The *in vitro* activity of amoxycillin with clavulanic acid against clinically significant bacteria

A multicentre study


Summary

A multicentre study to evaluate the activity of amoxycillin when combined with clavulanic acid against clinical bacterial isolates was performed in South Africa. Minimum inhibitory concentrations for amoxycillin alone and in combination with clavulanic acid were determined by agar dilution. The majority of amoxycillin-resistant *Klebsiella* and *Escherichia coli* strains became sensitive to amoxycillin in the presence of low concentrations of clavulanic acid. Beta-lactamase-producing strains of *Staphylococcus aureus* showed increased susceptibility to amoxycillin in the presence of clavulanic acid. Minimum bactericidal concentrations indicated that amoxycillin retains bactericidal activity in the presence of clavulanic acid.

Increasing numbers of bacteria resistant to β-lactam antibiotics are being encountered in clinical practice. Such resistance is often due to the production of β-lactamases. Clavulanic acid is a novel β-lactam which has relatively poor antibacterial activity but has been shown to inhibit bacterial β-lactamases. The combination of clavulanic acid with other β-lactam antibiotics which show higher antibacterial activity but less stability to β-lactamases offers a new approach to the chemotherapy of infections due to β-lactamase-producing bacteria.

A multicentre study was designed by the Antimicrobial Study Group of South Africa to evaluate the *in vitro* activity of amoxycillin combined with clavulanic acid against significant clinical bacterial isolates from various centres in South Africa. The study was carried out simultaneously in Bellville, CP, Bloemfontein, Cape Town, Durban, Johannesburg and Pretoria.

Materials and methods

Bacteria

A pool of organisms isolated from blood cultures was circulated to participating centres. Participants were requested to augment this with local isolates. A total of 223 strains were included in the final analysis. These included 166 *Enterobacteriaceae*, 11 *Pseudomonas aeruginosa*, 38 *Staphylococcus aureus* and 8 *Streptococcus faecalis*. Isolates were identified by standard methods.

Antibiotics

Standard reference powders of amoxycillin and clavulanic acid were supplied by Beecham Pharmaceuticals (Pty) Ltd.

Susceptibility testing

Minimum inhibitory concentrations (MICs) were determined according to the International Collaborative Study techniques. Agar MICs were determined using SAF agar (Mast Laboratories). Aliquots of the same batch of agar were distributed to each participating centre. Strains were tested against the following concentrations of clavulanic acid: 1, 2, 5 and 10 mg/L, in combination with two-fold dilutions of amoxycillin. The range of amoxycillin concentrations employed was 0.06 - 128 mg/L. One centre also determined MICs for clavulanic acid alone for some strains.

Minimum bactericidal concentrations (MBCs) were determined by a tube dilution technique using the same concentrations of amoxycillin and clavulanic acid in Mueller-Hinton broth. After 24 hours' incubation aliquots from tubes showing no visible growth were transferred onto agar plates and colony-forming units (CFUs) were counted. The MBC was defined as the lowest concentration of antibiotic resulting in a 99% or greater decrease in CFUs as compared with the initial broth inoculum.
Determination of ß-lactamase production

The production of ß-lactamase by bacterial strains was determined by individual methods using chromogenic cephalosporin 87/312 (Glaxo Research Ltd) as substrate.1

Results

The criteria for susceptibility to amoxycillin are depicted in Table I. Gram-negative bacilli with MICs > 128 mg/l indicated sensitivity. For Gram-positive cocci MICs of 8 mg/l were considered sensitive. None of the ampicillin-sensitive strains were positive for ß-lactamase production. The results obtained for amoxycillin-resistant strains are described further.

Effect of clavulanic acid on amoxycillin MICs

Of the 64 amoxycillin-resistant E. coli, 27%, 53% and 84% were sensitive to amoxycillin (MICs ≤ 8 mg/l) in the presence of 2.5, 5 and 10 mg/l clavulanic acid (Table III). Beta-lactamase production was demonstrated for 60 of these. The 4 strains in which ß-lactamase was not demonstrated remained resistant to amoxycillin in the presence of clavulanic acid.

Forty-five of 46 Klebsiella species were amoxycillin-resistant. In the presence of 1, 2.5, 5 and 10 mg/l clavulanic acid, 47%, 58%, 69% and 90% became sensitive to amoxycillin. Of 39 strains tested for ß-lactamase production 32 were positive, while a further 3 gave equivocal results (Table IV).
in the presence of 1, 2.5, 5 and 10 mg/l clavulanic acid respectively. Beta-lactamase production was clearly demonstrated for 33 of these strains (Table V).

**MICs for clavulanic acid alone**

The agar dilution MICs for clavulanic acid alone for some *E. coli*, *Klebsiella*, *Ps. aeruginosa* and *Staph. aureus* are presented in Table VI. With one exception, *E. coli* and *Klebsiella* were inhibited by clavulanic acid concentrations of 16 mg/l or higher. All 6 *Ps. aeruginosa* strains were highly resistant. *Staph. aureus* (9) were inhibited by 8 or 16 mg/l clavulanic acid.

For 4 salmonellae, 1 Proteus, 1 Citrobacter, 2 Providencia, 3 Enterobacter and 4 Serratia the MICs for clavulanic acid were all in the 16 - 64 mg/l range.

Thus, only for two-thirds of *Staph. aureus* and a single *Klebsiella* isolate did MICs for clavulanic acid fall within the range of concentrations used in the amoxycillin MIC and MBC determinations.

**Effect of clavulanic acid on amoxycillin MBCs**

MBCs were determined for 36 strains. MBCs were occasionally equal to but generally higher than the corresponding MICs determined by the broth dilution method. This difference was least in the presence of 5 and 10 mg/l clavulanic acid. Table VII provides comparative MICs and MBCs for amoxycillin alone and in the presence of clavulanic acid.

### Table VI. MICs For Clavulanic Acid Alone

<table>
<thead>
<tr>
<th>Organisms</th>
<th>No. of isolates tested</th>
<th>Cumulative No. of strains inhibited by indicated concentrations (mg/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>2</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>41</td>
<td>-</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>37</td>
<td>-</td>
</tr>
<tr>
<td><em>Ps. aeruginosa</em></td>
<td>6</td>
<td>-</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>6</td>
<td>-</td>
</tr>
</tbody>
</table>

### Table VII. Comparison of MICs and MBCs For Amoxycillin (AM) Alone and With Clavulanic Acid (CA) For 10 Strains

<table>
<thead>
<tr>
<th>Organism</th>
<th>MIC/MBC (mg/l)</th>
<th>AM only</th>
<th>CA 1 mg/l</th>
<th>CA 2.5 mg/l</th>
<th>CA 5 mg/l</th>
<th>CA 10 mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Staph. aureus</em></td>
<td>MIC</td>
<td>1</td>
<td>0.5</td>
<td>0.25</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>MBC</td>
<td>-</td>
<td>4</td>
<td>1</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td><em>Staph. aureus</em></td>
<td>MIC</td>
<td>0.5</td>
<td>0.25</td>
<td>0.25</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>MBC</td>
<td>0.25</td>
<td>0.25</td>
<td>0.125</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td><em>E. coli</em></td>
<td>MIC</td>
<td>0.25</td>
<td>0.25</td>
<td>0.125</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td></td>
<td>MBC</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>MIC</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td></td>
<td>MBC</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
<td>0.5</td>
</tr>
<tr>
<td><em>Klebsiella</em> spp.</td>
<td>MIC</td>
<td>1.1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>MBC</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
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</table>

**Discussion**

This study has shown that in the presence of low concentrations of clavulanic acid many local strains of otherwise amoxycillin-resistant bacteria became sensitive to amoxycillin. This enhanced susceptibility was most marked for *E. coli*, *Klebsiella* and *Staph. aureus*. Although only limited numbers of other bacteria were studied it is evident that a similar effect can be obtained with many amoxycillin-resistant Salmonellae and some Proteus species. The enhancement of the bacteriostatic and bactericidal activity of amoxycillin in the presence of clavulanic acid was progressive, increased enhancement being found with increasing concentrations of clavulanic acid.

This beneficial effect of clavulanic acid was not seen with strains of Enterobacter, Serratia and *Ps. aeruginosa*, which remained resistant to amoxycillin in the presence of up to 10 mg/l clavulanic acid.
These findings are consistent with those of other workers who have studied the effect of β-lactamase inhibitors such as clavulanic acid and penicillanic acid sulphone on the susceptibility of β-lactamase-producing bacteria to various β-lactam antibiotics, including amoxycillin. The moderate inoculum effect noted has also been described previously with other β-lactam antibiotics.

Beta-lactamase production was demonstrated in the majority of strains showing enhanced susceptibility to amoxycillin with clavulanic acid. However, centres sometimes differed in their interpretations of tests for β-lactamase production, and where differences were encountered the majority finding was accepted. In a recent study the reliability of single rapid tests for the detection of β-lactamase production by Enterobacteriaceae was questioned, so these differences are not unique to the study under discussion.

In addition to providing the opportunity to evaluate amoxycillin in combination with clavulanic acid against local clinical isolates, this multicentre study has promoted standardization of sensitivity testing in the participating centres in keeping with the aims of the Antibiotic Study Group of South Africa and has identified a technical problem for future improvement.

We thank Eli Lilly & Co. and Beecham Pharmaceuticals (Pty) Ltd for support and assistance.

REFERENCES

Terapie van urienweginfeksies by pasiënte met rugmurgbesering deur die toediening van amoksisillien en klavulanie suur

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Summary

Augmentin, a formulation of amoxycillin trihydrate 250 mg and sodium clavulanate 125 mg per tablet (A-CS) (Augmentin; Beecham), was used in treating 29 episodes of urinary tract infection occurring in 26 patients admitted to the Spinal Unit of the H. F. Verwoerd Hospital, Pretoria. Patients who had a urinary bacterial cell count of more than 10⁵ of the same amoxycillin-resistant organism before and after the oral administration of amoxycillin 500 mg 3 times a day for 48 hours, received 2 A-CS 375 mg tablets orally, 3 times a day at the start of a meal for 5 days. The 29 strains of amoxycillin-resistant organisms treated in this study were: Escherichia coli (11), Klebsiella pneumoniae (11), Proteus mirabilis (4), Enterobacter cloacae (2), and Staphylococcus epidermidis (1). The bacteriological success rate 24 hours after therapy was 100% and 8 days after therapy 69%, dependent on patient management. In patients on free drainage and managed with condoms a bacteriological success rate of 55,5% was recorded and in patients managed by intermittent catheterization a bacteriological success rate of 75% was recorded. Side-effects were minimal; 1 patient complained of dizziness and no instances of nausea or vomiting were reported. Haematological, renal and hepatic monitoring before and after A-CS-therapy revealed no drug-related toxicity.

Pasiënte en metodes

Die ontwikkeling van urienweginfeksies by pasiënte met rugmurgbeseringe is 'n gevaarlike toestand wat selfs dodelik kan wees. Die gebruik van 4-uurlike intermitterende katheterisatie, in plaas van 'n inblywende kater tydens die aanvanklike