Susceptibility testing of *Streptococcus mitis* group isolates

G. Bancescu, S. Dumitriu, A. Bancescu, C. Defta, M. Pana*, D. Ionescu, S. Alecu & M. Zamfirescu

*Chair of Microbiology, University of Medicine & Pharmacy "Carol Davila" & *National Reference Laboratory for Streptococci, Cantacuzino Institute, Bucharest, Romania

Received August 8, 2003

**Background & objectives:** Suppurative oral and maxillofacial infections are usually mixed infections due to aerobic and anaerobic bacteria, most frequently by oral streptococci and antimicrobial treatment is necessary for such infections. The aim of this study was to investigate the antimicrobial susceptibility of *Streptococcus mitis* group strains isolated from Romanian patients with different oral and maxillofacial infections.

**Methods:** Eighty-five isolates belonging to *S. mitis* group isolated from pus samples were identified at species level by the Rapid ID 32 STREP system. The E test was used to determine the susceptibilities of the isolates to penicillin, ampicillin, cefotaxime, erythromycin, clindamycin, chloramphenicol and tetracycline.

**Results:** Of the 151 samples studied, 85 isolates belonged to *S. mitis* group. The minimum inhibitory concentration (MIC) values (mg/l) ranged from 0.016-0.75 for penicillin, 0.016-2 for ampicillin, 0.016-1 for cefotaxime, 0.016-4 for erythromycin, 0.016-0.047 for clindamycin, 0.5-4 for chloramphenicol and 0.047-256 for tetracycline.

**Interpretation & conclusion:** The low susceptibility and the resistance to some commonly used antibiotics found in this study indicated a need for a careful surveillance of the susceptibility pattern of oral streptococci isolates of clinical significance. Clindamycin and chloramphenicol might be suitable alternative agents in treatment of oral and maxillofacial infections involving penicillin-resistant bacteria and in case of patients with hypersensitivity to beta-lactam antibiotics.

**Key words** Antimicrobial susceptibility - oral and maxillofacial infections - *Streptococcus gordonii* - *Streptococcus mitis* - *Streptococcus oralis* - *Streptococcus sanguis*

The frequency of suppurative oral and maxillofacial infections in Romania is high¹, and these are usually mixed infections produced by an association of aerobic and anaerobic bacteria. The most frequently isolated facultatively anaerobic microorganisms were the oral streptococci, in particular those belonging to *anginosus* (the former *Streptococcus milleri* group) and *mitis* groups. Although surgical drainage is of primary importance, administration of antimicrobial therapy becomes necessary in serious infections and their complications, particularly in immunocompromised patients. Antibiotics are often prescribed empirically and penicillin is still considered the drug of choice for the treatment of such diseases¹-⁴. However, the emerging resistance to beta-lactam antibiotics among viridans streptococci may represent a potential clinical problem in the management of therapy. Therefore, the aim of the present study was to test the antimicrobial susceptibility of *Streptococcus mitis* group isolates obtained from Romanian patients with different oral and maxillofacial infections.

**Material & Methods**

Pus samples were obtained primarily by needle aspiration and occasionally by swabs dipped into the
surgical wound from 151 patients presenting at the Emergency Service of the Oral and Maxillofacial Surgery Clinic, Bucharest, with pyogenic oral and maxillofacial infections (dento-alveolar abscess, periodontal abscess, fascial space abscess, pericoronitis, sialadenitis, osteomyelitis of the mandible and maxillary sinusitis). Gram stained smears and cultures were performed in all samples. Streptococcal strains were identified at species level using the Rapid ID 32 STREP system (BioMérieux, Marcy-l’Etoile, France). Isolates belonging to \textit{mitis} group were further tested for susceptibility to penicillin, ampicillin, cefotaxime, erythromycin, clindamycin, chloramphenicol and tetracycline by the E test (AB Biodisk, Solna, Sweden). \textit{S. pneumoniae} ATCC 49619 was used for quality control. The inoculum was standardized at 0.5 McFarland turbidity in order to give semiconfluent growth on Müller-Hinton agar (Difco Laboratories, Detroit, USA) supplemented with 5 per cent sheep blood. The plates were incubated at 35ºC for 24 h in 5 per cent CO\textsubscript{2} atmosphere, except for erythromycin and clindamycin when ambient atmosphere was required. Susceptibility breakpoint concentrations were those recommended by the National Committee for Clinical Laboratory Standards\textsuperscript{5}.

**Results**

Identification at species level of the streptococcal isolates from the pus samples indicated that 85 isolates belonged to \textit{mitis} group. Of these, 73 were \textit{S. oralis}, six were \textit{S. mitis}, five \textit{S. sanguis} and one isolate was \textit{S. gordonii}. Correlating the results of pus smears microscopy with those of primary cultures, \textit{S. oralis} was the only bacteria found to be involved in causing infection in seven abscess cases. The MICs values of antibiotics tested and the distribution of the isolates of each species within the three sensitivity groups are presented in Tables I, II.

The ranges of the minimum inhibitory concentrations (MICs) were: 0.016-0.75 mg/l for penicillin (78.8\% susceptible and 21.2\% intermediate susceptible isolates), 0.016-2 mg/l for ampicillin (93\% susceptible and 7\% intermediate susceptible isolates), 0.016-1 mg/l for cefotaxime (98\% susceptible and 2\% intermediate susceptible isolates), 0.016-4 mg/l for erythromycin mg/l (90.6\% susceptible, 3.5\% intermediate susceptible and 5.9\% resistant isolates), 0.016-0.047 mg/l for clindamycin (100\% susceptible isolates), 0.5-4 mg/l for chloramphenicol (100\% susceptible isolates) and 0.047-256 mg/l for tetracycline (48.2\% susceptible, 10.6\% intermediate susceptible and 41.2\% resistant isolates).

All isolates classified as intermediate susceptible to cefotaxime and/or to ampicillin were also found to be intermediate susceptible to penicillin. Regarding the association of resistance to antibiotics belonging to different classes, only one isolate of \textit{S. mitis} and one of \textit{S. sanguis} was found with low sensitivity to penicillin and resistance to tetracycline. In case of \textit{S. oralis} a single isolate with intermediate susceptibility to all beta-lactam antibiotics tested was found to be resistant to erythromycin, whereas another isolate was resistant to erythromycin and tetracycline.

**Discussion**

Though routine susceptibility testing of oral streptococci involved in oral and maxillofacial infections is usually not performed, it is necessary to monitor from time to time the susceptibility patterns of the clinical isolates belonging to these bacteria within a geographical area. This study was focused on the susceptibility testing of clinical isolates belonging to \textit{mitis} group, since these microorganisms are frequently isolated from oral and maxillofacial infections. Unfortunately, these are often unrecognized pathogens due to the lack and difficulties in their identification and therefore, little information is available on their antimicrobial susceptibility.

In this study the sensitivity of the isolates was determined by the E test which has been evaluated as an easy, accurate and standardized antimicrobial susceptibility method\textsuperscript{6-8}. Although most isolates were sensitive to the beta-lactam antibiotics tested, 21, 7 and 2 per cent isolates were intermediate susceptible to penicillin, ampicillin and cefotaxime, respectively. Isolates with reduced susceptibility to penicillin were found in each species, except for \textit{S. gordonii} represented by a single strain which was susceptible to all seven antimicrobial agents. Low susceptibility to ampicillin, eventually associated with reduced susceptibility to cefotaxime, was found only among \textit{S. oralis} and \textit{S. mitis} isolates. In contrast, another study reported more penicillin-resistant isolates among \textit{S. sanguis} from acute suppurative oral infections\textsuperscript{3}. Highly penicillin-and cephalosporin-resistant \textit{S. mitis}, \textit{S. oralis} and \textit{S. sanguis} isolates have been found in different countries\textsuperscript{7,9,11}. 

Although erythromycin is considered the drug of choice for patients known to be hypersensitive to penicillin\textsuperscript{4,8}, the 5.9 per cent resistant and 3.5 per cent intermediate sensitive isolates confirmed that the susceptibility to this macrolide could not be predicted before \textit{in vitro} testing. Several published reports\textsuperscript{10-14} have indicated much higher resistance rate (between 17-55\%) to erythromycin for \textit{S. mitis}, \textit{S. oralis} and \textit{S. sanguis} isolates mostly obtained from pus samples from oral and maxillofacial infections or in blood cultures. In contrast, resistance to clindamycin and chloramphenicol has been reported only occasionally among oral streptococci isolates\textsuperscript{7,10,11,13}. In the present study, all isolates were sensitive to both of...
these drugs. The data suggested that clindamycin and chloramphenicol were suitable alternative antimicrobial agents in treatment of suppurative oral and maxillofacial infections when penicillin-resistant bacteria were involved or in case the patient was allergic to penicillin.

In the present study, the resistance rate to tetracycline reached more than 40 per cent and that of intermediate susceptibility more than 10 per cent. The same high frequency of tetracycline-resistance among mitis group isolates has been found in other studies also\(^7,\)\(^11\). The results indicate that this drug is clearly not

<table>
<thead>
<tr>
<th>Antibiotic/species (no. of isolates)</th>
<th>S. oralis (73)</th>
<th>S. mitis (6)</th>
<th>S. sanguis (5)</th>
<th>S. gordonii (1)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Penicillin</td>
<td>60</td>
<td>13</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>68</td>
<td>5</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Cefotaxime</td>
<td>72</td>
<td>1</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Erythromycin</td>
<td>67</td>
<td>3</td>
<td>3</td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>73</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>73</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>33</td>
<td>8</td>
<td>32</td>
<td></td>
</tr>
</tbody>
</table>

Table II. Distribution of Streptococcus mitis group isolates within the three antimicrobial sensitivity groups
recommended in the therapy of pyogenic oral and maxillofacial diseases in which oral streptococci are involved.

In conclusion, the present findings indicated that the susceptibility of oral streptococci isolates of clinical importance should be periodically tested, particularly to the widely-used antibiotics, since there is considerable concern about the increase in the frequency of penicillin- and multi-resistant strains.

Acknowledgment

The authors thank their colleagues from the Oral and Maxillofacial Surgery Clinic, Bucharest, for their help in collecting samples.

References


Reprint requests: Dr G. Bancescu, Chair of Microbiology, University of Medicine & Pharmacy, "Carol Davila" Bucharest, Romania e-mail: adrianbancescu@hotmail.com