Comparison of ceftobiprole susceptibility testing using broth microdilution and gradient strip (Etest®)

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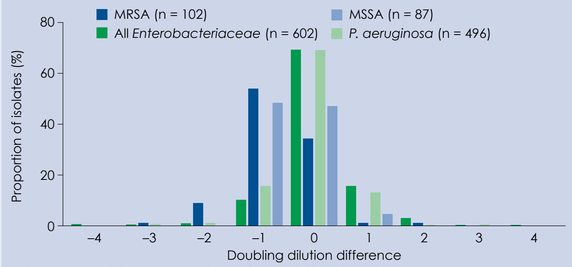
Revised abstract

Objectives: Ceftobiprole is a novel anti-methicillin-resistant *Staphylococcus aureus* (anti-MRSA) cephalosporin approved in Europe for the treatment of community-acquired pneumonia and hospital-acquired pneumonia (excluding ventilator-associated pneumonia). This study was carried out to confirm whether or not susceptibility testing by Etest® was comparable to the gold standard broth microdilution methodology.
Methods: A subset of 1287 isolates from a surveillance collection used previously to demonstrate the activity of ceftobiprole against clinical isolates from Europe and the Middle East was used (Rossolini *et al. J Antimicrob Chemother* 2011;66:151–9). Minimum inhibitory concentration (MIC) for ceftobiprole was determined by Clinical and Laboratory Standards Institute broth microdilution and by Etest® (bioMérieux) against 602 *Enterobacteriaceae*, 102 methicillin-resistant *S. aureus* (MRSA), 87 methicillin-susceptible *S. aureus* (MSSA) and 496 *Pseudomonas aeruginosa*. Absolute differences in MIC values were measured and MIC by broth was plotted against MIC by Etest® (MICs rounded to the nearest doubling dilution). Comparisons were also made according to European Committee on Antimicrobial Susceptibility Testing breakpoint category (*Enterobacteriaceae* [S ≤ 0.25 : R > 0.25 mg/L]; *S. aureus* [S ≤ 2 : R > 2 mg/L]) or pharmacokinetic/pharmacodynamic breakpoint category (*P. aeruginosa* [S ≤ 4 : R > 4 mg/L]). These were classified as no error, major error (false-resistant by Etest®) or very major error (false-susceptible by Etest®).
Results: For all isolates combined, 1235/1287 (96.0%) had an MIC by Etest® that differed by no more than one dilution from the broth MIC. This was consistent within the separate organism groups as shown in the Figure. All MSSA were susceptible to ceftobiprole (MIC ≤ 2 mg/L) with no discrepant results using Etest®. With MRSA, 9/102 (8.8%) were false-resistant by Etest® (major error). For *Enterobacteriaceae*, 4/602 (0.7%) were false-susceptible by Etest® (very major error) and 3/602 (0.5%) were false-resistant by Etest® (major error). For *P. aeruginosa*, no very major errors were observed and 4/496 (0.8%) were false-resistant by Etest® (major error).

Figure. MIC difference, broth microdilution versus Etest®.



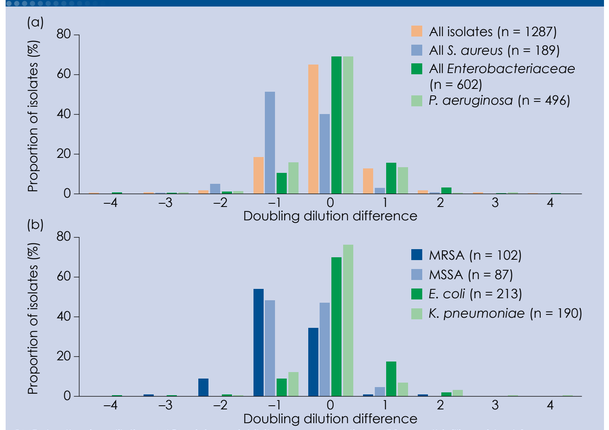
MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*.

Conclusions: MIC by Etest® compared very well with broth microdilution MIC. Etest® should therefore be a reliable device for clinical laboratories to determine ceftobiprole susceptibility with *Enterobacteriaceae*, *S. aureus* and *P. aeruginosa*.

Introduction and purpose

- Ceftobiprole medocaril is a novel cephalosporin approved in Europe for the treatment of adults with community-acquired pneumonia and hospital-acquired pneumonia (excluding ventilator-associated pneumonia).[1]
- Ceftobiprole (the active moiety of ceftobiprole medocaril) has broad-spectrum bactericidal activity against a wide range of pathogens, including both Gram-positive and Gram-negative bacteria, such as methicillin-susceptible *Staphylococcus aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), *Pseudomonas aeruginosa* and *Enterobacteriaceae*.[2]
- The Ceftobiprole Local Antibiotic Susceptibility Surveillance (CLASS) study was a comprehensive assessment of the *in vitro* activity of ceftobiprole and comparator antimicrobials against clinical isolates collected from a large number of local centres in Europe and the Middle East.[2]
  - Minimum inhibitory concentrations (MICs) were determined at local study centres using the ceftobiprole Etest® (bioMérieux, Marcy-l'Étoile, France). Any isolates that were classified as resistant to ceftobiprole at a local site plus a random selection of 10% of isolates from each site were retested at a central reference laboratory using broth microdilution (BMD) and Etest® methodologies.[3,4]
- The simplicity and convenience of the Etest® make it particularly useful in the clinical setting. Here, using data from the CLASS study, we aim to confirm whether the Etest® is comparable to the gold-standard BMD method for ceftobiprole susceptibility testing.

Figure 1. MIC doubling dilution difference between BMD and Etest® assays. (a) Main groups (all isolates, *Staphylococcus aureus*, *Enterobacteriaceae* and *Pseudomonas aeruginosa*). (b) Breakdown of organisms of interest (MRSA, MSSA, *Escherichia coli* and *Klebsiella pneumoniae*).



BMD, broth microdilution; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

Methods

- A total of 1287 clinical isolates from the CLASS study were analysed.
- MICs for ceftobiprole were determined by the Clinical Laboratory Standards Institute BMD method[4] and by the Etest® method[3] for:
  - 602 isolates of *Enterobacteriaceae* (213 *Escherichia coli* isolates, 190 *Klebsiella pneumoniae* isolates and 199 *Enterobacter* spp. isolates)
  - 189 isolates of *S. aureus* (102 MRSA and 87 MSSA)
  - 496 isolates of *P. aeruginosa*.
- MICs for the Etest® were rounded to the nearest doubling dilution and absolute differences in MIC values between the Etest® and BMD methods were calculated.
- Doubling dilution differences (DDDs) for BMD compared with Etest® were calculated as the proportion of isolates for which:
  - BMD and Etest® gave identical MICs (DDD of 0)
  - BMD gave a 2-, 4-, 8- or 16-fold *lower* MIC than Etest® (DDDs of –1, –2, –3 or –4, respectively)
  - BMD gave a 2-, 4-, 8- or 16-fold *higher* MIC than Etest® (DDDs of 1, 2, 3 or 4, respectively).
- The microbial susceptibilities as assessed by Etest® and BMD were compared according to the ceftobiprole European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoint for *Enterobacteriaceae* (susceptible [S] ≤ 0.25 : resistant [R] > 0.25 mg/L) and *S. aureus* (S ≤ 2 : R > 2 mg/L), or according to the pharmacokinetic/pharmacodynamic breakpoint for *P. aeruginosa* (S ≤ 4 : R > 4 mg/L).
  - False-resistance by Etest® compared with BMD was classified as a major error (ME).
  - False-susceptibility by Etest® compared with BMD was classified as a very major error (VME).

Results

- For all isolates combined, 1235/1287 (96.0%) had a MIC by Etest® that differed by no more than one doubling dilution from the MIC as assessed by BMD (Figure 1a). Similar results were observed for:
  - *Enterobacteriaceae*, 572/602 (95.0%)
  - *S. aureus*, 178/189 (94.2%)
  - *P. aeruginosa*, 485/496 (97.8%).
- The proportion of isolates for which the MICs differed by no more than one doubling dilution between Etest® and BMD was also consistent in the organism subgroups (Figure 1b):
  - MRSA, 91/102 (89.2%)
  - MSSA, 87/87 (100%)
  - *E. coli*, 205/213 (96.2%)
  - *K. pneumoniae*, 181/190 (95.3%).

Table 1. MIC as assessed by Etest® and BMD assay for: (a) MRSA; (b) MSSA; (c) *Enterobacteriaceae*; (d) *Pseudomonas aeruginosa*.

(a) MRSA (n = 102)

Etest® MIC (mg/L)	Number of isolates (n)					
4					9	2
2	1	7	31	21		
1	2	15	9			
0.5		2		1		
0.25	1	1				
	0.25	0.5	1	2	4	
	Broth microdilution MIC (mg/L)					

(b) MSSA (n = 87)

Etest® MIC (mg/L)	Number of isolates (n)						
4							
2							
1				1			
0.5		37	21	1			
0.25	4	20	3				
0.12							
	0.12	0.25	0.5	1	2	4	
	Broth microdilution MIC (mg/L)						

(c) *Enterobacteriaceae* (n = 602)

Etest® MIC (mg/L)	Number of isolates (n)													
> 32												1	3	223
32										1		1	3	8
16											1	2	3	7
8										2	12	8		1
4									3	7	3	2		
2									7	4	2			
1						1	1	4	4					
0.5				1	1		4	2						
0.25						2	4	3						
0.12				1	4	8	7							
0.06				43	56	15	4		1					
0.03	1	4		87	35	3								
0.015				1										
0.008		1												
	0.008	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	> 32
	Broth microdilution MIC (mg/L)													

(d) *P. aeruginosa* (n = 496)

Etest® MIC (mg/L)	Number of isolates (n)											
> 32								2	1	5	114	
32								3	19	7	2	
16								31	41	6	1	
8							4	79	14		1	
4						1	9	52				
2						7	37	24				
1					3	10	18					
0.5					1	1		1				
0.25												
0.12												
0.06	1											
	0.06	0.12	0.25	0.5	1	2	4	8	16	32	> 32	
	Broth microdilution MIC (mg/L)											

Breakpoint highlighted by superimposed black lines (MRSA, MSSA and *Enterobacteriaceae*: EUCAST breakpoint; *P. aeruginosa*: PK/PD breakpoint). Agreement between Etest® and BMD regarding susceptibility or resistance is shaded light green; isolates with identical MIC by Etest® and BMD are shaded dark green. Major errors and very major errors are shaded light and dark orange, respectively.
BMD, broth microdilution; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC, minimum inhibitory concentration; MRSA, methicillin-resistant *Staphylococcus aureus*; MSSA, methicillin-susceptible *S. aureus*; PD, pharmacodynamic; PK, pharmacokinetic.

- Overall, there was a clear correlation between the ceftobiprole MICs determined by the Etest® and BMD assay (Table 1).
  - For *S. aureus*, 178/189 isolates (94.2%) were susceptible to ceftobiprole by Etest® according to the EUCAST breakpoint.
  - All MSSA isolates were susceptible to ceftobiprole, with no discrepant results between the BMD and the Etest®.
  - For MRSA, 91/102 isolates (89.2%) were susceptible to ceftobiprole as assessed by Etest®, while the BMD identified 100/102 isolates (98.0%) as susceptible.

Table 2. Comparison of susceptibilities by BMD and Etest® based on EUCAST breakpoints.

Species	Number of isolates	Major error (false-resistant by Etest®), n (%)	Very major error (false-susceptible by Etest®), n (%)
All *Staphylococcus aureus*	189	9 (4.8%)	0
MRSA	102	9 (8.8%)	0
MSSA	87	0	0
All *Enterobacteriaceae* members	602	3 (0.5%)	4 (0.7%)
Escherichia coli	213	1 (0.5%)	0
Klebsiella pneumoniae	190	0	1 (0.5%)
Enterobacter spp.	199	2 (1.0%)	3 (1.5%)
Pseudomonas aeruginosa	496	4 (0.8%)	0

BMD, broth microdilution; EUCAST, European Committee on Antimicrobial Susceptibility Testing; MRSA, methicillin-resistant *S. aureus*; MSSA, methicillin-susceptible *S. aureus*.

  - For the *Enterobacteriaceae*, 280/602 isolates (46.5%) were susceptible to ceftobiprole as assessed by Etest®, similar to the assessment by BMD (279/602 [46.3%]).
  - For *P. aeruginosa*, 166/496 (33.5%) were susceptible to ceftobiprole as assessed by Etest®, similar to the BMD results (170/496 [34.3%]).
- For 1267/1287 isolates (98.4%), there was no ME or VME when classification by Etest® was compared with the gold standard BMD classification; overall, only four isolates were classified as false-susceptible by Etest® (VME), while 16 isolates were classified as false-resistant by Etest® (ME; Table 2).
  - No *S. aureus* isolates were identified as false-susceptible by Etest®, although 9/189 (4.8%) were false-resistant (all from the MRSA group).
  - For the *Enterobacteriaceae*, 4/602 (0.7%) isolates were identified as false-susceptible by Etest®, and 3/602 (0.5%) were identified as false-resistant by Etest®. The four VMEs for the *Enterobacteriaceae* occurred in *K. pneumoniae* (n = 1) and *Enterobacter* spp. (n = 3) isolates; the three MEs occurred in *E. coli* (n = 1) and *Enterobacter* spp. (n = 2) isolates.
  - For *P. aeruginosa*, no VMEs were observed, and 4/496 (0.8%) isolates were identified as false-resistant by Etest®.

Conclusions

- Ceftobiprole is approved in Europe for the treatment of adults with community-acquired pneumonia and hospital-acquired pneumonia (excluding ventilator-associated pneumonia). A convenient and reliable test for susceptibility to ceftobiprole would facilitate the use of this broad-spectrum cephalosporin in clinical practice.
- Overall, this study shows strong agreement between Etest® and BMD assay when determining ceftobiprole MICs and the susceptibility of Gram-positive and Gram-negative bacterial pathogens.
  - For all 1287 isolates tested, 96% of samples did not differ in MIC by more than one dilution between Etest® and BMD. There were no VMEs with the Etest® in *S. aureus* and *P. aeruginosa* isolates, and only four (0.7%) VMEs among the *Enterobacteriaceae* isolates.
- There is a high level of agreement between the MICs for ceftobiprole as assessed by the Etest® and gold-standard BMD assay. The Etest® thus offers a reliable and convenient alternative to the BMD method for determining the susceptibility of clinical pathogens to ceftobiprole.

References

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Disclosures

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