

Comparative In Vitro Activities of Amoxicillin-Clavulanate against Aerobic and Anaerobic Bacteria Isolated from Antral Puncture Specimens from Patients with Sinusitis

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Received 21 September 1998/Returned for modification 9 November 1998/Accepted 14 December 1998

By an agar dilution method, the antimicrobial susceptibilities of antral sinus puncture isolates were studied. Pneumococci were generally susceptible to amoxicillin, azithromycin, and clarithromycin, but 17% of pneumococcal isolates were resistant to cefuroxime. *Haemophilus influenzae* isolates were resistant to amoxicillin and clarithromycin. β -Lactamase production occurred in 69% of *Prevotella* species. One-third of *Peptostreptococcus magnus* isolates were resistant to azithromycin and clarithromycin. Cefuroxime had limited activity against *Prevotella* species and *P. magnus*. Levofloxacin was active against most isolates except peptostreptococci. Amoxicillin-clavulanate was active against all isolates, with the MIC at which 90% of the isolates were inhibited being ≤ 1 $\mu\text{g/ml}$.

Choosing appropriate antimicrobial therapy for acute and chronic sinusitis has been complicated by the recent development of beta-lactam and macrolide resistance in pneumococci (3, 14), β -Lactamase production in *Prevotella* species, and penicillin resistance due to altered penicillin-binding proteins in peptostreptococci (2, 8, 12). Chronic sinusitis involves anaerobes including peptostreptococci, fusobacteria, and *Prevotella* species in >50% of cases (1, 4). In order to evaluate the potential efficacy of various oral antimicrobial agents currently used in the therapy of sinusitis, we studied recent aerobic and anaerobic clinical isolates from patients with sinusitis.

Strains were isolated from antral puncture specimens obtained from adult patients with sinusitis between 1994 and 1998 and were identified by standard criteria (9, 13). The numbers and species of clinical isolates tested are given in Table 1.

The suppliers of the following standard laboratory powders were as indicated: amoxicillin-clavulanate and amoxicillin, SmithKline Beecham Laboratories, Philadelphia, Pa.; azithromycin, Pfizer Inc., New York, N.Y.; levofloxacin, R. W. Johnson Pharmaceutical Research Institute, Raritan, N.J.; clarithromycin, Abbott Laboratories, Abbott Park, Ill.; and cefuroxime, Glaxo-Wellcome, Research Triangle Park, N.C.

Frozen cultures were transferred at least twice to ensure purity and good growth. Susceptibility testing was performed according to National Committee for Clinical Laboratory Standards guidelines (10, 11). *Streptococcus pneumoniae* ATCC 49619, *Haemophilus influenzae* ATCC 49247, *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Bacteroides fragilis* ATCC 25285 were tested simultaneously with the appropriate plates and environments. Brucella agar supplemented with hemin, vitamin K₁, and 5% laked sheep blood was the basal medium used for anaerobic species. Mueller-Hinton agar was used for staphylococci, Mueller-Hinton agar supplemented with 5% sheep blood was used for pneumococci and *Moraxella* isolates, and Mueller-Hinton agar supplemented with yeast extract, hematin, and NAD (*Haemophilus*

test medium) was used for *Haemophilus* species. Antimicrobial agents were reconstituted according to the manufacturers' instructions. Serial twofold dilutions of antimicrobial agents were prepared on the day of the test and added to the media at various concentrations (micrograms per milliliter).

The agar plates were inoculated with 10⁴ CFU per spot for aerobes and 10⁵ CFU for anaerobes by using a Steers replicator (Craft Machine Inc., Chester, Pa.). The MIC was defined as the lowest concentration of an agent that yielded no growth or a marked change in the appearance of growth as compared to the growth control plate. All peptostreptococci were tested and were negative for β -lactamase production by both cefinase disk and acidometric methods.

Because antimicrobial resistance is increasingly frequent, newer agents are being evaluated for the therapy of sinusitis (2, 14). Thornsberry et al. (14) tested 9,190 general clinical isolates of *S. pneumoniae* and found susceptibilities as follows: 67% to penicillin, 82% to amoxicillin clavulanate, 75% to cefuroxime, and 97% to levofloxacin. Doern et al. (3) studied 1,527 pneumococcal isolates collected in the 1994 to 1995 winter months from 30 U.S. reference centers and found 26% overall resistance to penicillin, but 39% of sinus aspirate isolates were penicillin resistant. They also noted that 12% of sinus aspirate isolates were resistant to cefuroxime. Our sinus isolates differed in that 94% were susceptible to amoxicillin, amoxicillin-clavulanate, azithromycin, and clarithromycin; 78% were susceptible to cefuroxime; and 100% were susceptible to levofloxacin. Our study did not include any pediatric strains, which may be more resistant than adult strains.

While the mechanism of resistance was not elucidated in their study, Thornsberry et al. (14) noted clarithromycin resistance in 29% of pneumococci. Our pneumococcal sinus isolates differed in that 94% were susceptible to azithromycin and clarithromycin, which was similar to the findings of Doern et al. (3), who found 10% resistance to azithromycin, clarithromycin, and erythromycin among pneumococci. The comparability of macrolide resistance of pneumococci may be related to the test system used by the investigators because of the potential effect of CO₂ in the incubation atmosphere for the E test and disk diffusion methods but not the agar or broth microdilution method (5), which we used.

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TABLE 1. Comparative in vitro activities of various agents against aerobic and anaerobic bacteria isolated from patients with sinusitis

Organism (no. of isolates)	Agent	MIC ($\mu\text{g/ml}$) ^a			Organism (no. of isolates)	Agent	MIC ($\mu\text{g/ml}$) ^a		
		Range	50%	90%			Range	50%	90%
<i>Haemophilus influenzae</i> (13)	Amoxicillin	4–16	16	16	Propionibacterium spp. ^e (23)	Amoxicillin	≤0.016–0.25	0.06	0.25
	Amox-Clav ^b	1–4	1	2		Amox-Clav	0.03–0.25	0.06	0.25
	Cefuroxime	0.5–2	1	2		Cefuroxime	0.06–0.5	0.125	0.5
	Levofloxacin	≤0.016	0.016	0.016		Levofloxacin	0.125–0.5	0.25	0.5
	Azithromycin	2–4	4	4		Azithromycin	0.03–>32	0.06	0.125
	Clarithromycin	8–32	16	32		Clarithromycin	0.016–>32	0.03	0.06
<i>Moraxella catarrhalis</i> (10)	Amoxicillin	0.06–8	2	8	Peptostreptococcus magnus (35)	Amoxicillin	0.06–0.5	0.25	0.25
	Amox-Clav	0.03–0.5	0.25	0.25		Amox-Clav	0.06–0.25	0.25	0.25
	Cefuroxime	0.25–1	1	1		Cefuroxime	1–16	4	8
	Levofloxacin	≤0.03	≤0.03	0.03		Levofloxacin	0.25–>16	0.5	1
	Azithromycin	0.06–0.25	0.06	0.25		Azithromycin	1–>32	2	4
	Clarithromycin	0.06–0.5	0.25	0.25		Clarithromycin	2–>32	2	4
<i>Staphylococcus aureus</i> (16)	Amoxicillin	1–16	2	16	Peptostreptococcus micros (12)	Amoxicillin	≤0.016–4	0.06	0.125
	Amox-Clav	0.25–2	0.5	2		Amox-Clav	≤0.016–1	0.125	1
	Cefuroxime	1–2	1	2		Cefuroxime	0.06–4	1	2
	Levofloxacin	0.125–0.5	0.125	0.5		Levofloxacin	0.25–0.5	0.25	0.5
	Azithromycin	0.25–>32	1	>32		Azithromycin	0.03–1	0.5	1
	Clarithromycin	0.125–>32	0.25	>32		Clarithromycin	≤0.016–2	0.5	1
<i>Streptococcus pneumoniae</i> (18)	Amoxicillin	≤0.016–2	≤0.016	0.5	Peptostreptococcus spp. ^f (14)	Amoxicillin	≤0.016–0.25	0.06	0.25
	Amox-Clav	≤0.016–2	≤0.016	0.5		Amox-Clav	≤0.016–0.5	0.125	0.25
	Cefuroxime	≤0.016–2	≤0.016	1		Cefuroxime	0.125–32	0.5	16
	Levofloxacin	0.125–1	1	1		Levofloxacin	0.25–4	0.5	4
	Azithromycin	≤0.016–4	0.03	0.03		Azithromycin	0.125–>32	2	4
	Clarithromycin	≤0.016–0.5	≤0.016	0.03		Clarithromycin	0.03–>32	2	>32
<i>Fusobacterium</i> spp. ^c (16)	Amoxicillin	≤0.016–2	0.06	0.5	<i>Veillonella</i> spp. (14)	Amoxicillin	≤0.016–4	1	4
	Amox-Clav	≤0.016–0.25	0.03	0.25		Amox-Clav	≤0.016–4	1	2
	Cefuroxime	≤0.016–0.5	0.06	0.5		Cefuroxime	0.5–16	8	16
	Levofloxacin	0.125–1	1	1		Levofloxacin	0.125–4	0.25	0.25
	Azithromycin	0.125–4	2	4		Azithromycin	1–>32	4	16
	Clarithromycin	2–32	16	32		Clarithromycin	2–>32	16	>32
<i>Prevotella</i> spp. ^d (17)	Amoxicillin	0.03–16	2	8	Other anaerobes ^g (8)	Amoxicillin	0.125–>32	4	
	Amox-Clav	0.03–2	0.25	1		Amox-Clav	0.03–4	0.25	
	Cefuroxime	0.06–>32	8	>32		Cefuroxime	≤0.016–>32	8	
	Levofloxacin	0.25–2	0.5	1		Levofloxacin	0.5–>16	1	
	Azithromycin	0.125–16	0.25	16		Azithromycin	0.03–16	1	
	Clarithromycin	0.06–8	0.125	4		Clarithromycin	0.125–>32	4	

^a 50% and 90%, MICs at which 50 and 90% of isolates tested, respectively, are inhibited.

^b Amox-Clav, amoxicillin-clavulanate.

^c Includes *F. nucleatum* ($n = 12$), *F. necrophorum* ($n = 2$), *F. naviforme* ($n = 1$), and other *Fusobacterium* species, ($n = 1$).

^d Includes *P. melaninogenica* ($n = 9$), *P. buccae* ($n = 3$), *P. intermedia* ($n = 1$), and other *Prevotella* species ($n = 1$).

^e Includes *P. acnes* ($n = 10$), *P. avidum* ($n = 3$), *P. granulosum* ($n = 3$), and other *Propionibacterium* species ($n = 7$).

^f Includes *P. anaerobius* ($n = 3$), *P. asaccharolyticus* ($n = 6$), *P. prevotii* ($n = 2$), and other *Peptostreptococcus* species ($n = 3$).

^g Includes *Bilophila wadsworthia* ($n = 1$), *Eubacterium lentum* ($n = 1$), *Bacteroides fragilis* ($n = 2$), *Bacteroides uniformis* ($n = 1$), *Bacteroides ureolyticus* ($n = 1$), and other *Bacteroides* species ($n = 2$).

Thornsberry et al. (14) noted 67 and 58% of *H. influenzae* were amoxicillin and clarithromycin susceptible, respectively. In our study, all *H. influenzae* isolates (100%) were β -lactamase producers and resistant to amoxicillin but susceptible to amoxicillin-clavulanate. Almost all were resistant to clarithromycin but susceptible to cefuroxime, azithromycin, and levofloxacin. All of our *S. aureus* isolates were β -lactamase producers, and 44% were also resistant to both macrolides.

The transition of acute into chronic sinusitis may be related to the emergence of resistant anaerobes (2, 8, 12) such as *Prevotella* species, fusobacteria, and peptostreptococci. Sixty-five percent (11 of 17) of our *Prevotella* isolates were resistant to amoxicillin (MIC $\geq 1 \mu\text{g/ml}$), as were 57% of the *Veillonella* species (for which there is no amoxicillin breakpoint, but the

ampicillin breakpoint is $\leq 0.5 \mu\text{g/ml}$ for susceptibility). All anaerobes were susceptible to amoxicillin-clavulanate. No Food and Drug Administration or National Committee for Clinical Laboratory Standards interpretive criteria have been established for the activity of levofloxacin, azithromycin, clarithromycin, or cefuroxime against anaerobic bacteria. Using concentrations similar to those established for most aerobes, overall 8% of anaerobes were resistant to levofloxacin (MIC $> 2 \mu\text{g/ml}$), especially some peptostreptococci. In contrast, 59% of *Prevotella* species, 66% of *Peptostreptococcus magnus*, and 86% of *Veillonella* species isolates were resistant to cefuroxime (MIC $> 4 \mu\text{g/ml}$). All *P. magnus* and fusobacteria isolates and 71% of *Veillonella* species isolates were resistant to clarithromycin (MIC $\geq 2 \mu\text{g/ml}$). Azithromycin was often more active

than clarithromycin against anaerobes but still had poor activity against 27% of fusobacteria, 29% of *P. magnus* (for 33 of 35, the MICs were ≥ 2 $\mu\text{g/ml}$), and 64% of *Veillonella* species isolates. The effect of CO_2 in the atmosphere of incubation has been shown to decrease the activity of macrolides against anaerobes but the clinical relevance has not been determined (6, 7).

Levofloxacin also had reasonably good activity with the exception of some peptostreptococci. Amoxicillin-clavulanate was the most active agent against the broad spectrum of aerobic and anaerobic bacteria tested.

We thank Yumi Warren and Kerin Tyrell for technical assistance and Judee H. Knight and Alice E. Goldstein for various forms of assistance.

This study was funded, in part, by an educational grant from Smith-Kline Beecham.

REFERENCES

1. Brook, I., P. Yocum, and E. H. Frazier. 1996. Bacteriology and beta-lactamase activity in acute and chronic maxillary sinusitis. *Arch. Otolaryngol. Head Neck Surg.* **122**:418–422.
2. Brook, I., E. H. Frazier, and P. A. Foote. 1996. Microbiology of the transition from acute to chronic maxillary sinusitis. *J. Med. Microbiol.* **45**:372–375.
3. Doern, G. V., A. Brueggemann, H. P. Holley, Jr., and A. M. Rauch. 1996. Antimicrobial resistance of *Streptococcus pneumoniae* recovered from outpatients in the United States during winter months of 1994 to 1995: results of a 30-center national surveillance study. *Antimicrob. Agents Chemother.* **40**:1208–1213.
4. Frederick, J., and A. I. Braude. 1974. Anaerobic infection of the paranasal sinuses. *N. Engl. J. Med.* **290**:135–137.
5. Gerardo, S. H., D. M. Citron, M. C. Claros, and E. J. C. Goldstein. 1996. Comparison of Etest to broth microdilution method for testing *Streptococcus pneumoniae* susceptibility to levofloxacin and three macrolides. *Antimicrob. Agents Chemother.* **40**:2413–2415.
6. Goldstein, E. J. C., V. L. Sutter, Y. Y. Kwok, R. P. Lewis, and S. M. Finegold. 1981. Effect of carbon dioxide on the susceptibility of anaerobic bacteria to erythromycin. *Antimicrob. Agents Chemother.* **20**:705–708.
7. Goldstein, E. J. C., and V. L. Sutter. 1983. Effect of carbon dioxide on erythromycin. *Antimicrob. Agents Chemother.* **23**:325–327.
8. Murdoch, D. A. 1998. Gram-positive anaerobic cocci. *Clin. Microbiol. Rev.* **11**:81–120.
9. Murray, P. R., E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Tenover. 1995. *Manual of clinical microbiology*, 6th ed. American Society for Microbiology, Washington, D.C.
10. National Committee for Clinical Laboratory Standards. 1997. *Methods for antimicrobial susceptibility testing of anaerobic bacteria*, 4th ed. Approved standard. NCCLS publication no. M11-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
11. National Committee for Clinical Laboratory Standards. 1997. *Method for dilution antimicrobial susceptibility testing for bacteria that grow aerobically*, 4th ed. Approved standard. NCCLS publication no. M7-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
12. Rasmussen, B. A., K. Bush, and F. P. Tally. 1997. Antimicrobial resistance in anaerobes. *Clin. Infect. Dis.* **24**(Suppl. 1):S110–S120.
13. Summanen, P., E. J. Baron, D. M. Citron, C. A. Strong, H. M. Wexler, and S. M. Finegold. 1993. *Wadsworth anaerobic bacteriology manual*, 5th ed. Star Publishing Co., Belmont, Calif.
14. Thornberry, C., P. Ogilvie, J. Khan, Y. Mauriz, and The Laboratory Investigator Group. 1997. Surveillance of antimicrobial resistance in *Streptococcus pneumoniae*, *Haemophilus pneumoniae*, and *Moraxella catarrhalis* in the United States in 1996–1997 respiratory season. *Diagn. Microbiol. Infect. Dis.* **29**:249–257.