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Reevaluation of the cefepime minimal inhibitory concentrations and disk diffusion test zone diameter relationship for a worldwide collection of Enterobacteriaceae enriched for extended-spectrum β-lactamase–producing organisms

Sudha Pottumarthy[a], Lalitagauri M. Deshpande[a], Helio S. Sader[a,*], Ronald N. Jones[a,b]

[a]*JMI Laboratories, North Liberty, IA 52317, USA*

[b]*Tufts University School of Medicine, Boston, MA 02111, USA*

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Abstract

To reassess the validity of existing susceptibility breakpoint criteria and to propose alternative breakpoint criteria for disk diffusion testing at lower susceptible MIC breakpoints, we analyzed a contemporary global collection of Enterobacteriaceae isolates (350) strains enriched for extended-spectrum β-lactamase (ESBL) producers (68 strains, 19.4%). The majority of the isolates (88.3% of the entire collection and 83.8% of the ESBL subset) were from bloodstream infections. Cefepime minimal inhibitory concentrations (MICs) were determined by broth microdilution methods and compared with the results obtained from disk diffusion testing for the entire collection of Enterobacteriaceae and for the ESBL subset alone. The regression coefficient was excellent for both scattergrams (r = 0.92–0.94). The intermethod categorical agreement remained excellent for the current breakpoints (susceptible at ≤8 μg/mL or ≥18 mm and resistant at ≥32 μg/mL or ≤14 mm) published by the National Committee for Clinical Laboratory Standards at 94.0%. The 2 alternative interpretive criteria considered at lower MIC breakpoints (i.e., susceptible as ≤4 μg/mL and ≥21 mm and susceptible as ≤2 μg/mL and ≥24 mm) did not compromise the intermethod test categorical accuracy, which remained excellent at 96.9% and 94.0%, respectively. Adopting the existing breakpoint criteria that remain accurate for ESBL-producing strains or any one of the above two alternative sets of breakpoint criteria analyzed would be acceptable, with excellent intermethod concordance between the MIC and disk diffusion results.

Keywords: Cefepime; MICs; Disk diffusion; NCCLS; Interpretive criteria; ESBL

1. Introduction

Cefepime is a fourth-generation cephalosporin with high antimicrobial potency and wide spectrum of activity (Elan Pharmaceuticals, 2004; Fuchs et al., 1985; Sanders, 1993). The distinctive efficacy of cefepime is attributable to its zwitterionic nature, resulting in rapid drug penetration into the Gram-negative cell, higher affinity for multiple essential penicillin-binding proteins, and high stability to hydrolysis by many Gram-negative β-lactamases, which is most apparent with the Bush group 1, Class C enzymes (Fuchs et al., 1985; Sanders, 1993). Nevertheless, cefepime can be hydrolyzed by extended-spectrum β-lactamases (ESBLs), although the degree of hydrolysis varies with the nature of the enzyme (Queenan et al., 2004).

The necessity to detect ESBL-producing strains became evident with increasing reports of clinical failures among infections caused by ESBL-producing strains treated with some third-generation cephalosporins (Bradford, 2001; Paterson et al., 2001). The clinical failures occurred even when the cephalosporin used had a minimal inhibitory concentration (MIC) in the susceptible range (often with a MIC at ≤8 μg/mL) using the existing breakpoints (Paterson et al., 2001). To overcome this deficiency, the National Committee for Clinical Laboratory Standards (NCCLS, 2005) has standardized specific screening and confirmatory tests for the detection of ESBL-producing strains of *Escherichia coli* and *Klebsiella* spp., with a recommendation to report ESBL-producing strains as resistant to all cephalosporins and monobactams. However, the process

* Corresponding author. Tel.: +1-319-665-3370; fax: +1-319-665-3371.
E-mail address: helio-sader@jmilabs.com (H.S. Sader).

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described above fails to address the entire problem in (1) recognizing the production of ESBLs in other enteric Gram-negative bacteria, such as *Enterobacter* spp., *Proteus* spp., and *Serratia* spp. (Bradford, 2001; Thomson, 2001), (2) the ability to recognize other enzymatic (β-lactamases other than ESBLs) and nonenzymatic (permeability and efflux changes) resistance mechanisms that can also lead to elevated MIC results to clinical failure, and (3) the ability to detect the presence of ESBLs when the clavulanic acid inhibitory effect is masked by the presence of inhibitor-resistant β-lactamases such as those of Bush Group 1 Class C or the presence of inhibitor-resistant Bush Group 2br enzymes (Thomson, 2001). Consequently, there is a need to reevaluate the susceptibility breakpoints for cephalosporins and many other β-lactams for MIC testing and the corresponding disk diffusion diameters tailored to predict favorable clinical response (Thomson, 2001; Paterson et al., 2001). To this end, we have evaluated the cefepime regression relationships using a global collection of Enterobacteriaceae isolates (2003) enriched for ESBL producers to reassess the validity of current disk diffusion test intermethod criteria and determine possible alternative breakpoints for disk diffusion testing at lower cefepime MIC breakpoint values that may be necessary to more accurately predict clinical success or failure.

2. Materials and methods

2.1. Strain collection

A contemporary international collection of 350 clinical isolates of Enterobacteriaceae collected in the SENTRY Antimicrobial Surveillance Program in 2003 were analyzed. The organisms included *E. coli* (74), *Klebsiella* spp. (79), *Enterobacter* spp. (43), *Citrobacter* spp. (29), *Serratia* spp. (32), *Proteus mirabilis* (30), *Salmonella* spp. (20), *Shigella* spp. (15), *Morganella morganii* (10), and others (18). The majority of isolates (293, 88.3%) were from blood stream infections, with the remainder being from urine (29, 8.3%), gastrointestinal infections (22, 6.3%), and documented skin and soft tissue infections (6, 1.7%). The collection included 68 (19.4%) ESBL producers: *Klebsiella pneumoniae* (43), *E. coli* (20), *P. mirabilis* (3), and *K. oxytoca* (2). Bloodstream culture isolates constituted 83.8% (57) of the ESBL producers with the remainder from urine (7, 10.3%) and documented skin and soft tissue infections (4, 5.9%).

2.2. Susceptibility testing methods

Cefepime MIC values and inhibitory zone diameters were determined by the reference broth microdilution and standardized disk diffusion methods in accordance with NCCLS (2003a, 2003b, 2005) guidelines. The quality control was assured by concurrent testing *E. coli* ATCC 25922, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923 and 29213 strains. All results were within published quality control limits (NCCLS, 2005).

2.3. Analysis of the results

Results of the disk diffusion test (zones of inhibition in mm) were compared with those of the reference broth microdilution method (MICs). The data were plotted as scattergrams and the least squares method was used to calculate the regression equation. The interpretive criteria used to calculate the intermethod agreement included the current NCCLS (2005) criteria for broth microdilution method (susceptible at ≤8 μg/mL, resistant at ≥32 μg/mL) and the corresponding disk diffusion method criteria (susceptible at ≥18 mm, resistant at ≤14 mm); as well as 2 alternative criteria: (1) susceptible at ≤4 μg/mL, resistant at ≥16 μg/mL for broth microdilution method and susceptible at ≥21 mm, resistant at ≤17 mm for the disk diffusion method, and (2) susceptible at ≤2 μg/mL, resistant at ≥8 μg/mL for broth microdilution method, and susceptible at ≥24 mm, resistant at ≤20 mm for the disk diffusion method.

3. Results

Cefepime had excellent activity against the Enterobacteriaceae strains' overall (susceptibility rates 74.7–100.0% by

Table 1
Distribution of tested species and cefepime potency (MIC_{50}, MIC_{90}, and ranges) for 350 Enterobacteriaceae enriched for ESBL-producing strains (68); only organism groups with ≥10 isolates were tabulated

Organism (no. tested)	MIC (μg/mL)			% susceptible[a]
	MIC_{50}	MIC_{90}	Range	
All *Citrobacter* (29)	0.03	0.25	≤0.016 to 4	100.0
C. freundii (14)	0.03	2	≤0.016 to 4	100.0
C. koseri (12)	≤0.016	0.03	≤0.016 to 0.06	100.0
All *Enterobacter* (43)	0.03	2	≤0.016 to 8	100.0
E. aerogenes (12)	0.03	0.12	≤0.016 to 8	100.0
E. cloacae (28)	0.06	4	≤0.016 to 8	100.0
E. coli (74)	0.03	>32	≤0.016 to >32	83.8[b]
All *Klebsiella* (79)	1	>32	≤0.016 to >32	74.7[b]
K. pneumoniae (71)	2	>32	≤0.016 to >32	74.6[b]
M. morganii (10)	≤0.016	0.06	≤0.016 to 8	100.0
P. mirabilis (30)	0.03	0.06	0.03 to >32	96.7[b]
Salmonella spp. (20)	0.03	0.06	≤0.016 to 0.25	100.0
Serratia (32)	0.06	1	0.03 to 4	100.0
Shigella spp. (15)	0.03	0.12	0.03 to 4	100.0
MIC range	≤0.016 to 2	0.03 to >32	≤0.016 to >32	–

[a] Susceptibility rate using M100-S15 (NCCLS, 2005) criteria at ≤8 μg/mL.
[b] Species containing ESBL-producing strains.