

Correlation of In Vitro Susceptibility Results for Amoxicillin-Clavulanate and Ampicillin-Sulbactam Tested against *Escherichia coli*

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The results of amoxicillin-clavulanate (AUG) and ampicillin-sulbactam (A/S) susceptibility testing by three different susceptibility testing methods, the MicroScan, Etest, and Kirby-Bauer methods, for 61 consecutive isolates of ampicillin-resistant *Escherichia coli* from different patients were compared. There was poor correlation of results for the two agents, the most and least marked discrepancies being observed by the MicroScan method (86.9% susceptible to AUG and 4.9% susceptible to A/S) and the Kirby-Bauer method (39.4% susceptible to AUG and 32.8% susceptible to A/S), respectively. More organisms were susceptible to AUG than A/S, regardless of the susceptibility testing methodology. The results from a College of American Pathologists survey with one *E. coli* isolate tested at different institutions also indicated greater susceptibility to AUG than to A/S. These agents are thought to be equally efficacious clinically. The discrepancies observed among methods for each antimicrobial inhibitor combination and the discrepancies observed between the two agents by each testing method suggest that the breakpoints for these agents need to be reevaluated.

The National Committee for Clinical Laboratory Standards (NCCLS) considers amoxicillin-clavulanate (AUG) and ampicillin-sulbactam (A/S) essentially equivalent agents that “need not be duplicated in testing because interpretive results are usually similar and clinical efficacy comparable” (4). They are considered to have “an almost identical spectrum of activity and interpretive results, and for which cross-resistance and susceptibility are nearly complete” (4). However, we observed a frequent lack of concordance of the results of AUG and A/S against *Escherichia coli*. The results from a recent College of American Pathologists (CAP) survey also suggested a lack of correlation between AUG and A/S for an isolate of ampicillin-resistant *E. coli*, regardless of the susceptibility test methodology used by participating laboratories (1). As a result of these findings, we undertook a more extensive analysis of the in vitro susceptibilities of *E. coli* isolates to these agents. (A portion of these data was presented previously [6].)

A total of 61 consecutive ampicillin-resistant *E. coli* isolates (1 per patient) obtained from clinical specimens collected at the Warren G. Magnuson Clinical Center at the National Institutes of Health was tested. The sources of isolates were urine (48 isolates), blood (5 isolates), skin lesions (3 isolates), sputum (2 isolates), abscesses (2 isolates), and biopsy (1 isolate). The isolates were maintained frozen at -70°C in tryptic soy broth with 15% glycerol (Remel, Lenexa, Kans.) and were subcultured at least twice on Trypticase soy agar with 5% sheep blood (Remel) before testing. MicroScan Gram Negative BP Combo-8 panels (Dade MicroScan, Inc., West Sacramento, Calif.) were inoculated according to the manufacturer's instructions. With the same 0.5 McFarland suspension used to inoculate the MicroScan plates, the surfaces of two 100-mm Mueller-Hinton plates (Remel) were inoculated with a swab by evenly streaking in three directions. After the plates were allowed to dry for approximately 5 min, AUG (20 and 10 μg of

amoxicillin and clavulanate, respectively) and A/S (10 and 10 μg of ampicillin and sulbactam, respectively) antibiotic disks (Becton Dickinson, Sparks, Md.) were placed on the surface of one plate and AUG and A/S Etest strips (AB Biodisk, Culver City, Calif.) were placed on the surface of the other plate. Purity check plates were inoculated for each suspension, and colony counts were performed on randomly selected suspensions to verify proper inoculum concentrations. MicroScan panels and Mueller-Hinton plates were incubated for 18 to 20 h at 35°C in ambient air, while purity and colony count plates were incubated at 35°C in 5% CO_2 . MicroScan panels were read with an autoSCAN-4 (Dade MicroScan, Inc.) according to the manufacturer's instructions, and the results were confirmed visually. The results of disk diffusion testing were obtained according to NCCLS guidelines (4). Etest MICs were determined according to the manufacturer's instructions. NCCLS guidelines were used for the interpretation of disk diffusion and MIC testing results (5). *E. coli* ATCC 25922 was inoculated as a quality control for each susceptibility system each time that testing was performed. Susceptibility test results were extracted from the 1994 CAP bacteriology survey (1), in which the efficacies of AUG and A/S against one isolate of ampicillin-resistant *E. coli* were tested at different institutions by disk testing and MIC methods (1); these data are compiled in tabular form in Table 1.

Susceptibility test results for all 61 isolates tested by the

TABLE 1. Results from participants in 1994 CAP bacteriology survey^a

Method	Antibiotic	No. of participants	No. (%) of participants reporting the following results:		
			Sensitive	Intermediate	Resistant
MIC	AUG	26	27 (78.7)	28 (15.8)	29 (5.5)
	A/S	30	31 (1.3)	32 (31.7)	33 (67.0)
Disk	AUG	34	35 (16.8)	36 (63.4)	37 (19.8)
	A/S	38	39 (8.7)	40 (45.6)	41 (45.6)

^a Set D-C, specimen D-15, for *E. coli* (see reference 1).

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TABLE 2. Comparative analysis of AUG and A/S susceptibility results

Test	No. of isolates ^a (%) tested with AUG			No. of isolates ^a (%) tested with A/S		
	Sensitive ^b	Intermediate ^c	Resistant ^d	Sensitive ^e	Intermediate ^f	Resistant ^g
MicroScan	53 (86.9)	6 (9.8)	2 (3.3)	3 (4.9)	24 (39.4)	34 (55.7)
Etest	33 (54.1)	23 (37.7)	5 (8.2)	3 (4.9)	22 (36.1)	36 (59.0)
Kirby-Bauer	24 (39.4)	31 (50.8)	6 (9.8)	20 (32.8)	14 (22.9)	27 (44.3)

^a $n = 61$ ampicillin-resistant *E. coli* isolates.

^b MIC, $\leq 8/4$ $\mu\text{g/ml}$; zone diameter, >17 mm.

^c MIC, $16/8$ $\mu\text{g/ml}$; zone diameter, 14 to 17 mm.

^d MIC, $>16/8$ $\mu\text{g/ml}$; zone diameter, ≤ 13 mm.

^e MIC, $\leq 8/4$ $\mu\text{g/ml}$; zone diameter, >14 mm.

^f MIC, $16/8$ $\mu\text{g/ml}$; zone diameter, 12 to 14 mm.

^g MIC, $>16/8$ $\mu\text{g/ml}$; zone diameter, ≤ 11 mm.

MicroScan, Etest, and Kirby-Bauer methods are summarized in Table 2. By MicroScan testing, 53 (86.9%) isolates were susceptible to AUG, while 3 (4.9%) isolates were susceptible to A/S. A total of 2 (3.3%) isolates were resistant to AUG, and 34 (54.7%) isolates were resistant to A/S. Overall, by MicroScan testing, the susceptibility results for 58 (95.0%) of the 61 isolates did not correlate.

By the Etest, 33 (54.1%) isolates were susceptible to AUG, while 3 (4.9%) isolates were susceptible to A/S. A total of 5 isolates (8.2%) were resistant to AUG, and 36 (59.0%) isolates were resistant to A/S. Overall, by the Etest, the results for 53 (86.9%) isolates did not correlate.

By the Kirby-Bauer method, 24 (39.4%) isolates were susceptible to AUG, while 20 (32.8%) isolates were susceptible to A/S. A total of 6 (9.8%) isolates were resistant to AUG, and 27 (44.3%) isolates were resistant to A/S. Overall, by the Kirby-Bauer method, the results for 33 (54.1%) isolates did not correlate.

With regard to the CAP isolate, by MIC testing, 617 participants (78.7% of those testing for AUG) found it to be susceptible to AUG and 9 participants (1.3% of those testing for A/S) found the isolate to be susceptible to A/S. By disk testing, 28 participants (16.8% of those testing for AUG) found the isolate to be susceptible to AUG and 12 participants (8.7% of those testing for A/S) found it to be susceptible to A/S.

Jones and Barry compared MICs of A/S and AUG and concluded that although these two beta-lactamase inhibitor-beta-lactam combination drugs appeared overall to have comparable activities against members of the *Enterobacteriaceae* family, nevertheless there were sufficient discrepancies between the results for AUG and A/S that the two agents should be tested separately. At the breakpoints that these authors used, five of their *E. coli* isolates were susceptible to AUG but resistant to A/S (3). In a multiinstitutional survey, Jochimsen et al. analyzed the results of clinical trials and in vitro susceptibility data for A/S for members of the *Enterobacteriaceae* (2). These authors found a disparity of $>20\%$ between in vitro testing results and clinical outcome; patients were often cured or improved with A/S therapy when in vitro susceptibility could not be demonstrated. These authors suggested a number of changes in susceptibility testing procedures, including changing

the NCCLS breakpoints to achieve better correlation between in vivo and in vitro results for A/S (2).

Our data demonstrate that, for *E. coli*, there is poor correlation between the results for AUG and A/S, with the in vitro susceptibility results for one agent being poorly predictive of the results for the other agent. The results did not correlate for 58 isolates (95.0%) by the MicroScan method, for 53 isolates (86.9%) by the Etest method, and for 33 isolates (54.1%) by the Kirby-Bauer method. While the greatest discrepancies for these two agents were observed by MicroScan testing, the results were nearly as discrepant by the Etest method. The CAP proficiency test results (1) for one *E. coli* isolate are in general agreement with those from our multiorganism study. In addition, clinical trials (2) suggest in vivo response to A/S with isolates showing in vitro resistance. If, in fact, AUG and A/S are of equivalent clinical efficacies, it appears that the susceptibility testing breakpoints for these agents need to be reevaluated.

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