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$bla_{CTX\text{-}M\text{-}15}$ carried by IncF-type plasmids is the dominant ESBL gene in *Escherichia coli* and *Klebsiella pneumoniae* at a hospital in Ghana

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ABSTRACT

Escherichia coli and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases (ESBLs) are among the most multidrug-resistant pathogens in hospitals and are spreading worldwide. Horizontal gene transfer and spread of high-risk clones are involved in ESBL dissemination. Investigation of the resistance phenotypes of 101 consecutive clinical *E. coli* (n = 58) and *K. pneumoniae* (n = 43) isolated at the Komfo Anokye Teaching Hospital in Ghana over 3 months revealed 63 (62%) with an ESBL phenotype. All 63 had a $bla_{CTX\text{-}M}$ gene, and sequence analysis showed that 62 of these were $bla_{CTX\text{-}M\text{-}15}$. $bla_{CTX\text{-}M\text{-}15}$ was linked to IS*Ecp1* and orf477Δ in all isolates, and most isolates also carried bla_{TEM}, *aac(3)-II*, *aacA4*cr, and/or $bla_{OXA\text{-}30}$ genes on IncF plasmids. XbaI/pulsed-field electrophoresis showed heterogeneity among isolates of both species, suggesting that $bla_{CTX\text{-}M\text{-}15}$ dissemination is caused by horizontal gene transfer rather than clonal spread of these species in Ghana.

1. Introduction

β-Lactam resistance mediated by extended-spectrum β-lactamases (ESBLs) has become a serious public health problem in recent decades, especially in *Escherichia coli* and *Klebsiella pneumoniae* (Oteo et al., 2010; Paterson et al., 2003). These enzymes are capable of hydrolyzing most broad-spectrum β-lactams and monobactams but not cephamycins, and most of them are susceptible to β-lactam inhibitors such as clavulanic acid (Bush and Jacoby, 2010). Coresistance to aminoglycosides and fluoroquinolones is often associated with these ESBL genes (Partridge et al., 2011). Plasmid-encoded ESBLs are mostly of the TEM, SHV, or CTX-M type, with CTX-M enzymes becoming the predominant ESBL type in *E. coli* and *K. pneumoniae* over the last decade (Rossolini et al., 2008). CTX-M enzymes are a group of class A ESBLs comprising over 150 allelic variants (http://www.lahey.org/studies/other.asp#table1) that can be divided into 5 major phylogenetic groups (CTX-M-1, CTX-M-2, CTX-M-8, CTX-M-9, and CTX-M-25) on the basis of their amino acid sequences. CTX-M-15, which belongs to the CTX-M-1 group, is the most widely disseminated (Rossolini et al., 2008). Horizontal gene transfer via IncF-type plasmids (Novais et al., 2007; Partridge et al., 2011; Villa et al., 2010) and globally disseminated *E. coli* clones such as 025:H4-ST131 (Peirano and Pitout, 2010) are associated with the success of $bla_{CTX\text{-}M\text{-}15}$. CTX-M-9 group genes are very common in some European and Asian countries (Ruiz de Alegria et al., 2011; Shin et al., 2011; Titelman et al., 2011), with CTX-M-2 and CTX-M-8 more prevalent in South America (Climaco et al., 2010; Rossolini et al., 2008).

Spread of $bla_{CTX\text{-}M\text{-}15}$ constitutes a significant threat to public health, as isolates carrying this gene are mostly also resistant to several different classes of antimicrobial agents. Understanding the local epidemiology of ESBL genes, particularly $bla_{CTX\text{-}M\text{-}15}$, is thus important for effective screening and patient management. CTX-M–type β-lactamases have been reported in Africa (Ndugulile et al., 2005; Rafai et al., 2015; Soge et al., 2006); however, epidemiological data on ESBL β-lactamases in Ghana are very limited. In this study, we investigated the resistance phenotypes, resistance genes and associated plasmids, and relatedness of clinical isolates of *E. coli* and *K. pneumoniae* at the Komfo Anokye Teaching Hospital (KATH) in Ghana.

2. Materials and methods

2.1. Identification and susceptibility testing

A total of 101 consecutive isolates of *E. coli* (n = 58) and *K. pneumoniae* (n = 43), each from a clinical infection (sepsis or urinary tract infection) of a different patient, were collected from May to July 2013 at the KATH, the second largest hospital in Ghana (~1500 beds). Species were identified by routine diagnostic methods with API 20E (bioMérieux, Marcy l'Etoile, France) at KATH. They were stored at −70 °C in CRYOBANK™ medium (Copan Diagnostics, Murrieta, CA,

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USA) and transported to the Centre for Infectious Diseases and Microbiology, Westmead Hospital (Australia). Species identification was verified using MALDI-TOF MS (Microflex LT, Bruker, Germany) (Kok et al., 2011).

Susceptibilities to antimicrobial agents were determined using the NMIC-203 card on the Phoenix system (BD Diagnostic Systems, Sparks, MD, USA) and interpreted according to M100-S24 guidelines (CLSI, 2014). Fosfomycin, ceftazidime, cefotaxime, and ceftriaxone MICs were also determined for selected isolates by E-test (AB Biodisk, Solna, Sweden). Phenotypic detection of carbapenem-hydrolyzing enzymes was carried out using the Carba NP test, as previously described (Nordmann et al., 2012).

2.2. Detection of antibiotic resistance genes

Bacterial isolates were screened for the presence of various antibiotic resistance genes by multiplex PCR/reverse line blot (mPCR/RLB) hybridization using a previously described set of targets (Ginn et al., 2014) plus a further 4 that were optimized and validated (Table S1). Control isolates (Table S2) known to carry 1 or 2 of the new target genes were obtained from local surveillance studies or were kindly provided by J. Bell (SA Pathology, Adelaide, Australia) or Bruno Gonzalez-Zorn (Universidad Complutense de Madrid, Spain). Optimization and validation were carried out using these 7 isolates plus the control isolates listed in Ginn et al. (2013) Table 2 and the original set of 22 probes at the concentrations listed in Ginn et al. (2013) Table 1 plus the 4 new probes at 1, 3, 5, 7, or 10 mmol/L. The final concentrations listed in Table S1 were selected to produce relatively consistent spot intensities across all targets. Additional simplex PCR was used to confirm selected results from mPCR/RLB and to amplify $bla_{\text{CTX-M-1}}$ and $bla_{\text{CTX-M-9}}$ group genes (Zong et al., 2008) for sequencing.

2.3. Pulsed-field gel electrophoresis (PFGE), hybridization, and plasmid analysis

PCR-based replicon typing (PBRT) (Carattoli et al., 2005) with additional primers for FII, FII_K (Villa et al., 2010), and IncX (Johnson et al., 2012) plasmids was used to detect different plasmid replicons. S1 nuclease (Promega, Madison, WI, USA) digestion and PFGE (Barton et al., 1995; Partridge et al., 2011) and Southern hybridization (Partridge et al., 2015) were carried out for all isolates containing $bla_{\text{CTX-M-15}}$. Probes for IncFII, IncFIA, $IncFII_K$, and $bla_{\text{CTX-M-15}}$ were prepared using published primers (Villa et al., 2010; Zong et al., 2008) and a PCR DIG Probe Synthesis Kit (Roche, Mannheim, Germany). Images were obtained on a ChemiDoc™ MP System (Bio-Rad Laboratories, Richmond, CA, USA).

2.4. Clonal relationships

The relatedness of isolates was determined by PFGE analysis of XbaI-digested genomic DNA (Han et al., 2013). Electrophoresis was carried out for 20 h at 14 °C with pulse times ranging from 6 to 36 s at 6 V/cm with Bio-Rad CHEF MAPPER apparatus (Bio-Rad Laboratories). Restriction profiles of isolates were analyzed using BioNumerics version 7.10 fingerprinting software (Applied Maths, Sint-Martens-Latem, Belgium). Cluster analysis of the Dice similarity indices was performed to generate a dendrogram based on the unweighted pair group according to Tenover's criteria (Tenover et al., 1995). Isolates with ≥80% Dice similarity indices were considered closely related. For the detection of ST131, $bla_{\text{CTX-M-15}}$ *E. coli* isolates were screened by PCR for O16 and O25b variants followed by amplification and sequencing of *fimH* and *fumC* alleles (Johnson et al., 2009; Johnson et al., 2013; Johnson et al., 2014; Weissman et al., 2012).

3. Ethical considerations

This study was approved by Committee on Human Research, Publication and Ethics of the School of Medical Sciences, Kwame Nkrumah University of Science and Technology, and the KATH, Ghana. The isolates obtained from patient samples were assigned arbitrary numbers.

4. Results

4.1. Antimicrobial resistance genes and phenotypes

Antimicrobial susceptibility profiles revealed widespread resistance to antibiotics commonly used in the clinical setting (Tables S3 and S4). About half of the *E. coli* isolates (n = 30/58) and the majority of *K. pneumoniae* (n = 33/43) were nonsusceptible to third-generation cephalosporins, and generally, these isolates were also resistant to non–β-lactam agents, especially gentamicin, tobramycin, and ciprofloxacin. All *E. coli* and *K. pneumoniae* isolates were susceptible to carbapenems (MICs ETP ≤0.5 μg/mL, IPM ≤1 μg/mL, MEM ≤0.5 μg/mL; Tables S3 and S4), and no carbapenemase genes were identified by mPCR/RLB nor was any carbapenemase activity detected by the CarbaNP test.

All *E. coli* isolates nonsusceptible to ceftriaxone (n = 30) and/or ceftazidime (n = 27) had a $bla_{\text{CTX-M}}$ gene ($bla_{\text{CTX-M-15}}$ = 29, $bla_{\text{CTX-M-27}}$ = 1; Fig. 1 and Table S3). All *K. pneumoniae* isolates nonsusceptible to ceftriaxone and ceftazidime (n = 33) had $bla_{\text{CTX-M-15}}$ (Fig. 2 and Table S4). IS*Ecp1* was located upstream of $bla_{\text{CTX-M}}$ in all 63 isolates, with 48 bp between the left end of IS*Ecp1* and the start of $bla_{\text{CTX-M-15}}$ (n = 62) and 42 bp between IS*Ecp1* and $bla_{\text{CTX-M-27}}$ (n = 1). mPCR/RLB detected different combinations of the bla_{TEM}, $bla_{\text{OXA-30}}$, *aacA4*cr, and *aac(3)-II* genes, all commonly associated with $bla_{\text{CTX-M-15}}$ (Partridge et al., 2011), in *E. coli* (Fig. 1) and *K. pneumoniae* (Fig. 2). *qnrB* genes were also quite common in *K. pneumoniae* isolates with $bla_{\text{CTX-M-15}}$, and other plasmid-mediated quinolone resistance genes (*qnrA*, *qnrS*) were detected in few isolates. All 24 *E. coli* and all 32 *K. pneumoniae* isolates resistant to gentamicin had an *aac(3)-II* gene, with an *aadB* gene also present in 1 of these *K. pneumoniae* isolates plus another with intermediate gentamicin resistance. All 25 *E. coli* with an *aacA4*cr variant were resistant to ciprofloxacin, but some isolates without this gene were also resistant, and 2 isolates had *qnrS*, but no other *qnr* genes were detected. Unlike *E. coli*, *aacA4*cr was not predictive of ciprofloxacin resistance in *K. pneumoniae*. While *qnrB* genes were relatively common (n = 12/43) in *K. pneumoniae*, they did not correlate perfectly with ciprofloxacin nonsusceptibility, and *qnrS* (n = 6) and *qnrA* (n = 1) genes were less common.

4.2. Plasmid characteristics

IncFII (n = 41/58), IncFIA (n = 43/58), and IncFIB (n = 48/58) were the most common replicon types found in *E. coli* isolates (Fig. 1), while $IncFII_K$ was the predominant replicon type among *K. pneumoniae* isolates (n = 31/43) (Fig. 2). S1 nuclease/PFGE revealed various numbers and sizes (~23–240 kb) of plasmids among *E. coli* and *K. pneumoniae* isolates (Figs. S1–S8). In general, more plasmid bands were detected by S1/PFGE than replicons amplified by PBRT. In *E. coli*, $bla_{\text{CTX-M-15}}$ was located on a multireplicon IncFII/FIA/FIB plasmid in 18 isolates, an IncFIA/FIB plasmid in 2 isolates, and an FIA plasmid in 1 isolate. Among *K. pneumoniae*, $bla_{\text{CTX-M-15}}$ was identified only on $IncFII_K$ plasmids (n = 21/31 $IncFII_K$-positive isolates). $bla_{\text{CTX-M-15}}$ was also found in the *E. coli* (n = 4) or *K. pneumoniae* (n = 7) chromosome. In 2 *E. coli* and 2 *K. pneumoniae* isolates, $bla_{\text{CTX-M-15}}$ was on the chromosome in addition to an IncF plasmid.

4.3. Polyclonal spread of Enterobacteriaceae carrying $bla_{\text{CTX-M}}$ genes in Ghana

PFGE analysis showed genetic heterogeneity among *E. coli* isolates, revealing 35 distinct pulsotypes using a Dice coefficient of ≥80%