High Prevalence of CTX-M Type Extended-Spectrum Beta-Lactamase Genes and Detection of NDM-1 Carbapenemase Gene in Extraintestinal Pathogenic Escherichia coli in Cuba

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Abstract: Increase of extraintestinal pathogenic Escherichia coli (ExPEC) showing resistance to beta-lactams is a major public health concern. This study was conducted as a first molecular epidemiological study on ExPEC in Cuba, regarding prevalence of extended-spectrum beta-lactamas (ESBLs) and carbapenemase genes. A total of 306 ExPEC isolates collected in medical institutions in 16 regions in Cuba (2014–2018) were analyzed for their genotypes and presence of genes encoding ESBL, carbapenemase, plasmid-mediated quinolone resistance (PMQR) determinants by PCR and sequencing. The most common phylogenetic group of ExPEC was B2 (49%), followed by D (23%), A (21%), and B1 (7%). Among ESBL genes detected, blaCTX-M was the most common and detected in 61% of ExPEC, with blaCTX-M-15 being dominant and distributed to all the phylogenetic groups. NDM-1 type carbapenemase gene was identified in two isolates of phylogenetic group B1-ST448. Phylogenic group B2 ExPEC belonged to mostly ST131 (or its single-locus variant) with O25b allele, harboring blaCTX-M-27, and included an isolate of emerging type ST1193. aac (6’)-Ib-cr was the most prevalent PMQR gene (40.5%), being present in 54.5% of CTX-M-positive isolates. These results indicated high prevalence of CTX-M genes and the emergence of NDM-1 gene among recent ExPEC in Cuba, depicting an alarming situation.

Keywords: extraintestinal pathogenic E. coli (ExPEC); extended-spectrum beta-lactamase (ESBL); carbapenemase; NDM-1; Cuba

1. Introduction

Escherichia coli is the most representative Gram-negative bacteria in the intestinal tracts of humans and animals as a commensal organism. However, infectious diseases in humans are caused by a group of E. coli strains, e.g., pathogenic E. coli, which is classified into diarrheagenic E. coli and extraintestinal pathogenic E. coli (ExPEC). While both pathogenic E. coli are distributed globally, ExPEC has been
described as the common cause of community-acquired urinary tract infections and bloodstream infections [1]. Among the four major phylogenetic groups (i.e., A, B1, B2, D), ExPEC strains mainly belong to group B2, and to a lesser extent, group D. Although ExPEC consists of strains with many lineages, only a subset of lineages represented by sequence type (ST) 131 (ST131) is responsible for majority of infections in humans and considered a pandemic multiresistant clone [2].

Acquisition of drug resistance by ExPEC, as well as spread of multidrug-resistant ExPEC, has been established as a global public health concern. Most importantly, increased prevalence of the \textit{E. coli} resistant to beta-lactam antibiotics raises morbidity and mortality with social costs [3,4]. Beta-lactamases that represent the most common cause of resistance to this class of antimicrobials have been classified into four major groups; penicillinas, cephalosporinas, extended-spectrum beta-lactamases (ESBLs), and carbapenemases. ESBLs hydrolyze all the beta-lactams except carbapenems and cephams, and are inactivated by beta-lactamase inhibitors such as clavulanic acid, sulbactam or tazobactam. ESBLs consist of three main types, TEM, SHV, and CTX-M, among which CTX-M is dominant and has become disseminated globally, with CTX-M-14 and CTX-M-15 being the major genotypes [5]. In addition to ESBL, AmpC beta-lactamases are also responsible for resistance to broad-spectrum cephalosporins [6]. Particularly, plasmid-mediated AmpC (pAmpC) carried by \textit{E. coli} and other \textit{Enterobacteriaceae} species has become a major clinical concern due to its resistance traits and transferability [7]. Carbapenem resistance in \textit{Enterobacteriaceae} is primarily mediated by carbapenemases, which have been classified into Ambler class A (KPC, IMI, GES types), B (NDM, IMP, VIM types), and D (OXA types) enzymes [8]. Among them, class B carbapenemases (metallo beta-lactamases) exhibit a broad spectrum of activity to all penicillins, cephalosporins and carbapenems except for aztreonam. NDM is recognized as an emerging class B carbapenemase, and NDM-producing \textit{E. coli} have been reported around the world [9].

Fluoroquinolone-resistant \textit{E. coli} has been increasing as a uropathogen globally, following the extensive use of this antibiotics, often associated with the increasing trend of ESBL-producing ExPEC [10]. Resistance to fluoroquinolone is caused primarily by occurrence of mutation(s) in GyrA subunit of DNA gyrase and ParC subunit of topoisomerase IV. In addition, plasmid-mediated quinolone resistance (PMQR) determinants represented by Qnr, aac (6’)-Ib-cr, and QepA confer reduced susceptibility to fluoroquinolones [11].

In Cuba, drug resistance and prevalence of beta-lactamase genes in ExPEC has been scarcely studied to date, and mechanism of fluoroquinolone resistance has never been analyzed. Although only an available report showed prevalence of TEM and SHV for hospital isolates collected from 2002 to 2004 [12], there has been no report in Cuba regarding prevalence and types of beta-lactamase genes and genotypes of \textit{E. coli} showing resistance to beta-lactams. The present study was conducted to investigate drug resistance, prevalence and genetic characteristics of recent ExPEC isolates harboring ESBL, pAmpC, carbapenemase genes and PMQR genes from whole regions in Cuba. We report here high prevalence of ST131 ExPEC carrying CTX-M-15 or CTX-M-27 genes, and first identification of NDM-1 gene in ST448 \textit{E. coli} in Cuba.

\textbf{2. Results}

Among a total of 306 \textit{E. coli} isolates, the dominant phylogenetic group was B2 (49%), followed by group D (23%) and A (21%) (Table 1). Resistance rates to 18 antimicrobials in the decreasing order are as follows: nalidixic acid (77.4%), norfloxacin (77.1%), cefotaxime (76.1%), ciprofloxacin (76.1%), cefuroxime (73.9%), ceftazidime (70%), cefepime (68.3%), trimethoprim-sulfamethoxazole (67%), aztreonam (53%), gentamicin (44.7%), tobramycin (40.7%), cefoxitin (22%), piperacillin-tazobactam (17%), amikacin (6.9%), fosfomycin (5.5%), meropenem (5.2%), imipenem (2.3%) and colistin (0.7%).

The most prevalent beta-lactamase gene was \textit{bla}_{CTX-M} (61.1%), followed by \textit{bla}_{TEM} (31.7%) while the pAmpC gene (\textit{bla}_{CMV-2}) and carbapenemase gene (\textit{bla}_{NDM-1}) were detected in four (1.4%) and two (0.7%) isolates, respectively (Table 1). The two NDM-1-positive isolates were derived from patients in different provinces, although information of their overseas travel history was not available. Most CTX-M gene belonged to CTX-M-1 group which was found in 54.9% of all isolates and accounted
for 90% of blaCTX-M genes. CTX-M-1 group beta-lactamase gene was detected in all the phylogenetic groups at the rate of 52–59% (phylogenetic group A, 57.8%; B1, 52.4%; B2, 52%; D, 59%). Most of blaCTX-M-9 group was found in phylogenetic group B2 isolates, while blaCTX-M-2 group was detected in two isolates of group A. TEM gene was distributed to all the phylogenetic groups. More than half of TEM gene (54.6%, 53/97) was associated with CTX-M gene. Accordingly, 17.3% of ExPEC isolates had both blaTEM and blaCTX-M. CMY-2 and NDM-1 genes were identified in four and two isolates, respectively. Carbapenemase genes encoding VIM, IMP, KPC, and OXA-48 were not detected.

Table 1. Prevalence of beta-lactamase genes and plasmid-mediated quinolone resistance (PMQR) genes in E. coli isolates (n = 306, 2014–2018).

| Beta-Lactamase Gene (Genotype) / PMQR Determinant | Number of Isolates in Phylogenetic Group (%) | Total |
|-----------------------------------------------|---------------------------------------------|-------|
|                                               | A (n = 64) | B1 (n = 21) | B2 (n = 150) | D (n = 71) | (n = 306) |
| CTX-M                                         |            |            |             |            |          |
| CTX-M-1 group                                 | 39 (60.9)  | 11 (52.4)  | 94 (62.7)   | 43 (60.6)  | 187 (61.1) |
| CTX-M-2 group                                 | 37 (57.8)  | 11 (52.4)  | 78 (52)     | 42 (59.2)  | 168 (54.9) |
| CTX-M-9 group                                 | 2 (3.1)    | 0           | 0           | 0           | 2 (0.7)   |
| TEM                                           | 0           | 0           | 16 (10.7)   | 1 (1.4)    | 17 (5.6)  |
| CTX-M *3 + TEM                                | 16 (25)    | 7 (33.3)   | 47 (31.3)   | 27 (38.0)  | 97 (31.7) |
| NDM-1                                         | 0           | 2 (9.5)    | 0           | 0           | 2 (0.7)   |
| CMY-2                                         | 1 (1.6)    | 3 (14.3)   | 0           | 0           | 4 (1.3)   |
| PMQR                                          |            |            |             |            |          |
| aac (6′)-Ib-cr                                | 27 (42.2)  | 8 (38.1)   | 71 (47.3)   | 18 (25.4)  | 124 (40.5) |
| qnrB                                          | 16 (25)    | 3 (14.3)   | 5 (3.3)     | 17 (23.9)  | 41 (13.4) |
| qnrD                                          | 1 (1.6)    | 0           | 1 (0.7)     | 0           | 2 (0.7)   |
| qnrS                                          | 4 (6.3)    | 1 (4.8)    | 2 (1.3)     | 2 (2.8)    | 9 (2.9)   |
| oqxAB                                         | 2 (3.1)    | 0           | 0           | 0           | 2 (0.7)   |
| CTX-M *4 + aac (6′)-Ib-cr                     | 22 (34.4)  | 7 (33.3)   | 57 (38)     | 16 (22.5)  | 102 (33.3) |
| O25b allele                                   | 0           | 0           | 106 (70.7)  | 0           | 106 (34.6) |

*1 Following genes were not detected in any isolate: qnrA, qnrC and qepA. *2 CTX-M-27, 16 isolates; CTX-M-14, 1 isolate. *3 CTX-M-1 group gene, except for CTX-M-9 group gene detected in four isolates (three and one isolate of phylogenetic group B2 and D, respectively). *4 CTX-M-1 group gene, except for CTX-M-9 group gene detected in one isolate (phylogenetic group B2).

Five PMQR genes (aac (6′)-Ib-cr, qnrB, qnrD, qnrS, and oqxAB) were detected, with aac (6′)-Ib-cr being the most common (40.5% of all the isolates), followed by qnrB and qnrS (Table 1). Detection rate of aac (6′)-Ib-cr was significantly higher among phylogenetic group B2 (47%) (p < 0.05), than group A (42%), B1 (38%), and D (25%). O25b allele was detected in only phylogenetic group B2 with a rate of 70.7% (106/150). Isolates positive for both aac (6′)-Ib-cr and blaCTX-M accounted for 33% (n = 102) of all isolates. Among CTX-M-positive isolates, prevalence of aac (6′)-Ib-cr was 54.5%, which was higher than those of qnrB and qnrS (Table S1).

Genotypes of E. coli (ST, fimH) and beta-lactamases were determined for a total of 71 ExPEC isolates consisting of 16, 11, 30, and 14 isolates of phylogenetic groups A, B1, B2, and D, respectively, and summarized in Table 2. These isolates were selected from different provinces and different specimens, in each year, and included ExPEC harboring one of the six CTX-M genes (group 1, blaCTX-M-15, blaCTX-M-32, blaCTX-M-55; group 2, blaCTX-M-27; group 9, blaCTX-M-14, blaCTX-M-27), and those with TEM (blaTEM-1), CMY (blaCMY-2), or NDM (blaNDM-1). Common STs of phylogenetic group A isolates were ST10, ST410, and their single-locus variant (SLV) and double-locus variant (DLV), and these isolates harbored mostly blaCTX-M-15 and any of the PMQR genes. ST448 was detected in only phylogenetic group B1 ExPEC, and two ST448 isolates possessed NDM-1 gene. Isolates with ST405 and its variants were commonly identified among phylogenetic group D. Except for only a single isolate of ST1193, all the phylogenetic group B2 isolates belonged to ST131 or its SLVs (five STs), and harbored blaCTX-M-15 or blaCTX-M-27. Eighteen isolates were positive for O25b allele, and classified into fimH-30 type. Among the five ST131 SLV detected in phylogroup B2, three STs (ST5716, ST5717, ST5718) were newly identified in the present study.
Table 2. Genotypes and antimicrobial resistance profile of representative *E. coli* strains of phylogenetic group A, B1, B2 and D isolated in Cuba (n = 71).

| Strain ID (IPK) | Year  | Specimen  | Patient Age, Sex | Province | Phylogenetic Group | ST *2   | Allelic Profile *3 | O25b Allele *4 | fimH Type *4 | Beta-Lactamase Gene | PMQR *5 Gene | Antimicrobial Resistance Profile *6 |
|-----------------|-------|-----------|------------------|----------|--------------------|--------|--------------------|---------------|--------------|---------------------|--------------|-------------------------------------|
| 25              | 2014  | wound     | Adult, F         | LH       | A                  | ST1463 | 6-95-4-222-7-7-7  | ND            | ND          | β-lactam-1, β-lactam-2 | qnrB         | CAZ, CTX, CXM, FOX, TOB          |
| 86              | 2014  | blood     | Adult, F         | LH       | A                  | ST5715 (ST410 SLV) | 6-4-12-1-20-18-73 | ND            | ND          | β-lactam-1 | qnrB                   | CAZ, CTX, CXM, ATM, NAL, CIP, NOR, GEN, TOB, AMK, SXT |
| 110             | 2015  | peritoneal fluid | Adult, M       | LH       | A                  | ST167 (ST10 SLV) | 10-11-4-8-8-12-2 | ND            | ND          | β-lactam-15, aac (6')-Ib-cr, qnrB | TZIP, CTX, CXM, FOX, ATM, NAL, CIP, NOR, GEN, SXT |
| 118             | 2015  | wound     | Adult, M         | SC       | A                  | ST1488 (ST10 SLV) | 10-11-4-8-8-8-73 | ND            | ND          | β-lactam-15, β-lactam-2 | qnrS         | CTX, CXM, TOB, AMK          |
| 119             | 2015  | respiratory tissue | Adult, M       | VC       | A                  | ST1488 (ST10 SLV) | 10-11-4-8-8-8-73 | ND            | ND          | β-lactam-15, aac (6')-Ib-cr | CTX, CXM, ATM, NAL, CIP, NOR, GEN, SXT |
| 145             | 2015  | urine     | Adult, F         | HG       | A                  | ST4238 (ST10 SLV) | 10-11-4-8-8-9-2   | ND            | ND          | β-lactam-15, β-lactam-1 | qnrB         | CTX, CXM, FEP, ATM, GEN, SXT   |
| 152             | 2015  | wound     | Adult, F         | CF       | A                  | ST10 (ST10 Cpx)    | 10-11-4-8-8-8-2   | ND            | ND          | β-lactam-15 | qnrS                   | CTX, CXM, ATM         |
| 121             | 2015  | wound     | Adult, F         | PR       | A                  | ST156 (ST156 Cpx)  | 6-29-32-16-11-8-44 | ND            | ND          | β-lactam-15, β-lactam-1 | qnrB         | CTX, CXM, FEP, ATM, GEN, SXT   |
| 107             | 2015  | urine     | Adult, F         | LH       | A                  | ST166 DLV | 52-746-55-53-40-422-43 | ND            | ND          | qnrD                   | CXM                |
| 192             | 2016  | respiratory tissue | Adult, M         | HG      | A                  | ST1437 | 10-27-5-8-8-1-2  | ND            | ND          | β-lactam-32 | qnrB                   | CAZ, CTX, FEP, NAL, CIP, GEN, ATM |
| 204             | 2016  | placenta  | Adult, F         | MT       | A                  | ST1421 | 8-7-1-8-8-8-2   | ND            | ND          | β-lactam-32 | qnrB                   | CAZ, CTX, FEP, NAL, CIP, GEN, ATM |
| 266             | 2016  | urine     | Adult, F         | LH       | A                  | ST410  | 6-4-12-1-20-18-7 | ND            | ND          | β-lactam-1 | qnrB                   | FOX, FEP, CIP, NOR, NAL, SXT |
| 290             | 2016  | urine     | Child, F         | HG       | A                  | ST735 SLV | 92-11-4-8-8-9-295 | ND            | ND          | qnrB                   | CIP, NOR, NAL                  |
| 75              | 2018  | urine     | Child, M         | LH       | A                  | ST410 DLV | 6-4-281-1-20-12-7 | ND            | ND          | β-lactam-15, aac (6')-Ib-cr, qnrB | NAL, CIP, NOR, SXT    |
| 543             | 2018  | blood     | Adult, M         | SC       | A                  | ST410  | 6-4-12-1-20-18-7 | ND            | ND          | β-lactam-15 | qnrS                   | TZIP, CAZ, CTX, FEP, FOX, NAL, NAL, CIP, MEM, IPM, GEN, AMK, SXT |
| 556             | 2018  | blood     | Child, M         | LH       | A                  | ST410 DLV | 6-4-281-1-20-12-7 | ND            | ND          | β-lactam-15, β-lactam-1 | qnrB         | CAZ, SXT               |
| 17              | 2014  | urine     | Adult, F         | SS       | B1                 | ST156  | 6-29-32-16-11-8-44 | ND            | ND          | β-lactam-1 | qnrB                   | CAZ, SXT               |
Table 2. Cont.

| Strain ID (IPK) | Year | Specimen | Age, Sex | Province | Phenylogenetic Group | ST * 2 | Allelic Profile * 3 | O25b Allele * 4 | fimH Type * 4 | Beta-Lactamase Gene | PMQR * 5 | Antimicrobial Resistance Profile * 6 |
|----------------|------|----------|----------|----------|----------------------|--------|---------------------|----------------|--------------|---------------------|----------|-------------------------------------|
| 101            | 2015 | respiratory tissue | Adult, M | LH | B1 | ST641 (ST86 Cpx) | 9-6-3-33-131-24-8-7 | ND | ND | blaCTX-M-15, aac (6)′-Ib-cr, qnrB | CTX, CXM, ATM |
| 182            | 2016 | urine | Adult, M | SC | B1 | ST448 | 6-6-5-16-11-8-7 | ND | ND | blaNDM-1, blaCTX-M-15 | CXM, CTX, FOX, FEP, NAL, CIP, NOR, MEM, IPM |
| 184            | 2016 | kidney | Adult, M | VC | B1 | ST448 | 6-6-5-16-11-8-7 | ND | ND | blaNDM-1, blaCTX-M-15 | CXM, CTX, FEP, NAL, CIP, FOF, TOB, ATM, MEM, IPM |
| 191            | 2016 | sputum | Adult, M | HG | B1 | ST162 (ST469 Cpx) | 9-65-51-9-13-6 | ND | ND | blaCTX-M-15 | CXM, CTX, FEP, NAL, CIP, FOF, TOB, ATM |
| 216            | 2016 | wound | Child, F | SC | B1 | ST23 (ST23 Cpx) | 6-4-12-1-20-13-7 | ND | ND | blaCTX-M-15, aac (6)′-Ib-cr, qnrS | CTX, CIP, NOR, SXT |
| 152            | 2018 | urine | Adult, F | LH | B1 | ST224 | 6-4-33-16-11-8-6 | ND | ND | blaCMV-2, aac (6)′-Ib-cr | CTX, CIP, NOR, SXT |
| 185            | 2018 | urine | Adult, M | LH | B1 | ST448 | 6-6-5-16-11-8-7 | ND | ND | blaCTX-M-15, aac (6)′-Ib-cr | CAZ, CTX, FEP, CIP, MEM, GEN, SXT |
| 232            | 2018 | skin | Adult, F | LH | B1 | ST448 | 6-6-5-16-11-8-7 | ND | ND | blaCTX-M-15, aac (6)′-Ib-cr, qnrS | CAZ, CTX, FEP, FOF, CIP, MEM, AMK, SXT |
| 373            | 2018 | skin | Adult, F | LH | B1 | ST448 | 6-6-5-16-11-8-7 | ND | ND | blaCTX-M-15, aac (6)′-Ib-cr | TZIP, CAZ, CTX, FOX, FEP, CIP, MEM, AMK, SXT |
| 544            | 2018 | blood | Adult, M | SC | B1 | ST4173 | 6-6-32-16-9-7-6 | ND | ND | blaCTX-M-15, aac (6)′-Ib-cr | CAZ, CTX, FEP, FOF, CIP, SXT |
| 19             | 2014 | blood | Adult, F | GT | B2 | ST5718 (ST131 SLV) | 36-40-9-13-17-11-25 | - | ND | qnrD | SXT |
| 45             | 2014 | endotracheal tube | Adult, M | SC | B2 | ST131 | 53-40-47-13-36-28-29 | O25b fimH-30 blaCTX-M-27, aac (6)′-Ib-cr, qnrS | CAZ, CTX, CXM, ATM, CIP, NOR |
| 68             | 2014 | respiratory tissue | Adult, M | LH | B2 | ST131 | 53-40-47-13-36-28-29 | O25b fimH-30 blaCTX-M-27 | CAZ, CTX, CXM, FEP, ATM, NAL, CIP, NOR, MEM, IPM, CST, TOB, SXT |
| 69             | 2014 | respiratory tissue | Adult, M | LH | B2 | ST131 | 53-40-47-13-36-28-29 | - | ND | blaCTX-M-27, aac (6)′-Ib-cr, qnrB | CAZ, CTX, CXM, FEP, ATM, NAL, CIP, NOR, MEM, IPM, CST, TOB, SXT |
| 89             | 2014 | tracheal aspirate | Adult, M | LH | B2 | ST131 | 53-40-47-13-36-28-29 | - | ND | blaCTX-M-15, aac (6)′-Ib-cr | TZIP, CAZ, CTX, ATM, NAL, CIP, NOR, MEM, GEN, TOB, SXT |
Table 2. Cont.

| Strain ID (IPK) | Year | Specimen | Patient | Age, Sex | Province | Phylogenetic Group | ST | Allelic Profile | Allelic Profile | Allelic Profile | Allelic Profile | PMQR | Gene | Beta-Lactamase Gene | Antimicrobial Resistance Profile |
|----------------|------|----------|---------|----------|----------|--------------------|----|----------------|----------------|----------------|----------------|-------|------|-------------------|---------------------------------|
| 104            | 2015 | wound    | Adult, M| LH       | B2       | ST131              | 53-40-47-13-36-28-29 | -   | ND             | blαCTX-M-15       | aac (6')-Ib-cr | CTX, CMX, FEP, ATM, NAL, CIP, NOR, MEM, GEN, TOB, SXT |
| 117            | 2015 | wound    | Adult, F| SC       | B2       | ST131              | 53-40-47-13-36-28-29 | O25b | fimH-30         | blαCTX-M-15       | aac (6')-Ib-cr | CTX, CMX, ATM, NAL, CIP, NOR, GEN |
| 130            | 2015 | urine    | Adult, F| VC       | B2       | ST5717 (ST131 SLV) | 53-40-47-13-36-28-29-73 | -   | ND             | blαCTX-M-15       | aac (6')-Ib-cr | CAZ, CTX, CMX, FEP, ATM, NAL, CIP, NOR, SXT |
| 151            | 2015 | catheter | Adult, M| CF       | B2       | ST131              | 53-40-47-13-36-28-29 | O25b | fimH-30         | blαCTX-M-27       | CTX, CMX, FEP, NAL, CIP, NOR, SXT |
| 168            | 2015 | urine    | Adult, F| HG       | B2       | ST5716 (ST131 SLV) | 53-24-47-13-36-28-29 | O25b | fimH-30         | blαCTX-M-15       | aac (6')-Ib-cr | CTX, CMX, FEP, NAL, CIP, NOR, TOB, SXT |
| 177            | 2016 | respiratory tissue | Adult, M | VC | B2 | ST131 | 53-40-47-13-36-28-29 | O25b | fimH-30         | blαCTX-M-27       | aac (6')-Ib-cr | CAZ, CMX, CTX, FEP, NAL, CIP |
| 186            | 2016 | urine    | Child, M| CF       | B2       | ST131              | 53-40-47-13-36-28-29 | O25b | fimH-30         | blαCTX-M-27       | CAZ, CMX, CTX, FEP, NAL, CIP |
| 203            | 2016 | catheter | Adult, F| MT       | B2       | ST131              | 53-40-47-13-36-28-29 | O25b | fimH-30         | blαCTX-M-27       | SXT, CMX, CAZ, CTX, FEP, NAL, CIP |
| 222            | 2016 | urine    | Adult, F| LH       | B2       | ST131              | 53-40-47-13-36-28-29 | O25b | fimH-30         | blαCTX-M-15       | aac (6')-Ib-cr | CMX, CTX, FOX, FEP, NAL, CIP, MEM, IPM, TOB, AMK, SXT |
| 234            | 2016 | wound    | Adult, M| LH       | B2       | ST131              | 53-40-47-13-36-28-29 | -   | ND             | blαCTX-M-15       | aac (6')-Ib-cr | GEN, CTX, CIP |
| 283            | 2016 | endotracheal tube | Adult, M | LH | B2 | ST131 | 53-40-47-13-36-28-29 | O25b | fimH-30         | blαCTX-M-27       | CAZ, CTX, NAL, CIP, NOR, SXT |
| 288            | 2016 | urine    | Child, F| HG       | B2       | ST3185 (ST131 SLV) | 92-40-47-13-36-28-29 | O25b | fimH-30         | blαCTX-M-15       | qnrS            | CTX, FEP, NAL, CIP |
| 324            | 2016 | blood    | Adult, M| LH       | B2       | ST131              | 53-40-47-13-36-28-29 | -   | ND             | blαCTX-M-15       | aac (6')-Ib-cr | CAZ, FEP, NAL, CIP, NOR, MEM, IPM, GEN, SXT, CST |
| 37             | 2018 | urine    | Adult, F| LH       | B2       | ST131              | 53-40-47-13-36-28-29 | O25b | fimH-30         | blαCTX-M-27       | CAZ, ATM, CIP, NOR, SXT |
| 149            | 2018 | urine    | Adult, M| LH       | B2       | ST131              | 53-40-47-13-36-28-29 | O25b | fimH-30         | blαCTX-M-27       | CAZ, CIP, NOR, SXT |
| 194            | 2018 | urine    | Adult, F| LH       | B2       | ST3223 (ST131 SLV) | 10-40-47-13-36-28-29 | O25b | fimH-30         | blαCTX-M-15       | aac (6')-Ib-cr | CAZ, ATM, CIP, NOR |
| Strain ID (IPK) | Year | Specimen | Patient Age, Sex | Province 1 | Phylogenetic Group | ST * 2 | Allelic Profile * 3 | O25b Allele * 4 | fimH Type * 4 | Beta-Lactamase Gene | PMQR * 5 | Antimicrobial Resistance Profile * 6 |
|----------------|------|----------|------------------|------------|------------------|--------|------------------|----------------|---------------|------------------|----------|-------------------------------|
| 330            | 2018 | urine    | Adult, M         | LH         | B2               | ST131  | 53-40-47-13-36-28-29 | O25b          | fimH-30      | bla\(_{\text{CTX-M-15}}\) | Aac (6\(^{-}\))-Ib-cr, qnrB | ATM, CIP, NOR, SXT |
| 398            | 2018 | wound    | Child, F         | CF         | B2               | ST131  | 53-40-47-13-36-28-29 | O25b          | fimH-30      | bla\(_{\text{CTX-M-27}}\) |                    | CAZ, CTX, FEP, CIP, SXT |
| 401            | 2018 | endotracheal tube | Child, M     | CF         | B2               | ST131  | 53-40-47-13-36-28-29 | O25b          | fimH-30      | bla\(_{\text{CTX-M-15}}\) |                    | CAZ, CTX, FEP, CIP, SXT |
| 417            | 2018 | skin     | Adult, F         | LH         | B2               | ST131  | 53-40-47-13-36-28-29 | -             | ND           | bla\(_{\text{CTX-M-27}}\) |                    | CAZ, CTX, FEP, CIP, SXT |
| 506            | 2018 | skin     | Adult, F         | LH         | B2               | ST131  | 53-40-47-13-36-28-29 | O25b          | fimH-30      | bla\(_{\text{CTX-M-27}}\) |                    | CAZ, CTX, FEP, CIP, SXT |
| 528            | 2018 | sputum   | Adult, M         | IJ         | B2               | ST131  | 53-40-47-13-36-28-29 | -             | ND           | bla\(_{\text{CTX-M-15}}\) | aac (6\(^{-}\))-Ib-cr | CAZ, CTX, FEP, CIP, SXT |
| 602            | 2018 | urine    | Adult, F         | IJ         | B2               | ST131  | 53-40-47-13-36-28-29 | O25b          | fimH-30      | bla\(_{\text{CTX-M-27}}\), bla\(_{\text{TEM-1}}\) |                    | CAZ, NAL, CIP, SXT |
| 610            | 2018 | urine    | Adult, F         | IJ         | B2               | ST131  | 53-40-47-13-36-28-29 | -             | ND           | bla\(_{\text{CTX-M-27}}\) |                    | CAZ, NAL, CIP, SXT |
| 629            | 2018 | lochia   | Adult, F         | GT         | B2               | ST1193 | 14-14-10-200-17-7-10 | -             | ND           | bla\(_{\text{CTX-M-27}}\) |                    | CAZ, CTX, FEP, CIP, SXT |
| 15             | 2014 | wound    | Child, F         | HG         | D                | ST5162 (ST405 SLV) | 35-37-29-25-4-5-2 | ND           | ND           | bla\(_{\text{TEM-1}}\) | qnrB | TXP, CAZ, CTX, CXM, FOX, FEP, ATM, NAL, CIP, NOR, GEN, TOB, SXT |
| 65             | 2014 | respiratory tissue | Adult, F     | LH         | D                | ST405  | 35-37-29-25-4-5-73 | ND           | ND           | bla\(_{\text{CTX-M-15}}\) |                    | TZP, CAZ, CTX, CXM, FOX, FEP, ATM, NAL, CIP, NOR, GEN, TOB, SXT |
| 174            | 2015 | cerebrospinal fluid | Adult, M     | LH         | D                | ST405  | 35-37-29-25-4-5-73 | ND           | ND           | bla\(_{\text{CTX-M-55}}\) | aac (6\(^{-}\))-Ib-cr | TZP, CAZ, CTX, FOX, FEP, FOFOF, GEN, CIP, SXT |
| 217            | 2016 | urine    | Adult, F         | LH         | D                | ST3496 (ST405 DLV) | 35-37-29-382-4-8-73 | ND           | ND           | bla\(_{\text{CTX-M-15}}\) |                    | SXT, CXM, CTX, FOX, FEP, NAL, CIP, NOR, FOFOF, SXT |
| 258            | 2016 | urine    | Child, F         | LH         | D                | ST405  | 35-37-29-25-4-5-73 | ND           | ND           | bla\(_{\text{CTX-M-14}}\) |                    | CFZ, NAL, CIP, SXT |
| 261            | 2016 | cerebrospinal fluid | Adult, F     | AT         | D                | ST349  | 34-36-39-87-67-16-4 | ND           | ND           | qnrS            |                    | SXT |
Table 2. Cont.

| Strain ID (IPK) | Year | Specimen | Patient Age, Sex | Province 1 | Phylogenetic Group | ST 2 | Allelic Profile 3 | O25b Allele 4 | fimH Type 4 | Beta-Lactamase Gene | PMQR 5 | Gene | Antimicrobial Resistance Profile 6 |
|----------------|------|----------|------------------|------------|-------------------|------|------------------|--------------|-------------|----------------------|--------|------|----------------------------------|
| 274            | 2016 | blood    | Adult, M         | SC         | D                 | ST648| 92-4-78-96-78-58-2| ND           | ND          | blaCTX-M-15, blaTEM-1 | qnrS   |       | TZP, CAZ, CTX, FOX, FEP, ATM, CIP, NOR, NAL, SXT |
| 135            | 2017 | urine    | Adult, F         | SC         | D                 | ST405| 35-37-29-25-4-5-73| ND           | ND          | blaCTX-M-15             | aac(6')-Ib-cr |       | TZP, CTX, FOX, FEP, NAL, CIP, MEM, GEN, SXT |
| 517            | 2017 | lung tissue | Child, M        | CF         | D                 | ST405| 35-37-29-25-4-5-73| ND           | ND          | blaCTX-M-15             |       |       | CAZ, FOX, FEP, NAL, CIP, MEM, IPM, GEN, TEI, SXT |
| 148            | 2018 | urine    | Adult, F         | LH         | D                 | ST69 (ST69 Cplx) | 32-33-27-6-5-5-4 | ND           | ND          | blaTEM-1               |        |       | NAL                |
| 537            | 2018 | urine    | Adult, F         | SC         | D                 | ST405| 35-37-29-25-4-5-73| ND           | ND          | blaTEM-1               |        |       | NAL, CIP, GEN          |
| 605            | 2018 | urine    | Adult, F         | IJ         | D                 | ST69 (ST69 Cplx) | 21-35-27-6-5-5-4 | ND           | ND          | blaTEM-1               |        |       | NAL, SXT            |
| 622            | 2018 | blood    | Adult, F         | IJ         | D                 | ST68 | 33-26-2-31-5-16-19 | ND           | ND          | blaCTX-M-15, blaTEM-1 |        |       | TZP, CTX, GEN, AMK, SXT |
| 630            | 2018 | blood    | Adult, F         | IJ         | D                 | ST69 (ST69 Cplx) | 21-35-27-6-5-5-4 | ND           | ND          | blaTEM-1               |        |       | GEN, NAL             |

*1 Abbreviations of provinces: AT, Artemisa; CF, Cienfuegos; GT, Guantánamo; HG, Holguín; IJ, Isla de la Juventud; LH, La Habana; MT, Matanzas; PR, Pinar del Río; SC, Santiago de Cuba; SS, Sancti Spíritus; VC, Villa Clara. *2 SLV, single-locus variant; DLV, double-locus variant; Cplx, complex. *3 Underline represents variant locus number of SLV or DLV shown in the left column. *4 ND, not determined; −, negative. *5 Plasmid-mediated quinolone resistance. *6 Abbreviations of antimicrobials: AMK, amikacin; ATM, aztreonam; CAZ, ceftazidime; CIP, ciprofloxacin; CST, colistin; CTX, cefotaxime; CXM, cefuroxime; FEP, cefepime; FOF, fosfomycin; FOX, cefoxitin; GEN, gentamicin; IPM, imipenem; MEM, meropenem; NAL, nalidixic acid; NOR, norfloxacin; SXT, trimethoprim-sulfamethoxazole; TOB, tobramycin; TZP, piperacillin-tazobactam.
Mutations in QRDR in GyrA and ParC were analyzed for 39 isolates showing resistance to quinolones (Table S2). In most isolates, mutations were detected in both proteins. S83L, D87N mutations in GyrA and S80I, E84V mutations in ParC were the most commonly identified. Among the 22 isolates with double mutations in both GyrA and ParC, 14 isolates harbored aac (6′)-Ib-cr. Two isolates belonging to phylogenetic group B2 showed resistance to colistin (MIC, 16 and 8 mg/µL), while mcr-1, mcr-2, mcr-3 genes were not detected by PCR.

3. Discussion

In the present study, we observed high prevalence of E. coli positive for CTX-M gene (61%) as well as those resistant to ceftazidime (70%) and cefotaxime (76.1%). In a surveillance report from 11 Latin American countries (2011–2014), documented rates of CLSI ESBL screening phenotype in E. coli ranged from 14.7% (Brazil) to 69.9% (Mexico), while overall rate was 37.7% [13]. These rates were higher than previous surveillance (2008–2010) in the four Latin American countries representing ESBL rates as 18.1–48.4% [14]. Comparing these CTX-M-positive rates with those in our present study (2014–2018), Cuba is considered one of the countries showing highest prevalence of ESBL in E. coli among Latin America. The early study in Cuba (2002–2004, Havana city) reported that ESBL phenotype rate was 10%, associated with resistance rates to cefotaxime as 14.1% [12]. Accordingly, ESBL-producing E. coli is suggested to have increased drastically during the past decade in Cuba, similarly to the increasing trend in other Latin American countries.

In Cuba, CTX-M type beta-lactamase is considered to be virtually a predominant ESBL, because all the TEM genes analyzed were assigned to non-ESBL genotype (TEM-1). Among the CTX-M type beta-lactamase genes, blaCTX-M-15 was the dominant type, as reported in the United States [15] and Canada [16], and distributed to all the phylogenetic groups. Phylogenetic group B2 included ST131 E. coli with O25b allele harboring blaCTX-M-15, which is known as the pandemic clonal group of multidrug resistant ExPEC [17], was revealed to be prevalent in Cuba in our present study. Furthermore, it was notable that blaCTX-M-27 was detected in B2-ST131 isolates showing higher incidence than blaCTX-M-15. ST131 E. coli with blaCTX-M-27 has been rapidly increasing in the past decade in Asia, Europe, and north America [18]. We found unusually high prevalence of ST131 ExPEC with blaCTX-M-27, suggesting the need for further monitoring of this clone. In addition, rare CTX-M-types, blaCTX-M-32 and blaCTX-M-55 were detected in phylogenetic group A isolates. CTX-M-55 has been reported as an emerging ESBL type among humans, animals, and the environment. [19], while blaCTX-M-32 is often associated with cattle and meat products [20]. Recently in Cuba, blaCTX-M-32 was identified in plasmid of an E. coli strain isolated from a healthy pig [21]. Hence, there may be a possibility that E. coli having CTX-M-32 and -55 genes might be derived from animal or environmental origin.

Since the first identification of NDM-1 in 2008, NDM-type carbapenemase has attracted worldwide attention because of its rapid dissemination among Gram-negative bacteria that caused infections or colonization in human and animals, and also those distributed to environments [22]. Main reservoir of NDM producers is regarded as South Asia, while secondary reservoir is considered the Balkan regions and the Middle East [23]. Among NDM variants that have been discriminated into more than 20 types, NDM-1 is the most common globally, and distributed mainly to specific clones (53 STs) of E. coli with ST101, ST167, ST131, ST405, ST40, and ST648 being more frequently identified. In Latin America, prevalence of carbapenem-resistant E. coli has been extremely low [13,24], and E. coli carrying NDM gene has been rarely reported [25]. Only isolates of ST10 and ST617 E. coli from nosocomial outbreaks were reported to harbor blanDM-1 in Mexico [25,26]. In our present study, NDM-1 gene was identified in two ExPEC isolates of phylogenetic group B1-ST448 that were isolated from sporadic infections in 2016, representing NDM-detection rate of 0.7% (2/306). This is the first report of NDM-1 gene in E. coli in Cuba, although we identified blanDM-1 in a rare Acinetobacter species, A. soli in 2011 [27]. It was also remarkable in the present study that blanDM-1 was detected in a rare E. coli clone, ST448. This clone harboring NDM gene has been identified in only limited reports in Asia (The Middle East and India) and Europe (Spain, Poland), and associated with various NDM-types, with NDM-5 being...
common [22,28,29]. Furthermore, KPC-3 and VIM-1-producing ST448 E. coli was reported in Spain, as a unique multiresistant clone [30]. Although it is not certain whether the Cuban NDM-producing ST448 E. coli was derived from Europe or autochthonous infection, it is necessary to carefully monitor the trend of this novel clone.

While phylogenetic group B2 isolates were mostly assigned into ST131 or its variant, it was worthy of note that a single isolate (IPK-629) belonged to ST1193, which is described as an emerging clone [31]. ST1193 belongs to the ST14 clonal complex, and shows fluoroquinolone resistance, while resistance profiles were variable depending on isolates [31]. This E. coli clone was documented to show temporal prevalence trend in the US [32], and ST1193 having CTX-M-14 and CTX-M-15 genes were reported in Germany [33], and only the latest report from France described CTX-M-27 gene-positive ST1193 E. coli [34]. IPK-629 in our present study was isolated from lochia in 2018, and had CTX-M-27 gene, and showed resistance to ceftazidime, ciprofloxacin, and trimethoprim-sulfamethoxazole. ST1193 E. coli was suggested to have evolved through frequent gain or loss of resistance gene cassettes [31], and thus, possible to change in its resistance profiles and epidemiological features in the future. Therefore, detection of ST1193 in Cuba may be a concern for the control of ExPEC.

In the present study, high resistance rate was noted also against ciprofloxacin (76.1%), associated with mutations in GyrA and ParC and prevalence of PMQR gene aac (6′)-Ib-cr. This finding suggests substantial progress in resistance of E. coli to fluoroquinolone in Cuba. It has been described that PMQR gene aac (6′)-Ib-cr is often located on plasmids of the IncF family with bla\textsubscript{CTX-M-15} [11]. Considerable rate of aac (6′)-Ib-cr among CTX-M-positive E. coli (54.5%) observed in our present study suggest coexistence of these genes on plasmids, which may imply the spread of ESBL associated with quinolone and aminoglycoside resistance, leading to dissemination of multidrug resistant E. coli. While qnrB has been implicated in ISCR-1-linked genes encoding CTX-M and carbapenemases on plasmids [35], prevalence of qnrB was low among CTX-M-positive isolates in our study. Furthermore, among nine qnrS-positive isolates, only two isolates had TEM genes, although association of qnrS1 with bla\textsubscript{TEM} was documented [35]. These findings suggest the presence of an uncommon or novel type of plasmid containing qnr genes among ExPEC.

Our present study revealed high prevalence of CTX-M type ESBL gene represented by bla\textsubscript{CTX-M-15} in ExPEC in Cuba, associated with progress of quinolone resistance, and described also first identification in E. coli of NDM-1 type carbapenemase gene. These findings could be attributable to the presumptive frequent use of third generation cephalosporins and carbapenems in Cuba. Along with continuous surveillance of antimicrobial resistance, a systematic investigation for the usage, i.e., frequency and number of individual antimicrobials used in this country would be necessary for the effective control of antimicrobial resistance in ExPEC.

4. Materials and Methods

4.1. Bacterial Isolates

A total of 306 non-duplicate clinical isolates of E. coli derived from patients with extraintestinal infections were analyzed. These isolates were collected from hospitals in 16 regions (provinces) in Cuba during a period between 2014 and 2018. The main source of the isolates was urine (49.7%), followed by blood (13.4%), wound (10.8%), tracheal aspirate (9.5%), skin (5.6%), cerebrospinal fluid (2.9%), catheter tip (2.3%), and others (5.8%). Bacterial identification was performed by conventional method. Gram-negative rods grown on MacConkey agar were identified by colonial morphology, motility and a series of biochemical tests (indole test, citrate utilization, urease test, oxidase reaction, lysine and ornithine test and sugar fermentation). Further, E. coli was confirmed by PCR targeting adk using primers employed in multilocus sequence typing (MLST) of this bacterial species [36].
4.2. Susceptibility Testing

Minimum inhibitory concentration (MIC) was measured for 18 antimicrobial agents (amikacin, aztreonam, cefazidime, ciprofloxacin, colistin, cefotaxime, cefuroxime, cefepime, fosfomycin, cefoxitin, gentamicin, imipenem, meropenem, nalidixic acid, norfloxacin, sulfamethoxazole/trimethoprim, tobramycin, piperacillin-tazobactam). E-test was employed for beta-lactams, while disc diffusion test for other antibiotics except for colistin. Broth microdilution test was used for colistin, and also for imipenem and meropenem for confirmation of MIC. Susceptibility to all the antimicrobials except for nalidixic acid was judged according to EUCAST guideline [37]. CLSI guideline [38] was employed for nalidixic acid, of which breakpoint was not assigned in EUCAST guideline.

4.3. Molecular Detection of Beta-Lactamase Genes and Plasmid-Mediated Quinolone Resistance (PMQR) Genes

For all the E. coli isolates, presence of beta-lactamase genes \( \text{bla}_{\text{CTX-M}}, \text{bla}_{\text{TEM}}, \text{bla}_{\text{SHV}} \) was examined by multiplex PCR as described previously [39], and four \( \text{bla}_{\text{CTX-M}} \) subgroups (group 1, 2, 9 and 8/25/26) were discriminated by multiplex PCR assay [40]. For all the isolates showing resistance to imipenem and/or meropenem, presence of carbapenemase genes (\( \text{bla}_{\text{NDM}}, \text{bla}_{\text{VIM}}, \text{bla}_{\text{IMP}}, \text{bla}_{\text{KPC}}, \) and \( \text{bla}_{\text{OXA-48}} \)) were confirmed by multiplex/uniplex PCR using primers and conditions as described previously [41]. Plasmid-mediated AmpC beta-lactamase genes consisting of six families were detected by multiplex PCR according to the scheme described by Perez-Perez and Hanson [42]. Nucleotide sequences of full-length \( \text{bla}_{\text{TEM}}, \text{bla}_{\text{CTX-M}}, \) carbapenemase genes (\( \text{bla}_{\text{NDM}}, \)) and AmpC genes (\( \text{bla}_{\text{CMY}} \)) were determined directly from PCR products with primers listed in Table S3, using the BigDye Terminator v3.1 Cycle Sequencing kit (Applied Biosystems, Foster City, CA, USA) on an automated DNA sequencer (ABI PRISM 3100). Subtypes of beta-lactamase genes were determined by using standard nucleotide BLAST (Basic Local Alignment Search Tool) available at the NCBI website [43]. Identification of plasmid-mediated quinolone resistance (PMQR) genes (\( \text{aac(6')-Ib-cr}, \text{qnrA}, \text{qnrB}, \text{qnrC}, \text{qnrD}, \text{qnrS}, \text{oqxAB} \) and \( \text{qepA} \)) was also performed by multiplex PCR using primers and conditions as described previously [44].

4.4. Genetic Analysis of E. coli

Four main phylogenetic groups of E. coli (A, B1, B2, and D) were discriminated by triplex PCR method described by Clermont et al. [45]. Sequence type (ST) of E. coli based on Achtman scheme of MLST was assigned by determination of partial sequence of seven housekeeping genes (\( \text{adk}, \text{fumC}, \text{gyrB}, \text{icd}, \text{mdh}, \text{purA} \) and \( \text{recA} \)) [36]. Presence of O25b allele was confirmed by PCR as described previously [46], and isolates with O25b allele were further analyzed for genotype based on \( \text{fimH} \) (type 1 fimbrial adhesin gene) by PCR and direct sequencing [47], using the FimTyper 1.0 web-based tool. Presence of mutation in quinolone-resistance determining region (QRDR) of DNA gyrase (GyrA) and topoisomerase IV (ParC) was analyzed for selected quinolone-resistant isolates by PCR and direct sequencing. Primer sequences for PCR are as follows: \( \text{gyrA} \), forward 5'-ACGTACTAGGCAATGACTGG-3', reverse 5'-AGTCACTGGCTGATGAA-3'; \( \text{parC} \), forward 5'-TGATGGATGTCTGA-3', reverse 5'-CTCAAATACAGCTGGAATA-3'. For isolates showing resistance to colistin, detection of \( \text{mcr} \) genes was attempted by PCR, as described previously [24].

4.5. GenBank Accession Numbers

The nucleotide sequence of beta-lactamase genes encoding TEM-1, CTX-M-2, -14, -15, -27, -32, and -55, and CMY-2 were deposited in the GenBank database under accession numbers MH900520-MH900529.

Supplementary Materials: The following are available online at http://www.mdpi.com/2076-0817/9/1/65/s1, Table S1: Coexistence of beta-lactamase genes and PMQR genes in E. coli isolates, Table S2: Mutations in QRDR of GyrA and ParC in ExPEC isolates showing quinolone resistance, Table S3: Primers used for sequencing in this study.
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References
1. Riley, L.W. Pandemic lineages of extraintestinal pathogenic Escherichia coli. Clin. Microbiol. Infect. 2014, 20,
380–390. [CrossRef] [PubMed]
2. Manges, A.R.; Geum, H.M.; Guo, A.; Edens, T.J.; Fibke, C.D.; Pitout, J.D.D. Global Extraintestinal Pathogenic
Escherichia coli (ExPEC) Lineages. Clin. Microbiol. Rev. 2019, 32, e00135-18. [CrossRef] [PubMed]
3. Giske, C.G.; Monnet, D.L.; Cars, O.; Carmeli, Y. Clinical and economic impact of common multidrug-resistant
gram-negative bacilli. Antimicrob. Agents Chemother. 2008, 52, 813–821. [CrossRef] [PubMed]
4. Pitout, J.D.; Laupland, K.B. Extended-spectrum beta-lactamase-producing Enterobacteriaceae: An emerging
public-health concern. Lancet Infect. Dis. 2008, 8, 159–166. [CrossRef]
5. Bevan, E.R.; Jones, A.M.; Hawkey, P.M. Global epidemiology of CTX-M beta-lactamases: Temporal and
geographical shifts in genotype. J. Antimicrob. Chemother. 2017, 72, 2145–2155. [CrossRef]
6. Thomson, K.S. Extended-spectrum-beta-lactamase, AmpC, and Carbapenemase issues. J. Clin. Microbiol.
2010, 48, 1019–1025. [CrossRef]
7. Jacoby, G.A. AmpC Beta-Lactamases. Clin. Microbiol. Rev. 2009, 22, 161–182. [CrossRef]
8. Nordmann, P.; Dortet, L.; Poirel, L. Carbapenem resistance in Enterobacteriaceae worldwide. Clin. Microbiol.
Perspect. Infect. Dis. 2012, 20, 821–830. [CrossRef]
9. Dalhoff, A. Global fluoroquinolone resistance epidemiology and implications for clinical use. Interdiscip.
Perspect. Infect. Dis. 2012, 2012, 976273. [CrossRef]
10. Poirel, L.; Madec, J.Y.; Lupo, A.; Schink, A.K.; Kieffer, N.; Nordmann, P.; Schwarz, S. Antimicrobial Resistance in
Escherichia coli. Microb. Spectr. 2018, 6. [CrossRef] [PubMed]
11. González Mesa, L.; Ramos Morí, A.; Nadal Becerra, L.; Morffi Figueroa, J.; Hernández Robledo, E.;
Alvarez, A.B.; Marchena Bequer, J.J.; González Alemán, M.; Villain Plous, C. Phenotypic and molecular
identification of extended-spectrum beta-lactamase (ESBL) TEM and SHV produced by clinical isolates
Escherichia coli and Klebsiella spp. in hospitals. Rev. Cubana Med. Trop. 2007, 59, 52–58. [PubMed]
12. Sader, H.S.; Castanheira, M.; Farrell, D.J.; Flamm, R.K.; Mendes, R.E.; Jones, R.N. Tigecycline antimicrobial
activity tested against clinical bacteria from Latin American medical centres: Results from SENTRY
Antimicrobial Surveillance Program (2011–2014). Int. J. Antimicrob. Agents 2016, 48, 144–150. [CrossRef]
[PubMed]
13. Gales, A.C.; Castanheira, M.; Jones, R.N.; Sader, H.S. Antimicrobial resistance among Gram-negative bacilli
isolated from Latin America: Results from SENTRY Antimicrobial Surveillance Program (Latin America,
2008–2010). Diagn. Microbiol. Infect. Dis. 2012, 73, 354–360. [CrossRef]
14. Chandramohan, L.; Revell, P.A. Prevalence and molecular characterization of extended-spectrum-beta-
lactamase-producing Enterobacteriaceae in a pediatric patient population. Antimicrob. Agents Chemother.
2012, 56, 4765–4770. [CrossRef]
15. Denisuik, A.J.; Karlowsky, J.A.; Adam, H.J.; Baxter, M.R.; Lagacé-Wiens, P.R.S.; Mulvey, M.R.; Hoban, D.J.;
Zhanel, G.G. Canadian Antimicrobial Resistance Alliance (CARA) and CANWARD. Dramatic rise in the
proportion of ESBL-producing Escherichia coli and Klebsiella pneumoniae among clinical isolates identified in
Canadian hospital laboratories from 2007 to 2016. J. Antimicrob. Chemother. 2019, 74 (Suppl. 4), iv64–iv71.
[CrossRef]
17. Nicolas-Chanoine, M.H.; Bertrand, X.; Madec, J.Y. *Escherichia coli* ST131, an intriguing clonal group. *Clin. Microbiol. Rev.* 2014, 27, 543–574. [CrossRef]

18. Matsumura, Y.; Pitout, J.D.; Gomi, R.; Matsuda, T.; Noguchi, T.; Yamamoto, M.; Peirano, G.; DeVinney, R.; Bradford, P.A.; Motyl, M.R.; et al. Global *Escherichia coli* Sequence Type 131 Clade with *bla*CTX-M-27 Gene. *Emerg. Infect. Dis.* 2016, 22, 1900–1907. [CrossRef]

19. Hu, X.; Gou, J.; Guo, X.; Cao, Z.; Li, Y.; Jiao, H.; He, X.; Ren, Y.; Tian, F. Genetic contexts related to the diffusion of plasmid-mediated CTX-M-55 extended-spectrum beta-lactamase isolated from *Enterobacteriaceae* in China. *Ann. Clin. Microbiol. Antimicrob.* 2018, 17, 12. [CrossRef]

20. Tamang, M.D.; Nam, H.M.; Gurung, M.; Jang, G.C.; Jung, S.C.; Park, Y.H.; Lim, S.K. Molecular characterization of CTX-M β-lactamase and associated addiction systems in *Escherichia coli* circulating among cattle, farm workers, and the farm environment. *Appl. Environ. Microbiol.* 2013, 79, 3898–3905. [CrossRef]

21. Hernández-Fillor, R.E.; Brillante, M.; Espinosa, I.; Perreten, V. Complete Circular Genome Sequence of a Multidrug-Resistant *Escherichia coli* Strain from Cuba Obtained with Nanopore and Illumina Hybrid Assembly. *Microbiol. Resour. Announc.* 2019, 8, e01269-19.

22. Dadashi, M.; Yaslanifard, S.; Hajikhani, B.; Kabir, K.; Owlia, P.; Goudarzi, M.; Hakemivala, M.; Darban-Sarokhalil, D. Frequency distribution, genotypes and prevalent sequence types of New Delhi metallo-β-lactamase-producing *Escherichia coli* among clinical isolates around the world: A review. *J. Glob. Antimicrob. Resist.* 2019, 19, 284–293. [CrossRef]

23. Dortet, L.; Poirel, L.; Nordmann, P. Worldwide dissemination of the NDM-type carbapenemases in Gram-negative bacteria. *BioMed Res. Int.* 2014, 2014, 249856. [CrossRef] [PubMed]

24. Li, J.; Shi, X.; Yin, W.; Wang, Y.; Shen, Z.; Ding, S.; Wang, S. A Multiplex SYBR Green Real-Time PCR Assay for the Detection of Three Colistin Resistance Genes from Cultured Bacteria, Feces, and Environment Samples. *Front. Microbiol.* 2017, 8, 2078. [CrossRef] [PubMed]

25. Torres-González, P.; Bobadilla-Del Valle, M.; Tovar-Calderón, E.; Leal-Vega, F.; Hernández-Cruz, A.; Martínez-Gamboa, A.; Niembro-Ortega, M.D.; Sifuentes-Osorio, J.; Ponce-de-León, A. Outbreak caused by *Enterobacteriaceae* harboring NDM-1 metallo-β-lactamase carried in an IncFII plasmid in a tertiary care hospital in Mexico City. *Antimicrob. Agents Chemother.* 2015, 59, 7080–7083. [CrossRef] [PubMed]

26. Bocanegra-Ibarias, P.; Garza-González, E.; Morfín-Otero, R.; Barrios, H.; Villarreal-Treviño, L.; Rodríguez-Noriega, E.; Garza-Ramos, U.; Petersen-Morfin, S.; Silva-Sanchez, J. Molecular and microbiological report of a hospital outbreak of NDM-1-carrying *Enterobacteriaceae* in Mexico. *PLoS ONE* 2017, 12, e0179651. [CrossRef] [PubMed]

27. Quiñones, D.; Carvajal, I.; Perez, Y.; Hart, M.; Perez, J.; Garcia, S.; Salazar, D.; Ghosh, S.; Kawaguchiyama, M.; Aung, M.S.; et al. High prevalence of *bla*OXA-23 in *Acinetobacter* spp. and detection of *bla*NDM-1 in *A. soli* in Cuba: Report from National Surveillance Program (2010–2012). *New Microbes New Infect.* 2015, 7, 52–56. [CrossRef]

28. Alsharaphy, S.A.; Gharout-Sait, A.; Muggeo, A.; Guillard, T.; Cholley, P.; Brasme, L.; Bertrand, X.; Moghram, G.S.; Touati, A.; De Champs, C. Characterization of Carbapenem-Resistant *Enterobacteriaceae* Clinical Isolates in Al Thawra University Hospital, Sana’a, Yemen. *Microb. Drug Resist.* 2019. [CrossRef]

29. Pitart, C.; Solé, M.; Roca, I.; Román, A.; Moreno, A.; Vila, J.; Marco, F. Molecular characterization of *bla*NDM-5 carried on an IncFII plasmid in an *Escherichia coli* isolate from a nontraveler patient in Spain. *Antimicrob. Agents Chemother.* 2015, 59, 659–662. [CrossRef] [PubMed]

30. Porres-Osante, N.; Azcona-Gutierrez, J.M.; Rojo-Bezares, B.; Undabeitia, E.; Torres, C.; Sáenz, Y. Emergence of a multiresistant KPC-3 and VIM-1 carbapenemase-producing *Escherichia coli* strain in Spain. *J. Antimicrob. Chemother.* 2014, 69, 1792–1795. [CrossRef]

31. Johnson, T.J.; Elnekave, E.; Miller, E.A.; Munoz-Aguayo, J.; Flores Figueroa, C.; Johnston, B.; Nielsen, D.W.; Logue, C.M.; Johnson, J.R. Phylogenomic Analysis of Extraintestinal Pathogenic *Escherichia coli* Sequence Type 1193, an Emerging Multidrug-Resistant Clonal Group. *Antimicrob. Agents Chemother.* 2018, 63, e01913-18. [CrossRef] [PubMed]

32. Johnson, J.R.; Johnston, B.D.; Porter, S.B.; Clabots, C.; Bender, T.L.; Thuras, P.; Trott, D.J.; Cobbold, R.; Mollinger, J.; Ferrieri, P.; et al. Rapid Emergence, Subsidence, and Molecular Detection of *Escherichia coli* Sequence Type 1193-fimH64, a New Disseminated Multidrug-Resistant Commensal and Extraintestinal Pathogen. *J. Clin. Microbiol.* 2019, 57, e01664-18. [CrossRef] [PubMed]
33. Valenza, G.; Werner, M.; Eisenberger, D.; Nickel, S.; Lehner-Reindl, V.; Höller, C.; Bogdan, C. First report of the new emerging global clone ST1193 among clinical isolates of extended-spectrum β-lactamase (ESBL)-producing Escherichia coli from Germany. J. Glob. Antimicrob. Resist. 2019, 17, 305–308. [CrossRef] [PubMed]

34. Birgy, A.; Madhi, F.; Jung, C.; Levy, C.; Cointe, A.; Bidet, P.; Hobson, C.A.; Bechet, S.; Sobral, E.; Vuthien, H.; et al. Diversity and trends in population structure of ESBL-producing Enterobacteriaceae in febrile urinary tract infections in children in France from 2014 to 2017. J. Antimicrob. Chemother. 2019, 75, 96–105. [CrossRef] [PubMed]

35. Jacoby, G.A.; Strahilevitz, J.; Hooper, D.C. Plasmid-mediated quinolone resistance. Microbiol. Spectr. 2014, 2. [CrossRef] [PubMed]

36. Wirth, T.; Falush, D.; Lan, R.; Colles, F.; Mensa, P.; Wieler, H.; Reeves, P.R.; Maiden, M.C.; Ochman, H.; et al. Sex and virulence in Escherichia coli: An evolutionary perspective. Mol. Microbiol. 2006, 60, 1136–1151. [CrossRef] [PubMed]

37. The European Committee on Antimicrobial Susceptibility Testing (EUCAST). Clinical Breakpoints-Bacteria v 6.0; 2016. Available online: http://www.eucast.org/clinical_breakpoints/ (accessed on 15 January 2020).

38. Clinical and Laboratory Standards Institute (CLSI). Performance Standards for Antimicrobial Susceptibility Testing; Twenty-Fourth Informational Supplement, M100-S24; Clinical and Laboratory Standards Institute (CLSI): Wayne, PA, USA, 2014.

39. Monstein, H.J.; Ostholm-Balkhed, A.; Nilsson, M.V.; Nilsson, M.; Dornbusch, K.; Nilsson, L.E. Multiplex PCR amplification assay for the detection of blaSHV, blaTEM and blaCTX-M genes in Enterobacteriaceae. APMIS 2007, 115, 1400–1408. [CrossRef]

40. Xu, L.; Ensor, V.; Gossain, S.; Nye, K.; Hawkey, P. Rapid and simple detection of blaCTX-M genes by multiplex PCR assay. J. Med. Microbiol. 2005, 54, 1183–1187. [CrossRef]

41. Poirel, L.; Walsh, T.R.; Cuvillier, V.; Nordmann, P. Multiplex PCR for detection of acquired carbapenemase genes. Diagn. Microbiol. Infect. Dis. 2011, 70, 119–123. [CrossRef]

42. Pérez-Pérez, F.J.; Hanson, N.D. Detection of plasmid-mediated AmpC beta-lactamase genes in clinical isolates by using multiplex PCR. J. Clin. Microbiol. 2002, 40, 2153–2162. [CrossRef]

43. Clermont, O.; Bonacorsi, S.; Bingen, E. Rapid and simple determination of the Escherichia coli phylogenetic group. Appl. Environ. Microbiol. 2000, 66, 4555–4558. [CrossRef] [PubMed]

44. Clermont, O.; Lavollay, M.; Vimont, S.; Deschamps, C.; Forestier, C.; Branger, C.; Denamur, E.; Arlet, G. The CTX-M-15-producing Escherichia coli diffusing clone belongs to a highly virulent B2 phylogenetic subgroup. J. Antimicrob. Chemother. 2008, 61, 1024–1028. [CrossRef] [PubMed]

45. Johnson, J.R.; Tchesnokova, V.; Johnston, B.; Clabots, C.; Roberts, P.L.; Billig, M.; Riddell, K.; Rogers, P.; Qin, X.; Butler-Wu, S.; et al. Abrupt emergence of a single dominant multidrug-resistant strain of Escherichia coli. J. Infect. Dis. 2013, 207, 919–928. [CrossRef]