Background: *Burkholderia pseudomallei* is the causative agent of melioidosis, a life-threatening infection that occurs predominantly in Southeast Asia and northern Australia. It is a laboratory Risk Group 3 organism in most countries and a potential biothreat ('Tier 1 Select Agent'). Although melioidosis requires intensive antimicrobial treatment, standardised antimicrobial susceptibility testing (AST) guidelines are currently lacking. In this study, we aimed to establish MIC and zone diameter distributions on which to set epidemiological cut-off (ECOFF) values for *B. pseudomallei* using EUCAST methodology for non-fastidious organisms.

Materials/methods: Between November 2018-January 2019, *B. pseudomallei* clinical isolates (16-70 per centre) were tested at eight study centres against antimicrobials listed in Table 1 by the EUCAST disk diffusion method and broth microdilution (BMD). Quality control of the antimicrobial disks (Oxoid, Basingstoke, UK) and BMD panels (Merlin Diagnostika, Bornheim-Hersel, Germany) was performed at the EUCAST Development Laboratory (EDL) before they were shipped to participating centres where this was repeated before testing was started. Guidance on performance of the tests and interpretation of results was provided by EDL. Each centre tested clinical isolates together with four QC strains (*Escherichia coli* ATCC 25922, *E. coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853 and *Staphylococcus aureus* ATCC 29213). Aggregated results were analysed according to EUCAST SOP 10.0 ‘MIC distributions and the setting of ECOFF values’ and MIC and zone diameter ECOFFs were determined by ECOFFinder program (www.eucast.org/mic_distributions_and_ecoffs) and visual estimation.

Results: Disk diffusion and MIC results (n=373) for *B. pseudomallei* were collected from eight centres. Isolates were from non-consecutive clinical cases, including isolates selected deliberately because of in vitro resistance to the agents under test. The ECOFFs are listed in Table 1.

Conclusions: In this multi-centre study, we have validated the use of standard MIC and disk diffusion methodology for AST of *B. pseudomallei* and determined MIC and zone diameter ECOFFs for *B. pseudomallei* against eight antimicrobials. The ECOFFs can be used to distinguish between wild-type (WT) and non-WT isolates and can also serve as background data to set clinical MIC breakpoints. Once these have been determined, zone diameter breakpoints can be set using the correlations established in this study.
Table 1. Epidemiological cut-off (ECOFF) values for *Burkholderia pseudomallei* on disk diffusion and minimum inhibitory concentration (MIC) data based on 373 observations for each antimicrobial agent.

<table>
<thead>
<tr>
<th>Antimicrobial agents</th>
<th>ECOFFs</th>
<th>% Non-wild-type organisms¹</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (mg/L)</td>
<td>Zone diameter (mm)</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>8</td>
<td>23</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>Imipenem</td>
<td>2</td>
<td>30</td>
</tr>
<tr>
<td>Meropenem</td>
<td>2</td>
<td>26</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>2</td>
<td>23</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>8</td>
<td>21</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>8</td>
<td>22</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>4</td>
<td>21</td>
</tr>
</tbody>
</table>

¹ The proportions of non-wild-type organisms appear spuriously high because a disproportionate number of isolates with *in vitro* resistance to the agents under test were included.