Susceptibility of haemolytic oral enterococci to eight antibiotics *in vitro*

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In vitro tests by the agar dilution method for sensitivity to eight antibiotics were made with 92 strains of haemolytic enterococci isolated from oral infections. All strains were highly sensitive to azidocillin and ampicillin (minimum inhibitory concentration 0.5—2.0 mcg/ml). The antibacterial activity of benzylpenicillin was observed at 1.0—4.0 mcg/ml and that of phenoxymethylpenicillin between 2 and 8 mcg/ml. Most strains were sensitive to erythromycin (0.5—8.0 mcg/ml) while 70 per cent of the strains were sensitive to tetracycline (0.5—2.0 mcg/ml). The strains investigated were all resistant to cephalexine (64—128 mcg/ml) and the minimal inhibitory concentration of lincomycin varied between 16 and 128 mcg/ml.

Key-words: Microbial sensitivity tests; antibiotics; Streptococcus faecalis

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Enterococci can cause infections in humans such as urinary tract infections (*Freedman*, 1963), bacterial endocarditis (*Cherubin & Neu*, 1971), and oral infections (*Möller*, 1966). Results of tests for antibiotic susceptibility have recently been reported by several investigators (*Toala et al.*, 1969; *Moellering et al.*, 1970; *Bengtsson et al.*, 1972). The sources of these clinical isolates of enterococci have been urine, blood, faeces, sputum and skin. Hitherto no study has been carried out to test the antibiotic sensitivity of enterococci isolated from oral infections. This report presents the results of *in vitro* tests of eight antibiotics against isolates of oral enterococci.

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MATERIAL AND METHODS

Identification of the strains. The enterococci used in this investigation were isolated from patients with oral infections (periodontitis apicalis and pulpitis) as recently described (*Nord & Wadström*, 1973). Only haemolytic strains which occurred in pure cultures were used in this study. The strains were identified as *Streptococcus faecalis* according to *Deibel* (1964) by the following criteria: no catalase or oxidase production; no motility; fermentation of glucose; serological group D; growth at 10°C and 45°C, growth at pH 9.6; growth in 6.5 per cent NaCl; resistance to tellurite; growth on 10 per cent and 40 per cent bile; hydrolysis of

aesculine, arginine and hippurate; degradation of gelatin; producion of acid from maltose, trehalose, salicin, glycerol, mannitol, sorbitol, sucrose and glucose; no production of acid from arabinose and raffinose. Only one isolate of each species from each patient was included in the study.

Antibiotic susceptibility tests. The tests of *in vitro* susceptibility were carried out on PDM-antibiotic sensitivity medium (Bio-Disk, Stockholm, Sweden) with the agar dilution method according to *Ericsson & Sherris* (1971). The composition of the antibiotic sensitivity medium was: peptone 6.0 g/l, casein (acid hydrolyzed) 3.0 g/l, yeast extract 1.4 g/l, glucose 3.8 g/l, NaCl 1.0 g/l, Na_2HPO_4 3.0 g/l,sodium acetate trihydrate 1.0 g/l, $MgSO_4$. $7H_2O$ 20 mg/l, $FeCl_3$/20 mg/l, L-cysteine hydrochloride 20 mg/l, L-tryptophan 20 mg/l, vitamine B_{12} 10 μg/l, vitamine K 10 μg/l, cocarboxylase 10 mg/l, peridoxine hydrochloride 3 mg/l, Ca-d-pantothenate 3 mg/l, nicotinamide 3 mg/l, riboflavin 15 mg/l, biotin 3 μg/l, choline chloride 7.5 mg/l, adenine 10 mg/l, guanine 10 mg/l, xanthine 10 mg/l, uracil 10 mg/l, starch 2 g/l and agar 10 g/l, pH 7.3—7.4. An undiluted inoculum from an 18 hours' culture on blood agar was applied by the inocula replicating method described by *Steers, Foltz* and *Graves* (1959), and the results were read after incubation for 18 hours at 37°C. The replicator delivered 0.002 ml of the culture containing approximately 10^4 bacteria.

Antibiotics used. Dilutions of antibiotics from stock solutions were freshly prepared in 0.01 M phosphate buffer, pH 7.0, for each experiment. The stock solutions contained 1280 mcg/ml active substance for all tested antibiotics and were kept frozen at -20°C (*Ericsson & Sherris*, 1971). The solutions were only thawed once. The following antibiotics were tested: D-azidobenzylpenicillin sodium salt batch 20 (azidocillin), aminobenzylpenicillin batch 186 (ampicillin) and tetracycline phosphate 19 (tetracycline) were obtained from Astra, Södertälje, Sweden; Erythromycin estolate lot DE 16452 (erythromycin) and cephalexine monohydrate lot 454—226—20 (cephalexine) were provided from Lilly, Windlesham, Surrey, England; Lincomycin hydrochloride monohydrate no. 6542 (lincomycin) from Upjohn, Kalamazoo, USA; Phenoxymethylpenicillin potassium salt 15-204-1 (penicillin V) and benzylpenicillin sodium salt 58703 (penicillin G) from Kabi, Stockholm, Sweden.

RESULTS

The results of the susceptibility tests are shown graphically in Figure 1. As can be seen from the figure the minimum inhibiting concentration of azidocillin and ampicillin was lower (0.5—2.0 mcg/ml) than for benzylpenicillin (1.0—4.0 mcg/ml) and phenoxymethylpenicillin (2.0—8.0 mcg/ml) for all strains tested. The strains were found to be resistant to cephalexine (64—128 mcg/ml). Most strains were sensitive to tetracycline but showed a wide variation in sensitivity to this antibiotic (0.5—$\geq$128 mcg/ml). The strains showed a similar pattern to erythromycin (0.5 — $\geq$128 mcg/ml) as to tetracycline. The minimal inhibitory concentration of lincomycin varied between 8 and $\geq$128 mcg/ml.

DISCUSSION

Successful treatment of bacterial infections in the jaws, which are mainly of

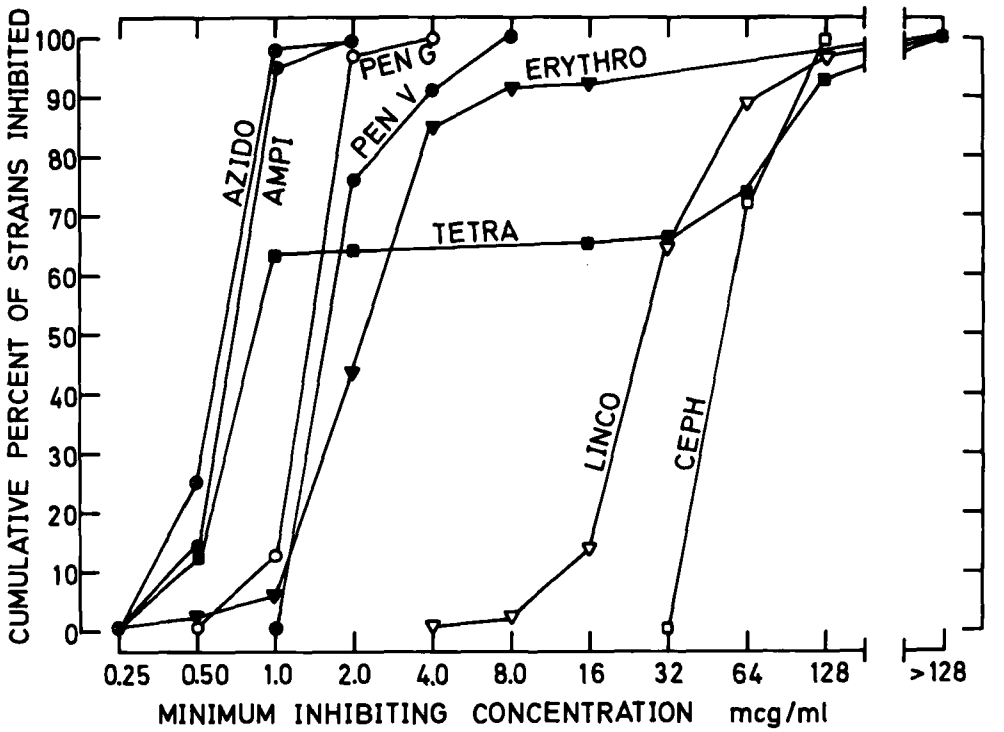

Fig. 1.
Antibiotic susceptibility of the oral haemolytic enterococci to eight antibiotics.

AZIDO = azidocillin;
AMPI = ampicillin;
PEN G = benzylpenicillin;
PEN V = phenoxymethylpenicillin;
ERYTHRO = erythromycin;
TETRA = tetracycline;
LINCO = lincomycin;
CEPH = cephalexine;

dental origin, may require the use of a proper antibiotic. With local treatment of infected root canals with different disinfectants, enterococci have proved to be particularly resistant (*Engström & Frostell*, 1964). Thus, in some cases the difficulty of obtaining sterile conditions in the treatment of infected root canals is probably caused by selection of particularly resistant bacteria such as enterococci. In these cases the treatment should be directed specifically against the isolated microorganism by determining its sensitivity to different antibiotics. It is also very important to emphasize that the use of antibiotics for treatment of oral infections should be based on clear clinical indications.

Patients with rheumatic or congenital heart disease who undergo certain dental procedures may develop bacterial endocarditis. In such cases the prevention of the transitory bacteremia by antibiotic prophylaxis is important. Since viridans streptococci and enterococci are the bacteria most commonly implicated in bacterial endocarditis after surgical procedures (*Cherubin & Neu*, 1971), prophylaxis should be directed specifically against them. Therefore, we tested different

antibiotics of relevant use by oral administration. Penicillin, the first of the antibiotics that came into general therapeutic use, is still the best in the treatment of infections caused by gram-positive bacteria. Penicillin is not toxic in ordinary doses, but the allergy reactions are a more serious problem with penicillin than with most other antibiotics. Reactions usually follow an injection but they have also been caused by oral administration (*Fox*, 1965). Ampicillin is slightly less active than benzylpenicillin against most gram-positive bacteria but sligthly more active against enterococci. However, ampicillin has more sideeffects than other penicillins. Apart from occasional gastric intolerance, rashes were reported and found to be more be more common than with other penicillins (*Shapiro et al.*, 1969).

The enterococcus strains studied in this investigation were more sensitive to ampicillin and azidocillin than to benzylpenicillin and phenoxymethylpenicillin. *Tunevall & Frisk* (1967) and *Sonne & Jawetz* (1968) also reported similar results for enterococci isolated from other infections. *S. faecalis* is also generally more susceptible than *S. faecium* to penicillins, cephalosporins and erythromycin (*Toala et al.* 1969). *Bengtsson et al.* (1972) reported recently two cases of enterococcal septicemia, which were successfully treated with azidocillin. However, there is no significant difference between the clinical effect of phenoxymethylpenicillin and azidocillin in the treatment of scarlet fever (*Bengtsson, Holmgren & Tunevsll*, 1969).

The cephalosporins, cephalothin and cephaloridine are not absorbed from the alimentary tract and therefore cannot be administered orally. However, with cephalexine it is now possible to give this cephalosporin by oral route. Cephalexine has been reported to give high concentration in bone and has been used for treatment of osteomyelitis (*Kanyuck et al.*, 1971). However, the strains tested were resistant to cephalexine which should thus probably never be used in enterococcal infections.

With known allergy to penicillins and cephalosporins the use of erythromycin for streptococcal infections is recommended (*Garrod & O'Grady*, 1971). However, erythromycin against enterococcal infections is of limited value since some strains are resistant to erythromycin and unsuccessful treatment with erythromycin in enterococcal septicemia has been reported (*Bengtsson et al.*, 1972).

About one third of the strains tested were resistant to tetracycline while the other two thirds were quite sensitive. Thus enterococci shows variation in sensitivity to tetracycline and also its analogues (*Toala et al.*, 1969). Since tetracyclines produce sideeffects such as staining of teeth of children, liver damage, and gastrointestinal disturbances, they are of limited use in the treatment of oral infections in humans.

Lincomycin closely resembles erythromycin in its activity against most gram-positive bacteria (*Garrod & O'Grady*, 1971). Enterococci are unique amongst these species in being resistant to lincomycin and clindamycin (*McGehee et al.*, 1968). Moreover, lincomycin in combination with streptomycin had no effect on enterococcal endocarditis (*deYcaza, Waisbreu & Goodman*, 1967).

It is very important to emphasize that the use of antibiotics for treatment of oral infections should be based on clinical and bacteriological indications. The difference in antibiotic susceptibility of the various isolates of enterococci as well as most other bacterial species makes anti-

biotic sensitivity testing important in these infections.

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