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

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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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