non-typeable plasmid, next to $bla_{CMY-2}$ on an 80 kb IncK plasmid and $bla_{CTX-M-1}$ on a 100 kb IncI1 plasmid. The IncI1 plasmid was further typed by plasmid multilocus sequence typing (pMLST) as sequence type (ST) 7.[4] The coexistence of *qnrS1* and $bla_{SHV-12}$ has been reported on IncN plasmids in *Klebsiella* isolates from Italy.[5] Nevertheless, to our knowledge, we describe the first *E. coli* isolate harbouring *qnrS1* and $bla_{SHV-12}$ on a single non-typeable 45 kb plasmid. The presence of $bla_{CMY-2}$ on an IncK plasmid and $bla_{CTX-M-1}$ on an IncI1 plasmid was previously identified in *E. coli* isolates from Dutch broiler chickens.[2] Moreover, IncI1 plasmids of ST7 harbouring $bla_{CTX-M-1}$ are frequently detected amongst ESBL-producing *E. coli* from Dutch broiler chickens (pMLST databases: http://pubmlst.org/plasmid/). Yet, we report the first coexistence of $bla_{CTX-M-1}$, $bla_{SHV-12}$ and $bla_{CMY-2}$ genes next to *qnrS1* in an *E. coli* isolated from animals. In the *E. coli* isolate from a veal calf (no. 77.01), *qnrS1* was located on an IncX2 plasmid, which has recently been described in *E. coli* from healthy animals in Nigeria.[6] In the *E. coli* isolate from a broiler chicken (no. 74.21), *qnrB19* was also identified on an InX2 plasmid. The presence of *qnrB19* has been reported in *E. coli* isolated from animals on ColE,[6] IncN[7] and IncR[8] plasmids, but not on IncX2.

Our results demonstrate the presence of *qnr* genes on two different types of plasmids in *E. coli* isolated from animals. These findings indicate the emergence of PMQR genes in the commensal flora of food-producing animals in the Netherlands. The remarkable finding of the coexistence of three different cephalosporinase genes on three different plasmids in a single *E. coli* isolate demonstrates the complexity of the plasmid-mediated dissemination of β-lactamase and PMQR genes in Enterobacteriaceae.

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## Transparency declarations

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# Characteristics of CTX-M ESBL-producing *Escherichia coli* isolates from the Lao People's Democratic Republic, 2004–09

**Nicole Stoesser[1–4]*, Derrick W. Crook[3,4], Catrin E. Moore[1,2], Rattanaphone Phetsouvanh[1,2], Vilada Chansamouth[1], Paul N. Newton[1–3] and Nicola Jones[1,3]**

*[1]Wellcome Trust–Mahosot Hospital–Oxford Tropical Medicine Research Collaboration, Mahosot Hospital, Vientiane, Lao People's Democratic Republic; [2]Centre for Clinical Vaccinology and Tropical Medicine, Churchill Hospital, Oxford, UK; [3]Nuffield Department of Clinical Medicine, Oxford University, John Radcliffe Hospital, Oxford, UK; [4]National Institute for Health Research, Oxford Biomedical Research Centre Programme, John Radcliffe Hospital, Oxford, UK*

*Corresponding author. Tel: +44-1865-221226; Fax: +44-1865-764192; E-mail: nicole.stoesser@ndm.ox.ac.uk

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Sir,

Antimicrobial resistance in common Gram-negative organisms such as *Escherichia coli* is a major threat to global health, and particularly relevant to clinical management in resource-poor settings, where access to appropriate antibiotics may be limited by cost. An example of this is the Lao Peoples' Democratic Republic (Laos), which has some of the poorest health indicators in the south-east Asia region,[1] and borders several countries reporting high rates of CTX-M-mediated extended-spectrum β-lactamase (ESBL) and multidrug resistance in *E. coli*.[2] We used a combination of genotypic and

phenotypic testing to characterize ESBL-producing *E. coli* isolated at the Mahosot Hospital in Vientiane, April 2004–09.

Bloodstream *E. coli* isolates at the Mahosot Hospital have been routinely tested for ESBL production since 2000; urine and pus isolates have been tested since 2006. Speciation of isolates was done using the API-20E or mini-API (bioMérieux, France). Screening and confirmatory testing of isolates for ESBL production and additional antibiotic susceptibility testing for the study were carried out in accordance with published guidelines (CLSI and BSAC methodologies). Additional antibiotics tested included ciprofloxacin, gentamicin, trimethoprim, nitrofurantoin, meropenem and amikacin. DNA was prepared from boiled cell suspensions and subjected to PCR analysis for $bla_{CTX\text{-}M}$.[3] The resulting 504 bp amplicon (of the 876 bp $bla_{CTX\text{-}M}$ gene) was sequenced and genetic homologues identified by querying the National Centre for Biotechnology Information (NCBI) nucleotide database. Multilocus sequence typing of *E. coli* was carried out in accordance with an established scheme (http://mlst.ucc.ie/). Statistical analyses were undertaken with Stata/SE 11.1 software. Ethical approval was granted by the National Ethical Committee for Health Research, Government of the Lao PDR (Laos) and the Oxford Tropical Research Ethics Committee (UK).

Fifty-four ESBL-producing *E. coli* were identified during the study period from blood ($n$=18/197; 9%), urine ($n$=23/354; 6%) and pus ($n$=11/76; 14%) samples culturing *E. coli*, consistent with the general epidemiology of extra-intestinal pathogenic *E. coli* (ExPEC) infections. For two samples the source was not confirmed. All ESBL-producing *E. coli* isolates harboured $bla_{CTX\text{-}M}$, the invariable presence of which is similar to other molecular epidemiological studies carried out in Asia.[4] There was an increase in the proportion of all microbiological specimens culturing *E. coli* during the study period (2.9% to 4.5%; Fisher's exact test, $P$=0.02), and the proportion of ESBL-producing *E. coli* more than tripled since their first isolation in 2004 (3.9% to 13.3%; Fisher's exact test, $P$=0.04). While a survey in only one hospital represents a singular snapshot of the overall epidemiology, this study suggests the expansion of CTX-M ESBLs in *E. coli* in Vientiane occurred relatively late, given that the CTX-M gene was first identified in 1991 and high rates of ESBL-producing *E. coli* were reported in Asia as early as 1998–2002.

Considerable multidrug resistance was found amongst ESBL-producing *E. coli* isolates, with 66% displaying resistance to a further three classes of antibiotic (ciprofloxacin, trimethoprim and gentamicin). The rate of ciprofloxacin resistance (91%) was substantially higher than that in the 2008 Study for Monitoring Antimicrobial Resistance Trends (SMART) survey of ESBL-producing Enterobacteriaceae isolates in the Asia-Pacific region (64%),[2] and showed no association with year of isolation, with ciprofloxacin resistance being the norm in ESBL-producing *E. coli* in Laos since 2004. No carbapenem resistance was found in this survey; only one isolate was resistant to amikacin.

CTX-M-14-like enzymes were most common [including CTX-M-14/18, -17, -21, -24, -46, -47, -48, -49, -50, -83 and -104; $n$=22 (41%)], with CTX-M-15-like [including CTX-M-28, -82 and -88; $n$=15 (28%)], CTX-M-27 [$n$=12 (22%)] and CTX-M-55-like [including CTX-M-57, -69 and -79; $n$=5 (9%)] variants being identified in descending order of frequency. This mimics to some degree the distribution seen in Thailand and China, where the appearance of ESBLs in *E. coli* pre-dates that seen in this study in Laos, suggesting plausible transmission networks between these countries sharing land borders.

**Table 1.** Number of ESBL-producing *E. coli* isolates by ST per year; annual periods run from 1 April of one year to 31 March of the following year

| ST | 2004–05 | 2005–06 | 2006–07 | 2007–08 | 2008–09 | Total |
|---|---|---|---|---|---|---|
| 12 | | | | 1 | | 1 |
| 38 | | 1 | | | 1 | 2 |
| 69 | | | | 2 | | 2 |
| 88 | | | 1 | | | 1 |
| 95 | | 1 | | | | 1 |
| 101 | | | | | 1 | 1 |
| 131 | | 1 | 4 | 4 | 8 | 17 |
| 167 | | | | 2 | 2 | 4 |
| 209 | | | | | 1 | 1 |
| 354 | 1 | | | 3 | 1 | 5 |
| 405 | 1 | | 1 | 2 | | 4 |
| 410 | | | | | 3 | 3 |
| 648 | | 1 | | 2 | 7 | 10 |
| 744 | | | | | 1 | 1 |
| 1340 | | | | | 1 | 1 |
| Total | 2 | 4 | 6 | 16 | 26 | 54 |

Fifteen different sequence types (STs) were identified among the ESBL-producing *E. coli* isolates (Table 1). While a pandemic global lineage, ST-131, was the most frequently identified ST ($n$=17/54; 31%), of particular interest was the finding that ST-648 was the second most common ($n$=10/54; 19%). ESBL-producing ST-648 has been identified to date in only a handful of human clinical isolates, wild birds and poultry,[5] suggesting the potential for zoonotic transmission. Poultry farming is common in Laos, with 95% being of the 'backyard', small-holding variety.[6] A further bird-associated strain (ST-1340), which has not been found in human clinical samples before, was also found in this study. ST-648 was significantly associated with CTX-M-15-like enzymes (Fisher's exact test, $P$<0.0001).

This study describes the emergence and expansion since 2004 of ESBL-producing *E. coli* in Vientiane, Laos, and the invariable presence of the CTX-M gene. Local surveillance has the capacity to demonstrate discrete features of ESBL-producing *E. coli* molecular epidemiology. The diverse range of host bacterial genotypes and CTX-M variants identified in this study support the notion that higher-resolution approaches, such as those afforded by whole genome sequencing technology, are required to gain a thorough understanding of the epidemiology of this resistance problem.

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## Transparency declarations

None to declare.

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# Importation of KPC-2-producing *Escherichia coli* from India

**Anaïs Potron[1], Laurent Poirel[1], Delphine Verdavaine[2] and Patrice Nordmann[1]***

[1]*Service de Bactériologie-Virologie, INSERM U914 'Emerging Resistance to Antibiotics', Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, 78 rue de Général Leclerc, K.-Bicêtre, France;* [2]*Centre Hospitalier 'René Pleven', Dinan, France*

*Corresponding author. Tel: +33-1-45-21-36-32; Fax: +33-1-45-21-63-40; E-mail: nordmann.patrice@bct.aphp.fr

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Sir,

The production of carbapenem-hydrolysing β-lactamases is increasingly reported in Enterobacteriaceae. Among the different types of carbapenemases, the emergence of the Ambler class A KPC-type β-lactamases is of great concern, since those enzymes hydrolyse all β-lactams with the exception of cephamycins. Enterobacterial isolates producing KPC-type β-lactamases were reported in many areas in the USA and subsequently worldwide.[1] The rapid dissemination of KPC enzymes among different enterobacterial species is related to the localization of $bla_{KPC}$ genes on transferable broad host range plasmids and their association with a transposon.[1] This dissemination has also been linked with a 'successful' international clone of KPC-producing *Klebsiella pneumoniae* of sequence type (ST) 258.[2]

Early in 2011, a middle-aged patient was transferred from a hospital in Mumbai, India, to the hospital of Dinan, France. The patient suffered from pleurisy due to *Streptococcus pneumoniae* for which he had received a combination of imipenem, vancomycin and piperacillin/tazobactam in India. Upon admission, a rectal swab revealed the presence of a multidrug-resistant *Escherichia coli* (designated strain GRU) with reduced susceptibility to carbapenems. No secondary local transmission occurred at the Dinan hospital following the rapid implementation of strict infection control measures.

The antibiogram determined by the disc diffusion method and MICs determined by Etest (AB bioMérieux, Solna, Sweden) and interpreted according to the CLSI guidelines[3] revealed that *E. coli* strain GRU was resistant to all penicillins and expanded-spectrum cephalosporins, to ertapenem (MIC >32 mg/L) and to meropenem (MIC 8 mg/L) and was of intermediate susceptibility to imipenem (MIC 1.5 mg/L). The isolate was susceptible to tetracycline and fosfomycin, and MICs of tigecycline and colistin were 1 and 0.5 mg/L, respectively. However, it was resistant at a high level to all fluoroquinolones (MICs >256 mg/L). Molecular investigations performed as described previously[1] identified the $bla_{KPC-2}$ gene. Isolate GRU also harboured the $bla_{TEM-1}$ and $bla_{OXA-1}$ genes. Plasmid location of the $bla_{KPC-2}$ gene was confirmed by electroporation of a plasmid DNA preparation obtained by the Kieser method into *E. coli* TOP10 with selection on Trypticase soy plates containing ampicillin (100 mg/L).[1] Molecular and phenotypic analysis of the *E. coli* transformant confirmed that $bla_{KPC-2}$ was located on an ~20 kb plasmid. The $bla_{KPC-2}$-positive plasmid was non-typeable using PCR-based replicon typing.[4] No other antibiotic resistance marker was co-transferred. PCR mapping performed as described[1] showed that the $bla_{KPC-2}$ gene was part of the Tn*4401* transposon. It is noteworthy that *E. coli* GRU additionally harboured a gene encoding the 16S rRNA methylase ArmA, conferring high-level resistance to all aminoglycosides (MICs of gentamicin, netilmicin, kanamycin and tobramycin >256 mg/L). Interestingly, KPC-2- and ArmA-producing *Enterobacter cloacae* and *K. pneumoniae* isolates have been reported in China and Poland.[5,6] Multilocus sequence typing (MLST) performed according to the protocol described on the *E. coli* MLST web site (http://www.pasteur.fr/recherche/genopole/PF8/mlst/EColi.html) showed that *E. coli* GRU belonged to ST101, recently reported to be the most frequent NDM-1-producing *E. coli* clone in the UK and Pakistan.[7] That study reported a KPC-producing *E. coli* originating from India. It remains to be determined to what extent the spread of KPC-type enzymes will contribute to the problem of carbapenem resistance in India, which currently is commonly regarded as reflecting the dissemination of the NDM-1 carbapenemase.[8]

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