# Comparative activity of ampicillin and seven cephalosporins against group D streptococci

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SYNOPSIS Minimum inhibitory concentrations have been determined for ampicillin and seven cephalosporins against 93 strains of group D streptococci isolated recently from clinical material. Ampicillin was much the most active compound (modal MIC = 1·6 $\mu$g/ml); cephaloridine, cephacetrile, and cefazolin had a modal MIC of 25 $\mu$g/ml, while corresponding figures for cephalothin, cephradine, cephalexin, and cefoxitin were 50, 100, 200, and 800 $\mu$g/ml, respectively. Thus, none of the newer cephalosporins is an improvement in respect to activity against enterococci over existing compounds, and ampicillin remains overwhelmingly the $\beta$-lactam antibiotic of choice for the treatment of infections by such organisms. Pharmacokinetic considerations, however, indicate that certain cephalosporins, for instance, cephaloridine, cefazolin, and cephanone, may be worthy of further study in view of possible synergy with aminoglycoside antibiotics.

Strains of streptococci belonging to Lancefield group D, commonly called enterococci or faecal streptococci, differ markedly from other streptococci pathogenic to man by being relatively resistant to many commonly used antibiotics (Garrod, Lambert, and O'Grady, 1973). Thus, Toala, McDonald, Wilcox, and Finland (1969), who determined the sensitivities of some 382 strains of enterococci to 21 antibiotics, found that only ampicillin, benzylpenicillin, and vancomycin inhibited virtually all strains at a concentration of 6·3 $\mu$g/ml. The advent of several new cephalosporins in the past few years (Hamilton-Miller and Brumfitt, 1974) has raised the question as to whether any of these newer compounds show useful activity against enterococci, especially in view of two recent reports. The first of these, by Isenberg, Painter, Sampson-Scherer, and Siegel (1973), suggests that, on the basis of disk tests, cephacetrile is considerably more active than cephalothin against group D streptococci, and the second is a statement (unsupported by any documentation) by Neiss (1973) that cephradine is more active than is cephalexin against enterococci. In view of the above, it was felt that a study of the antibacterial activity *in vitro* of established cephalosporins (cephalothin, cephaloridine, and cephalexin) in comparison with that of newer compounds (cefazolin, cephacetrile, cephradine, and cefoxitin),

using ampicillin as reference compound, would help to clarify the situation.

## Methods and Materials

### BACTERIAL STRAINS

These were taken from amongst isolates made from clinical material sent to the routine microbiology laboratory of this hospital. The series does not consist of consecutive isolates, but strains were taken at random over the course of about three months at the end of 1973 and the beginning of 1974; it can thus be regarded as representative of clinically isolated enterococci from a general hospital in Great Britain.

It is the practice in our laboratory to identify enterococci by virtue of their ability to grow well on agar containing 40% (w/v) bile (Facklam and Moody, 1970), and it was from such plates that the strains comprising this series were taken. The isolates came in roughly equal proportions from urine, high vaginal swabs, and wound swabs. They were first subcultured to MacConkey agar, and those showing characteristic pinpoint magenta colonies were entered as confirmed enterococci. Strains were speciated by virtue of their ability to grow in nutrient broth containing 6·5% (w/v) NaCl, their fermentative activity on sorbitol and arabinose, and growth in the presence of tellurite (Cowan and Steel, 1965). *Strept. faecalis* strains were further subdivided on

the basis of their haemolytic activity and their action on gelatin-charcoal discs: gelatinase-negative, non-haemolytic strains were classed as var *faecalis*; gelatinase-positive, non-haemolytic strains as var *liquefaciens*; and β-haemolytic, gelatinase-variable strains as var *zymogenes* (Cruickshank, 1968). Strains were stored on blood agar at 4°.

## MIC DETERMINATIONS

Strains were inoculated into brain heart infusion (BHI) broth (Difco) containing 4% (v/v) lysed horse blood, and incubated overnight at 37°. Cultures were diluted one hundred fold in water and plated on to BHI agar + 4% lysed horse blood containing serial doubling dilutions of antibiotic. Inoculation was performed using a multiple inoculating device which delivers some 3 μl each of 25 individual bacterial suspensions simultaneously from loops. Separate determinations indicated that about 16 ng dry weight of each organism was inoculated. Taking the figures of Luria (1960) for the dimensions of *Strept. faecalis* and the density of dried bacteria, it can be calculated that this inoculum is equivalent to about 40 000 individual steptococci. The plates were incubated at 37°C, and read after 18 and 42 hours.

Experience showed that the same result was obtained at both time intervals but that plates were much easier to read after 42 hours. Growth was scored as positive if more then one distinct colony was observed in an inoculation area; slight confluent growth and multiple microcolonies visible by both reflected and transmitted light were also scored as positive. Results were cumulated and processed to determine arithmetic mean, mode, and median value for each antibiotic, and also plotted on semilogarithmic paper to calculate, by interpolation, that concentration (C50) which would inhibit 50% of the strains.

## ANTIBIOTICS

The following compounds were used: sodium ampicillin (Beecham Research Laboratories), cepha-

loridine and cephalexin (Glaxo Research Laboratories), sodium cephalothin (Lilly Research Laboratories), cefazolin (Fujisawa Pharmaceuticals), cephacetrile (Ciba Pharmaceuticals), cephradine (Smith, Kline & French Laboratories), and sodium cefoxitin (Merck, Sharp & Dohme).

## Results

### CLASSIFICATION OF ORGANISMS

Of the 93 strains of group D streptococci isolated, most, 52 (56%), were *Strept. faecalis* var *faecalis*; 23 (24·7%) strains were var *zymogenes* (19 were gelatinase-negative), and 11 (11·8%) var *liquefaciens*. The remaining seven strains were divided between *Strept. faecium* (2) and *Strept. durans* (5). These figures are in marked contrast to those reported from the Boston City Hospital (Toala *et al*, 1969), where the *liquefaciens* and *zymogenes* varieties together made up 72·5% of the total isolates; here the corresponding figure is 36·5%.

### SENSITIVITIES TO AMPICILLIN AND CEPHALOSPORINS

The results are shown in table I and summarized in table II. As no clear-cut species differences were discernible, the data in table II are for all the strains. The close coincidence in the numerical values for the mean, mode, and median MIC for each antibiotic

| Antibiotic | Mean | Median (μg/ml) | Mode | C50 |
|---|---|---|---|---|
| Cefoxitin | 741·1 | 800 | 800 | 540 |
| Cephalexin | 222·6 | 200 | 200 | 150 |
| Cephradine | 108·9 | 100 | 100 | 72 |
| Cephalothin | 42·3 | 50 | 50 | 30 |
| Cefazolin | 35·2 | 25 | 25 | 21 |
| Cephacetrile | 22·2 | 25 | 25 | 16 |
| Cephaloridine | 20·8 | 25 | 25 | 15 |
| Ampicillin | 1·9 | 1·6 | 1·6 | 1·25 |

Table II   *Summary of antibiotic activity against enterococci*

| Species | Cefoxitin | | | Cephalexin | | | | Cephradine | | | | Cephalothin | | | | Cefazolin | | | | Cephacetrile | | | Cephaloridine | | | | Ampicillin | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | 800 | 400 | 200 | 400 | 200 | 100 | 50 | 200 | 100 | 50 | 25 | 100 | 50 | 25 | 12·5 | 100 | 50 | 25 | 12·5 | 50 | 25 | 12·5 | 25 | 12·5 | 6·3 | 3·1 | 1·6 | 0·8 | 0·4 |
| *Strept. faecalis* | | | | | | | | | | | | | | | | | | | | | | | | | | | | | |
| var *faecalis* | 47 | 5 | | 12 | 37 | 3 | | 10 | 39 | 3 | | 1 | 36 | 15 | | 1 | 24 | 27 | | 2 | 37 | 13 | 43 | 9 | | | 14 | 37 | 1 |
| var *zymogenes* | 19 | 2 | 2 | 2 | 18 | 3 | | 3 | 15 | 5 | | | 13 | 10 | | | 4 | 17 | 2 | | 20 | 3 | 16 | 7 | | | 5 | 14 | 4 |
| var *liquefaciens* | 10 | 1 | | 1 | 9 | 1 | | | 8 | 3 | | | 8 | 3 | | | 2 | 9 | | 1 | 7 | 3 | 7 | 4 | | | 3 | 7 | 1 |
| | | | | | | | | | | | | | | | | | | | | | | | | 2 | | 1 | 1 | | 1 |
| *Strept. faecium* | 1 | | 1 | | 1 | | 1 | | 1 | | 1 | 1 | | | 1 | | | 1 | | | 3 | 2 | 4 | 1 | | | 1 | 4 | |
| *Strept. durans* | 4 | 1 | | 1 | 2 | 1 | 1 | 2 | 2 | 1 | | | 4 | 1 | | | 3 | 2 | | | | | | | | | | | |
| Totals | 81 | 9 | 3 | 16 | 67 | 8 | 2 | 15 | 65 | 12 | 1 | 1 | 62 | 29 | 1 | 2 | 33 | 56 | 2 | 3 | 67 | 23 | 70 | 22 | 1 | 23 | 63 | 6 | 1 |

Table I   *Minimum inhibitory concentrations of eight antibiotics against enterococci*[1]

[1]Number of strains with indicated minimum inhibitory concentration (μg/ml)

| Antibiotic | Route | Mean Peak Serum Level ($\mu g/ml$) | Reference |
|---|---|---|---|
| Cefazolin | Intramuscular | 64 | Gold *et al* (1973) |
| Cefoxitin | Intramuscular | 22·5 | Kosmidis *et al* (1973) |
| Cephacetrile | Intramuscular | 22·7 | Hodges *et al* (1973) |
| Cephalexin | Oral | 32 | Griffith and Black (1968) |
| Cephaloridine | Intramuscular | 27 | Turck *et al* (1967) |
| Cephalothin | Intramuscular | 22·8 | Klein *et al* (1964) |
| Cephanone | Intramuscular | 56·6 | Meyers *et al* (1972) |
| Cephapirin | Intramuscular | 24 | Axelrod *et al* (1972) |
| Cephradine | Oral | 24·2 | Zaki *et al* (1974) |

Table III   *Mean peak blood levels of cephalosporins after 1 g dose*

shows that we are dealing with a normal, symmetrically distributed population of organisms.

## Discussion

The results obtained here closely resemble those reported in similar studies, carried out in the USA and involving only a few cephalosporins, by Toala *et al* (1969) and Matsen and Coghlan (1972). Sensitivity patterns of enterococci thus appear to be similar on each side of the Atlantic. Seneca (1973), in a more recent survey of the activity of seven cephalosporins against organisms isolated from the urinary tract, confined his attention to Gram-negative organisms, so the present study supplements his. Our results confirm the two statements referred to in the introduction, that cephacetrile is more active than cephalothin, and cephradine than cephalexin, against enterococci, but the clinical significance of such facts is of extremely dubious significance, due to the high values of the MIC observed.

Two further cephalosporins which have proceeded as far as pharmacokinetic studies in man are cephanone and cephapirin. We were unable to test these compounds, but other workers have shown that their activity against enterococci is akin to that of cephalothin (Axelrod, Meyers, and Hirschman, 1971; Meyers, Hirschman, and Nicholas, 1972). Thus, none of the newer cephalosporins tested appear to show outstanding activity against enterococci, and none remotely approach ampicillin in this respect. Before dismissing these compounds, however, it is important to compare MICs with therapeutically attainable concentrations (table III). From these figures it can be seen that serum antibiotic levels inhibitory to enterococci are likely to be attainable *in vivo* for cephaloridine, cefazolin, and cephanone, and possibly for cephacetrile as well. When urinary concentrations are considered an entirely different picture emerges; due to the relatively short half-lives of the cephalosporins in man, urinary excretion of a given dose is virtually complete after about six hours, so that average concentrations in the urine over this period can be expected to be of the order of 2 mg/ml after a 1 g dose. Such concentrations of any of the antibiotics tested here would be inhibitory for all the enterococci tested. Choice of treatment for infections of the biliary tract must take into account that, of the compounds tested, only cefazolin and ampicillin are excreted to any substantial degree in the bile.

For one specific enterococcal infection, subacute endocarditis, combination therapy with benzylpenicillin and streptomycin is favoured by clinicians (Standiford, de Maine, and Kirby, 1970). Such a regimen may well be hazardous if the patient is allergic to penicillins, and the markedly lower incidence of reactions in such patients to cephalosporins makes the latter an attractive potential alternative (Simon, 1967). On the basis of the findings in the present paper, a study of synergism between aminoglycosides on one hand, and cephaloridine, cefazolin, and cephanone on the other, may give valuable guidelines to alternative treatment regimens for systemic enterococcal infections in the penicillin-sensitive patient.

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