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

David M LIVERMORE[1,2*], Michaela DAY[1], Paul CLEARY[3], Katie L HOPKINS[1], Mark A TOLEMAN[4], David W WAREHAM[5], Camilla WIUFF[6], Michel DOUMITH[1] and Neil WOODFORD[1]

1. *Antimicrobial Resistance and Healthcare-Associated Infections Reference Unit, PHE National Infection Service, London, United Kingdom*
2. *Norwich Medical School, University of East Anglia, Norwich, United Kingdom*
3. *PHE Field Service, Liverpool, United Kingdom*
4. *Cardiff University, Heath Park Campus, Cardiff, United Kingdom*
5. *Blizard Institute, Queen Mary University London, United Kingdom*
6. *Health Protection Scotland, Glasgow, United Kingdom*

**Running Head:** OXA-1 and penicillin/inhibitor resistance

***Corresponding author:**

Bob Champion Research & Educational Building,
James Watson Road,
University of East Anglia,
Norwich Research Park,
NORWICH, NR4 7UQ

Telephone: +44(0)1603 597568
E-mail: d.livermore@uea.ac.uk

**Abstract**

**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 hypothesised that this variability related to co-production of OXA-1 penicillinase. **Methods.** During a national study we collected 293 ESBL *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) and $\textit{bla}_{\text{CTX-M-15}}$ (present in 229 isolates, 78.2%); over half the isolates (159/293, 54.3%) were ST131 with $\textit{bla}_{\text{CTX-M-15}}$. $\textit{bla}_{\text{OXA-1}}$ was found in 149 ESBL producers (50.9%) and $\textit{bla}_{\text{TEM-1/191}}$ 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 $\textit{bla}_{\text{OXA-1}}$ and reduced susceptibility to penicillin/inhibitor combinations, whereas there was no significant association with co-carriage of $\textit{bla}_{\text{TEM-1/191}}$. For piperacillin/tazobactam the mode MIC rose from 2 mg/L in the absence of $\textit{bla}_{\text{OXA-1}}$ to 8-16 mg/L in its presence; for co-amoxiclav the shift was smaller, from 8 to 16 mg/L, but crossed the breakpoint. $\textit{bla}_{\text{OXA-1}}$ was strongly associated with co-carriage also of *aac(6')-Ib-cr,* which compromises amikacin and tobramycin. **Conclusion.** Co-carriage of OXA-1, a penicillinase with weak affinity for inhibitors, is a major arbiter 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 in-patient 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 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) hyper-production of target β–lactamases[18,19] and (iii) impermeability.[20] We explored the role of OXA-1 enzyme in a national collection of genomically-sequenced ESBL *E. coli* from bloodstream infections.

## Materials and Methods

### *Isolates*

Isolates were from human bloodstream infections and were collected in 2013-2014 during a national study comparing ESBL *E. coli* from human and non-human sources. Collecting sites in London (1 hospital), East Anglia (5 hospitals), Northwest England (2 hospitals), Wales (2 hospitals) and Scotland (2 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 sub-cultured to agar slopes and sent to PHE Colindale. On receipt, their identity was confirmed by MALDI-ToF (Bruker Daltonics, Bremen, Germany) and $bla_{\text{CTX-M}}$ genes were sought by PCR,[21] with isolates found positive accepted as ESBL producers. Isolates lacking $bla_{\text{CTX-M}}$ were screened for $bla_{\text{TEM}}$ and $bla_{\text{SHV}}$ 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 IsoSensitest agar (Oxoid).[24] Tazobactam was used at a fixed 4 mg/L and clavulanate at a fixed 2 mg/L, in keeping with current EUCAST guidance.

*WGS*

DNA libraries were prepared using the NexteraXT method and sequenced to >30X coverage with a standard 2x100 base 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 the 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 calling format (VCF) file generated by SAMtools mpileup.[27] This process was automated into the 'Genefinder' pipeline created by PHE Bioinformatics (M. Doumith, PHE, unpublished). 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 chi-square tests to assess significance of associations at 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 Northwest 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 >2 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*$_{CTX-M-27}$, 20 had *bla*$_{CTX-M-14}$, four had *bla*$_{CTX-M-1}$, three had *bla*$_{CTX-M-3}$ and one had *bla*$_{CTX-M-9}$. Three isolates had *bla*$_{SHV-12}$ and one had *bla*$_{SHV-31}$ both of which encode recognised SHV-ESBLs; one isolate, with an ESBL phenotype, solely had *bla*$_{TEM-117}$ and eight, all carrying other well-known ESBL determinants, also had *bla*$_{TEM-191}$, 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*$_{TEM-1}$ was present in 129/293 isolates (or 137/293 if those with TEM-191 were included, 46.8%) and *bla*$_{OXA-1}$ (or, in one case, a variant with a conservative Ile187Leu modification) was found in 149/293 (50.9%). *bla*$_{TEM-1}$

accompanied many different ESBL genes but *bla*$_{OXA-1}$ was always together with *bla*$_{CTX-M-15}$ along, in one isolate, with *bla*$_{CTX-M-14}$. Two isolates had acquired *bla*$_{CMY}$/*ampC* genes together with their ESBLs, and two had *bla*$_{OXA-9}$. Among the ST131 isolates, the great majority (159/188, 84.6%) had *bla*$_{CTX-M-15}$, though 24 had *bla*$_{CTX-M-27}$ and 5 had *bla*$_{CTX-M-14}$ alone or in combination; 116 had *bla*$_{OXA-1}$ whilst 76 had *bla*$_{TEM-1/191}$.

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 for piperacillin/tazobactam and amoxicillin/clavulanate.

Whenever *bla*$_{OXA-1}$ was present, alone or together with *bla*$_{TEM-1/191}$, the MIC distributions of penicillin/inhibitor combinations were raised, with the mode increasing from 2 mg/L to 8 or (depending on the particular sub-set) 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*$_{OXA-1}$-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*$_{TEM-1/191}$ whereas the proportion susceptible fell to 67/91 (73.6%) among those with an ESBL plus *bla*$_{OXA-1}$ and to 33/58 (56.9%) for those with an ESBL plus both *bla*$_{OXA-1}$ and *bla*$_{TEM-1/191}$. 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*$_{TEM-1/191}$ whilst these 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 both *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*$_{CMY}$ gene, 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-acetyl transferase 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*$_{\text{OXA-1}}$ also had *aac(6')-Ib-cr*, compared with 1/144 of those that lacked *bla*$_{\text{OXA-1}}$. Other resistance genes associated with *bla*$_{\text{OXA-1}}$ 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*$_{\text{OXA-1}}$ (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*$_{\text{OXA-1}}$. The association between *bla*$_{\text{OXA-1}}$ and *aac(6')-Ib-cr* remained clear when ST131 isolates were considered alone, but *aac(3')-IIa, aadA5, sul1*, *dfrA17* and *tet*(A) also were widespread among ST131 isolates with *bla*$_{\text{CTX-M-27}}$ alone or with *bla*$_{\text{CTX-M-15}}$ combined with either or both of *bla*$_{\text{TEM}}$ and/or *bla*$_{\text{OXA-1}}$. *strA/B* and *sul2* genes remained negatively associated with *bla*$_{\text{OXA-1}}$ 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 on 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*$_{\text{OXA-1}}$-positive compared with *bla*$_{\text{OXA-1}}$–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 bacteria, let alone using those characterised 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 *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 *E. coli* lacking OXA-1 enzyme were almost all clearly within the susceptible range for piperacillin/tazobactam, at around 2+4 mg/L, and narrowly within it for amoxicillin/clavulanate, clustering at 4-8 mg/L. A few individual isolates lay outside these generalisations, 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 for the inhibitor combinations and carriage of *bla*$_{OXA-1}$ were 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 carried 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, they 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 7,457-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*$_{\text{CTX-M-15}}$. Similar organisation 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 UTI patients, giving accurate results 24-48h 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*$_{OXA-1}$, *bla*$_{CTX-M}$, *aac(6')-Ib-cr* trio should give a steer towards early carbapenem use in the severely ill patient, whilst the absence of *bla*$_{OXA-1}$ should increase the confidence with which penicillin/inhibitor combinations might be used.

**Acknowledgements**

The research team would like to thank the following people for assisting in collection of isolates: Jo Seale, Lynette Phee and Benny Cherian (Barts Health NHS Trust); Mark Reacher and Iain Roddick (PHE East of England Field Epidemiology Services); Nicholas Brown and Estee Torok (Addenbrookes Hospital, Cambridge); Emma Meader (Norfolk & Norwich University Hospital); Sally Millership and Debbie Orriss (Princess Alexandra Hospital, Harlow); Richard Kent and Sara Ginwalla (Ipswich Hospital); Stephen Barrett, Marilyn Meyers, Charlotte Jude and Alison Westran (Southend Hospital); Charlotte Rawstrone, Malcolm Armstrong and Clare Langan (Manchester Royal Infirmary); Victoria Travis, Caroline Stubbs and Lorraine Bolton (Lancashire Teaching Hospitals NHS Trust); Julian Bendle and Julia Lewis (Royal Gwent Hospital) and Mandy Wootton (Public Health Wales); Brian Jones (Glasgow Royal Infirmary): Kristján Orr Helgason and Alan Gibb (Royal Infirmary Edinburgh).

**Funding.**

This paper is based on independent research commissioned and funded by the NIHR Policy Research Programme ("Defining Reservoirs of ESBL-Producing *E. coli* and the Threat Posed to Personal, Animal and Public Health in the UK"; Policy

Research Programme-funded Independent Research,' contract PRP 041/0039.) The views expressed in the publication are those of the authors and not necessarily those of the NHS, the NIHR, the Department of Health, 'Arms'-Length Bodies or other government departments.

**Transparency declaration**

**DML**: Advisory Boards or ad-hoc consultancy Accelerate, Achaogen, Adenium, Allecra, AstraZeneca, Basilea, BioVersys, Centauri, Integra-Holdings, Meiji, Melinta, Nordic, Pfizer, Roche, Shionogi, Taxis, T.A.Z., Tetraphase, VenatoRx, Wockhardt, Zambon, Zealand. Paid lectures – Astellas, bioMerieux, Beckman Coulter, Cardiome, Cepheid, Merck, Pfizer and Nordic. Relevant shareholdings and options – Dechra, GSK, Merck, Perkin Elmer, Pfizer and T.A.Z amounting to <10% of portfolio value. **DW:** Advisory Boards or ad-hoc consultancy for Pfizer, Merck and Shionogi. **All other authors:** none to declare. However, PHE's AMRHAI Reference Unit has received financial support for conference attendance, lectures, research projects or contracted evaluations from numerous sources, including: Accelerate Diagnostics, Achaogen Inc, Allecra Therapeutics, Amplex, AstraZeneca UK Ltd, AusDiagnostics, Basilea Pharmaceutica, Becton Dickinson Diagnostics, bioMérieux, Bio-Rad Laboratories, The BSAC, Cepheid, Check-Points B.V., Cubist Pharmaceuticals, Department of Health, Enigma Diagnostics, European Centre for Disease Prevention and Control, Food Standards Agency, GlaxoSmithKline Services Ltd, Helperby Therapeutics, Henry Stewart Talks, IHMA Ltd, Innovate UK, Kalidex Pharmaceuticals, Melinta Therapeutics, Merck Sharpe & Dohme Corp, Meiji Seika Pharma Co., Ltd, Mobidiag, Momentum Biosciences Ltd, Neem Biotech, NIHR, Nordic Pharma Ltd, Norgine Pharmaceuticals, Rempex Pharmaceuticals Ltd, Roche, Rokitan Ltd, Smith & Nephew UK Ltd, Shionogi & Co. Ltd, Trius Therapeutics, VenatoRx Pharmaceuticals, Wockhardt Ltd., and the World Health Organization.

## References


1. Cooke J, Stephens P, Ashiru-Oredope D *et al*. Longitudinal trends and cross-sectional analysis of English national hospital antibacterial use over 5 years (2008-13): working towards hospital prescribing quality measures. *J Antimicrob Chemother* 2015; **70:** 279-85.
2. Schuetz AN, Reyes S, Tamma PD. Point-Counterpoint: piperacillin-tazobactam should be used to treat infections with extended-spectrum-β–lactamase-positive organisms. *J Clin Microbiol* 2018; **56:** e01917-17.
3. Poirel L, Gniadkowski M, Nordmann P. Biochemical analysis of the ceftazidime-hydrolysing extended-spectrum β–lactamase CTX-M-15 and of its structurally related β–lactamase CTX-M-3. *J Antimicrob Chemother* 2002; **50:**1031-4.
4. Bush K, Macalintal C, Rasmussen BA *et al.* Kinetic interactions of tazobactam with β–lactamases from all major structural classes. *Antimicrob Agents Chemother* 1993; **37:** 851-8.
5. Drawz SM, Bonomo RA. Three decades of β–lactamase inhibitors. *Clin Microbiol Rev* 2010; **23:** 160-201.
6. Kalp M, Bethel CR, Bonomo RA *et al.* Why the extended-spectrum β–lactamases SHV-2 and SHV-5 are "hypersusceptible" to mechanism-based inhibitors. *Biochemistry* 2009; **48:** 9912-20.
7. Hoban DJ, Nicolle LE, Hawser S *et al.* Antimicrobial susceptibility of global inpatient urinary tract isolates of *Escherichia coli*: results from the Study for Monitoring Antimicrobial Resistance Trends (SMART) program: 2009-2010. *Diagn Microbiol Infect Dis* 2011; **70:** 507-11.
8. Lob SH, Hackel MA, Hoban DJ *et al*. Activity of ertapenem against Enterobacteriaceae in seven global regions-SMART 2012-2016. *Eur J Clin Microbiol Infect Dis* 2018; **37:** 1481-9.
9. Karlowsky JA, Hoban DJ, Hackel MA *et al.* Resistance among Gram-negative ESKAPE pathogens isolated from hospitalized patients with intra-abdominal and urinary tract infections in Latin American countries: SMART 2013-2015. *Braz J Infect Dis* 2017; **21:** 343-8

10. Tamma PD, Rodriguez-Bano J. The use of non-carbapenem β-lactams for the treatment of extended-spectrum β-lactamase infections. *Clin Infect Dis* 2017; **64:** 972-80.

11. Leclercq R, Cantón R, Brown DF *et al.* EUCAST expert rules in antimicrobial susceptibility testing. *Clin Microbiol Infect* 2013; **19:** 141-60.

12. Delgado-Valverde M, Torres E, Valiente-Mendez A et al. Impact of the MIC of piperacillin/tazobactam on the outcome for patients with bacteraemia due to Enterobacteriaceae: the bacteraemia-MIC project. *J Antimicrob Chemother* 2016; **71:** 521-30.

13. Retamar P, López-Cerero L, Muniain MA *et al.* Impact of the MIC of piperacillin-tazobactam on the outcome of patients with bacteremia due to extended-spectrum-β-lactamase-producing *Escherichia coli. Antimicrob Agents Chemother* 2013; **57:** 3402-4.

14. Harris PNA, Tambyah PA, Lye DC *et al.* Effect of piperacillin-tazobactam vs meropenem on 30-day mortality for patients with *E coli* or *Klebsiella pneumoniae* bloodstream infection and ceftriaxone Resistance: a randomized clinical Trial. J*AMA* 2018; **320:** 984-94.

15. Babini GS, Yuan M, Hall LM *et al.* Variable susceptibility to piperacillin/tazobactam amongst *Klebsiella* spp. with extended-spectrum β–lactamases. *J Antimicrob Chemother* 2003; **51:** 605-12.

16. Sugumar M, Kumar KM, Manoharan A *et al.* Detection of OXA-1 β-lactamase gene of *Klebsiella pneumoniae* from blood stream infections (BSI) by conventional PCR and in-silico analysis to understand the mechanism of OXA mediated resistance. *PLoS One* 2014; **9:** e91800.

17. Gatermann S, Marre R. Comparative in vitro activities of amoxicillin-clavulanate, ampicillin-sulbactam and piperacillin-tazobactam against strains of *Escherichia coli* and *Proteus mirabilis* harbouring known β–lactamases. Infection. 1991; **19:** 106-9.

18. French GL, Shannon KP, Simmons N. Hospital outbreak of *Klebsiella* pneumoniae resistant to broad-spectrum cephalosporins and β–lactam-β–lactamase inhibitor combinations by hyperproduction of SHV-5 β–lactamase. *J Clin Microbiol* 1996; **34:** 358-63

19. Livermore DM. Determinants of the activity of β–lactamase inhibitor combinations. *J Antimicrob Chemother* 1993; **31 Suppl A:** 9-21.

20. Rice LB, Carias LL, Hujer AM *et al*. High-level expression of chromosomally encoded SHV-1 β-lactamase and an outer membrane protein change confer resistance to ceftazidime and piperacillin-tazobactam in a clinical isolate of *Klebsiella pneumoniae*. *Antimicrob Agents Chemother* 2000; **44:** 362–7.

21. Woodford N, Fagan EJ, Ellington MJ. Multiplex PCR for rapid detection of genes encoding CTX-M extended-spectrum β–lactamases. *J Antimicrob Chemother* 2006; **57:** 154-5.

22. Fang H, Ataker F, Hedin G *et al.* Molecular epidemiology of extended-spectrum β–lactamases among *Escherichia coli* isolates collected in a Swedish hospital and its associated health care facilities from 2001 to 2006. *J Clin Microbiol* 2008; **46:** 707-12

23. Legrand P, Fournier G, Buré A et al. Detection of extended broad-spectrum β–lactamases in Enterobacteriaceae in four French hospitals. *Eur J Clin Microbiol Infect Dis* 1989; **8:** 527-9.

24. Anon. A guide to sensitivity testing. report of the Working Party on Antibiotic Sensitivity Testing of the British Society for Antimicrobial Chemotherapy. *J Antimicrob Chemother* 1991; **27 Suppl D:** 1-50.

25. VelvetOptimiser. http://bioinformatics.net.au/software.velvetoptimiser.shtml.

26. Tewolde R, Dallman T, Schaefer U *et al.* MOST: a modified MLST typing tool based on short read sequencing. *Peer J.* 2016; 4: e2308.

27. McArthur AG, Waglechner N, Nizam F *et al.* The comprehensive antibiotic resistance database. *Antimicrob Agents Chemother* 2013; **57:** 3348-57.

28. Nicolas-Chanoine MH, Bertrand X *et al. Escherichia coli* ST131, an intriguing clonal group. *Clin Microbiol Rev* 2014; **27:** 543-74.

29. Bevan ER, Jones AM, Hawkey PM. Global epidemiology of CTX-M β-lactamases: temporal and geographical shifts in genotype. *J Antimicrob Chemother* 2017; **72:** 2145-55.

30. Zeil C, Widmann M, Fademrecht S *et al.* Network analysis of sequence-function relationships and exploration of sequence space of TEM β-lactamases *Antimicrob Agents Chemother* 2016; **60:** 2709-17.

31. Aubert D, Poirel L, Chevalier J *et al.* Oxacillinase-mediated resistance to cefepime and susceptibility to ceftazidime in *Pseudomonas aeruginosa. Antimicrob Agents Chemother* 2001; **45:** 1615-20.

32. Torres E, López-Cerero L, Rodríguez-Martínez JM *et al.* Reduced susceptibility to cefepime in clinical isolates of Enterobacteriaceae producing OXA-1 β–lactamase. *Microb Drug Resist* 2016; **22:** 141-6.

33. Livermore DM, Mushtaq S, Warner M *et al.* Potential of high-dose cefepime/tazobactam against multiresistant Gram-negative pathogens. *J Antimicrob Chemother* 2018; **73:**126-33.

34. Woodford N, Ward ME, Kaufmann ME *et al.* Community and hospital spread of *Escherichia coli* producing CTX-M extended-spectrum β–lactamases in the UK. *J Antimicrob Chemother* 2004; **54:** 735-43.

35. Woodford N, Carattoli A, Karisik E *et al.* Complete nucleotide sequences of plasmids pEK204, pEK499, and pEK516, encoding CTX-M enzymes in three major *Escherichia coli* lineages from the United Kingdom, all belonging to the international O25:H4-ST131 clone. *Antimicrob Agents Chemother* 2009; **53:** 4472-82.

36. Boyd DA, Tyler S, Christianson S *et al.* Complete nucleotide sequence of a 92-kilobase plasmid harboring the CTX-M-15 extended-spectrum β–lactamase involved in an outbreak in long-term-care facilities in Toronto, Canada. *Antimicrob Agent Chemother* 2004; **48:** 3758-64.

37. Schmidt K, Stanley KK, Hale R *et al.* Evaluation of multiplex tandem PCR (MT-PCR) assays for the detection of bacterial resistance genes among Enterobacteriaceae in clinical urines. *J Antimicrob Chemother* 2018, in press.

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

| | No isolates with indicated MIC (mg/L) | | | | | | | | Total | % Susceptible at 8 mg/L |
|---|---|---|---|---|---|---|---|---|---|---|
| | **≤1** | **2** | **4** | **8** | **16** | **32** | **64** | **>64** | | |
| **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 | 100.0 |
| CTX-M-14 | | 3 | 2 | | | | | | 5 | 100.0 |
| 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 | 74.2 |
| CTX-M-15; CTX-M-3;OXA-1 | | | | 1 | | | | | 1 | - |
| CTX-M-15;CTX-M-14;OXA-1 | | | 1 | | | | | | 1 | - |
| **Total** | **0** | **8** | **25** | **34** | **13** | **5** | **2** | **2** | **91** | **73.6** |

**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 | - |

**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 | | | | 2 | - |
| 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 | 23.9 |
| 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** | **12** | **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% LCI | 95% UCI | p value | Relative risk of MIC > 8 mg/L | 95% LCI | 95% UCI | p value |
| All ESBL *E. coli* isolates | OXA-1[a] | 6.49 | 3.03 | 13.88 | <0.001 | 2.34 | 1.85 | 2.96 | <0.001 |
| | 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 *E. coli* isolates | OXA-1 | 12.10 | 3.01 | 48.61 | <0.001 | 2.43 | 1.73 | 3.41 | <0.001 |
| | 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) | | | |

LCI = lower confidence interval; UCI = upper confidence interval; p values shown are for chi-square 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

**Table 3.** Aminoglycoside and fluoroquinolone resistance among major ST131 groups

| | No 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-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% LCI | 95% UCI | p value | Relative risk of OXA-1 presence | 95% LCI | 95% UCI | p value |
| *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 |

LCI = lower confidence interval; UCI = upper confidence interval; p values shown for chi-square tests except where indicated.

'OXA-1' includes one isolate with an Ile187Leu sequence variant.