Activity of Amoxicillin/Clavulanic Acid and 16 Comparator Agents Against Respiratory Isolates, Collected Worldwide in 2000

Application to a new pharmacokinetically enhanced amoxicillin/clavulanic acid (2000/125 mg b.i.d.) formulation

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Revised Abstract

Background: *Streptococcus pneumoniae, Haemophilus influenzae, Moraxella catarrhalis* and *Streptococcus pyogenes* are major respiratory tract pathogens, many of which have exhibited increasing resistance to antimicrobial agents. **Methods:** MIC susceptibility testing was performed on 9179 isolates collected from 95 centers in North America, Europe, Australia, and Hong Kong by broth microdilution, according to NCCLS recommended procedures, using amoxicillin/clavulanic acid and 16 comparator antimicrobial agents. Results were interpreted according to NCCLS (where applicable) and PK/PD breakpoints based on oral dosing regimens. **Results:** A total of 97.3% of *S. pneumoniae* were susceptible to the new amoxicillin/clavulanic acid formulation (2000/125 mg b.i.d.) based on a PK/PD susceptible breakpoint of ≤4 µg/ml for the amoxicillin component. Penicillin intermediate and resistant rates were 13% and 16.5%, respectively. Macrolide resistant rates were 25%. While 21.9% of *H. influenzae* were β-lactamase producing, >99% of isolates were susceptible to amoxicillin/clavulanic acid, cefixime, ciprofloxacin and levofloxacin at PK/PD breakpoints. The most active agents against *M. catarrhalis* were amoxicillin/clavulanic acid, macrolides, cefixime, ciprofloxacin, levofloxacin and doxycycline. Some resistance was evident with macrolides (13%) and doxycycline (MIC_{90} 8 mg/L), but most agents were very active against *S. pyogenes*. **Conclusion:** In the clinical context of RTIs, the most active agents tested, based on MIC_{90}/%, for all the organisms tested in this study were amoxicillin/clavulanic acid, ceftriaxone and levofloxacin.

Introduction

This study was conducted to provide *in vitro* susceptibility data for amoxicillin/clavulanic acid, for a new amoxicillin/clavulanic acid 2000/125 mg b.i.d. formulation, and 16 comparator agents against key respiratory tract pathogens, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis* and *Streptococcus pyogenes*.

Materials and Methods

Antimicrobial Agents

The following antimicrobial agents were tested: penicillin, amoxicillin/clavulanic acid, amoxicillin, ampicillin, cefaclor, cefixime, cefprozil, ceftriaxone, cefuroxime, azithromycin, clarithromycin, erythromycin, ciprofloxacin, levofloxacin, clindamycin, doxycycline, and trimethoprim/sulfamethoxazole.

Isolates

A total of 3714 *S. pneumoniae*, 3793 *H. influenzae*, 972 *M. catarrhalis* and 700 *S. pyogenes* isolates were collected globally from 95 sites (Table 1).

Table 1. Number of Each Organism Tested by Country

Country	*S. pneumoniae*	*H. influenzae*	*M. catarrhalis*	*S. pyogenes*
Australia	156	233	28	53
Belgium	233	177	114	50
Canada	294	289	0	50
France	292	298	72	51
Germany	303	223	105	50
Hong Kong	102	280	0	7
Italy	276	295	106	64
Mexico	281	281	0	47
Netherlands	300	288	100	49
Spain	240	234	28	47
Sweden	290	297	95	47
UK	299	300	100	50
USA	648	598	224	135
Totals	3714	3793	972	700

Isolates were collected primarily from cultures of blood, sputum, bronchoalveolar lavage (BAL), middle ear fluid, nasopharyngeal swabs or aspirates, paranasal sinuses, and throat specimens (*S. pyogenes* only) between May 1999 and December 2000.

Testing Sites

Testing was performed by GR Micro (London, UK), Laboratory Specialists, Inc. (Westlake, OH) and Case Western Reserve University (Cleveland, OH).

Identification

The testing sites confirmed identification of each isolate, following standard methods.

MIC Determination

Susceptibility testing was conducted by broth microdilution MIC determination, according to NCCLS recommended procedures using custom dried plates (TREK, Westlake, OH).[1] Inoculum size was determined for each isolate tested and testing accepted if 3–7×10^5 cfu/ml. The counts were used by the laboratories at the onset of the study to determine the volume of a 0.5 McFarland organism suspension to be added to the broth.

The microdilution trays were reconstituted with Haemophilus Test Medium (HTM) broth (PML) (*H. influenzae*), cation adjusted Mueller–Hinton (MH) broth supplemented with 3% lysed horse blood (CAMHB-LHB) (MicroScan) (*S. pneumoniae* and *S. pyogenes*) and cation-adjusted MH broth (MicroScan) (*M. catarrhalis*). For each isolate, a suspension equivalent to a 0.5 McFarland Standard was prepared in saline, and 25–30 µl (*H. influenzae*), 200 µl (*S. pneumoniae*), 40–100 µl (*S. pyogenes*) or 50–200 µl (*M. catarrhalis*), was added to 25 ml of broth. Dried trays were then inoculated with 100 µl into each well using an Autoinoculator (TREK). Trays were incubated under ambient conditions at 35°C for 20–24 h and the lowest concentration showing no growth was read as the MIC.

Quality control organisms specified by NCCLS, including *S. pneumoniae* ATCC 49619, *H. influenzae* ATCC 49247 and 49766, *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 and 35218 were tested on each day of testing.

Data Analysis

MIC_{50}, MIC_{90} and susceptibility rates were determined for all agents tested. NCCLS interpretive criteria were used to determine susceptibility rates,[2] with the following exceptions:

1. *S. pneumoniae, S. pyogenes* and *M. catarrhalis* amoxicillin/clavulanic acid susceptible breakpoint of ≤4/2 µg/ml was used, based on pharmacokinetic/pharmacodynamic (PK/PD) breakpoint of new amoxicillin/clavulanic acid 2000/125 mg b.i.d. formulation.
2. PK/PD breakpoints were used for cefixime, ciprofloxacin and doxycycline against *S. pneumoniae* as no NCCLS criteria exist.
3. *H. influenzae* and *S. pyogenes* susceptibility rates were calculated based on both NCCLS and PK/PD breakpoints.
4. PK/PD breakpoints were used for *M. catarrhalis* as no NCCLS criteria exist.
5. For trimethoprim/sulfamethoxazole, *S. pneumoniae* NCCLS breakpoints were used for *M. catarrhalis*.

PK/PD breakpoints were based on standard dosing regimens and criteria appropriate to each agent. For β-lactams, erythromycin and clarithromycin, these breakpoints were based on drug concentrations in serum present for 40–50% of the dosing interval, while for azithromycin, fluoroquinolones and doxycycline, they were based on 24-h AUC/MIC ratio of 25 (Table 2).[3-5]

Table 2. NCCLS and PK/PD Susceptible Breakpoints (µg/ml)

Antimicrobial	NCCLS[a]			PK/PD (all organisms)
	S. pneumoniae	*H. influenzae*	*S. pyogenes*	
Penicillin	≤0.06	–	≤0.12	–
Amox/clav 2000/125 mg	–	–	–	≤4/2
Amox/clav	≤2/1	≤4/2	–	≤2/1
Amoxicillin	≤2	–	–	≤2
Cefaclor	≤1	≤8	–	≤0.5
Cefixime	–	≤1	–	≤1
Cefprozil	≤2	≤8	–	≤1
Ceftriaxone	≤0.5	≤2	≤0.5	–
Cefuroxime	≤1	≤4	–	≤1
Azithromycin	≤0.5	≤4	≤0.5	≤0.12
Clarithromycin	≤0.25	≤8	≤0.25	≤0.25
Erythromycin	≤0.25	–	≤0.25	≤0.25
Ciprofloxacin	–	≤1	–	≤1
Levofloxacin	≤2	≤2	≤2	≤2
Clindamycin	≤0.25	–	≤0.25	–
Doxycycline	–	–	–	≤0.25
Trim/sulfa	≤0.5/9.5	≤0.5/9.5	–	–

Amox/clav, amoxicillin/clavulanic acid; trim/sulfa, trimethprim/sulfamethoxazole.
[a]Reference 2

Results

- The highest prevalences (>20%) of *S. pneumoniae* penicillin resistance were in France, Hong Kong, Mexico, Spain and the USA. The lowest prevalences (<5%) were in The Netherlands, UK and Germany (Figure 1).
- A total of 28 *S. pneumoniae* had levofloxacin MICs >4 µg/ml; the majority were from Hong Kong and the USA (Figure 2).
- *In vitro* activity against *S. pneumoniae*, based on susceptibility rates from most active to least active, was levofloxacin, amoxicillin/clavulanic acid (2000/125 mg), amoxicillin ± clavulanic acid, clindamycin, ceftriaxone, cefprozil, ciprofloxacin, cefuroxime, doxycycline, azithromycin, clarithromycin, cefixime, erythromycin, trimethoprim/sulfamethoxazole and cefaclor (Tables 3 and 4).

Figure 1. Percent Penicillin Susceptible, Intermediate and Resistant *S. pneumoniae* by Country

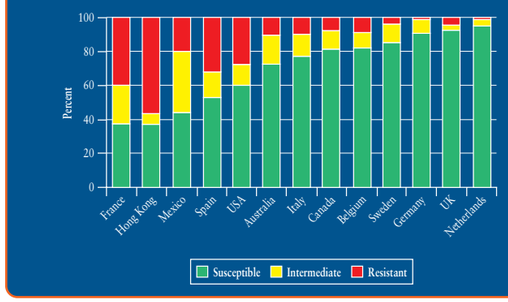

Figure 2. Percent *S. pneumoniae* with Levofloxacin MICs >4 µg/ml by Country (Countries not Shown are 0%)

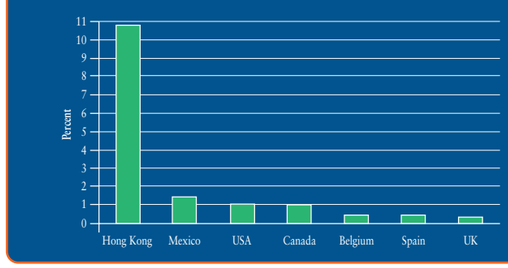

Figure 3. Percentage of β-Lactamase Positive *H. influenzae* by Country

Table 3. Cumulative MIC Frequency Distribution, MIC_{50}, MIC_{90} and Susceptibility Rates of *S. pneumoniae* (n = 3714)

Antimicrobial	Susceptible BP		Cumulative percentage of isolates at MIC (µg/ml):													MIC_{50} (µg/ml)	MIC_{90} (µg/ml)	% S
	PK/PD	NCCLS	0.015	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64			
Penicillin	–	≤0.06	43.7	65.5	70.5	74.7	78.3	80.3	83.5	92.2	99.4	100				0.03	2	70.5
Amox/clav 2000/125 mg	≤4/2	–	4.0	64.6	72.5	75.8	79.1	80.6	84.3	93.5	97.3	99.3	100			0.03/0.015	2/1	97.3
Amox/clav	≤2/1	≤2/1	4.0	64.6	72.5	75.8	79.1	80.6	84.3	93.5	97.3	99.3	100			0.03/0.015	2/1	93.5
Amoxicillin	≤2	≤2	5.5	64.5	72.6	75.6	78.6	80.3	84.2	93.8	97.3	99.2	100			0.03	2	93.8
Cefaclor	≤0.5	≤1						10.6	47.8	72.3	78.4	80.3	81.5	100		2	32	47.8
Cefixime[a]	≤1	–			0.3	4.4	57.3	68.7	73.7	100						0.25	2	73.7
Cefprozil	≤1	≤2				31.6	68.6	76.3	79.0	81.0	82.6	87.9	96.8	100		0.25	16	81.0
Ceftriaxone	–	≤0.5	26.7	65.1	72.7	76.5	79.9	83.7	95.0	99.1	99.8	99.9	100			0.03	1	83.7
Cefuroxime	≤1	≤1	1.9	38.6	63.9	69.0	73.9	77.6	79.5	81.4	87.5	97.8	99.3	100		0.06	8	79.5
Azithromycin	≤0.12	≤0.5	0.5	5.1	58.9	72.8	73.7	74.0	74.5	76.3	79.7	83.2	84.3	84.5	100	0.06	64	74.0
Clarithromycin	≤0.25	≤0.25	6.2	66.8	73.0	73.6	73.9	74.4	75.4	78.9	81.5	84.1	84.7	85.0	100	0.03	64	73.9
Erythromycin	≤0.25	≤0.25	1.3	23.2	71.6	73.2	73.6	74.0	74.4	76.4	79.8	83.2	84.5	84.9	100	0.06	64	73.6
Ciprofloxacin[a]	≤1	–	0.1	0.1	0.2	0.3	0.8	14.4	79.6	95.9	98.9	100				1	2	79.6
Levofloxacin	≤2	≤2	0.1	0.1	0.1	0.1	0.7	41.8	96.7	99.0	99.2	99.4	100			1	1	99.0
Clindamycin	–	≤0.25	0.7	6.1	62.0	83.9	84.6	85.0	85.4	100						0.06	2	84.6
Doxycycline[a]	≤0.25	–			8.0	44.6	76.7	78.9	79.6	80.5	84.1	93.8	100			0.25	8	76.7
Trim/sulfa	–	≤0.5		0.2	2.6	30.0	62.4	68.3	73.7	78.2	90.7	98.2	99.6	99.8	100	0.25/4.75	4/76	68.3

%S, % susceptible; % intermediate; % resistant; amox/clav, amoxicillin/clavulanic acid; trim/sulfa, trimethoprim/sulfamethoxazole. [a]PK/PD breakpoints used for susceptibility rate

- The MIC_{90}s for the macrolides tested were all 64 µg/ml against *S. pneumoniae*. The susceptibility of *S. pneumoniae* was ≤74% to azithromycin, clarithromycin and erythromycin (Tables 3 and 4).
- The highest prevelences (>29%) of β-lactamase production by *H. influenzae* were in France, Hong Kong and the USA. The lowest prevalences (<5%) were in Italy, The Netherlands and Germany (Figure 3).
- Amoxicillin/clavulanate 2000/125 mg, amoxicillin/clavulanic acid, cefixime and the fluoroquinolones tested were the most active agents against *H. influenzae* based on susceptibility rates (Table 5).
- Using PK/PD breakpoints, <2% of *H. influenzae* were susceptible to the macrolides tested. Only 2.1% of these isolates were susceptible to cefaclor (Table 5).
- The percentage of β-lactamase-negative ampicillin-resistant *H. influenzae* strains was 0.3%.
- The majority of *M. catarrhalis* (93.5%) were β-lactamase producers. Amoxicillin/clavulanic acid MICs for all *M. catarrhalis* were ≤4 µg/ml.
- MIC_{50}, MIC_{90} and susceptibility rates for *H. influenzae*, *M. catarrhalis* and *S. pyogenes* for all antimicrobial agents tested are shown in Tables 4 and 5.

Table 4. MIC_{50} and MIC_{90} (µg/ml) of all Organisms Tested

Antimicrobial	*S. pneumoniae* (n = 3714)		*H. influenzae* (n = 3793)		*M. catarrhalis* (n = 972)		*S. pyogenes* (n = 700)	
	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}	MIC_{50}	MIC_{90}
Amox/clav[a]	0.03/0.015	2/1	0.5/0.25	1/0.5	0.12/0.06	0.25/0.12	0.015/0.008	0.03/0.015
Amoxicillin	0.03	2	0.5	32	4	16	0.015	0.03
Ampicillin	–	–	0.25	32	4	16	0.12	0.12
Penicillin	0.03	2	–	–	–	–	0.015	0.015
Cefaclor	2	32	4	8	2	4	0.5	0.5
Cefixime	0.25	2	0.06	0.06	0.25	0.5	0.12	0.12
Cefprozil	0.25	16	2	8	2	8	0.12	0.12
Ceftriaxone	0.03	1	0.015	0.015	0.25	1	0.015	0.03
Cefuroxime	0.06	8	1	2	1	4	0.015	0.03
Azithromycin	0.06	64	1	2	0.03	0.03	0.012	4
Clarithromycin	0.03	64	8	8	0.06	0.12	0.03	4
Erythromycin	0.06	64	4	8	0.12	0.25	0.06	8
Ciprofloxacin	1	2	0.008	0.015	0.03	0.06	0.5	2
Levofloxacin	1	1	0.015	0.015	0.03	0.06	0.5	1
Clindamycin	0.06	2	–	–	–	–	0.06	0.12
Doxycycline	0.25	8	0.5	1	0.25	0.25	0.12	8
Trim/sulfa	0.25/4.75	4/76	0.12/2.38	8/152	0.25/4.75	1/19	0.12/2.38	1/19

Amox/clav, amoxicillin/clavulanic acid; trim/sulfa, trimethprim/sulfamethoxazole
[a]Applies to both formulations of amoxicillin/clavulanic acid

Table 5. Susceptibility Rates for All Organisms Tested

Antimicrobial	Susceptible (%)					
	S. pneumoniae (n = 3714)	*H. influenzae* (n = 3793)		*M. catarrhalis* (n = 972)	*S. pyogenes* (n = 700)	
	NCCLS[a]	NCCLS	PK/PD	PK/PD	NCCLS	PK/PD
Amox/clav 2000/125 mg	97.3	99.8	98.7	100	–	100
Amox/clav	93.5	99.8	98.7	100	–	100
Amoxicillin	93.8	–	79.0	33.3	–	100
Ampicillin	–	78.8	–	–	99.9	–
Penicillin	70.5	–	–	–	100	–
Cefaclor	47.8	90.7	2.1	14.6	–	91.9
Cefixime	73.7[a]	99.7	99.7	99.6	–	99.3
Cefprozil	81.0	92.7	21.9	20.4	–	100
Ceftriaxone	83.7	99.9	–	–	99.9	–
Cefuroxime	79.5	97.9	77.1	52.4	–	100
Azithromycin	74.0	99.7	1.9	98.2	86.7	84.0
Clarithromycin	73.9	90.0	0.6	98.2	86.7	86.7
Erythromycin	73.6	N/A	0.8	96.1	87.1	87.1
Ciprofloxacin	79.6[a]	99.9	99.9	100	–	90.1
Levofloxacin	99.0	99.9	99.9	100	100	100
Clindamycin	84.6	–	–	–	97.1	–
Doxycycline	76.7[a]	N/A	30.8	93.9	N/A	85.3
Trim/sulfa	68.3	83.2	–	–	N/A	–

Amox/clav, amoxicillin/clavulanic acid; trim/sulfa, trimethprim/sulfamethoxazole
[a]PK/PD breakpoints used for cefixime, ciprofloxacin and doxycycline

Conclusions

- Amoxicillin/clavulanic acid (2000/125 mg b.i.d.) and levofloxacin were the most active agents tested against *S. pneumoniae*.
- *S. pneumoniae* resistance to fluoroquinolones is emerging, with the highest prevalances of levofloxacin isolates with MICs ≥4 µg/ml observed in Hong Kong (11%), Mexico (1%), Canada (1%) and the United States (1%).
- The macrolides showed poor activity against *S. pneumoniae* with MIC_{90}s of 64 µg/ml and susceptibility rates of ≤74%.
- Amoxicillin/clavulanic acid continues to be very active against β-lactamase-producing *H. influenzae* strains (99.6% susceptible).
- Using PK/PD breakpoints <2% of *H. influenzae* were susceptible to the macrolides.
- The percentage of β-lactamase-negative ampicillin-resistant *H. influenzae* strains remains extremely low (0.3%).
- The majority of *M. catarrhalis* worldwide are β-lactamase producers and amoxicillin/clavulanic acid remains active with a MIC_{90} of 0.25/0.12 µg/ml and all MICs ≤4 µg/ml.
- Although some resistance is evident with the macrolides and doxycycline, most agents (including amoxicillin/clavulanic acid) continue to be very active against *S. pyogenes*.
- Amoxicillin/clavulanic acid continues to be one of the most active oral agents against respiratory pathogens worldwide.

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