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Comparison of Etest to Broth Microdilution for Testing of Susceptibility of *Pseudomonas aeruginosa* to Ceftolozane-Tazobactam

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The emergence and spread of multidrug-resistant (MDR) *Pseudomonas aeruginosa* are a significant burden to health care systems due to poor patient outcomes, serious infection control implications, and limited antibiotic effectiveness (1, 2). Ceftolozane-tazobactam is a novel beta-lactam–beta-lactamase inhibitor combination that retains activity against MDR *P. aeruginosa*, but current Food and Drug Administration (FDA) labels are only for complicated urinary tract and intra-abdominal infections (3). In the era of increasing antibacterial resistance, with few therapeutic options available, accurate and timely susceptibility testing is essential for improving patient outcomes (4). High rates of very major errors (VME), or false susceptibilities, have been reported with *P. aeruginosa* for piperacillin-tazobactam with automated systems and Etests (5, 6). The effects of VME on ceftolozane-tazobactam susceptibility testing are currently unknown.

Kirby-Bauer ceftolozane-tazobactam disks are FDA approved for testing against *P. aeruginosa*, but their utility is limited due to the lack of reported MICs. MICs are often necessary in severe infections (e.g., MDR *P. aeruginosa*) to allow for pharmacodynamic optimization of therapy. Clinical and Laboratory Standards Institute (CLSI) breakpoints for ceftolozane-tazobactam are as follows: susceptible, ≤4/4 μg/ml; intermediate, 8/4 μg/ml; and resistant, ≥16/4 μg/ml, respectively (7). Etest strips are designated research use only (RUO). The limitations of RUO susceptibility testing and the history of VME with *P. aeruginosa* and piperacillin-tazobactam prompted this investigation into susceptibility testing with ceftolozane-tazobactam.

We evaluated 90 meropenem-nonsusceptible (MIC, ≥4 μg/ml) *P. aeruginosa* isolates obtained from bronchoalveolar lavage, blood, peritoneal fluid, or bone biopsy specimen cultures from January 2015 to August 2016 using both Etest and broth microdilution (BMD). All isolates were stored at −20°C in tryptic soy broth with 10% glycerol (BBL, Sparks, MD). Isolates were subcultured overnight on blood agar plates (BBL; Trypticase soy agar [TSA II] with 5% sheep blood [SB]). They were then suspended in 0.85% saline to match the turbidity of a 0.5 McFarland standard. The same suspension was used to perform BMD as described below and to inoculate a Mueller-Hinton plate. Etest strips were aseptically placed onto the agar surface, and the plates were incubated for 24 h at 35°C in ambient air. Etest results were compared to BMD, which was performed concurrently per CLSI standards (8). Panels for BMD were prepared in-house with ceftolozane-tazobactam (Merck & Co.) using cation-adjusted Mueller-Hinton II broth. The MIC was read after 16 to 20 h of incubation at 35°C in ambient air and was determined by the lowest concentration of ceftolozane-tazobactam that completely inhibited visible growth in a well (8). *Escherichia coli* ATCC 35218 and *P. aeruginosa* ATCC 27853 were used as quality control organisms and were within the range daily (7). All very major error discrepancies were confirmed by repeat testing with both BMD and Etests.

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TABLE 1 Comparison of MICs of ceftolozane-tazobactam against *P. aeruginosa* ($n = 90$) via Etest and broth microdilution

Method	% of isolates meeting breakpoint[a]			No. positive/total no. (%)				
	S	I	R	EA[b]	CA[c]	VME[d]	ME[e]	mE[f]
Etest	92.2	1.1	6.7	68/90 (75.5)	80/90 (88.8)	6/12 (50)	2/76 (2.6)	3/90 (3.3)
Broth microdilution	84.4	2.2	13.3					

[a]Clinical and Laboratory Standards Institute (CLSI) breakpoints for ceftolozane-tazobactam: susceptible (S), ≤4/4 μg/ml; intermediate (I), 8/4 μg/ml; and resistant (R), ≥16/4 μg/ml, respectively.
[b]EA, essential agreement; agreement within ±1 2-fold dilution of the Etest to BMD.
[c]CA, categorical agreement; agreement of interpretative results (susceptible, intermediate, and resistant) between Etest and BMD.
[d]VME, very major error; susceptible by Etest and resistant by BMD.
[e]ME, major error; resistant by Etest and susceptible by BMD.
[f]mE, minor error; reported as intermediate by Etest when susceptible or resistant by BMD or vice versa.

Overall, we observed a significant rate of discrepancy between the Etest and BMD results (Table 1). Susceptibility rates of *P. aeruginosa* isolates were 92.2% by Etest and 84.4% by BMD. VME were found in 50% (6/12) of results, and major errors (ME) were found in 2.6% (2/76) of results; minor errors were found in 3.3% of results. Essential agreement (EA) and categorical agreement (CA) were 75.5% and 88.8%, respectively.

This study highlights a significant issue regarding RUO susceptibility testing for ceftolozane-tazobactam. Rates of VME plus major errors (ME) observed were above the 3% threshold to be classified as an equivalent testing agent (7). Performance rates for EA and CA were below the threshold of ≥90% (9). This is concerning, as ceftolozane-tazobactam susceptibility testing is generally performed when there are few alternative agents. Given the potential for negative patient outcomes associated with major susceptibility errors, our institution no longer uses ceftolozane-tazobactam Etests to determine MICs. With unacceptable mortality rates in MDR *P. aeruginosa* infections and time to optimal therapy being a significant marker for better outcomes, inappropriate utilization of antibiotics in these scenarios is expected to have a negative impact on patient outcomes (10, 11). We support the idea that prompt and accurate susceptibility testing for new antimicrobials is of critical importance to effectively treat these infections (4).

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REFERENCES

1. Centers for Disease Control and Prevention. 2013. Antibiotic resistance threats in the United States. Centers for Disease Control and Prevention, Atlanta, GA. http://www.cdc.gov/drugresistance/pdf/ar-threats-2013-508.pdf. Accessed 2 September 2016.
2. Nathwani D, Raman G, Sulham K, Menon V. 2014. Clinical and economic consequences of hospital-acquired resistant and multidrug-resistant Pseudomonas aeruginosa infections: a systematic review and meta-analysis. Antimicrob Resist Infect Control 3:32. https://doi.org/10.1186/2047-2994-3-32.
3. Papp-Wallace KM, Bonomo RA. 2016. New β-lactamase inhibitors in clinic. Infect Dis Clin North Am 30:441–464. https://doi.org/10.1016/j.idc.2016.02.007.
4. Humphries RM, Hindler JA. 2016. Emerging resistance, new antimicrobial agents... but not tests! The challenge of antimicrobial susceptibility testing in the current US regulations. Clin Infect Dis 63:83–88. https://doi.org/10.1093/cid/ciw201.
5. Burns JL, Saiman L, Whittier S, Krewinski J, Liu Z, Larone D, Marshall SA, Jones RN. 2001. Comparison of two commercial systems (Vitek and MicroScan-WalkAway) for antimicrobial susceptibility testing of Pseudomonas aeruginosa isolate in cystic fibrosis patients. Diagn Microbiol Infect Dis 39:257–260. https://doi.org/10.1016/S0732-8893(01)00234-6.
6. Gagliotti C, Sarti M, Sabia C, Gargiulo R, Rossolini GM, Carillo C, Cassani C, Cipolloni AP, Pedna F, Rossi MR, Incerti SS, Testa G, Venturelli C, Moro ML. 2011. Accuracy of automated and manual susceptibility testing of *Pseudomonas aeruginosa* to piperacillin and piperacillin-tazobactam. New Microbiol 34:97–99.
7. CLSI. 2016. Performance standards for antimicrobial susceptibility testing; 26th informational supplement. CLSI M100S. CLSI, Wayne, PA.
8. CLSI. 2015. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 10th ed. CLSI M07. CLSI, Wayne, PA.
9. Clark RB, Lewinski MA, Loeffelholz MJ, Tibbetts RJ. 2009. Cumitech 31A, Verification and validation of procedures in the clinical microbiology laboratory. Coordinating ed, Sharp SE. ASM Press, Washington, DC.
10. Micek ST, Lloyd AE, Ritchie DJ, Reichley RM, Fraser VJ, Kollef MH. 2005. Pseudomonas aeruginosa bloodstream infection: importance of appropriate initial antimicrobial treatment. Antimicrob Agents Chemother 49:1306–1311. https://doi.org/10.1128/AAC.49.4.1306-1311.2005.
11. Kollef MH. 2000. Inadequate antimicrobial treatment: an important determinant of outcome for hospitalized patients. Clin Infect Dis 31:S131–S138. https://doi.org/10.1086/314079.

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RETRACTION

Retraction for Flynt et al., "Comparison of Etest to Broth Microdilution for Testing of Susceptibility of *Pseudomonas aeruginosa* to Ceftolozane-Tazobactam"

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Volume 55, no. 1, p. 334–335, 2017, https://doi.org/10.1128/JCM.01920-16. We hereby retract this article. In our paper we described discrepant results between ceftolozane-tazobactam Etests and broth microdilution. However, when repeat testing on the same isolates was done for a second, larger study recently published by R. M. Humphries, J. A. Hindler, P. Magnano, A. Wong Beringer, R. Tibbetts, and S. A. Miller (J Clin Microbiol 56:e01633-17, 2018, https://doi.org/10.1128/JCM.01633-17) we could not replicate the initial results. Upon review of the procedure used for broth microdilution it was determined that the tazobactam concentrations were not prepared according to CLSI guidelines (Clinical Laboratory Standards Institute, *Performance Standards for Antimicrobial Susceptibility Testing*, 26th ed, CLSI supplement M100S, 2016). We hypothesized that this may have contributed to the high MICs and very major errors. In order to test this hypothesis, we repeated the broth microdilution in the original laboratory using the same procedure as initially performed; however, we could not replicate the errors. Based on this outcome, a second hypothesis was that perhaps the bacteria possessed a resistance mechanism at the time of initial testing but that upon repeated subculture the mechanism was lost; however, we are unable to test this hypothesis.

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