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Molecular characterization of carbapenem-resistant Escherichia coli and Acinetobacter baumannii in the Lao People's Democratic Republic

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Sir,

Global dissemination of carbapenemases among Gram-negative bacteria is a growing public health concern. Therapeutic options for these organisms are often limited, with alternative agents such as tigecycline and colistin having potentially less favourable efficacy and toxicity profiles.1,2 Furthermore, these agents are expensive and not readily available in resource-constrained settings. In the Lao People's Democratic Republic (Laos), carbapenemases are not yet on the national list of essential drugs, although in Vientiane the high prevalence of ESBL-producing Enterobacterales3,4 has yet to be addressed. In Vientiane, the Lao People's Democratic Republic (Laos), carbapenems are not widely available, and not readily available in resource-constrained settings. In resource-constrained settings, the high prevalence of ESBL-producing Enterobacterales has yet to be addressed. In Vientiane, the Lao People's Democratic Republic (Laos), carbapenems are not widely available, and not readily available in resource-constrained settings.

The Microbiology Laboratory, Mahosot Hospital, Vientiane, Laos, receives clinical samples from several hospitals in Vientiane and other provinces, undertakes AST using CLSI methodology and participates in the UK National External Quality Assessment (UK NEQAS) scheme for AST. Since 2010, isolates of Enterobacterales and Acinetobacter spp. displaying resistance to three or more first-line agents have been routinely tested against meropenem using 10 μg discs (Oxoid, Basingstoke, UK). In 2017, 280/428 Escherichia coli, 67/208 Klebsiella pneumoniae and 35/111 Acinetobacter spp. isolates underwent meropenem susceptibility testing. The first carbapenem-resistant E. coli was identified in 2015. A second isolate (Patient 2) was sent to the Oxford Genomics Centre (University of Oxford, Oxford, UK), where WGS using the Illumina HiSeq 250 platform identified it as E. coli ST410 carrying blaNDM-5, prompting the current review.

Laboratory records were retrospectively reviewed for meropenem- or imipenem-resistant Enterobacteriales (from 1 January 2010 to 31 December 2017) and Acinetobacter spp. (from 1 January to 31 December 2017). All CRE and CRAB were retrieved from storage at −80 °C and their identity was confirmed using API 20E Enterobacterales and API 20NE for Acinetobacter spp. (bioMérieux, Basingstoke, UK). Phenotypic susceptibilities were confirmed by disc diffusion according to CLSI 2018 standards and breakpoints,3 and the modified carbapenem inactivation method (mCIM)6 was used to detect carbapenemase production. Clinical and demographic data were obtained from review of hospital charts. The isolates were sent for further characterization at the Centre for Infections and Global Health, Nuffield Department of Medicine, University of Oxford, Oxford, UK, to determine antimicrobial susceptibility testing (AST) and surveillance networks are not well established.

Four CRE isolates, all E. coli, were identified from four patients from two hospitals in Vientiane (1 from 2015, 1 from 2016 and 2 from 2017) (Table 1). Two were isolated from urine, one from a wound swab, and one from a blood culture. All were resistant to all ß-lactams tested as well as to ciprofloxacin, gentamicin, co-trimoxazole and tetracycline. Three isolates were susceptible to amikacin, and both urinary isolates were also susceptible to fosfomycin and nitrofurantoin, but the bloodstream isolate was only susceptible to doxycycline and nitrofurantoin. None of the patients had documented prior exposure to carbapenems. The mCIM test confirmed carbapenemase production and molecular analysis identified a New Delhi MBL (NDM) gene in all four isolates. NDM subtype was not determined.

Meropenem resistance was confirmed in 22 non-duplicate Acinetobacter spp. isolates in 2017, all of which contained blaOXA-51-like genes intrinsic to A. baumannii (Table 51, available as Supplementary data at JAC Online). All isolates were also resistant to ceftriaxone, ceftazidime, imipenem, ciprofloxacin and tetracycline.
Eighteen (81.8%) were susceptible to amikacin. Two isolates were not susceptible to any agents tested. All CRAB produced the OXA-23-like carbapenemase, with two additionally carrying an NDM carbapenemase gene. Most CRAB (19/22) were from endotracheal aspirates from the adult ICU at Mahosot Hospital, but, as isolates were not further characterized, we could not determine whether this reflected cross-infection in the ICU or the emergence of multiple independent strains. Although colistin susceptibility was not tested phenotypically, mcr-1/-2 genes were not detected in either species.

To the best of our knowledge, this is the first report of carbapenemase-producing E. coli and A. baumannii in Laos. While our results are from a single laboratory and therefore may not be representative of the epidemiology of carbapenem resistance nationally, Mahosot Hospital is a tertiary referral centre from other provinces and the Microbiology Laboratory also receives specimens from hospitals in several provinces, comprising an informal surveillance network. Molecular findings are consistent with reports from Thailand and Vietnam, where carbapenem resistance in A. baumannii and Enterobacterales appears to be predominantly related to OXA-23-like carbapenemases and NDM carbapenemases, respectively.\(^5\)

In summary, this study demonstrates the presence of OXA-23-like and NDM carbapenemases in Laos. Given the increasing use of carbapenems, lack of established infection control protocols, and limited access to alternative therapeutic agents in Laos, this is of grave concern. Efforts to prevent further dissemination of these organisms in Laos must be prioritized.

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Transparency declarations
None to declare.

Supplementary data
Table S1 is available as Supplementary data at JAC Online.

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### Table 1. Clinical and microbiological details of four carbapenem-resistant E. coli isolated at Mahosot Microbiology Laboratory

| Parameter                          | Patient 1              | Patient 2              | Patient 3              | Patient 4              |
|-----------------------------------|------------------------|------------------------|------------------------|------------------------|
| Age (years)                       | 33                     | 50                     | 55                     | 56                     |
| Sex                               | female                 | male                   | male                   | female                 |
| Specimen                          | abdominal wound swab   | urine                  | blood culture          | urine                  |
| Ward                              | August 2015            | September 2016         | July 2017              | July 2017              |
| Reason for admission/clinical syndrome | wound infection and liver abscess post-cholecystectomy | urology right perinephric abscess post-renal tract surgery in Savannakhet province for calculi | urology biliary sepsis, underlying cholangiocarcinoma | urology pyelonephritis associated with ureteric calculus |
| Phenotypic AST results susceptible intermediate resistant | AMK, CHL, FOF\(^a\) NIT\(^b\) | AMK, FOF, NIT | DOX, NIT\(^a\) | AMK, FOF, NIT |
| Acquired carbapenemase genes detected | bl\(\text{a}_{\text{NDM}}\) | bl\(\text{a}_{\text{NDM}}\) | bl\(\text{a}_{\text{NDM}}\) | bl\(\text{a}_{\text{NDM}}\) |
| Carbapenem exposure prior to specimen collection | no                     | no                     | no                     | no                     |
| Status at discharge               | alive, well            | alive, well            | alive, re-admitted August 2017 with recurrent fever | moribund |

AMK, amikacin; AMC, amoxicillin/clavulanate; AMP, ampicillin; CAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; GEN, gentamicin; CPD, cepodoxime; CRO, ceftriaxone; DOX, doxycycline; NIT, nitrofurantoin; FOF, fosfomycin; IPM, imipenem; MEM, meropenem; SXT, trimethoprim/sulfamethoxazole; TET, tetracycline.

\(^a\)Zone diameter interpreted according to CLSI criteria for urinary isolates.

\(^b\)Previously confirmed as bl\(\text{a}_{\text{NDM-5}}\) by WGS.
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