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**Ping Yi Grace Lee**
London School of Hygiene & Tropical Medicine
**Samuel Addo Akwetey**
University of Development Studies
**Humphrey P. K. Addy**
Kwame Nkrumah University of Science and Technology
**Elvis Quansah**
University of Skills Training and Entrepreneurial Development
**Thanh Le-Viet**
Quadram Institute
**David Baker**
Quadram Institute
**Richael Odarkor Mills**
University of Cape Coast
**Isaac Dadzie**
University of Cape Coast
**Ebenezer Foster-Nyarko**
Ebenezer.Foster-Nyarko2@lshtm.ac.uk

London School of Hygiene & Tropical Medicine

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# Convergent chromosomal integration of blaCTX-M-15 via ISEcp1 in Klebsiella pneumoniae from Southern Ghana

Ping Yi Grace Lee[1], Samuel Addo Akwetey[2], Humphrey P. K. Addy[3], Elvis Quansah[4], Thanh Le-Viet[5], David Baker[5], Richael Odarkor Mills[5§], Isaac Dadzie[6§], Ebenezer Foster-Nyarko[1§#]

[1] Department of Infection Biology, London School of Hygiene & Tropical Medicine, Keppel Street, London, UK

[2] Department of Clinical Microbiology, School of Medicine, University of Development Studies, Tamale, Ghana

[3] Department of Biochemistry and Biotechnology, College of Science, Kwame Nkrumah University of Science and Technology, Kumasi, Ghana

[4] Department of Biological Sciences Education, University of Skills Training and Entrepreneurial Development, Mampong-Ashanti, Ghana

[5] Quadram Institute Biosciences, Norwich Research Park, Norwich, NR4 7UQ, UK

[6] Department of Medical Laboratory Technology, University of Cape Coast, Ghana

[§] These authors contributed equally.

[#] Corresponding author; email Ebenezer.Foster-Nyarko2@lshtm.ac.uk

## Abstract

**Background:** Antimicrobial resistance (AMR) is a critical global health threat, with *Klebsiella pneumoniae* among the leading contributors to AMR-associated mortality in sub-Saharan Africa. Chromosomal integration of extended-spectrum beta-lactamase genes, particularly *bla*$_{\text{CTX-M-15}}$, enables stable vertical transmission of resistance independent of plasmid maintenance, yet the mechanisms and genomic contexts of these events remain poorly characterised in isolates from Ghana.

**Methods:** We analysed 103 hybrid nanopore-Illumina assemblies of *K. pneumoniae* isolates from five Ghanaian tertiary hospitals using AMRFinderPlus, ISEScan, IntegronFinder, and ICEfinder. Flanking regions (±20 kb) of chromosomal *bla*$_{\text{CTX-M-15}}$ loci were extracted for BLAST comparison against the NCBI nucleotide database, mapped onto the *K. pneumoniae* HS11286 reference genome, and placed in phylogenetic context using core-genome multilocus sequence typing (cgMLST).

**Results:** Chromosomal *bla*$_{\text{CTX-M-15}}$ was identified in 8/103 isolates (7.8%; 7 as *bla*$_{\text{CTX-M-15}}$, 1 untyped), spanning six sequence types (ST101, ST48, ST39, ST6613-1LV, ST307, ST36-2LV). ISEcp1 (IS1380 family) was consistently located upstream of *bla*$_{\text{CTX-M-15}}$ (-15) in seven isolates, with partial retention of TnpA transposase sequence (1,076-1,285 bp). Three isolates from distinct STs shared an identical integration site within the major facilitator superfamily transporter gene KPHS_05650. In three isolates, the ISEcp1- *bla*$_{\text{CTX-M-15}}$ unit was embedded within a Tn3-family backbone. Two isolates showed integration contexts highly homologous to plasmid-borne sequences (IncI1 and IncF replicons) circulating across diverse Enterobacterales species, suggesting recent plasmid-to-chromosome mobilisation.

**Conclusions:** Chromosomal *bla*$_{\text{CTX-M-15}}$ integration in Ghanaian *K. pneumoniae* arises via recurrent, independent ISEcp1-mediated transposition from plasmid precursors across multiple high-risk lineages, including convergent insertion at a shared chromosomal hotspot in three unrelated sequence types. Their presence in globally disseminated STs (ST101, ST307, ST48) underscores the need for surveillance that distinguishes chromosomal from plasmid localisation to track this stable, vertically inherited resistance reservoir.



**Keywords:** *Klebsiella pneumoniae*; *bla*$_{\text{CTX-M-15}}$; Chromosomal integration; ISEcp1; Extended-spectrum beta-lactamase; Antimicrobial resistance; Ghana; Hybrid genome assembly; Mobile genetic elements

## Background

Antimicrobial resistance (AMR) is one of the most serious global health threats: an estimated 4.95 million deaths were associated with bacterial AMR in 2019, of which 1.27 million were directly attributable, with Western sub-Saharan Africa bearing the highest attributable mortality burden of any region (27.3 deaths per 100,000) [1]. Resistance to broad-spectrum and last-resort beta-lactams is of particular concern within this burden, and *Klebsiella pneumoniae* is a leading vehicle for this specific threat, accounting for 19.9% of AMR-attributable deaths in sub-Saharan Africa in 2019, the highest of any pathogen in the region [1]. The World Health Organization classified *K. pneumoniae* as a Critical Priority Pathogen, explicitly citing strains resistant to third-generation cephalosporins and carbapenems [2].

*K. pneumoniae* is genomically diverse and clinically important, and its acquired AMR follows a markedly lineage-concentrated distribution, disproportionately carried by a small number of "high-risk" clones such as ST258, ST307, ST15 and ST101 [3,4,5,6]. Extended-spectrum beta-lactamases (ESBLs), particularly CTX-M-type enzymes, have displaced the earlier TEM- and SHV-derived enzymes to become the dominant beta-lactam resistance mechanism worldwide [7,8,9]. Of these, CTX-M-15 is the most globally prevalent ESBL and the most consistently recurring resistance determinant across high-risk *K. pneumoniae* lineages, frequently co-occurring with carbapenemases in ST307 [9,10].

*bla*$_{\text{CTX-M-15}}$ genes originated as chromosomal beta-lactamases intrinsic to *Kluyvera spp.* and were subsequently mobilised onto plasmids [11,12]; a trajectory *bla*$_{\text{CTX-M-15}}$ has since partially reversed. Mobile genetic elements, principally the insertion sequence ISEcp1, periodically move *bla*$_{\text{CTX-M-15}}$ back from plasmids into bacterial chromosomes via a characteristic one-ended transposition mechanism, and can additionally provide promoter activity for the captured gene [13,14,15,16,17]. Chromosomal integration has important clinical consequences: it ensures vertical transmission independent of plasmid maintenance and stable persistence even without antibiotic selection, reducing the fitness burden of plasmid carriage while entrenching resistance within a lineage [18,19,20]. To date, only three multi-isolate reports describe chromosomal *bla*$_{\text{CTX-M-15}}$ in *K. pneumoniae*: clonal expansions of ST48 and ST307 in Korea, ST101 in a German hospital outbreak, and ST1 in Barcelona; alongside sporadic single-isolate case reports from the USA and Uruguay [20,21,22,23,24].

Detecting these events is constrained by a technical surveillance gap. Short-read-only sequencing, still the dominant AMR surveillance strategy, cannot span the repetitive IS elements that typically flank *bla*$_{\text{CTX-M-15}}$, so assemblies routinely fragment precisely at the chromosome/plasmid boundary, leaving genomic context, and with it plasmid-versus-

chromosome location, unresolved. Hybrid long- and short-read assembly overcomes this limitation, producing complete genomes with unambiguous gene assignment [25,26].

In Ghana, ESBL-producing *K. pneumoniae* is prevalent (regional estimate 9.3% in West Africa [27]; hospital-based surveys report ESBL carriage of 43.5–73%, predominantly CTX-M-mediated [28,29,30,31]), but genomic investigations have focused on virulence, resistance phenotype and plasmid epidemiology without resolving chromosomal from plasmid-borne resistance at the mechanistic level [32,28,29,30,31]. A recent genomic survey of 103 *K. pneumoniae* isolates from five tertiary hospitals in Southern Ghana found 78% of isolates to carry $bla_{\text{CTX-M-15}}$, including eight isolates with chromosomally located $bla_{\text{CTX-M}}$, but the mechanisms and genomic contexts of these integration events were not investigated [33].

Using hybrid Nanopore–Illumina assemblies from this same collection, which allow clear resolution of chromosomal versus plasmid loci, we characterise the mobile genetic elements, insertion sites, and phylogenetic distribution of chromosomal $bla_{\text{CTX-M}}$ integration in this population, and place these findings in global context.

## Methods

### Isolates, genome assembly and quality control

A total of 103 *K. pneumoniae* isolates were collected prospectively from five tertiary hospitals across Southern Ghana as part of the study by Mills et al. [33]. Each isolate was sequenced using both Oxford Nanopore MinION (R9.4.1 flow cells; Guppy v4.0.14, Super Accurate basecalling) and Illumina NextSeq 500 platforms, and hybrid genome assemblies were constructed following the protocol of Wick et al. [34], as described previously [33]. This study used only the chromosomal assemblies from this collection; all downstream resistance-gene localisation, MGE annotation, and phylogenetic reconstruction were restricted to chromosomal sequences.

Assembly quality was characterised with QUAST v5.3.0 [35] against thresholds adapted from the Institut Pasteur BIGSdb *Klebsiella* genome-quality criteria [36] (≤500 contigs, genome length 4.7–6.3 Mb, GC content 56.1–58.1%, coverage ≥40×), for reporting rather than as an inclusion filter: neither AMR gene calling nor coordinate-based MGE and integration-site analysis requires a complete, closed assembly, so no isolate was excluded on these grounds. All 103 assemblies were therefore retained for downstream analysis. Assemblies were re-annotated with Bakta v1.12.0 [37] (genus *Klebsiella*, species *pneumoniae*). Chromosome-only composition was independently verified using MOB-suite v3.1.9 [38,39],

cross-referenced against AMRFinderPlus's own chromosome/plasmid context calls, confirming that no plasmid contigs were present in the dataset.

### Resistance gene identification

AMRFinderPlus v4.2.7 [40] was run on Bakta-annotated assemblies (organism *Klebsiella pneumoniae*, extended “plus” database), producing per-isolate resistance-gene calls used to characterise chromosomal resistance-gene distribution across the collection.

### Mobile genetic element detection

Insertion sequences were annotated with ISEScan v1.7.3 [41]. Integrons were detected with IntegronFinder v2.0.6 [42,43] (local-max option; gene prediction via Prodigal v2.6.3 [44]). ICEs and integrative mobilisable elements (IMEs) were identified with ICEfinder v2.0 [45] in Single mode (multi-contig assemblies were merged into single-contig FASTA files for compatibility). Outputs from all three tools were summarised across the full collection and, for the subset of isolates carrying chromosomal *bla*$_{\text{CTX-M}}$, integrated with the resistance-gene calls above into a single comparative table.

### Deep characterisation of ESBL integration contexts

Flanking regions (±20 kb) of chromosomal *bla*$_{\text{CTX-M}}$ loci were extracted with seqkit v2.13.0 [46] and interrogated with two complementary BLASTn v2.17.0 [47] analyses. First, extracted IS1380 sequences were compared against the ISEcp1 reference sequence (GenBank AY044436[48]) to confirm species-level identity. Second, the full flanking sequences were queried against the NCBI nucleotide (nt) database [49] to test for plasmid homology; hits annotated as plasmid origin with ≥90% identity were classified as evidence of plasmid-to-chromosome mobilisation.

The precise chromosomal integration site of each event was determined by aligning flanking regions to the *K. pneumoniae* HS11286 reference genome (GenBank CP003200.1[50]) with minimap2 v2.30 [51,52] (asm5 preset), processing alignments with samtools v1.23.1 [53], and inspecting CIGAR soft-clipping patterns to localise integration breakpoints.

### Phylogenetic analysis

The cgMLST phylogenetic tree was inferred in Pathogenwatch v21.3.0 from a concatenated alignment of 1972 core genes (2,172,367 bp) comprising the Pathogenwatch core-gene library for *K. pneumoniae*, with midpoint rooting (phangorn v2.11.1) and visualisation in ggtree v3.0.4. Kleborate v3.2.4 [54] (kpsc preset) was used to generate MLST assignments

matching the original study's approach; chromosomal *bla*$_{CTX-M}$ status and sequence type were overlaid onto the tree.

### Global comparison

BLAST outputs from the flanking-region analysis above were further interrogated, irrespective of replicon or molecule type, to identify similar integration configurations among public genomes. Corresponding public assemblies were downloaded via the NCBI datasets CLI v18.32.0 [49] (498 candidate assemblies resolved; 490 available for download, 7 subsequently suppressed by NCBI, 1 superseded). Kleborate was run on the 490 downloaded assemblies as above, producing MLST, clonal group, and chromosomal ESBL status for comparison.

### Data analysis and visualisation

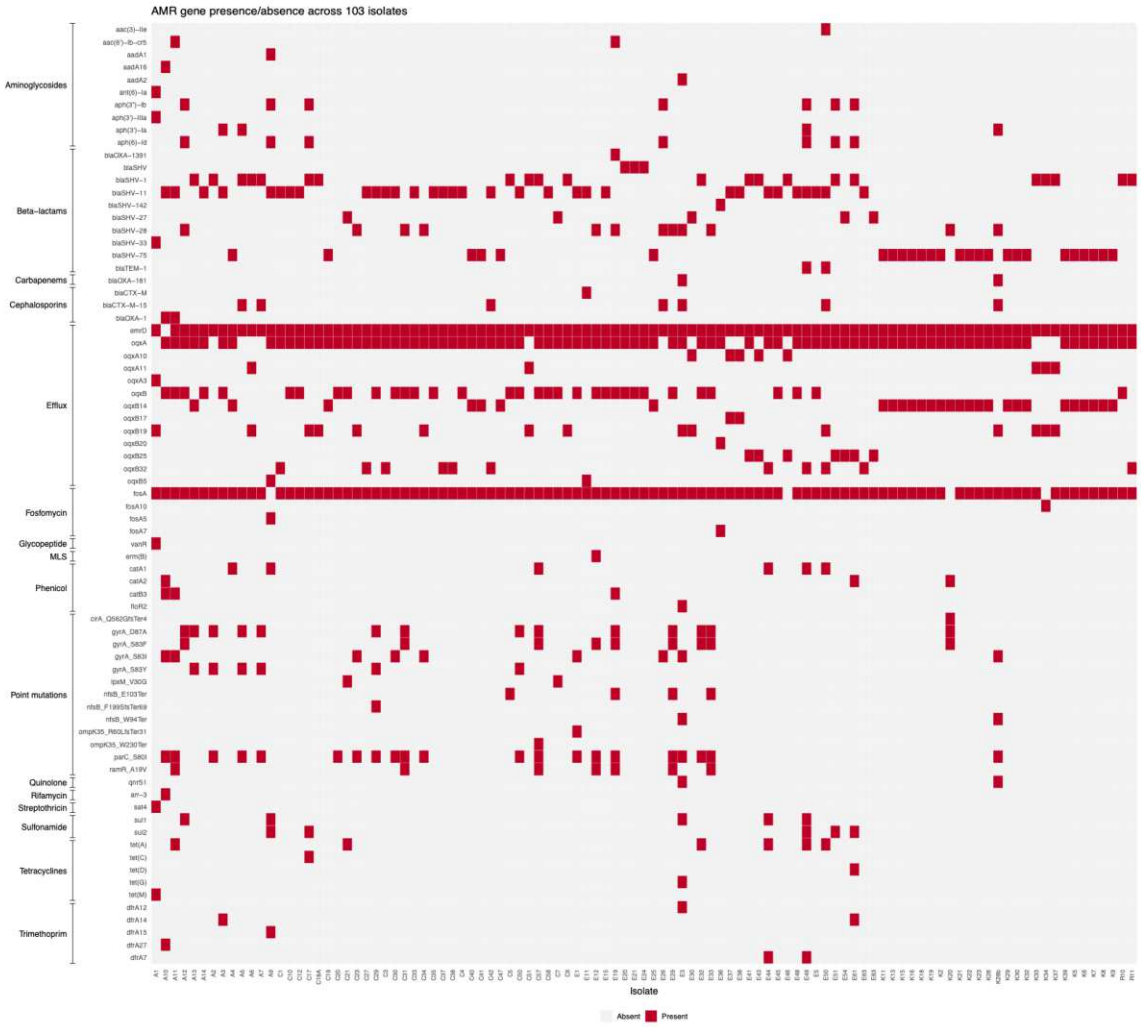
All downstream processing and visualisation were performed in R v4.5.2 [55] using tidyverse v2.0.0 [56] and ggplot2 v4.0.3 [57]. Linear gene-arrow maps used gggenes v0.7.0 [58]; de-overlapped coordinate plotting used ggrepel v0.9.8 [59]; the annotated phylogeny used ggtree v4.0.4 [60] and tidytree v0.4.8 [61]; and the geographic distribution plot used maps v3.4.3 [62]. BLAST outputs were filtered to exclude subject sequences shorter than 20 kb, removing bare single-gene GenBank submissions of the ISEcp1– *bla*$_{CTX-M-15}$ cassette, which structurally cannot carry informative source metadata, before ranking by query coverage.

## Results

### Chromosomal resistance gene distribution

Analysis of 103 *K. pneumoniae* chromosomal assemblies identified 73 distinct chromosomally carried AMR genes across 26 antibiotic subclasses (**Supplementary Figure 1**). Beta-lactam determinants showed the clearest gradient of clinical relevance: *bla*$_{SHV}$ alleles were present in 102/103 (99%) isolates, predominantly narrow-spectrum, species-intrinsic variants (*bla*$_{SHV-11}$, 32/103, 31%; *bla*$_{SHV-75}$, 26/103, 25%; *bla*$_{SHV-1}$, 22/103, 21%), against which a smaller subset of ESBL-associated SHV variants was also detected (*bla*$_{SHV-27}$, 5/103; *bla*$_{SHV-28}$, 12/103; *bla*$_{SHV-33}$, 1/103; *bla*$_{SHV-142}$, 1/103; untyped, 3/103). Acquired ESBL and carbapenem-resistance determinants comprised *bla*$_{OXA-1}$ (2/103, 2%), *bla*$_{CTX-M-15}$ (7/103, 7%), untyped *bla*$_{CTX-M}$ (1/103, 1%), and *bla*$_{OXA-181}$ (2/103, 2%; accompanied by porin mutations ompK35 R60Lfs*31 and ompK35 W230* in the same isolates). Other resistance classes (quinolones, aminoglycosides, tetracycline, chloramphenicol, sulfonamide, trimethoprim, tigecycline, rifamycin, streptothricin, vancomycin, macrolide/lincosamide/streptogramin B) were detected only sporadically (≤24% of isolates).

Efflux (emrD, 99%; oqxAB, 96%) and fosfomycin-resistance (fosA and variants, 99%) determinants were near-ubiquitous but reflect core-genome content intrinsic to the species rather than an acquired-resistance signal.




***Supplementary Figure 1.*** *AMR gene presence/absence across 103 chromosomal K. pneumoniae assemblies. Heatmap of 75 distinct acquired AMR genes identified by AMRFinderPlus, grouped by antibiotic class/subclass. Each row represents one isolate (n = 103) and each column one AMR gene; presence/absence indicated by fill colour. Porin loss-of-function mutations are annotated separately from acquired genes.*

## Mobile genetic element landscape

Across the 103 isolates, 175 IS elements spanning 12 families were identified by ISEScan (**Figure 1**), dominated by IS3 (50 copies), ISNCY (48) and IS5 (30). IntegronFinder identified 12 integron annotations, 24 *attC* sites, 33 integron-associated proteins, and 6 integron

integrases (intI); ICEfinder identified 140 ICE/IME elements across 83/103 isolates (64 IME; 76 ICE, all T4SS-type) (**Supplementary File 1**).

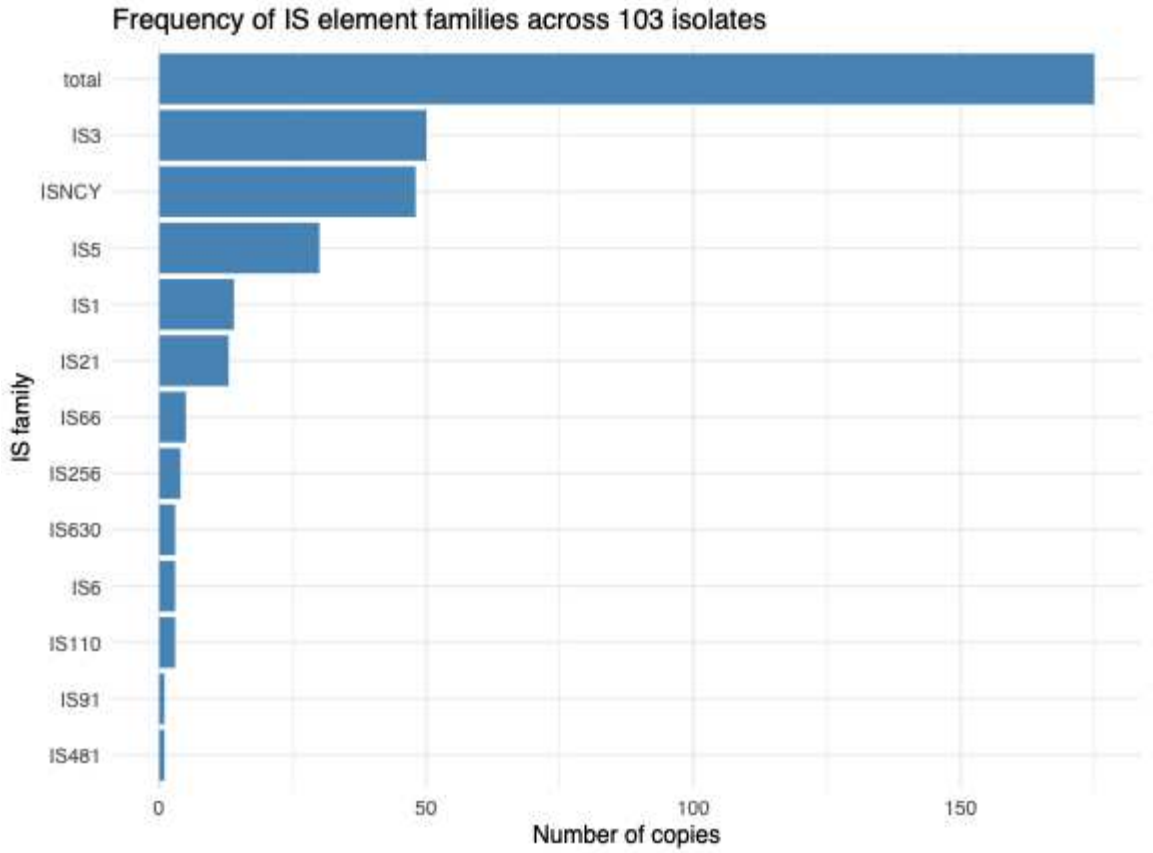



***Figure 1.*** *Frequency of IS element families across 103 isolates. Horizontal bar chart showing total IS element copies identified by ISEScan for each of the 12 IS families detected, ranked in descending order of abundance (total n = 175 copies).*

## MGE context of chromosomal *bla*$_{CTX-M}$

Eight isolates carried chromosomal *bla*$_{CTX-M}$ (7.8% of the collection), spanning six sequence types. Seven of the eight (all except isolate A5) carried at least one IS element within 20 kb of *bla*$_{CTX-M}$ (**Table 1**); IS1380 was in all seven, with additional families (IS6, IS1, IS3, IS5) detected in a subset. None of the eight isolates harboured integrons, ICEs, or IMEs within the flanking regions. One isolate (E11) carried a chromosomal *bla*$_{CTX-M}$ gene that could not be resolved to variant level and is reported as *bla*$_{CTX-M}$ (untyped).

***Table 1.*** *Mobile genetic element context of the eight isolates carrying chromosomal bla*$_{CTX-M}$

| Isolate | Gene | IS elements nearby | Integrons nearby | ICEs nearby |
|---|---|---|---|---|
| **A5** | *bla*$_{\text{CTX-M-15}}$ | none detected | none detected | none detected |
| **A7** | *bla*$_{\text{CTX-M-15}}$ | IS1380 | none detected | none detected |
| **C42** | *bla*$_{\text{CTX-M-15}}$ | IS1380 | none detected | none detected |
| **E11** | *bla*$_{\text{CTX-M}}$ (untyped) | IS1380 | none detected | none detected |
| **E26** | *bla*$_{\text{CTX-M-15}}$ | IS1380, IS6, IS1, IS5 | none detected | none detected |
| **E3** | *bla*$_{\text{CTX-M-15}}$ | IS1380, IS6, IS1, IS3, IS5 | none detected | none detected |
| **E50** | *bla*$_{\text{CTX-M-15}}$ | IS1380 | none detected | none detected |
| **K28b** | *bla*$_{\text{CTX-M-15}}$ | IS1380, IS6, IS1, IS5 | none detected | none detected |

ISEScan annotated the flanking elements as IS1380 in all seven isolates, classified as partial copies; BLAST comparison against the ISEcp1 reference confirmed 100% identity. Six isolates (A7, C42, E11, E26, E3, K28b) carried an identical 1,123 bp truncated copy, while E50 harboured a longer, less-truncated 1,372 bp copy; both truncated forms retained substantial portions (1,076 bp and 1,285 bp, respectively) of the TnpA transposase coding sequence.

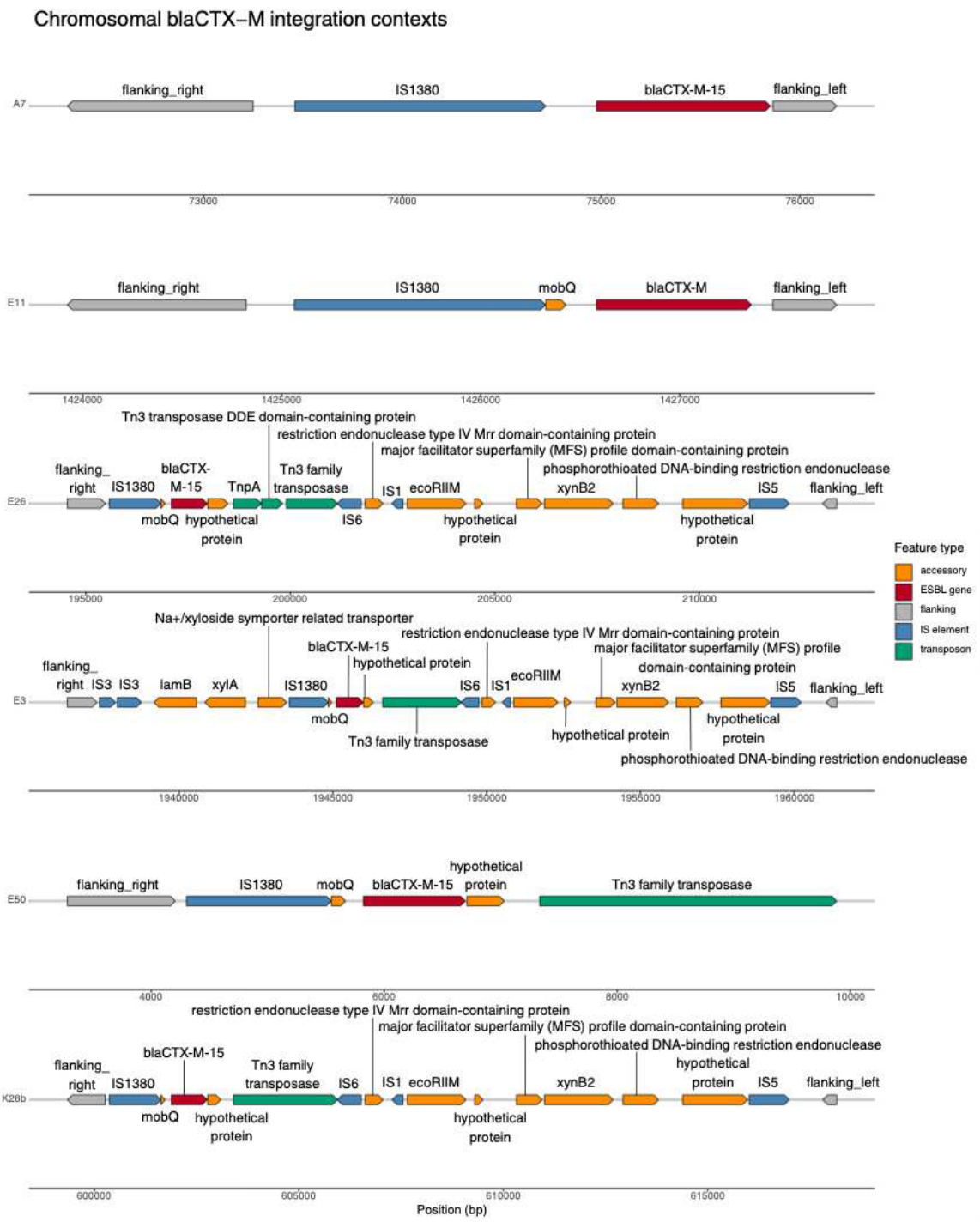
BLAST comparison of flanking sequences against the NCBI nt database identified plasmid-homologous hits for two isolates only. For E26, 585/889 hits (66%) mapped to plasmids spanning six species (*E. coli*, *K. pneumoniae*, *K. variicola*, *Salmonella enterica*, *Serratia nevei*, *Shigella sonnei*), including p$_{\text{CTX-M-15}}$_d1-58, pIITR008_75, pEC_L46 and pEC_L8, with IncI1 and IncF replicon types evident. For E50, 234/276 hits (85%) mapped to plasmids across five species, again dominated by p$_{\text{CTX-M-15}}$_d1-58 and pIITR008_75 with the same IncI1/IncF replicon types.

## Integration structure

*bla*$_{CTX-M}$ was encoded on the reverse strand in five isolates (A7, E11, E26, E3, K28b) and the forward strand in two (C42, E50), with ISEcp1 functionally upstream of *bla*$_{CTX-M}$ in all seven. In A7, C42, E11 and E50 the structure was a simple ISEcp1–*bla*$_{CTX-M}$ unit without a Tn3-family backbone; in E26, E3 and K28b, the unit was nested within a Tn3-family backbone carrying transposase and resolvase genes (**Figure 2**; C42 omitted from the figure, as its 446 bp contig break downstream of *bla*$_{CTX-M}$ leaves too short a flank to construct an informative map). Isolate E50 was a partial exception: a Tn3-family transposase lay immediately downstream, but the contig terminated only 33 bp beyond it, precluding confirmation of an enclosing backbone; E50 nonetheless carried the least-truncated ISEcp1 copy of any isolate, the clearest single-event reference case for the mechanism.

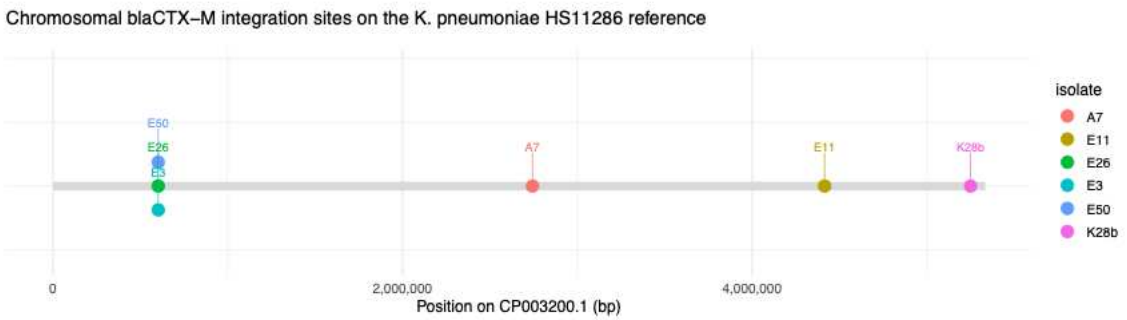
## Chromosomal insertion sites

Integration occurred at diverse chromosomal loci (**Supplementary Figure 2**). Three isolates from unrelated sequence types—E26, E3 and E50 (ST36-2LV, ST307 and ST6613-1LV, respectively)—converged on an identical site within the MFS transporter gene KPHS_05650 (602,688 bp; 821 bp from its 3′ end). Isolate E11 integrated within the putative NAD(P)H-dependent xylose reductase gene KPHS_43790, 78 bp from its 3′ end. A7 and K28b integrated in intergenic regions (between KPHS_27370/KPHS_27380, and KPHS_52270/KPHS_52280, respectively). Insertion sites could not be resolved for C42 (contig break 446 bp downstream of *bla*$_{CTX-M-15}$) or A5 (its 3,560 bp contig terminates only 48 bp after *bla*$_{CTX-M-15}$).




247

248 ***Figure 2.*** *Structural context of chromosomal bla$_{CTX-M}$ integration in six focal isolates (A7,*
249 *E11, E26, E3, E50, K28b; C42 omitted owing to a contig break yielding an uninformative*
250 *flank). Linear gene-arrow maps of the annotated flanking regions, coloured by feature type:*
251 *ESBL gene (red), IS element (blue), transposon (green), accessory gene (orange), flanking*
252 *sequence (grey). X-axis coordinates are local to each isolate's own contig.*




***Supplementary Figure 2*** *Chromosomal location of bla$_{CTX-M}$ integration sites relative to the K. pneumoniae HS11286 reference genome (GenBank CP003200.1). Integration sites for the six isolates with resolvable coordinates are plotted along the linear chromosome; E26, E3 and E50 converge on an identical coordinate (602,688 bp, within KPHS_05650) and are vertically offset for visual clarity. C42 and A5 are excluded (insertion sites unresolved).*

## Phylogenetic context

The eight chromosomal *bla*$_{CTX-M}$ isolates spanned six sequence types (A5/A7, ST101; E11, ST48; C42, ST39; E50, ST6613-1LV; E3/K28b, ST307; E26, ST36-2LV) and were not confined to a single cgMLST clade (**Figure 3**), instead falling into three loosely clustered, phylogenetically unrelated groups: A5/A7 (both ST101), C42/E50 (ST39 and its sister ST6613-1LV), and E3/K28b/E26 (ST307/ST307/ST36-2LV). No clade encompassed more than three of the eight isolates.

## Global context

Among the seven flanking-region BLAST comparisons against NCBI nt (A5 was excluded, its 3,560 bp contig leaving an insufficient flanking window), only C42 produced hits exceeding a 70% query-coverage threshold (**Table 2**): 65 subject sequences exceeded 70% coverage of C42's 21,322 bp query, with the top hit spanning 93.6% of the window against a chromosome annotated as ST101 (**Supplementary File 2**). Across the wider, replicon-agnostic global comparison, 452 public genomes carried matching integration contexts, concentrated in South Korea (83), the USA (41), China (32), Germany (22), Taiwan (20) and Bulgaria (20); Ghana itself was represented by three genomes, providing a degree of internal validation (**Figure 4**). Country metadata was available for 285/452 (63%) genomes.

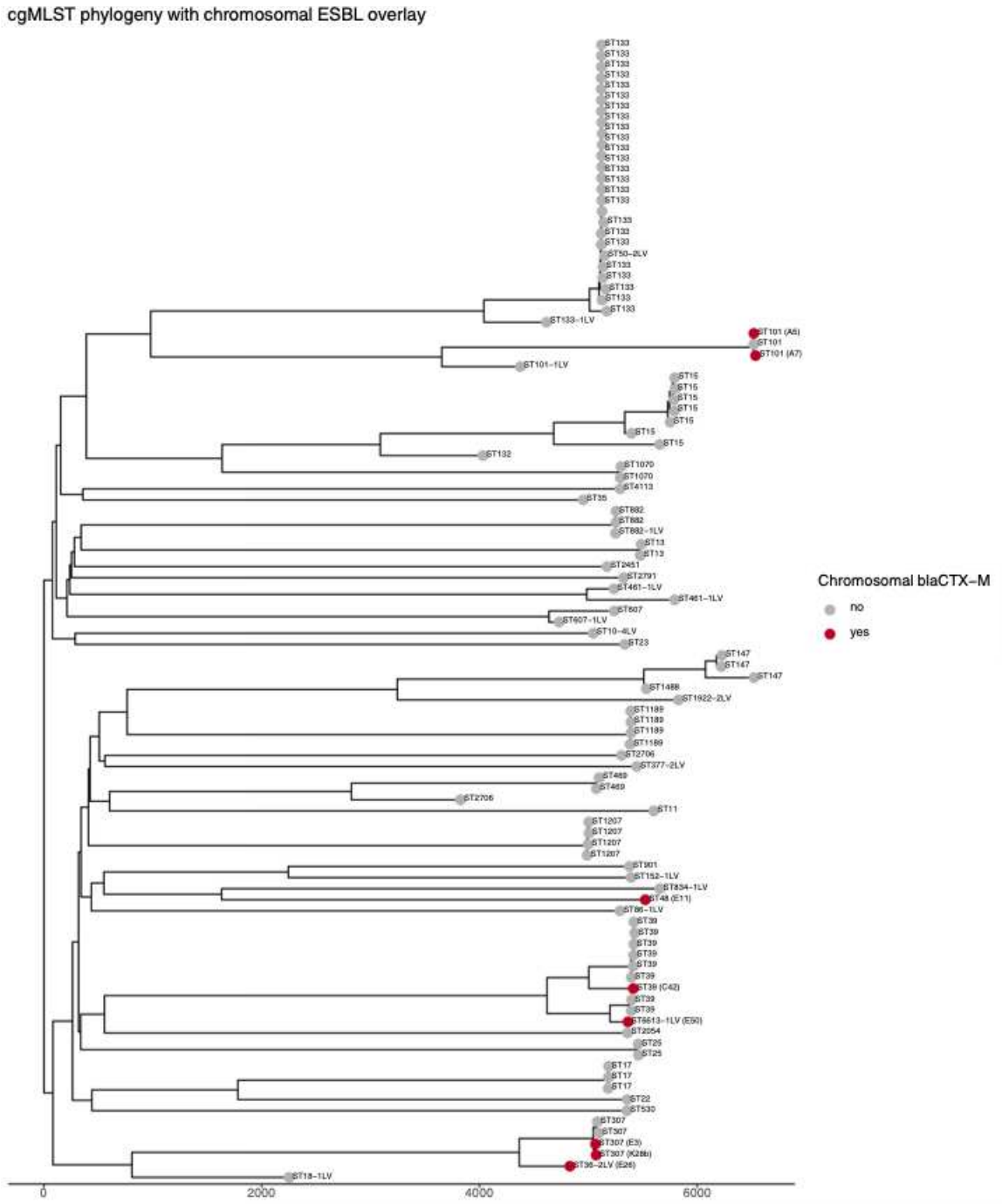
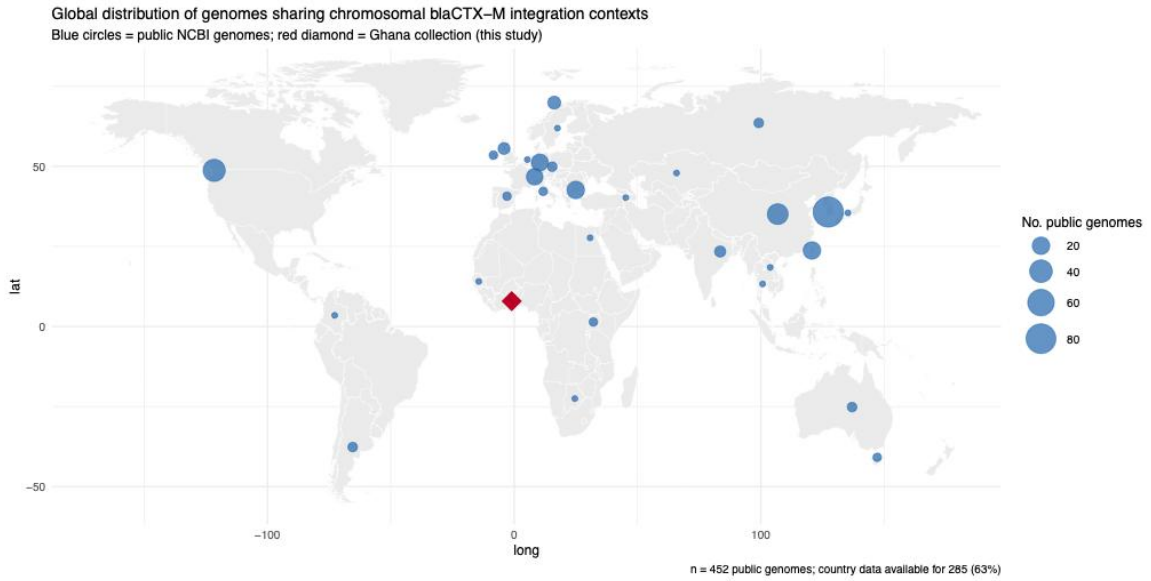



**Figure 3.** cgMLST phylogeny of the 103-isolate collection with chromosomal *bla*$_{\text{CTX-M}}$ status overlaid. Tips are labelled by sequence type and coloured by chromosomal *bla*$_{\text{CTX-M}}$ status (positive/negative); the eight chromosomal *bla*$_{\text{CTX-M}}$-carrying isolates are additionally labelled by isolate ID.




285

286 **Figure 4.** Global distribution of public genomes sharing chromosomal *bla*$_{CTX-M}$ integration
287 contexts. World map showing geographic origin of 452 public NCBI nt genomes returned by
288 BLAST comparison of the Ghanaian integration contexts, for the 285 (63%) genomes with
289 available country metadata. Circle size scaled to genome count per country; the Ghanaian
290 isolate collection is marked separately.

291 ***Table 2.*** *Top NCBI nucleotide (nt) BLAST match per isolate for the extracted ±20 kb bla*$_{CTX-M}$
292 *flanking region.*

| Isolate | Accession | Identity (%) | Query length (bp) | Subject length (bp) | Query coverage |
|---|---|---|---|---|---|
| **A7** | CP025576.1 | 99.91 | 40,876 | 5,277,535 | 0.478 |
| **C42** | CP031368.2 | 99.61 | 21,322 | 5,453,528 | 0.936 |
| **E11** | CP153003.1 | 99.48 | 40,780 | 5,379,284 | 0.449 |
| **E26** | CP067924.1 | 99.90 | 25,505 | 52,618 | 0.282 |
| **E3** | CP099672.1 | 100.00 | 40,876 | 5,349,357 | 0.448 |
| **E50** | OW967765.1 | 99.98 | 9,917 | 5,530,992 | 0.586 |
| **K28b** | CP052239.1 | 99.76 | 40,876 | 5,256,349 | 0.380 |

## Discussion

This study aimed to characterise the mechanisms and genomic contexts underlying chromosomal *bla*$_{CTX\text{-}M\text{-}15}$ integration in *K. pneumoniae* from Southern Ghana, using hybrid Nanopore–Illumina assemblies to resolve chromosomal from plasmid-borne resistance genes at a level of detail unattainable from short-read data alone. Chromosomal *bla*$_{CTX\text{-}M}$ was identified in eight of 103 isolates (7.8%), spanning six sequence types. In seven of these eight — all but A5 — ISEcp1 was consistently positioned upstream of *bla*$_{CTX\text{-}M}$, with partial retention of the TnpA transposase. Three of these seven isolates showed the unit embedded within a Tn3-family backbone, and two showed flanking sequence highly homologous to circulating plasmids, consistent with recent plasmid-to-chromosome mobilisation. Integration occurred at diverse chromosomal loci, including a site shared by three isolates from otherwise unrelated lineages, and the phylogenetic distribution of these events across multiple sequence types indicated convergent, independent acquisition rather than clonal spread of a single pre-integrated lineage. Matching integration contexts were identified in public genomes from multiple continents, suggesting this is not a Ghana-specific phenomenon.

### Mechanism of integration: conservation of ISEcp1–*bla*$_{CTX\text{-}M\text{-}15}$ across plasmids and chromosomes

Seven of the eight focal isolates (all except A5) carried ISEcp1 upstream of *bla*$_{CTX\text{-}M}$, confirming ISEcp1-mediated mobilisation as the canonical mechanism for chromosomal CTX-M integration, consistent with prior observations from Korea and Germany [20,21]. This specificity is notable against the backdrop of the wider MGE landscape: although 175 IS elements spanning 12 families, 12 integrons, and 140 ICE/IME elements were detected across 83/103 isolates, the focal isolates uniformly harboured ISEcp1 upstream of *bla*$_{CTX\text{-}M}$ with no integrons or ICEs in the flanking regions, indicating a dedicated mechanism rather than a nonspecific reflection of population-wide element prevalence.

Both truncated ISEcp1 forms retained substantial portions of the TnpA transposase coding sequence (1,076 bp and 1,285 bp), suggesting partial rather than complete loss of the transposase body. This is noteworthy: previous reports of other ISEcp1-mobilised beta-lactamases (e.g., *bla*$_{ACC\text{-}1}$) have described more extensive transposase truncation following chromosomal integration [63], whereas the isolates here retained significant transposase sequence, suggesting these elements may remain potentially mobilisable despite partial truncation.

A structural distinction separated isolates into a simple ISEcp1–*bla*$_{CTX-M}$ unit (A7, C42, E11, E50) versus a nested Tn3-backbone structure (E26, E3, K28b), suggesting two layers of mobility: ISEcp1 responsible for the initial positioning of *bla*$_{CTX-M}$, and the Tn3 backbone potentially facilitating movement of the broader multi-gene block in a subset of isolates. Such nested structures have been described previously in ESBL contexts [64,65,66], but their occurrence within chromosomally integrated units in African isolates has not previously been documented systematically.

The BLAST results for E26 and E50 show the IS1380–*bla*$_{CTX-M}$ unit to be highly conserved and essentially identical to plasmid-borne copies circulating across multiple Enterobacterales species (p$_{CTX-M-15}$_d1-58, pIITR008_75, pEC_L46, pEC_L8), consistent with these being well-characterised epidemic plasmid backbones implicated in interspecies dissemination of *bla*$_{CTX-M}$ alleles [65,67,68]. The presence of IncI1 and IncF replicon types among these hits is consistent with the epidemiological dominance of these families in ESBL outbreaks and with *K. pneumoniae*'s general preference for IncF-borne *bla*$_{CTX-M-15}$ [4,69,70,71]. Together, these findings support a plasmid-precursor origin for the chromosomal IS1380– *bla*$_{CTX-M-15}$ elements in E26 and E50, reflecting ongoing movement of resistance determinants from plasmids into chromosomes—a mechanism that, in this respect, confirms existing knowledge established in Korean and German cohorts [20,21]. The novelty here lies not in the mechanism itself but in the population-level pattern of its emergence in Ghana, discussed next.

## Convergent evolution versus clonal spread

The eight chromosomal *bla*$_{CTX-M}$ isolates were distributed across six sequence types rather than clustered within one, indicating that chromosomal integration has arisen through convergent evolution via repeated, independent ISEcp1-mediated transposition events rather than clonal expansion of a single pre-integrated lineage. The identical integration site within KPHS_05650 in three isolates from three different sequence types (E26, E3, E50) reinforces this interpretation: repeated, independent transposition into the same genomic site across distantly related lineages suggests either a local sequence feature favouring ISEcp1 insertion, or, more plausibly, convergent selection for a locus where fitness costs are minimised while resistance is stably maintained.

This pattern contrasts with the single clonal expansions documented in Korea (ST48, ST307) [20], Germany (ST101) [21], and Barcelona (ST1) [22]. The absence of extensive clonal clustering in the Ghanaian dataset instead suggests chromosomal integration may be an ongoing, dynamic process occurring across multiple lineages, with implications for

surveillance: convergent integration across diverse sequence types implies the phenomenon is not lineage-restricted and may emerge in additional high-risk clones under continued selective pressure. Integrations were detected here in the established high-risk lineages ST48, ST307 and ST101, alongside two sequence types (ST39 and ST6613-1LV) not previously recorded as carriers of chromosomally integrated *bla*$_{\text{CTX-M-15}}$, and a further extension of the ST307-associated phenomenon (previously documented in Korea [20]) to the closely related ST36-2LV. Taken together with the shared insertion hotspot not previously reported in the literature, our findings suggest ongoing, convergent chromosomal *bla*$_{\text{CTX-M-15}}$ in a sub-Saharan African setting.

### Insertion site diversity and fitness constraints

ISEcp1 is not known to target a single conserved locus; it inserts at diverse chromosomal sites, disproportionately in intergenic regions or plausibly dispensable genes, consistent with fitness cost, rather than target sequence specificity, as the main constraint [20,21,72,73,74,75,76]. The insertion sites here align with this picture: A7 and K28b integrated in intergenic regions, minimising disruption of coding sequence; E11 integrated within a putative NAD(P)H-dependent xylose reductase gene (KPHS_43790), where disruption likely imposes a modest fitness cost to carbohydrate metabolism [77]; and E26, E3 and E50 converged on the MFS transporter gene KPHS_05650, whose disruption may reduce efflux capacity while the inserted element drives beta-lactam resistance [78]. The repeated, independent convergence on KPHS_05650 across unrelated sequence types suggests either a favoured insertion target or a selective advantage to integration at this site, perhaps via reduced efflux-associated fitness cost or a transcriptionally favourable genomic neighbourhood.

### Clinical implications

Chromosomal integration ensures vertical transmission independent of plasmid maintenance and stable persistence even without antibiotic selection [18,19], facilitating clonal expansion of high-risk lineages carrying *bla*$_{\text{CTX-M}}$ [20]. Although only eight of 103 isolates in this collection carried chromosomal *bla*$_{\text{CTX-M}}$, their presence in high-risk sequence types (ST101, ST307, ST48) already associated with global dissemination and hospital-acquired infection highlights a clinically relevant, stable, vertically inherited resistance reservoir that may expand under continued antibiotic pressure. Combined with BLAST matches to homologous plasmids from multiple species in public databases, this underscores the need for genomic surveillance that distinguishes chromosomal from plasmid localisation of resistance genes, rather than reporting gene presence alone.

## Limitations

Several limitations bear on interpretation. C42's elevated NCBI BLAST hit count may reflect two confounds rather than a uniquely conserved integration context: its query window is shorter (~21 kb versus ~40 kb for intact-contig isolates, owing to a contig break, which mechanically eases the 70% coverage threshold), and C42 belongs to the globally disseminated, database-overrepresented ST101 lineage. Its strongest individual match (93.6% coverage) nonetheless indicates genuine conservation of the upstream flank, *bla*$_{\text{CTX-M-15}}$, and adjacent IS element across ST101 genomes; C42's raw hit count is simply not directly comparable to isolates with full-length windows. Assembly fragmentation also limited resolution of insertion sites for A5 and C42, and the identities of IS6, IS5 and IS1 elements co-located with *bla*$_{\text{CTX-M}}$ in E26, E3 and K28b rely on ISEScan's family-level classification rather than independent BLAST confirmation. Comparisons drawn with the German ST101 outbreak and Korean lineages [20,21] are at the sequence-type and mechanistic level only: Mshana et al.'s reported insertion site is annotated on a different reference genome (K. pneumoniae 342, between KPK_0275 and KPK_0276), so whether it corresponds to the same chromosomal site described here cannot be confirmed. This analysis was scoped to ESBL genes; two isolates additionally carried chromosomal *bla*$_{\text{OXA-181}}$ carbapenemase, whose integration mechanism was not characterised here. Finally, the eight identified integration events, while reflecting the genuine rarity of the phenomenon in this population, constrain statistical power for population-level inference; larger, longitudinal surveys; ideally paired with functional validation of the fitness-advantage hypothesis via plasmid-curing and competitive-growth assays, will be needed to determine whether the sequence-type distribution and insertion-site preferences observed here generalise across Ghanaian or wider West African *K. pneumoniae* populations.

## Conclusions

This study confirms ISEcp1-mediated transposition as the mechanism underlying chromosomal *bla*$_{\text{CTX-M-15}}$ integration in *K. pneumoniae*, consistent with prior reports from Korea and Germany. What is new is the pattern by which this mechanism has taken hold in a previously uncharacterised sub-Saharan African setting: rather than the single clonal expansions documented elsewhere, chromosomal integration in this Ghanaian collection arose independently and convergently across six unrelated sequence types, including three isolates converging on a single, previously unreported insertion site (KPHS_05650) and two sequence types (ST39 and ST6613-1LV) not previously recorded as carriers of this phenotype. Considered alongside the presence of these integrations in globally disseminated

lineages (ST101, ST307, ST48), this points to an active, ongoing process of chromosomal fixation rather than a historical, geographically confined event, positioning Ghana, and by extension the wider West African region, as a setting where this stable, vertically inherited resistance reservoir may be actively emerging. These findings underscore the importance of genomic surveillance that distinguishes chromosomal from plasmid localisation, particularly given the region's high ESBL prevalence and substantial neonatal infection burden: chromosomally integrated resistance genes may persist and spread even without antibiotic selection, entrenching resistance independent of ongoing prescribing pressure.

## Declarations

### Ethics approval and consent to participate

Ethical approval for the collection and whole-genome sequencing of the 103 Klebsiella pneumoniae isolates analysed in this study was granted by the institutional review boards of the participating hospital laboratories in Southern Ghana, as reported in the original study [33]. This study is a secondary analysis of publicly available, anonymised genomic data; accordingly, the LSHTM Research Governance & Integrity Office reviewed it and assessed that it did not require separate ethical approval from the LSHTM MSc Research Ethics Committee (ethics ref: 33345, approved 23 March 2026).

### Consent for publication

Not applicable.

### Availability of data and materials

Whole-genome sequencing data for the 103 isolates were deposited by Mills et al. (2024) under NCBI BioProject PRJNA1052100. Analysis scripts and pipelines are available at https://github.com/efosternyarko/kpn-chromosomal-esbl-ghana.

### Competing interests

The authors declare that they have no competing interests.

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## Authors' contributions

E.F.-N. conceived and supervised the study, and contributed to bioinformatic pipeline development. PYGL performed the bioinformatic analyses, interpreted the results, and drafted the manuscript. E.F.-N. critically reviewed and edited the manuscript. All authors read and approved the final manuscript.

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## Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFile1integronICEIMEsummary.csv
- SupplementaryFile2NCBIntBLASTmatches.csv