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Etest® versus broth microdilution for ceftaroline MIC determination with *Staphylococcus aureus*: results from PREMIUM, a European multicentre study

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Objectives: To compare the concordance of ceftaroline MIC values by reference broth microdilution (BMD) and Etest (bioMérieux, France) for MSSA and MRSA isolates obtained from PREMIUM (D372SL00001), a European multicentre study.

Methods: Ceftaroline MICs were determined by reference BMD and by Etest for 1242 MSSA and MRSA isolates collected between February and May 2012 from adult patients with community-acquired pneumonia or complicated skin and soft tissue infections; tests were performed across six European laboratories. Selected isolates with ceftaroline resistance in broth (MIC >1 mg/L) were retested in three central laboratories to confirm their behaviour.

Results: Overall concordance between BMD and Etest was good, with >97% essential agreement and >95% categorical agreement. Nevertheless, 12 of the 26 MRSA isolates found resistant by BMD scored as susceptible by Etest, with MICs ≤1 mg/L, thus counting as very major errors, whereas only 5 of 380 MRSA isolates found ceftaroline susceptible in BMD were miscategorized as resistant by Etest. Twenty-one of the 26 isolates with MICs of 2 mg/L by BMD were then retested twice by each of three central laboratories: BMD MICs of 2 mg/L were consistently found for 19 of the 21 isolates. Among 147 Etest results for these 21 isolates (original plus six repeats per isolate) 112 were >1 mg/L.

Conclusions: BMD and Etest have good overall agreement for ceftaroline against *Staphylococcus aureus*; nevertheless, reliable Etest-based discrimination of the minority of ceftaroline-resistant (MIC 2 mg/L) MRSA is extremely challenging, requiring careful reading of strips, ideally with duplicate testing.

Introduction

Ceftaroline is a new cephalosporin with broad activity against common Gram-positive and Gram-negative pathogens including MRSA.[1,2] It has proved superior to vancomycin plus aztreonam in complicated skin and soft tissue infections (cSSTI) and to ceftriaxone in community-acquired pneumonia (CAP).[1–3] On the basis of these trials, ceftaroline, administered as its fosamil ester, was approved for treatment of these infections by the US FDA in October 2010,[4] and by the EMA in August 2012.[5]

EUCAST categorizes *Staphylococcus aureus* with ceftaroline MICs of ≤1 mg/L as susceptible and those with MICs >1 mg/L as resistant.[6] This might present a detection challenge, because the 'tail' of the ceftaroline MIC distribution for MRSA extends to 2 mg/L.[7] It was shown previously that routine diagnostic laboratories, at least in the UK,[8] are poorly able to distinguish isolates with MICs >1 mg/L from more susceptible organisms using either disc or gradient tests, though better discrimination was achieved using discs in a study in Sweden.[9] In the present analysis we compare Etest versus standard broth microdilution (BMD) for ceftaroline

MIC determination. Moreover, we sought to examine whether discrimination of isolates with MICs of 2 mg/L was achieved with these methods. This analysis was undertaken in the course of PREMIUM (D372SL00001), a multicentre European survey that evaluated the activity of ceftaroline.

Materials and methods

Clinical isolates

Consecutive clinical isolates from cSSTI and CAP in patients aged ≥18 years were collected from February to May 2012 at 58 laboratories in: Belgium (10 sites), Italy (16), Portugal (6), Spain (15), Switzerland (2) and the UK (9). For CAP, samples were taken from sputum, bronchoalveolar lavage (BAL), tracheal/bronchial aspirate, bronchoscopic protected brush specimen, blood culture or pleural fluid. For cSSTI, fine needle aspiration puncture/biopsy was preferred, although good-quality swabs were also permitted. Collected isolates were then sent to the central laboratory in the corresponding country, along with information about the microorganism and the source patient, recorded in an electronic Case Report Form. The study was approved by the Ethics Committee (ref. 336/1, Ramón y Cajal University Hospital, Madrid, Spain).

Antimicrobial susceptibility testing

Organisms were tested for susceptibility to ceftaroline by BMD and Etest (bioMérieux, France) at six central laboratories. Each of these laboratories tested isolates from their respective countries using the same methods and quality control *S. aureus* ATCC 25923 isolate. BMD MICs were determined using Sensititre plates (ThermoFisher Scientific, UK) with Mueller–Hinton broth, as indicated by both EUCAST and CLSI specifications.[6,10,11] The concentration range for ceftaroline was 0.008–4 mg/L. Etest had ceftaroline gradients from 0.002 and 32 mg/L, which were used on Mueller–Hinton agar from the laboratories' local suppliers. MICs were analysed with respect to EUCAST criteria, with ≤1 mg/L considered susceptible and MIC >1 mg/L resistant.[6]

Data analysis

S. aureus isolates with MIC values obtained by both methods were included in the analysis. Etest MICs were rounded up to the next concentration of the standard doubling dilution scale when necessary. Concordance criteria included: essential agreement (EA) (i.e. agreement within one doubling dilution between methods), categorical agreement (CA) (agreement as susceptible or resistant), major errors (susceptible by BMD but resistant by Etest) and very major errors (VMEs; resistant by BMD but susceptible by Etest).[12] MIC distributions were compared by regression of log MICs using WHONET software.[13]

Repetitive study: selective retesting of clinical isolates

Initial analyses revealed MICs >1 mg/L by BMD for 26 MRSA isolates. To confirm these values, 21 of these isolates were retested twice by both methods at the three central laboratories in Italy, Spain and the UK, with results recorded by different operators. The remaining five isolates were not available for reanalysis. Additionally, two isolates with MIC 1 mg/L were also retested as controls.

Results

MIC agreement: BMD versus Etest

A total of 1242 *S. aureus* isolates (836 MSSA and 406 MRSA) were tested by both methods. For MSSA, the MIC_{50}/MIC_{90} by BMD was 0.25/0.25 mg/L (range ≤0.008–1 mg/L) and 0.25/0.25 mg/L by Etest (range 0.008–1 mg/L). For MRSA, the corresponding values were 0.5/1.0 mg/L (range 0.125–4 mg/L) and 0.5/1.0 mg/L (range 0.016–4 mg/L) by BMD and Etest, respectively. EA was 97.1% for MSSA and 97.3% for MRSA, whereas CA was 100% for MSSA and 95.8% for MRSA. Rounded-up MICs by Etest were lower than by BMD in 308 (24.8%) cases, higher in 150 (12.1%) cases and identical in 784 (63.1%) cases. MIC correlation across the MIC spectrum was $r=0.37$ (MSSA) and $r=0.62$ (MRSA) (Figure 1).

Error rates

All MSSA isolates were found susceptible by both BMD and Etest (Table 1). Among MRSA isolates, 17/406 (4.2%) were counted as resistant by Etest but susceptible by BMD or vice versa. Crucially, among the 26 isolates found resistant by BMD, with MICs of 2–4 mg/L, 12 were found susceptible (i.e. MIC ≤1 mg/L) by Etest, equating to a very major error rate of 46.2%. The major error rate (susceptible by BMD but resistant by Etest) was much lower, at 5/380 cases (1.3%).

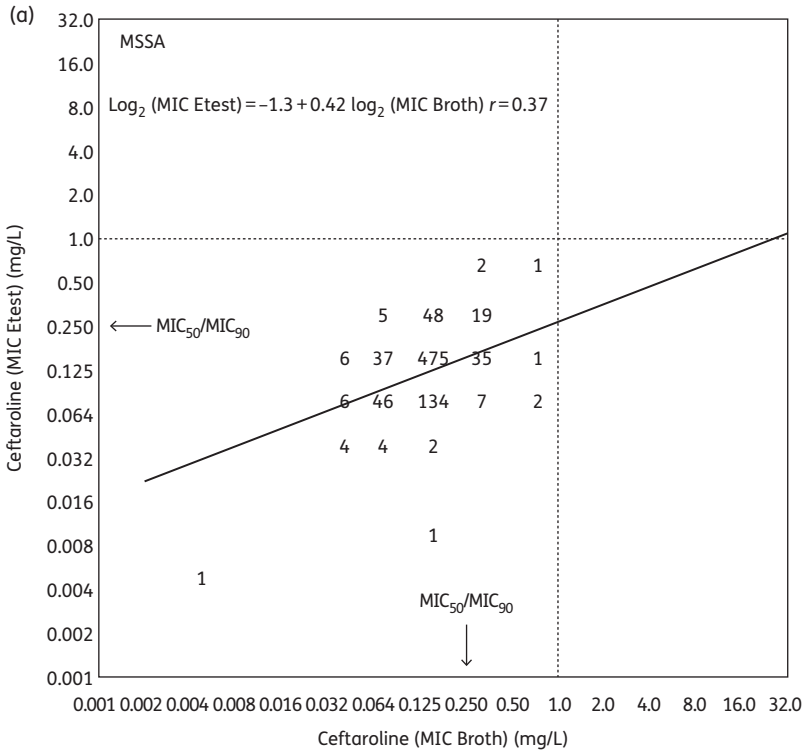
Repetitive study

To further examine reproducibility and discrimination for isolates with raised MICs, we selected 21 isolates with MIC 2 mg/L and two with MIC 1 mg/L by BMD in the original analysis (Table 1). These were then retested twice in each of three different central laboratories, giving seven results per method per isolate in total (six in the repetitive study plus the original data). Nineteen isolates gave consistent MICs of 2 mg/L by BMD, irrespective of where and when tested. Among the 147 corresponding Etest results (7×21), 112 indicated resistance (MIC >1 mg/L) and 35 susceptibility (MIC 1 mg/L). Only 2 of the 19 isolates with consistent broth MICs of 2 mg/L had a majority of Etest results at 1 mg/L, though 17 had a majority of results ≥1.5 mg/L, rounded to 2 mg/L. Nine of the 19 had no results at 1 mg/L (Table 1).

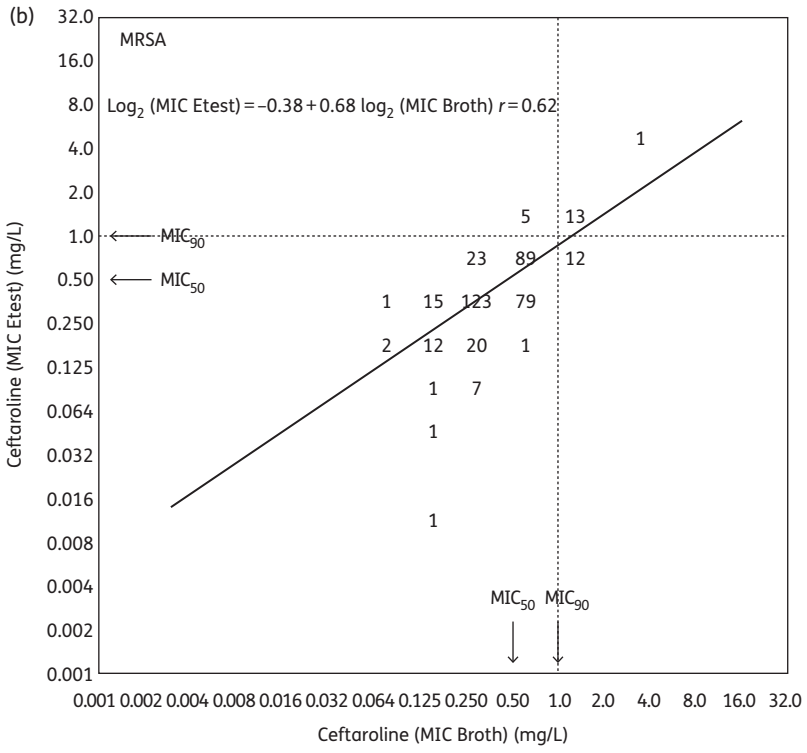
Discussion

Ceftaroline is one of a very few broad-spectrum agents with a spectrum including MRSA (the only others are tigecycline and ceftobiprole), and it is important that laboratories are able to test it reliably. Although automated systems or disc testing are the routine methods of susceptibility testing in most diagnostic laboratories, gradient strips such as Etest are widely used for low-throughput, high-precision work, when a more precise estimate of the MIC is sought.

This study was therefore designed to compare Etest and standard BMD methods for ceftaroline MIC determination, and sought to assess whether Etest could reliably detect *S. aureus* isolates with MICs >1 mg/L, which count as resistant according to EUCAST criteria,[6] though as intermediate on CLSI and FDA breakpoints.[10] This detection is inherently challenging, as MICs for these isolates are almost invariably 2 mg/L (as in 25/26 cases here, Figure 1), and they might represent the tail of a normal distribution for MRSA isolates with WT PBP2a protein,[7] though recent whole-genome sequence analysis shows that isolates with ceftaroline MICs of ~2–4 mg/L often have amino acid substitutions in the non-penicillin-binding domain of PBP2a.[14]



Ceftaroline-susceptible *S. aureus* = MIC ≤1 mg/L.



Ceftaroline-susceptible *S. aureus* = MIC ≤1 mg/L.

Figure 1. Comparison of ceftaroline MICs by BMD and Etest for (a) MSSA and (b) MRSA (analysis is based on initial results and excludes those of retesting).

Table 1. Variability of repeat tests across centres

Country	Original test centre	Original BMD test MIC result	Total number of tests with described MIC by BMD in the repetitive study				Final BMD MIC value	Original Etest MIC result	Number of tests with described MIC by Etest in the repetitive study								Final Etest MIC result
			0.25	0.5	1.0	2.0			0.19	0.25	0.38	0.5	0.75	1.0	1.5	2.0	
Spain	3404–46	2				6	2	1						3	3		1
Spain	3408–14	1		4	2		0.5	0.5			2	2		2			0.5
Spain	3409–64	2				6	2	2						1	3	2	2
Spain	3411–33	1	2			4	2	1	2			2		2			1
Portugal	3502–106	2				6	2	1.5							4	2	2
Portugal	3502–115	2				6	2	1.5						2	4		2
Portugal	3503–14	2			4	2	1	0.75					2	4			1
Italy	3901–006	2				6	2	1							3	3	2
Italy	3901–049	2				6	2	1.5							4	2	2
Italy	3901–032	2				6	2	1						2	4		2
Italy	3907–006	2				6	2	1						5	1		1
Italy	3907–10	2				6	2	1.5							6		2
Italy	3907–48	2				6	2	1						2	4		2
Italy	3909–081	2				6	2	1.5							4	2	2
Italy	3909–154	2				6	2	2						1		5	2
Italy	3909–164	2				6	2	1.5							5	1	2
Italy	3909–206	2				6	2	1.5							2	4	2
Italy	3909–244	2				6	2	1							3	3	2
Italy	3914–023	2				6	2	1.5							6		2
Italy	3914–40	2				6	2	1						2	4		2
Italy	3920–004	2				6	2	2							2	4	2
Italy	3923–021	2			2	4	2	1						2	2	2	2
Italy	3920–045	2				6	2	1.5							4	2	2

A total of seven tests were carried out per isolate (original result plus six in the repetitive study). Tests were performed at the original centre shown and repeated twice at three central laboratories in Spain, Italy and the UK. The mode was taken as the final MIC when results across tests differed by ≤2 doubling dilutions, and the median if results differed by >2 dilutions. An MIC >1 mg/L is resistant according to EUCAST criteria. MIC values are expressed in mg/L.

Such substitutions are prevalent in, for example, South Korea, China and Thailand.[15]

In general, agreement between Etest and BMD MIC results is good, although there are some systematic biases toward higher or (mostly) lower MICs by Etest for particular microorganism–antibiotic combinations.[16–19] In the current study, Etest returned slightly lower MICs than BMD for *S. aureus* and, although EA and CA between the two methods exceeded 95%, almost half (12/26, 46.2%) of the MRSA isolates found resistant by BMD were scored as susceptible, with MICs of 1 mg/L, by Etest in the original testing. These findings, which were based on pooled results from the six national laboratories, led us to retest 23 isolates, 21 of them with initial MICs of 2 mg/L by BMD, at three central laboratories. Each site tested each isolate twice by each method, with different staff scoring the Etest and broth results. BMD MICs of 2 mg/L were found for 19 isolates in all repeats at all sites, supporting the view that their resistance was 'real'. For 17, Etest MICs of >1.5 mg/L (2 mg/L after rounding) were obtained in a majority of the seven tests performed per isolate. This experience suggests that, with careful reading, it is possible to reduce the proportion of cases where low-level ceftaroline resistance is missed by Etest, thus decreasing potential VME results. Performance may also be improved if multiple Etests are run per isolate, albeit at additional cost of time and materials. Reproducibility of MIC values with gradient tests from other manufacturers also showed no clear distinction between isolates with MIC results of 1 and 2 mg/L.[19]

In conclusion, ceftaroline achieves robust *in vitro* antibacterial activity against the great majority of *S. aureus* from cSSTI and CAP in the European countries surveyed. Nevertheless, MICs of 2 mg/L, signifying resistance on EUCAST criteria or intermediate status on CLSI and FDA criteria, are seen for a small minority of isolates by BMD. Detection of these non-susceptible organisms by Etest is challenging, even under reference laboratory testing, as here, requiring experience and diligence on the part of the individual reader. We cannot rule out heteroresistance and/or protein amino acid substitutions in the non-penicillin-binding domain of PBP2a of these isolates.[14,15,20]

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