Aminoglycoside-resistant enterococci

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SUMMARY

Thirty-four recent clinical isolates of *Streptococcus faecalis* were tested for sensitivity to amoxycillin, benzylpenicillin, streptomycin, kanamycin, gentamicin, tobramycin, and amikacin. Amoxycillin was two- to four-fold more active than benzylpenicillin and all strains were inhibited by low concentrations of the penicillins. The aminoglycosides were less active against the enterococci than were the penicillins and a significant number of strains were insensitive or relatively insensitive to one or more of the aminoglycosides. Thus, eight (23%) strains showed a high level of resistance to streptomycin and kanamycin (MIC >5000 μg/ml) but were sensitive to gentamicin, tobramycin, and amikacin. In addition, two strains of *Strep. faecalis*, isolated at different hospitals from patients who had received topical gentamicin therapy, were relatively resistant to gentamicin (MIC 250 to 500 μg/ml) and were less sensitive also to the other aminoglycosides. Bactericidal synergy was demonstrated by amoxycillin/aminoglycoside combinations against the enterococci, provided that the test strain of *Strep. faecalis* was sensitive to the aminoglycoside in the combination. An exception to this was the combination of amoxycillin plus amikacin which was not synergistic against kanamycin-resistant strains of *Strep. faecalis* although these organisms were sensitive to amikacin in the growth inhibition tests. The gentamicin-resistant strains showed variable responses to amoxycillin/aminoglycoside combinations in tests for bactericidal synergy and were generally less sensitive than typical strains of *Strep. faecalis*.

The aminoglycoside group of antibiotics is relatively inactive against enterococci and these compounds are not usually considered for the treatment of enterococcal infections. However, combinations of aminoglycosides with penicillins can be shown to produce bactericidal synergy against many strains of enterococci, and combined penicillin/aminoglycoside therapy is recommended for the treatment of severe infections, notably enterococcal endocarditis (Garrod et al., 1973). Synergism is not always demonstrated *in vitro* by combinations of penicillins and aminoglycosides and is dependent upon the level of resistance of the organism to the aminoglycoside. Thus a number of strains of enterococci are highly resistant to streptomycin or kanamycin, and combinations of penicillin and streptomycin or penicillin and kanamycin are not synergistic against these strains (Standiford et al., 1970; Moelerring et al., 1971; Russell and Sutherland, 1975).

The newer aminoglycosides, gentamicin and tobramycin, are more active *in vitro* than streptomycin and kanamycin against enterococci (Moelerring et al., 1973; Russell and Sutherland, 1975; Iannini et al., 1976), and amikacin, a semisynthetic derivative of kanamycin, is as active as kanamycin against these organisms (Iannini et al., 1976). Enterococci that are resistant to high levels of streptomycin or kanamycin are sensitive to these new aminoglycosides, and combined therapy with the new compounds and penicillins has been proposed for enterococcal infections (Moelerring et al., 1971; Moelerring et al., 1973; Ruhen and Darrell, 1973; Russell and Sutherland, 1975; Iannini et al., 1976).

There have been no reports in the literature of enterococci resistant to gentamicin, tobramycin or amikacin, but during a study in this laboratory of bacteria isolated from various hospitals during December 1975 it was observed that three cultures of *Strep. faecalis* were relatively resistant to gentamicin and tobramycin. It was also noted that amikacin failed to produce bactericidal synergy in combination with penicillins against kanamycin-resistant strains of *Strep. faecalis*. This report describes the sensitivities of recent isolates of *Strep. faecalis* to penicillins, aminoglycosides, and combinations of the compounds.
Material and methods

**ANTIBIOTICS**

The penicillins tested were amoxycillin trihydrate and sodium benzylpenicillin (Beecham Pharmaceuticals) and the aminoglycosides were streptomycin sulphate (Glaxo Laboratories Ltd), kanamycin sulphate (Winthrop Laboratories), gentamicin sulphate (Roussel Laboratories Ltd), tobramycin sulphate (Eli Lilly and Co Ltd), and amikacin base (Bristol Laboratories).

**CULTURES**

The strains of enterococci tested were clinical isolates collected from three hospitals during December 1975 which had been cultured from a variety of sources (blood, urine, and wounds). The organisms were identified as group D streptococci by serotyping and were classified as strains of *Strep. faecalis* according to the criteria of Deibel (1964).

**MINIMUM INHIBITORY CONCENTRATIONS**

Antibacterial activity was measured by serial dilution of the antibiotics in 18 ml volumes of 5% blood agar (Blood Agar Base, Oxoid; Defibrinated Horse Blood, Wellcome). Agar plates were inoculated with 0.001 ml of an undiluted overnight culture of the test strain delivered with a multiple inoculating device (Dynatech Laboratories). Minimum inhibitory concentrations (MIC) were measured after incubation at 37°C for 18 hours.

**MINIMUM BACTERICIDAL CONCENTRATIONS OF AMOXYCILLIN/AMINOGLYOSIDE COMBINATIONS**

Serial dilutions of amoxycillin were made in 4.5 ml volumes of nutrient broth (Nutrient broth No. 2, Oxoid), and 0.5 ml volumes of selected concentrations of the aminoglycosides were added to each tube. The aminoglycoside concentrations were selected as being levels attainable in the blood after usual dosage, and at these concentrations the compounds failed to demonstrate bactericidal activity against the test organisms. The tubes were inoculated with 0.03 ml of an overnight broth culture (approximately 10^8 cells) and incubated at 37°C for 18 hours. A loopful of culture was taken from each tube not showing visible growth and streaked onto blood agar containing penicillinase (Difco). The plates were incubated overnight at 37°C and the minimum bactericidal concentration (MBC) was recorded as the lowest concentration of antibiotic from which subculture failed to yield viable colonies.

**BACTERICIDAL ACTIVITY**

Tubes of nutrient broth (10 ml) containing known concentrations of the antibiotics were inoculated with 0.03 ml of an 18-hour broth culture and incubated at 37°C. Samples were taken at intervals and 0.02 ml volumes of suitable dilutions were pipetted onto blood agar containing penicillinase. Colonies were counted after incubation at 37°C for 24 hours and the number of viable bacteria was estimated.

**Results**

**MINIMUM INHIBITORY CONCENTRATIONS**

Results in Table 1 show the distribution of the minimum inhibitory concentrations of amoxycillin, benzylpenicillin, and various aminoglycosides against 34 recent clinical isolates of *Strep. faecalis*. Amoxycillin was the most active of the compounds and was two- to four-fold more active than benzylpenicillin. All cultures were sensitive to low concentrations of the penicillins. The aminoglycosides were notably less active than the penicillins, and the descending order of activity was tobramycin, gentamicin, kanamycin, amikacin, and streptomycin. Eight strains were highly resistant to both streptomycin and kanamycin (MIC values > 5000 μg/ml) but these strains showed no increase in resistance to gentamicin, tobramycin, or amikacin. Another strain was highly resistant to streptomycin but was sensitive to kanamycin and the other compounds.

Three strains of *Strep. faecalis* were notably less sensitive to gentamicin than were the majority of strains, and the sensitivities of these organisms to the

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Minimum inhibitory concentration (μg/ml) and number of strains</th>
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<tr>
<td></td>
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<tr>
<td>Amoxycillin</td>
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<td>Kanamycin</td>
<td>5</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>5</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>5</td>
</tr>
<tr>
<td>Amikacin</td>
<td>1</td>
</tr>
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</table>

Table 1: Distribution of minimum inhibitory concentrations of amoxycillin, benzylpenicillin, and aminoglycosides against 34 strains of Streptococcus faecalis

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Table 2  Minimum inhibitory concentrations of amoxycillin, benzylpenicillin, and aminoglycosides against clinical isolates and laboratory-selected strains of Strep. faecalis resistant to gentamicin or streptomycin

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Minimum inhibitory concentration (µg/ml)</th>
<th>Laboratory-selected strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Clinical isolates</td>
<td>C90 S res© C90 G res©</td>
</tr>
<tr>
<td></td>
<td>C134 C135 W464 Control© C36</td>
<td></td>
</tr>
<tr>
<td>Amoxycillin</td>
<td>0.25 0.25 0.25 0.25 0.25 0.25 0.25</td>
<td>0.25 0.25 0.25</td>
</tr>
<tr>
<td>Benzylpenicillin</td>
<td>0.5 0.5 1.25 1.25 1.25 1.25 1.25</td>
<td>1.0 0.5 0.5 0.25</td>
</tr>
<tr>
<td>Streptomycin</td>
<td>500 500 1250 &gt;5000</td>
<td>50 &gt;5000 1250</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>1250 1250 1250 &gt;5000</td>
<td>50 50 500</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>250 250 500 500</td>
<td>10 10 250</td>
</tr>
<tr>
<td>Tobramycin</td>
<td>125 125 250 250</td>
<td>10 125 10 100</td>
</tr>
<tr>
<td>Amikacin</td>
<td>500 500 1250 50</td>
<td>125 125 2500</td>
</tr>
</tbody>
</table>

1*Streptomycin, kanamycin resistant.

Table 2 shows the minimum inhibitory concentrations (MIC) of amoxycillin, benzylpenicillin, and aminoglycosides against clinical isolates and laboratory-selected strains of Strep. faecalis resistant to gentamicin or streptomycin. The antibiotic concentrations were determined in vitro in the presence of each drug. The results for the clinical isolates were similar to those for the laboratory-selected strains. The MIC values for amoxycillin and benzylpenicillin were lower than those for streptomycin, kanamycin, and amikacin. The MIC values for gentamicin were comparable to those for streptomycin, kanamycin, and amikacin. The MIC values for tobramycin were higher than those for gentamicin, streptomycin, and amikacin. The MIC values for amikacin were the highest of all the aminoglycosides tested.

**Bacterial synergism studies**

The results of tests to determine the minimum inhibitory concentrations (MIC) of combinations of amoxycillin and the aminoglycosides against a number of clinical isolates of Strep. faecalis are shown in Table 3. Amoxycillin was ineffective in these tests (MIC >100 µg/ml), but in combination with sub-lethal concentrations of the aminoglycosides, amoxycillin MBC values (50 µg/ml) against the aminoglycoside-sensitive strain of Strep. faecalis (T1089) were not greatly in excess of inhibitory concentrations (ca 10 µg/ml). Against the streptomycin-resistant strain C119, the amoxycillin/streptomycin combination was not bactericidal but the other combinations were as effective against this strain as against the sensitive strain, T1089. Similarly, the amoxycillin/streptomycin and amoxycillin/kamycin combinations failed to demonstrate bactericidal activity against the streptomycin/kamycin-resistant strain of Strep. faecalis C36. Unexpectedly, the MIC of the amoxycillin/amikacin combination was in excess of 100 µg amoxycillin/ml + 20 µg amikacin/ml, although the organism showed typical sensitivity to amikacin in the growth inhibition tests (MIC 50 µg/ml). Similar results were obtained in tests with the other seven kanamycin-resistant strains isolated in this study, and amoxycillin/amikacin and benzyl-
penicillin/amikacin combinations failed to produce synergistic bactericidal effects against any of the kanamycin-resistant (amikacin-sensitive) strains of *Strep. faecalis*. There was no evidence of cross-resistance with the amoxicillin/gentamicin and amoxicillin/tobramycin combinations against the kanamycin-resistant strain *Strep. faecalis* C36, and both combinations produced marked bactericidal activity against this strain and other kanamycin-resistant enterococci.

The gentamicin-resistant isolates responded in a more variable fashion to amoxicillin/aminoglycoside combinations in the bactericidal tests compared with gentamicin-sensitive strains. In the MBC tests, sensitive strains gave a sharp end-point so that no growth was observed on subculture from combinations containing amoxicillin at concentrations of 5-0 μg/ml or more. With the gentamicin-resistant strains there was no growth from cultures containing 5-0 μg amoxicillin/ml or 10 μg amoxicillin/ml in combination with the aminoglycosides but growth sometimes occurred in subcultures from tubes containing higher concentrations of amoxicillin. In the viable count tests, gentamicin-sensitive strains of *Strep. faecalis* were always sterilised by combinations of 2-5 μg amoxicillin plus 50 μg gentamicin/ml whereas, with the gentamicin-resistant strains, on some occasions the cultures were made sterile, but on repeat tests viable bacteria were often recovered (Table 4). Similar variable responses were observed with combinations containing other aminoglycosides.

**Discussion**

The results reported here for a limited number of recent clinical isolates of *Strep. faecalis* show the prevalence of a relatively high proportion of strains with reduced sensitivity to aminoglycoside antibiotics. Strains resistant to streptomycin or kanamycin demonstrated a high level of resistance to the compounds but were sensitive to gentamicin, tobramycin or amikacin. In contrast, the strains with reduced sensitivity to gentamicin showed a moderate level of resistance to gentamicin and to the other aminoglycosides. Enterococci with a high level of resistance to streptomycin have been known for some time (Havard *et al.*, 1959), and more recent reports have described a relatively high incidence of resistance to streptomycin and kanamycin (Standiford *et al.*, 1970).
The characteristics of the gentamicin-resistant clinical isolates, namely, the moderate level of resistance and degree of cross-resistance with other aminoglycosides, were markedly similar to those of the resistant strains selected in vitro after subculture in the presence of gentamicin. These findings, coupled with the failure to demonstrate conjugal transfer of resistance or to eliminate resistance with curing agents, suggest that the strains might have arisen in vivo by selection as a result of therapy with topical gentamicin, as has been reported for *Pseudomonas aeruginosa* (Snelling et al., 1971; Holmes et al., 1974) and, more recently, for *Staphylococcus aureus* (Porthouse et al., 1976; Warren and Roberts, 1976).

Therapy with benzylpenicillin and streptomycin has often been recommended as the treatment of choice for severe enterococcal infections but awareness of the increasing incidence of streptomycin and kanamycin-resistant strains has led to an increased interest in the activity of combinations of penicillins with newer aminoglycosides, namely, gentamicin, tobramycin, and amikacin. The finding of strains that are apparently less sensitive to these compounds emphasises the need for appropriate laboratory tests for the selection of the most suitable penicillin/aminoglycoside combination for therapy of enterococcal infections.

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**References**


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