

Context-dependent contribution of blaOXA-1 to piperacillin-tazobactam susceptibility in bloodstream *Escherichia coli*: a genomic and phenotypic cohort study

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Abstract

OXA-1 beta-lactamase is a determinant of reduced piperacillin-tazobactam susceptibility in *Escherichia coli*, but its independent contribution outside ESBL-enriched populations is unclear. We screened 2257 *E. coli* bloodstream isolates from the Spanish PROBAC cohort for *bla*_{OXA-1} by PCR; positive isolates underwent long-read sequencing, susceptibility testing, expression, dosage, and conjugation analyses. *bla*_{OXA-1} was detected in 169 isolates (7.5%), mainly ST131 (65.1%) and ST88 (19.5%); chromosomal in 101, plasmid-borne in 66, dual-location in two. MICs were heterogeneous, with reduced susceptibility when *bla*_{OXA-1} was the sole beta-lactamase. Higher expression correlated with higher MIC and was modified by clonal background: ST88 isolates had high MICs despite moderate expression, linked to *ompF* disruption. In multivariable models, *bla*_{OXA-1} expression, ST88/*ompF* disruption, and *bla*_{TEM-1B} co-carriage were independently associated with higher MIC. *bla*_{OXA-1} in invasive *E. coli* is thus a context-dependent determinant of piperacillin-tazobactam susceptibility, shaped by gene integrity, dosage, expression, genetic environment, co-carried beta-lactamases, and outer-membrane permeability.

Introduction

Antimicrobial resistance in *Escherichia coli* is a growing global concern, particularly in bloodstream infections, where multidrug-resistant strains increasingly limit therapeutic options. β -Lactamase production remains one of the main resistance mechanisms in Enterobacterales, with CTX-M-type extended-spectrum β -lactamases (ESBLs) predominating worldwide and frequently disseminating through plasmid-mediated horizontal transfer (1).

Although CTX-M enzymes have been extensively studied, *bla*_{OXA-1} has gained increasing clinical relevance despite not encoding a classical ESBL. OXA-1 hydrolyses aminopenicillins and ureidopenicillins and is poorly inhibited by clavulanic acid and tazobactam, thereby compromising β -lactam/ β -lactamase inhibitor combinations such as amoxicillin-clavulanate (AMC) and PTZ (2–4). OXA-1 production has also been associated with reduced susceptibility to cefepime (FEP), although this phenotype is often context-dependent and influenced by co-existing resistance mechanisms (5).

The clinical significance of OXA-1 was highlighted by the MERINO trial and subsequent genotype-stratified analyses, in which elevated PTZ MICs and co-carriage of OXA-1 with ESBL enzymes were associated with poorer outcomes (6, 7). However, most available evidence comes from ESBL-enriched or resistance-selected populations, making it difficult to distinguish the independent contribution of *bla*_{OXA-1} from the broader multidrug-resistant backgrounds in which it is usually found (3, 4).

The genetic context of *bla*_{OXA-1} is central to understanding its epidemiology and phenotypic impact. *bla*_{OXA-1} is commonly embedded in integron-derived multidrug-resistance regions, frequently linked to *bla*_{CTX-M-15}, *aac(6')-Ib-cr*, *catB3*, and *IS26*-mediated rearrangements. These regions can be carried on *IncF* plasmids but may also become integrated into the chromosome, particularly in successful *E. coli* lineages such as ST131 (8–11). Beyond gene presence alone, promoter architecture, plasmid copy

number, co-carried β -lactamases, and outer-membrane permeability defects may further modulate MICs (12, 13).

We performed an integrated genomic and phenotypic characterization of *bla*_{OXA-1}-positive *E. coli* bloodstream isolates from a large, unselected national cohort. Using long-read sequencing and complementary phenotypic assays, we resolved *bla*_{OXA-1} location, plasmid mobility, local genetic context, expression, and chromosomal modifiers to determine how *bla*_{OXA-1} contributes to resistance phenotypes, with particular focus on PTZ.

Results

Among 2257 *E. coli* bloodstream isolates, *bla*_{OXA-1} was detected in 169, corresponding to a prevalence of 7.5%. The gene was chromosomal in 101 isolates, plasmid-borne in 66, and present in both locations in two. *bla*_{OXA-1} carriage was strongly structured by clonal background: ST131 accounted for 110 isolates (65.1%) and ST88 for 33 (19.5%), with phylogroup B2 predominating. Core-genome phylogeny showed that *bla*_{OXA-1}-positive isolates clustered mainly according to sequence type, particularly ST131 and ST88 (Fig. 1A). Co-carriage of β -lactamase genes was common, especially *bla*_{CTX-M-15} (94 [55.6%]) and *bla*_{TEM-1B} (30 [17.8%]); however, 59 isolates (34.9%) carried *bla*_{OXA-1} as the only β -lactamase (Fig. 1B). The complete resistome of the collection is provided in the Supplementary Table S2.

The plasmid analysis showed that *bla*_{OXA-1}-bearing plasmids were predominantly *IncF*. A total of 68 *bla*_{OXA-1}-containing plasmids were characterised from 66 plasmid-borne isolates and two dual-location isolates. Most plasmids belonged to the *IncF* incompatibility group (64 [94.1%]), and pMLST identified [F2:A1:B-] (25 [36.8%]) and [F36:A4:B1] (14 [20.6%]) as the predominant profiles. MOB-suite classified 57 plasmids (83.8%) as conjugative, five (7.4%) as mobilizable, and six (8.8%) as non-mobilizable (Fig. 2A). Conjugation assays showed broad variability in transfer frequency, with no significant differences across predicted mobility categories (Kruskal-Wallis $p = 0.51$; Fig. 2B). Additional plasmid backbone comparison identified conserved *IncF* plasmid groups, including a [F36:A4:B1] backbone shared by isolates from different sequence types, supporting cross-lineage circulation of related *bla*_{OXA-1}-bearing plasmids (Supplementary Figure S1).

The local genetic context of *bla*_{OXA-1} further linked clonal background with genomic location. Six recurrent genetic environments were identified, together with a heterogeneous group of unassigned structures. Their distribution across sequence types and genomic compartments is summarized in Fig. 3A. The three predominant environments accounted for most isolates: E1 (56 [33.1%]), E2 (44 [26.0%]), and E3 (34 [20.1%]). Less frequent environments included E4 (6 [3.6%]), E5 (3 [1.8%]), and E6 (3 [1.8%]), while 23 isolates (13.6%) were assigned to a heterogeneous Others group. E1 and E2 were both dominated by ST131 but differed in genomic compartment, with E1 mainly plasmid-borne and E2 mainly chromosomal. In contrast, E3 was associated with ST88 and was predominantly chromosomal. E3

corresponded to a conserved class 1 integron-derived context in which *bla*_{OXA-1} was located as the first cassette, 131–132 bp downstream of the Pc promoter (Fig. 3B) (14, 15).

Two isolates carrying non-intact *bla*_{OXA-1} sequences were excluded from expression, dosage, and downstream phenotype analyses, leaving 167 isolates with intact *bla*_{OXA-1}. Normalized *bla*_{OXA-1} expression measured by RT-ddPCR varied substantially across the collection (median 3.15 [IQR 2.10–5.36]; mean 4.75 [SD 7.71]), indicating marked heterogeneity in transcriptional output among isolates carrying the same β -lactamase gene. Expression differed according to genomic location: plasmid-borne *bla*_{OXA-1} showed higher expression than chromosomal *bla*_{OXA-1} (median 5.26 [IQR 3.66–7.51] vs 2.50 [1.88–3.40]; $p < 0.001$, Fig. 4A), while the two dual-location isolates showed intermediate values (3.13 and 5.88). Sequencing-depth-based *bla*_{OXA-1} dosage estimates were generally close to one copy per genome equivalent (median 1.01 [IQR 0.74–1.30]; 90th percentile 1.88; 95th percentile 2.62; Fig. 4B) but were higher in plasmid-borne than chromosomal isolates (median 1.30 vs 0.82). Copy-number ratio correlated with normalized expression ($p < 0.001$; Supplementary Figure S2).

PTZ, AMC, and FEP MICs were determined by broth microdilution (Fig. 5A–C). *bla*_{OXA-1}-positive isolates showed broad β -lactam MIC distributions, with MIC50 values of 16/4 mg/L for PTZ, $\geq 128/2$ mg/L for AMC, and 16 mg/L for FEP; MIC90 values were 128/4, $\geq 128/2$, and 512 mg/L, respectively. PTZ and AMC MIC distributions were similar regardless of chromosomal or plasmid *bla*_{OXA-1} location. FEP MICs were numerically lower among plasmid-borne *bla*_{OXA-1} isolates than among chromosomal isolates (MIC50, 4 vs 64 mg/L), although this difference did not reach statistical significance ($p = 0.066$). PTZ MICs differed significantly across genetic environments (Kruskal-Wallis $p < 0.001$; Fig. 5D), with the highest values observed in the ST88-associated E3 environment (median 96 mg/L), compared with E1 and E2 (median 16 and 8 mg/L, respectively; both pairwise $p < 0.001$). Extended antimicrobial susceptibility profiles are provided in Supplementary Table S3.

To investigate the high PTZ MICs observed in ST88/E3 isolates (Fig. 5D), we examined chromosomal variants in genes potentially involved in β -lactam susceptibility. Among all genes analysed, only *ompF* showed recurrent disruption of the coding sequence in ST88: 31 of 33 mappable ST88 isolates carried an identical 5 bp GGTC A tandem duplication in *ompF* (c.241_245dup). This duplication is predicted to shift the reading frame from amino acid 83 and introduce a premature stop codon at amino acid 175, altering the native OmpF that includes the L3 constriction-loop region (Supplementary Figure S3). Of the remaining ST88 isolates, one retained an apparently intact *ompF* reading frame, whereas one showed a highly divergent *ompF* allele that could not be confidently classified as intact. A summary of the genetic variants identified across all analysed candidate genes is provided in Supplementary Table S4.

We next assessed whether PTZ MICs were primarily explained by co-carriage of the major companion β -lactamases *bla*_{CTX-M-15} and *bla*_{TEM-1B} (Fig. 5F). Among the 55 isolates with intact *bla*_{OXA-1} as the only detected β -lactamase, PTZ MICs were frequently above the susceptible breakpoint (41 of 55 [74.5%]; median 32 mg/L, IQR 12–128). After excluding isolates with disrupted *ompF*, this pattern persisted,

although attenuated (17 of 30 [56.7%]; median 16 mg/L, IQR 8–28). PTZ MICs did not differ significantly between this group and isolates co-carrying *bla*_{CTX-M-15} without *bla*_{TEM-1B} (median 16 mg/L, IQR 8–32; *p* = 0.53), whereas isolates co-carrying both *bla*_{CTX-M-15} and *bla*_{TEM-1B} showed higher PTZ MICs (median 48 mg/L, IQR 28–64; *p* = 0.001).

Finally, we integrated these variables in multivariable linear regression models of log₂(PTZ MIC). In a lineage-based model, *bla*_{OXA-1} expression, ST88 lineage, and *bla*_{TEM-1B} co-carriage retained independent associations with higher MICs: expression coefficient + 0.77 per doubling of expression, *p* < 0.001; ST88 coefficient + 2.11 log₂ dilutions, *p* < 0.001; and *bla*_{TEM-1B} coefficient + 0.72, *p* < 0.05. Copy-number ratio, plasmid location, ST131, and *bla*_{CTX-M-15} were not independently associated with MIC in this model. The model explained 41% of the variance in PTZ MIC. In a sensitivity model replacing ST88 with *ompF* disruption, it showed a similarly strong association with higher MICs (coefficient + 1.96, *p* < 0.001), while *bla*_{OXA-1} expression and *bla*_{TEM-1B} remained significant. Including both ST88 and *ompF* disruption in the same model attenuated both terms, consistent with their tight coupling in this dataset.

Discussion

This study provides a genomic and phenotypic characterization of bloodstream *Escherichia coli* carrying *bla*_{OXA-1} in a large, unselected national cohort. The central finding is that *bla*_{OXA-1} should not be viewed only as a companion gene of ESBL-producing backgrounds. Instead, our data supports a direct contribution of OXA-1 to PTZ susceptibility, detectable even in isolates in which *bla*_{OXA-1} was the only acquired β-lactamase identified. However, this effect was not fixed: it was shaped by gene integrity, expression, β-lactamase background, clonal lineage, genomic location, and outer-membrane permeability.

The prevalence of *bla*_{OXA-1} in this unselected bloodstream cohort was 7.5%, lower than reported in AMC-resistant or ESBL-enriched *E. coli* collections, where *bla*_{OXA-1} is expected to be over-represented (3, 4, 16–18). This difference highlights the importance of studying *bla*_{OXA-1} outside resistance-selected populations. ST131 was the dominant lineage, consistent with the epidemiology of multidrug-resistant extraintestinal *E. coli*, whereas ST88 emerged as a second biologically relevant background. Previous Spanish studies had already linked ST88 to OXA-1-producing *E. coli* and reduced susceptibility to AMC or FEP; our data extend these observations by connecting ST88 to a recurrent chromosomal *bla*_{OXA-1} environment, frequent *ompF* disruption, and high PTZ MICs (5, 19).

Resolving the chromosomal or plasmid location of *bla*_{OXA-1} was essential to interpret its persistence. Plasmid-borne *bla*_{OXA-1} can circulate across lineages, whereas chromosomal integration can stabilize resistance modules within successful clonal backgrounds. These routes are probably connected rather than independent, because insertion sequences, transposons, and integron-derived structures can mediate movement of *bla*_{OXA-1}-containing modules between plasmids and chromosomes. Our data supports this dynamic model: most *bla*_{OXA-1}-bearing plasmids were *IncF*, and conjugation confirmed

transferability in a subset, but chromosomal *bla*_{OXA-1} was also frequent, particularly in ST131- and ST88-associated environments. Thus, *bla*_{OXA-1} persistence in invasive *E. coli* cannot be attributed to plasmid spread alone but also reflects inter-replicon mobilization and chromosomal stabilization within defined lineages. The discordance between predicted plasmid mobility and measured conjugation frequency further reinforces that transfer potential depends on more than the presence of mobility genes alone (20–22).

The genetic environments helped connect epidemiology with mechanism. The dominant ST131-associated environments differed mainly by genomic location, whereas ST88/environment 3 represented a distinct chromosomal, class 1 integron-derived context. These findings are consistent with previous work showing that *bla*_{OXA-1} can be embedded in multidrug-resistance regions shaped by mobile elements, including *IS26*-associated rearrangements and amplification events (8, 23, 24). In our cohort, however, the same resistance gene was not associated with a single genomic solution, but with multiple lineage- and compartment-specific configurations.

The expression data explain why the genomic context was phenotypically relevant. Normalised *bla*_{OXA-1} expression varied substantially and was higher in plasmid-borne than chromosomal copies. Sequencing-depth-based dosage estimates correlated with expression, supporting gene dosage as one contributor to transcriptional variation. Nevertheless, dosage and genomic location alone did not explain PTZ MICs once expression and lineage-linked variables were considered. This is in keeping with previous observations that β -lactamase copy number and expression can increase β -lactam MICs, but that their phenotypic effect depends on the broader genetic background (8, 13).

A key result is that elevated PTZ MICs were not restricted to isolates co-producing CTX-M-15. Among isolates with intact *bla*_{OXA-1} as the only acquired β -lactamase, reduced PTZ susceptibility was frequent, and this persisted after excluding isolates with disrupted *ompF*. Moreover, after *ompF*-disrupted isolates were removed, MICs did not differ significantly between *bla*_{OXA-1}-only isolates and isolates co-carrying *bla*_{CTX-M-15} without *bla*_{TEM-1B}. In contrast, isolates carrying both *bla*_{CTX-M-15} and *bla*_{TEM-1B} showed higher MICs. These findings suggest that OXA-1 itself can contribute meaningfully to the PTZ phenotype, while additional β -lactamases may further increase MICs through cumulative hydrolytic burden. This interpretation is aligned with *post-hoc* analyses of the MERINO trial, in which OXA-1 co-carriage and elevated PTZ MICs were linked to poorer outcomes among patients treated with PTZ (6, 25).

Outer-membrane permeability was the other major modifier. ST88 isolates had high PTZ MICs despite not being the highest *bla*_{OXA-1} expressers, and most carried a recurrent *ompF* frameshift predicted to disrupt native OmpF. The multivariable models supported this interpretation: *bla*_{OXA-1} expression, *bla*_{TEM-1B} co-carriage, and ST88 retained independent associations with higher PTZ MIC, while replacing ST88 with *ompF* disruption captured a similar signal. These results support a model in which OXA-1-mediated hydrolysis and reduced permeability act together to raise PTZ MICs, consistent with known interactions between β -lactamase activity and outer-membrane porin disruption (12, 26).

This study has limitations. The cohort reflects bloodstream *E. coli* collected in Spain during 2016-17, and temporal or geographic changes could affect *bla*_{OXA-1} prevalence, lineage distribution, and plasmid backgrounds. Plasmid backbone similarity supports relatedness but does not prove direct transmission. Sequencing-depth-based dosage should be interpreted as relative *bla*_{OXA-1} abundance, particularly for plasmid-borne genes, rather than as an exact molecular copy number per cell. Finally, although the candidate variant analysis identified a strong ST88-associated *ompF* signal, additional regulatory, physiological, or epistatic determinants of PTZ MIC may remain undetected.

In conclusion, *bla*_{OXA-1} in invasive *E. coli* is best understood as a clinically relevant, context-dependent resistance determinant. Its persistence reflects both clonal expansion and plasmid-mediated dissemination, while its phenotypic impact on PTZ depends on gene integrity, expression, accompanying β -lactamases, and host permeability. These findings support moving beyond simple presence-or-absence interpretation of *bla*_{OXA-1} and integrating genomic context when assessing its clinical relevance.

Methods

Study design and isolates

A total of 2257 *E. coli* clinical isolates were obtained from the PROBAC study, a multicenter cohort study in 26 Spanish hospitals (18 of them tertiary and 8 community hospitals) between 1st October 2016 and 31st March 2017, including all episodes of bloodstream infections in patients aged ≥ 14 years (27). The collection was screened for *bla*_{OXA-1} by real-time PCR and positive isolates were rechecked by conventional PCR (Supplementary Table S1).

Long-read whole-genome sequencing and assembly

Total DNA from *bla*_{OXA-1} positives isolates was extracted using the Dneasy Blood & Tissue Kit (Qiagen, Hilden, Germany) following the manufacturers recommendations. DNA was quantified using Qubit 3 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA). Bacterial whole genome sequencing was performed using MinION (FLO-MIN106 R10.4.1) flow cell platform (Oxford Nanopore technology, United Kingdom). Nanopore reads were quality filtered with Filtlong and used for *de novo* genome assembly with Autocycler (28). Autocycler generated a consensus assembly from multiple long-read assemblies, which was subsequently polished with two rounds of Medaka (Oxford Nanopore Technologies). Final assembly quality was assessed using QUAST (29).

Genomic analysis

Assemblies were annotated with Bakta (software: v1.11.0 | database: v6.0.0). The presence of antimicrobial resistance (AMR) genes was identified using ResFinder (v4.6.0)(30). Integron finder was used to characterize integrons (v2)(31). Resistance gene co-occurrence patterns were derived from the ResFinder output after manual harmonization of gene names.

Multilocus sequence typing was performed using MLST v2.0 (32). Phylogroup assignment was performed using ClermontTyping (33). A core-genome alignment was generated with Roary v3.13.0 and used to infer a maximum-likelihood phylogeny with RAxML. Recombination was assessed using ClonalFrameML v1.13 (34–36). The resulting tree and metadata annotations were visualized using iTOL (37). Genetic environment comparisons were visualized using clinker (38).

The genomic location of *bla*_{OXA-1} was assigned from the assembled replicon carrying the gene. Copies located on chromosomal contigs were classified as chromosomal, whereas copies located on plasmid contigs were classified as plasmid borne. Isolates carrying *bla*_{OXA-1} in both locations were classified as dual location isolates. Plasmid contigs were further characterized using PlasmidFinder v2.0.1/database v2.2, pMLST, and MOB-suite v3.1.9 for incompatibility typing, plasmid sequence typing, and predicted mobility classification, respectively (39,40).

Phylogenetic clusters were defined from the core-genome tree using a pairwise genetic distance threshold of 0.001. Isolates not assigned to one of the main named clusters were grouped as “other” for visualization and annotation purposes.

To identify additional genomic determinants associated with PTZ MIC, and a selection of genes involved in β -lactam resistance was analyzed. It included porins and permeability-associated genes such as *ompF*, *ompC* and *phoE*; efflux pumps: *acrA*, *acrB* and *tolC*; regulators: *marA*, *marR*, *soxR*, *soxS* and *robA*; PBPs-related genes: *ftsI*, *mrcA*, *mrcB* and *dacA-D*; envelope stress responses: *cpxA*, *cpxR*, *phoP* and *phoQ* and peptidoglycan recycling genes: *ampD*, *ampE*, *ampG* and *ampH*.

Coding sequences were extracted from annotated assemblies and compared with reference sequences from *E. coli* K-12 MG1655. Nucleotide and protein alignments were inspected for non-synonymous substitutions, frameshifts, indels, premature stop codons, and severe truncations. *ompF* status was classified as intact or disrupted/loss-of-function according to the presence of frameshift mutations, severe truncation, or absence of a complete coding sequence.

Antimicrobial susceptibility testing

The susceptibility to piperacillin (Sigma-Aldrich, USA) in combination to tazobactam (Sigma-Aldrich), amoxicillin (Sigma-Aldrich) together with clavulanate (Santa Cruz Biotechnology, USA) and cefepime (USP, USA) was determined following EUCAST guidelines using broth microdilution method (41). PTZ concentrations ranged from 0.25/4 to 256/4 mg/L, AMC from 0.125/2 to 128/2 mg/L and FEP from 0.016 to 1024 mg/L. *E. coli* ATCC 25922 and ATCC 35218 (TEM-1 positive) were used as control strains. The assays were conducted in triplicate, and the susceptibility was interpreted using EUCASTv24 clinical

breakpoints (42). Susceptibility to a wider antibiotic panel (NMDR2, Beckman Coulter) was also performed from MicroScan automated testing.

Conjugation assays

The capacity of plasmids carrying *bla*_{OXA-1} to be transferred was assessed in conjugation assays using the *E. coli* J53 strain (azide-resistant) as the recipient. Briefly, pairs of clinical isolates (donors) and *E. coli* J53 strain (azide-resistant as recipient) were grown overnight in Mueller-Hinton broth (MHB). Initial bacterial concentration was adjusted to an OD₆₀₀ of 0.5. Each conjugation assay was serially diluted and plated onto Mueller-Hinton agar (MHA) containing 150 mg/L azide alone and onto MHA supplemented with 150 mg/L azide with 50 mg/L amoxicillin. Colony-forming units (CFU) were counted, and the conjugation frequency was calculated as the ratio of transconjugant bacteria (those growing on azide + amoxicillin plates) to the total recipient *E. coli* J53 population (growing on azide-only plates).

Expression of *bla*_{OXA-1} gene

RNA from *bla*_{OXA-1} positives isolates was extracted using the RNeasy Mini Kit (Qiagen, Hilden, Germany) following manufacture's recommendations. After extraction, RNA was treated with TURBO DNA-free Kit to reduce residual genomic DNA contamination. RNA concentration was first determined using a Qubit fluorometer with the Qubit RNA BR Assay Kit using Qubit 3 Fluorometer (Thermo Fisher Scientific, Waltham, MA, USA), and sample purity was assessed by measuring absorbance ratios (A260/280 and A260/230) on a NanoDrop ND-1000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA).

Quantification of *bla*_{OXA-1} expression was then performed by one-step reverse-transcription droplet digital PCR (RT-ddPCR) using the QX200 Droplet Digital PCR System (Bio-Rad) and the QX Manager Software 2.3.1. Primers and hydrolysis probes used for *bla*_{OXA-1} detection, confirmation, and expression analysis are listed in Supplementary Table S1.

Absolute copy numbers of *bla*_{OXA-1} and *gyrB* were calculated with QX Manager Software 2.3.1 and expressed as normalized *bla*_{OXA-1} per *gyrB*.

Statistical analysis

Categorical variables were compared using Fisher's exact or χ^2 tests, continuous variables using Mann-Whitney U or Kruskal-Wallis tests with Dunn's post-hoc comparisons when appropriate, and correlations using Spearman's rank correlation. Multivariable linear regression models used log2-transformed piperacillin-tazobactam MIC as the outcome and included biologically selected predictors. Sensitivity models replaced ST88 lineage with *ompF* disruption, and model performance was reported using R².

Declarations

Declaration of generative AI and AI-assisted technologies in the manuscript preparation process

During the preparation of this work, the authors used Claude to improve the fluency and readability of the English language. The authors reviewed and edited the output as needed and took full responsibility for the content of the published article.

Code availability

This study did not generate custom code. All analyses used publicly available published software and pipelines, with versions specified in Methods.

Data Availability

The genome data have been deposited in the NCBI under Bioproject PRJNA150133.

Author Contributions

CSC, MRP, JMRM, JRB and FDP conceptualized the study. CSC and FDP wrote the first draft of the manuscript. CSC and MRP did the bioinformatic and statistical analyses. CSC, MCL and AV did the phenotypic assays. JRB, AP and FDP provided the biological samples. All authors provided input to the overall direction and content of the manuscript and read, contributed to, and approved the final manuscript. All authors had full access to all the data in the study and had final responsibility for the decision to submit for publication. CSC and FDP accessed and verified the data.

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Competing Interests

We declare no competing interests.

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Figures

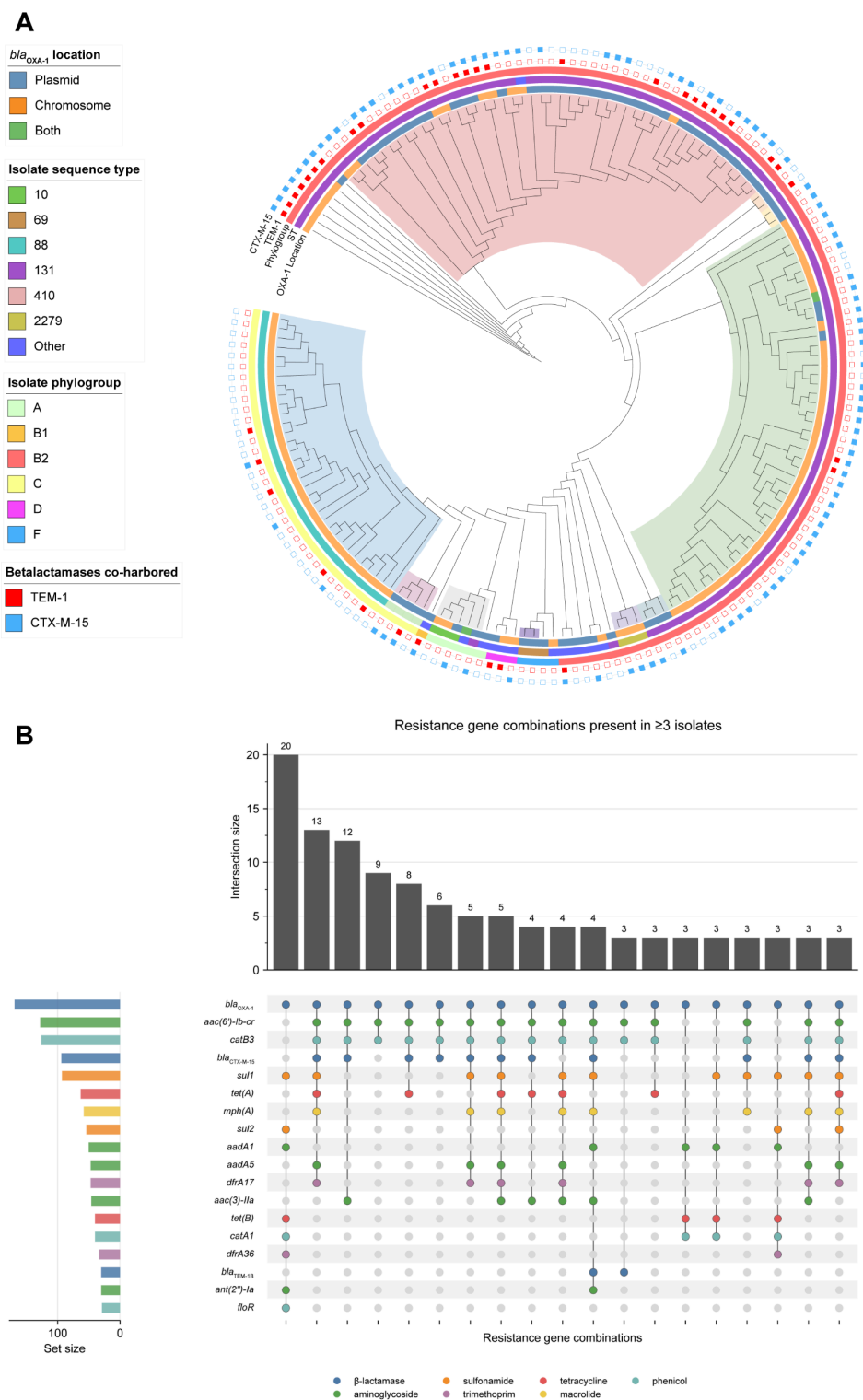


Figure 1

Clonal structure and resistance gene backgrounds of *bla*_{OXA-1}-positive *Escherichia coli*.

A Maximum-likelihood phylogenetic tree of *bla*_{OXA-1}-positive *E. coli* clinical isolates based on core-genome alignment. Annotation rings indicate *bla*_{OXA-1} genomic location, sequence type, phylogroup, and co-harboured β -lactamases. B UpSet plot showing resistance gene combinations present in ≥ 3 isolates.

Vertical bars indicate the number of isolates sharing each gene combination; horizontal bars indicate total set size for each resistance gene. Coloured dots indicate gene presence and are coloured according to antimicrobial resistance class.

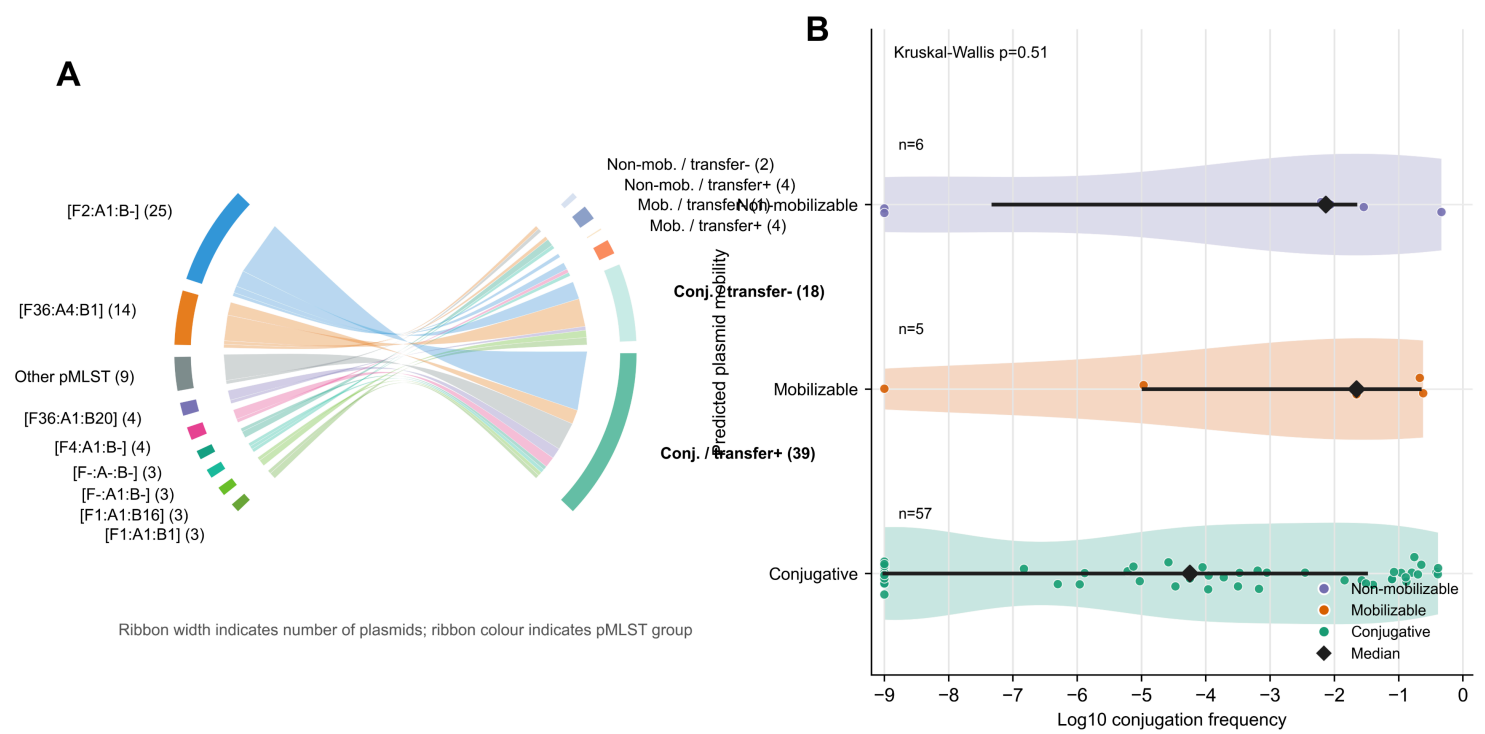


Figure 2

Transferability and backbone relatedness of *bla*_{OXA-1}-bearing plasmids

A, Distribution of *bla*_{OXA-1}-bearing plasmids according to pMLST profile, MOB-suite predicted mobility, and experimental conjugation outcome. Ribbon width indicates the number of plasmids. B, Distribution of conjugation frequencies by MOB-suite predicted mobility category. Frequencies are shown on a log₁₀ scale; points indicate individual plasmids, violins show the distribution, black bars indicate the interquartile range, and diamonds indicate the median. Values at 10⁻⁹ correspond to no detectable transfer and were treated as censored at ≤10⁻⁹.

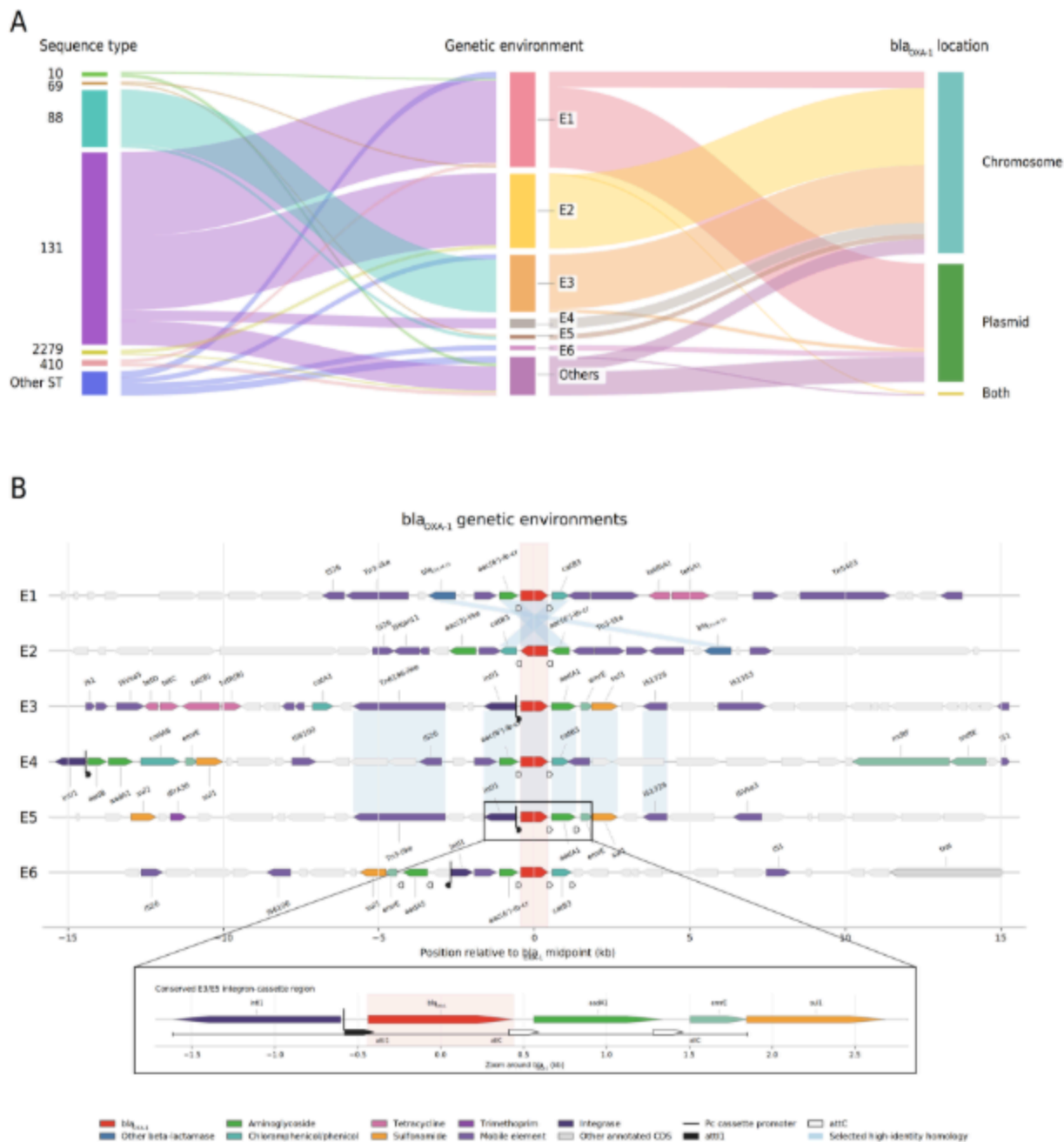


Figure 3

Clonal, genomic, and structural organization of *bla*_{OXA-1} genetic environments

A Sankey diagram showing the distribution of *bla*_{OXA-1}-positive isolates across sequence type, genetic environment, and genomic location. Flow width indicates the number of isolates in each category. B Representative genetic architectures of the six *bla*_{OXA-1} environments. Arrows indicate annotated genes or mobile genetic elements, with arrow direction indicating coding strand. Shaded links connect homologous features between environments. The lower inset shows the conserved class 1 integron architecture shared by the main integron-associated environments, including attI/attC sites and the proximal Pc promoter region upstream of *bla*_{OXA-1}.

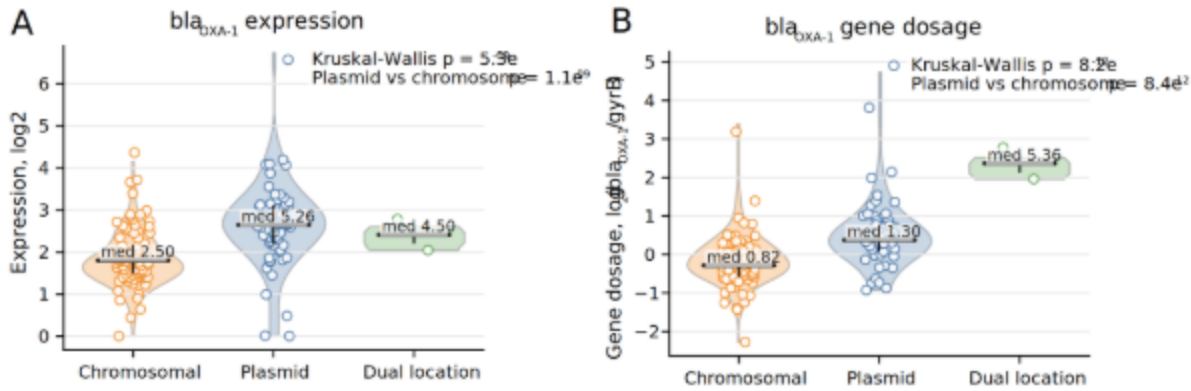


Figure 4

***bla*_{OXA-1} gene dosage and expression according to genomic location.**

A Normalised *bla*_{OXA-1} expression measured by RT-ddPCR according to genomic location. Isolates are grouped as chromosomal, plasmid-borne, or dual-location *bla*_{OXA-1}. Median values are indicated for each group. P values correspond to global Kruskal-Wallis tests and pairwise comparison between chromosomal and plasmid-borne isolates. B Sequencing-depth-based *bla*_{OXA-1} dosage estimates among isolates carrying intact *bla*_{OXA-1}, expressed as the log-transformed coverage ratio between *bla*_{OXA-1} and the single-copy housekeeping gene *gyrB*.

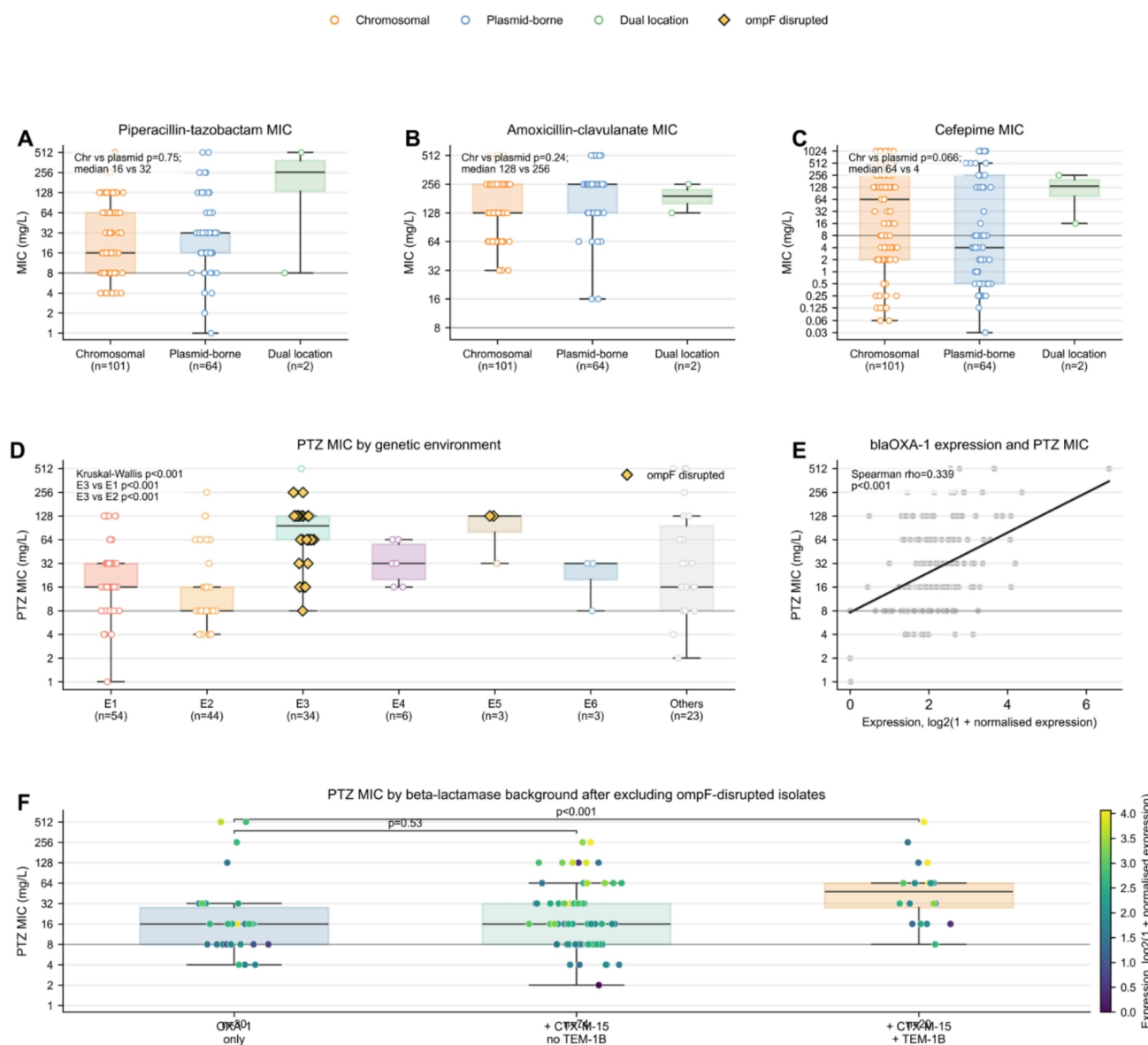


Figure 5

Antimicrobial susceptibility and determinants of piperacillin-tazobactam MIC in *bla*_{OXA-1}-positive *Escherichia coli*.

A-C Broth microdilution MIC distributions for piperacillin-tazobactam, amoxicillin-clavulanate, and cefepime according to *bla*_{OXA-1} genomic location. **D** Piperacillin-tazobactam MICs according to *bla*_{OXA-1} genetic environment; highlighted points indicate isolates with disrupted *ompF*. **E** Association between normalised *bla*_{OXA-1} expression and piperacillin-tazobactam MIC. **F** Piperacillin-tazobactam MICs according to β -lactamase background after exclusion of isolates with disrupted *ompF*. Boxes indicate median and IQR; individual points represent isolates. P values are shown for the indicated comparisons.

Supplementary Files

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