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What is This?
Penicillin-Resistant Streptococci from the Saliva

RICHARD K. WATASE,* ARTHUR N. BAHN, and CARL HAGA

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SYNOPSIS IN INTERLINGUA

STREPTOCOCCOS PENICILLINO-RESISTENTE ISOLATE AB LE SALIVA DE PATIENTES CON FEBRE RHEUMATIC.—Le presenta de streptococcos penicillin-resistente in le cavitate oral representa un periculo potential pro le salute general de patientes con febre rheumatic e congenite morbo cardiac quando illes se subjice a un tractamento dental. Esseva studiate le saliva de patientes con febre rheumatic sub tractamento penicillinc a diurne dosage oral. Le streptococcos hemolytic alpha e nonhemolytic esseva identificate per le tests morphologic e biochimic de uso in le microbiologia diagnostic. Le resultatos del studio monstra que 45 ex 58 patientes rheumatic (77,5 pro cento) habeva disveloppate lineas penicillin-resistente de streptococcos, in comparation con solmente 7 del 58 patientes de controlo (12 pro cento).

Oral daily administration of antibiotics to rheumatic fever patients may cause the development of antibiotic resistant oral microorganisms. In prophylactic treatment of rheumatic fever, penicillin is the most commonly used antibiotic. The induction of penicillin-resistant streptococci in the oral cavity has been noted following administration of this drug, and it may be surmised that the presence of such organisms in the oral cavity may constitute a hazard to patients with rheumatic or congenital heart conditions who undergo dental treatment. Such a hazard might be the subsequent development of subacute bacterial endocarditis caused by a transient bacteremia of antibiotic resistant streptococci from the oral cavity.

To counteract this hazard, it has been recommended that rheumatic fever patients be given prophylactic antibiotics prior to severe dental manipulation, such as dental extraction. The level of antibiotic in the blood stream will destroy any residual microorganism not phagocytized from a transient bacteremia of oral origin. If penicillin is administered and penicillin-resistant streptococci enter the blood stream, prophylactic penicillin treatment will be of little protective value against the penicillin-resistant streptococci. The purpose of this investigation is to determine whether penicillin-resistant alpha-hemolytic and nonhemolytic streptococci are present in the saliva of rheumatic fever patients on a daily regimen of oral penicillin.

Materials and Methods

Fifty-eight children from the outpatients’ clinic of Children’s Memorial Hospital, Chicago, who had a history of rheumatic fever and were on a prophylactic regimen of 400,000 units of daily oral penicillin, were studied. The age range of the children was 6 to 15 years. A control group of 58 healthy children of the same age group with no history of penicillin therapy for at least 3 months or longer, was also used. The majority of the control group had not received penicillin for over a year or longer.

Paraffin-stimulated saliva was obtained from the patients. The serial dilution method was employed in which varying amounts of penicillin were added to a series of tubes containing selective culture medium for streptococci. Streptosel broth was used as the selective culture medium. The concentrations of penicillin G used in this study were from 1 to 10 units per milliliter in 10 ml. of broth.

To each of the 10 tubes, 0.1 ml. of the saliva sample was added. Tube 1 had 1

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unit of penicillin, tube 2 had 2 units, and so on. The tubes were incubated at 37° C. for 24 hours. After 24 hours, contents of these tubes with bacterial growth, indicating some penicillin resistance, were streaked on trypticase-soy blood agar plates and incubated at 37° C. for 24 hours. The isolated colonies were subcultured on trypticase-soy blood agar and the isolates were identified by the morphologic, serologic, and biochemical tests used in diagnostic microbiology.8

Results

The number of penicillin-resistant isolates of microorganisms that were isolated from the saliva of the rheumatic patients as compared to the control healthy group is shown (Table 1).

Penicillin-resistant microorganisms were isolated from 57 of the 58 rheumatic patients. Only one patient had no penicillin-resistant microorganisms. Forty-five rheumatic patients had penicillin-resistant streptococci, or 77.6 per cent of the total 58 patients. Thirty patients, or 51.7 per cent, showed growth of yeasts; six patients, or 10.3 per cent, showed staphylococcal growth.

In contrast, in the control group, 33 penicillin-resistant isolates were found in 29 patients, two patients showing 3 isolates each. In the other 29 patients, or 50 per cent of the control group, no penicillin-resistant microorganisms were found. In only seven of the 58 patients were penicillin-resistant streptococci present.

An identification of the penicillin-resistant microorganisms and the degree of their resistance to penicillin is shown (Table 2). *Streptococcus mitis* made up the majority with 26 isolates, and with an average resistance to 6.7 units of penicillin. The *Streptococcus salivarius* isolates were divided into two groups: those that fermented inulin and were classified as *Str. salivarius* according to the characteristics listed in Bergey’s Manual,8 and those that produced a highly mucoid colony and did not ferment inulin, referred to here as *Str. salivarius* variant. There were 3 isolates of *Str. salivarius* that did ferment inulin; these had an average penicillin resistance of 4.6 units. The majority of *Str. salivarius* isolates (16) did not ferment inulin and had an average penicillin resistance of 2.5 units. There were only 2 isolates of *Streptococcus faecalis*, and both had resistance at least to 10 units. Further analysis revealed resistance in excess of 12.5 units. There were 4 isolates of a streptococcus identified as *Streptococcus sanguis*, causing alpha-hemolysis on blood plates and gumdrop colonies on the *mitis-salivarius* agar. The average unit resistance to penicillin was 4.7. There were five isolates of streptococci in which no definite identification could be made. These had gamma-hemolysis reaction on blood plates, pinhead colonies on the *mitis-salivarius* agar, and an average penicillin resistance of 4.6 units. There was no Group A beta-hemolytic streptococcus, *Streptococcus pyogenes*. The total number of penicillin-resistant strep-

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**TABLE 1**

**PENICILLIN-RESISTANT MICROORGANISMS ISOLATED FROM THE SALIVA**

<table>
<thead>
<tr>
<th>Microorganism</th>
<th>Isolates from 58 Rheumatic Patients on Daily Oral Penicillin</th>
<th>Isolates from 58 Control Patients</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Number</td>
<td>Per Cent</td>
</tr>
<tr>
<td>Streptococcus</td>
<td>56*</td>
<td>57.7</td>
</tr>
<tr>
<td>Yeasts</td>
<td>30</td>
<td>30.9</td>
</tr>
<tr>
<td>Staphylococcus</td>
<td>6</td>
<td>6.1</td>
</tr>
<tr>
<td>Others</td>
<td>5†</td>
<td>5.1</td>
</tr>
<tr>
<td>Total</td>
<td>97</td>
<td></td>
</tr>
</tbody>
</table>

* Fifty-six isolates were found in 45 patients. In every other instance, except (†), 1 isolate was found per patient.
† Four gram-negative rods and 1 gram-negative coccus.
‡ Three gram-positive rods, 2 gram-negative rods, and 1 gram-negative coccus.
§ Thirty-three isolates were found in 29 patients. Two patients had 3 isolates each, the remaining 27 had 1 isolate each.
Penicillin-resistant streptococci were isolated from the saliva of rheumatic patients. There were 7 penicillin-resistant streptococci in the saliva of the control group (Table 3). All species had an average penicillin-resistance of 1 unit. There were 3 isolates of Str. mitis, 2 isolates of Str. salivarius, 1 isolate of Str. faecalis, and 1 gamma-hemolytic pinhead streptococcus, and no beta-hemolytic streptococcus, Str. pyogenes. In contrast to the experimental group, the penicillin resistance in the control group was decidedly low.

**Discussion**

Although the oral cavity is the logical site of origin of the bacteremia that follows dental manipulation, and although it is generally considered that penicillin-resistant, nonhemolytic streptococci from extraoral sites are of little significance in the etiology of subacute bacterial endocarditis, there have been relatively few investigations on penicillin-resistant salivary and oral microorganisms.

Krumwiede has found that the penicillin resistance of nonhemolytic streptococci isolated from the throats of children receiving prophylactic penicillin was increased; however, the resistance was not great. Garrod and Waterworth have demonstrated that large numbers of penicillin-resistant microorganisms appear after penicillin therapy, employing the cylinder or “cup method” instead of the dilution method. Barrow and Naiman have demonstrated that patients given penicillin therapy had oral microorganisms resistant to penicillin. Eagle, Fleischman, and Musselman stated that organisms that need a high concentration of penicillin for bactericidal effect in vitro will need similarly high doses of penicillin in vivo for therapeutic effectiveness in the blood stream.

The rheumatic fever patients on daily oral penicillin are excreting minute concentrations of penicillin into the oral cavity. It has been shown that, in the saliva, the average penicillin excreted after parenteral administration of 400,000 units of fortified penicillin is 0.0125, the peak level being reached in the first hour. This sublethal dose of penicillin could suppress the normal streptococcal flora in the mouth and allow the overgrowth of penicillin-resistant strains. These strains would constitute a therapeutic problem if penicillin were employed as the drug of choice.

Of interest was the observation that 45 out of 58 rheumatic patients, or 77.6 per cent of those tested, had penicillin-resistant strains of streptococcus. Only 7 of the 58 controls, or 12 per cent, had resistant strain of streptococcus. There was a difference in the selection of penicillin-resistant mutant streptococci in the group of rheumatic fever patients on daily oral penicillin and the control group with no penicillin therapy.

In the control group 29 patients, or 50 per cent, had no penicillin-resistant mutants, compared with only one patient in the rheumatic fever group. It appears that daily

### Table 2

**Penicillin-Resistant Streptococci Isolated from the Saliva of Rheumatic Patients on Daily Oral Penicillin**

<table>
<thead>
<tr>
<th>Species of Streptococcus</th>
<th>Average Resistance to Penicillin</th>
<th>Range of Resistance to Penicillin</th>
</tr>
</thead>
<tbody>
<tr>
<td>Str. mitis</td>
<td>6.7</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Str. salivarius (inulin ferment)</td>
<td>4.6</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Str. salivarius var. (no inulin ferment)</td>
<td>2.5</td>
<td>1 to 7</td>
</tr>
<tr>
<td>Str. faecalis</td>
<td>10.0</td>
<td>10</td>
</tr>
<tr>
<td>Str. sanguis*</td>
<td>4.7</td>
<td>3 to 10</td>
</tr>
<tr>
<td>Streptococcus†</td>
<td>4.6</td>
<td>1 to 10</td>
</tr>
<tr>
<td>Str. pyogenes</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* Alpha-hemolysis; gumdrop appearance.
† Gamma-hemolysis; pinhead appearance.
oral administration of penicillin is necessary for the continued presence of penicillin-resistant mutants in the oral cavity.

The degree of penicillin resistance of the streptococci in the control group was much lower than in the rheumatic fever group. All 7 streptococcal isolates from the control group grew sparsely and had resistance to only as much as 1 unit of penicillin. In one patient with *Streptococcus mitis*, there was only 1 colony on the blood plate. This colony represented a 1:10 dilution and is equivalent to 10 isolates per milliliter of saliva. In three patients, there were only 2 colonies on each of the blood plates. The mutants that were isolated were few. Increased resistance to penicillin appears to be favored by daily administration of the antibiotic.

The penicillin-resistant species of streptococci in the rheumatic fever group was entirely different from those from the control group. There were many streptococcal colonies that were resistant to as much as 10 units of penicillin. *Streptococcus mitis* and *Streptococcus salivarius* were the most prevalent resistant microorganisms. *Streptococcus mitis* and *Streptococcus salivarius* are the most commonly reported streptococci in the mouth and throat.14–16 Both of these organisms also were frequently isolated from patients with subacute bacterial endocarditis.17, 18

*Streptococcus faecalis* was cultured in two patients. It is mainly an organism of the intestine and is secondarily found in the mouth.19 The organisms isolated from the patients were resistant to 10 units of penicillin. This confirms the findings of others who have stated that *Streptococcus faecalis* is relatively resistant.1, 20

The streptococcus that showed alphahemolysis on the blood agar plate and gumdrop appearance on *mitis-salivarius* agar was tentatively identified as *Streptococcus sanguis*. This is inconclusive identification, because a positive characterization of *Streptococcus sanguis* is far more complicated.21–23 There were four strains of these organisms that resisted an average of 4.7 units of penicillin. Investigators17, 22, 23 think that *Streptococcus sanguis* occurs infrequently in the oral cavity, regardless of its high incidence in subacute bacterial endocarditis patients.

This study measured penicillin resistance only up to 12.5 units. Some of the isolates had penicillin resistance to more than 10 units. It will require large amounts of penicillin to attain a blood level of more than 10 units per milliliter of blood in patients infected with streptococci resistant to more than 10 units of penicillin.

Bender and others24 and Bender and Pressman25 have advocated the use of penicillin-streptomycin combination as prophylaxis in rheumatic patients. They have stated that the drug combination is more effective than penicillin alone. In 1961, however, the Food and Drug Administration placed a label on penicillin-streptomycin combinations drugs as “not for pediatric use.”26 This type of combination regimen may be effective for the adult rheumatic patient.

It is sometimes recommended that penicillin therapy be given 24 and 48 hours before dental manipulation. The merit of this treatment is not known, since it has never been determined whether resistant strains of streptococci will develop with such
therapy. Parenterally administered procaine penicillin is eliminated from the bloodstream within 24 hours. Therefore, it would not be effective to treat patients having rheumatic or congenital heart conditions with penicillin 24 and 48 hours prior to dental manipulation. Administration of the antibiotic immediately before dental treatment in rheumatic fever patients, so that a blood level is attained that will be adequate for the resistant streptococci, appears to be advisable.

It has been found in this study that resistant strains of streptococci may flourish in the oral cavity when daily oral penicillin is administered. Resistance may be enhanced by concentrations of the drug too minute to inhibit growth. This theory may explain the emergence of resistant strains. Rheumatic patients on daily oral penicillin excrete minute amounts of penicillin in the oral cavity. This may cause a suppression of the susceptible streptococcal flora and may allow the overgrowth of penicillin-resistant strains. It also is possible that simultaneous spontaneous mutation may occur.

Therapy for these rheumatic patients may be directed either toward the use of an antibiotic drug that will be effective against these penicillin-resistant streptococci or toward the use of a higher dosage of penicillin that will overcome the level of penicillin resistance of the streptococci.

Summary

An investigation was performed to study the occurrence of penicillin-resistant streptococci in the saliva of 58 rheumatic patients treated with daily oral penicillin, as compared to 58 control patients not on penicillin therapy.

Forty-five of the 58 rheumatic patients (77.6 per cent) had penicillin-resistant isolates of streptococci, as compared to only seven of the 58 control patients (12.0 per cent).

The degree of penicillin-resistance of the streptococci in the control group was not as high as that of the rheumatic group. All 7 streptococcal isolates from the control group failed to grow in the presence of greater than 1 unit of penicillin. The rheumatic group had penicillin resistance to 10 units or more.

*Streptococcus mitis* and *Streptococcus salivarius* were the most prevalent microorganisms recovered in the rheumatic group. There were 26 isolates of *Streptococcus mitis* and 19 isolates of the inulin and non-inulin fermenting *Streptococcus salivarius*.

References


