

Supplementary Information

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

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Assay	Target	Forward primer (5'-3')	Reverse primer (5'-3')	Probe (5'-3')	Probe Label
Real-time PCR screening	<i>bla</i> _{OXA-1}	ACACAATACATATCAACTTCGC	TCCGCATCAAATGCCATAAGTG	NA	NA
Conventional PCR confirmation	<i>bla</i> _{OXA-1}	ACACAATACATATCAACTTCGC	AGTGTGTTTAGAATGGTGATC	NA	NA
RT-ddPCR expression	<i>bla</i> _{OXA-1}	CTGTATGGGAAAACGGTGCAG	CCCCAAGTTTCCTGTAAGTGC	5'-CCTTACAAAACGGATGGTTTGAAGGGT-3	FAM/BHQ1
RT-ddPCR reference gene	<i>gyrB</i>	ACGAAGCGATGGATCAGTACC	TCATTTTCTGCGTCGCGTTG	5'-CAACGCTGCACACCAACGCC-3'	HEX/BHQ1

Supplementary Table S1. Primers and probes used for *bla*_{OXA-1} detection and RT-ddPCR expression analysis.

Primer and hydrolysis probe sequences used for *bla*_{OXA-1} screening, conventional PCR confirmation, and RT-ddPCR quantification of *bla*_{OXA-1} expression relative to *gyrB*. NA indicates not applicable.

Gene or curated locus	Antimicrobial class	n isolates with full-length high-confidence hit	% isolates with full-length high-confidence hit	n reported	n partial confidence	Median coverage (%)	Minimum coverage (%)	Median identity (%)	Minimum identity (%)	Reference accessions
<i>aac(6')-Ib-cr</i>	aminoglycoside	127	75,1	128	1	100	100	100	99,807	DQ303918;EF636461
<i>aadA1</i>	aminoglycoside	50	29,6	50	0	100	100	100	99,747	JQ414041;JQ480156;JX185132
<i>aadA5</i>	aminoglycoside	45	26,6	47	2	99,873	99,873	99,873	99,747	AF137361
<i>aac(3)-IIa</i>	aminoglycoside	44	26	46	2	100	100	100	100	CP023555
<i>ant(2'')-Ia</i>	aminoglycoside	30	17,8	30	0	100	100	100	100	X04555
<i>aph(3'')-Ib</i>	aminoglycoside	21	12,4	22	1	100	100	100	99,751	AF321551
<i>aph(6)-Id</i>	aminoglycoside	15	8,9	20	5	100	100	100	99,88	AF024602;CP000971;M28829
<i>aac(3)-IIId</i>	aminoglycoside	7	4,1	7	0	100	100	99,884	99,884	EU022314
<i>aadA2b</i>	aminoglycoside	5	3	5	0	100	100	99,744	96,282	D43625
<i>aadA2</i>	aminoglycoside	3	1,8	3	0	100	100	99,874	99,874	JQ364967
<i>aadA13</i>	aminoglycoside	2	1,2	2	0	100	100	100	100	AY713504
<i>aadA9</i>	aminoglycoside	2	1,2	2	0	100	100	97,013	97,013	AJ420072
<i>aac(3)-IV</i>	aminoglycoside	1	0,6	1	0	100	100	100	100	DQ241380
<i>aph(4)-Ia</i>	aminoglycoside	1	0,6	1	0	100	100	100	100	V01499

<i>bla</i>_{OXA-1}	beta-lactamase	167	98,8	169	2	100	99,88	100	99,88	HQ170510
<i>bla</i>_{CTX-M-15}	beta-lactamase	94	55,6	94	0	100	100	100	100	AY044436
<i>bla</i>_{TEM-1B}	beta-lactamase	30	17,8	30	0	100	100	100	99,768	AY458016
<i>bla</i>_{TEM-1D}	beta-lactamase	5	3	5	0	100	100	100	100	AF188200
<i>bla</i>_{CTX-M-1}	beta-lactamase	3	1,8	3	0	100	100	100	100	DQ915955
<i>bla</i>_{TEM-1C}	beta-lactamase	3	1,8	3	0	100	100	100	99,884	FJ560503
<i>bla</i>_{TEM-1A}	beta-lactamase	2	1,2	2	0	100	100	100	100	HM749966
<i>bla</i>_{CTX-M-14}	beta-lactamase	1	0,6	1	0	100	100	100	100	AF252622
<i>bla</i>_{SHV-12}	beta-lactamase	1	0,6	1	0	100	100	100	100	KF976405
<i>bla</i>_{TEM-30}	beta-lactamase	1	0,6	2	0	100	100	100	100	AJ437107
<i>bla</i>_{TEM-54}	beta-lactamase	1	0,6	1	0	100	100	100	100	AF104442
<i>lnu</i>(F)	lincosamide	2	1,2	2	0	100	100	100	100	EU118119
<i>mph</i>(A)	macrolide	58	34,3	58	0	100	99,669	100	99,89	D16251
<i>mph</i>(B)	macrolide	3	1,8	3	0	100	100	100	100	D85892
<i>erm</i>(B)	macrolide	1	0,6	1	0	100	100	99,864	99,864	JN899585
<i>mef</i>(B)	macrolide	1	0,6	1	0	100	100	100	100	FJ196385
<i>mef</i>(C)	macrolide	1	0,6	1	0	100	100	99,918	99,918	AB571865
<i>mph</i>(G)	macrolide	1	0,6	1	0	100	100	100	100	AB571865
<i>catA1</i>	phenicol	38	22,5	40	2	100	99,242	99,848	99,847	V00622
<i>floR</i>	phenicol	29	17,2	29	0	99,918	99,918	98,105	98,105	AF118107
<i>cmlA1</i>	phenicol	9	5,3	9	0	100	100	99,762	99,762	M64556
<i>catB3</i>	phenicol	6	3,6	126	120	100	100	100	100	AJ009818;U13880

qnrS1	quinolone	5	3	5	0	100	100	100	100	AB187515
qnrB1	quinolone	2	1,2	2	0	100	100	100	100	DQ351241
qnrB19	quinolone	1	0,6	1	0	100	100	100	100	EU432277
qnrS2	quinolone	1	0,6	1	0	100	100	100	100	DQ485530
ARR-3	rifampicin	2	1,2	2	0	100	100	100	100	JF806499
sul1	sulfonamide	93	55	93	0	100	99,881	100	99,881	AM040449;U12338
sul2	sulfonamide	54	32	54	0	100	100	100	99,877	AY034138;HQ840942
sul3	sulfonamide	6	3,6	6	0	100	100	100	100	AJ459418
tet(A)	tetracycline	62	36,7	63	1	100	100	100	94,917	AF534183;AJ517790
tet(B)	tetracycline	39	23,1	40	1	100	100	100	99,917	AF326777;AP000342
dfrA17	trimethoprim	47	27,8	47	0	100	100	100	100	FJ460238
dfrA36	trimethoprim	33	19,5	33	0	100	100	100	99,83	CP038791
dfrA14	trimethoprim	9	5,3	9	0	100	100	100	100	KF921535
dfrA1	trimethoprim	3	1,8	3	0	100	100	99,789	99,789	AF203818;X00926
dfrA12	trimethoprim	3	1,8	3	0	100	100	100	100	AM040708

Supplementary Table S2. Curated acquired antimicrobial resistance genes detected among *bla*_{OXA-1}-positive *Escherichia coli* isolates.

The table summarises acquired resistance gene calls after curation for alignment coverage and identity. Full-length high-confidence hits were defined as gene calls with $\geq 99\%$ coverage and $\geq 90\%$ nucleotide identity. Partial-confidence hits are reported separately from full-length high-confidence calls and were not interpreted as complete genes. When multiple allele names mapped to the same genomic locus, they were collapsed into a single curated locus. Percentages were calculated using the 169 *bla*_{OXA-1}-positive isolates as denominator.

Antibiotic class	Antibiotic	n tested	S	I	R	ATU	S (%)	I (%)	R (%)	ATU (%)
Penicillin	Ampicillin	169	1	0	168	0	0.6	0.0	99.4	0.0
Beta-lactam/beta-lactamase inhibitor	Amoxicillin/clavulanic acid	169	4	0	165	0	2.4	0.0	97.6	0.0
Penicillin	Ticarcillin	169	1	0	168	0	0.6	0.0	99.4	0.0
Beta-lactam/beta-lactamase inhibitor	Piperacillin/tazobactam	169	80	0	89	0	47.3	0.0	52.7	0.0
Cephalosporin	Cefuroxime	169	40	0	129	0	23.7	0.0	76.3	0.0
Cephameycin	Cefoxitin	169	157	0	12	0	92.9	0.0	7.1	0.0
Cephalosporin	Ceftazidime	169	69	0	100	0	40.8	0.0	59.2	0.0
Beta-lactam/beta-lactamase inhibitor	Ceftazidime/avibactam	169	169	0	0	0	100.0	0.0	0.0	0.0
Cephalosporin	Cefepime	169	32	26	111	0	18.9	15.4	65.7	0.0
Beta-lactam/beta-lactamase inhibitor	Ceftolozan/tazobactam	169	164	0	5	0	97.0	0.0	3.0	0.0
Monobactam	Aztreonam	169	72	56	41	0	42.6	33.1	24.3	0.0
Carbapenem	Ertapenem	169	169	0	0	0	100.0	0.0	0.0	0.0
Carbapenem	Imipenem	169	169	0	0	0	100.0	0.0	0.0	0.0
Carbapenem	Meropenem	169	169	0	0	0	100.0	0.0	0.0	0.0
Aminoglycoside	Gentamicin	169	95	0	74	0	56.2	0.0	43.8	0.0
Aminoglycoside	Tobramycin	169	18	0	151	0	10.7	0.0	89.3	0.0
Aminoglycoside	Amikacin	169	127	0	42	0	75.1	0.0	24.9	0.0
Fluoroquinolone	Ciprofloxacin	169	7	0	161	1	4.1	0.0	95.3	0.6
Fluoroquinolone	Levofloxacin	169	9	0	160	0	5.3	0.0	94.7	0.0
Tetracycline derivative	Tigecycline	169	169	0	0	0	100.0	0.0	0.0	0.0

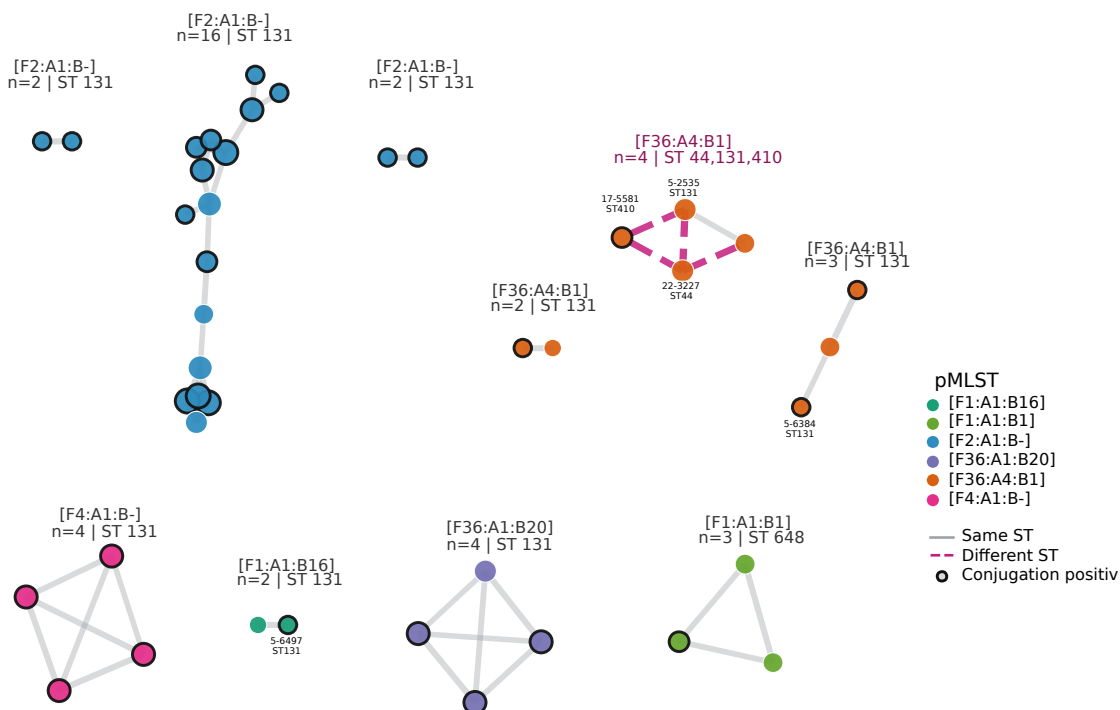
Penicillin	Ampicillin	169	1	0	168	0	0.6	0.0	99.4	0.0
Beta-lactam/beta-lactamase inhibitor	Amoxicillin/clavulanic acid	169	4	0	165	0	2.4	0.0	97.6	0.0
Penicillin	Ticarcillin	169	1	0	168	0	0.6	0.0	99.4	0.0
Beta-lactam/beta-lactamase inhibitor	Piperacillin/tazobactam	169	80	0	89	0	47.3	0.0	52.7	0.0
Cephalosporin	Cefuroxime	169	40	0	129	0	23.7	0.0	76.3	0.0
Cephameycin	Cefoxitin	169	157	0	12	0	92.9	0.0	7.1	0.0
Folate pathway inhibitor combination	Trimethoprim/sulfamethoxazole	169	71	0	98	0	42.0	0.0	58.0	0.0
Fosfomycin	Fosfomycin	169	141	0	28	0	83.4	0.0	16.6	0.0
Polymyxin	Colistin	169	166	0	3	0	98.2	0.0	1.8	0.0

Supplementary Table S3. MicroScan antimicrobial susceptibility profiles of *bla*_{OXA-1}-positive bloodstream *Escherichia coli* isolates.

Gene	Functional category	Isolates with non-synonymous variants	Total synonymous variant events	Total non-synonymous variant events	Total indel events	Isolates with indels	Non-synonymous variants (isolate count)
<i>ompF</i>	Outer-membrane permeability / porin	26	593	160	687	38	D306E (24); D52N (24); K229N (14); E51V (6); G48D (6)
<i>ompC</i>	Outer-membrane permeability / porin	58	1268	826	1379	51	R352H (48); L235F (47); L235M (47); A212G (45); V214I (45)
<i>phoE</i>	Outer-membrane permeability / porin	52	1153	164	60	2	V17A (49); V306I (49); S258T (48); D48A (6); A203S (5)
<i>acrA</i>	Major efflux system	46	553	47	0	0	A104T (42); A167S (2); A117T (1); A167T (1); R5S (1)
<i>acrB</i>	Major efflux system	54	2195	102	0	0	N1043S (54); N596H (46); V557L (1); V791I (1)
<i>tolC</i>	Major efflux system	52	1456	281	866	1	T233A (47); G114E (2); A22V (1); D433A (1); E23A (1)
<i>marA</i>	Global regulator of permeability/efflux	49	232	49	0	0	N127S (49)

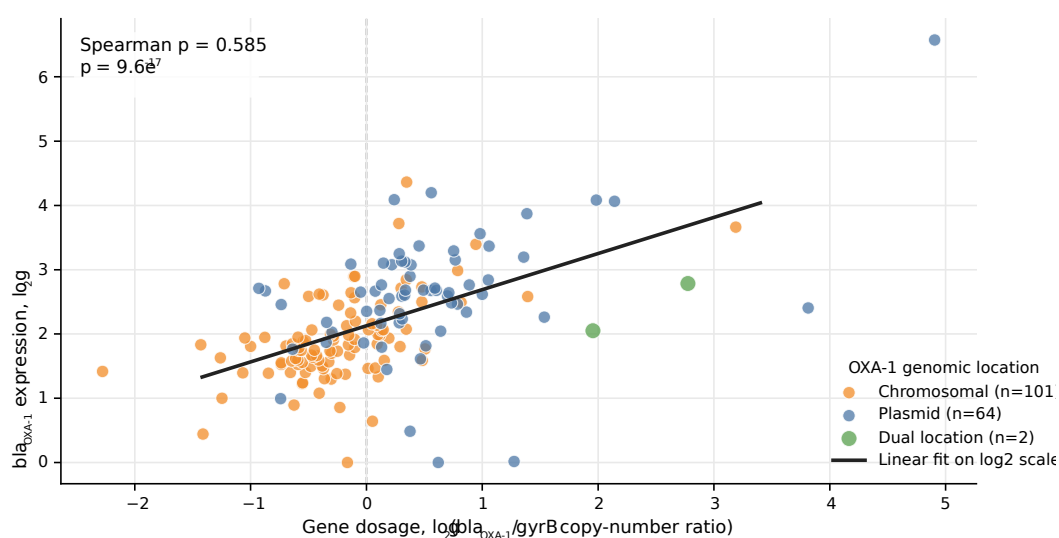
marR	Global regulator of permeability/efflux	161	342	1518	4344	18	L94P (144); V8A (47); G61E (38); A19V (34); W76L (34)
soxR	Global regulator of permeability/efflux	50	481	54	169	2	R74G (42); T38S (7); A111T (2); C122Y (1); G121D (1)
soxS	Global regulator of permeability/efflux	1	294	1	0	0	R58H (1)
robA	Global regulator of permeability/efflux	6	650	12	0	0	A171S (5); Q20H (5); S146P (1); V64A (1)
ftsI	PBP / cell-wall synthesis-remodelling	51	1351	101	0	0	T233A (49); V332I (44); D149E (5); Q227H (2); A485V (1)
mrcA	PBP / cell-wall synthesis-remodelling	57	184	2422	0	0	L165R (50); A117V (49); H240R (49); Q340R (49); Q345R (49)
mrcB	PBP / cell-wall synthesis-remodelling	59	5182	175	912	55	E836D (54); D591E (53); N604H (49); V782I (6); M799I (3)
dacA	PBP / cell-wall synthesis-remodelling	0	876	0	432	6	
dacB	PBP / cell-wall synthesis-remodelling	56	1280	197	291	1	A269T (54); T121A (52); I27V (37); P182Q (37); V18I (4)
dacC	PBP / cell-wall synthesis-remodelling	9	680	9	0	0	M383V (6); K69N (1); P339L (1); T337M (1)
dacD	PBP / cell-wall synthesis-remodelling	54	27	787	420	21	V268A (52); A219V (51); L209S (51); T215M (51); A36V (42)
cpxA	Envelope stress response	51	944	109	428	1	S457* (51); S457T (51); H366N (2); S315G (2); A112T (1)
cpxR	Envelope stress response	53	546	53	98	49	T149S (53)
phoP	Envelope stress response	43	594	43	0	0	L44I (40); M175T (3)
phoQ	Envelope stress response	9	1234	12	339	1	L467M (4); A482T (3); R6H (2); S138T (2); R219H (1)
ampD	Peptidoglycan recycling / AmpC-related pathway	54	341	178	0	0	A9V (47); R143C (42); A176V (41); N149K (41); V178I (3)
ampE	Peptidoglycan recycling / AmpC-related pathway	54	707	183	369	1	H106R (54); A195V (53); C45G (53); A60T (4); A191G (2)
ampG	Peptidoglycan recycling / AmpC-related pathway	54	1359	69	9	1	I436V (49); E420A (7); V401I (5); D413G (4); L90M (2)
ampH	Peptidoglycan recycling / AmpC-related pathway	55	205	1458	980	5	L3R (54); P62R (49); L151R (47); P150L (47); Y103C (47)

Supplementary Table S4. Targeted chromosomal variant analysis of genes potentially associated with piperacillin-tazobactam susceptibility.



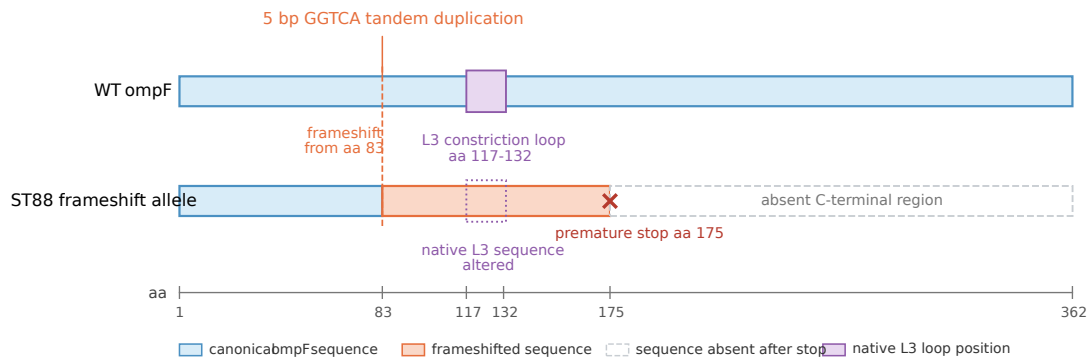
Supplementary Figure S1. Backbone similarity network of *bla*_{OXA-1}-bearing plasmids.

Nodes represent long-read-resolved plasmid sequences and are coloured by pMLST profile. Edges connect plasmids sharing high backbone similarity, defined as $\geq 85\%$ aligned coverage and $\geq 99\%$ nucleotide identity. Grey edges connect plasmids from the same host sequence type, whereas dashed magenta edges connect plasmids from different sequence types. Black-outlined nodes indicate plasmids from isolates with detectable conjugation.



Supplementary Figure S2. Association between *bla*_{OXA-1} gene dosage and expression.

Scatter plot showing sequencing-depth-based *bla*_{OXA-1} dosage estimates against normalised *bla*_{OXA-1} expression measured by RT-ddPCR among isolates carrying intact *bla*_{OXA-1} (n=167). Gene dosage is expressed as the log-transformed *bla*_{OXA-1}/*gyrB* copy-number ratio, and expression is shown on a log-transformed scale. Points are coloured according to *bla*_{OXA-1} genomic location. The line indicates the linear fit on the log₂ scale. Spearman correlation: $\rho=0.585$, $p=9.6\times10^{-17}$.



Supplementary Figure S3. Predicted effect of the recurrent ST88-associated *ompF* frameshift.

Schematic representation of wild-type *ompF* and the ST88-associated *ompF* frameshift allele carrying a 5 bp GGTC A tandem duplication (*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 L3 constriction-loop region of OmpF.