

# Comparison of EUCAST and CLSI ceftaroline minimum inhibitory concentration (MIC) for *H. influenzae* and *S. pneumoniae*

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## Abstract

**Objectives:** Ceftaroline, the active metabolite of ceftaroline fosamil, is a new cephalosporin with activity *in vitro* against a range of organisms including methicillin resistant *Staphylococcus aureus* (MRSA). Ceftaroline fosamil is indicated in the EU for treatment of complicated skin and soft tissue infections and community-acquired pneumonia (CAP). EUCAST and CLSI MIC methods for fastidious organisms use different media. The objective of this study was to compare ceftaroline MIC results using EUCAST and CLSI methods for 2 CAP pathogens, *Streptococcus pneumoniae* and *Haemophilus influenzae*.

**Methods:** Antimicrobial susceptibility testing was performed in 2011 using CLSI and EUCAST broth microdilution methods on a collection of global stock strains, consisting of 124 *S. pneumoniae* and 52 *H. influenzae*. Quality control strains, *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247 and *H. influenzae* NCTC 8468 were also tested. MIC plates, containing ceftaroline concentrations of 0.001 – 1 mg/L, were prepared by Laboratory Specialists, Inc. (LSI). Cation adjusted Mueller Hinton broth (CAMHB) + 5% lysed horse blood was used for testing *S. pneumoniae* in the CLSI test method and the same media with the addition of 20 mg/L beta-NAD was used for *S. pneumoniae* and *H. influenzae* in the EUCAST method. Haemophilus testing media (HTM) was used in the CLSI method for testing of *H. influenzae*.

**Results:** *S. pneumoniae* ceftaroline MICs using EUCAST and CLSI methods ranged from 0.004-1 mg/L. *H. influenzae* ceftaroline MICs using EUCAST and CLSI methods ranged from 0.004-0.06 and 0.004-0.12 mg/L, respectively. All MIC results were within one doubling dilution for all strains tested. The distribution of dilution differences for both species is shown in Table 1. Ceftaroline MIC results for the quality control strains, *S. pneumoniae* ATCC 49619 and *H. influenzae* ATCC 49247, were within the CLSI established ranges of 0.008-0.015 mg/L and 0.03-0.12 mg/L, respectively. For *H. influenzae* NCTC 8468, ceftaroline MIC was 0.008 mg/L by EUCAST and 0.004 mg/L by CLSI.

Organism	MIC difference (EUCAST – CLSI)		
	-1 dilution	same	+1 dilution
<i>S. pneumoniae</i>	4	110	10
<i>H. influenzae</i>	1	25	26

**Conclusion:** There was excellent correlation of ceftaroline MIC results between CLSI and EUCAST methods among 124 *S. pneumoniae* and 52 *H. influenzae* tested, with a skewing of slightly higher EUCAST MIC results towards +1 dilution.

## Methods

**Isolates:** 124 *S. pneumoniae* (selected based on resistance to penicillin) and 52 *H. influenzae* were frozen stock strains from clinical sources, collected over several years from a variety of global geographical areas.

**Testing Site:** Laboratory Specialists, Inc., Westlake, OH.

**MIC Method:** Broth Microdilution according to CLSI/EUCAST/ISO method with broth being only variable that was different as follows:

	EUCAST	CLSI
<i>S. pneumoniae</i>	Cation adjusted Mueller Hinton + 5% Lysed Horse Blood + 20 mg/L β-NAD (MH-F broth)	Cation adjusted Mueller Hinton + 5% Lysed Horse Blood (CAMHB+5%LHB)
<i>H. influenzae</i>	Cation adjusted Mueller Hinton + 5% Lysed Horse Blood + 20 mg/L β-NAD (MH-F broth)	Haemophilus Test Medium (HTM)

Figure 1 (A-B): *Streptococcus pneumoniae* and *Haemophilus influenzae* ceftaroline MIC distributions

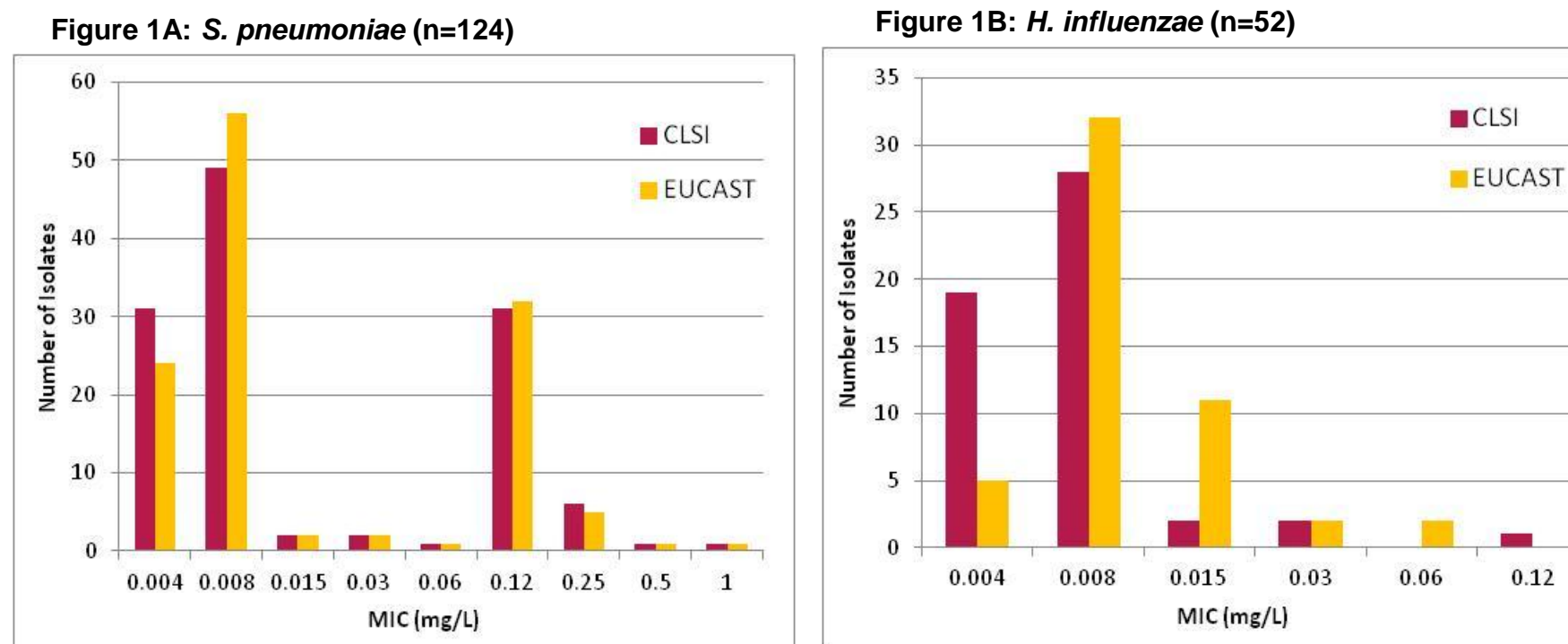
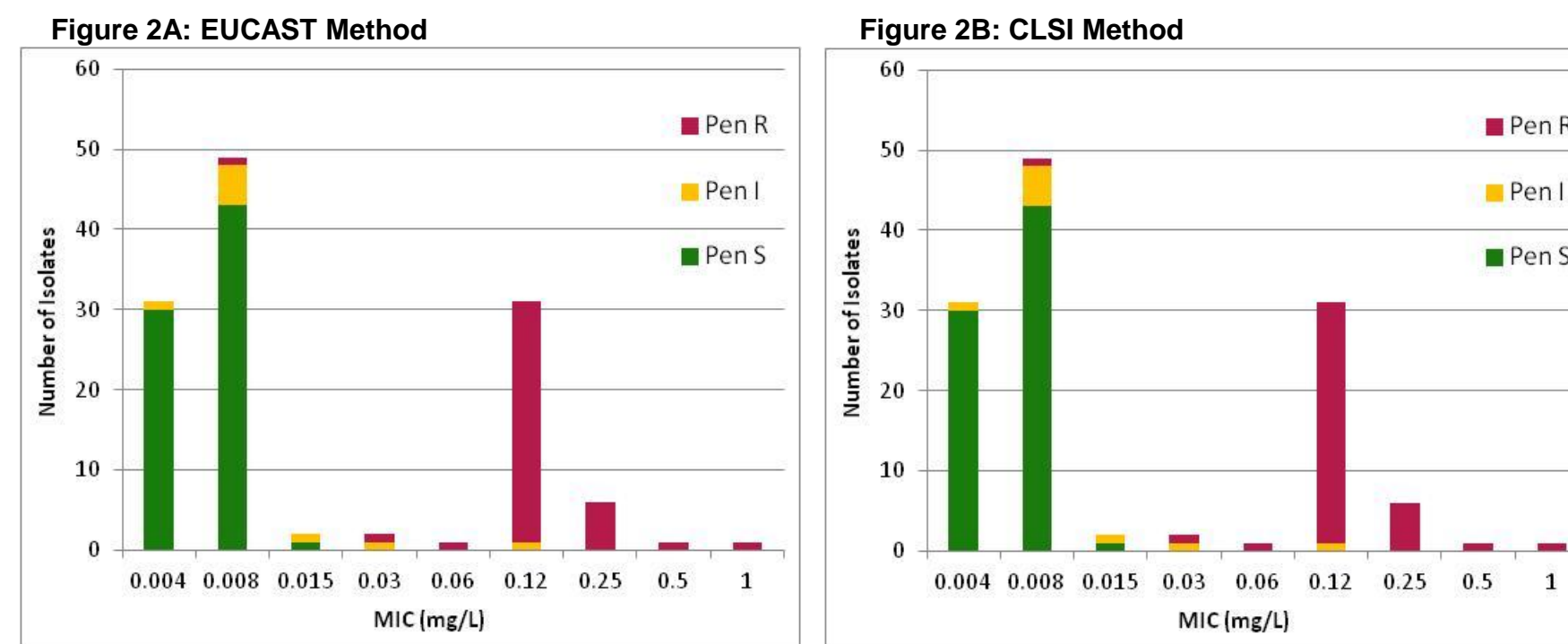


Figure 2 (A-B): *Streptococcus pneumoniae* ceftaroline MIC distributions by susceptibility to benzylpenicillin (using EUCAST non-meningitis breakpoints S≤0.06, R>2 mg/L)



Strain	EUCAST MIC (mg/L)	CLSI MIC (mg/L)	EUCAST expected range (mg/L)	CLSI expected range (mg/L)
<i>H. influenzae</i> ATCC 49247	0.06	0.06	NA	0.03-0.12
<i>H. influenzae</i> NCTC 8468	0.008	0.004	In Process	NA
<i>S. pneumoniae</i> ATCC 49619	0.015	0.008	0.008-0.03	0.008-0.03
	0.015	0.015		
	0.015	0.015		
	0.015	0.015		

## Results

### *Streptococcus pneumoniae*

- For 88.7% (110/124) of *S. pneumoniae* strains, EUCAST and CLSI MIC results were the same (Figure 1A).
- For 3.2% (4/124) of *S. pneumoniae*, EUCAST MIC results were one dilution lower than CLSI MIC results.
- For 8.1% (10/124) of *S. pneumoniae*, EUCAST MIC results were one dilution higher than CLSI MIC results.
- There was no difference in the visible growth of *S. pneumoniae* in the 2 media.

### *Haemophilus influenzae*

- For 48.1% (25/52) of *H. influenzae*, EUCAST and CLSI MIC results were the same (Figure 1B).
- For 1.9% (1/52) of *H. influenzae*, EUCAST MIC results were one dilution lower than CLSI MIC results.
- For 50.0% (26/52) of *H. influenzae*, EUCAST MIC results were one dilution higher than CLSI MIC results.
- H. influenzae* growth was visibly more apparent in the EUCAST media; MIC endpoints were easier to determine.

## Conclusions

- S. pneumoniae* ceftaroline MIC results were similar for both EUCAST and CLSI methods. The addition of NAD to CAMHB+5% LHB did not impact MIC results or growth of *S. pneumoniae*.
- H. influenzae* ceftaroline MIC results for EUCAST and CLSI methods were within 1 doubling dilution, but skewed slightly higher for EUCAST. Visible growth of *H. influenzae* was better in MH F broth compared to HTM.

## References

- EUCAST: [http://www.eucast.org/antimicrobial\\_susceptibility\\_testing](http://www.eucast.org/antimicrobial_susceptibility_testing)
- CLSI. 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 8<sup>th</sup> ed. Approved standard, M7-A8, Clinical Laboratory and Standards Institute, Wayne, PA.
- CLSI 2013. Performance standards for antimicrobial susceptibility testing, 23<sup>rd</sup> ed. Approved standard, M100-S23, Clinical Laboratory and Standards Institute, Wayne, PA
- ISO: SO 20776-1 (2006) Clinical laboratory testing and in vitro diagnostic test systems - Susceptibility testing of infectious agents and evaluation of performance of antimicrobial susceptibility test devices; <http://www.iso.org>.

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