were the most prevalent pAmpC genes and *qnr* and *qepA* genes (22.2%) were the most common fluoroquinolone resistance genes (TEM, SHV and AmpC and *qnr* genes were not sequenced).

The majority of genes were isolated from *E. coli*, where SHV was the most prevalent. *bla*<sub>CTX-M-55</sub> was only detected in *Citrobacter* spp. While *bla*<sub>CTX-M-79/-101/-164</sub> were identified in *E. coli*. Resistance gene content ranged from 0-9 in several permutations and combinations (Figure 1).

Based on ERIC-PCR profiles (Figure 1), the *E. coli* isolates had similarities in banding patterns varying from 4 to 15 fragments, ranging in size from 0.5 to 20 kb in length and allowed the differentiation of the 21 *E. coli* isolates into 14 ERIC-types which were grouped into seven clusters (C1-7), with the majority of the

**PCR amplification results**

PCR results indicated that the predominant ESBL gene was *bla*<sub>SHV</sub> (85.0%), followed by *bla*<sub>CTX-M</sub> (62.9%) and *bla*<sub>TEM</sub> (44.4%). DNA sequencing of a random selection of CTX-M amplicons identified *bla*<sub>CTX-M-55/-79/-101/-164</sub> with *bla*<sub>CTX-M-164</sub> being most common (Table 2). *bla*<sub>MOX</sub> (33.3%) and *bla*<sub>TEM</sub> (33.3%) were the most prevalent pAmpC genes and *qnr* and *qepA* genes (22.2%) were the most common fluoroquinolone resistance genes (TEM, SHV and AmpC and *qnr* genes were not sequenced).

| Table 2. Antibiotic Resistance Genes Identified. |
|-----------------------------------------------|
| **Country** | **Code** | **Species** | **Source** | **β-lactam genes** | **Plasmid-mediated fluoroquinolone genes** |
|-------------|----------|-------------|------------|---------------------|------------------------------------------|
|             |          |             | CTX-M | SHV | TEM | CMY | MOX | FOX | DHA | qnrA | qnrB | qnrD | qnrS | qepA |
| Brazil      | B17c     | *E. coli*   | Carcass | +   | -   | -   | -   | -   | -   | +    | -    | -    | -    | -    |
|             | B18c     | *E. coli*   | Carcass | +   | +   | +   | +   | +   | +   | -    | -    | -    | -    | -    |
|             | B23d     | *C. freundii* | Thaw | -   | -   | -   | -   | -   | -   | -    | -    | -    | -    | -    |
|             | M15d     | *E. cloacae* | Thaw | -   | -   | -   | -   | -   | -   | -    | -    | -    | -    | -    |
|             | M27c     | *E. coli*   | Carcass | -   | -   | -   | -   | -   | -   | -    | -    | -    | -    | -    |
|             | M27d     | *E. coli*   | Thaw | -   | -   | -   | -   | -   | -   | -    | -    | -    | -    | -    |
|             | M30d     | *E. coli*   | Thaw | +   | +   | +   | +   | +   | +   | -    | -    | -    | -    | -    |
|             | M32c     | *E. coli*   | Carcass | +   | +   | +   | +   | +   | +   | +    | -    | -    | -    | -    |

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isolates being found in cluster 3 (Figure 1; C3). Most of the Brazilian and Mozambican samples were observed in clusters C3-5 while South African samples shared more similarity.

The ERIC-PCR fingerprint profiles of the four *Citrobacter* spp. isolates are shown in Figure 2. Isolates exhibited banding patterns varying from 5 to 18 fragments of 0.5 to 20 kb in length and the *C. freundii* isolates were not clonal. Although the CTX-M and SHV genes were identified in (3/4) 75% of these isolates, variation was observed in pAmpC and quinolone resistance gene content.

**Discussion**

Plasmid-mediated antibiotic resistance to third-generation cephalosporins, cephamycins and fluoroquinolones is increasingly being reported in meat products such as chicken [21—23] which is the most popular animal protein in Mozambique. Susceptibility testing results showed high levels of resistance to the antibiotics tested. Resistance to ampicillin was 100%, followed by 89% to ceftriaxone, 74% to cefotaxime, 70% to ciprofloxacin, 67% to ceftazidime and 22% to cefoxitin. These results were similar to a study on poultry from retail outlets in Hannover, Germany, which had been imported from Italy, where 100% resistance was observed to ampicillin while resistance
to cefotaxime was higher at 94% and resistance to ceftazidime was lower at 30% [21]. The resistance recorded for ceftriaxone was 90% in a study carried out in Owerri, Nigeria, which investigated the presence of ESBL-producing E. coli from poultry, but, in contrast to our study, 100% resistance to ceftazidime and cefotaxime was observed [22]. In an Italian study, the resistance of bacteria isolated from 163 broiler chickens to cefotaxime and ceftazidime were also higher at 91.7% for both antibiotics [23].

The ESBL frequency of 13.6% in this study was lower than that reported in a study that characterized PMQR determinants, β-lactamases, plasmids and clonality among commensal E. coli isolated from 100 healthy chickens at slaughterhouse in Ibadan, Nigeria where ESBL-positive strains were isolated from 15% (15/100) of the chickens [24] as well as a study in Anhui Province, China where 49.5% (100/202) of chickens yielded ESBL-positive E. coli isolates [16]. The majority of ESBL-producing Enterobacteriaceae were isolated in South African chicken, followed by Mozambican and Brazilian chicken. This may be related to selection pressure of antibiotics used as growth promoters, for prophylaxis and metaphylaxis in South Africa (D. Petty, personal communication, May 14, 2016). E. coli was the predominant bacterial species isolated, which is consistent with the observation that E. coli is one of ESBL-producing bacteria that is often isolated in greater numbers in food-producing animals [25]. Similar results were found in healthy broiler chickens in Germany, where E. coli and E. cloacae were identified [21].

The β-lactam antibiotic susceptibility profiles were corroborated by the ESBLs and pAmpC genes identified in 78.9% (15/19) of the South African isolates, 60.0% (3/5) of Mozambican isolates and 33% (1/3) of Brazilian isolates (Table 1). Examples of anomalies were E. coli A25c which carried the TEM, MOX and DHA genes but was sensitive to ceftazidime and cefoxitin, E. coli A23c which carried only the TEM gene but was resistant to all the β-lactams tested and E. coli M32c, B18c and B23d which all carried the CTX-M genes but were sensitive to cefotaxime. These anomalies point to silent or minimally expressed genes in the main while the expression of ESBLs and pAmpCs belonging to other families is also possible.

Fluoroquinolone resistance was evident only in the South African isolates. Resistance to ciprofloxacin was 70.4%, lower than that found in E. coli from retail broiler chicken in Italy (88.8%) [23] and higher than in E. coli (39%) from German retail outlets selling poultry of Italian origin [21]. In this study, a higher prevalence of qepA (22.2%), qnrB (18.5%) and qnrS (18.5%) genes was detected compared to the study in Nigeria where the prevalence was lower at 3.1%, 4.2% and 9.4%, respectively [24] while the study in Anhui Province, China, reported qmS as the most prevalent with none of the isolates being positive for qnrA, qnrB and qepA genes [16]. Six isolates exhibited resistance in the absence of qnr and qep genes indicating alternative mechanisms of resistance such as chromosomal mutations in the quinolone resistance determining regions of the gyrA, gyrB, parC and/or parE target genes or efflux [25,26].

Co-carriage of ESBL, pAmpC and plasmid-mediated quinolone resistance genes observed in this study has been similarly observed in a study on retail broiler chicken in Italy [23], albeit not at the same level. ESBL, AmpC and PMQR determinants were detected at a frequency of 98.5%, 11.2% and 91%, respectively in the Italian study, while in our study ESBL and PMQR determinants were detected at a lower frequency and AmpC frequency was higher at 23%. In several studies related to the ESBL-producing bacteria in chicken, the gene most frequently isolated is blactxM [21,27], which differs from this study where the most common ESBL gene was blashv, blactxM-M3, blactxM-M79, blactxM-T16 and blactxM-M164 genes were definitively identified in this study, different from other studies where blactxM-1 was most prevalent [10,11,27,28]. CTX-M-79 and CTXM-101 were found in Northeast China (Heilongjiang, Liaoning, Jilin) and in the Jiangsu province in a study characterizing ESBLs in E. coli from chickens, although with lower frequencies of 3.6% and 0.5%, respectively [29].

Among the three countries, E. coli with blactxM-M55, blactxM-79 and blactxM-T101 genes were only detected in South African isolates while the blactxM-M164 gene was detected in two South African and one Brazilian isolates. blactxM-M55 was only detected in C. freundii isolates from South African chickens. To our knowledge, this is the first report of the blactxM-M164 in chicken.

The CTX-M ESBLs found in our study are dissimilar to those isolated in humans as evident from a non-systematic literature review of research published in 2008-2012, which described the prevalence of CTX-M 1, 3, 9, 14a, 14b, 15, 27 and 28 in Enterobacteriaceae from hospital and community settings in Africa [30]. Other studies have, in contrast, showed similarity between ESBLs isolated from E. coli in chicken meat and humans [11].

Overall, the ERIC-PCR dendrogram showed extensive diversity of E. coli isolates, however, there
were some isolates that demonstrated similarity, especially those of South African origin. Mozambican *E. coli* isolates shared some similarity with South African and Brazilian isolates. Interestingly, only South African *E. coli* isolates carried plasmid-mediated quinolone resistance genes while isolates from all three origins harbored ESBL and pAmpC genes in different permutations and combinations. There was no correlation between the ERIC-PCR profiles and resistance genes identified. Isolates with similar profiles demonstrated different resistance gene content, e.g., M27D (SHV) and B18C (CTX-M, TEM, CMY, MOX and DHA) in cluster C3 and A10D (CTX-M, SHV, TEM, MOX, FOX, DHA, *qnrB*, *qnrS* and *qepA*) and A12C (CTX-M, SHV and TEM) in cluster C1. CTX-M and SHV genes were amplified from isolates belonging to all clusters, however the *qnr* and *qepA* genes were identified in South African isolates clustering in C1-3.

**Conclusion**

This study describes the presence of β-lactam and fluoroquinolone-resistant bacteria in chickens consumed in Mozambique. This represents a food safety concern as the Mozambican population is exposed to a reservoir of plasmid-mediated, and hence mobile β-lactam and quinolone resistance genes via imported, and to a lesser extent, locally produced poultry.

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### Annex – Supplementary Items

**Supplementary Table 1. Primers and Annealing Temperatures.**

| Gene | Sequence (5’ to 3’) | Annealing temp (°C) | Reference |
|------|---------------------|---------------------|-----------|
| CTX-M | F - GGTAAAAAATCACTGCGTC  
R - TGGTGACGAATTTAGCCGC | 53 | [17] |
| TEM  | F - AAAATTCTTGAGACG  
R - TTACCAATGCTTAATCA | 60 | [17] |
| SHV  | F - TTAACCTCCTGGTAGCCA  
R - GATTGCTGATTTCGCC | 56 | [17] |
| CMY  | F - GATCCCTTGGACTCTTCAG  
R - TAAAACCAGGTTCCCAATAGC | 50 | [18] |
| MOX  | F - GCTGCTCAAGGAGCACAGGAT  
R - CACATGGACATAGGTGTGGTG | 50 | [18] |
| FOX  | F - CACCAGAGAATAACCAT  
R - ATGGGACGCCTGAAACT | 50 | [18] |
| DHA  | F - AACTTTCACAGGTGTGCTGGGT  
R - CCGTACGCAACTGGCTTTTG | 50 | [18] |
| qnrB | F - GGAATCGAAATTCGCCACTG  
R - TTTGCCTCGCCAGTCGAA | 49 | [17] |
| qnrD | F - AGATCAATTACGGGAATA  
R - AACAGCTGAAAGCGCCTG | 49 | [19] |
| qnrS | F - CACTTTGATGGTCGACAGT  
R - CAAACTAACCAGTGTGGT | 49 | [20] |
| qepA | F - CGATGAGCAAGCAGCAAGG  
R - CTACGGGGCTCAAGCAGTGGG | 49 | [17] |

F: Forward; R: Reverse.