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COMMENTARY

The Search for a Practical Method for Colistin Susceptibility Testing: Have We Found It by Going Back to the Future?

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ABSTRACT Polymyxins are relied upon for the treatment of carbapenem-resistant Gram-negative bacterial infections, but polymyxin resistance is increasing. Only broth microdilution is recommended for polymyxin susceptibility testing, but this method is impractical for most clinical microbiology laboratories. An article in this issue of the *Journal of Clinical Microbiology* (P. J. Simner, Y. Bergman, M. Trejo, A. A. Roberts, R. Marayan, T. Tekle, S. Campeau, A. Kazmi, D. Bell, S. Lewis, P. D. Tamma, R. Humphries, and J. A. Hindler, J Clin Microbiol 57:e01163-18, 2019, https://doi.org/10.1128/JCM.01163-18) found that colistin broth disk elution, a method that requires only colistin disks and broth, had excellent performance compared to broth microdilution for all strains except *mcr*-positive *Escherichia coli* strains.

The polymyxins (i.e., colistin, polymyxin B) were first recognized to have broad-spectrum activity against Gram-negative bacteria in the 1940s and were used therapeutically over the following two decades (1, 2). However, they were abandoned as systemic antimicrobial agents in the 1970s, after they were shown to be highly nephrotoxic and neurotoxic and as agents with more favorable side effect profiles became available (2, 3). Unfortunately, with the emergence of carbapenem-resistant Gram-negative bacteria that are resistant to all other classes of antimicrobial agents, polymyxins have been increasingly relied upon as agents of last resort (2). Nationwide data demonstrate a nearly 3-fold increase in the use of colistin from 2006 to 2012 (4). Even with the availability of newly approved antimicrobial agents for carbapenem-resistant *Enterobacteriaceae* and *Pseudomonas aeruginosa*, there is continued need for polymyxins because the novel agents are active against only a subset of carbapenem-resistant Gram-negative bacteria and because the high costs of these new agents may limit their use in areas of the world where the need is the greatest (5).

Isolates of *P. aeruginosa* and *Acinetobacter baumannii* with colistin MICs of ≥4 μg/ml are considered to be resistant to colistin by the Clinical and Laboratory Standards Institute (CLSI) (6) and the European Committee on Antimicrobial Susceptibility Testing (EUCAST) (7). EUCAST also considers *Enterobacteriaceae* with colistin MICs of ≥4 μg/ml to be resistant, whereas CLSI designates these organisms non-wild type. In the rest of this commentary, I refer to organisms with colistin MICs of ≥4 μg/ml as resistant. These cutoffs for resistance are supported by pharmacokinetic/pharmacodynamic analyses that demonstrate that even with the highest tolerable dosages of colistin, patients are very unlikely to attain exposures that are correlated with efficacy when MICs are ≥4 μg/ml (8).

Unfortunately, colistin resistance among carbapenem-resistant Gram-negative bacteria has become common. In two multicenter analyses of carbapenem-resistant *Klebsiella pneumoniae* from U.S. medical centers, 13% to 16% of isolates were colistin resistant (9, 10). Colistin resistance has become even more problematic in other countries. For example, a multicenter study from Italy demonstrated that 43% of carbapenemase-producing *K. pneumoniae* isolates were colistin resistant (11). Although

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colistin resistance is not as common as it is with *K. pneumoniae*, colistin resistance has also emerged among *A. baumannii* isolates (12) and, to a lesser degree, among *P. aeruginosa* isolates (13). The colistin resistance mechanisms identified in these studies have almost exclusively been chromosomally mediated, but the emergence of a newly identified plasmid-mediated mechanism of colistin resistance, *mcr-1* (and its variants), further threatens the activity of these last-resort agents (14).

It is critical that clinical microbiology laboratories be able to identify carbapenem-resistant Gram-negative bacteria that are resistant to colistin, so that patients do not receive this nephrotoxic therapy when it is unlikely to be effective. Unfortunately, there are no U.S. Food and Drug Administration (FDA)-cleared diagnostic tests for colistin or polymyxin B susceptibility testing, and thus, neither agent is on automated susceptibility panels that are used by most clinical microbiology laboratories. This lack of an FDA-cleared test is partially because the FDA does not have any breakpoints for the polymyxins, and thus, manufacturers cannot validate the performance of their panels to correctly classify susceptible and resistant strains against a reference method (15).

Most clinical microbiology laboratories rely on disk diffusion or gradient diffusion susceptibility testing methods for agents that are not on automated panels. Unfortunately, the polymyxins are large cationic molecules that diffuse poorly in these diffusion-based assays. It is thus not surprising that neither disk diffusion nor gradient diffusion tests can accurately detect colistin resistance. Tan and Ng compared the performance of multiple colistin disk diffusion methods to that of the reference agar dilution method and demonstrated that the majority of colistin-resistant isolates were called falsely susceptible by all disk diffusion methods (16). Because of these high rates of very major errors (in which an isolate is called susceptible when it is resistant by the reference method), no CLSI or EUCAST disk diffusion breakpoints exist for the polymyxins. Widely used gradient diffusion tests, such as Etest (bioMérieux) and MIC test strips (MTS; Liofilchem), also fail to identify colistin-resistant organisms, with very major error rates being 35 to 53% (9, 17). Based on the poor performance of these commonly used methods, CLSI and EUCAST do not recommend using disk diffusion or gradient diffusion for colistin susceptibility testing and instead recommend using broth microdilution (BMD). Unfortunately, reference broth microdilution is not a practical method for the majority of clinical microbiology laboratories. Premade Sensititre broth microdilution panels (Thermo Fisher) perform well compared to the reference broth microdilution method (18), but even the use of these premade panels requires substantial manual labor that is beyond the capacity of many clinical laboratories and unnecessarily duplicates susceptibility testing for the other agents that are on these panels. The lack of a simple, easy-to-perform colistin susceptibility test has left most clinical microbiology laboratories in an unenviable position where they cannot provide clinicians with an accurate assessment of colistin susceptibility.

In this issue of the *Journal of Clinical Microbiology*, Simner and colleagues report the performance of the colistin broth disk elution (CBDE) test for assessing the susceptibility of Gram-negative bacteria to colistin (19). In this method, 0, 1, 2, and 4 10-μg colistin disks are placed in four different 10-ml cation-adjusted Mueller-Hinton broth tubes, respectively. These tubes are then incubated at room temperature to allow the colistin to elute from the disks, leading to presumed colistin concentrations of 0, 1, 2, and 4 μg/ml in these tubes, respectively. The organisms are then inoculated into these tubes and incubated overnight, and the MIC is read as the lowest concentration where turbidity is not observed. This test was evaluated on 172 *Enterobacteriaceae*, *A. baumannii*, and *P. aeruginosa* isolates, including 38 isolates that were colistin resistant, by comparing the results of CBDE to those of the gold standard of broth microdilution (BMD). They found a categorical agreement of 98%, an essential agreement of 99% (MIC within a single doubling dilution), and no major errors (in which the organism is called resistant when it is susceptible by the reference method). They did find an 8% very major error rate, and this was because three of the six *Escherichia coli* isolates with *mcr-1* that had MICs of 4 μg/ml by BMD (resistant) had MICs of 2 μg/ml by CBDE (susceptible).

Broth disk elution was first reported as an antimicrobial susceptibility testing

method 45 years ago (20). It was primarily evaluated for anaerobes, where it demonstrated good performance in this setting compared to agar dilution (21), and was previously a method approved by the National Committee for Clinical Laboratory Standards (NCCLS; the organization that preceded CLSI) for anaerobes. However, the NCCLS rescinded its approval of this method because of performance issues for *Bacteroides fragilis* isolates when the MIC was near the breakpoint and challenges in assessing turbidity when partial disintegration of the disks occurred (22, 23). This method eventually fell out of favor, and a national survey in 2006 did not identify any clinical microbiology laboratories that used broth disk elution for anaerobic susceptibility testing (24).

The results from Simner et al. (19) are highly encouraging because they suggest that this simple test can reliably identify *Enterobacteriaceae*, *A. baumannii*, and *P. aeruginosa* isolates that have chromosomally mediated colistin resistance, the most common mechanism of colistin resistance (25). Furthermore, this test requires only colistin disks and Mueller-Hinton broth, supplies that can easily be obtained by any clinical microbiology laboratory, including those in resource-limited settings, where colistin may be needed the most and where colistin resistance may be the most prevalent.

While these preliminary results are encouraging, limitations of this study merit attention. First, although the study was performed at two sites, the vast majority of isolates were tested at only a single site. As the authors note, a true multicenter study that assesses a larger number of colistin-resistant isolates is needed before this method can be widely recommended. Second, the CBDE test had difficulty in identifying *E. coli* isolates that were colistin resistant because of *mcr-1*, which led to the 8% very major error rate in the study. This very major error rate of 8% would likely have been much lower if a more representative sample of organisms were used. Currently, *mcr* is identified in only 3% of colistin-resistant *Enterobacteriaceae* worldwide (25), yet it was present in 16% of colistin-resistant *Enterobacteriaceae* in the study. Furthermore, only a single *mcr*-positive *P. aeruginosa* isolate has been reported, and *mcr*-positive *A. baumannii* isolates have yet to be reported (26). The poor performance in isolates with *mcr* is not surprising because these isolates often have colistin MICs of 2 to 4 μg/ml, values that straddle the current breakpoint (18). These findings are reminiscent of performance issues when the broth elution method was used for *B. fragilis* isolates with MICs near the breakpoint for these organisms (22). Based on these considerations, the authors recommend performing reference broth microdilution on isolates with colistin MICs of 2 μg/ml by CBDE. For most clinical microbiology laboratories, this would require sending the isolate to a reference lab, which would mean the result would not be obtained in a clinically actionable time frame.

As an alternative, it may be worthwhile to assess the actual colistin concentrations in the tube of broth with four colistin disks and experiment with using 3.5 disks in the last tube if the colistin concentration is found to be $>$4 μg/ml with four disks. A colistin susceptibility testing method called Polymyxin NP, a colorimetric assay that is similar to Carba NP and that uses a colistin concentration of 3.75 μg/ml in the final well, has been developed in Europe and may be more accurate in identifying colistin-resistant isolates with *mcr* (27). However, as with Carba NP, Polymyxin NP requires the frequent preparation of specialized reagents, which may not be practical for laboratories where the need for colistin susceptibility testing is sporadic.

Lastly, given that polymyxin B may offer a more favorable pharmacokinetic-pharmacodynamic profile and may be less nephrotoxic than colistin (28, 29), it would be valuable to know how the broth disk elution method works for polymyxin B. Although the categorical agreement between colistin and polymyxin B is nearly 99% for Gram-negative bacteria, approximately one-third of colistin-resistant isolates will have a lower polymyxin B MIC than colistin MIC (30).

In summary, there is an urgent need for a simple, practical, and inexpensive colistin susceptibility testing method, so that laboratories can reliably identify isolates that cause infections that are highly unlikely to respond to this toxic antimicrobial agent. The broth disk elution test, which repurposed an antiquated method that had been

largely abandoned for decades, may be the method that we have been looking for to detect resistance to an antimicrobial agent that was also abandoned for decades and that is now used as an agent of last resort.

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