ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1983, p. 31–38
0066-4804/83/070031-08$02.00/0

Vol. 24, No. 1

In Vitro Evaluation of Augmentin by Broth Microdilution and Disk Diffusion Susceptibility Testing: Regression Analysis, Tentative Interpretive Criteria, and Quality Control Limits

PETER C. FUCHS,[1]* ARTHUR L. BARRY,[2] CLYDE THORNSBERRY,[3] THOMAS L. GAVAN,[4] AND RONALD N. JONES[5]

Department of Pathology, St. Vincent Hospital and Medical Center, Portland, Oregon 97225[1]; Clinical Microbiology Laboratory, University of California, Davis, Medical Center, Sacramento, California 95817[2]; Antimicrobics Investigations Section, Centers for Disease Control, Atlanta, Georgia 30333[3]; Department of Microbiology, The Cleveland Clinic Foundation, Cleveland, Ohio 44106[4]; and Department of Pathology, Kaiser Foundation Laboratories (Oregon Region), Clackamas, Oregon 97015[5]

Received 7 February 1983/Accepted 2 May 1983

Augmentin (Beecham Laboratories, Bristol, Tenn.), a combination drug consisting of two parts amoxicillin to one part clavulanic acid and a potent beta-lactamase inhibitor, was evaluated in vitro in comparison with ampicillin or amoxicillin or both for its inhibitory and bactericidal activities against selected clinical isolates. Regression analysis was performed and tentative disk diffusion susceptibility breakpoints were determined. A multicenter performance study of the disk diffusion test was conducted with three quality control organisms to determine tentative quality control limits. All methicillin-susceptible staphylococci and *Haemophilus influenzae* isolates were susceptible to Augmentin, although the minimal inhibitory concentrations for beta-lactamase-producing strains of both groups were, on the average, fourfold higher than those for enzyme-negative strains. Among the *Enterobacteriaceae*, Augmentin exhibited significantly greater activity than did ampicillin against *Klebsiella pneumoniae, Citrobacter diversus, Proteus vulgaris*, and about one-third of the *Escherichia coli* strains tested. Bactericidal activity usually occurred at the minimal inhibitory concentration. There was a slight inoculum concentration effect on the Augmentin minimal inhibitory concentrations. On the basis of regression and error rate-bounded analyses, the suggested interpretive disk diffusion susceptibility breakpoints for Augmentin are: susceptible, ≥18 mm; resistant, ≤13 mm (gram-negative bacilli); and susceptible, ≥20 mm (staphylococci and *H. influenzae*). The use of a beta-lactamase-producing organism, such as *E. coli* Beecham 1532, is recommended for quality assurance of Augmentin susceptibility testing.

The increasing prevalence of clinical bacteria producing plasmid-mediated beta-lactamases has progressively restricted the usefulness of beta-lactamase-susceptible penicillins such as ampicillin and amoxicillin. When either of these drugs is combined with a potent beta-lactamase inhibitor such as clavulanic acid, the penam is protected from the beta-lactamase and is thus free to exert its normal antimicrobial activity (4, 8, 13, 20). Clavulanic acid inhibits beta-lactamases by virtue of its strong affinity for and irreversible binding to certain beta-lactamases, especially plasmid-mediated enzymes (19). Various combinations of beta-lactam antibiotics with clavulanic acid have been shown to exhibit synergism against beta-lactamase-producing bacteria (4, 8, 13, 20). Noteworthy among these bacteria are beta-lactamase-producing staphylococci, *Haemophilus influenzae, Neisseria gonorrhoeae*, and several *Enterobacteriaceae* spp. The combination of amoxicillin plus clavulanic acid at a 2-to-1 ratio is known as Augmentin (Beecham Laboratories, Bristol, Tenn.). The potential usefulness of this compound has been demonstrated in several preliminary clinical studies (3, 7, 9, 12, 18).

The purposes of this study were to (i) assess the susceptibility of a wide variety of clinical bacterial isolates to Augmentin by both broth microdilution and disk diffusion methods, (ii) evaluate the bactericidal activity of Augmentin, (iii) determine the effect of inoculum size on the activity of this drug, (iv) perform a regression analysis on the susceptibility data from which tentative susceptible and resistant breakpoints would be derived, and (v) evaluate the performance of standard quality control (QC) organisms with this drug.

MATERIALS AND METHODS

Drugs. A laboratory reference preparation of Augmentin (formerly BRL 25000) consisting of two parts amoxicillin trihydrate to one part potassium clavulanate was provided by Beecham Laboratories. Standard reference powders of ampicillin, amoxicillin, and clavulanic acid were also provided separately by Beecham Laboratories. Augmentin disks containing 20 μg of amoxicillin plus 10 μg of clavulanic acid were prepared and provided by Difco Laboratories, Detroit, Mich. Ampicillin disks (10 μg) were commercially prepared (Difco).

Bacteria. A total of 555 selected clinical isolates were collected from the Cleveland Clinic Foundation, Cleveland, Ohio; Kaiser Foundation Hospital, Clackamas, Oreg.; Northwestern Memorial Hospital, Chicago, Ill.; St. Francis Hospital, Wichita, Kans.; St. Vincent Hospital and Medical Center, Portland, Oreg.; University of California, Davis, Medical Center, Sacramento, Calif.; and the Centers for Disease Control, Atlanta, Ga. These isolates were selected as representative of clinically significant bacteria and have been used in previous studies of new beta-lactam antibiotics (5, 10). The organisms (see Tables 1 and 2) were used in susceptibility and regression analysis studies. For staphylococcal regression analysis, the 69 *Staphylococcus aureus* strains in the above group were supplemented with 50 *S. aureus*, 40 *Staphylococcus epidermidis*, and 10 *Staphylococcus saprophyticus* clinical isolates from the St. Vincent Hospital and Medical Center and the Kaiser Foundation Hospital. For inoculum size effect and bactericidal studies, 110 isolates representing 11 commonly encountered genera were utilized (see Table 3). For assessing the performance of QC organisms with Augmentin, two commonly used QC strains, *Escherichia coli* ATCC 25922 and *S. aureus* ATCC 25923, were utilized, as well as a beta-lactamase-producing strain of *E. coli* (Beecham 1532; Beecham Laboratories).

Antimicrobial susceptibility testing. The minimal inhibitory concentrations (MICs) were determined at the Centers for Disease Control and the University of California, Davis, Medical Center by broth microdilution procedures described previously (10, 11, 16). Concentrations of antimicrobial agents tested were serial twofold dilutions as follows: clavulanic acid, 16 to 0.06 μg/ml; ampicillin and amoxicillin, 64 to 0.06 μg/ml; amoxicillin-clavulanic acid (Augmentin), 64-32 to 0.015-0.008 μg/ml. Test panels were prepared by a local media manufacturer (Prepared Media Laboratory, Tualatin, Oreg.), frozen at −70°C, and shipped to the participating laboratories. The inoculum size was approximately 5×10^5 CFU/ml. Ten percent of the isolates were tested in parallel in both laboratories as a quality assurance measure. Disk diffusion susceptibility testing was conducted by the same two laboratories on the same organisms by a method previously outlined by the National Committee for Clinical Laboratory Standards (NCCLS) (15). The results of the disk and dilution tests were compared by regression analysis by the method of least squares adapted for computer computation and the error rate-bounded method (14).

Determinations of bactericidal activity were performed by subculturing 5 μl from each microtiter well onto blood agar plates with a multiple-inoculum replicating device. Endpoints were read at 24 h as the lowest concentration yielding no more than 0.1% (≤2 colonies) survivors.

Inoculum concentrations evaluated for inoculum size effect were 10^3, 5×10^5, and 10^7 CFU/ml.

For evaluating the performance of QC organisms, the currently recommended (NCCLS) disk diffusion method and QC strains for gram-negative and gram-positive organisms were used (15). In addition, beta-lactamase-producing *E. coli* Beecham 1532 was tested to evaluate the effect of the clavulanic acid component of Augmentin disks. Nine laboratories participated in this phase, including the aforementioned laboratories plus the following individuals from clinical microbiology laboratories: J. Matsen, University of Utah, Salt Lake City, Utah; L. B. Reller, University of Colorado, Denver, Colo.; and S. Brown, Good Samaritan Hospital, Portland, Oreg. Ten different lots of Mueller-Hinton agar were tested, nine by individual laboratories, and the tenth serving as a common cross-over QC lot for all participants. These included three lots from BBL Microbiology Systems, Cockeysville, Md., four lots from Difco, two lots from GIBCO Diagnostics, Madison, Wis., and one lot from Acumedia Laboratories, Baltimore, Md. Each laboratory tested the same three different lots of commerically prepared Augmentin disks (BBL and Difco), and assay potencies of which were a mean of 116% of the stated potency. Each laboratory contributed a minimum of 55 tests per disk for each QC organism, 50 with its individual Mueller-Hinton agar lot and 5 with the common cross-over Mueller-Hinton agar lot. Statistical determinations included mean zone diameter, standard deviation, individual test control, and accuracy and precision control by methods previously described (6, 15, 22).

RESULTS

The MICs of Augmentin, ampicillin, and clavulanic acid for 555 clinical isolates are shown in Tables 1 and 2. Among the *Enterobacteriaceae*, Augmentin exhibited the greatest synergistic effect against *Klebsiella pneumoniae*, *Citrobacter diversus*, and *Proteus vulgaris*, rendering virtually all ampicillin-resistant strains susceptible to the conbination drug. Among the other *Enterobacteriaceae*, Augmentin showed either a variable response (as with, e.g., *E. coli* and *Enterobacter agglomerans*) or no appreciable increased activity as compared with ampicillin. *Acinetobacter calcoaceticus* subsp. *anitratus* was the only gram-negative bacillus tested that showed any susceptibility to clavulanic acid alone. The susceptibility of individual *Acinetobacter* isolates to Augmentin generally equalled the susceptibility to the more active of the two drugs, indicating no synergistic activity with the combination. Among *Pseudomonas* spp., Augmentin exhibited activity against only *Pseudomonas stutzeri*, but this activity was no greater than that of ampicillin alone.

With the exception of methicillin-resistant staphylococci, the gram-positive cocci were sus-

ceptible to Augmentin. Beta-lactamase-producing staphylococci required 1-0.5 to 8-4 μg of Augmentin per ml for inhibition of growth, and enterococci were generally inhibited by 1-0.5 μg/ml. All others were inhibited by substantially lower concentrations. All beta-lactamase-producing *H. influenzae* strains were resistant to ampicillin and were inhibited by Augmentin, but the MICs were two to four times higher than those for ampicillin-susceptible strains. *Neisseria meningitidis* was highly susceptible to Augmentin and to each of its components individually.

There was a moderate effect of inoculum concentration on Augmentin MICs (Fig. 1), with a <0.5-$\log_2$ dilution interval between MICs with inocula of 10^3 and 5×10^5/ml. There was a 1.0-$\log_2$ dilution step difference in MICs between tests in which inoculum concentrations of 5×10^5 and 1×10^7 CFU/ml were used. Six organisms (representing three *Enterobacteriaceae* spp.) required 3 $\log_2$ concentrations more Augmentin with an inoculum of 1×10^7/ml than with 5×10^5/ml. Interestingly, the MBCs for these six organisms with an inoculum of 5×10^5 CFU/ml were identical to the MICs at this concentration. This was comparable to the inoculum size effect with ampicillin alone. With an inoculum size of 5×10^5/ml, the bactericidal activity of Augmentin against all isolates tested equalled its inhibitory activity.

The correlation of Augmentin MICs and disk diffusion zone diameter with bacteria other than staphylococci and *H. influenzae* is shown in Fig. 2. Regression analysis calculated on points in the MIC range of 2 to 64 μg/ml yielded a regression coefficient of -0.26 with a correlation coefficient of -0.89. With susceptible and resistant MIC breakpoints for Augmentin of ≤8-4 and ≥32-16 μg/ml, respectively, both regression analysis and the error rate-bounded methods indicated corresponding zone diameter breakpoints of ≥18 and ≤13 mm, with indeterminant zone diameters of 14 to 17 mm.

All *H. influenzae* strains, including 24 beta-lactamase-producing strains, were susceptible to Augmentin at ≤2 μg/ml. Augmentin MICs for beta-lactamase producers ranged from 0.5-0.25 to 2-1 μg/ml, whereas those for ampicillin-susceptible strains ranged from 0.06-0.03 to 0.5-0.25 μg/ml. The ampicillin zone diameter susceptible breakpoint for *H. influenzae* of ≥20 mm (4) correctly separated beta-lactamase producers from enzyme-deficient strains. When the same ≥20-mm susceptible breakpoint was utilized for Augmentin, the beta-lactamase-producing strains fell in the susceptible category.

All staphylococci were susceptible (MIC, ≤8.0 μg/ml) to Augmentin. Augmentin was significantly more active than ampicillin against beta-lactamase-producing staphylococci which were intermediate or resistant to ampicillin but susceptible to Augmentin. The added clavulanic acid exerted no significant effect on the beta-lactamase-negative strains.

With respect to QC organisms, *P. aeruginosa* ATCC 27853 was not tested, since no inhibitory zones would be expected with either ampicillin or Augmentin. The data obtained from the nine participating laboratories with the two NCCLS QC strains, *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, as well as *E. coli* Beecham 1532, are summarized in Table 3.

DISCUSSION

Among the *Enterobacteriaceae*, the synergistic effect of the amoxicillin-clavulanic acid combination that constitutes Augmentin was most apparent with *K. pneumoniae*, *C. diversus*, and *P. vulgaris*. Whereas some *E. coli* and *E. agglomerans* strains responded in a similar fashion, many did not, and showed no greater response to Augmentin than to ampicillin. The other *Enterobacteriaceae* and *Pseudomonas* spp. were generally resistant to both ampicillin and Augmentin at the concentrations tested. These findings are consistent with those of previous reports (9, 12) and also correlate with the recognized inhibitory activity of clavulanic acid against Richmond-Sykes types II, III, IV, and V beta-lactamases but not against type I enzymes (17). Because the *P. mirabilis* isolates in this series were all non-beta-lactamase producing and hence susceptible to ampicillin, we were unable to demonstrate the Augmentin synergy described by others against this species (4, 8, 13, 20).

Since the pharmacokinetics of amoxicillin in Augmentin are essentially the same as those of amoxicillin alone (1), the MIC correlate (≤8 μg/ml) for the disk diffusion susceptible breakpoint of ampicillin, as recommended by the NCCLS (16), was chosen for the MIC susceptible breakpoint for the amoxicillin component of Augmentin for gram-negative bacteria. Based on susceptible and resistant Augmentin breakpoints of ≤8-4 and ≥32-16 μg/ml, respectively, the corresponding zone diameter breakpoints calculated by regression and error rate-bounded methods are ≥18 and ≤13 mm, with an indeterminant range of 14 to 17 mm. The very-major and major error rates were each less than 1% with these breakpoints, and the minor-error rate was only 4.1%. That the zone diameter breakpoint for Augmentin is greater than that for ampicillin is a reflection of the 20-μg content of amoxicillin in the Augmentin disk, as compared with 10 μg in the standard ampicillin disk. Early clinical trials with urinary tract infections indi-

TABLE 1. MICs of augmentin, ampicillin, and clavulanate against gram-negative bacilli

Organism (no. of strains)	Drug[a]	MIC (μg/ml)[b]			% Strains with MIC (μg/ml) of:	
		Range	50%	90%	≤8	≤32
Escherichia coli (30)	AUG	1.0–>64	4.0	32	77	93
	AM	2.0–>64	4.0	>64	67	70
	CA	>16	>16	>16	0	
Klebsiella pneumoniae (30)	AUG	1.0–64	2.0	8.0	97	97
	AM	2.0–>64	32	>64	3	73
	CA	>16	>16	>16	0	
Enterobacter cloacae (22)	AUG	2.0–>64	64	>64	14	18
	AM	1.0–>64	>64	>64	9	23
	CA	>16	>16	>16	0	
Enterobacter aerogenes (22)	AUG	1.0–64	64	64	5	5
	AM	8.0–>64	>64	>64	5	5
	CA	16	>16	>16	0	
Enterobacter agglomerans (10)	AUG	0.5–>64	2.0	>64	80	80
	AM	0.5–>64	16	32	40	90
	CA	>16	>16	>16	0	
Enterobacter gergoviae (2)	AUG	>64	>64	>64	0	0
	AM	>64	>64	>64	0	0
	CA	>16	>16	>16	0	
Serratia marcescens (29)	AUG	32–>64	>64	>64	0	3
	AM	16–>64	>64	>64	0	28
	CA	>16	>16	>16	0	
Citrobacter diversus (11)	AUG	2.0–8.0	2.0	2.0	100	100
	AM	32–64	32	64	0	63
	CA	>16	>16	>16	0	
Citrobacter freundii (12)	AUG	8.0–64	32	64	8	75
	AM	4.0–64	16	64	17	75
	CA	>16	>16	>16	0	
Morganella morganii (11)	AUG	64–>64	>64	>64	0	0
	AM	16–>64	>64	>64	0	9
	CA	>16	>16	>16	0	
Proteus mirabilis (30)	AUG	0.5–1.0	0.5	1.0	100	100
	AM	0.5–2.0	1.0	1.0	100	100
	CA	>16	>16	>16	0	
Proteus vulgaris (12)	AUG	1.0–32	4.0	8.0	92	100
	AM	1.0–>64	>64	>64	25	33
	CA	>16	>16	>16	0	
Providentia rettgeri (12)	AUG	0.5–>64	64	>64	8	25
	AM	0.25–>64	32	>64	17	50
	CA	>16	>16	>16	0	
Providentia stuartii (24)	AUG	16–>64	64	>64	0	8
	AM	16–>64	64	>64	0	38
	CA	>16	>16	>16	0	
Acinetobacter calcoaceticus subsp. *anitratus* (18)	AUG	2–64	16	64	44	78
	AM	4–64	32	64	11	72
	CA	4–16	>16	>16	39	

TABLE 1. *Continued*

Organism (no. of strains)	Drug[a]	MIC (μg/ml)[b]			% Strains with MIC (μg/ml) of:	
		Range	50%	90%	≤8	≤32
Pseudomonas aeruginosa (30)	AUG	32–>64	64	>64	0	3
	AM	>64	>64	>64	0	0
	CA	>16	>16	>16	16	
Pseudomonas sp.[c] (38)	AUG	1–>64	64	>64	39	47
	AM	1–>64	>64	>64	32	32
	CA	8–>16	>16	>16	3	

[a] AUG, Augmentin; AM, ampicillin; CA, clavulanic acid.
[b] MICs given for amoxicillin only. 50% and 90%, MICs inhibiting 50 and 90% of strains respectively.
[c] Includes *P. acidovorans* (4) *P. cepacia* (5), *P. fluorescens* (7), *P. maltophilia* (4), *P. putida* (6), and *P. stutzeri* (12).

cate that many of the infections caused by beta-lactamase-producing *Enterobacteriaceae* strains respond when treated with Augmentin (9, 12, 17, 21). Since (i) levels of Augmentin in urine may be ≥100-fold than those in serum, (ii) the suggested breakpoints are based on achievable serum levels, and (iii) no published studies on Augmentin therapy of non-urinary tract infections caused by beta-lactamase-producing *Enterobacteriaceae* strains are currently available, we consider the above interpretive criteria to be tentative at this time.

Our data on *H. influenzae* supports that of others (2, 18) in that beta-lactamase-producing strains are both resistant to ampicillin and susceptible to Augmentin. Although the Augmentin

TABLE 2. MICs of Augmentin, ampicillin, and clavulanic acid for gram-positive cocci, *H. influenzae*, and *N. meningitidus*

Organism (no. of strains)[a]	Drug[b]	MIC (μg/ml)[c]			% Strains with MIC of 8 μg/ml
		Range	50%	90%	
Streptococcus pyogenes (20)	AUG	0.03	0.03	0.03	100
	AM	0.125	0.125	0.125	100
	CA	8.0–>16	>16	>16	5
Streptococcus pneumoniae (20)	AUG	0.03–0.06	0.125	0.25	100
	AM	0.125–4.0	0.125	0.25	100
	CA	16–>16	16	>16	0
Streptococcus faecalis (30)	AUG	0.5–8.0	1.0	1.0	100
	AM	0.5–16.0	1.0	1.0	97
	CA	>16	>16	>16	0
Staphylococcus aureus (MR) (12)	AUG	8.0–64	16	32	8
	AM	16–>64	32	>64	0
	CA	>16	>16	>16	0
Staphylococcus aureus (MS) (57)	AUG	0.06–8.0	0.5	8.0	100
	AM	0.125–64	1.0	16	88
	CA	8–>16	>16	>16	2
Haemophilus influenzae (BL^+) (24)	AUG	0.5–2.0	1.0	2.0	100
	AM	32–>64	>64	>64	0
	CA	NT[d]	NT	NT	NT
Haemophilus influenzae (BL^-) (24)	AUG	0.06–2.0	0.25	0.25	100
	AM	0.12–2.0	0.12	0.25	100
	CA	NT	NT	NT	NT
Neisseria meningitidis (25)	AUG	≤0.06–0.12	≤0.06	0.12	100
	AM	≤0.06–0.12	≤0.06	0.12	100
	CA	0.12–8.0	0.5	1.0	100

[a] MR and MS, Methicillin resistant and susceptible, respectively; BL^+ and BL^-, beta-lactamase positive and negative, respectively.
[b] AUG, Augmentin; AM, ampicillin; CA, clavulanic acid.
[c] MIC given for amoxicillin only. 50% and 90%, MIC inhibiting 50 and 90% of strains, respectively.
[d] NT, Not tested.

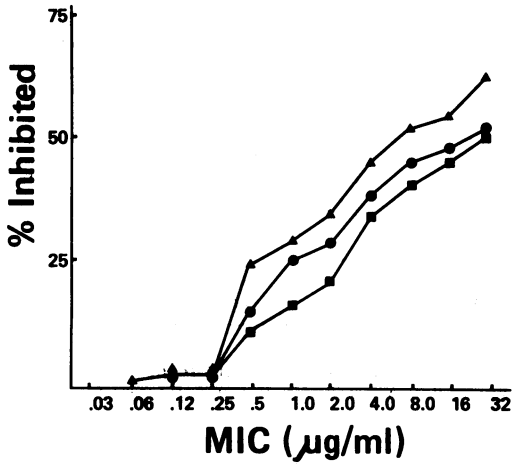

FIG. 1. Effect of inoculum density on Augmentin MICs (expressed as concentration of amoxicillin component) for 110 bacterial strains (14 species). ▲, 10^3 CFU/ml; ●, 10^5 CFU/ml; ■, 10^7 CFU/ml.

MICs for beta-lactamase-producing strains were, on the average, fourfold higher than those for non-beta-lactamase producers, the MICs were all ≤2-1 μg/ml and thus equivalent to the 2-μg/ml susceptible breakpoint for ampicillin (10). Since our *H. influenzae* population contained no Augmentin-resistant strains, we have tentatively chosen a zone diameter of ≥20 mm as the susceptible breakpoint for *H. influenzae* to stay in conformity with the current ampicillin breakpoint (15). All strains tested fell into this zone diameter category. Because the clinical correlative data is limited at this time, a tentative stance is recommended.

The staphylococci are another group of organisms that require separate consideration, not only because of their variable beta-lactamase production, but also because some are resistant to the penicillinase-resistant penicillins. Although the MICs of Augmentin are lower and the Augmentin disk inhibitory zone diameters are larger for methicillin-resistant *S. aureus* than those of ampicillin, the changes are modest. In light of the data on other beta-lactam antibiotics with methicillin-reisistant staphylococci, we recommend that such organisms be considered resistant to Augmentin, irrespective of susceptibility test results.

With both beta-lactamase-producing *S. aureus* and *S. epidermidis*, a substantial reduction in MICs were observed with Augmentin as compared with ampicillin. As with *H. influenzae*, the Augmentin MICs for beta-lactamase-producing strains were approximately fourfold higher than for beta-lactamase-negative strains, but all were

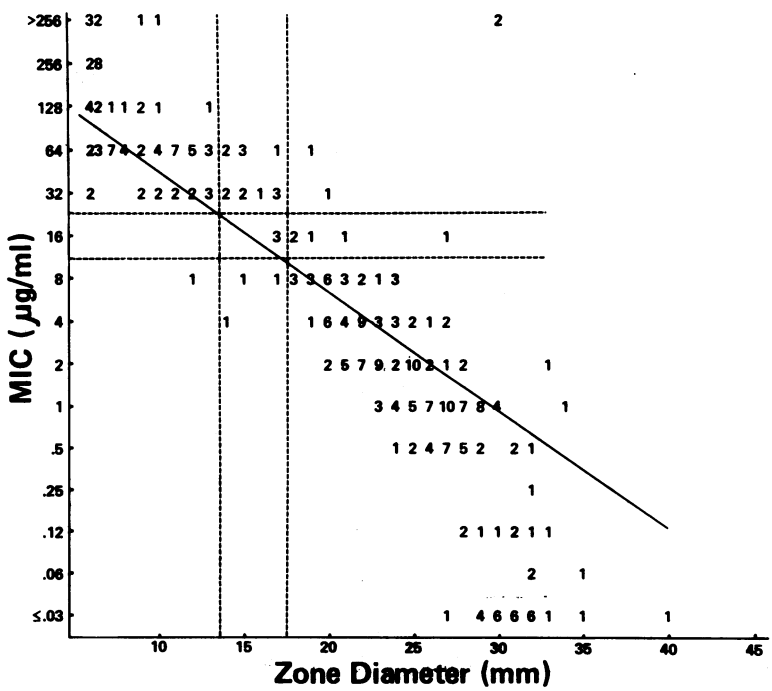

FIG. 2. Augmentin MIC (expressed as concentration of amoxicillin component) and disk (20 μg of amoxicillin–10 μg of clavulanic acid) diffusion zone diameter correlation with 412 organisms (excluding staphylococci and *H. influenzae*). ——, Regression line; ------, proposed susceptible and resistant breakpoints.

TABLE 3. Combined zone diameter results of QC organisms in nine laboratories and estimated control limits for monitoring precision and accuracy of inhibitory zone diameters

Organism	Drug[a]	Disk content (μg)	No. of tests	Zone diam (mm)					Precision control (mm)[c]		% Out of control range
				Mean	Medium	Range	Individual daily test[b]	Accuracy control	Maximum	Avg	
S. aureus	AM	10	445	31.9	32	25–38	29–35	30–34	7	3.5	3.1
ATCC 25923	AUG	20–10	1,323	32.0	33	25–38	28–36	29.3–34.7	9	4.7	4.6
E. coli	AM	10	450	19.3	20	16–24	17–21	12.7–20.3	4	2.3	0.7
ATCC 25922	AUG	20–10	1,350	22.2	22	15–27	19–25	20–24	7	3.5	0.7
E. coli	AM	10	400	6	6						
Beecham 1532	AUG	20–10	1,340	20.4	20	17–23	18–22	19.7–21.3	4	2.3	2.8

[a] AM, Ampicillin; AUG, augmentin.

[b] Mean of five consecutive values.

[c] Range of five consecutive values (maximum minus minimum values) should not exceed the maximum listed, and the mean should fall within the accuracy control range. In continuing series with ranges of five consecutive value groups, the average range should not exceed the listed value.

inhibited by ≤8/4 μg/ml and thus would be considered susceptible to Augmentin. Again, as with *H. influenzae*, no methicillin-susceptible, augmentin-resistant staphylococci were encountered in the population studied, and thus it is tenuous to select a zone diameter breakpoint. An Augmentin inhibitory zone diameter breakpoint of ≥18 or ≥20 mm (staphylococci and *H. influenzae*, respectively) would adequately encompass the susceptible strains in this study. Since an intermediate category should not be recognized with staphylococci or *H. influenzae*, it seems reasonable to select ≥20 mm as the susceptible breakpoint. Again, the sparcity of reported clinical data precludes definitive recommendations at this time.

With respect to ampicillin, there were seven *S. aureus* strains whose zone diameters of inhibition fell in the intermediate zone; all were beta-lactamase producers and hence should be considered resistant. In light of this as well as previous experience, we question the validity of the concept of an intermediate category for ampicillin (or penicillin) against staphylococci as currently recommended by the NCCLS (15).

Using previously described methods (6, 15, 22), we calculated various disk diffusion susceptibility control limits for Augmentin and ampicillin with three QC organisms. With both *S. aureus* ATCC 25923 and *E. coli* ATCC 25922, the calculated limits for ampicillin were somewhat narrower than those recommended by the NCCLS (15) but fell within those limits. These results served as a control and validate the results obtained with Augmentin. The mean inhibitory zone diameters produced by Augmentin disks (with 20 μg of amoxicillin) were either essentially the same (*S. aureus*) or slightly lower (*E. coli*) than those obtained with 10-μg ampicillin disks.

The problem with using these two strains for QC of Augmentin susceptibility testing is that they are both non-beta-lactamase producers, and hence the results reflect only the amoxicillin component of the drug. For the control of the clavulanic acid component, a beta-lactamase-producing organism, such as *E. coli* Beecham 1532, must be used. When tested in the nine participating laboratories, this organism was not inhibited by ampicillin (6-mm zone diameters), but a median inhibitory zone diameter of 20 mm was obtained with Augmentin. Since clavulanic acid is the less stable of the two Augmentin components, it appears to be important to use a beta-lactamase-positive organism, such as *E. coli* Beecham 1532, for quality assurance, at least periodically if not routinely. Conversely, because *E. coli* may lose its beta-lactamase during storage, it is equally important to confirm the ampicillin resistance of *E. coli* Beecham 1532 before Augmentin quality assurance data is interpreted.

LITERATURE CITED

1. **Adam, D., I. DeVisser, and P. Koeppe.** 1982. Pharmacokinetics of amoxicillin and clavulanic acid administered alone and in combination. Antimicrob. Agents Chemother. 22:353–357.
2. **Boon, R. J., A. S. Berle, K. R. Comber, C. V. Pierce, and R. Sutherland.** 1982. Distribution of amoxicillin and clavulanic acid in infected animals and efficacy against experimental infections. Antimicrob. Agents Chemother. 22:369–375.
3. **Crokaert, F., M. P. Van der Linden, and E. Yourassowsky.** 1982. Activities of amoxicillin and clavulanic acid combination against urinary tract infections. Antimicrob. Agents Chemother. 22:346–349.
4. **Dumon, L., P. Adriaens, J. Anné, and H. Eysson.** 1979. Effect of clavulanic acid on the minimum inhibitory concentration of benzylpenicillin, ampicillin, carbenicillin, or cephalothin against clinical isolates resistant to beta-lactam antibiotics. Antimicrob. Agents Chemother. 15:315–317.
5. **Fuchs, P. C., A. L. Barry, C. Thornsberry, R. N. Jones, T. L. Gavan, and H. M. Sommers.** 1980. Cefotaxime: in

vitro activity and tentative interpretive standards for disk susceptibility testing. Antimicrob. Agents Chemother. 18:88–93.

6. **Gavan, T. L., R. N. Jones, A. L. Barry, P. C. Fuchs, E. H. Gerlach, J. M. Matsen, L. B. Reller, C. Thornsberry, and L. D. Thrupp.** 1981. Quality control limits for ampicillin, carbenicillin, mezlocillin, and piperacillin disc diffusion susceptibility tests: a collaborative study. J. Clin. Microbiol. 14:67–72.
7. **Goldstein, F. W., M. P. Kitzis, and J. F. Acar.** 1979. Effect of clavulanic acid and amoxycillin formulation against beta-lactamase producing gram-negative bacteria in urinary tract infections. J. Antimicrob. Chemother. 5:705–709.
8. **Hunter, P. A., K. Coleman, J. Fisher, and D. Taylor.** 1980. In vitro synergistic preparation of clavulanic acid, with ampicillin, amoxycillin and ticarcillin. J. Antimicrob. Chemother. 6:455–470.
9. **Iravani, A., and G. A. Richard.** 1982. Treatment of urinary tract infection with a combination of amoxicillin and clavulanic acid. Antimicrob. Agents Chemother. 22:672–677.
10. **Jones, R. N., P. C. Fuchs, A. L. Barry, T. L. Gavan, H. M. Sommers, and E. H. Gerlach.** 1980. Cefoperazone (T-1551), a new semisynthetic cephalosporin: comparison with cephalothin and gentamicin. Antimicrob. Agents Chemother. 17:743–749.
11. **Jones, R. N., P. C. Fuchs, T. L. Gavan, E. H. Gerlach, A. C. Barry, and C. Thornsberry.** 1977. Cefuroxime, a new parenteral cephalosporin: collaborative in vitro susceptibility comparison with cephalothin against 5,887 clinical bacterial isolates. Antimicrob. Agents Chemother. 12:47–50.
12. **Martinelli, R., A. Alberto da Silva Lopes, M. M. M. G. de Oliveira, and H. Rocha.** 1981. Amoxicillin-clavulanic acid in treatment of urinary tract infection due to gram-negative bacteria resistant to penicillin. Antimicrob. Agents Chemother. 20:800–802.
13. **Matsura, M., H. Nakazawa, T. Hashimoto, and S. Mitsuhashi.** 1980. Combined antibacterial activity of amoxicillin with clavulanic acid against ampicillin-resistant strains. Antimicrob. Agents Chemother. 17:908–911.
14. **Metzler, C. M., and R. M. Dehaan.** 1974. Susceptibility of anaerobic bacteria: statistical and clinical considerations. J. Infect. Dis. 130:588–594.
15. **National Committee for Clinical Laboratory Standards.** 1979. Performance standards for antimicrobial disc susceptibility tests. Approved standard M2-A2, 2nd ed. National Committee for Clinical Laboratory Standards, Villanova, Pa.
16. **National Committee for Clinical Laboratory Standards.** 1982. Standard methods for dilution antimicrobial susceptibility tests for bacteria which grow aerobically. Proposed standard M7-T. National Committee for Clinical Laboratory Standards, Villanova, Pa.
17. **Neu, H. C., and K. P. Fu.** 1978. Clavulanic acid, a novel inhibitor of beta-lactamase. Antimicrob. Agents Chemother. 14:650–655.
18. **Ninane, G., J. Joby, M. Kraytman, and P. Piot.** 1978. Broncho-pulmonary infection due to beta-lactamase-producing *Branhamella catarrhalis* treated with amoxycillin-/clavulanic acid. Lancet ii:257.
19. **Reading, C., and M. Cole.** 1977. Clavulanic acid: a beta-lactamase-inhibiting beta-lactam from *Streptomyces clavuligerus*. Antimicrob. Agents Chemother. 11:852–857.
20. **Wise, R., J. M. Andrews, and K. A. Bedford.** 1978. In vitro study of clavulanic acid in combination with penicillin, amoxycillin, and carbenicillin. Antimicrob. Agents Chemother. 13:389–393.
21. **Yogev, R., C. Melick, and W. J. Kabat.** 1981. In vitro and in vivo synergism between amoxicillin and clavulanic acid against ampicillin-resistant *Haemophilus influenzae* type b. Antimicrob. Agents Chemother. 19:993–996.
22. **Zar, J. H.** 1974. Biostatistical analysis. Prentice-Hall, Inc., Englewood Cliffs, N.J.