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Title: Interpreting *Burkholderia pseudomallei* disc diffusion susceptibility test results by the EUCAST method

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COMMENTARY

In some parts of the tropics, melioidosis is an important public health problem and diagnostic laboratories frequently encounter *Burkholderia pseudomallei*. Most of these laboratories use disc diffusion for antimicrobial susceptibility testing but have had difficulty with *B. pseudomallei* because of a lack of internationally accepted criteria to interpret the results. The European Committee on Antimicrobial Susceptibility Testing (EUCAST) has recently published guidelines for interpretation of disc diffusion testing for *B. pseudomallei*, which include the adoption of the new EUCAST interpretative categorisation of susceptibility and resistance[1]. As a consequence, laboratories have been faced with reporting antimicrobial agents of proven effectiveness for melioidosis treatment as 'I' (Susceptible, increased exposure) instead of 'S' (Susceptible), creating confusion for clinicians. In this commentary we briefly explain the background to these changes, affirm that the recommended antibiotics with appropriately high doses can be used against *B. pseudomallei* reported as 'I' by the EUCAST method, and suggest how laboratories should use the new criteria to inform clinical practice.

Melioidosis is a severely neglected disease caused by the soil saprophyte, *Burkholderia pseudomallei*, which modelling suggests may kill as many as 89,000 people worldwide each year[2,3]. Although microbiology laboratories in Europe and the United States of America rarely encounter *B. pseudomallei*, in some tropical countries this is a daily occurrence, particularly during the rainy season. Although the antimicrobial susceptibility of *B. pseudomallei* is relatively predictable, variations can occur and resistance can also emerge during treatment[3]. Laboratories are therefore obliged to test isolates for their susceptibility *in vitro* in order to guide their clinical users about patient management. Most laboratories in the developing tropical world where melioidosis is endemic rely on disc diffusion to undertake antimicrobial susceptibility testing (AST), since automation is not readily available and routinely determining minimum inhibitory concentrations (MICs) for a range of antibiotics is simply not practical or affordable. However, although *B. pseudomallei* grows readily under the standard conditions used for disc diffusion susceptibility testing, until recently there were no published and internationally accepted criteria to enable *B. pseudomallei* to be classified as susceptible (S), intermediate (I) or resistant (R). The Clinical and Laboratory Standards Institute (CLSI), whose methods are widely used in the USA and elsewhere, have only ever published MIC-based criteria for *B. pseudomallei*[4], whilst EUCAST did not include *B. pseudomallei* in their guidance before 2020. The treatment of melioidosis has a strong evidence base[5], and many laboratories have developed their own interpretative criteria, usually adapted from members of the family Enterobacteriaceae or more closely related species such

as *Burkholderia cepacia* or *Pseudomonas aeruginosa*[6,7], that resulted in the vast majority of wild-type strains of *B. pseudomallei* being reported as 'S' to agents that are known to be effective in treatment; ceftazidime and carbapenems during the initial parenteral phase and trimethoprim-sulfamethoxazole during the oral eradication phase. This approach was purely a pragmatic response by laboratories in melioidosis-endemic areas to the absence of internationally recognised criteria for *B. pseudomallei*, and the precise breakpoints used varied from laboratory to laboratory and were never officially endorsed by CLSI.

It was in this context that a number of us embarked on a collaborative project with EUCAST, the results of which were published recently, to develop and validate criteria for disc diffusion using standard EUCAST methodologies[1]. Unbeknownst to us initially, this coincided with work undertaken by EUCAST to change the meanings of the different criteria, particularly 'I'. Previously, this was usually taken to mean 'intermediate' and thus agents reported as 'I' were usually avoided by clinicians for treatment in favour of agents reported as 'S'.

The published definitions of the new EUCAST criteria are as follows[8]:

- S - Susceptible, standard dosing regimen: A microorganism is categorised as "Susceptible, standard dosing regimen", when there is a high likelihood of therapeutic success using a standard dosing regimen of the agent.
- I - Susceptible, increased exposure*: A microorganism is categorised as "Susceptible, Increased exposure*" when there is a high likelihood of therapeutic success because exposure to the agent is increased by adjusting the dosing regimen or by its concentration at the site of infection.
- R - Resistant: A microorganism is categorised as "Resistant" when there is a high likelihood of therapeutic failure even when there is increased exposure.

The relevant zone diameters for *B. pseudomallei* are shown in Table 1

Table 1. EUCAST interpretative criteria for disc diffusion AST of *B. pseudomallei*[8]

	Zone diameter breakpoints (mm)		
	S ≥	**I**	**R <**
Amoxicillin-clavulanic acid	50	22-49	22
Ceftazidime	50	18-49	18
Imipenem	29	N/A	29
Meropenem	24	N/A	24
Tetracycline	50	23-49	23
Chloramphenicol	50	22-49	22
Trimethoprim-sulfamethoxazole	50	17-49	17

N/A: Not applicable

These changes, which took effect across the board and not just for *B. pseudomallei*, were well justified and consulted on extensively (see https://www.eucast.org/publications_and_documents/consultations/). They effectively changed the system from having two levels of resistant to having two levels of susceptible. However, since *B. pseudomallei* is less susceptible gram for gram to many agents than some other bacterial species, the practical consequence for several antibiotics, such as ceftazidime, has been to shift all wild-type isolates of *B. pseudomallei* from the 'S' category into the 'I' category. We are aware that this has already led to considerable confusion amongst both laboratory staff and clinicians. This has resulted in some laboratories declining to adopt the new system, and to clinicians switching antibiotics unnecessarily, for example from ceftazidime to meropenem, on the basis of a laboratory report of 'I' (Susceptible, increased exposure) for ceftazidime, when treating patients with melioidosis. The intention of EUCAST was always that the adoption of the new criteria should be accompanied by a period of intensive education of clinical users to help them to understand the new system but, in the areas of the tropics where melioidosis is common, interactions between laboratory staff and clinicians are often less frequent than they are in Europe and so this has been difficult to achieve.

We would encourage all laboratories to adopt the new criteria, which are the only internationally validated criteria for testing *B. pseudomallei* by disc diffusion. However, we also felt it important to explain the system in this commentary.

In summary, clinicians should not change their prescribing practice when treating melioidosis on the basis of a laboratory report that says 'I' based on disc diffusion using the new EUCAST criteria.

We would refer readers who wish to learn more about the evidence base for the treatment of melioidosis to a review of this topic[5] and to the treatment guidelines used in Darwin, Australia, where overall melioidosis mortality has now been reduced to below 10%[6]. These have recently been updated and represent a gold standard for management of patients with melioidosis, although in some countries the durations and doses may be difficult to afford. Key points are as follows:

- The doses of ceftazidime and trimethoprim-sulfamethoxazole used in melioidosis are higher than the usual standard dosing regimens of those agents, consistent with the EUCAST concept of 'I' (Susceptible, increased exposure).
- Ceftazidime remains the most appropriate first line treatment during the initial parenteral phase, even when it is reported as 'I' (which will be the majority of cases), as long as appropriately high doses of ceftazidime are used[5,6].
- Meropenem should be reserved for patients with sepsis admitted to Intensive Care Units or for patients who fail to respond to ceftazidime.
- Trimethoprim-sulfamethoxazole should be used for the eradication phase when it is reported as 'I' (in the majority of patients), unless there are contraindications to its use or clinically relevant toxicity occurring during treatment. Isolates should not be reported as 'R' on the basis of disc diffusion, but MICs should be determined (by gradient diffusion such as E test or a dilution method) and the isolate should only be reported as 'R' if the MIC is more than 4mg/l[9].
- In order to provide clarity for clinicians, laboratories may wish to add a comment to reports when an isolate is reported as 'I', for example 'Susceptible but requires high doses'.

There are several excellent resources on the EUCAST web site that explain the new system in greater detail, and we would particularly recommend the following webinar that specifically relates to the new definitions of S, I and R - https://youtu.be/QX5jtbpsbgI.

In the longer run, we would encourage greater harmonisation between the approaches and terminology used by both EUCAST and CLSI, with consideration given to ensuring that this leads to laboratory reports that are readily understood by clinicians who may not be familiar with the finer details of AST.

Transparency declaration

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Author contributions

DABD wrote the first draft. All authors edited the drafts and approved the final version.

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