Epidemiology of Plasmids in *Escherichia coli* and *Klebsiella pneumoniae* with Acquired Extended Spectrum Beta-Lactamase Genes Isolated from Chronic Wounds in Ghana

Frederik Pankok 1, Stefan Taudien 1, Denise Dekker 2*, Thorsten Thye 3, Kwabena Oppong 4, Charity Wiafe Akenten 4, Maike Lamshöft 5,5,5, Anna Jaeger 3, Martin Kaase 3, Simone Scheithauer 1, Konstantin Tanida 6, Hagen Frickmann 6,7, Jürgen May 3,5,5, and Ulrike Loderstädt 1,*

1 Institute for Infection Control and Infectious Diseases, University Medical Center Göttingen, 37075 Göttingen, Germany; frederik.pankok@med.uni-goettingen.de (F.P.); stefan.taudien@med.uni-goettingen.de (S.T); martin.kaase@med.uni-goettingen.de (M.K.); simone.scheithauer@med.uni-goettingen.de (S.S.)
2 Department of Implementation Research, Bernhard Nocht Institute for Tropical Medicine Hamburg, 20359 Hamburg, Germany; dekker@bnitm.de
3 Department of Infectious Disease Epidemiology, Bernhard Nocht Institute for Tropical Medicine Hamburg, 20359 Hamburg, Germany; thye@bnitm.de (T.T.); lamshoeft@bnitm.de (M.L.); anna.jaeger@bnitm.de (A.J.); may@bnitm.de (J.M.)
4 Kumasi Centre for Collaborative Research in Tropical Medicine (KCCR), Kumasi 039-5028, Ghana; Oppong.kwabena@presbyuniversity.edu.gh (K.O.); danquah@kccr.de (C.W.A.)
5 German Center for Infection Research (DZIF), Partner Site Hamburg-Lübeck-Borstel-Riems, 80331 Munich, Germany
6 Department of Microbiology and Hospital Hygiene, Bundeswehr Hospital Hamburg, External Site at the Bernhard Nocht Institute for Tropical Medicine Hamburg, 20359 Hamburg, Germany; Konstantin.tanida@gmail.com (K.T.); frickmann@bnitm.de (H.F.)
7 Institute for Medical Microbiology, Virology and Hygiene, University Medicine Rostock, 18057 Rostock, Germany
8 Tropical Medicine II, University Medical Center Hamburg-Eppendorf (UKE), 20251 Hamburg, Germany

*Correspondence: ulrike.loderstaedt1@med.uni-goettingen.de; Tel.: +49-551-3965-709

Abstract: Little information is available on the local epidemiology of mobile genetic elements such as plasmids harboring acquired beta-lactamase genes in Western African Ghana. In the present study, we screened for plasmids in three *Escherichia coli* and four *Klebsiella pneumoniae* isolates expressing extended spectrum beta-lactamases (ESBL) mediated by the *bla*CTX-M-15 gene from chronically infected wounds of Ghanaian patients. Bacterial isolates were subjected to combined short-read and long-read sequencing to obtain the sequences of their respective plasmids. In the *bla*CTX-M-15-gene-carrying plasmids of the four ESBL-positive *K. pneumoniae* isolates, IncFIB/IncFII (*n* = 3) and FIA (*n* = 1) sequences were detected, while in the *bla*CTX-M-15-gene-carrying plasmids of the three ESBL-positive *E. coli* isolates, IncFIA/IncFIB (*n* = 2) and IncFIB (*n* = 1) sequences were found. The three IncFIB/IncFII sequence-containing plasmids were almost identical to a *K. pneumoniae* plasmid reported from France. They belonged to the clonal lineages ST17, ST36 and ST39 of *K. pneumoniae*, suggesting transversal spread of this obviously evolutionary successful plasmid in Ghana. Other resistance gene-encoding plasmids observed in the assessed Enterobacterales harbored IncFIA/IncR and IncFII sequences. International spread was confirmed by the high genetic similarity to resistance-mediating plasmids published from Asia, Australia, Europe and Northern America, including a *bla*CTX-M-15-gene-carrying plasmid isolated from a wild bird in Germany. In conclusion, the study contributed to the scarcely available information on the epidemiology of third-generation cephalosporine resistance-mediating plasmids in Ghana. Furthermore, the global spread of resistance-mediating plasmids provided hints on the evolutionary success of individual resistance-harboring plasmids by transversal spread among *K. pneumoniae* lineages in Ghana.

Keywords: chronic wound infection; *Klebsiella pneumoniae*; *Escherichia coli*; plasmid; resistance genes; mobile genetic element; Enterobacterales; Ghana; phylogeny
1. Introduction

In recent years, multidrug resistance has become a major concern in sub-Saharan Africa. It has made bacterial infections increasingly difficult to treat, especially those associated with Gram-negative pathogens [1–6]. Acquired antimicrobial drug resistance in Gram-negative bacteria is typically mediated by mobile genetic elements such as plasmids, whose horizontal spread is driven by conjugation-based transmission [7]. Their persistence in bacterial clones is influenced by both fitness costs for the bacterial hosts [8] as well as by compensatory mutations [7,9]. The latter comprise, e.g., mutations in intergenic regions and the selection of genes involved in anaerobic metabolism [10].

In Enterobacterales such as *Escherichia coli* and *Klebsiella pneumoniae*, multidrug resistance is frequently mediated by epidemic resistance plasmids of incompatibility (Inc) groups such as IncFII, IncA/C, IncL/M, IncN and IncI1, which carry genes for extended-spectrum beta-lactamases (ESBLs), AmpC beta-lactamases and carbapenemases [11–38]. Next-generation sequencing-based approaches have been introduced early for the identification of plasmid sequences [39–41].

Epidemiological information on the spread and distribution of resistance-mediating plasmids in bacteria in Ghana is scarce besides individual approaches such as a study on the diversity of plasmids in Ghanaian gonococci from the beginning of the 1980s [42]. Regional spread of a trimethoprim resistance gene cassette via a successful transposable element was reported for *Escherichia coli* strains isolated in Ghana between 2006 and 2008 [43]. The conjugation-based transfer of *bla* <sub>TEM</sub>-gene-mediated ESBL expression could be shown for two-thirds of *bla* <sub>TEM</sub> gene-positive Enterobacterales isolated at a Ghanaian tertiary hospital [44]. In Ghanaian salmonellae comprising ESBL-positive strains mediated by the beta-lactamase genes *bla* <sub>TEM</sub>-<sub>ESBL</sub>, *bla* <sub>CTX-M</sub>, IncN-type, IncFII(S)/IncFIB(S)/IncQ1-type, IncX1-type and TrfA/IncHI2/IncHI2A-type plasmids have been reported [45]. In an *Escherichia coli* isolate of the ST410 sequence type, the IncHI-type transferrable plasmid *p*2189-NDM was described, carrying the resistance genes *bla* <sub>NDM</sub>-1, *bla* <sub>CTX-M</sub>-15, *aadA1*, *aac(6’)-Ib*, *sul3*, *dfrA12* and *cmlA1* [46]. In *Klebsiella pneumoniae* isolates from a teaching hospital, *IncFIB*(K)-type and *ColRNAI*-type plasmids harbored resistance genes such as *bla* <sub>CTX-M</sub>-15, *bla* <sub>SHV</sub>-11, *bla* <sub>TEM</sub>-1B, *bla* <sub>OXA</sub>-1, *ac(3)-IIa*, *strB*, *strA*, *aadA16*, *qnrB66*, *oqxA* and *oqxB*, [47]. Various other epidemiological studies conducted in Ghana provide information on locally abundant resistance mechanisms without further addressing transposable genetic elements [48–53].

Recently, a predominance of Gram-negative rod-shaped bacteria was identified in chronic wounds in rural Ghana [54] with only low to moderate resistance rates compared to other reports from Ghanaian hospitals [55]. Among the Enterobacterales, a minority of three *E. coli* and four *K. pneumoniae* expressing the *bla* <sub>CTX-M</sub>-15 gene with a resulting ESBL-phenotype [56] were identified.

In the present study, the mobile genetic elements within those ESBL-producing Enterobacterales from chronic wounds of Ghanaian patients were assessed. By doing so, the so far scarce knowledge on the local epidemiology of plasmids mediating acquired antimicrobial resistance in Enterobacterales from Ghana was investigated.

2. Results

From seven Enterobacterales isolates from chronic wounds in Ghana as characterized in the methods chapter, 28 plasmid sequences were detected in four assessed ESBL-positive *K. pneumoniae* strains and in three *E. coli* strains. The sizes of the recorded plasmid contigs ranged from 1538 to 224,675 base pairs (Table 1). GenBank accession numbers and typing results applying the software Plasmidfinder 2.0 and mob-typer are shown in Table 1. The most frequently detected PlasmidFinder 2.0 and mob-typer matches for Inc sequences comprised IncFII (*n* = 5), IncFIA (*n* = 4), IncFIB (*n* = 4), IncFIC (*n* = 1), IncP1 (*n* = 1) and IncR (*n* = 1) sequences in 9 out of 16 plasmid sequences from ESBL-positive *K. pneumoniae* isolates. The ESBL-encoding *bla* <sub>CTX-M</sub>-15 genes were carried on plasmids of the IncFIB/IncFII type in three out of four *K. pneumoniae* strains and in another instance on an IncFIA type.
plasmid. In the plasmids from the three ESBL-positive E. coli, IncFIB (n = 4), IncFII (n = 2), IncFIC (n = 1) and IncY (n = 1) sequences were detected in 7 out of 12 plasmid sequences. The ESBL-encoding \( \text{bla}_{\text{CTX-M-15}} \) genes were located on plasmids of the IncFIA/IncFIB/IncFIC-like type, the IncFIA/IncFIB/IncFII type and the IncFIB-type in E. coli strains. The \( \text{bla}_{\text{CTX-M-15}} \)-carrying plasmids of both K. pneumoniae and E. coli strains are visualized in Figure 1. As suggested by the mob-typer software, predicted mobilities of the plasmids comprised the following categories: conjugative (n = 8, including 4 \( \text{bla}_{\text{CTX-M-15}} \) gene harboring plasmids), mobilizable (n = 11, including 2 \( \text{bla}_{\text{CTX-M-15}} \) gene harboring plasmids) and non-mobilizable (n = 9, including 1 \( \text{bla}_{\text{CTX-M-15}} \) gene harboring plasmid) (Table 1).

Table 1. Identified plasmids with information on size, typing results based on PlasmidFinder-2.0 and mob-typer, predicted mobility based on mob-typer and encoded resistance genes. Resistance genes occurring with more than one copy are marked with (*).

| Species and Isolate Number/MLST Type of the Isolate/GenBank Accession Number | Plasmid Id and GenBank Accession Number | Plasmid Size | Typing Results Based on PlasmidFinder-2.0 and Mob-Typer | Predicted Mobility Based on Mob-Typer | Resistance Genes on the Plasmid |
|---|---|---|---|---|---|
| K. pneumoniae Iso00073/ST39/CP095150 | pIsso00073_01; CP095151 | 219,384 | IncFIB, IncFII, rep_cluster_2183 conjugative | Conjugative | aac(3)-IIa, \( \text{bla}_{\text{TEM-1B}} \)*, \( \text{bla}_{\text{CTX-M-15}} \)*, sul2, aph(3')-Ib, aph(3')-Ia, aph(3')-Ib, \( \text{bla}_{\text{SHV-187}} \)*, \( \text{catA2}\)-like, tet(D), aac(6')-Ib-cr, ARR-3, \( \text{dfrA27} \), \( \text{aadA1} \), \( \text{qacE} \), \( \text{sul1} \), \( \text{qnrB2} \) |
| K. pneumoniae Iso00073/ST39/CP095150 | pIsso00073_02; CP095152 | 92,025 | IncFIA, IncR mobilizable | Mobilizable | \( \text{dfrA1} \), \( \text{aadA1} \), \( \text{qacE} \), \( \text{sul1} \), \( \text{tet(D)} \), \( \text{dfrA27} \), \( \text{aadA1} \), \( \text{qacE} \)*, \( \text{qnrB6} \), \( \text{dfrA27} \), \( \text{bla}_{\text{TEM-1B}} \), \( \text{bla}_{\text{CTX-M-15}} \), \( \text{bla}_{\text{SHV-187}} \)*, \( \text{catA1} \) |
| K. pneumoniae Iso00073/ST39/CP095150 | pIsso00073_03; CP095153 | 82,442 | IncFII, IncFIA, IncFIC conjugative | Conjugative | \( \text{erm(B)} \), \( \text{mph(A)} \), \( \text{bla}_{\text{TEM-1B}} \)* |
| K. pneumoniae Iso00073/ST39/CP095150 | pIsso00073_04; CP095154 | 4350 | ColRNAI_rep_cluster_1987 non-mobilizable | Non-mobilizable | - |
| K. pneumoniae Iso00100/ST152/CP095145 | pIsso0100_01; CP095146 | 224,675 | IncFIB, IncFII, rep_cluster_2183 conjugative | Conjugative | \( \text{dfrA1} \), \( \text{aadA1} \), \( \text{qacE} \), \( \text{sul1} \), \( \text{tet(D)} \), \( \text{dfrA27} \), \( \text{aadA1} \), \( \text{qacE} \)*, \( \text{qnrB6} \), \( \text{dfrA27} \), \( \text{bla}_{\text{TEM-1B}} \), \( \text{bla}_{\text{CTX-M-15}} \), \( \text{bla}_{\text{SHV-187}} \)*, \( \text{catA1} \) |
| K. pneumoniae Iso00100/ST152/CP095145 | pIsso0100_02; CP095147 | 109,388 | FIA, rep_cluster_1418 mobilizable | Mobilizable | \( \text{mph(A)} \), \( \text{aph(3')-Ia} \), \( \text{sul1} \), \( \text{qacE} \)*, \( \text{qnrB6} \), \( \text{dfrA27} \), \( \text{bh}_{\text{TEM-1B}} \), \( \text{bh}_{\text{CTX-M-15}} \), \( \text{bh}_{\text{SHV-187}} \)*, \( \text{catA1} \) |
| K. pneumoniae Iso00100/ST152/CP095145 | pIsso0100_03; CP095148 | 8282 | ColRNAI_rep_cluster_1857 non-mobilizable | Non-mobilizable | - |
| K. pneumoniae Iso00100/ST152/CP095145 | pIsso0100_04; CP095149 | 4642 | Col440I, ColRNAI_rep_cluster_1987 non-mobilizable | Non-mobilizable | - |
| K. pneumoniae Iso00199/ST17/CP095140 | pIsso0199_01; CP095141 | 221,581 | IncFIB, IncFII, rep_cluster_2183 conjugative | Conjugative | \( \text{aac(3)-IIa} \), \( \text{bla}_{\text{TEM-1B}} \)*, \( \text{bla}_{\text{CTX-M-15}} \)* |
| K. pneumoniae Iso00199/ST17/CP095140 | pIsso0199_02; CP095142 | 52,096 | IncP1 conjugative | Conjugative | - |
| K. pneumoniae Iso00199/ST17/CP095140 | pIsso0199_03; CP095143 | 4420 | rep_cluster_2358 non-mobilizable | Non-mobilizable | - |
| K. pneumoniae Iso00199/ST17/CP095140 | pIsso0199_04; CP095144 | 3643 | ColRNAI_rep_cluster_1857 non-mobilizable | Non-mobilizable | - |
| Species and Isolate Number/MLST Type of the Isolate/GenBank Accession Number | Plasmid Id and GenBank Accession Number | Plasmid Size | Typing Results Based on PlasmidFinder-2.0 and Mob-Typer | Predicted Mobility Based on Mob-Typer | Resistance Genes on the Plasmid |
|---|---|---|---|---|---|
| **K. pneumoniae Iso00267/ST36/CP095132** | plso00267_01; CP095133 | 194,916 | IncFIB, IncFil, rep_cluster_2183 | conjugative | aac(3)-Ila, blaTEM-1B, blaCTX-M-15 |
| | plso00267_02; CP095134 | 70,936 | IncFIA, rep_cluster_1418 | mobilizable | tet(D), catA2-like, aph(6)-Id, aph(3\')-Ib, sul2, aac(6\')-Ib-cr, ARR-3, dfrA27, aadA16, qacE, sul1 |
| | plso00267_03; CP095135 | 9294 | ColRNAI_rep_cluster_1857 | mobilizable | - |
| | plso00267_04; CP095136 | 5835 | Col(Ye4449) | mobilizable | - |
| **E. coli Iso00041/ST2/CP095155** | plso00041_01; CP095156 | 174,508 | IncFIA, IncFIB, IncFIC | conjugative | catB3, blaCMA-1, aac(6\')-Ib-cr, sitABCD, blaCTX-M-35, tet(B), catA1, mph(A), sul1, qacE, aadA5, dfrA17, aac(3)-IId, blaTEM-1B |
| | plso00041_02; CP095157 | 5164 | IncFIB, rep_cluster_2131 | mobilizable | - |
| | plso00041_03; CP095158 | 2348 | IncFil, Col(IGRK) | non-mobilizable | - |
| | plso00041_04; CP095159 | 1538 | Col(MG828) | mobilizable | - |
| **E. coli Iso00225/ST506/CP095137** | plso00225_01; CP095138 | 133,313 | Col156, IncFIA, IncFIB, IncFil, rep_cluster_2131 | conjugative | tet(A), aph(6)-Id, aph(3\')-Ib, sul2, mph(A), sul1, qacE, aadA5, dfrA17 |
| | plso00225_02; CP095139 | 110,850 | IncFIB, rep_cluster_488 | non-mobilizable | blaCTX-M-15 |
| **E. coli Iso000270/ST2/CP095125** | plso00270_01; CP095126 | 99,933 | IncY | non-mobilizable | - |
| | plso00270_02; CP095127 | 94,223 | rep_cluster_1704 | non-mobilizable | - |
| | plso00270_03; CP095128 | 64,118 | IncFIA, IncFIB, IncFil | mobilizable | catA1, blaCTX-M-15-tet(B) |
| | plso00270_04; CP095129 | 5164 | rep_cluster_2131 | mobilizable | - |
| | plso00270_05; CP095130 | 3007 | rep_cluster_2350 | mobilizable | - |
| | plso00270_06; CP095131 | 2255 | Col(MG828) | non-mobilizable | - |

Resistance genes were located on 8 out of 16 plasmids of the four ESBL-positive K. pneumoniae isolates, as well as on 4 out of 12 plasmids of the three ESBL-positive E. coli isolates, as detailed in Table 1. In three out of four instances in K. pneumoniae, the blaCTX-M-15 gene was associated with the aminoglycoside-mediating gene aac(3)-Ila and the narrow-spectrum beta-lactamase gene blaTEM-1B on the same plasmid. One blaCTX-M-15 gene harboring plasmid in K. pneumoniae was associated with multiple resistance genes comprising macrolide resistance-mediating mph(A); aminoglycoside resistance-mediating aph(3\')-Ia, aph(3\')-Ib, aph(6)-Id, aac(3)-Ila and aadA16; sulfonamide resistance-mediating sul1 and sul2; disinfectant resistance-mediating qacE; quinolone resistance-mediating qnrB6;
trimethoprim-resistance-mediating \textit{dfrA27}; rifampicin resistance-mediating \textit{ARR-3}; fluoroquinolone and aminoglycoside-resistance-mediating \textit{aac(6')-Ib-cr}; the narrow-spectrum beta-lactamases \textit{bla}_{\text{TEM}-1B} and \textit{bla}_{\text{OXA-1}}; and phenicol resistance-mediating \textit{catB3}. In the three ESBL-positive \textit{E. coli} strains, the \textit{bla}_{\text{CTX-M-15}} gene was the only resistance gene located on the plasmid in one instance. In another instance, it was associated with phenicol resistance-mediating \textit{catA1} and tetracycline resistance-mediating \textit{tet(B)}. In the third strain, an association with phenicol resistance-mediating \textit{catA1} and \textit{catB3}, the narrow-spectrum beta-lactamases \textit{bla}_{\text{OXA-1}} and \textit{bla}_{\text{TEM}-1B}, fluoroquinolone and aminoglycoside resistance-mediating \textit{aac(6')-Ib-cr}, disinfectant resistance-mediating \textit{sitABCD} and \textit{qacE}, tetracycline resistance-mediating \textit{tet(B)}, macrolide resistance-mediating \textit{mph(A)}, sulfonamide resistance-mediating \textit{sul1}, aminoglycoside resistance-mediating \textit{aadA5} and \textit{aac(3)-IId}, as well as trimethoprim resistance-mediating \textit{dfrA17}, was recorded.

**Figure 1.** Visualization of the \textit{bla}_{\text{CTX-M}}-gene-carrying plasmids. Upper row: Plasmids detected in \textit{K. pneumoniae}. Lower row: Plasmids detected in \textit{E. coli}. Genes located on the forward and reverse strand are colored in light and dark grey, respectively. \textit{Bla} genes are shown in red. Genes coding for replication-associated proteins and marking the start gene of the sequence are shown in green. PlasmidFinder matches are shown in purple.

Of note, three out of four \textit{bla}_{\text{CTX-M-15}}-gene-carrying plasmids from \textit{K. pneumoniae} isolates of different clonal lineages showed a very high genetic similarity to a plasmid isolated from a \textit{K. pneumoniae} isolate from France (Appendix A, Tables A1 and A2). This similarity was confirmed by a BlastN comparison of all \textit{bla}_{\text{CTX-M-15}} gene harboring plasmids from the present study, confirming the high genetic similarity of the plasmids \textit{pIs00073}_{01}, \textit{pIs00100}_{02} and \textit{pIs00199}_{01} from the \textit{K. pneumoniae} clonal complex isolates ST39, ST17 and ST36, respectively (Table 2). In the mob-typer analysis, these plasmids were characterized as conjugative (Table 1).

As indicated by another BlastN search (Appendix A), similar resistance-carrying plasmids to the ones from the present study have been globally isolated and sequenced in Europe, Asia, Australia and North America. The international isolations were performed not only from human samples but also from a sample taken from a wild bird, as reported from Germany (Appendix A).
### Table 2. Sequence homology as assessed by pairwise blastN analysis of blaCTX-M-gene containing plasmids/query coverages.

|            | K. pneumoniae | E. coli |
|------------|---------------|---------|
|            | Iso00073      | Iso00073_01 | pIso00073_01 |
|            | Iso00199      | Iso00199_01 | pIso00199_01 |
|            | Iso00267      | Iso00267_01 | pIso00267_01 |
|            | Iso00100      | Iso00100_02 | pIso00100_02 |
|            | Iso00041      | Iso00041_01 | pIso00041_01 |
|            | Iso00225      | Iso00225_02 | pIso00225_02 |
|            | Iso00270      | Iso00270_03 | pIso00270_03 |
| **K. pneumoniae** |           | 100%      | 83%      |
|            |             | 99%       | 83%      |
|            |             | 92%       | 92%      |
|            |             | 21%       | 21%      |
| **E. coli** |             | 15%       | 15%      |
|            |             | 2%        | 2%       |
|            |             | 23%       | 23%      |
| **Klebsiella pneumoniae** | 100% | 83% | 9% | 11% | 1% | 7% |
| **Escherichia coli** | 99% | 83% | 9% | 11% | 1% | 7% |
| **K. pneumoniae** | 92% | 92% | 12% | 11% | 1% | 8% |
| **Escherichia coli** | 21% | 25% | - | 35% | 2% | 14% |

### 3. Discussion

The study was conducted to add epidemiological information on the local epidemiology of third-generation cephalosporine resistance-mediating plasmids in Enterobacterales isolated from chronic wounds in Ghana. To do so, four *K. pneumoniae* and three *E. coli* strains carrying the ESBL-mediating *bla*CTX-M-15 genes were chosen from a previous study [54,56]. Wound isolates were chosen due to their likely etiological relevance for human infections, although etiologically irrelevant colonization cannot be completely ruled out at primarily non-sterile sampling sites such as superficial wounds in contact with the environment. Phenotypical strain characteristics did not affect the choice, which included all isolated *bla*CTX-M-15-gene-carrying Enterobacterales from the previous assessment. The strains were subjected to combined long-read and short-read sequencing to identify resistance-encoding plasmids and to compare the results with previous assessments.

In concordance with our results, IncFIB-type plasmids found in *K. pneumoniae* isolates from Ghana associated with the *bla*CTX-M-15 gene had been previously described in 2019 by Agyepong and colleagues [47]. In addition to the previously published results, we detected three plasmids with IncFIB/IncFII sequences that had previously been reported from France (GenBank accession number LR991402.1). Very similar, although not completely identical, plasmids were found in Ghanaian *K. pneumoniae* strains of the clonal lineages ST17, ST36 and ST39, suggesting the horizontal transmission of this plasmid within *K. pneumoniae* strains in Ghana, as also confirmed by mobility prediction with the mob-typer software. Of note, ESBL-positive ST39 *K. pneumoniae* strains have previously been reported to be highly prevalent in pigs and abattoir workers in Cameroon [57]. A *bla*CTX-M-15-gene-carrying plasmid of the FIA type found in another *K. pneumoniae* strain was previously described by Canadian scientists (GenBank accession number CP023950.1), confirming its international spread. Plasmids of the incompatibility groups IncFIA, IncFIB and IncFII carrying *blaCTXM-15* genes have also been described from Eastern African Tanzania [58]. In Tanzanian *K. pneumoniae* strains, in particular, a *blaCTXM-15*-gene-harboring plasmid of the incompatibility group IncFIILK5/IncR has been associated with highly efficient horizontal transfer [59].

The description of plasmids carrying IncFIA/IncFIB/IncFIC, IncFIA/IncFIB/IncFI and IncFIB sequences associated with *blaCTX-M-15* genes in *E. coli* strains is new and adds to the available information on the epidemiology of plasmids encoding resistance against third-generation cephalosporines in Gram-negative pathogens in Ghana [46,47]. Interestingly, genetically highly similar plasmid sequences have been reported from Australia (GenBank accession number LR890289.1), the United Kingdom (UK) [60] and Germany [61] before, comprising a human *E. coli* isolate from the UK and an *E. coli* strain isolated from a wild bird in Germany. It has recently been suggested [62] that not only international travel but also bacterial spread by migrating birds might contribute to the distribution of resistant bacterial isolates and their resistance-encoding plasmids. A single isolation from a bird cannot be considered as definitive proof because contamination from human sources remains an option but is nevertheless in line with this hypothesis. Interestingly, plasmids of the incompatibility group IncY have been linked to *blaCTXM-15* gene-carriage in Tanzanian
E. coli strains [63], while an IncY plasmid from one of the assessed Ghanaian E. coli strains did not harbor any resistance-associated genes.

Resistance against several antibiotic drugs other than beta-lactams, which had been phenotypically observed for the assessed Ghanaian Enterobacterales, was shown to be caused by co-occurring plasmids. In K. pneumoniae, such plasmids comprised the types IncFIA, IncFIA/IncR, IncFIA/IncFIC/IncFII and IncFIB/IncFII, while in E. coli, an IncFIA/IncFIB/IncFII-type plasmid encoded multiple resistance genes. Resistance-gene-carrying IncFIC plasmids in Africa have also been described in multidrug-resistant Salmonella enterica isolated in Kenya [64]. In contrast, CoRNASI-type plasmids, which were associated with antimicrobial resistance in Ghanaian K. pneumoniae strains in a previous study [47], did not encode resistance genes in our assessment.

As reported previously [39], linking of different contigs on the same plasmid can be challenging due to technical limitations of the sequencing technology. With focus on this technical issue, double sequencing with short-read (Illumina) and long-read (Nanopore) technologies was performed, followed by hybrid assembly of both data sets. Furthermore, evidence of plasmid replicon sequences was secured using three different methods (PlasmidFinder, MOB typing and BlastN versus the SRST2 database [65]) for all contigs. Furthermore, the plasmid nature of these shorter contigs is supported by normalized depths from 1.3 to 12.1 with an average of 4.2, as calculated by the Unicyler assembler (chromosome set to 1.0). Admittedly, these procedures do not provide 100% proof but were considered as sufficient justification for reporting the contigs as plasmids.

The study has a few limitations. First and most important, the number of ESBL-positive isolates available from the previous study [56] for inclusion in the plasmid assessment was considerably low. Accordingly, a regional representativeness cannot be ensured, although partial matching with previously published results from Ghana could be demonstrated [47]. Second, although the inclusion of etiologically relevant strains was aspired to by including strains from a wound infection study [54,56] instead of screening isolates, etiological relevance of the included strains is not definitely assured because the discrimination of causative infectious agents and colonizing bacterial flora is challenging at primarily non-sterile sites such as chronic wounds. Third, the postulated horizontal spread of plasmid-mediated third-generation cephalosporine resistance in Ghana was not confirmed by conjugation experiments. Such approaches would have been beyond both the scope and the financial options of this investigator-initiated, solely epidemiological study.

4. Materials and Methods
4.1. Sample Collection, Bacterial Culture, Antibiotic Susceptibility Testing and Whole Genome Sequencing

A total of seven ESBL-positive Enterobacterales carrying the blaCTX-M-15 gene were selected from a previous study on bacterial isolates from chronic wounds of patients from rural Ghana [54,56]. In summary, the strains were isolated from patients ≥15 years with infected chronic wounds at the Outpatient Department (OPD) of the Agogo Presbyterian Hospital in the Asante Akim North District of rural Ghana. Antibiotic resistance, as indicated in the Appendix A below, was assessed by the disk diffusion method and interpreted following the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines v6.0 (http://www.eucast.org (accessed on 31 January 2016)). Both species identity and antibiotic susceptibility had been confirmed using the VITEK2 system (bioMérieux, Nütingen, Germany), as described elsewhere [54,56].

Following nucleic acid extraction applying the MasterPure Complete DNA and RNA Purification Kit (LGC standards GmbH, Wesel, Germany), DNA was sent for whole genome sequencing (WGS) to BGI Europe, Denmark, Copenhagen. There, a BGISEQ-500 device was used for sequencing, generating 2 × 150 bp paired-end reads with an aimed coverage of 100×. Short-read archive (SRA) accession numbers of the obtained sequences are indicated in Table 1, linking to the original raw data as uploaded for public use to the short-read archive (SRA, NCBI) under the accession number PRJNA699140 [56]. Details on the chosen isolates are provided in Appendix A.
In addition, 1 µg DNA was sent to BGI Genomics C, Ltd., for long-read sequencing. In detail, sequencing analysis was performed on a FornmethION (Nanopore) device using the flow-cell version R.9.4.1. Base-calling was performed with the Guppy software (https://nanoporetech.com, last accessed on 19 May 2022) applying the high-accuracy (HAC) model. After sequencing, about 3 mb of data was obtained from each sample.

4.2. Assessment of the Whole Genome Sequencing Data for Plasmids

Assembly of long-read sequences by Flye, v2.9 (https://github.com/fenderglass/Flye, last accessed on 30 March 2022) using (i) unfiltered fastq reads, (ii) fastq reads > 3 kb and (iii) fastq reads > 3 kb with subsequent polishing by Medaka v1.5.0 (https://github.com/nanoporetech/medaka, last accessed on 30 March 2022) did not reveal significant differences with respect to the assembled contigs. However, as expected, hybrid assemblies of long- and short-read sequences were of remarkably higher quality due to the resolution of sequencing errors in homo-nucleotide stretches (data not shown), a shortcoming of the nanopore technology [66]. Therefore, hybrid assemblies were performed by Unicycler [67] using unfiltered Oxford nanopore long-reads and BGI paired-end short-reads (after fastqc, trimmomatic). This resulted in assemblies containing one chromosome contig (K. pneumoniae 5.3–5.4 Mb; E. coli 4.7–5.0 Mb) and up to six plasmids per genome. Furthermore, the assembled plasmid contigs were analyzed by blastN (https://blast.ncbi.nlm.nih.gov/Blast.cgi, last accessed on 30 March 2022) vs. “Bacteria (taxid:2)” [68]. Results were filtered for the highest query coverage (as shown in Appendix A). Annotation was conducted by RAST (http://rast.nmpdr.org, last accessed on 30 March 2022). Analysis of the assembled genomes for resistance genes was conducted by ResFinder (http://cge.cbs.dtu.dk/services/ResFinder/—version 4.1, last accessed on 30 March 2022), with a nucleotide identity threshold of 90% and a minimum match length of 60%. Detection of plasmids was performed by PlasmidFinder, version 2.0.1 (https://cge.cbs.dtu.dk/services/PlasmidFinder-2.0/), last accessed on 30 March 2022) [39,69] and by the mob-typer software (https://github.com/phac-nml/mob-suite, last accessed on 14 April 2022) to identify matching incompatibility types [70] and to predict the mobility of the plasmids. As a third procedure, BlastN versus the SRST2 database [65] was conducted to ensure evidence of plasmid replicon sequences for all contigs, which were named IsoXXXXX_pXX. The sizes of the plasmids were based on the Unicycler results (short- and long-read hybrid assembly), which represents the most reliable approach currently available. Regarding the position of the bla\textit{CTX-M-15} genes within the plasmid sequences, the ResFinder results were independently confirmed by PROKKA annotation and Abricate [71] analysis, using the NCBI AMR Finder Plus Database, of the contigs of the above-mentioned hybrid assemblies (data not shown [72]). The ResFinder output provided the coordinates of the detected genes within the analyzed nucleotide sequences. For visualization purposes, the plasmid nucleotide sequences were annotated via RASTk using default settings [73]. The generated merged GenBank files were visualized using the tool DNAplotter [74].

4.3. Ethical Clearance

Ethical clearance was provided by the Committee on Human Research, Publications and Ethics, School of Medical Science, Kwame Nkrumah University of Science and Technology in Kumasi, Ghana (approval number CHRPE/AP/078/16).

5. Conclusions

Despite the above-mentioned limitations, which narrow the interpretability of the results, the study adds to the scarcely available data on the epidemiology of plasmids encoding third-generation cephalosporine resistance in ESBL-positive Enterobacteriales in Western African Ghana. At least for \textit{K. pneumoniae}, an individual conjugative \textit{bla}\textit{CTX-M-15}-gene-carrying plasmid was found in three out of four assessed strains of different clonal lineages, suggesting successful vertical transmission in Ghana. Furthermore, the assessment exemplarily demonstrated the international spread of such resistance-mediating plasmids...
in times of globalization, affected, e.g., by human travelling and the migration of wild birds. Future assessments should comprise more sampling sites, clinical conditions and geographic locations in Ghana to provide more robust and conclusive epidemiological data compared to the present hypothesis-forming study.

**Author Contributions:** U.L., F.P., S.T. and H.F. designed and coordinated this study. F.P. and S.T. performed the bioinformatic analysis. H.F. and U.L. wrote the first draft of this manuscript. F.P., S.T., D.D., T.T., K.O., C.W.A., M.L., A.J., M.K., S.S., K.T., H.F., J.M. and U.L., jointly supported the interpretation of the results, writing and editing the manuscript. All authors have read and agreed to the published version of the manuscript.

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**Institutional Review Board Statement:** The study was conducted according to guidelines of the Declaration of Helsinki. The Committee on Human Research, Publications and Ethics, School of Medical Science, Kwame Nkrumah University of Science and Technology in Kumasi, Ghana, approved this study (approval number CHRPE/AP/078/16).

**Informed Consent Statement:** Informed consent was obtained from all study participants.

**Data Availability Statement:** All relevant data have been provided in the paper. Raw data are available via the links indicated in the paper and can also be provided by the authors on reasonable request.

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

**Appendix A**

Table A1. Plasmids carrying antimicrobial resistance genes and disinfectant resistance genes and genetic similarity according to BlastN matching with previously published plasmids in the NCBI database, as well as the previous sites of detection.

| Strain/Sequence Type (ST)/GenBank Accession Number of Genomic DNA | Plasmid Number; GenBank Accession Number | Best Hit with Respect to the Query Coverage | Query Coverage (%) | Nucleotide Identity (%) | Geographic Site of NCBI Sequence Submission (Bacterial Species and Source) [Reference] |
|---|---|---|---|---|---|
| *K. pneumoniae* Iso00073/ST39/CP095150 | pIs00073_01; CP095151 LR991402.1 | 97% | 99.99% | France (*K. pneumoniae*, source: unknown) [none] |
| | pIs00073_02; CP095152 CP063009.1 | 94% | 99.99% | Russian Federation (*K. pneumoniae*, source: human) [75] |
| | pIs00073_03; CP095153 CP054171.1 | 95% | 99.86% | India (*K. pneumoniae*, source: human) [none] |
| *K. pneumoniae* Iso00100/ST152/CP095145 | pIs00100_01; CP095146 CP065826.1 | 79% | 99.95% | United States of America, (*K. pneumoniae*, source: human) [none] |
| | pIs00100_02; CP095147 CP029501.1 | 80% | 99.48% | Canada (*K. pneumoniae*, source: human) [none] |
| *K. pneumoniae* Iso00199/ST17/CP095140 | pIs00199_01; CP095141 LR991402.1 | 96% | 99.99% | France (*K. pneumoniae*, source: unknown) [none] |
| *K. pneumoniae* Iso00267/ST36/CP095132 | pIs00267_01; CP095133 LR991402.1 | 94% | 99.99% | France (*K. pneumoniae*, source: unknown) [none] |
| | pIs00267_02; CP095134 CP016810.1 | 72% | 99.97% | United States of America, (*K. pneumoniae*, source: human) [none] |
Table A1. Cont.

| Strain/Sequence Type (ST)/GenBank Accession Number of Genomic DNA | Plasmid Number; GenBank Accession Number | Best Hit with Respect to the Query Coverage | Query Coverage (%) | Nucleotide Identity (%) | Geographic Site of NCBI Sequence Submission (Bacterial Species and Source) [Reference] |
|---|---|---|---|---|---|
| **E. coli Iso00041/ST2/CP095155** | pIs00041_01; CP095156 | LR890289.1 | 99% | 99.98% | Australia (E. coli, source: unknown) [none] |
| **E. coli Iso00225/ST2/CP095138** | pIs00225_01; CP095138 | CP088462.1 | 100% | 100% | South Korea (E. coli, source: human) [none] |
| **E. coli Iso00270/ST2/CP095125** | pIs00270_03; CP095128 | MW390712.1 | 100% | 100% | United Kingdom (E. coli, source: human) [60] |

Table A2. Strain-specific details of the 7 Enterobacteriales included in the screening for mobile genetic elements mediating antimicrobial resistance. Further details are provided elsewhere [56].

| Species and Strain Number (73) | Sequence Type | Acquired Antimicrobial Resistance Genes | Recorded Phenotypic Resistance Against Apart From Penicillins and Cephalosporines * | Short-Read Archive (SRA) Accession Number |
|---|---|---|---|---|
| **Klebsiella pneumoniae (73)** | ST39 | blaTEM-1B, blaCTX-M-15, sul1, fosA, dfrA27, erm(B), mph(A), tet(D), oqxB, oqxA, aac(6′)-Ib-cr, aprB2, catA2-like, aadA16, aac(3)-Ila, aph(3′)-Ib, aph(6)-Id | gentamicin, ciprofloxacin, moxifloxacin, trimethoprim/sulfamethoxazole | SRR13617236 |
| **Klebsiella pneumoniae (100)** | ST152 | blaCTX-M-15, blaOXA-1, blaTEM-1B, sul2, sul1, dfrA1, dfrA27, mph(A), aac(6′)-Ib-cr, oqxB, qnrB6, oqxA, catB3, catA1, ARR-3, aac(3)-Ila, aph(6)-Id, aph(3′)-Ib, aadA1, aadA16, aph(3′)-Ia | gentamicin, ciprofloxacin, moxifloxacin, trimethoprim/sulfamethoxazole | SRR13617311 |
| **Klebsiella pneumoniae (199)** | ST17 | blaCTX-M-15, blaTEM-1B, sul2, sul1, fosA-like, dfrA16, oqxA, oqxB, aadA2b, aac(3)-Ila | gentamicin, trimethoprim/sulfamethoxazole | SRR13617280 |
| **Klebsiella pneumoniae (267)** | ST36 | blaCTX-M-15, blaTEM-1B, sul2, sul1, fosA, dfrA27, tet(D), aac(6′)-Ib-cr, oqxA, oqxB, catA2-like, ARR-3, aph(6)-Id, aph(3′)-Ib, aadA16, aac(3)-Ila | gentamicin, trimethoprim/sulfamethoxazole | SRR13617257 |
| **Escherichia coli (41)** | ST2 | blaOXA-1, blaTEM-1B, blaCTX-M-15, sul1, dfrA17, mph(A), tet(B), aac(6′)-Ib-cr, catB3, catA1, aac(3)-IId, aadA5, mdf(A) | gentamicin, ciprofloxacin, moxifloxacin, trimethoprim/sulfamethoxazole | SRR13617294 |
| **Escherichia coli (225)** | ST506 | blaTEM-1D, blaCTX-M-15, sul1, sul2, dfrA17, mph(A), tet(A), catA1, aadA5, aph(6)-Id, aph(3′)-Ib, mdf(A)-like | moxifloxacin, trimethoprim/sulfamethoxazole | SRR13617270 |
| **Escherichia coli (270)** | ST2 | blaCTX-M-15, tet(B), catA1, mdf(A) | ciprofloxacin, moxifloxacin | SRR13617256 |

* Only resistance according to EUCAST is recorded, while intermediate susceptibility has been attributed to the susceptibility group. All strains were phenotypically resistant against ampicillin, ampicillin/sulbactam, piperacillin/tazobactam, cefuroxime, cefuroxime axetil, cefpodoxime, cefotaxime and cefazidime. Other tested antimicrobial drugs comprised ertapenem, imipenem, meropenem, gentamicin, ciprofloxacin, moxifloxacin, tigecycline and trimethoprim/sulfamethoxazole.
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