In vitro activity of ceftaroline against *Klebsiella* spp. as evaluated by Broth Microdilution and Etest

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Abstract

Objectives: Ceftaroline is an advanced generation cephalosporin with activity against Gram-positive and Gram-negative organisms including the enterobacteriaceae. As ceftaroline is new to the market, automated susceptibility testing panels do not contain ceftaroline, and if susceptibility testing is warranted, institutions utilize Etests in order to determine minimum inhibitory concentrations (MICs). Previous analyses have demonstrated that for some organism/antimicrobial combinations discrepancies exist between methodologies leading to organisms being called falsely susceptible or resistant based on methodology. We completed this analysis to see if any discrepancy exists for ceftaroline and *Klebsiella spp.*

Methods: 107 unique patient *Klebsiella* isolates (83 *K.pneumoniae*, 24 *K.oxytoca*) were collected at the Detroit Medical Center. Each isolate underwent susceptibility testing via Etest and BMD and the results were analyzed for concordance.

Results: Overall there was excellent concordance between methodologies. Out of 72 non-ESBL producing *k.pneumoniae* 52 (72%) were susceptible via BMD and 55 (72%) via Etest. For *k.oxytoca* 21/22 (95%) of the non-ESBL producing isolates were susceptible by both methodologies (Table 1). Out of the 87 isolates that could be analyzed minor errors occurred in 4% of isolates, there were no major errors, and the very major error rate was 3%. In all three of the major errors the organism was highly susceptible via Etest, but resistant via BMD. Encouragingly, when comparing methodologies, 83/87 (95%) isolates were within one dilution of each other.

Conclusions: Utilizing Etests for determining susceptibility clinical *Klebsiella* isolates should be considered an appropriate method. Further analysis into the isolates with very major errors is warranted

Introduction

- Ceftaroline is an advanced generation cephalosporin with activity against both Gram-positive bacteria, including Methicillin-resistant *Staphylococcus aureus* (MRSA) and Gram-negative bacteria, including more susceptible strains of enterobacteriaceae
- A potential area for use of ceftaroline is for treatment of polymicrobial infections due to both MRSA and susceptible enterobacteriaceae
- Ceftaroline testing is not currently available on automated susceptibility testing panels; and if a minimum inhibitory concentration (MIC) for ceftaroline is desired it must be completed via Etest
- Ceftaroline Etest is only approved for *S. aureus* MIC testing; and for research use only for *Klebsiella spp.*
- Significant MIC discrepancies between MIC testing methodologies have been reported for other antimicrobials for certain pathogens, and Etest typically reports higher MICs compared to broth microdilution (BMD).
- Comparisons between the readily available Etest, and the gold standard, BMD, are warranted

Purpose:

To determine if any discrepancies exist for ceftaroline activity against *Klebsiella spp.* as determined by Etest compared to BMD.

Methods

107 consecutive, unique patient *Klebsiella spp.* strains collected from patient samples (blood, urine, lung) (83 *K.pneumoniae*, 24 *K.oxytoca*) were included

All isolates tested for susceptibility by BMD and Etest

- FDA breakpoints of ≤ 0.5 (S), 1 (I), and ≥ 2 (R) were used for susceptibility determination
- All ESBL-producers were resistant by both methods and removed from further analysis (n = 11 *K. pneumoniae*, 2 *K.oxytoca*)

Susceptibility testing was performed on 94 remaining isolates (72 *K.pneumoniae*, 22 *K.oxytoca*)

Isolates without defined endpoints for either Etest or BMD were excluded from further analysis

87 remaining isolates were analyzed for concordance between testing methods (65 *K.pneumoniae*, 22 *K.oxytoca*)

- Negative numbers for concordance indicate that MIC, as determined by BMD, was lower compared to MIC as determined by Etest
- Positive numbers for concordance indicate that MIC determined by BMD was higher than that determined by Etest
- Minor error: isolates interpreted as intermediate by Etest, that are either susceptible or resistant by BMD
- Major error: isolate interpreted as false-resistant by Etest, that are susceptible by BMD
- Very major error: isolate interpreted as false susceptible by Etest, that are resistant by BMD

Results

Non-ESBL *K.pneumoniae* ceftaroline susceptibility (n=72)

	Susceptible	Intermediate	Resistant	MIC50	MIC90	Range
BMD	52 (72)	2 (3)	18 (25)	0.25	16	(0.03- >16)
Etest	55 (76)	2 (3)	15 (21)	0.25	8	(0.016 - >32)

Percent susceptible listed as n (%)

Non-ESBL *K. oxytoca* ceftaroline susceptibility (n=22)

	Susceptible	Intermediate	Resistant	MIC50	MIC90	Range
BMD	21 (95)	0 (0)	1 (5)	0.25	0.5	(0.06 - >16)
Etest	21 (95)	0 (0)	1 (5)	0.25	0.5	(0.06 - >32)

Percent susceptible listed as n (%)

K. pneumoniae concordance (n=65)

BMD versus Etest n (%) MIC variation (log2 dilutions)							
Strains with an Etest MIC (mg/L) of	-2	-1	0	+1	+2	+3	≥+4
0.016	-	-	1 (100)	-	-	-	-
0.03	-	-	-	1 (100)	-	-	-
0.06	-	1 (50)	1 (50)	-	-	-	-
0.125	1 (3.2)	12 (38.7)	11 (35.4)	6 (19.3)	-	-	1 (3.2)
0.25	-	8 (44.4)	7 (38.9)	1 (5.6)	-	-	2 (11.1)
0.5	-	-	1 (50)	1 (50)	-	-	-
1	-	1 (50)	-	1 (50)	-	-	-
2	-	1 (33.3)	-	2 (66.7)	-	-	-
4	-	-	1 (50)	1 (50)	-	-	-
8	-	-	1 (20)	1 (20)	-	-	-

Listed as n (%)

K.oxytoca concordance (n=22)

BMD versus Etest n (%) MIC variation (log2 dilutions)			
Strains with an Etest MIC (mg/L) of	-1	0	+1
0.06	-	1(100)	-
0.125	-	3 (100)	-
0.25	2 (28)	2 (28)	3 (43)
0.5	3 (30)	7 (70)	-
1	-	-	-
2	-	1(100)	-

Listed as n (%)

Error rates

	Minor Error	Major Error	Very Major Error
K.pneumoniae	2 (3)	0 (0)	3 (5)
K. oxytoca	0 (0)	0 (0)	0 (0)

Listed as n (%)

Conclusions

- Excellent concordance was seen between MIC testing methodologies.
  - Etest and BMD test results were within one dilution of one another for 83/87 (95%) isolates.
- There were three very major errors where, interestingly, the isolate was susceptible to ceftaroline, as determined by Etest, but resistant as determined by BMD. Further analysis of these isolates is warranted.
- Etest should be considered to be a reliable method for determining ceftaroline susceptibility for *Klebsiella spp.*

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