459

Susceptibility Patterns for Amoxicillin/Clavulanate Tests Simulating Licensed Formulations and Pharmacokinetic Relationships: Does the 2:1 Ratio MIC Accurately Reflect Activity Against ß-Lactamase Producing *H. influenzae* and *M. catarrhalis*?

S POTTUMARTHY, HS SADER, TR FRITSCHE, RN JONES
JMI Laboratories, North Liberty, IA, USA

IDSA 2005

JMI Laboratories
North Liberty, IA, USA
www.jmilabs.com
319.665.3370, fax 319.665.3371
ronald-jones@jmilabs.com

ABSTRACT

Background: Current amoxicillin/clavulanate (A/C) formulations have concentration ratios ranging from 2:1 to 16:1 and result in serum PK ratios of 2:1 to 9:1. Ability of the reference 2:1 A/C ratio testing to accurately reflect susceptibility (S) to the various A/C formulations and PK ratios was re-evaluated.
Methods: A/C was tested by the CLSI broth microdilution method against *H. influenzae* (HI; 330 strains, 300 ß-lactamase positive [BL+]) and *M. catarrhalis* (MCAT; 40 strains, 30 BL+, 16 BRO-1 and 14 BRO-2). A/C was tested in 8 ratios reflecting formulation and PK (4, 5, 7, 9, 14 and 16:1; 0.5 and 2 µg/ml fixed inhibitor conc.) and compared to 2:1 results.
Results: A/C tested at 2:1 ratio had excellent activity against all strains (100.0% S for HI and $MIC_{90} \leq 0.5$ µg/ml for MCAT). For BL- strains of HI and MCAT, all A/C ratio test results were unchanged compared to the 2:1 ratio MICs. However, for BL+ HI and MCAT, at ratios ≥ 4:1 there was a significant shift of the MIC scatterplot compared to 2:1 ratio MIC, resulting in a 2-fold increase in MIC_{50} and MIC_{90}, most prominent for HI and MCAT BRO-1. For BL+ HI this resulted in a 10-fold increase in isolates with a MIC at the S breakpoint (BP; 4/2 µg/ml), 19 MICs each at 14:1 and 16:1 versus 2 at 2:1 ratio, indicating that target attainment from PK/PD may be compromised at higher ratios. Both fixed C concentration MIC tests produced MICs equal to or lower than 2:1 MIC results; however, the C_{max} for C was only 1.5 - 2.2 mg/L (1.7 - 2.0, formulations) questioning the PK/PD validity of C level used at the current CLSI BP.
Conclusions: A/C 2:1 ratio test MIC results for BL+ HI and MCAT strains cannot be extrapolated to reflect S for formulations with ratios 4:1 to 16:1. Availability of contemporary PK/PD calculations will enable the standardizing authorities to re-assess A/C MIC testing to reflect current practice and predict favorable outcomes.

INTRODUCTION

Resistance rates of community-acquired respiratory pathogens, predominantly *Streptococcus pneumoniae,* to existing antimicrobial agents has escalated to an alarming rate worldwide. Additionally, the licensure and widespread use of the 7-valent pneumococcal conjugate vaccine (PVC7) has resulted in a major change in the epidemiology of respiratory tract infections in children. Evaluating the changes in microbiology of acute otitis media (AOM) before (1992-1998) and after (2000-2003) community-wide routine implementation of PVC7 in central Kentucky, investigators observed that the proportion of *S. pneumoniae* as a cause of AOM decreased from 48.0 to 31.0% while non-typeable *H. influenzae* increased from 41.0 to 56.0%. Among *H. influenzae*, the prevalence of ß-lactamase-producing strains increased from 56.0 to 64.0%.

To preserve its efficacy and utility in face of these resistance threats, the initial adult formulation of amoxicillin/clavulanate with a 2:1 ratio, has been adjusted over the last 25 years in response to changes in recommendations for treatment of infections due to pneumococcal strains with elevated penicillin/amoxicillin MIC values. These formulations with increasing concentrations of amoxicillin only include: amoxicillin/clavulanate 4:1 ratio (500/125 mg); 7:1 ratio (875/125 mg); and 8:1 ratio (1000/125 mg). The most recent formulations have still higher concentrations of amoxicillin, in ratios of 16:1 for adults (2000/125 mg, Augmentin XR® [AXR]) and 14:1 for children (600/42.9 mg in 5 ml, Augmentin ES-600® [AES]).

While the ratio of amoxicillin/clavulanate in the formulations now available for clinical use ranges from 2:1 to 16:1, the Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards [NCCLS]) continues to recommend only the 2:1 ratio of amoxicillin/clavulanate for in vitro susceptibility testing. The purpose of this study was to determine if the reference 2:1 ratio used to test amoxicillin/clavulanate accurately reflects the in-vitro susceptibility to all the currently available formulations. We determined the minimum inhibitory concentrations (MIC) of amoxicillin/clavulanate in eight combinations: ratios ranging from 4:1 to 16:1 (simulating the ratios in the licensed formulations and/or resulting serum pharmacokinetic [PK] values) and two alternative fixed inhibitor concentrations for a preselected collection of beta-lactamase-positive and -negative strains of *H. influenzae* and *M. catarrhalis,* and compared results to MIC values obtained using the reference 2:1 test ratio.

MATERIALS AND METHODS

Strain collection. A contemporary collection of 370 strains of *H. influenzae* (330) and *M. catarrhalis* (40) were analyzed. The bacterial strains were isolated primarily from community-acquired respiratory tract infections (CARTI; 99.5%) in medical centers participating in the SENTRY Antimicrobial Surveillance Program in North America over a three year period, 2002-2004. The *H. influenzae* subset consisted of 300 (90.9%) ß-lactamase-positive and 30 (9.1%) ß-lactamase-negative strains. *M. catarrhalis* strains included 30 (75.0%) ß-lactamase-positive strains, among which, 16 (53.3%) were BRO-1 enzyme producers and 14 (46.7%) were BRO-2 producers.

Susceptibility testing methods. ß-lactamase characterization was determined using nitrocefin disks (Remel, Lenexa, KS, USA). Minimal inhibitory concentrations (MICs) of six antimicrobial agents, ampicillin (*H. influenzae* only), penicillin (*M. catarrhalis* only), amoxicillin/clavulanate, cefdinir, cefprozil and cefuroxime were determined by the CLSI standardized broth microdilution method, and the results interpreted according to CLSI/NCCLS guidelines.

Susceptibility testing of amoxicillin/clavulanate at varying ratios/fixed concentrations. In addition to the standard 2:1 ratio, MIC testing of amoxicillin/clavulanate was also determined at additional ratios of: 4:1 (formulation 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); and 5:1 (PK of 7:1 formulation) and 9:1 (PK of two formulations 14:1 and 16:1). Amoxicillin/clavulanate was also tested at a fixed inhibitor concentration of 2 µg/ml (average maximum achievable serum concentration [C_{max}] of all formulations) and 0.5 µg/ml (C_{max}/4 of all formulations) of clavulanate. MIC values of amoxicillin alone and clavulanate alone were also determined. All MIC results were compared with the benchmark, amoxicillin/clavulanate 2:1 (250/125 mg Q 8 h) test ratio.

RESULTS

- The rank order of antimicrobial activity by percentage susceptible against ß-lactamase-positive *H. influenzae* strains was amoxicillin/clavulanate (2:1 ratio; 100.0%), followed by cefuroxime (99.3%) > cefdinir (99.0%) > cefprozil (77.3%; Table 1).
- Cefdinir was ≥ four-fold more potent than cefuroxime against both subsets of *H. influenzae* and all three subsets of *M. catarrhalis* strains analyzed.
- Clavulanate tested alone had minimal or no inherent antimicrobial activity detectable against ß-lactamase-negative and ß-lactamase-positive *H. influenzae* isolates (MIC_{50}, ≥ 16 µg/ml; Table 2).
- For ß-lactamase-negative *H. influenzae* strains, all amoxicillin/clavulanate ratios (2:1 to 16:1) evaluated had identical MIC_{50} (0.5 µg/ml) and MIC_{90} (1 µg/ml) values.
- ß-lactamase-positive *H. influenzae* isolates consistently had ≥ two-fold higher MIC values when tested at amoxicillin/clavulanate ratios > 2:1 compared to the reference 2:1 ratio test.
- There was a general shift of the central tendency of the MIC, with the MIC range increasing from ≤ 0.06 - 4 µg/ml (2:1 ratio) to 0.25 - 8 µg/ml (16:1 ratio). This resulted in a ten-fold increase in the number of isolates with an MIC value at the susceptible breakpoint of 4/2 µg/ml (19 each at 14:1 and 16:1 versus 2 at 2:1 ratio); and the percentages of isolates with MIC values at or one dilution lower than the susceptible breakpoint (4/2 µg/ml) increased significantly, from 5.0% (15 strains) at 2:1 to a dramatic 32-33% (96-99 strains) at 14:1 and 16:1 ratios.

Table 1. In vitro activity of five ß-lactam antimicrobials tested against 370 strains of *H. influenzae* and *M. catarrhalis* isolated in the United States (2002 - 2004).

Organism (no. tested)/antimicrobial agent	MIC (µg/ml) 50%	90%	Range	% by category[a] Susceptible	Resistant
H. influenzae					
ß-lactamase-negative (30)					
Ampicillin	1	1	≤0.5->4	90.0	10.0
Amoxicillin/Clavulanate	1	2	0.12-4	100.0	0.0
Cefdinir	0.5	1	0.12-4	93.3	-[b]
Cefprozil	4	16	0.5-16	86.7	0.0
Cefuroxime	2	4	0.5-8	93.3	0.0
ß-lactamase-positive (300)					
Ampicillin	>4	>4	1->4	0.0[c]	100.0
Amoxicillin/Clavulanate	1	2	0.12-4	100.0	0.0
Cefdinir	0.25	0.5	0.06-2	99.0	-
Cefprozil	4	>16	≤0.12->16	77.3	11.7
Cefuroxime	1	2	0.12-8	99.3	0.0
M. catarrhalis					
ß-lactamase-negative (10)					
Penicillin	≤0.03	0.06	≤0.03-0.12	100.0[c]	0.0
Amoxicillin/Clavulanate	≤0.06	≤0.06	≤0.06	-	-
Cefdinir	0.06	0.12	0.06-0.12	-	-
Cefprozil	0.5	0.5	0.25-0.5	-	-
Cefuroxime	0.25	0.5	0.12-0.5	-	-
ß-lactamase-positive BRO-1 (16)					
Penicillin[c]	>4	>4	>4	0.0[c]	100.0
Amoxicillin/Clavulanate	0.25	0.5	0.12-0.5	-	-
Cefdinir	0.25	0.5	0.12-0.5	-	-
Cefprozil	4	8	2-16	-	-
Cefuroxime	2	4	1-4	-	-
ß-lactamase-positive BRO-2 (14)					
Penicillin[c]	4	4	1-4	0.0[c]	100.0
Amoxicillin/Clavulanate	0.12	0.25	0.12-0.25	-	-
Cefdinir	0.12	0.12	0.06-0.25	-	-
Cefprozil	0.5	1	0.5-2	-	-
Cefuroxime	1	1	0.5-2	-	-

a. Susceptible and resistant categories established by using CLSI/NCCLS criteria (M100-S15).
b. - = Breakpoints have not been established by the CLSI/NCCLS.
c. Susceptibility and resistance rates based on ß-lactamase test results.

- For the fixed inhibitor concentration tests (0.5 and 2 µg/ml of clavulanate) the MIC_{90} values of *H. influenzae* strains remained unaltered at 1 µg/ml compared to the standard 2:1 test ratio.
- Clavulanate had detectable antimicrobial activity against ß-lactamase-negative and ß-lactamase-positive (BRO-1 and BRO-2 producers) *M. catarrhalis* isolates (MIC range, 2 - 8 µg/ml).
- Ten ß-lactamase-negative *M. catarrhalis* isolates showed no differences in the MIC values at the seven amoxicillin/clavulanate ratios (2:1 to 16:1) or the two fixed inhibitor concentrations tested (MIC_{90}, ≤ 0.06 µg/ml).
- BRO-1 and -2 ß-lactamase-producing *M. catarrhalis* isolates consistently had ≥ two-fold higher MIC values when testing amoxicillin/clavulanate at ratios ≥ 4:1 compared to the reference 2:1 ratio.
- There was a general shift of the central tendency of the *M. catarrhalis* MIC distribution, with the MIC range increasing from 0.12 - 0.25 µg/ml (2:1) to 0.25 - 1 µg/ml (9:1,14:1 and 16:1 ratios) for BRO-1 producers; and from ≤ 0.06 - 0.12 µg/ml (2:1 ratio) to ≤ 0.06 - 0.25 µg/ml (5:1 to 16:1 ratios) for BRO-2 producers.
- Fixed inhibitor concentrations (0.5 and 2 µg/ml of clavulanate) resulted in a two-fold decrease in the MIC_{90} values from 0.25 (2:1 ratio) to 0.12 µg/ml for the *M. catarrhalis* BRO-1 producers.

Table 2. Comparisons of amoxicillin/clavulanate MIC values when tested in various in vitro ratios (seven) and fixed inhibitor concentrations (0.5 and 2 µg/ml) against commonly isolated ß-lactamase-producing or non-producing respiratory pathogens.

Organism (no. tested)	In vitro test used	A/C MIC (µg/ml) occurrences at: ≤0.06	0.12	0.25	0.5	1	2	4	8	≥16
H. influenzae										
ß-lactamase-negative (30)										
	Amoxicillin	-	-	6	6	4	14	-	-	-
	2:1	2	4	5	5	13	1	-	-	-
	4:1	2	2	7	5	13	1	-	-	-
	5:1	2	2	7	5	13	1	-	-	-
	7:1	2	2	7	5	13	1	-	-	-
	9:1	2	2	7	5	13	1	-	-	-
	14:1	1	3	7	5	12	2	-	-	-
	16:1	1	4	6	5	12	2	-	-	-
	Fixed 0.5	-	-	13	3	11	3	-	-	-
	Fixed 2	-	2	9	3	7	9	-	-	-
	Clavulanate	-	-	-	-	-	-	-	-	30
ß-lactamase-positive (300)										
	Amoxicillin	-	-	-	-	-	1	4	19	276
	2:1	2	25	81	122	55	13	2	-	-
	4:1	-	13	46	116	82	41	2	-	-
	5:1	-	5	32	114	96	47	6	-	-
	7:1	-	2	24	98	106	58	9	-	-
	9:1	-	1	24	85	113	67	10	-	-
	14:1	-	-	16	64	121	80	19	-	-
	16:1	-	-	16	62	123	77	19	3	-
	Fixed 0.5	-	-	227	20	40	12	1	-	-
	Fixed 2	-	11	172	56	39	21	1	-	-
	Clavulanate	-	-	-	-	-	-	-	9	291
M. catarrhalis										
ß-lactamase-negative (10)										
	Amoxicillin	10	-	-	-	-	-	-	-	-
	2:1	10	-	-	-	-	-	-	-	-
	4:1	10	-	-	-	-	-	-	-	-
	5:1	10	-	-	-	-	-	-	-	-
	7:1	10	-	-	-	-	-	-	-	-
	9:1	10	-	-	-	-	-	-	-	-
	14:1	10	-	-	-	-	-	-	-	-
	16:1	10	-	-	-	-	-	-	-	-
	Fixed 0.5	10	-	-	-	-	-	-	-	-
	Fixed 2	10	-	-	-	-	-	-	-	-
	Clavulanate	-	-	-	-	-	3	7	-	-
ß-lactamase-positive BRO-1 (16)										
	Amoxicillin	-	-	1	10	4	1	-	-	-
	2:1	-	13	3	-	-	-	-	-	-
	4:1	-	6	9	1	-	-	-	-	-
	5:1	-	4	10	2	-	-	-	-	-
	7:1	-	1	13	2	-	-	-	-	-
	9:1	-	-	14	1	1	-	-	-	-
	14:1	-	-	12	3	1	-	-	-	-
	16:1	-	-	10	5	1	-	-	-	-
	Fixed 0.5	1	12	3	-	-	-	-	-	-
	Fixed 2	5	11	-	-	-	-	-	-	-
	Clavulanate	-	-	-	-	-	3	5	8	-
BRO-2 (14)										
	Amoxicillin	2	1	11	-	-	-	-	-	-
	2:1	7	7	-	-	-	-	-	-	-
	4:1	4	10	-	-	-	-	-	-	-
	5:1	3	10	1	-	-	-	-	-	-
	7:1	3	10	1	-	-	-	-	-	-
	9:1	3	10	1	-	-	-	-	-	-
	14:1	2	9	3	-	-	-	-	-	-
	16:1	2	7	5	-	-	-	-	-	-
	Fixed 0.5	6	7	1	-	-	-	-	-	-
	Fixed 2	9	5	-	-	-	-	-	-	-
	Clavulanate	-	-	-	-	-	3	9	2	-

CONCLUSIONS

- Our results indicate that for ß-lactamase-positive *H. influenzae* and *M. catarrhalis* isolates, the results of the standard 2:1 ratio testing cannot be extrapolated to reflect the susceptibility to other licensed formulations with amoxicillin/clavulanate ratios of 4:1 to 16:1.
- Re-evaluation of the amoxicillin/clavulanate susceptibility testing methods to accurately reflect the contemporary clinical practice formulations should be considered by standardizing authorities, including the CLSI/NCCLS, with combined analyses using PK/PD and target attainment simulations.
- A consensus decision needs to be reached on whether the in vitro susceptibility testing of amoxicillin/clavulanate correlates best with clinical outcome when performed by fixed inhibitor concentration method (ticarcillin/clavulanate and piperacillin/tazobactam), or by a ratio method (ampicillin/sulbactam).

SELECTED REFERENCES

Block SL, Hedrick J, Harrison CJ, Tyler R, Smith A, Findlay R, Keegan E. (2004) Community-wide vaccination with the heptavalent pneumococcal conjugate significantly alters the microbiology of acute otitis media. *Pediatric Infectious Disease Journal* 23:829-833.

Black S, Shinefield H, Baxter R, Austrian R, Bracken L, Hansen J, Lewis E, Fireman B (2004) Postlicensure surveillance for pneumococcal invasive disease after use of heptavalent pneumococcal conjugate vaccine in northern California Kaiser Permanente. *Pediatric Infectious Disease Journal* 23:485-489.

Clinical and Laboratory Standards Institute (2005) *Performance standards for antimicrobial susceptibility testing, 15th informational supplement M100-S15.* Wayne, PA:CLSI.

National Committee for Clinical Laboratory Standards (2003) *Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6.* Wayne, PA:NCCLS.

White AR, Kaye C, Poupard J, Pypstra R, Woodnutt G, Wynne B (2004) Augmentin® (amoxicillin/clavulanate) in the treatment of community-acquired respiratory tract infection: a review of the continuing development of an innovative antimicrobial agent. *Journal of Antimicrobial Chemotherapy* 53, S1:i3-i20.