

Disk susceptibility testing of *Burkholderia pseudomallei*



David Dance, Vanaporn Wuthiekanun, Direk Limmathurotsakul and Joanne Letchford

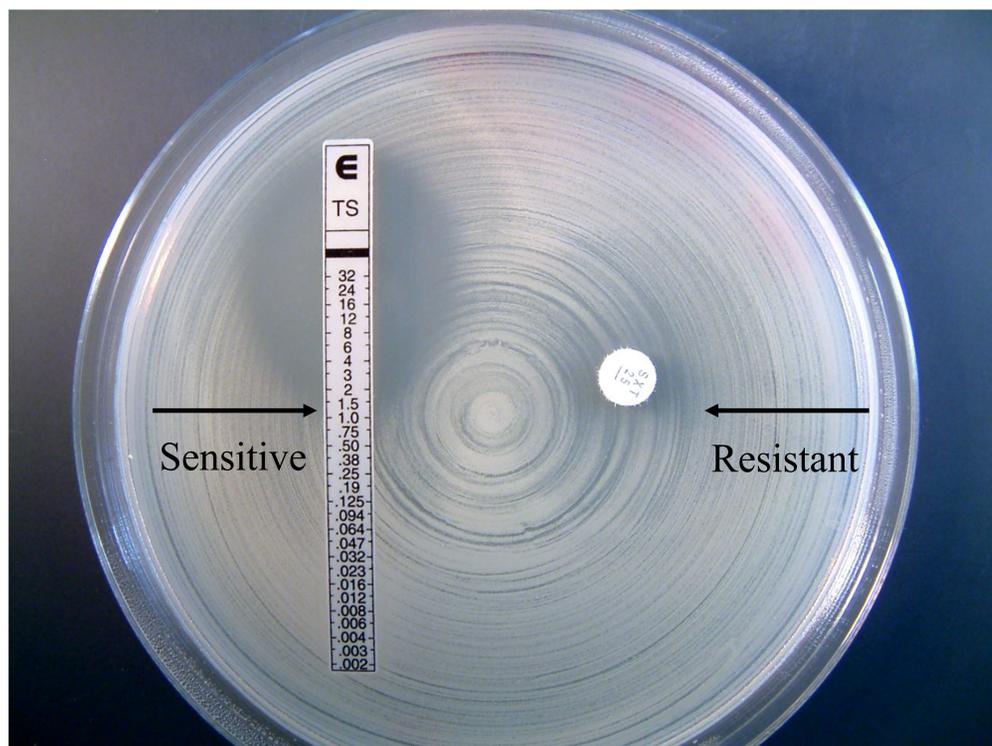
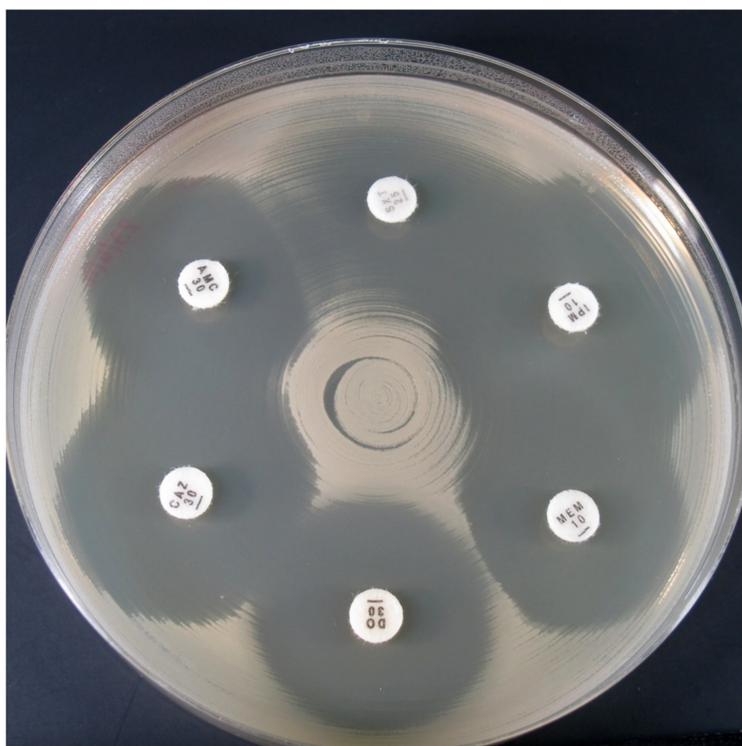
Method (based on CLSI M02-A12 Standard)

1. Perform the antimicrobial susceptibility testing in a biological safety cabinet (BSC).
2. Prepare inoculum of *B. pseudomallei* isolated colonies selected from an 18-24 h non-selective agar plate to get a concentration of approximately $1-2 \times 10^8$ CFU/mL (usually equivalent to a 0.5 McFarland standard).
3. Apply the bacterial inoculum onto the surface of Mueller Hinton agar.
4. Dispense preferred set of antimicrobial disks (e.g. CAZ, MEM, AMC, DO and SXT).
5. Incubate plates at 35°C for 20-24 h.
6. The resulting zones of inhibition need to be uniformly circular with confluent lawn of growth.
7. Since no zone diameter breakpoints for interpretive categories have been published by Clinical and Laboratory Standards Institute (CLSI) for disk diffusion testing of *B. pseudomallei*, interpret the size of the zones of inhibition by referring to breakpoints of *Pseudomonas aeruginosa* or *Enterobacteriaceae* (see below).

Table 1. Zone diameter Breakpoints for Interpretive Categories (R, I, S) for *B. pseudomallei*

Antimicrobial Agent	Code	Disk Content (µg)	Zone Diameter Breakpoints (mm) <i>Burkholderia pseudomallei</i>			Adapted from CLSI M100 27 th Ed. breakpoints for:
			<u>R</u>	<u>I</u>	<u>S</u>	
Amoxicillin-clavulanic acid	AMC	20/10	≤13	14-17	≥18	<i>Enterobacteriaceae</i>
Ceftazidime	CAZ	30	≤14	15-17	≥18	<i>P. aeruginosa</i>
Doxycycline	DO	30	≤10	11-13	≥14	<i>Enterobacteriaceae</i>
Meropenem	MEM	10	≤15	16-18	≥19	<i>P. aeruginosa</i>
Trimethoprim-sulfamethoxazole	SXT	1.25/23.75	≤10	11-15	≥16	<i>Enterobacteriaceae</i>
Trimethoprim-sulfamethoxazole	SXT	Etest MIC	≥ 4 µg/mL	–	≤ 2 µg/mL	<i>B. cepacia</i> Etest

Caution needs to be exercised in interpreting zone diameter for trimethoprim-sulfamethoxazole (SXT) as disk diffusion tends to overcall resistance. **Etest is a more accurate alternative and should be undertaken for any isolate that appears resistant or intermediate by disk diffusion.**



Note. Quality Assurance procedures, including regular testing of recommended Quality Control strains (E.coli ATCC 25922 (CAZ, DO, SXT), E.coli ATCC 35218 (AMC), *P. aeruginosa* ATCC 27853 (MER)) are important to achieve reliable results.