non-typeable plasmid, next to blaCTX-M-1 on a 100 kb IncI1 plasmid and
blaCTX-M-1 on a 80 kb IncI1 plasmid. The IncI1 plasmid was
further typed by plasmid multilocus sequence typing (pMLST) as sequence
type (ST) 7. The coexistence of qnrS1 and blaSHV-12 has been reported on
IncN plasmids in Klebsiella isolates from Italy. Nevertheless, to our knowledge, we describe the first E. coli isolate harbouiring qnrS1 and blaSHV-12 on a single non-typeable
45 kb plasmid. The presence of blaCTX-M-1 on an IncI1 plasmid and
blaCTX-M-1 on an IncI1 plasmid was previously identified in E. coli isolates from Dutch broiler chickens. Moreover, IncI1 plasmids of
ST7 harbouring blaCTX-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 blaCTX-M-1, blaSHV-12 and blaCMY-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. In the E. coli isolate from a broiler chicken (no. 74.21), qnrB19 was also identified on an IncX2 plasmid. The presence of qnrB19 has been reported in E. coli isolated from animals on CoE, IncN6 and IncR8 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

None to declare.

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PCR analysis for DNA was prepared from boiled cell suspensions and subjected to microcin, trimethoprim, nitrofurantoin, meropenem and amikacin. According to published guidelines (CLSI and BSAC methods), antibiotic susceptibility testing for the study was 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-M}. The resulting 504 bp amplicon (of the 876 bp bla_{CTX-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-M}, the invariable presence of which is similar to other molecular epidemiological studies carried out in Asia. 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%), 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, -59 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       |         |         |         | 2     |
| 38        | 1       |         | 1       | 1       |         | 3     |
| 69        |         |         | 2       | 2       |         | 4     |
| 88        |         |         | 1       |         | 1       | 2     |
| 95        |         |         |         | 1       |         | 1     |
| 101       |         |         |         | 1       | 1       | 2     |
| 131       | 1       | 4       | 4       | 8       | 17      | 33    |
| 167       |         |         |         | 2       |         | 4     |
| 209       |         |         |         | 1       | 1       | 2     |
| 354       | 1       |         | 3       | 1       | 5       | 10    |
| 405       | 1       |         | 1       | 2       | 4       | 8     |
| 410       |         |         |         |         | 3       | 3     |
| 668       | 1       |         | 2       | 7       | 10      | 20    |
| 744       |         |         |         | 1       | 1       | 2     |
| 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, suggesting the potential for zoonotic transmission. Poultry farming is common in Laos, with 95% being of the ‘backyard’, smallholding variety. 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
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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 blaKPC genes on transferable broad host range plasmids and their association with a transposon.3 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 guidelines3 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 previously1 identified the blaKPC-2 gene. Isolate GRU also harboured the blaTEM-1 and blaOXA-1 genes. Plasmid location of the blaKPC-2 gene was confirmed by electrophoresis 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 blaKPC-2 was located on an ~20 kb plasmid. The blaKPC-2-positive plasmid was non-typeable using PCR-based replicon typing.4 No other antibiotic resistance marker was co-transferred. PCR mapping performed as described1 showed that the blaKPC-2 gene was part of the Tn4401 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/PPB/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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