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Reply to Gonzales-Luna et al

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To The Editor—We thank Gonzales-Luna and colleagues [1] for their comments. We agree that laboratories must have access to accurate and standardized methods for antimicrobial susceptibility testing (AST) results to be clinically meaningful. The reference method for performing *Clostridioides difficile* AST is agar dilution according to Clinical and Laboratory Standards Institute (CLSI) guidelines [2]. The CLSI method for performing AST for anaerobic bacteria recommends that 5 μg/mL of hemin be incorporated into agar dilution plates and that the hemin stock solution should be protected from light and stored at 4°C–8°C for no longer than 1 month [2]. The susceptibility testing done by Gargis et al [3] was performed according to these guidelines, and the hemin stock solution was protected from light.

Nevertheless, we read with interest the research in recent years [4–6] related to heme-dependent metronidazole resistance, including the reported association between isolates characterized as heme dependent and metronidazole resistant and the presence of a T to G mutation (P*nimB*^{G}) in the −10 promoter region of the nitroimidazole reductase gene, *nimB* [5]. While Olaitan et al [5] found that not all heme-dependent metronidazole-resistant isolates contained the P*nimB*^{G} mutation, Olaitan et al [5] indicate that most do; therefore, the presence of P*nimB*^{G} may be predictive of resistance. We determined that the *nimB* mutation was present in 20% of our study isolates (116 of 593), of which 99% (115 of 116) belonged to RT027 (Table 1). The remaining isolate was RT014, the only RT014 isolate containing the P*nimB*^{G} mutation among the 65 evaluated.

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Disclaimer. The findings and conclusions in this letter are those of the authors and do not necessarily represent the views of the Centers for Disease Control and Prevention or the Agency for Toxic Substances and Disease Registry.

Potential conflicts of interest. L. C. M. reports participation on a data safety monitoring board or advisory board for the Division of Microbiology and Infectious Disease, National Institutes of Health (protocol 19–0021: A Randomized, Double-blinded Evaluation of CRS3123 in Patients with Non-severe to Severe *Clostridium difficile* Infection). All other authors report no potential conflicts. All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

The Centers for Disease Control and Prevention (CDC) data set included a total of 137 RT027 isolates, and 84% (115 of 137) contained the *nimB* mutation. A summary of the presence and absence of P*nimB*^{G} among the CDC study isolates is provided in Table 1, along with corresponding CDC minimum inhibitory concentration (MIC) data. Using the CLSI reference method, the CDC metronidazole MIC distribution for RT027 isolates with the P*nimB*^{G} mutation (0.25–8 μg/mL) was shifted toward higher MICs than the MIC distribution for RT027 isolates without the P*nimB*^{G} mutation (0.25–2 μg/mL) ($P < .01$).

AST is performed to monitor resistance trends over time and to provide clinically meaningful data that allow clinicians to predict the in vivo success or failure of antimicrobial therapy. To ensure consistency, the use of standardized AST methods (media, incubation time, inoculum, etc) is critical. Agar dilution as described by CLSI is currently the recommended reference method for *C. difficile* AST. Although genotypic methods can serve as a tool for detecting specific resistance genes or mutations, their ability to accurately predict phenotypic resistance must be validated using established reference methods. We encourage Gonzales-Luna et al to bring their AST method and observations on the *nimB* mutation to CLSI for further evaluation of its relevance in vivo and correlation to clinical outcome. Given the rapid ongoing evolution of pathogen resistance, it is essential that public health and academia join forces to detect and characterize emerging resistance, investigating every credible threat to the fullest degree possible. In this light we appreciate the authors' comments and contribution to this evolving field.

References

1. Gonzales-Luna A, Dureja C, Eubank TA, Garey KW, Hurdle JG. Surveillance of *Clostridioides difficile* antimicrobial resistance in the United States. Clin Infect Dis 2023; 76:2038–9. [PubMed: 36883582]
2. Clinical and Laboratory Standards Institute. Methods for antimicrobial susceptibility testing of anaerobic bacteria. 9th ed. CLSI M11. Wayne, PA: Clinical and Laboratory Standards Institute, 2018.
3. Gargis AS, Karlsson M, Paulick AL, et al. Reference susceptibility testing and genomic surveillance of *Clostridioides difficile*, United States, 2012–17. Clin Infect Dis 2023; 76:890–6. [PubMed: 36208202]
4. Wu X, Shen WJ, Deshpande A, et al. The integrity of heme is essential for reproducible detection of metronidazole-resistant *Clostridioides difficile* by agar dilution susceptibility tests. J Clin Microbiol 2021; 59:e0058521.
5. Olaitan AO, Dureja C, Youngblom MA, et al. Decoding a cryptic mechanism of metronidazole resistance among globally disseminated fluoroquinolone-resistant *Clostridioides difficile*. bioRxiv [Preprint]. September 26, 2022. Available from: https://www.biorxiv.org/content/10.1101/2022.09.23.509282v2.
6. Gonzales-Luna AJ, Olaitan AO, Shen WJ, et al. Reduced susceptibility to metronidazole is associated with initial clinical failure in *Clostridioides difficile* infection. Open Forum Infect Dis. 2021; 8:ofab365. [PubMed: 34381844]
7. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 32nd ed. CLSI M100. Wayne, PA: Clinical and Laboratory Standards Institute, 2022.
8. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters: version 13.0, valid from 1 January 2023. Available at: https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_13.0_Breakpoint_Tables.pdf. Accessed 30 January 2022.

Table 1.

Presence and Absence of P*nimB*^{G} Mutation Among Centers for Disease Control and Prevention (CDC) Study Isolates and Corresponding CDC Minimum Inhibitory Concentration Data on Metronidazole Susceptibility

MIC Obtained With CLSI Agar Dilution Method, μg/mL[a]	Isolates, No.			
	P*nimB*^{G} Mutation Present (n = 116)	P*nimB*^{G} Mutation Absent (n = 477)	RT027 With P*nimB*^{G} Mutation (n = 115)	RT027 Without P*nimB*^{G} Mutation (n = 22)
≤0.25	0	5	0	0
0.25	2	136	2	6
0.5	25	175	25	10
1	50	108	50	5
2	30	46	29	1
4	8	7	8	0
8	1	0	1	0

Abbreviations: CLSI, Clinical and Laboratory Standards Institute; MIC, minimum inhibitory concentration.

[a]The susceptibility breakpoint for metronidazole according to CLSI is ≤8 μg/mL [7]. The susceptibility breakpoint for metronidazole according to the European Committee on Antimicrobial Susceptibility Testing is ≤2 μg/mL [8]. Kuiper's test was used to compare the empirical distribution of metronidazole MICs for RT027 isolates with or without the P*nimB*^{G} mutation.