LETTER TO THE EDITOR

ANTIBACTERIAL ACTIVITY OF VARIOUS ANTIBIOTICS AGAINST ORAL STREPTOCCI ISOLATED IN THE ORAL CAVITY

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A total of 550 oral streptococci: 270 *Streptococcus mitis*, 110 *Streptococcus sanguis*, 90 *Streptococcus anginosus*, 50 *Streptococcus mutans*, 30 *Streptococcus salivarius*, were isolated from dental plaque and gengival crevices of patients and tested for their susceptibility to 12 β-lactam antibiotics and to 5 non-β-lactam antibiotics, using the microdiluition method. Overall, a reduced susceptibility to penicillin was recorded in 13.4% of cases. The percentage of strains resistant to penicillin appeared significantly higher in *S. mitis* (24%) than in *S. sanguis* (19%), in *S. mutans* (14%) and in *S. salivarius* (10%). No levels of penicillin resistance were shown by 90 strains of *S. anginosus*. In susceptibility test to antibiotics, imipenem was the most active molecule tested, confirming its general good activity against oral streptococci. Also third generation cephalosporins such as ceftriaxone and fourth generation cephalosporins such as cefepime, showed good activity. Chinolones, glycopeptides and rifampicin confirmed a good activity against oral streptococci.

Oral streptococci are an important part of the normal pharyngeal flora of humans and animals. They may be found in upper respiratoty tract, female genital tract, gastrointestinal tract, but they are most prevalent in the oral cavity (1). Oral streptococci, on average, represent 28% of the microrganisms isolated from dental plaque, 29% of flora isolated from gengival crevices, 45% isolated from the tongue and 46% from the saliva (2). The various species, however, are not distributed uniformly in the oral cavity. Many studies have confirmed the prevalence of *S. salivarius* on the tongue and the prevalence of *S. mitis* on the buccal mucosa and the association of *S. mutans* and *sanguis* with dental structures (3).

The pathogenicity of oral streptococci is related by their ability to produce endocarditis. Extracellular polysaccharides, especially dextran, play an important role in adhesion and invasion of cardiac valves. The data obtained by the clinical observations demonstrated that, after bacteremia caused by dextran-producing streptococci, there is a higher incidence of infective endocarditis than when bacteremia is caused by non-dextran-producing streptococci (4).

In addition to cause infective endocarditis, several species of viridans streptococci, such as *S. mutans*, have a strong association with the development of dental caries. This organism is acquired early

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through horizontal and vertical trasmission from mother to infant (5). Laboratory experiments have shown that caries develop in the germ-free animals after infection by *S. mutans*. However, colonization of dental surfaces and production of caries occurs only in the presence of dietary sucrose (6-7). Cariogenic properties of *S. mutans* are related to ability to adhere on teeth and to production of acid from dietary sugars (8).

Although oral streptococci were generally considered to be uniformly susceptible to penicillin, strains showing penicillin resistance were reported since the 1962 in the gengival flora of patients receiving penicillin prophylaxis for reumatic fever (9).

In recent years, resistance has emerged as a significant problem, especially resistance to penicillin and other β-lactam agents. Some strains of oral streptococci exhibit high levels of resistance to penicillin (10).

Significant levels of resistance have been observed also against aminoglycosides (11), but only rare isolates have been referred as resistant to chloramphenicol and vancomycin.

The purpose of this study is to identify oral streptococci from dental plaque and dental crevices of patients and to evaluate the susceptibility to β-lactam and non-β-lactam antibiotics to prevent the risk of infective endocarditis.

MATERIALS AND METHODS

Microrganisms

A total of 550 oral streptococci were isolated from the oral cavity of patients and tested for antimicrobial susceptibility. The patients (aged 30 to 68 years, 58% males and 42% female), not frequently exposed to antibiotics, were from private dental surgeries. Informed consent was obtained from each patient.

The identification of oral streptococci was performed according to standard methods (12), comprising also colony morphology, fermentation of different carbohydrates, pyruvate utilization, sodium ippurate hydrolysis and hydrolysis of starch. Also the PCR method was used for identification of colonies. Five species were recognized, i.e. *S. mitis*, *S. sanguis*, *S. anginosus*, *S. salivarius* and *S. mutans*.

Sampling procedure

Samples were taken from the gengival crevice of posterior teeth by aseptic insertion of two sterile paper points (Johnson and Johnson, Slough, UK) into the gengival crevice. Samples from dental plaque were collected by passing a sterile swab over the dental arch, previously dried of saliva by a jet of compressed air. The samples were immediately transferred into 2 ml of sterile saline solution and sent to a laboratory for isolation and identification.

Antimicrobial agents and susceptibility testing

The following antibiotics were used: penicillin (P), amoxycillin (AML), ampicillin (AMP), amoxycillin – clavulanate (AMC), piperacillin (PRL), imipenem (IPM), cefotaxime (CTX), cefpodoxime (CPD), cefuroxime (CXM), ceftibuten (CFT), cefepime (FEP), ceftriaxone (CRO), clindamycin (DA), tetracicline (TE), levofloxacin (LEV), vancomycin (VA) and rifampicin (RD), (Sigma Aldrich).

The Minimal Inhibitory Concentrations (MICs) were determined by the microdilution method using cation-adjusted Mueller Hinton broth (Oxoid), supplemented with lysed horse blood (final concentration, 3%) as recommended by the Clinical and Laboratory Standards Institute (CLSI) (13). The inoculum was prepared by suspending several colonies from an overnight blood agar culture, in sterile 0.9% saline solution and adjusting the turbidity to 0.5 McFarland standard. The suspension was further diluted within 15 min to provide a final concentration of bacteria of $5x10^5$ CFU/ml for each strain. The plates were covered with plastic tape and incubated at 35°C for 18 to 24 h. MIC was defined as the lowest concentration of antibiotic which inhibits visible bacterial growth. Strains were classified for their susceptibility or resistance in accordance with the breakpoints recommended by the CLSI; in addition, available EUCAST breakpoints were also used (14).

RESULTS

Among the isolated 550 oral streptococci, the following species were identified: 270 *S. mitis* strains, 110 *S. sanguis*, 90 *S. anginosus*, 50 *S. mutans*, 30 *S. salivarius*. The overall prevalence of penicillin resistance and susceptibility is reported in Fig. 1. *S. mitis* showed susceptibility to penicillin in 76% of cases and resistance in 24% of cases. *S. mitis* was the species most frequently isolated (60% of all strains). *S. sanguis* (24.4% of isolated strains) showed susceptibility to penicillin in 81% of cases and resistance in 19%. *S. mutans* (11.1% of strains) showed susceptibility to penicillin in 86% of tested strains and resistance in 14% of strains. No levels

Table I. *In vitro activities (%) of β-lactam and non-β-lactam antibiotics against oral streptococci group isolated from oral cavity.*

Antibiotic	S (%)	R(%)	MIC50	MIC 90	RANGE
P	87	13	2	2	0.12-8
AML	86	14	2	4	0.06-16
AMP	86	14	2	4	0.06-16
AMC	86	14	2	4	0.06-16
PRL	84	16	4	8	0.03-16
IPM	90	10	1	2	0.03-4
CTX	89	11	2	1	0.03-16
CRO	89	11	2	1	0.03-8
FEP	89	11	2	1	0.03-16
CPD	83	17	4	8	0.03-16
CXM	86	14	2	4	0.03-16
CFT	46	54	32	<32	0.25->32
DA	82	18	1	128	0.12-128
TE	66	34	1	32	0.06-128
LEV	100	0	1	1	0.25-2
VA	100	0	0.25	0.5	0.03-1
RD	100	0	<0.015	0.03	<0.015-0.5

S: susceptible, R: resistant
MIC_{50} and MIC_{90} values at which 50 and 90% of strains are inhibited, expressed in μg/ml.

of penicillin resistance was showed by 90 strains of *S. anginosus* (20% of isolated). *S. salivarius* (6.6 of all isolated) showed susceptibility against penicillin in 90% of strains and resistance in 10% of isolated strains.

The percentage of strains resistant to penicillin was significantly higher in *S. mitis* (24%) than in *S. sanguis* (19%), or in *S. mutans* (14%) or in *S. salivarius* (10%). All groups of isolated strains showed susceptibility to penicillin in 86.6% of cases, and resistance in 13.4%. The values of MICs against β-lactam and non-β-lactam antibiotics are reported in Table I.

In the group of β-lactams, several antibiotics were more active or equivalent to P. These included: IPM, CTX, CRO and FEP. IPM was more active than P, whereas CTX and CRO showed a comparable activity. Antibiotics such as AML, AMP, AMC and PRL, showed a slighly lower activity than P, while CFT was nearly inactive.

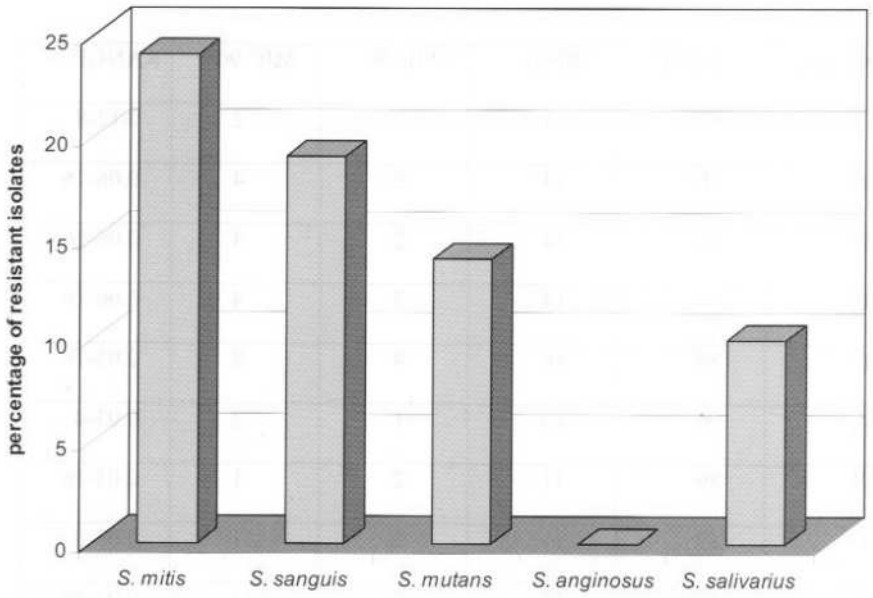

Fig. 1. *Percentage of penicillin-resistant isolates within each oral streptococcal species.*

Significant levels of resistance were found towards DA and TE. The quinolone, the glycopeptide and the RD exhibited a good activity against all isolates.

DISCUSSION

β-lactam antibiotics are the most commonly prescribed chemoprophylactic agents in general dental practices. However, resistance to penicillin among oral streptococci is increasing (15). The number of resistant oral streptococci is greater in people frequently exposed to antibiotics (16), although these bacteria may also be found in healthy subjects who have not been recently treated with an antimicrobial (17).

The British Society for Antimicrobial Chemotherapy recommend a short course of prophylaxis to limit the selection of resistant strains (18).

In our study we observed a significant level of penicillin resistance (13.4%) in oral streptococcal clinical isolates. The high prevalence of resistance to penicillin of the *S. mitis* (24%), is similar to that which has been previously observed in South Africa and Spain (19-20).

Several *in vitro* studies, have demonstrated the capability to transfer penicillin resistance determinants among related species (21). These mechanisms, together with selective antibiotic pressure, may play an important role in the emergence and spread of penicillin resistance in oral streptococci.

In our study, imipenem was the most active molecule, confirming the general good activity characteristic of this carbapenem. Also third and fourth generation cephalosporins showed a good activity. Chinolones, glycopeptides and RD confirm a good activity against oral streptococci.

In conclusion, our study demonstrates significant levels of penicillin-resistance in oral streptococci isolated in dental patients. Resistant strains showed various degrees of diminished susceptibility to β-lactams, to DA and to member of tetracyclines, the most active β-lactams were: IPM, CTX, CRO and FEP.

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