Table 1
Minimum inhibitory concentrations (MICs) of fosfomycin and various carbapenems, duration of the post-antibiotic effect (PAE), frequency of mutation, results of synergy testing, and frequency of various resistance mechanisms for fosfomycin and carbapenems alone and in combination for 70 clinical carbapenem-resistant *Pseudomonas aeruginosa* (CR-PA).

| Antibiotic(s) | MIC (μg/mL) | PAE (h) | Mutation frequency | Percentage of isolates showing synergy (n) | Percentage of isolates positive for carbapenem resistance mechanism (n) |
|--------------|-------------|---------|--------------------|------------------------------------------|--------------------------------------------------|
| FOS          | 128         | 1       | TNTIC              | 3.1 × 10−9                               | 2.1 × 10−10                                      |
|              |             |         |                    |                                          | 2.7 × 10−11                                      |
| Carbenpenems |             |         |                    |                                          | 3.2 × 10−9                                      |
| MEM          | 4           | 2       | <2.9 × 10−9        | 7.3 × 10−9                               | 3.3 × 10−9                                      |
|              |             |         |                    |                                          | <1.4 × 10−9                                      |
| IPM          | 2           | 2       | <2.9 × 10−9        | TNTIC                                    | TNTIC                                            |
|              |             |         |                    |                                          | <1.4 × 10−9                                      |
|              |             |         |                    |                                          | 5.2 × 10−9                                      |
| DOR          | 2           | 2       | <2.9 × 10−9        | 7.3 × 10−9                               | 2.4 × 10−9                                      |
|              |             |         |                    |                                          | <1.4 × 10−9                                      |
|              |             |         |                    |                                          | 5.2 × 10−9                                      |
| Combinations |             |         |                    |                                          |                                                  |
| FOS+MEM      | 32/0.5      | 2       | 6.8 × 10−9        | 4.9 × 10−9                               | 3.1 × 10−9                                      |
|              |             |         |                    |                                          | 3.6 × 10−9                                      |
| FOS+IPM      | 16/0.5      | 2       | 7.2 × 10−9        | <2.9 × 10−9                              | 7.9 × 10−9                                      |
|              |             |         |                    |                                          | 10.9 × 10−9                                     |
| FOS+DOR      | 16/0.5      | 2       | 7.2 × 10−9        | <2.9 × 10−9                              | 7.9 × 10−9                                      |
|              |             |         |                    |                                          | 10.9 × 10−9                                     |

FOS, fosfomycin; MEM, meropenem; IPM, imipenem; DOR, doripenem; TNTIC, too numerous to count.

a For selected clinical CR-PA strains (CM06, SI19, CH35 and UB45) and the standard strain PA01.
b Among isolates showing synergy.

CR-PA infection. Further in vivo studies should be performed on combination efficacy and pharmacokinetic aspects.

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Benjaporn Kunakovichaya
Krit Thirapannethee
Piyatip Khuntayaporn
Department of Microbiology, Faculty of Pharmacy, Mahidol University, 447 Sri Ayutthaya Road, Rajathevi, Bangkok 10400, Thailand

Preecha Montakantikul
Department of Pharmacy, Faculty of Pharmacy, Mahidol University, 447 Sri Ayutthaya Road, Rajathevi, Bangkok 10400, Thailand

Mullika Traidej Chomnawang

Department of Microbiology, Faculty of Pharmacy, Mahidol University, 447 Sri Ayutthaya Road, Rajathevi, Bangkok 10400, Thailand

*Corresponding author. Tel.: +66 2 644 8690; fax: +66 2 644 8692. E-mail address: mullika.tra@mahidol.ac.th* (M.T. Chomnawang)

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Trimethoprim/sulfamethoxazole resistance in clinical isolates of *Burkholderia pseudomallei* from Thailand

Sir,

*Burkholderia pseudomallei* is the cause of melioidosis, a serious infection associated with a mortality rate of 14–43% [1]. Recommended antimicrobial therapy is >10 days of parenteral ceftazidime or a carbapenem, followed by oral trimethoprim/sulfamethoxazole (SXT; co-trimoxazole) to complete up to 20 weeks of therapy [2]. A previous evaluation of 1976 clinical *B. pseudomallei* isolated from patients in northeast Thailand between 1992 and 2003 reported that SXT resistance was detected in 13% of isolates [3]. Subsequent studies have reported much lower rates of SXT resistance for isolates from Laos (0.8%), Australia (0.4%) and Cambodia (0%) [4]. Here we report the results of a re-evaluation of SXT resistance in Thailand. Second-line oral treatment in patients infected with SXT-resistant *B. pseudomallei* or in whom SXT is contraindicated is amoxicillin/clavulanic acid (AMC) [1], thus we also evaluated the susceptibility of SXT-resistant isolates to AMC and doxycycline (DOX), which is used less frequently as an alternative to SXT.

SXT susceptibility to doxycycline was determined by Etest (bioMérieux, Marcy-l’Étoile, France) [3], with reading of the minimum inhibitory concentration (MIC) at the 80% inhibition point. Interpretative standards for the Etest were based on Clinical and Laboratory Standards Institute (CLSI) guidelines for broth microdilution, which classifies SXT MICs of ≤2/38 mg/L as susceptible and ≥4/76 mg/L as resistant [5]. *Escherichia coli* ATCC 25922 was used as the control. For SXT-resistant isolates, the Etest was used to define susceptibility to trimethoprim (TMP) alone and sulfamethoxazole (SMX) alone. Susceptibility testing to AMC and DOX was also performed using the Etest, in which the MIC was read at the point of no visible growth. *Escherichia coli* ATCC 35218 was used as a control for AMC, and *E. coli* ATCC 25922 was used as a control for TMP, SMX and DOX.
Two isolate collections were evaluated. The first was drawn from a retrospective study of 3270 patients with culture-proven melioidosis at Sappasitiprasong Hospital in northeast Thailand between 2004 and 2012. A single isolate was used per patient (the first positive culture). All isolates had been stored at −80 °C and subculture recovered isolates from 3038 patients. The Etest SXT MIC ranged from 0.016 mg/L to ≥32 mg/L [MIC 50 (concentration that inhibits 50% of bacterial isolates) = 0.19 mg/L and MIC 90 (concentration that inhibits 90% of bacterial isolates) = 0.75 mg/L; interquartile range 0.094–0.25 mg/L (Fig. 1). Ten isolates (0.3%) were resistant to SXT, with an annual resistance rate ranging from 0% to 0.7%. As this is considerably lower than that reported by us previously for B. pseudomallei isolated from patients presenting to the same hospital between 1992 and 2003 [3], we re-evaluated this original collection. Previously, 258/1976 isolates were assigned as SXT-resistant based on the Etest [3], of which 255 could be recovered from frozen stocks. Etest could only confirm SXT resistance in 13 (5.1%) of these 255 isolates (Supplementary Table S1). The 23 SXT-resistant isolates from both collections were resistant to TMP and SMX when tested as separate agents (Supplementary Table S1). All SXT-resistant isolates were susceptible to AMC, but only 21 isolates (91%) were susceptible to DOX.

The CLSI recommends the broth microdilution method as the standard method for MIC testing of B. pseudomallei [5]. This is impractical for such a large study collection and was therefore used to verify Etest results for a subset of isolates. These were all 13 isolates from the 1992–2003 collection that were classified as resistant in both studies as well as 15 randomly selected isolates from the 1992–2003 collection with discrepant results between the two studies. Escherichia coli ATCC 25922 was used as the control. This demonstrated complete concordance of results between broth dilution and Etest performed in this study, confirming that the previous study had overestimated resistance. The most likely explanation for the erroneous results in the original study is error in reading the 80% inhibition point. This is inherently subjective and a minor difference in the interpretation of MIC results that are close to the breakpoint can lead to false classification as resistance. The majority (68%) of MICs for isolates that were erroneously defined as resistant in the previous study were 3 mg/L or 4 mg/L, which is consistent with a minor upshift in the MIC value but a large error in susceptibility classification [3]. Inhibition zones frequently have diffuse edges, and reading against a black background aided technical observation in this study.

Our finding that 99.7% of clinical B. pseudomallei isolates were susceptible to SXT is comparable with rates reported from Laos (99.2%), Australia (99.6%) and Cambodia (100%) [4], which indicate that primary SXT resistance in B. pseudomallei is uncommon. Our study also confirmed that SXT-resistant B. pseudomallei were susceptible to AMC, the current second-line drug of choice.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.ijantimicag.2015.01.006.

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Changing antimicrobial resistance patterns and trends of Shigella isolates in Ningbo, mid-east China, 2005–2013

Sir

Presently, the increase in antimicrobial-resistant Shigella isolates has been greatly crippling the treatment of shigellosis. Due to the great variations in antimicrobial resistance patterns of Shigella, monitoring antimicrobial resistance of these micro-organisms is necessary to aid in the selection of appropriate antimicrobials. This study included 226 clinical Shigella isolates (1.1%) recovered from a total of 21,500 outpatients with diarrhea during two time periods (2005–2007 and 2010–2013) in Ningbo, mid-east China. All isolates were identified according to biochemical characteristics and serotyping. Antimicrobial susceptibility testing was conducted by the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines [1] using the following antimicrobial disks (Oxoid Ltd., Basingstoke, UK): ampicillin (10 μg); cefotaxime (30 μg); aztreonam (10 μg); ciprofloxacin (5 μg); gentamicin (10 μg); and trimethoprim/sulfamethoxazole (SXT) (1.25/23.75 μg). Antimicrobial susceptibility testing results were interpreted according to CLSI guidelines [2]. Strains resistant to three or more drugs of chemically unrelated classes were defined as multidrug-resistant [3].

Overall, the proportion of strains resistant was 83.2% for ampicillin, 72.6% for SXT, 47.8% for cefotaxime, 44.2% for ciprofloxacin, 38.9% for aztreonam and 28.3% for aztreonam (Table 1). All isolates resistant to cefotaxime were positive for extended-spectrum β-lactamase (ESBL). The rates of multidrug resistance and resistance to all six antimicrobials tested were 53.1% and 10.6%, respectively.

Comparison between the two time periods (2005–2007 and 2010–2013) showed significant increases in the resistance prevalence to cefotaxime (32.8–67.3%; P < 0.01), gentamicin (17.2–42.9%; P < 0.01) and aztreonam (20.3–63.3%; P < 0.01) as well as a minor increase in resistance to ampicillin (78.1–89.8%; P = 0.02), whereas there were no significant changes in resistance rates to ciprofloxacin (40.6–49.0%; P > 0.05) and SXT (71.9–73.5%; P > 0.05). In particular, obvious upward trends were seen in the prevalence of multidrug resistance (39.1–71.4%; P < 0.01) and resistance to all six antimicrobials tested (3.1–20.4%; P < 0.01) (Table 1). In addition, the top three most predominant resistance patterns accounted for 55.1% of 98 isolates during 2010–2013, which was statistically greater than the 38.3% of 128 isolates during 2005–2007 (P < 0.01), showing a clustering trend in resistance patterns for recent years.

These findings also showed certain inconsistency with resistance data in the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) released by the US Centers for Disease Control and Prevention (CDC) for the following drugs [4]: ciprofloxacin (44.2% in this study vs. 2% in NARMS); cefotaxime (47.8% in this study vs. 1.1% in NARMS); and a minor increase in ampicillin resistance in this study compared with a downward trend in NARMS (from 70.7% in 2005 to 25.5% in 2012).

In summary, the present study demonstrated the rapid spread of antibiotic-resistant Shigella strains in recent years, posing a serious challenge because the treatment of this disease depends greatly on timely and effective use of antimicrobials.

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