

IN-VITRO ACTIVITY OF SULBACTAM-AMPICILLIN COMBINATION

F. MOOSDEEN, PhD, MRCPATH; and V.K.E. LIM, MBBS, MRCPATH

Department of Microbiology, Faculty of Medicine, Universiti Kebangsaan Malaysia, Kuala Lumpur.

Summary

The activity of sulbactam (a beta-lactamase inhibitor) in combination with ampicillin against 415 recent clinical isolates was determined using a standard agar dilution technique. The majority of the organisms tested were ampicillin-resistant. In combination with 5 mg/l sulbactam, ampicillin at ≤ 16 mg/l inhibited 46.7% of strains of *Proteus* sp., *Enterobacter* sp., *Acinetobacter calcoaceticus*, *Shigella* sp. and *Salmonella* sp.. It was less active against *Klebsiella* sp. and *E. coli*. The combination was also found to be active against methicillin-sensitive *Staphylococcus aureus*, although less active against methicillin-resistant *Staphylococcus aureus*. There was no activity against *Pseudomonas aeruginosa*. It was also found that there was poor correlation between the results of disc sensitivity testing using a 10 ug:10 ug combination and that of MIC determination.

Keywords: Sulbactam-ampicillin activity, Enterobacteriaceae *Staphylococcus aureus*, *Pseudomonas aeruginosa*.

INTRODUCTION

The production of beta-lactamases by various Gram-positive and Gram-negative organisms contributes much to the resistance of these organisms to beta-lactam antibiotics. Various beta-lactamase inhibitors have been discovered. However, only those that possess poor intrinsic anti-bacterial activity are used as true inhibitors in combination with another beta-lactam compound. Such true inhibitors include clavulanic acid, halopenicillanic acids and sulbactam, a sulphone penicillanic acid. These compounds actively inhibit cell free enzymes and have been shown to potentiate the activity of enzyme labile beta-lactam antibiotics when used in combination.^{1,2,3}

This study was undertaken to determine the activity of sulbactam in combination with ampicillin against organisms isolated from clinical specimens of patients at the Kuala Lumpur General Hospital. Susceptibility measured as minimal inhibitory concentration (MIC) was also correlated with the results of disc susceptibility tests using a 1:1 combination (10 ug + 10 ug) of sulbactam and ampicillin.

MATERIALS AND METHODS

A total of 415 isolates of both Gram-positive and Gram-negative organisms were obtained from clinical specimens of patients at the Kuala Lumpur General Hospital. They comprised 100 strains of *E. coli*, 95 strains of *Klebsiella* sp., 52 strains of *Enterobacter* sp., 23 strains of *Pseudomonas aeruginosa*,

13 strains of *Pseudomonas* sp. (non-aeruginosa), 46 strains of *Proteus* sp., 17 strains of *Acinetobacter calcoaceticus*, 13 strains of *Salmonella* sp., 7 strains of *Shigella* sp., 4 strains of *Kluyvera* sp. and 45 strains of *Staphylococcus aureus*, 20 of which were methicillin-resistant strains. The majority of these isolates (> 70%) were resistant to ampicillin (>8 mg/l).

The minimal inhibitory concentration of ampicillin alone, sulbactam alone and ampicillin-inhibitor combinations were determined. The MIC was measured by the standard plate agar method using Diagnostic Sensitivity Test agar (Oxoid) and doubling dilutions of ampicillin or sulbactam ranging from 0.5 mg/l to 1024 mg/l. The MIC to the sulbactam-ampicillin combination was determined in two fashions. In the first, a fixed amount of sulbactam (5 mg/l) was added to various concentrations of ampicillin. This concentration was used as it is a concentration which is achievable *in vivo*. In the other, concentrations of sulbactam : ampicillin in the ratio of 1 : 2 were used. This 1 : 2 ratio was used due to formulation. Approximately 10^5 colony-forming units of each organism was inoculated onto the plates using a Denley multiple inoculator. Plates were incubated at 37°C for 18 hours and the MIC taken as the lowest concentration of ampicillin or ampicillin-sulbactam which inhibited growth.

Susceptibility tests using discs with known concentrations of ampicillin (10 ug) and

sulbactam-ampicillin (10 ug + 10 ug) were also performed using the same medium. Plates were inoculated with standardised inocula of bacteria which yielded semi-confluent growth after incubation. Zone sizes were measured after 18 hours of incubation at 37°C. Zone sizes were plotted against the MIC of ampicillin in combination with 5 mg/l sulbactam and the correlation calculated.

RESULTS.

Table 1 summarises the effect of the addition of sulbactam to ampicillin. Sulbactam-ampicillin was active against strains of *Enterobacter* sp., *Acinetobacter calcoaceticus*, *Shigella* sp. and *Salmonella* sp. with MIC₅₀s of 16 – 32 mg/l. In a few strains the MIC of ampicillin was further reduced to below 16 mg/l. However MIC₉₀s were around 1024 mg/l with the exception of *Shigella* sp. with a MIC₉₀ of 32 mg/l. The sulbactam-ampicillin combination was less active against strains

of *E. coli* and *Klebsiella* sp. Only about 20% of these strains had their MICs of ampicillin reduced to below 16 mg/l by sulbactam.

Sulbactam-ampicillin was more active against staphylococci. The combination of sulbactam-ampicillin against methicillin-resistant *Staphylococcus aureus* resulted in a MIC₉₀ of 8 mg/l. However, against methicillin-sensitive, penicillin-resistant *Staphylococcus aureus*, MICs of ampicillin were reduced to 0.25 – 1.0 mg/l by the addition of sulbactam. *Pseudomonas aeruginosa* strains were totally unaffected by the sulbactam-ampicillin combination.

The decrease in ampicillin MIC when sulbactam was added to ampicillin in a 1:2 ratio generally correlated well with the decrease seen when sulbactam was used in a fixed concentration. However, in strains with high MICs to ampicillin : sulbactam combinations (greater than 64 mg/l ampicillin plus 32 mg/l sulbactam) no decrease in ampicillin MIC

TABLE 1
EFFECT OF **SULBACTAM** (AT 5 **MG/L**) ON THE MIC OF AMPICILLIN
AGAINST VARIOUS HOSPITAL BACTERIAL ISOLATES.

Organism (No)	Ampicillin			Ampicillin/sulbactam		
	Range (mg/l)	MIC 50 (mg/l)	MIC 90 (mg/l)	MIC 50 (mg/l)	MIC 90 (mg/l)	No. inhibited by 16 mg/l ampicillin
<i>E. coli</i> (100)	8->1024	>1024	>1024	1024	>1024	16/100
<i>Klebsiella</i> (95)	32->1024	>1024	>1024	>1024	>1024	21/95
<i>Enterobacter</i> (52)	8->1024	128	>1024	16	>1024	25/52
<i>Acinetobacter</i> (17)	32->1024	>1024	>1024	16	>1024	10/17
<i>Proteus</i> (46)	32->1024	>1024	>1024	128	>1024	19/46
<i>Ps. aeruginosa</i> (23)	-	>1024	>1024	>1024	>1024	0/23
<i>Pseudomonas</i> sp. (13)	512->1024	>1024	>1024	512	>1024	1/13
<i>Kluyvera</i> (4)	-	>1024	>1024	>1024	>1024	0/4
<i>Shigella</i> (7)	128-256	128	128	16	32	4/7
<i>Salmonella</i> (13)	-	>1024	>1024	32	>1024	5/13
<i>Staph. aureus</i> (20) (methicillin resistant)	64-128	64	128	8	16	20/20
<i>Staph. aureus</i> (25) (methicillin sensitive)	0.5-32	4	>32			25/25*

*Range to which MIC was decreased: 0.25 – 1.0 mg/l

was observed with the addition of a fixed concentration (5 mg/l) of sulbactam. Such strains included isolates of *E. coli*, *Acinetobacter calcoaceticus* and *Klebsiella* sp.. *Pseudomonas aeruginosa* strains were not inhibited even by concentrations as high as 512 mg/l ampicillin plus 256 mg/l sulbactam. Strains which were inhibited by low ampicillin concentrations after the addition of 5 mg/l of sulbactam were also inhibited by the 1:2 combination at concentrations of less than 8 mg/l . 16 mg/l. In certain strains, however, this inhibition occurred at sulbactam concentrations approaching that of its (sulbactam) own MIC (result not shown).

Figure 1 shows the scatter diagram of all the strains tested with sulbactam-ampicillin disc zone sizes versus the MIC of ampicillin when used in combination with 5 mg/l of sulbactam. There was poor correlation between MIC of ampicillin and the zone sizes obtained ($r = -0.7$). At an ampicillin MIC of 16 mg/l, zone diameters vary from 10.5 mm to 21.5 mm. A few strains which had MICs of ampicillin between 128 mg/l to 1024 mg/l had zone diameters greater than 16 mm.

DISCUSSION

Sulbactam inhibited the beta-lactamases of many strains of Enterobacteriaceae, although there were differences in the extent of inhibition as shown by the decrease in the MIC of ampicillin. This inconsistency in activity has been previously observed.⁴ Sulbactam at 5 mg/l used in combination with ampicillin was more effective against strains of *Enterobacter* sp., *Acinetobacter calcoaceticus*, *Proteus* sp., *Shigella* sp. and *Salmonella* sp. than against *E. coli*, *Klebsiella* and *Kluyvera*. *Pseudomonas* was not affected. The activity of ampicillin against *Staphylococcus aureus* was enhanced. There was an eight fold reduction in the MIC of ampicillin for methicillin-sensitive and methicillin-resistant strains.

The lack of susceptibility of some strains may be due to the penetration inefficiency of sulbactam into bacterial cells. The permeability index of sulbactam has been shown to be 77.0 compared to 12.3 of clavulanic acid.¹ Using a 1:2 combination of sulbactam-ampicillin a high concentration of sulbactam was needed to achieve a slight reduction in ampicillin MIC. The concentrations of sulbactam used reached

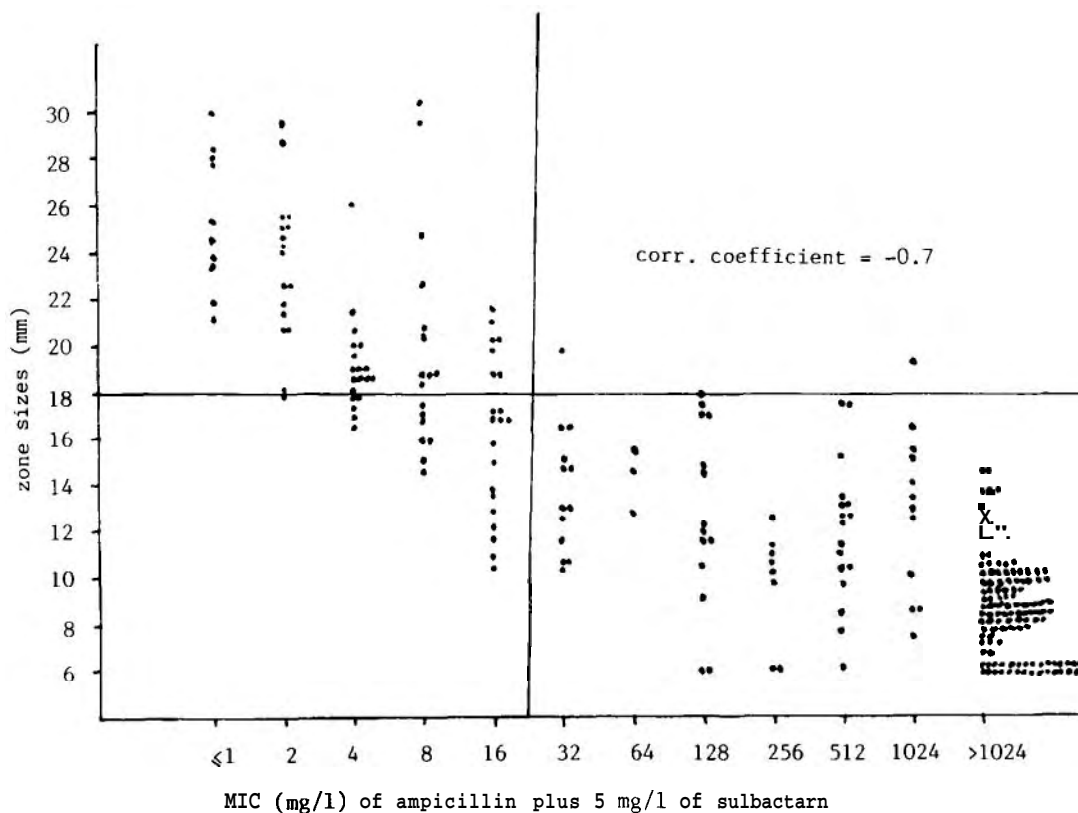


FIG. 1: Scatter diagram of zone sizes of ampicillin-sulbactam (10 ug + 10 ug) in relation to MIC of ampicillin (combined with 5 mg/l. of sulbactam) against ampicillin-resistant Enterobacteriaceae.

the MIC of sulbactam itself; therefore inhibition could be due to the intrinsic activity of sulbactam itself on the bacterial cell. The ability of an inhibitor to penetrate the bacterial cell and its ability to inhibit particular beta-lactamases produced by the various strains are factors contributing to the effectiveness of the inhibitor.

There was a significant variation in the zone sizes obtained in disc testing using sulbactam-ampicillin discs (concentration of 10 ug + 10 ug). A few strains which had MICs of ampicillin (in combination with sulbactam) greater than 16 mg/l had zone sizes greater than 16 or 17 mm. If this is taken as the "breakpoint" then such strains would be considered sensitive on disc testing. These variations could be due to the concentration of sulbactam used on the disc (i.e. 10 ug) which may not correlate well with the MIC test employing 5 mg/l of sulbactam. A breakpoint of 18 mm or greater to denote "sensitive" could be reasonable and would include those strains with MIC of ampicillin \leq 8 mg/l in the ampicillin-inhibitor concentration. However a few strains with MIC of ampicillin \geq 16 mg/l would still be included. The Kirby-Bauer/National Committee for Control of Laboratory Standards (NCCLS) method defined a disc zone size of "sensitive" as >14 mm (MIC \leq 8mg/l).⁶ This criterion can be used if the NCCLS method is strictly adhered to, otherwise new zone size criteria would have to be worked out when other methods of disc susceptibility testing are employed. Further studies on a larger sample of ampicillin-resistant bacterial strains to determine the most appropriate ratio of ampicillin: sulbactam to be used in the discs would be useful.

ACKNOWLEDGEMENTS

Our thanks are due to Cik Halijah Hj Yunus for technical assistance and Pfizer (Malaysia) Sdn Bhd for providing us a grant.

REFERENCES

1. Neu HC, Fu KP. Clavulanic acid, a novel inhibitor of beta-lactamases. *Antimicrob Agents Chemother* 1978; 14: 650-5.
2. English AR, Retsema JA, Girard AE, Lynch JE, Barth WE. CP-45899, a beta-lactamase inhibitor that extends the antibacterial spectrum of beta-lactams: initial bacteriological characterisation. *Antimicrob Agents Chemother* 1978 14: 414-9.
3. Aswapokee N, Neu HC. A sulphone beta-lactam compound which acts as a beta-lactamase inhibitor. *J Antibiotic* 1978; 31: 1238-44.
4. Fass RJ. Inconsistency of synergy between the beta-lactamase inhibitor CP-45889 and beta-lactam antibiotics against multiple drug-resistant Enterobacteriaceae and *Pseudomonas* species. *Antimicrob Agents Chemother* 1986; 19: 361-3.
5. Reading C, Farmer T. The inhibition of periplasmic beta-lactamase in *E. coli* by clavulanic acid and other beta-lactamase inhibitors. In: Progress and perspectives on beta-lactamase inhibition: A review of Augmentin. New York: Postgraduate Medicine Customs Communication, 1984: 163-8.
6. Pfizer International. Data on file, 1985.