Legionella antibiotic susceptibility testing: is it time for international standardisation and evidence-based guidance?

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Sir,

International guidelines, Epidemiological Cut off values (ECOFF), well validated methodologies and control strains validated in multiple laboratories are all absent for *Legionella*. Several antibiotic susceptibility methods that are described in the literature for legionellae (Table 1) show a wide variation in MIC values. Currently, gradient MIC strip testing on BCYE agar is the methodology recommended by EUCAST[1]. There are a number of technical caveats with this method including the sequestration of antibiotics by the charcoal present in the media which accounts for the documented rise in the MIC results [2,3]. Recent work by Portal *et al.* (submitted) demonstrated that gradient strip testing and BCYE agar dilution methodologies gave higher MIC values than the microbroth dilution method used in

Vandewalle-Capo *et al.*,[4] (2017). Portal *et al.* (submitted) describes the use of a charcoal-free solid media for *Legionella* that generated concordant results to MIC values when compared to those observed with the broth microdilution method. This finding provides a reliable alternative to micro-broth dilution for testing *Legionella* susceptibility which is also more applicable on a routine basis.

International treatment recommendations for patients infected with *Legionella* infection are also inconsistent, often providing differing guidelines and regimens[5–7] and employing variable defined breakpoints for assigning susceptibility/resistance phenotypes. Treatment is confounded by the relative penetration potential of different antibiotics to the infection site and its ability to gain access to the intracellular location of *Legionella* infections. Historically, antibiotic resistance in *Legionella* has not been a concern. However, reports of the *lpeAB* efflux pump[8] and single point somatic mutations in *L. pneumophila*[9] have increased. Moreover, a recently documented novel resistance mechanism in *L. longbeachae*[10] highlights the need for standardisation and validation at an international level.

Due to the lack of comparable data and the varied approaches and methodologies in use across the globe to address this topic, the international *Legionella* community makes several recommendations:

1) Gradient strip testing on BCYE agar to be discontinued as the recommended EUCAST methodology, due to higher MIC results when compared to microbroth dilution.
2) BCYE agar not to be used for serial antibiotic dilution determination of *Legionella* due to higher MIC results and antibiotic sequestration.
3) Future studies to employ microbroth dilution or concordant methodologies such as charcoal-free media (e.g. LASARUS) as the gold standard for determination of susceptibility of *Legionella*.

4) The *Legionella* community to identify and validate a panel of clinical and environmental reference strains for MIC determination. Three strain to be evaluated in a multicentre site study:

   a. *Legionella pneumophila* strain W872, Serogroup 1, monoclonal subgroup Benidorm (culture collections: NCTC12821, DSM27564, CCUG67715, WDCM00205);

   b. *Legionella pneumophila*, strain Philadelphia 1, Serogroup 1, monoclonal subgroup Philadelphia (culture collections: NCTC11192, ATCC33152; CCUG9568; CIP103854; DMZ7513; JCM7571; WDCM00107);

   c. *Legionella pneumophila* strain Paris, Serogroup 1, monoclonal subgroup Philadelphia (culture collections: NCTC 11192; ATCC 33152; CIP107-629-T French National Reference Center for *Legionella*)

5) The *Legionella* community to develop a consensus standard operating procedure, define ECOFF levels and develop consensus on antibiotic testing of strains of clinical relevance.

6) Antimicrobial susceptibility testing for *Legionella* using valid methods to be encouraged as part of a global surveillance for the emergence of resistance in this micro-organism

7) Phenotypically-resistant *Legionella* strain to be comprehensively analysed using whole genome sequencing and other complementary methods in order to identify new and emerging mechanisms underlying resistance in legionellae

Table 1. Documented legionellae susceptibility testing with MIC.

Papers were included when they detailed: screening of clinically relevant antibiotics, screening of over 30 *Legionella* strains, use of *in vitro* model, published in English language, available from PubMed.

Bibliography

1. EUCAST. *Antimicrobial susceptibility testing of Legionella pneumophila*. 2016. Available at: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/General_documents/Legionella_guidance_document_20160909.pdf. Accessed October 8, 2019.

2. Ruckdeschel G. The in-vitro activity of moxifloxacin against Legionella species and the effects of medium on susceptibility test results. *J Antimicrob Chemother* 1999; **43**: 25–9. Available at: https://patentimages.storage.googleapis.com/41/b5/ca/69ffeea861af61/US8949899.pdf. Accessed November 25, 2019.

3. Bruin JP, Ijzerman EPF, den Boer JW, Mouton JW, Diederen BMW. Wild-type MIC distribution and epidemiological cut-off values in clinical Legionella pneumophila serogroup 1 isolates. *Diagn Microbiol Infect Dis* 2012; **72**: 103–8. Available at: https://linkinghub.elsevier.com/retrieve/pii/S0732889311003762. Accessed June 4, 2019.

4. Vandewalle-Capo M, Massip C, Descours G, *et al.* Minimum inhibitory concentration (MIC) distribution among wild-type strains of Legionella pneumophila identifies a subpopulation with reduced susceptibility to macrolides owing to efflux pump genes. *Int J Antimicrob Agents* 2017; **50**: 684–9. Available at: https://www.sciencedirect.com/science/article/pii/S0924857917302935?via%3Dihub. Accessed May 2, 2018.

5. NICE. *Pneumonia (community-acquired): antimicrobial prescribing NICE guideline*. 2019. Available at: www.nice.org.uk/guidance/ng138. Accessed December 13, 2019.

6. NICE. *Pneumonia (hospital-acquired): Antimicrobial prescribing NICE guideline*. 2019. Available at: www.nice.org.uk/guidance/ng139. Accessed December 13, 2019.

7. Lim WS, Baudouin S, George R, *et al.* British Thoracic Society guidelines for the management of community acquired pneumonia in adults: Update 2009. *Thorax* 2009; **64**.

8. Massip C, Descours G, Ginevra C, Doublet P, Jarraud S, Gilbert C. Macrolide resistance in Legionella pneumophila: The role of LpeAB efflux pump. *J Antimicrob Chemother* 2017; **72**: 1327–33. Available at: https://academic.oup.com/jac/article-abstract/72/5/1327/2964580. Accessed December 12, 2019.

9. Shadoud L, Almahmoud I, Jarraud S, *et al.* Hidden Selection of Bacterial Resistance to Fluoroquinolones In Vivo: The Case of Legionella pneumophila and Humans. *EBioMedicine* 2015; **2**: 1179–85.

10. Forsberg KJ, Patel S, Wencewicz TA, Dantas G. The Tetracycline Destructases: A Novel Family of Tetracycline-Inactivating Enzymes. *Chem Biol* 2015; **22**: 888–97.