Brief Report

First Report and Comparative Genomics Analysis of a \textit{bla}_{OXA-244}-Harboring \textit{Escherichia coli} Isolate Recovered in the American Continent

Deisy Abril 1, Ingrid Gisell Bustos Moya 2, Ricaurte Alejandro Marquez-Ortiz 1, Diego Fernando Josa Montero 2, Zayda Lorena Corredor Rozo 1, Isabel Torres Molina 2, Natasha Vanegas Gómez 1,3 and Javier Escobar-Perez 1,*

1 Bacterial Molecular Genetics Laboratory, Universidad El Bosque, Carrera 9 N°131A-02, Bogota D.C. 110121, Colombia; djabril@unbosque.edu.co (D.A.); ramarquezo@gmail.com (R.A.M.-O.); zcorredor@unbosque.edu.co (Z.L.C.R.); natashavanegas@yahoo.es (N.V.G.)
2 Grupo de Medicina Cardiovascular y especialidades de alta complejidad—Fundación Clínica Shaio, Bogota D.C. 110121, Colombia; ingrid.bustos@shaio.org (I.G.B.M.); diego.josa@shaio.org (D.F.J.M.); isabel.torres@shaio.org (I.T.M.)
3 The i3 institute, Faculty of Science University of Technology, Sydney PO Box 123, Australia
* Correspondence: escobarjavier@unbosque.edu.co or javiesco21@yahoo.com; Tel.: +57-1-6489000 (ext. 1179); Fax: +57-1-6252030

Received: 13 September 2019; Accepted: 26 October 2019; Published: 13 November 2019

Abstract: The carbapenemase OXA-244 is a derivate of OXA-48, and its detection is very difficult in laboratories. Here, we report the identification and genomic analysis of an \textit{Escherichia coli} isolate (28Eco12) harboring the \textit{bla}_{OXA-244} gene identified in Colombia, South America. The 28Eco12 isolate was identified during a retrospective study, and it was recovered from a patient treated in Colombia. The complete nucleotide sequence was established using the PacBio platform. A comparative genomics analysis with other \textit{bla}_{OXA-244}-harboring \textit{Escherichia coli} strains was performed. The 28Eco12 isolate belonged to sequence type (ST) 38, and its genome was composed of two molecules, a chromosome of 5,343,367 bp and a plasmid of 92,027 bp, which belonged to the incompatibility group IncY and did not harbor resistance genes. The \textit{bla}_{OXA-244} gene was chromosomally encoded and mobilized by an ISR1-related Tn6237 composite transposon. Notably, this transposon was inserted and located within a new genomic island. To our knowledge, this is the first report of a \textit{bla}_{OXA-244}-harboring \textit{Escherichia coli} isolate in America. Our results suggest that the introduction of the OXA-244-producing \textit{E. coli} isolate was through clonal expansion of the ST38 pandemic clone. Other isolates producing OXA-244 could be circulating silently in America.

Keywords: \textit{bla}_{OXA-244}; \textit{Escherichia coli}; carbapenems; resistance; Colombia

1. Introduction

The World Health Organization WHO has recognized carbapenem-resistant Enterobacteriaceae as pathogens with critical priority for the development of new antibiotics [1]. OXA-244, a carbapenemase belonging to the Class D family, is a derivate of OXA-48 and encoded by the \textit{bla}_{OXA-244} gene. Although there are multiple reports of OXA-48-producing isolates, reports of isolates harboring OXA-244 are less frequent, perhaps because their detection is difficult due to their reduced carbapenem activity. The \textit{bla}_{OXA-244} gene was initially described in 2011, within a \textit{Klebsiella pneumoniae} isolate, which was identified in Spain [2]. It has already been identified in \textit{Escherichia coli} isolates recovered from Germany [3], France [4,5], the United Kingdom [6], Southeast Asia [7], and Egypt [5]. The molecular characterization of some of these \textit{E. coli} isolates have shown that the majority of them belong to
sequence type (ST) 38, although recently other STs have been found (ST361, ST1722, and ST3541) [5]; and they contain other β-lactamases, such as TEM, CTX-M, and CMY. The bla_{OXA-244} gene is located in the chromosome within a truncated Tn1999.2 transposon, which is immersed into an ISRI1-based Tn6237 transposon [4,8]. Here, we provide a genomic analysis of an *Escherichia coli* isolate (28Eco12) containing the bla_{OXA-244} gene that was recovered from a patient in Colombia, South America. To our knowledge, this is the first report of a bla_{OXA-244}–harboring *Escherichia coli* isolate in America.

2. Results

The 28Eco12 isolate was identified from a retrospective study in Bogotá, Colombia (see Materials and Methods), and we decided to establish its complete genome to determine its resistome and mobile genetic platform distribution (IS content). The genome was composed of two molecules, a chromosome of 5,343,367 bp and a plasmid of 92,027 bp (p28Eco12), which belonged to the incompatibility group IncY and did not harbor resistance genes. The resistance-genes arsenal of the isolate was composed of *β*-lactamases, such as TEM, CTX-M, and CMY.

The in silico serotyping of the isolate was O102:H6. The 28Eco12 isolate was identified from a retrospective study in Bogotá, Colombia (see Materials and Methods), and we decided to establish its complete genome to determine its resistome and mobile genetic platform distribution (IS content). The genome was composed of two molecules, a chromosome of 5,343,367 bp and a plasmid of 92,027 bp (p28Eco12), which belonged to the incompatibility group IncY and did not harbor resistance genes. The resistance-genes arsenal of the isolate was composed of *β*-lactamases, such as TEM, CTX-M, and CMY.

The 28Eco12 isolate was composed of 5,343,367 bp and a plasmid of 92,027 bp (p28Eco12), which belonged to the incompatibility group IncY and did not harbor resistance genes. The resistance-genes arsenal of the isolate was composed of *β*-lactamases, such as TEM, CTX-M, and CMY. The ESBL–harboring *Escherichia coli* isolates (28Eco12) in this study belonged to sequence type (ST) 38, although recently other STs have been found (ST361, ST1722, and ST3541) [5]; and they contain other *β*-lactamases, such as TEM, CTX-M, and CMY. The bla_{OXA-244} gene is located in the chromosome within a truncated Tn1999.2 transposon, which is immersed into an ISRI1-based Tn6237 transposon [4,8]. Here, we provide a genomic analysis of an *Escherichia coli* isolate (28Eco12) containing the bla_{OXA-244} gene that was recovered from a patient in Colombia, South America. To our knowledge, this is the first report of a bla_{OXA-244}–harboring *Escherichia coli* isolate in America.

![Figure 1. BLASTn comparison of the blaOXA-244-containing *Escherichia coli* chromosomes.](image-url)

The figure shows a BLASTn comparison of the blaOXA-244-containing *Escherichia coli* chromosomes. The K-12 (GenBank accession number NC_000913), F8111-1SC3 (GenBank accession number NZ_CP024269), and 266917_2 (GenBank accession number NZ_CP026723.1) strains were used as references. At the more external circle is shown the localization of the resistance genes and their putative genetic platforms of mobilization. The positions of the seven identical ISR1 and five IS1-family (89% of identity) sequences are also indicated. The strain positions on the figure are as follow (internal to external) (sequence type/serotype): K12 (ST10/O16:H48), F8111-1SC3 (ST182/O169:H41), 86J1 (ST361/O9:H30) MKGU01, 62D3 (ST1722/O1:H25) MKGY01, 85H4 (ST3541/O53:H18) MKGW01, 73G4 (ST3541/O53:H18) MKGV01, 266917_2 (ST38/O51:H30), 35J9 (ST38/O102:H6) MKGX01, 69E6 (ST38/O102:H6) MKGZ01, 78B5 (ST38/O102:H6) MKGT01, and 28Eco12 (ST38/O102:H6) NZ_CP038505.
Using the complete genome sequence, the 28Eco12 isolate was found to have a close genetic relationship with the E. coli strain 266917_2 (ST38), described recently in the United Kingdom (90% coverage, 97% identity, GenBank accession number CP026723.1), which does not contain the bla\textsubscript{OXA-244} gene. The genomic comparative analysis revealed that the bla\textsubscript{OXA-244} gene was mobilized by the Tn6237 transposon, as it has previously been described in Escherichia coli strain VAL [4,8]. However, in the 28Eco12 isolate, the Tn6237 transposon was not inserted within the \Pi536 pathogenicity island, as was previously reported to \textsubscript{bla}OXA-48 [8], but into a new putative genomic island, inserted within the \textit{tRNA-sec} gene. Its insertion produced a 39 bp direct repeat sequence (TTCGACTCCTGTGATCTTCCGCCAATTAACATCTTCTGA). This event did not change the \textit{tRNA-sec} gene sequence (Figure 2).

![Figure 2. Comparison of the region where the \textit{bla}OXA-244 gene was inserted within \textit{Escherichia coli} 28Eco12 isolate. The red arrow corresponds to the \textit{bla}OXA-244 gene. The mobile genetics elements are shown in different colors. The putative genomic island is shown in purple and its insertion within the \textit{tRNA-sec} gene is indicated respect to the \textit{E. coli} strain 266917_2 (GenBank accession number CP026723.1), F8111-15C3 (GenBank accession number CP024269), 536-EC15 (GenBank accession number HG977710.1), and K-12 (GenBank accession number NC_000913). The blue rectangles correspond to the gene where the Tn6237 transposon was inserted (green arrows). The pallets represent the target-site duplications. The \textit{int} gene that encodes the phage integrase protein is shown. Blue shading between pairs of sequences indicates >90% of identity in a window of 400 bp. The scale bar indicates sequence length. The putative island was also present in the \textit{bla}OXA-244-negative enterotoxigenic \textit{E. coli} F8111-1SC3 isolate (GenBank accession number NZ_CP024269). Interestingly, the \textit{tRNA-sec} gene is a hot spot for DNA insertion, because it also serves as the insertion site of the L536 pathogenicity island in the uropathogenic strain \textit{E. coli} 536 [10]. These results suggest that the Tn6237 transposon is active and moves to different sites in the \textit{E. coli} chromosome. In addition, the isolate harbored 69 insertion sequences (IS) belonging to 17 different IS families (Table 1). Some of these present as single copy, partial form, or multiple copies. The most frequent IS families were IS1, IS200/IS605, ssgr, IS200, and IS3, with 13, 10, and 8 IS copies, respectively. Target site duplications (TSD) are signatures of transposition.
events, and among the 69 ISs, 25 presented TSDs and none were present within the E. coli F8111-1SC3 isolate, indicating that they were inserted by single-copy transposition. The TSD pattern analysis also revealed the presence of two composite transposons, the Tn6237 (mentioned previously) and a 15,730 bp IS26-made transposon, which was inserted within a gene that encodes a hypothetical protein and mobilizes the aph(3′)-Ib, aph(6)-Id, blaTEM-1b (two copies), catA1, sul2, and tetD genes. Notably, this IS26 transposon was also inserted within another putative genomic island, which was inserted into the trnA-leu gene. The comparative analysis suggested that this IS26 transposon was mobilized from a plasmid because it harbored the repA gene that corresponds to the incompatibility group IncQ-1 and possesses DNA fragments with a high percentage of identity to pD90-1 and pEC141 plasmids, which were identified in mcr-1-containing Salmonella enterica and E. coli strains, respectively [11]. With respect to the other resistance genes, the blaCTX-M gene was mobilized by ISEcp1 and an IS26 remnant, which were inserted within a gene that encodes a hypothetical protein.

Table 1. Insertion sequences identified in 28Eco12 isolate. Target site duplications (TSD) are shown in bold and underlined.

| IS Family | IS | Position | Right and Left Flanking Sequences | Comments |
|-----------|----|----------|-----------------------------------|----------|
| IS1       | IS1R 1 | 102025..102792 | TGAATTTGCT AAGAATGTT | Composite transposon harboring the blaOXA-244 gene. |
| IS1       | IS1R 2 | 123120..123887 | GGGGATTTGCT TGAATTTGCT |
| IS1       | IS1R 3 | 936063..936830 | CAGACAACG CAGACAACG |
| IS1       | IS1L 4 | 975280..976060 | GTCGCAACC TACAACCGTT |
| IS1       | IS1L 5 | 977300..978080 | GACAATGTC CAATCTGCT |
| IS1       | IS1R 6 | 1007836..1008603 | TGCTTTTCT TGCTTTTCT | Single IS transposition. IS inserted within an intergenic region |
| IS1       | IS1L 7 | 1001519..101266 | GCAATCAG TCGAATCAG |
| IS1       | IS1L 8 | 2087231..2087998 | GCGTTTTGGA GAAGAGTTC |
| IS1       | IS1L 9 | 3237236..3237910 | CTGCAAATC TACAACCGG |
| IS1       | IS1H 10 | 3266386..3267153 | CTGCAAATTCA TACAACCGG |
| IS1       | IS1H 11 | 5066636..5067399 | CCGGTAAAC CTTCTGATG |
| IS200     | IS200C 1 | 1127230..1127936 | TTTT TTTT |
| IS200     | IS200C 2 | 1690413..1691121 | TTTT TTTT |
| IS200     | IS200C 3 | 2442370..2443280 | TTAA TTAA |
| IS200     | IS200C 4 | 2481694..2482403 | TTTT TTTT |
| IS200     | IS200C 5 | 2990220..2990930 | AAAA AAAA |
| IS200     | IS200C 6 | 3058643..3059351 | AAAA AAAA |
| IS200     | IS200C 7 | 3060222..3060929 | AAAA AAAA |
| IS200     | IS200C 8 | 3271558..3272271 | GCAA AAAA |
| IS200     | IS200C 9 | 3939865..3940573 | AAAA AAAA |
| IS200     | IS200C 10 | 3994005..3994713 | AAAA AAAA |
| IS Family | IS     | Position          | Right and Left Flanking Sequences | Comments                                                                 |
|-----------|--------|-------------------|-----------------------------------|--------------------------------------------------------------------------|
| IS3       | IS600  | 3254256..3255501  | CAA  ACA                          | IS inserted within a genomic island                                      |
|           | IS6d1  | 949559..950499    | CAGTT -                          | IS (truncated) inserted within a putative prophage                        |
|           | IS6d1  | 3267154..3267978  | - GGT                            | IS (truncated) inserted within a genomic island                           |
|           | ISSb10 | 951719..952045    | - GTT                            | IS (truncated) inserted within a putative prophage                        |
|           | IS3    | 3259199..3260456  | TCAT  TTAA                       | IS inserted within a genomic island                                       |
|           | IS3    | 3236998..3237235  | - CTTC                           | IS (truncated) inserted within a genomic island                           |
|           | ISEc52 | 3249338..3250086  | - -                              | IS (truncated) inserted within a genomic island                           |
|           | ISEc52 | 3246586..3247067  | - -                              | IS (truncated) inserted within a genomic island                           |
|           | ISEc1  | 369367..369900    | - CCCT                           | IS (truncated, formerly Rhs-rearrangement hot-spots element)             |
| ISAs1     | ISEc1  | 2456311..2456957  | GATC -                           | IS (truncated, formerly Rhs-rearrangement hot-spots element)             |
|           | ISEc1  | 3675287..3676199  | TGGTAG  TCCCTGGGC                | IS (formerly Rhs-rearrangement hot-spots element)                         |
|           | ISEc1  | 3815490..3816780  | GATGATA  CCTGCTCA                | IS (formerly Rhs-rearrangement hot-spots element)                         |
|           | ISEc1  | 4160599..4161889  | TTCTTCCC  CACCTCAC               | IS (formerly Rhs-rearrangement hot-spots element)                         |
|           | ISEc1  | 5069737..5071026  | AGACCATG  GCAATGTCAG             | IS (formerly Rhs-rearrangement hot-spots element)                         |
| IS6       | IS26   | 4500893..4501712  | AAATCATG  ATATCAAG               | Composite transposon harboring the **bla**TEM-1B (two copies), **catA1**, **aph(6′)-id**, **aph(3′)-ib**, sul2, and trtD genes. |
|           | IS26   | 4503629..4504448  | ATATCGGC  GGTAAATC               |                                                                            |
|           | IS26   | 4509192..4510011  | CCGGCAAT  GTAAGCTG               |                                                                            |
|           | IS26   | 4513665..4514484  | ACCATTGG  CGCTGCGG               |                                                                            |
|           | IS26   | 4515814..4516633  | CAACAGGG  AAATCATG               |                                                                            |
|           | IS609  | 3979710..3980457  | CTCA  ATAA                       | IS inserted within the **yjiI** gene                                      |
|           | IS609  | 4689442..4691189  | TGTG  ATAA                       | IS inserted within an intergenic region                                    |
|           | IS609  | 2110766..2111379  | - -                              | IS (truncated) inserted within the **yedK** gene                          |
|           | ISEc46 | 2191062..2192824  | TCAT  CTAA                       | IS inserted within an intergenic region                                    |
| IS200/IS605 | IS1397 | 1214273..1215704  | TCAA  TCAA                       | Single IS transposition within an intergenic region                        |
| IS3:ssgr IS150 | IS1397 | 1368490..1369921  | TGCC  TGCC                       | Single IS transposition within an intergenic region                        |
|           | IS150  | 259853..261295    | AAG  AAG                         | Single IS transposition within an intergenic region                        |
|           | IS150  | 2414087..2415529  | GTT  GTT                         | Single IS transposition. IS inserted within a genomic island              |
| IS3:ssgr IS2 | IS2   | 937126..938456    | GTGGT  TTGTC                      | IS inserted within a putative prophage                                      |
|           | IS2    | 966497..967827    | CCCGCC  ACGGT                      | IS inserted within a putative prophage                                      |
|           | IS2    | 2027528..2038588  | CTTTTT  CTTTT                      | Single IS transposition. IS inserted within a genomic island              |
|           | IS2    | 4799912..4800262  | AAAAC -                           | IS (truncated) inserted within a putative prophage                        |
| IS100Kyp  | IS100Kyp | 2015511..2017464 | TTTGT  TTTGT                      | Single IS transposition. IS inserted within a genomic island              |
|           | IS100Kyp | 3273162..3275115  | GTGATAAC  GATAACAT                | IS inserted within a genomic island                                        |
|           | IS100Kyp | 4582722..4584675  | TTCAGATG  AGATGAT                  | IS inserted within a putative prophage                                      |
Table 1. Cont.

| IS Family | IS       | Position | Right and Left Flanking Sequences | Comments                                    |
|-----------|----------|----------|-----------------------------------|---------------------------------------------|
| IS66      | IS682    | 924827–926816 | - CATGTATC                        | IS (truncated) inserted within a putative prophage |
|           | ISe22    | 923252–924827 | ACAGAAGG                           | IS (truncated) inserted within a putative prophage |
|           | ISCro1   | 946022–948720 | TTTTATCT TTTTATCT                | Single IS transposition. IS inserted within a putative prophage |
| IS2          | IS629    | 570569–571878 | AATT                               | IS inserted within the acrF gene               |
|            | IS1203   | 971759–973088 | GATTAACCG GTAATATC                | IS inserted within a putative prophage         |
|            | IS3_ssgr_IS51 |          |                                    |                                             |
| ISKox3     | IS30     | 970324–971101 | - ATGTATCA                        | IS (truncated) inserted within a putative prophage |
| IS38       | IS481    | 2022594–2024315 | AAAAGT ACTTTT                     | Single IS transposition. IS inserted within a genomic island (inverted TSD) |
| IS40       | IS30D    | 950498–951718 | GT GT                             | Single IS transposition. IS inserted within a putative prophage |
| IS10A      | IS4      | 105162–106490 | GGCGCAGGC GTGCTGAAC               | IS inserted into ISI-composite transposon     |
| IS380      | IS1380   | 326913–330008 | TTTA TTTA                         | Single IS transposition. IS inserted within a hypothetical gene |
| IS705      | IS110    | 1568363–1569809 | TT TT                            | Single IS transposition. IS inserted within a hypothetical gene |

3. Discussion

In this study, we perform the first report of an *Escherichia coli* isolate carrying the *blaOXA-244* gene in Colombia, South America. These *blaOXA-244*-positive isolates are less frequent (or perhaps they circulate but are not detected) by their difficult detection and clonal dissemination. The multiresistant 28Eco12 isolate harbored only the phage-like IncY plasmid p28Eco12, which is genetically related to the plasmids p266917_2_02 (88% coverage, 99% identity, GenBank accession number CP026725.1), p1303_95 (91% coverage, 99% identity, GenBank accession number CP009168.1), p1 of *Salmonella enterica* strain ty3-243 (90% coverage, 93% identity, GenBank accession number LT905089.1), and the *blaKPC*-containing pCRKP-59-KPC (89% coverage, 94% identity, GenBank accession number KX928752.1). Although this plasmid does not transport resistance genes, it appears to be conserved in almost all *blaOXA-244*-containing *E. coli* strains included in our analysis, and its permanence is perhaps caused by the presence of the PI *phd-doc* toxin-antitoxin system that participates in host post-segregational killing [12]. Currently, there is limited knowledge about this phage-like IncY-plasmid family (for instance, the 37% of their ORFs is encoding for hypothetical proteins), but it is also becoming a genetic platform to transport important resistance genes, such as *blaCTX-M-15* and *mcr-1*; the latter confers resistance to colistin [13].

All resistant genes were chromosomally located and mobilized by active composite transposons, such as Tn6237, which has moved to different sites in the *E. coli* chromosome. In *E. coli*, the *blaOXA-244* gene was disseminated mainly by ST38 clone in Europe and Asia [3–7]. However, non-ST38 *E. coli* isolates are starting to appear in other countries, showing some genetic differences (Figure 1). As it is known that ISs have an important impact on genetic variability, genome structure and function, and foreign DNA acquisition, we try to decipher the potential of the 28Eco12 isolate to capture and move more resistance genes through an analysis of the IS content and their TSD and flanking-sequences patterns. Notably, this isolate has incorporated at least 69 ISs, showing a IS massive expansion process [14]; the ISs-belonging family IS1 was the most active, with fifteen copies, in which four copies probably were recently mobilized as single transposition events (unique copies) and two mobilized as a composite transposon and responsible of the *blaOXA-244* gene integration (Table 1). In spite of
finding five IS26 copies, only two of these were mobilized as a composite transposon and transported seven resistance genes. A study conducted by He et al. reported the IS26 participation in the plasmid reorganization from clinical strains [15]. The high IS content found in this multiresistant *E. coli* isolate indicates a high likelihood to acquire more resistance genes.

Finally, our institution searched for the presence of the *bla*OXA-244* gene within other carbapenem-resistant *E. coli* isolates from 2013 to the present day, but none were positive. Considering the time of the identification of the isolate, we believe that the *E. coli* isolate could have been acquired in the remittent institution, suggesting an inter-institution dissemination. No additional information could be obtained from the other institution.

4. Materials and Methods

The 28Eco12 isolate was identified from a retrospective study, conducted to characterize the molecular mechanisms in carbapenem-resistant *Enterobacteriaceae* isolates, which were recovered between 2013 and 2017, from a health institution in Bogotá, Colombia. The 28Eco12 isolate was recovered from a male patient, in September 2013, who was transferred from another health institution in the same city. The patient had suffered multiple traumas caused by a fall from a height of 20 m, and he required treatment in the intensive-care unit for eleven days. The patient was transferred to our institution, however, on the next day; the patient had fever, dysuria, urethral pain, leukocytosis, and urethral purulent secretion, suggesting a possible catheter-associated urinary tract infection. From a urine sample, the carbapenem-resistant *Escherichia coli* isolate 28Eco12 was identified, which was also resistant to ampicillin/sulbactam, cefotaxime, ceftriaxone, cefepime, and aztreonam. The Hodge Test was positive, and synergy and double-disc tests with boronic acid and EDTA were negative. The patient was treated with meropenem (2 g every 8 h) and colistin (100 mg every 8 h), and thirteen days later, he responded well to the treatment. No history of travel by him or his relatives was reported.

The complete genome sequence of the *bla*OXA-244-positive 28Eco12 isolate was obtained using the PacBio RS II platform (Pacific Biosciences of California, Inc., Menlo Park, CA, USA) and assembled through the previously reported procedure [16]. Briefly, sequencing reads were de novo assembled, using the HGAP 3 protocol, and manually verified using BWA-MEM (Burrows–Wheeler Aligner with maximal exact matches) [17] and Tablet v1.15.09.01 [18]. Misassembled terminal repeat overlap sequences were identified with Gepard (Genome Pair Rapid Dotter) [19] and trimmed manually. The genome was annotated using Prokka v1.11 [20], and the relevant regions were manually confirmed using BLASTn and BLASTp and edited in Artemis [21]. The resistance-gene arsenal was identified using ARIBA (https://github.com/sanger-pathogens/ariiba/wiki), ResFinder [22], CARD [23], and ARG-ANNOT databases [24]. The insertion sequences (IS) were found using ISsaga (http://issaga.bioutoul.fr/), and their flanking sequences were manually determined.

The study was approved by the ethics committee of the Shaio Clinic. The 28Eco12 complete genome sequenced in this study is available in the DDBJ/EMBL/GenBank public databases, under the accession numbers CP038505.1 and CP038506.1.

5. Conclusions

The isolates producing OXA-244 could be circulating in America and may not yet be identified, perhaps due to their very low frequency, very difficult detection, or weakness in antimicrobial resistance surveillance programs in some countries (such as Colombia). It is necessary to strengthen the surveillance of last-line antibiotic resistance and move toward the implementation of molecular and genomic tools for the detection of resistance genes in clinical settings.

**Author Contributions:** J.E.-P., I.G.B.M., and N.V.G. designed research; I.G.M.B., D.F.J.M., and I.T.M. identified the isolate, performed microbiological analysis, and interpreted the clinical characteristics of the patient; D.A., R.A.M.O., and Z.L.C.R. performed the molecular analysis and genome sequencing; D.A., R.A.M.-Ö., J.E.-P., and Z.L.C.R. performed the bioinformatics analysis; D.A., I.G.B.M., R.A.M.-Ö., N.V.G., and J.E.-P. interpreted the data; D.A., I.G.B.M., and J.E.-P. wrote the paper.
Funding: This research was partially funded by the Departamento Administrativo de Ciencia, Tecnología e Innovación, Colciencias (grant number 1308-777-58007), Vice Chancellery for Research of El Bosque University (grant number PCI63-2014), and Fundacion-Clinica Shaio.

Acknowledgments: We gratefully acknowledge the clinical laboratory for your technical assistance and to the Vice Chancellery for Research of El Bosque University (especially to Miguel Otero for your invaluable support).

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Tacconelli, E.; Carrara, E.; Savoldi, A.; Harbarth, S.; Mendelson, M.; Monnet, D.L.; Pulcini, C.; Kahlmeter, G.; Kluytmans, J.; Carmeli, Y.; et al. Discovery, research, and development of new antibiotics: The WHO priority list of antibiotic-resistant bacteria and tuberculosis. *Lancet Infect. Dis.* 2018, 18, 318–327. [CrossRef]

2. Oteo, J.; Hernandez, J.M.; Espasa, M.; Fleites, A.; Saez, D.; Bautista, V.; Perez-Vazquez, M.; Fernandez-Garcia, M.D.; Delgado-Iribarren, A.; Sanchez-Romero, I.; et al. Emergence of OXA-48-producing *Klebsiella pneumoniae* and the novel carbapenemases OXA-244 and OXA-245 in Spain. *J. Antimicrob. Chemother.* 2013, 68, 317–321. [CrossRef] [PubMed]

3. Valenza, G.; Nickel, S.; Pfeifer, Y.; Eller, C.; Krupa, E.; Lehner-Reindl, V.; Holler, C. Extended-spectrum-beta-lactamase-producing *Escherichia coli* as intestinal colonizers in the German community. *Antimicrob. Agents Chemother.* 2014, 58, 1228–1230. [CrossRef] [PubMed]

4. Potron, A.; Foirel, L.; Dortet, L.; Nordmann, P. Characterisation of OXA-244, a chromosomally-encoded OXA-48-like beta-lactamase from *Escherichia coli*. *Int. J. Antimicrob. Agents* 2016, 47, 102–103. [CrossRef] [PubMed]

5. Hoyos-Mallecot, Y.; Naas, T.; Bonnin, R.A.; Patino, R.; Glaser, P.; Fortineau, N.; Dortet, L. OXA-244-Producing *Escherichia coli* Isolates, a Challenge for Clinical Microbiology Laboratories. *Antimicrob. Agents Chemother.* 2017, 61, e00818-17. [CrossRef] [PubMed]

6. Findlay, J.; Hopkins, K.L.; Loy, R.; Douthitt, M.; Meunier, D.; Hill, R.; Pike, R.; Mustafa, N.; Livermore, D.M.; Woodford, N. OXA-48-like carbapenemases in the UK: An analysis of isolates and cases from 2007 to 2014. *J. Antimicrob. Chemother.* 2017, 72, 1340–1349. [CrossRef]

7. van Hattem, J.M.; Arcilla, M.S.; Bootsma, M.C.; van Genderen, P.J.; Goorhuis, A.; Grobusch, M.P.; Molhoek, N.; Oude Lashof, A.M.; Schultsz, C.; et al. Prolonged carriage and potential onward transmission of carbapenemase-producing Enterobacteriaceae in Dutch travelers. *Future Microbiol.* 2016, 11, 857–864. [CrossRef] [PubMed]

8. Beyrouthy, R.; Robin, F.; Delmas, J.; Gibold, L.; Dalmasso, G.; Dabboussi, F.; Hamze, M.; Bonnet, R. IS1R-mediated plasticity of IncL/M plasmids leads to the insertion of bla OXA-48 into the *Escherichia coli* Chromosome. *Antimicrob. Agents Chemother.* 2014, 58, 3785–3790. [CrossRef]

9. Wirth, T.; Falush, D.; Lan, R.; Colles, F.; Mensa, P.; Wieler, L.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]

10. Brzuszkiewicz, E.; Bruggemann, H.; Liesegang, H.; Emmerth, M.; Olschlager, T.; Nagy, G.; Albermann, K.; Wagner, C.; Buchrieser, C.; Emody, L.; et al. How to become a uropathogen: Comparative genomic analysis of extraintestinal pathogenic *Escherichia coli* strains. *Proc. Natl. Acad. Sci. USA* 2006, 103, 12879–12884. [CrossRef]

11. Wang, J.; Li, X.; Li, J.; Hurley, D.; Bai, X.; Yu, Z.; Cao, Y.; Wall, E.; Fanning, S.; Bai, L. Complete genetic analysis of a *Salmonella enterica* serovar Indiana isolate accompanying four plasmids carrying mcr-1, ESBL and other resistance genes in China. *Vet. Microbiol.* 2017, 210, 142–146. [CrossRef] [PubMed]

12. Yang, Q.E.; Walsh, T.R. Toxin-antitoxin systems and their role in disseminating and maintaining antimicrobial resistance. *FEMS Microbiol. Rev.* 2017, 41, 343–353. [CrossRef] [PubMed]

13. Zhang, C.; Feng, Y.; Liu, F.; Jiang, H.; Qu, Z.; Lei, M.; Wang, J.; Zhang, B.; Hu, Y.; Ding, J.; et al. A Phage-Like IncY Plasmid Carrying the mcr-1 Gene in *Escherichia coli* from a Pig Farm in China. *Antimicrob. Agents Chemother.* 2017, 61. [CrossRef] [PubMed]

14. Siguier, P.; Gourbeyre, E.; Chandler, M. Bacterial insertion sequences: Their genomic impact and diversity. *FEMS Microbiol. Rev.* 2014, 38, 865–891. [CrossRef] [PubMed]
15. He, S.; Hickman, A.B.; Varani, A.M.; Siguier, P.; Chandler, M.; Dekker, J.P.; Dyda, F. Insertion Sequence IS26 Reorganizes Plasmids in Clinically Isolated Multidrug-Resistant Bacteria by Replicative Transposition. *MBio* 2015, 6, e00762. [CrossRef] [PubMed]

16. Marquez-Ortiz, R.A.; Haggerty, L.; Olarte, N.; Duarte, C.; Garza-Ramos, U.; Silva-Sanchez, J.; Castro, B.E.; Sim, E.M.; Beltran, M.; Moncada, M.V.; et al. Genomic Epidemiology of NDM-1-Encoding Plasmids in Latin American Clinical Isolates Reveals Insights into the Evolution of Multidrug Resistance. *Genome Biol. Evol.* 2017, 9, 1725–1741. [CrossRef]

17. Li, H.; Durbin, R. Fast and accurate long-read alignment with Burrows-Wheeler transform. *Bioinformatics* 2010, 26, 589–595. [CrossRef] [PubMed]

18. Milne, I.; Stephen, G.; Bayer, M.; Cock, P.J.; Pritchard, L.; Cardle, L.; Shaw, P.D.; Marshall, D. Using Tablet for visual exploration of second-generation sequencing data. *Brief. Bioinform. 2013, 14, 193–202. [CrossRef]

19. Krumsiek, J.; Arnold, R.; Rattei, T. Gepard: A rapid and sensitive tool for creating dotplots on genome scale. *Bioinformatics* 2007, 23, 1026–1028. [CrossRef] [PubMed]

20. Seemann, T. Prokka: Rapid prokaryotic genome annotation. *Bioinformatics* 2014, 30, 2068–2069. [CrossRef]

21. Rutherford, K.; Parkhill, J.; Crook, J.; Horsnell, T.; Rice, P.; Rajandream, M.A.; Barrell, B. Artemis: Sequence visualization and annotation. *Bioinformatics* 2000, 16, 944–945. [CrossRef] [PubMed]

22. Zankari, E.; Hasman, H.; Cosentino, S.; Vestergaard, M.; Rasmussen, S.; Lund, O.; Aarestrup, F.M.; Larsen, M.V. Identification of acquired antimicrobial resistance genes. *J. Antimicrob. Chemother. 2012, 67, 2640–2644. [CrossRef] [PubMed]

23. McArthur, A.G.; Waglechner, N.; Nizam, F.; Yan, A.; Azad, M.A.; Baylay, A.J.; Bhullar, K.; Canova, M.J.; De Pascale, G.; Ejim, L.; et al. The comprehensive antibiotic resistance database. *Antimicrob. Agents Chemother. 2013, 57, 3348–3357. [CrossRef]

24. Gupta, S.K.; Padmanabhan, B.R.; Diene, S.M.; Lopez-Rojas, R.; Kempf, M.; Landraud, L.; Rolain, J.M. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. *Antimicrob. Agents Chemother. 2014, 58, 212–220. [CrossRef] [PubMed]

© 2019 by the authors. Licensee MDPI, Basel, Switzerland. This article is an open access article distributed under the terms and conditions of the Creative Commons Attribution (CC BY) license (http://creativecommons.org/licenses/by/4.0/).