Disk susceptibility testing of *Burkholderia pseudomallei*

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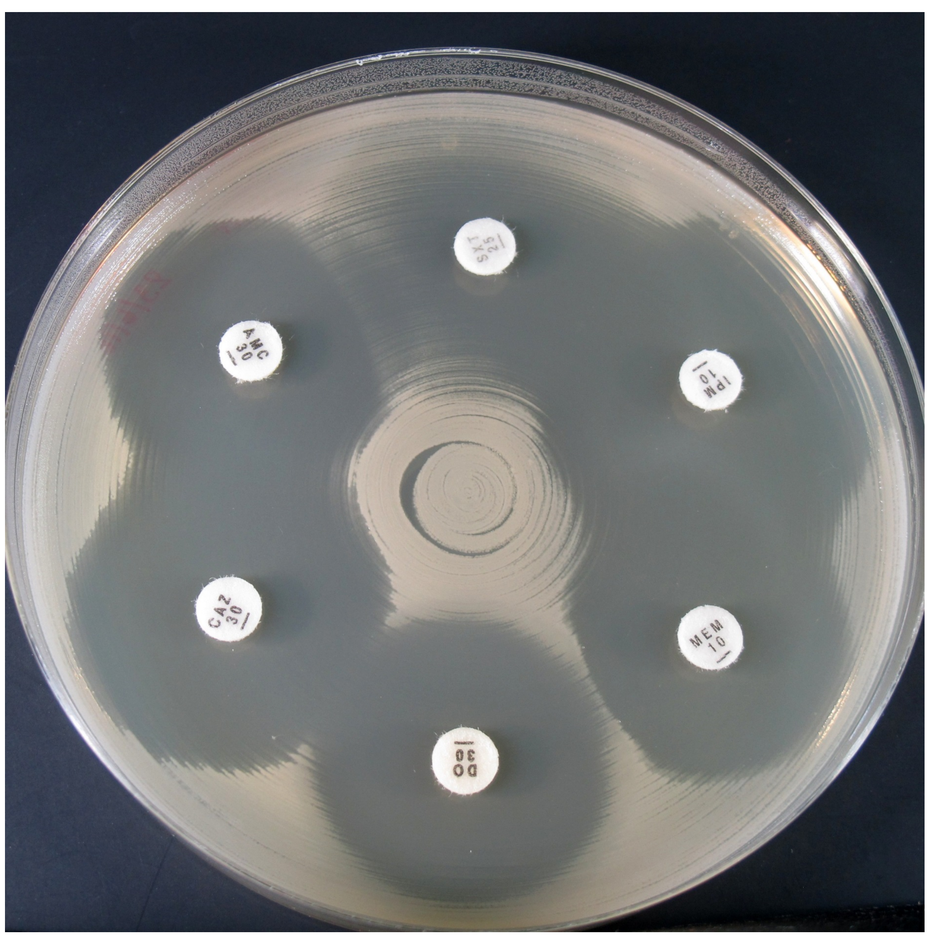
Method (based on CLSI guideline)

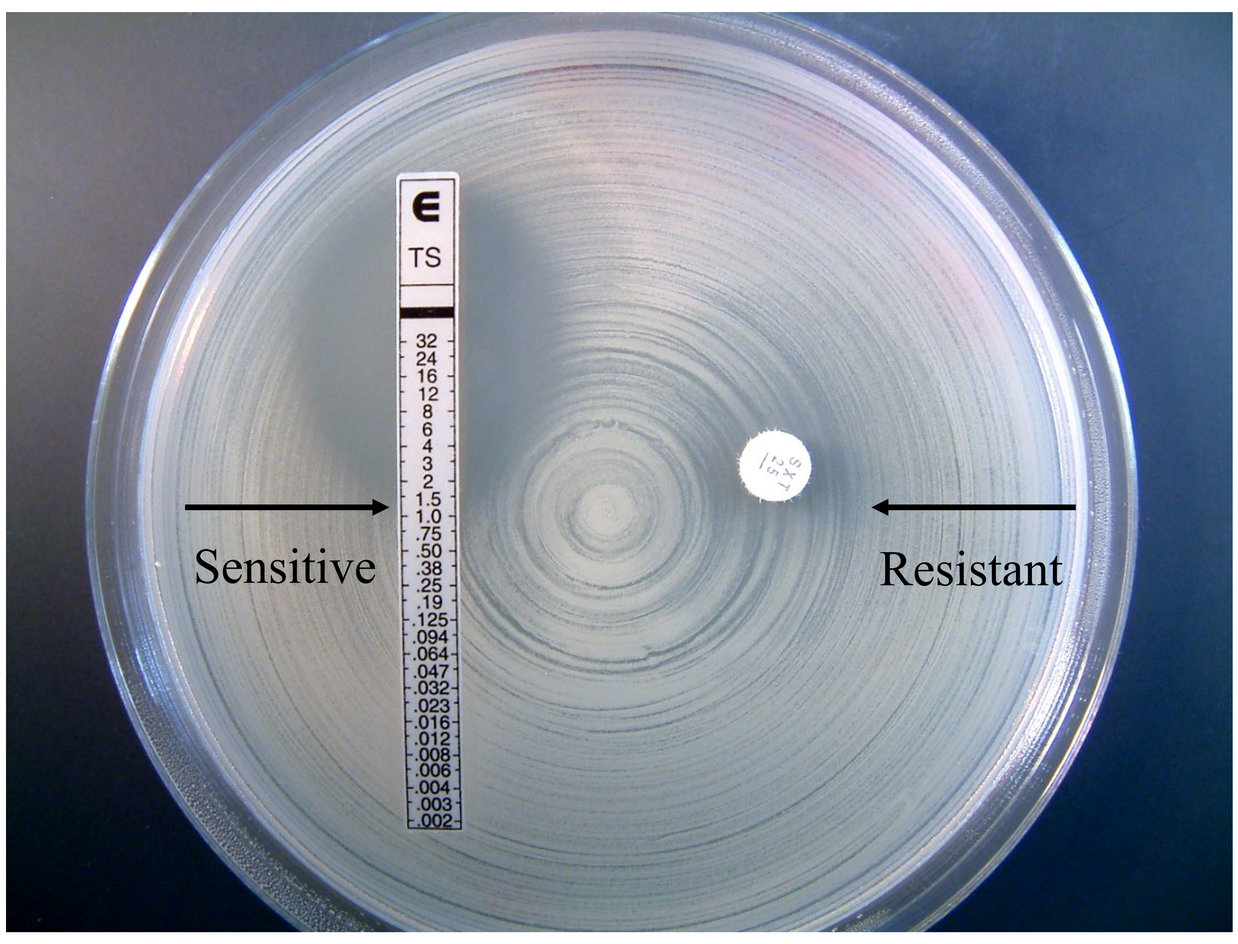
1. Perform the antimicrobial susceptibility testing in a biological safety cabinet (BSC).
2. Prepare inoculum of *B. pseudomallei* isolated colonies selected from an 18-24 h non-selective agar plate to get a concentration of approximately $1x10^8$ CFU/mL (usually equivalent to a 0.5 McFarland standard).
3. Apply the bacterial inoculum onto the surface of Mueller Hinton agar.
4. Dispense preferred set of antimicrobial disks (e.g. CAZ, MEM, IMP, AMC, DO and SXT).
5. Incubate plates at 35ºC for 18-24 h.
6. The resulting zones of inhibition need to be uniformly circular with confluent lawn of growth.
7. Since no interpretive guidelines 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 *Burkholderia cepacia*, *Pseudomonas aeruginosa* or Enterobacteriaceae (see below).

Table 1. Zone diameter Interpretive Standards for *B. pseudomallei*

Antimicrobial agent	Code	Disk Content (µg)	Zone Diameter Breakpoints (mm) *Burkholderia pseudomallei*		
			R	I	S
Amoxicillin-clavulanic acid	AMC	20/10	≤13	14-17	≥18
Ceftazidime	CAZ	30	≤14	15-17	≥18
Doxycycline	DO	30	≤12	13-15	≥16
Imipenem	IPM	10	≤13	14-17	≥18
Meropenem	MEM	10	≤12	13-15	≥16
Trimethoprim-sulfamethoxazole	SXT	1.25/23.75	≤10	11-15	≥16

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, are important to achieve reliable results.