Susceptibility Patterns for Amoxicillin/Clavulanate Tests Mimicking Newly Indicated Formulations and Pharmacokinetic Relationships: Does the 2:1 Ratio MIC Accurately Reflect Activity Against Beta-Lactamase-Producing *H. influenzae* and *M. catarrhalis*

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AMENDED ABSTRACT

Background: Amoxicillin/clavulanate (A/C) has recently undergone formulation changes (XR and ES-600) that represent 14:1 and 16:1 ratios of A/C. These ratios greatly differ from the 2:1 ratio used in initial tablets that produce a serum concentration ratio of approximately 9:1. The effects of these altered ratios on the S test was assessed.

Methods: Activity of A/C was determined by CLSI/NCCLS broth microdilution methods (M7-A6; 2003) against *H. influenzae* (HI; 19 strains, 12 beta-lactamase-positive [BL+]) and *M. catarrhalis* (MCAT; 23 strains, 16 BL+ including BRO-1 and -2). A/C was tested in 8 combinations reflecting formulation and serum PK ratios (4, 5, 7, 9, 14 and 16:1; 0.5 and 2 mg/L fixed clavulanate [C] conc.) and compared to 2:1 MIC results. Mueller-Hinton broth was modified to HTM for testing HI.

Results: The reference A/C (2:1) MICs for HI BL+ and BL- strains ranged from 0.5 - 4 and 0.25 - 2 mg/L, respectively. BL+ MCAT A/C MICs (MIC_{90}, 0.25 mg/L) were routinely >= 16-fold higher than BL- strains showing incomplete enzyme inhibition by C. All A/C MIC ratio test results were unchanged for BL- isolates compared to 2:1 ratio MICs. However, trends toward a 2-fold higher A/C MIC were observed for all ratio tests of > 5:1 and > 4:1 for BL+ HI and MCAT, respectively. Both C fixed concentration MIC tests were equal to or lower than 2:1 MICs, however the C_{max} for C was only 1.5 - 2.2 mg/L (1.7 - 2.0, new formulations) questioning the PK/PD validity of the C level at the current CLSI/NCCLS breakpoint (S at <= 4/2 mg/L).

Conclusions: The A/C MIC test using the 2:1 ratio was established prior to contemporary PK/PD calculations and before current altered formulations significantly modified drug ratios. At ratios of >= 4:1 the inhibition of BL+ strains was less efficient resulting in a 2-fold greater A/C MIC and reducing the probability of favorable clinical responses. Re-evaluation of A/C MIC testing should be considered by S test standard organizations.

INTRODUCTION

Amoxicillin/clavulanic acid (Augmentin®) has been used in clinical practice for several decades and is commonly prescribed for the treatment of respiratory tract infections. Indications include otitis media, sinusitis and community-acquired pneumonia caused by *H. influenzae* and *M. catarrhalis* including strains that produce ß-lactamase, as well as, *S. pneumoniae*. Numerous dosing formulations have been introduced to optimize bacteriological efficacy dependent upon patient age, infection site and the suspected or known susceptibility profile of the offending pathogen(s). For example, Augmentin® ES-600, a high-dose pediatric suspension, is available in the United States for the treatment of persistent and recurrent acute otitis media. High-dose and extended release adult formulations have also been approved for treating strains that have elevated penicillin or amoxicillin MIC values. Currently, there are five formulations available in the United States with amoxicillin/clavulanic acid ratios ranging from 2:1 to 16:1.

The purpose of this pilot study was to determine if the current 2:1 ratio used to test amoxicillin/clavulanic acid according to CLSI/NCCLS procedures is appropriate for all currently available formulations. We tested recent clinical isolates of *H. influenzae* and *M. catarrhalis* including ß-lactamase producing strains against seven ratios and at two fixed concentrations and then compared the MIC results to the standard test concentration ratio (2:1).

MATERIALS AND METHODS

Bacterial strains. A total of 42 isolates collected during 2004 were selected to be tested against seven amoxicillin/clavulanic acid ratios and two fixed inhibitor concentrations. Seven ß-lactamase-negative *H. influenzae* isolates with amoxicillin/clavulanic MIC values ranging from 0.25 - 2 mg/L and 12 ß-lactamase-positive (amoxicillin/clavulanic MIC range, 0.5 - 4 mg/L) strains were tested. Seven ß-lactamase-negative *M. catarrhalis* isolates were also selected which had amoxicillin/clavulanic MIC values of ≤ 0.016 mg/L. Among the 16 ß-lactamase-positive *M. catarrhalis* isolates tested, 13 strains were phenotypically BRO-1 and three were BRO-2 enzyme-producing isolates (amoxicillin/clavulanic MIC range, ≤ 0.016 - 0.25 mg/L).

ß-lactamase characterization was determined using nitrocefin disks (Remel, Lenexa, KS, USA). Five µg methicillin disks were used to characterize the type of BRO enzyme harboured in the *M. catarrhalis* isolates. A strain uninhibited by methicillin was determined to be a BRO-1 producer and a strain showing inhibition was characterized as a presumptive BRO-2 producing isolate.

Susceptibility test methods: MIC values were determined using CLSI/NCCLS broth microdilution methods in cation-adjusted Mueller-Hinton broth or Haemophilus Test Media. Methods were validated using *S. aureus* ATCC 29213, *E. coli* ATCC 25922, *E. faecalis* ATCC 29212 and *H. influenzae* ATCC 49247 quality control strains. Amoxicillin/clavulanic acid ratios were tested to simulate formulations found in the product package insert. The 2:1 ratio (250/125 mg Q 8 h) was the benchmark per CLSI/NCCLS procedures. Additionally, 4:1 (500/125 mg Q 12 h), 7:1 (875/125 mg Q 12 h); 14:1 (ES-600; 600/42.9 mg) and 16:1 (XR; 2000/125 mg) ratios were also tested. Two additional ratios (5:1 PK and 9:1 PKx2) and two fixed inhibitor concentrations (2 mg/L; average of clavulanate C_{max} of all formulations and 0.5 mg/L; clavulanate C_{max}/4 of all formulations) were tested, as well.

RESULTS

Table 1. Reference amoxicillin/clavulanic acid MIC (2:1 ratio) test results for the tested isolates (42 strains).

Organism (no. tested)	MIC (mg/L) 50%	90%	Range	% susceptible[a]
H. influenzae				
ß-lactamase-negative (7)	0.5	-	0.25-2	100.0
ß-lactamase-positive (12)	0.5	2	0.5-4	91.7
M. catarrhalis				
ß-lactamase-negative (7)	≤0.016	≤0.016	≤0.016	-
ß-lactamase-positive (16)[b]	0.12	0.25	≤0.016-0.25	-

a. Interpretive criteria of the CLSI/NCCLS, where indicated.
b. Includes BRO-1 (13 strains) and BRO-2 (three strains).

Table 2. Amoxicillin/clavulanic acid MIC distributions as compared to the reference 2:1 ratio CLSI/NCCLS [2003] test result when testing ß-lactamase-negative and -positive *H. influenzae* and *M. catarrhalis* isolates.

Organism (no. tested)	Testing protocol[a]	Variation in log_2 dilutions by test:[b] -3	-2	-1	Same	+1	+2
H. influenzae							
ß-lactamase-negative (7)	4:1	-	-	-	7	-	-
	5:1	-	-	-	7	-	-
	7:1	-	-	-	7	-	-
	9:1	-	-	-	7	-	-
	14:1	-	-	-	7	-	-
	16:1	-	-	-	7	-	-
	0.5	-	-	1	6	-	-
	2.0	-	-	2	5	-	-
ß-lactamase-positive (12)	4:1	-	-	-	9	3	-
	5:1	-	-	-	8	4	-
	7:1	-	-	-	4	8	-
	9:1	-	-	-	2	10	-
	14:1	-	-	-	2	10	-
	16:1	-	-	-	1	9	2
	0.5	-	-	4	8	-	-
	2.0	-	3	6	3	-	-
M. catarrhalis							
ß-lactamase-negative (7)	4:1	-	-	-	7	-	-
	5:1	-	-	-	7	-	-
	7:1	-	-	-	7	-	-
	9:1	-	-	-	7	-	-
	14:1	-	-	-	7	-	-
	16:1	-	-	-	7	-	-
	0.5	-	-	-	7	-	-
	2.0	-	-	-	7	-	-
ß-lactamase-positive (16)	4:1	-	-	-	11	5	-
	5:1	-	-	-	7	9	-
	7:1	-	-	-	6	10	-
	9:1	-	-	-	4	12	-
	14:1	-	-	-	3	12	1
	16:1	-	-	-	3	12	1
	0.5	-	-	3	12	1	-
	2.0	5	3	3	5	-	-

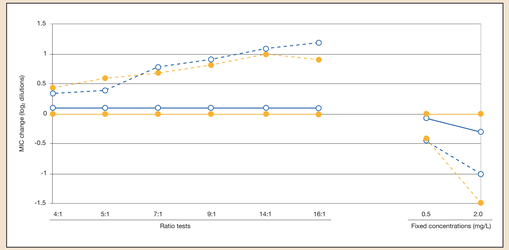
a. 4:1, 5:1, 7:1, 9:1, 14:1 and 16:1 indicate ratios of amoxicillin/clavulanic acid. 0.5 and 2.0 mg/L are tests with a fixed concentration of clavulanic acid.
b. +2 and +1 indicate higher amoxicillin/clavulanic acid MIC values compared to the reference test (2:1 ratio). Minus numbers illustrate lower MIC values in log_2 dilution steps.

- Table 1 shows the reference standard test ratio (2:1) MIC results for amoxicillin/clavulanic acid tested against *H. influenzae* and *M. catarrhalis* isolates (42 strains). The MIC_{50} for both ß-lactamase-producing and non-producing *H. influenzae* strains was 0.5 mg/L and susceptibility was 100.0% (ß-lactamase-negative) and 91.7% (ß-lactamase-positive). The MIC_{90} value for *M. catarrhalis* was ≤ 0.016 mg/L for ß-lactamase-negative isolates but 0.25 mg/L for ß-lactamase-positive isolates (≥ 16-fold greater).

- Table 2 lists the variation of the different test ratios and fixed concentrations compared to the standard test ratios (2:1) for the ß-lactamase-positive and -negative strains of *H. influenzae* and *M. catarrhalis*. There was generally no difference between the 2:1 test ratio and any other ratio or fixed concentration tested for the ß-lactamase-negative isolates. Higher amoxicillin/clavulanic acid MIC values were consistently noted for the higher ratios especially the 14:1 and 16:1 ratios when tested against the ß-lactamase-producing isolates.

- Figure 1 graphically shows the variations of the amoxicillin/clavulanic acid MIC results when tested at the ratios/fixed concentrations tested in this study. The fixed concentrations showed lower MIC values for the ß-lactamase-positive strains especially at a concentration of 2 mg/L. All ratios higher than the 2:1 (CLSI/NCCLS) test concentration had MIC values which increased as the ratio of amoxicillin to inhibitor increased.

Figure 1. Variations of the amoxicillin/clavulanic acid MIC using in vitro tests representing clinically available formulations and pharmacokinetic relationships compared to the current 2:1 ratio MIC results [CLSI/NCCLS] when testing *H. influenzae* (○- blue) and *M. catarrhalis* (○- orange). Broken lines indicate the ß-lactamase-positive and solid lines indicate ß-lactamase-negative strain results for each species.



CONCLUSIONS

- The newer formulations of amoxicillin/clavulanic acid including Augmentin® ES-600 and Augmentin® XR were developed to expand the PK/PD profile against increasingly resistant pathogens including penicillin-resistant pneumococci that commonly cause community-acquired pneumonia and other respiratory diseases.

- The current CLSI/NCCLS test ratio of amoxicillin/clavulanic acid (2:1) does not adequately reflect the higher ratios in the newer formulations, including Augmentin® XR (14:1) and Augmentin® ES-600 (16:1), among ß-lactamase-producing strains of *H. influenzae* and *M. catarrhalis*.

- Higher amoxicillin/clavulanic acid MIC values (1 - 2 log_2 dilutions) were found among the higher ratio formulations and lower MIC values (1 - 3 log_2 dilutions) were typical for the fixed concentration tests. Re-evaluation of the amoxicillin/clavulanic acid in vitro tests to more accurately reflect practice formulations should be considered by consensus organizations such as the CLSI/NCCLS.

SELECTED REFERENCES

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