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OXA-1 β-lactamase and non-susceptibility to penicillin/β-lactamase inhibitor combinations among ESBL-producing *Escherichia coli*

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Background: ESBL-producing *Escherichia coli* have expanded globally since the turn of the century and present a major public health issue. Their *in vitro* susceptibility to penicillin/inhibitor combinations is variable, and clinical use of these combinations against ESBL producers remains controversial. We hypothesized that this variability related to co-production of OXA-1 penicillinase.

Methods: During a national study we collected 293 ESBL-producing *E. coli* from bacteraemias, determined MICs by BSAC agar dilution, and undertook genomic sequencing with Illumina methodology.

Results: The collection was dominated by ST131 ($n = 188$ isolates, 64.2%) and $bla_{\text{CTX-M-15}}$ (present in 229 isolates, 78.2%); over half the isolates (159/293, 54.3%) were ST131 with $bla_{\text{CTX-M-15}}$. $bla_{\text{OXA-1}}$ was found in 149 ESBL producers (50.9%) and $bla_{\text{TEM-1/191}}$ in 137 (46.8%). Irrespective of whether all isolates were considered, or ST131 alone, there were strong associations ($P < 0.001$) between co-carriage of $bla_{\text{OXA-1}}$ and reduced susceptibility to penicillin/inhibitor combinations, whereas there was no significant association with co-carriage of $bla_{\text{TEM-1/191}}$. For piperacillin/tazobactam the modal MIC rose from 2 mg/L in the absence of $bla_{\text{OXA-1}}$ to 8 or 16 mg/L in its presence; for co-amoxiclav the shift was smaller, from 4 or 8 to 16 mg/L, but crossed the breakpoint. $bla_{\text{OXA-1}}$ was strongly associated with co-carriage also of *aac(6′)-Ib-cr*, which compromises amikacin and tobramycin.

Conclusions: Co-carriage of OXA-1, a penicillinase with weak affinity for inhibitors, is a major correlate of resistance to piperacillin/tazobactam and co-amoxiclav in *E. coli* and is commonly associated with co-carriage of *aac(6′)-Ib-cr*, which narrows aminoglycoside options.

Introduction

Penicillin/β-lactamase inhibitor combinations account for 20% of inpatient antibiotic use in UK hospitals[1] and for a greater proportion of parenteral use. Whilst these combinations are effective in many infections due to β-lactamase producers, debate persists on their efficacy against those with ESBLs, along with disagreements on breakpoints.[2]

Tazobactam and clavulanate inhibit TEM, SHV and CTX-M ESBLs,[3–5] in some cases more efficiently than classical penicillinases.[6] Nevertheless, surveys find that sizeable proportions of ESBL-producing *Escherichia coli* and *Klebsiella pneumoniae* are non-susceptible to piperacillin/tazobactam and amoxicillin/clavulanate, as are minorities of isolates with classical TEM and SHV penicillinases.[7–9] The issue is complicated by differing breakpoints for piperacillin/tazobactam between EUCAST ($S \leq 8$, $R > 16$ mg/L) and CLSI ($S \leq 16$, $R > 64$ mg/L) and different testing modalities for amoxicillin/clavulanate, where EUCAST advocates a fixed 2 mg/L clavulanate concentration but CLSI prefers a 2:1 amoxicillin/clavulanate ratio, giving breakpoints of 8+2 and 8+4 mg/L, respectively.

Clinical studies on the efficacy of penicillin/inhibitor combinations against ESBL producers have given contradictory results.[10] Both EUCAST and CLSI take the view of 'report as found',[11] and one bacteraemia study (not specifically of ESBL producers) found good outcomes for piperacillin/tazobactam against Enterobacteriaceae up to an MIC of 16 mg/L.[12] Another study, however, found good outcomes up to an MIC of 16 mg/L only if the bacteraemia had a urinary origin whereas there were high failure rates if the MIC was above 2 mg/L and the bacteraemia originated elsewhere.[13] The recent MERINO trial, investigating bacteraemia due to ceftriaxone-resistant, piperacillin/tazobactam-susceptible *E. coli* and *K. pneumoniae* found 12.3% 30 day mortality for patients

treated with piperacillin/tazobactam versus 3.7% for meropenem ($P = 0.002$).[14]

Reasons for variable resistance to penicillin/inhibitor combinations among ESBL producers are under-researched. Factors demonstrated for at least some isolates include: (i) production of multiple β-lactamases,[15] sometimes including poorly inhibited penicillinases such as OXA-1;[16,17] (ii) hyperproduction of target β-lactamases;[18,19] and (iii) impermeability.[20] We explored the role of the OXA-1 enzyme in a national collection of genomically sequenced ESBL-producing *E. coli* from bloodstream infections.

Materials and methods

Isolates

Isolates were from human bloodstream infections and were collected in the period 2013–14 during a national study comparing ESBL-producing *E. coli* from human and non-human sources. Collecting sites in London (one hospital), East Anglia (five hospitals), North-West England (two hospitals), Wales (two hospitals) and Scotland (two hospitals) incubated blood cultures on automated BacT/ALERT (bioMérieux, Basingstoke, UK) systems and performed identification and susceptibility testing according to local protocols. Consecutive isolates identified by these local methods as ESBL-producing *E. coli* were subcultured to agar slopes and sent to PHE Colindale, London, UK. On receipt, their identity was confirmed by MALDI-TOF MS (Bruker Daltonics, Bremen, Germany) and $bla_{CTX\text{-}M}$ genes were sought by PCR,[21] with isolates found positive accepted as ESBL producers. Isolates lacking $bla_{CTX\text{-}M}$ were screened for bla_{TEM} and bla_{SHV} by PCR[22] and, if positive, subjected to double-disc synergy tests between amoxicillin/clavulanate (20+10 μg; Oxoid, Basingstoke, UK) and each of cefepime, cefotaxime and ceftazidime (all 30 μg), with a positive result for any cephalosporin being taken to indicate ESBL activity.[23] Confirmation of ESBL production came from comprehensive susceptibility testing and sequencing, as below.

Antibiotics and susceptibility testing

Except for clavulanate (GlaxoSmithKline, Brentford, UK) and tazobactam (Alfa Aesar, Heysham, UK), antibiotics were obtained from Sigma, Poole, UK. MICs were determined by BSAC agar dilution using Iso-Sensitest agar (Oxoid).[24] Tazobactam was used at a fixed concentration of 4 mg/L and clavulanate at a fixed concentration of 2 mg/L, in keeping with current EUCAST guidance.

WGS

DNA libraries were prepared using the Nextera XT method and sequenced to >30× coverage with a standard 2×100 bp protocol on a HiSeq 2500 instrument (Illumina, San Diego, CA, USA). Reads were trimmed using Trimmomatic to remove low-quality data, then assembled into contigs using VelvetOptimiser[25] with *k*-mer values from 55 to 75. Strains were identified by mapping reads against ST-specific *E. coli* sequences using MOST software.[26]

Antibiotic resistance genes were sought in contigs by BLASTn, or by mapping reads against reference sequences in the Comprehensive Antibiotic Resistance Database and parsing the variant call format (VCF) file generated by SAMtools mpileup.[27] This process was automated into the 'Genefinder' pipeline created by PHE Bioinformatics (M. Doumith, PHE, unpublished data). The location of resistance determinants on assembled contigs was checked by BLASTn.

Statistics

We calculated relative risks and assessed potential interactions using the Woolf test for homogeneity. We used Pearson χ^2 tests to assess significance of associations at a *P* value equal to 0.05.

Results

ESBL confirmation and STs

Sixty-six ESBL producers were confirmed from bacteraemic patients in East Anglia, 55 from London, 61 from North-West England, 37 from Scotland and 74 from Wales, giving a geographically representative collection of 293 isolates. These isolates included 39 known STs, one non-typeable organism and five new STs. The well-known international ST131 lineage[28,29] dominated, with 188 representatives (64.2%); other STs with more than two representatives were ST38 ($n = 17$), ST648 ($n = 16$), ST405 ($n = 9$), ST73 ($n = 6$), ST69 ($n = 4$), ST636 ($n = 4$), ST95 ($n = 3$), ST10 ($n = 3$) and ST1193 ($n = 3$). CTX-M-15 β-lactamase was the predominant ESBL, with its gene present in 229 (78.2%) isolates, whereas 27 had $bla_{CTX\text{-}M\text{-}27}$, 20 had $bla_{CTX\text{-}M\text{-}14}$, 4 had $bla_{CTX\text{-}M\text{-}1}$, 3 had $bla_{CTX\text{-}M\text{-}3}$ and 1 had $bla_{CTX\text{-}M\text{-}9}$. Three isolates had $bla_{SHV\text{-}12}$ and one had $bla_{SHV\text{-}31}$, both of which encode recognized SHV-ESBLs; one isolate, with an ESBL phenotype, solely had $bla_{TEM\text{-}117}$ and eight, all carrying other well-known ESBL determinants, also had $bla_{TEM\text{-}191}$, encoding a TEM variant with an uncertain status, which was not counted as an ESBL here.[30] Four isolates carried two ESBL genes in combination; many more also carried genes for classical penicillinases along with those for ESBLs. In particular, $bla_{TEM\text{-}1}$ was present in 129/293 isolates (or 137/293 if those with TEM-191 were included, 46.8%) and $bla_{OXA\text{-}1}$ (or, in one case, a variant with a conservative Ile187Leu modification) was found in 149/293 (50.9%). $bla_{TEM\text{-}1}$ accompanied many different ESBL genes but $bla_{OXA\text{-}1}$ was always together with $bla_{CTX\text{-}M\text{-}15}$ along, in one isolate, with $bla_{CTX\text{-}M\text{-}14}$. Two isolates had acquired bla_{CMY}/*ampC* genes together with their ESBLs, and two had $bla_{OXA\text{-}9}$. Among the 188 ST131 isolates, the great majority (159/188, 84.6%) had $bla_{CTX\text{-}M\text{-}15}$, though 24 had $bla_{CTX\text{-}M\text{-}27}$ and 5 had $bla_{CTX\text{-}M\text{-}14}$ alone or in combination; 116 had $bla_{OXA\text{-}1}$, whilst 76 had $bla_{TEM\text{-}1/191}$.

The β-lactamase combinations found in the whole collection and among the ST131 isolates are detailed in Table 1, which also shows the corresponding MIC distributions of piperacillin/tazobactam and amoxicillin/clavulanate.

Whenever $bla_{OXA\text{-}1}$ was present, alone or together with $bla_{TEM\text{-}1/191}$, the MIC distributions of penicillin/inhibitor combinations were raised, with the mode increasing from 2 mg/L to 8 or (depending on the particular subset) 16 mg/L for piperacillin/tazobactam and from 4 or 8 to 16 mg/L for amoxicillin/clavulanate. These shifts in modal MIC were apparent for both the whole collection and for ST131, when this was reviewed separately. No such shift was seen when ESBLs were accompanied only by TEM-1/191 enzyme.

Whilst these $bla_{OXA\text{-}1}$-related MIC shifts were small in absolute terms, their effect was to move the peak of the distribution for piperacillin/tazobactam from within the susceptible range to around the breakpoint, whilst the mode for amoxicillin/clavulanate moved across the breakpoint. Overall, 62/63 (98.4%) isolates with ESBL genes alone were susceptible to piperacillin/tazobactam at 8 mg/L, as were 75/79 (94.9%) that had an ESBL gene together with only $bla_{TEM\text{-}1/191}$, whereas the proportion that were susceptible fell to 67/91 (73.6%) among those with an ESBL plus $bla_{OXA\text{-}1}$ and to 33/58 (56.9%) for those with an ESBL plus both $bla_{OXA\text{-}1}$ and $bla_{TEM\text{-}1/191}$. For amoxicillin/clavulanate, 44/63 (69.8%) were susceptible when the ESBL gene was present alone and 50/79 (63.3%) when it was accompanied by $bla_{TEM\text{-}1/191}$, whilst these

Table 1. β-Lactamase profiles and penicillin/inhibitor MICs for all ESBL-producing *E. coli* from bloodstream infections ($n = 293$) and ST131 isolates ($n = 188$)

	Number of isolates with indicated MIC (mg/L)									Percentage susceptible at 8 mg/L
	≤1	2	4	8	16	32	64	>64	Total	
Piperacillin/tazobactam										
all isolates with ESBL alone										
CTX-M-15	2	13	7	3		1			26	96.2
CTX-M-27	3	12	5	2					22	100.0
CTX-M-1	1	5							6	—
CTX-M-14		3	2						5	—
CTX-M-3		1							1	—
CTX-M-9		1							1	—
CTX-M-15; CTX-M-3			1						1	—
TEM-117-p*[a]		1							1	—
total	6	36	15	5	0	1	0	0	63	98.4
all isolates with ESBL plus TEM-1, no OXA-1										
CTX-M-15; TEM-1/191	6	19	20	3	2	1			51	94.1
CTX-M-14; TEM-1		6	4	3	1				14	92.9
CTX-M-27; TEM-1		2	2	1					5	—
CTX-M-1; TEM-1		2	1						3	—
SHV-12; TEM-1/191		2	1						3	—
CTX-M-3; TEM-1			1						1	—
CTX-M-24; TEM-1		1							1	—
CTX-M-1; OXA-9; SHV-31; TEM-1			1						1	—
total	6	32	30	7	3	1	0	0	79	94.9
all isolates with ESBL plus OXA-1, no TEM-1										
CTX-M-15; OXA-1[b]	2	8	24	33	13	5	2	2	89	75.3
CTX-M-15; CTX-M-3; OXA-1				1					1	—
CTX-M-15; CTX-M-14; OXA-1			1						1	—
total	2	8	25	34	13	5	2	2	91	75.8
all isolates with ESBL plus TEM-1 and OXA-1										
CTX-M-15; OXA-1; TEM-1/191		3	7	23	19	5			57	57.9
CTX-M-15; OXA-1; OXA-9; TEM-191-p*					1				1	—
total	0	3	7	23	20	5	0	0	58	56.9
all isolates with ESBL plus AmpC										
CTX-M-15; CMY-4-p*								1	1	—
CTX-M-15; CMY-42								1	1	—
total	0	0	0	0	0	0	0	2	2	0.0
major groups of ST131 isolates										
CTX-M-15	1	5	3	2		1			12	91.7
CTX-M-27	3	12	5	2					22	100.0
CTX-M-15; TEM-1/191	2	15	10	2		1			30	96.7
CTX-M-15; OXA-1	1	7	18	29	11	4	2	2	74	74.3
CTX-M-15; OXA-1; TEM-1/191		2	6	13	15	4			40	52.5
minor groups of ST131 isolates										
CTX-M-14		2	1						3	—
CTX-M-27; TEM-1		1		1					2	—
CTX-M-14; TEM-1		1							1	—
CTX-M-15; CTX-M-3			1						1	—
CTX-M-15; CTX-M-14; OXA-1			1						1	—
CTX-M-15; OXA-1; OXA-9; TEM-191-p*					1				1	—
CTX-M-3; TEM-1			1						1	—

Continued

Table 1. *Continued*

	Number of isolates with indicated MIC (mg/L)									Percentage susceptible at 8 mg/L
	≤1	2	4	8	16	32	64	>64	Total	
Amoxicillin/clavulanate										
all isolates with ESBL alone										
CTX-M-15		1	5	12	5	2	1		26	69.2
CTX-M-27			9	5	6	1	1		22	63.6
CTX-M-1			1	5					6	—
CTX-M-14				4	1				5	—
CTX-M-3				1					1	—
CTX-M-9				1					1	—
CTX-M-15; CTX-M-3					1				1	—
TEM-117-p*					1				1	—
total	0	1	15	28	14	3	2	0	63	69.8
all isolates with ESBL plus TEM-1, no OXA-1										
CTX-M-15; TEM-1/191			12	27	9	3			51	76.5
CTX-M-14; TEM-1				3	10	1			14	21.4
CTX-M-27; TEM-1			1	2	2				5	—
CTX-M-1; TEM-1				2	1				3	—
SHV-12; TEM-1/191				2	1				3	—
CTX-M-3; TEM-1					1				1	—
CTX-M-24; TEM-1				1					1	—
CTX-M-1; OXA-9; SHV-31; TEM-1					1				1	—
total	0	0	13	37	25	4	0	0	79	63.3
all isolates with ESBL plus OXA-1, no TEM-1										
CTX-M-15; OXA-1[b]			2	19	55	13			89	23.6
CTX-M-15; CTX-M-3; OXA-1					1				1	—
CTX-M-15; CTX-M-14; OXA-1					1				1	—
total	0	0	2	19	57	13	0	0	91	23.1
all isolates with ESBL plus TEM-1 and OXA-1										
CTX-M-15; OXA-1; TEM-1/191			1	5	33	18			57	10.5
CTX-M-15; OXA-1; OXA-9; TEM-191-p*				1					1	—
total	0	0	1	6	33	18	0	0	58	12.1
all isolates with ESBL plus AmpC										
CTX-M-15; CMY-42								1	1	—
CTX-M-15; CMY-4-p								1	1	—
total	0	0	0	0	0	0	0	2	2	0.0
major groups of ST131 isolates										
CTX-M-15			2	5	2	2	1		12	58.3
CTX-M-27			9	5	6	1	1		22	63.6
CTX-M-15; TEM-1/191			5	17	6	2			30	73.3
CTX-M-15; OXA-1			2	15	46	11			74	23.0
CTX-M-15; OXA-1; TEM-1/191			1	3	22	14			40	10.0
minor groups of ST131 isolates										
CTX-M-14				3					3	—
CTX-M-27; TEM-1			1		1				2	—
CTX-M-14; TEM-1				1					1	—
CTX-M-15; CTX-M-3					1				1	—
CTX-M-15; CTX-M-14; OXA-1					1				1	—
CTX-M-15; OXA-1; OXA-9; TEM-191-p*				1					1	—
CTX-M-3; TEM-1					1				1	—

[a]p*, enzyme is defined from a partial sequence, preventing confident precise matching.
[b]Includes one isolate with an OXA-1 sequence variant, with Ile187Leu.

Table 2. Risk of non-susceptibility to penicillin/β-lactamase inhibitor combinations in relation to the presence of secondary β-lactamases

		Piperacillin/tazobactam				Amoxicillin/clavulanate			
	Secondary β-lactamase	relative risk of MIC >8 mg/L	95% lower CI	95% upper CI	*P*	relative risk of MIC >8 mg/L	95% lower CI	95% upper CI	*P*
All ESBL-producing	OXA-1[a]	6.49	3.03	13.88	<0.001	2.34	1.85	2.96	<0.001
E. coli isolates ($n = 293$)	TEM-1/191	1.32	0.81	2.14	0.257	1.00	0.82	1.22	0.992
	OXA-1 + TEM-1/191	3.49	2.22	5.48	<0.001	1.72	1.47	2.02	<0.001
		(*P* value for homogeneity = 0.33)				(*P* value for homogeneity = 0.34)			
ST131 ESBL-producing	OXA-1	12.10	3.01	48.61	<0.001	2.43	1.73	3.41	<0.001
E. coli isolates ($n = 188$)	TEM-1/191	1.58	0.92	2.71	0.094	0.96	0.77	1.21	0.741
	OXA-1 + TEM-1/191	3.41	2.06	5.66	<0.001	1.57	1.31	1.89	<0.001
		(*P* value for homogeneity = 0.47)				(*P* value for homogeneity = 0.17)			

P values shown are for χ^2 tests except where indicated; *P* value for homogeneity indicates significance of interaction between OXA-1 and TEM-1 according to the Woolf test.
[a]Includes one isolate with an OXA-1 sequence variant, with Ile187Leu.

proportions fell to 21/91 (23.1%) for isolates with bla_{OXA-1} together with their ESBL gene and to 7/58 (12.1%) when both bla_{OXA-1} and $bla_{TEM-1/191}$ were present. When the ST131 organisms were considered alone, non-susceptibility to piperacillin/tazobactam at 8 mg/L was seen in 39/116 (33.6%) isolates where bla_{OXA-1} was present compared with 2/72 (2.8%) where it was absent; corresponding proportions for amoxicillin/clavulanate were 94/116 (81.0%) compared with 24/72 (33.3%), respectively.

Both for the whole collection and the ST131 isolates, the relative risks of non-susceptibility to penicillin/β-lactamase inhibitor combinations were highly significant for OXA-1 ($P < 0.001$) but non-significant for TEM-1/191 (Table 2). Although the modal MIC was one doubling dilution higher for the isolates that had both OXA-1 and TEM-1/191 than for those with only OXA-1, there was no statistical evidence of interaction between OXA-1 and TEM-1/191 to further augment resistance.

Occasional non-susceptibility to piperacillin/tazobactam was seen in isolates lacking bla_{OXA-1}, as in 1/26 with $bla_{CTX-M-15}$ alone (MIC 32 mg/L) and 4/65 with $bla_{CTX-M-14/15}$ together with bla_{TEM-1} (MICs 16–32 mg/L), also (unsurprisingly) in both isolates with acquired bla_{CMY} genes, neither of which had bla_{OXA-1}. On the other hand, 10/58 isolates with $bla_{CTX-M-15}$ plus both $bla_{TEM-1/191}$ and bla_{OXA-1} remained fully susceptible to piperacillin/tazobactam, with MICs of 2–4 mg/L.

Linkage of bla_{OXA-1}, aac(6′)-Ib and other resistance determinants

There was a striking association between the carriage of bla_{OXA-1} and of the aminoglycoside acetyltransferase determinant *aac(6′)-Ib*, which was almost always (146/148 cases) present as its *aac(6′)-Ib-cr* variant, encoding an enzyme that acetylates some fluoroquinolones as well as the normal aminoglycoside substrates. This association is illustrated both for the whole collection and for the major β-lactamase-defined subgroups of ST131 isolates in Table 3. Overall, 147 of the 149 isolates with bla_{OXA-1} also had *aac(6′)-Ib-cr*, compared with 1/144 of those that lacked bla_{OXA-1}. Other resistance genes associated with bla_{OXA-1} across the whole collection were *aac(3)-IIa*, *aadA5*, *sul1*, *dfrA17* and *tet*(A) (Table 4). *catB3*, encoding a chloramphenicol acetyltransferase, also was widely present in association with bla_{OXA-1} (not shown) but was truncated and surmised to be non-functional. Conversely, *sul2*, *strA*, *strB* and *aac(3)-IId* were more prevalent among isolates that lacked bla_{OXA-1}. The association between bla_{OXA-1} and *aac(6′)-Ib-cr* remained clear when ST131 isolates were considered alone, but *aac(3)-IIa*, *aadA5*, *sul1*, *dfrA17* and *tet*(A) remained widespread among ST131 isolates with $bla_{CTX-M-27}$ alone or with $bla_{CTX-M-15}$ combined with either or both of bla_{TEM} and/or bla_{OXA-1}. *strA/B* and *sul2* genes remained negatively associated with bla_{OXA-1} among the ST131 isolates (Table 4).

Resistance tracked with causative genes. Thus, 141/148 isolates with *aac(6′)-Ib-cr* were resistant to tobramycin and 69 had reduced susceptibility to amikacin, with MICs >4 mg/L, though non-susceptibility according to EUCAST criteria (MIC >8 mg/L) was seen for only 25/148. Tobramycin resistance was not, however, exclusive to isolates with *aac(6′)-Ib-cr*, also being associated with *aac(3)-II* variants when these were present independently of *aac(6′)-Ib-cr*. Overall non-susceptibility rates for bla_{OXA-1}-positive compared with bla_{OXA-1}-negative isolates were: tobramycin (MIC >2 mg/L), 94.6% versus 31.2%; amikacin (MIC >8 mg/L), 16.8% versus 2.8%; ciprofloxacin (MIC >0.25 mg/L), 97.2% versus 70.7%; tetracycline (MIC >8 mg/L), 83.4% versus 70.7%; sulphonamides (MIC >256 mg/L), 85.5% versus 76.4%; trimethoprim (MIC >2 mg/L), 89.6% versus 77.8%; and streptomycin (MIC >8 mg/L), 58.6% versus 71.1%. Truncated *catB3* was not associated with chloramphenicol resistance, confirming its non-functionality.

Discussion

Although a link between OXA-1 enzyme and reduced susceptibility or resistance to penicillin/inhibitor combinations has been suggested previously,[16,17] both for ESBL-producing and -non-producing Enterobacteriaceae, these assertions do not appear to have been tested with sizeable and geographically diverse collections of

Table 3. Aminoglycoside and fluoroquinolone resistance among major ST131 groups

		Number with:															
	n	*aac(6′)-1b*[a]	*aac(3)-IIa*	*aac(3)-IId*	*ant(2″)-Ia*	*aadA5*	*aadA1*	*aadA2*	*strA*	*strB*[b]	*dfrA17*	*dfrA12*	other *dfr*	*tet*(A)[c]	*sul1*	*sul2*	*catA1*
Whole collection (*n* = 293)																	
OXA-1 positive	149	147	88	7	6	113	6	9	25	26	113	10	14	121	122	31	19
OXA-1 negative	144	1	18	18	1	65	17	13	81	81	68	8	33	85	78	83	10
Major ST131 groups (*n* = 178 from a total of 188 ST131 isolates, see Table 1)																	
CTX-M-15	12	0	1	0	0	6	0	2	4	4	6	2	2	4	9	4	0
CTX-M-27	22	0	0	0	0	17	0	0	17	17	17	0	0	17	18	17	0
CTX-M-15; TEM-1	30	0	11	9	0	19	0	4	20	20	19	4	1	20	22	20	2
CTX-M-15; OXA-1	74	73	34	0	2	67	0	4	4	4	67	4	0	62	70	9	0
CTX-M-15; OXA-1; TEM-1	40	39	27	6	4	26	0	5	9	9	26	5	2	29	30	10	5

[a]Almost always (146/148 cases) as the *aac(6′)-Ib-cr* variant.
[b]Including *aph(6)-Id*.
[c]Including *tet*(A)-*1*.

Table 4. Relative likelihood of OXA-1 being present in relation to the presence of other resistance genes

	All *E. coli* isolates				ST131 *E. coli* isolates			
Resistance gene	relative risk of OXA-1 presence	95% lower CI	95% upper CI	*P*	relative risk of OXA-1 presence	95% lower CI	95% upper CI	*P*
aac(6′)-Ib	72.01	18.18	285.21	<0.001	37.00	9.43	145.18	<0.001
aac(3)-IIa	2.55	2.04	3.18	<0.001	1.79	1.44	2.23	<0.001
aadA5	1.97	1.48	2.62	<0.001	1.32	0.98	1.78	0.047
sul1	2.13	1.52	2.99	<0.001	1.38	0.94	2.03	0.058
dfrA17	1.94	1.45	2.60	<0.001	1.43	1.04	1.96	0.013
sul2	0.41	0.30	0.57	<0.001	0.39	0.26	0.57	<0.001
strA	0.36	0.25	0.51	<0.001	0.29	0.18	0.47	<0.001
strB	0.37	0.26	0.52	<0.001	0.29	0.18	0.47	<0.001
tet(A)	1.83	1.32	2.53	<0.001	1.43	1.04	1.95	0.012
aac(3)-IId	0.53	0.28	1.00	0.017	0.63	0.34	1.18	0.071

P values shown are for χ^2 tests.
'OXA-1' includes one isolate with an Ile187Leu sequence variant.

bacteria, let alone using those characterized by WGS. One study asserting this linkage only found OXA-1 in 12/59 piperacillin/tazobactam-resistant isolates and, since many of the remainder were resistant to carbapenems, it is likely that they had other mechanisms besides OXA-1 enzyme.[16]

Here we found that MICs of piperacillin/tazobactam for ESBL-producing *E. coli* with OXA-1 penicillinase clustered around or just above the 8+4 mg/L breakpoint, and that those of amoxicillin/clavulanate were narrowly above its 8+2 mg/L breakpoint. By contrast, and irrespective of whether they co-produced TEM-1 enzyme, MICs for ESBL-producing *E. coli* lacking OXA-1 enzyme were almost all clearly within the susceptible range for piperacillin/tazobactam, at around 2 mg/L, and narrowly within it for amoxicillin/clavulanate, clustering at 4 to 8 mg/L. A few individual isolates lay outside these generalizations, either: (i) lacking OXA-1 enzyme but being resistant to penicillin/β-lactamase inhibitor combinations; or (ii) possessing the gene for this enzyme and remaining susceptible. Anomalous resistance perhaps may reflect low permeability, up-regulated efflux, copious ESBL production or elevated expression of chromosomal AmpC; anomalous susceptibility may reflect high permeability, weak efflux or non-expression of bla_{OXA-1} or other genes. Nevertheless, the general relationship between raised MICs of the inhibitor combinations and carriage of bla_{OXA-1} was clear and individual anomalies were not pursued further.

It should be cautioned that the ESBL accompanying OXA-1 was always CTX-M-15, and we cannot be certain that identical behaviour would be seen with other ESBLs. However, there is no obvious

reason why the ESBL type should affect the poor inhibition of OXA-1, and CTX-M-15 is considerably the commonest ESBL in the UK and worldwide.[29] In the absence of OXA-1, modal MICs of the penicillin/inhibitor combinations were consistent irrespective of whether CTX-M-15 or another ESBL was produced.

These findings have clear implications for penicillin/inhibitor combinations but not for newer cephalosporin/inhibitor combinations (e.g. ceftolozane/tazobactam and ceftazidime/avibactam), as these use cephalosporins that are stable to OXA-1 enzyme. Cefepime is somewhat labile to OXA-1,[31,32] but prospective cefepime/tazobactam combinations appear to retain near universal activity against ESBL producers, many of which likely also carry OXA-1.[33]

The therapeutic challenges posed by bacteria carrying OXA-1 enzyme together with CTX-M-15 are exacerbated by frequent co-carriage of *aac(6′)-Ib* [almost always as its *aac(6′)-Ib-cr* variant], conferring resistance to tobramycin. AAC(6′)-Ib also acetylates amikacin and, although MICs for producers commonly remained below the breakpoint, current EUCAST advice remains to avoid the drug wherever this enzyme is present.[11] Resistance rates to ciprofloxacin, sulphonamides, trimethoprim and tetracycline also were slightly higher among OXA-1-positive than OXA-1-negative ESBL producers though, unlike for tobramycin and the penicillin/inhibitor combinations, these resistance rates were high in both groups.

Co-carriage of $bla_{\text{OXA-1}}$ with $bla_{\text{CTX-M-15}}$ has been previously established in UK variants of *E. coli* ST131, where it was associated with IncF plasmids pEK499 (117 536 bp) and pEK516 (64 471 bp).[34,35] Plasmid pEK516 had $bla_{\text{OXA-1}}$ and $bla_{\text{CTX-M-15}}$ separated by a 7457 bp region that encoded *catB4*, *aac(3)-IIa* and tunicamycin resistance genes; *aac(6′)-1b-cr* was immediately upstream of $bla_{\text{OXA-1}}$ and a class 1 integron containing *dfrA17*, *aadA5* and *sul1* genes was present 1.7 kb upstream of $bla_{\text{CTX-M-15}}$. A similar organization is seen in the common Canadian $bla_{\text{CTX-M-15}}$ plasmid pC15-1a.[36] In the case of pEK499, which differed from pEK516 in having an IS*26*-mediated deletion of *aac(3)-IIa* and the tunicamycin resistance genes, $bla_{\text{OXA-1}}$ and $bla_{\text{CTX-M-15}}$ were only 4037 bp apart. Given their earlier prevalence, and the similarity of the present resistance profiles it seems likely that the same or very similar plasmids to pEK499 and pEK516 remain prevalent in bloodstream ST131 *E. coli* from the UK. This could not be definitively proven here because the presence of multiple copies of IS*26* precluded assembly from short-read sequencing data; nevertheless, we could confirm that $bla_{\text{OXA-1}}$, *aac(6′)-1b-cr* and the truncated *catB3* were demonstrably linked on the same ∼2–3 kb contig in at least 139 of the 149 isolates that had both $bla_{\text{OXA-1}}$ and $bla_{\text{CTX-M-15}}$.

In conclusion, these data suggest that the frequent question, 'Are penicillin/inhibitor combinations active against ESBL producers?' is misplaced. The more pertinent query is, 'Does my ESBL-producing isolate also have OXA-1 enzyme?' The findings have implications for diagnostic development. We have shown elsewhere that multiplex tandem PCR can be used to seek bacterial resistance genes in urine from urinary tract infection (UTI) patients, giving accurate results 24–48 h before susceptibility test data become available.[37] A panel that targeted *E. coli* generically, *E. coli* ST131 specifically, $bla_{\text{OXA-1}}$, $bla_{\text{CTX-M}}$, *aac(6′)-1b*, common gentamicin resistance determinants and the *gyrA* mutations responsible for fluoroquinolone resistance has the potential to provide a useful guide for the treatment of patients being admitted to hospital with upper UTIs and urosepsis. Detection of ST131 and the $bla_{\text{OXA-1}}$, $bla_{\text{CTX-M}}$, *aac(6′)-Ib-cr* trio should give a steer towards early carbapenem use in the severely ill patient, whilst the absence of $bla_{\text{OXA-1}}$ should increase the confidence with which penicillin/inhibitor combinations might be used.

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