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Short Communication

In Vitro Susceptibilities of *Escherichia coli* and *Klebsiella* Spp. to Ampicillin-Sulbactam and Amoxicillin-Clavulanic Acid

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SUMMARY: Ampicillin-sulbactam (A/S) and amoxicillin-clavulanic acid (AUG) are thought to be equally efficacious clinically against the *Enterobacteriaceae* family. In this study, the in vitro activities of the A/S and AUG were evaluated and compared against *Escherichia coli* and *Klebsiella* spp. Antimicrobial susceptibility tests were performed by standard agar dilution and disc diffusion techniques according to the Clinical and Laboratory Standards Institute (CLSI). During the study period, 973 strains were isolated. Of the 973 bacteria isolated, 823 were *E. coli* and 150 *Klebsiella* spp. More organisms were found to be susceptible to AUG than A/S, regardless of the susceptibility testing methodology. The agar dilution results of the isolates that were found to be sensitive or resistant were also compatible with the disc diffusion results. However, some differences were seen in the agar dilution results of some isolates that were found to be intermediately resistant with disc diffusion. In *E. coli* isolates, 17 of the 76 AUG intermediately resistant isolates (by disc diffusion), and 17 of the 63 A/S intermediately resistant isolates (by disc diffusion) showed different resistant patterns by agar dilution. When the CLSI breakpoint criteria are applied it should be considered that AUG and A/S sensitivity in *E. coli* and *Klebsiella* spp. strains may show differences.

As in other members of the *Enterobacteriaceae* family, resistance to β-lactam antibiotics in *Escherichia coli* is primarily due to the production of β-lactamases. When β-lactamase inhibitors are added to the ampicillin and amoxicillin, there is a significant increase in susceptibility (1). The Clinical and Laboratory Standards Institute (CLSI, formerly National Committee for Clinical Laboratory Standards) considers amoxicillin-clavulanic acid (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'. They are considered to have 'an almost identical spectrum of activity and interpretive results, and for which cross-resistance and susceptibility are nearly complete' (2). However, investigators have observed a frequent lack of concordance of the results of AUG and A/S against *E. coli* (3).

In the present study the in vitro activities of the A/S and AUG were evaluated and compared against *E. coli* and *Klebsiella* spp.

E. coli and *Klebsiella* spp. strains isolated from various clinical materials (urine, blood, skin lesions, sputum, and periton) between January and June 2006 were used in the study. Organisms were identified by conventional methods and confirmed by API 20E (bioMerieux, Marcy l'Etoile, France). Susceptibility profiles were obtained by the standard disc diffusion and agar dilution methods. The results of disc diffusion testing and agar dilution were evaluated according to CLSI guidelines (2). Antimicrobial agents for agar dilution susceptibility testing were provided by SmithKline Beecham Pharmaceuticals, Madrid, Spain (ampicillin, amoxicillin, and clavulanate) and Pfizer S. A., Madrid, Spain (sulbactam). *E. coli* ATCC 25922 was inoculated as a quality control for each susceptibility system each time that testing was performed.

During the study period, 973 strains were isolated. Of these, 823 were *E. coli* and 150 *Klebsiella* spp. In *E. coli* isolates, by disc diffusion testing, 414 (50%) isolates were found to be susceptible to AUG, while 373 (45%) were susceptible to A/S. A total of 333 (40%) isolates were resistant to AUG, and 387 (47%) isolates were resistant to A/S. Overall, by disc diffusion testing, the susceptibility results for 206 (25%) of the 823 isolates did not correlate (Table 1). In *Klebsiella* spp. isolates, by disc diffusion testing, 57 (38%) isolates were susceptible to AUG, while 50 (33%) were susceptible to A/S. A total of 82 (55%) isolates were resistant to AUG, and 94 (63%) were resistant to A/S. Overall, by disc diffusion testing, the susceptibility results for 31 (20%) of the 150 isolates did not correlate (Table 1).

In *E. coli* isolates, by agar dilution testing, 418 (51%) isolates were found to be susceptible to AUG, while 386 (47%) were susceptible to A/S. A total of 346 (42%) isolates were resistant to AUG, and 391 (48%) were resistant to A/S. Overall, by agar dilution testing, the susceptibility results for 190 (23%) of the 823 isolates did not correlate (Table 2). In *Klebsiella* spp. isolates, by agar dilution testing, 57 (38%) were susceptible to AUG, while 51 (34%) were susceptible to A/S. A total of 87 (58%) isolates were resistant to AUG, and 94 (63%) were resistant to A/S. Overall, by agar dilution testing, the susceptibility results for 27 (18%) of the 150 isolates did not correlate (Table 2).

The agar dilution results for most of the isolates that were found to be sensitive or resistant were also compatible with the disc diffusion results. However, some differences were seen in the agar dilution results of some isolates that were found to be intermediate with regard to disc diffusion. In *E. coli*, 76 isolates were found to be intermediately resistant to AUG by disc diffusion. Incompatible results were found in 17 and compatible results in 59 of 76 AUG intermediately

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Table 1. Comparative analysis of AUG and A/S susceptibility results in *E. coli* and *Klebsiella* spp. isolates by disc difusion method

		AUG							
		S		I		R		Total (%)	
		E. coli	*Klebsiella* spp.	*E. coli*	*Klebsiella* spp.	*E. coli*	*Klebsiella* spp.	*E. coli*	*Klebsiella* spp.
A/S	S	322	41	16	3	35	6	373 (45)	50 (33)
	I	24	4	18	2	21	–	63 (8)	6 (4)
	R	68	12	42	6	277	76	387 (47)	94 (63)
	Total (%)	414 (50)	57 (38)	76 (10)	11 (7)	333 (40)	82 (55)	823	150

AUG, amoxicillin-clavulanic acid; A/S, ampicillin-sulbactam; S, sensitive; I, intermediate; R, resistant.

Table 2. Comparative analysis of AUG and A/S susceptibility results in *E. coli* and *Klebsiella* spp. isolates by agar dilution method

		AUG							
		S		I		R		Total (%)	
		E. coli	*Klebsiella* spp.	*E. coli*	*Klebsiella* spp.	*E. coli*	*Klebsiella* spp.	*E. coli*	*Klebsiella* spp.
A/S	S	330	42	21	3	35	6	386 (47)	51 (34)
	I	20	3	9	1	17	1	46 (5)	5 (3)
	R	68	12	29	2	294	80	391 (48)	94 (63)
	Total (%)	418 (51)	57 (38)	59 (7)	6 (4)	346 (42)	87 (58)	823	150

Abbreviations are in Table 1.

resistant isolates by agar dilution compared to disc diffusion. Sixty-three isolates were found to be intermediately resistant to A/S by disc diffusion in *E. coli*. Incompatible results were found in 17, and compatible results were found in 46 of 63 A/S intermediately resistant isolates by agar dilution compared to disc diffusion. In *Klebsiella* spp., 5 of the 11 A/S intermediately resistant isolates, and 1 of the 6 AUG intermediately resistant isolates showed different resistant patterns by agar dilution compared to disc diffusion.

The destruction of β-lactams by β-lactamases is the most important resistance mechanism in Gram-negative bacteria. The genes encoding β-lactamases can be located on the bacterial chromosome, on plasmids, or on transposons (1,4). Recently, an increasing number of β-lactamase genes have been discovered on integrons (5). More than 340 β-lactamase enzymes have been detected to date (4,6). In *Klebsiella* spp. and *E. coli* isolates, resistance to β-lactam-β-lactamase inhibitor combinations occur due to (i) modified outer membrane permeability, (ii) the emerging class A SHV and TEM-derived extended-spectrum β-lactamases (ESBLs) and inhibitor-resistant enzymes, (iii) non-TEM, non-SHV ESBLs, and class B metallo-β-lactamases and some of their novel inhibitors, (iv) plasmid and chromosomally encoded class C enzymes, and (v) the OXA-type and CTX-M ESBLs (4,6,7). The TEM-1 β-lactamase production levels depend upon the number of plasmid copies, the number of gene copies per plasmid, and the promoter efficiency (8,9). Various investigators have already pointed out the correlation between the level of resistance to inhibitor combinations and the amount of enzyme produced (10). Oliver et al. (11) have observed this correlation in their study.

Jones and Barry (12) have compared MICs of A/S and AUG, and have concluded that although these two β-lactamase inhibitor-β-lactam combination drugs appear overall to have comparable activities against members of the *Enterobacteriaceae* family, there are nevertheless sufficient discrepancies between the results for AUG and A/S that the two agents should be tested separately. Clavulanate has been previously described to be a better inhibitor of broad-spectrum plasmid-mediated β-lactamases than sulbactam (3,13). In our study, more organisms were found to be susceptible to AUG than A/S, regardless of the susceptibility testing methodology. With the CLSI criteria, discrepancies between both combinations were noted in our series, and thus A/S was found to be a bad predictor for AUG susceptibility; therefore, both antibiotics or, specifically, the one that is intended to be used as a therapeutic option must be tested. This phenomenon is probably related to the amount of enzyme that is produced by bacteria and/or the differential activities of clavulanate and sulbactam against various β-lactamases.

As a result, when the CLSI breakpoint criteria are applied, it should be considered that AUG and A/S sensitivity in *E. coli* and *Klebsiella* spp. isolations may show differences and that both AUG and A/S should be separately evaluated.

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