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Trimethoprim/sulfamethoxazole resistance in *Burkholderia pseudomallei*

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Sir,

With the recent publication of the MERTH study [1], trimethoprim/sulfamethoxazole (SXT) monotherapy will be used increasingly in the treatment of melioidosis during the eradication phase, and possibly as the only treatment for some mild infections. It is therefore important to know the prevalence of SXT resistance in *Burkholderia pseudomallei*. This is difficult to test in vitro, with disc diffusion testing overestimating resistance, and indistinct endpoints in all methods [2]. Even using the Etest method to estimate the minimum inhibitory concentration (MIC), SXT resistance rates as high as 24% in a year have occasionally been reported from Thailand [3]. Recent data from Northern Australia published in this journal [4] are consistent with our own experience in Southeast Asia and suggest that true resistance to SXT in *B. pseudomallei* is actually very rare.

Table 1
Source of isolates.

	Laos PDR	Cambodia
Blood culture	240	31
Respiratory	126	2
Pus/swab	228	115
Fluid	26	1
Total	620	149

As our own experience indicated a substantially lower prevalence of SXT resistance than the literature suggests, we reviewed routine data from our diagnostic laboratories in Laos (February 2003 to October 2012) and Cambodia (February 2006 to December 2012). Etest (bioMérieux, Basingstoke, UK) was performed according to the manufacturer's instructions on all isolates in Vientiane (Laos) and on all isolates that appeared non-susceptible by disc diffusion testing [Clinical and Laboratory Standards Institute (CLSI) M02-A11 method; zone diameter < 16 mm] in Siem Reap (Cambodia), as previously recommended [3]. The sources of the strains tested are shown in Table 1.

Of 769 sequential isolates, 99.2% (615/620) from Laos and 100% (149/149) from Cambodia were classified as susceptible to SXT according to CLSI criteria (trimethoprim MIC ≤ 2 mg/L). Three isolates had a trimethoprim MIC of 3 mg/L and two isolates had a trimethoprim MIC of 4 mg/L. The range of MICs is shown in Fig. 1.

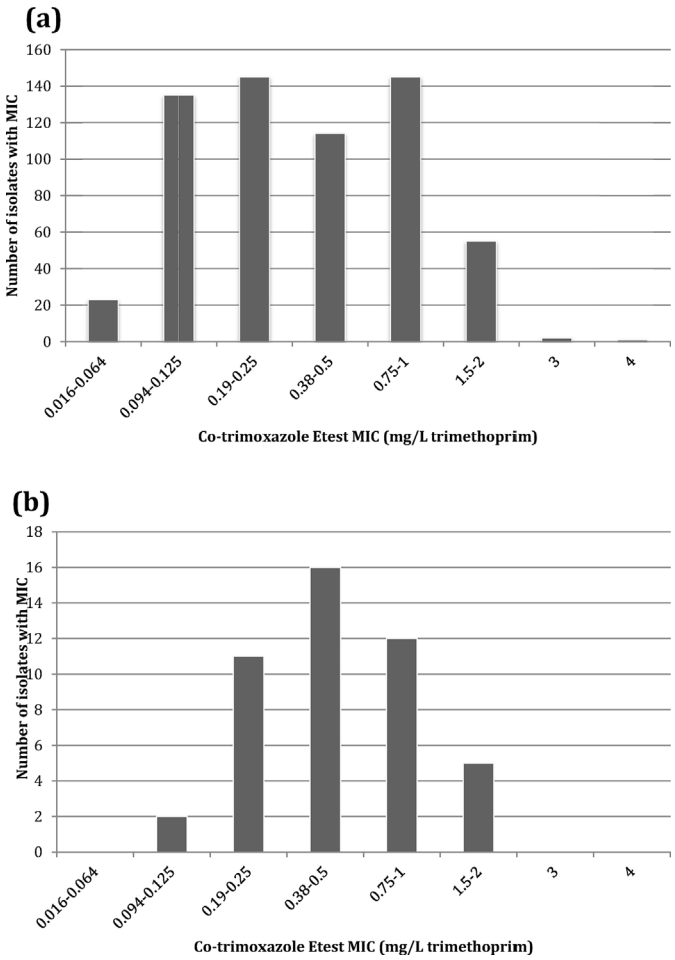

Fig. 1. Distribution of trimethoprim/sulfamethoxazole (co-trimoxazole) Etest minimum inhibitory concentrations (MICs) of clinical isolates of *Burkholderia pseudomallei* from (a) Laos and (b) Cambodia.

This confirms that primary resistance of *B. pseudomallei* to SXT is extremely uncommon and should rarely be a contraindication to SXT monotherapy. These results from Vientiane and Siem Reap closely mirror those of Crowe et al. in Darwin [4]. Although the CLSI currently only recommends broth microdilution testing for *B. pseudomallei* [5], many years of experience in melioidosis-endemic areas suggests that disc diffusion testing is reliable for all agents except SXT, for which Etest gives acceptable results. We think that the misleading data in the literature are due to the difficulty of interpreting endpoints in testing susceptibility of *B. pseudomallei* to SXT. We tried to follow the manufacturer's instructions and read the Etest at 80% inhibition, but this is a somewhat subjective endpoint. However, the really important thing that remains to be established is whether SXT MICs can predict the outcome of SXT monotherapy in melioidosis.

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Carbapenem-resistant *Enterobacter gergoviae* harbouring bla_{KPC-2} in Brazil

Sir,

Worldwide, *Klebsiella pneumoniae* carbapenemase (KPC) detection has been extensively reported every year, and the detection of new species capable of producing this enzyme is of great concern. KPC production is currently the main mechanism of resistance to carbapenems in Enterobacteriaceae strains [1]. In Brazil, reports of new species carrying the bla_{KPC} gene have been made in different regions [2]. However, there are rare reports of KPC-producing *Enterobacter gergoviae* strains; as far as we are aware, there are only two reports, both in the USA [3,4].

During routine bacteriological diagnosis in a public teaching hospital in Recife (Brazil), a bacterial isolate identified as *Enterobacter* spp. (named EG2) was selected. This isolate was derived from blood of a patient hospitalised in the intensive care unit (ICU). The patient was hospitalised in February 2008 with hepatic failure. During their hospital stay, the patient received piperacillin/tazobactam (TZP) [500 mg every 6 h (q6 h)] empirically for 2 days. After that, therapy was changed to imipenem (250 mg q6 h). Ten days later the patient died. On the same day, blood samples showed a carbapenem-resistant *Enterobacter* spp. isolate. Confirmation of the isolate identity by 16S ribosomal DNA sequencing revealed an *E. gergoviae* isolate. This isolate was used for further analyses.

Minimum inhibitory concentrations (MICs) were determined by broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) 2013 guidelines (M100-S23). Molecular analyses were performed through sequencing after specific PCR for bla_{KPC}, for the most common β-lactamase genes (bla_{CTX-M}, bla_{TEM} and bla_{SHV}) and the class 1 integron variable region. Furthermore, the genetic environment of the bla_{KPC-2} gene was analysed by specific PCR for the region between IS*Kpn*7 and bla_{KPC}, according to Cuzon et al. [1].

Plasmid DNA was obtained by a standard protocol and was used to transform *Escherichia coli* DH5α chemocompetent cells. Transformant cells were selected on Mueller–Hinton agar containing 2 μg/mL imipenem and 50 μg/mL ticarcillin. Plasmid typing was performed by PCR-based replicon typing (PBRT) as previously described by Carattoli et al. [5].

Results of susceptibility assays showed that isolate EG2 was multidrug-resistant (Table 1). Isolate EG2 harboured the bla_{KPC-2}, $bla_{CTX-M-15}$, bla_{SHV-5} and bla_{TEM-1} genes (Table 1). Moreover, the *dfrA22* gene, which confers resistance to trimethoprim, was present in the variable region of a class 1 integron. Plasmid analysis showed that EG2 had 133, 65, 25 and 2 kb plasmids. The presence of bla_{KPC-2}, $bla_{CTX-M-15}$ and bla_{SHV-5} genes was confirmed by PCR in transformant recipient cells, named T-EG2. Analysis of the plasmid profile from donor and recipient cells showed that they shared only a