Bacterial interference of Neisseria gonorrhoeae by α-haemolytic streptococci

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SUMMARY Fifty pharyngeal isolates of α-haemolytic streptococci were tested against 20 cervical isolates of Neisseria gonorrhoeae for bacterial interference in vitro using the lawn-spotting method. Forty-seven (94%) isolates of streptococci showed inhibitory activity toward N gonorrhoeae, although nine of these were inhibitory to only one isolate of N gonorrhoeae. Isolates of N gonorrhoeae varied widely in their sensitivity to streptococci; the most sensitive were inhibited by 40 isolates of streptococci and the least sensitive by only 14 isolates. Species of Streptococcus found to inhibit growth of N gonorrhoeae were S mitis, S MG intermedius, S sanguis II, S mutans, and S morbillorum.

Introduction

In a previous study of cervical flora in women with recent sexual contact with men infected with Neisseria gonorrhoeae,1 one woman, who had a negative culture result for N gonorrhoeae, was found to have a high population of α-haemolytic streptococci in the cervix. This isolate of α-haemolytic streptococci inhibited N gonorrhoeae in vitro. Since α-haemolytic streptococci are known to inhibit a variety of micro-organisms,2 including Neisseria species,3 we decided to survey a larger number of strains of each of these organisms for evidence of bacterial interference and of their prevalence and to establish whether or not there were differences in susceptibility between various isolates of N gonorrhoeae.

Materials and methods

BACTERIAL STRAINS

Fifty isolates of α-haemolytic streptococci were obtained from pharyngeal swabs from healthy men and women between the ages of 20 and 50 by culture on Casman’s sheep blood agar. Single-colony isolates were selected on the basis of their α-haemolytic activity; frequently more than one strain, as differentiated by colonial morphology, was isolated per person. Isolates were identified by the following tests using the scheme of Facklam: optochin sensitivity and bile solubility, bile esculin hydrolysis, growth in 6-5% NaCl, growth in 5% sucrose and agar and broth, hippurate hydrolysis, and use of mannitol, lactose, inulin, esculin, and raffinose. Cultures were frozen at −4°C in trypticase soy broth (Difco) containing 20% glycerol and also maintained on Casman’s sheep blood agar (Baltimore Biological Laboratories, Maryland, USA) at 4°C.

Isolates of N gonorrhoeae were obtained from the Houston Public Health Laboratory from cervical cultures of patients reporting with suspected gonococcal infections. Cervical swabs were immediately plated on to Thayer-Martin medium and incubated in CO₂ for 18-24 hours. Oxidase-positive colonies of Gram-negative diplococci were presumptively identified as N gonorrhoeae, subcultured on to chocolate agar, and incubated at 37°C in CO₂ jars. After 18-24 hours’ incubation, growth was used immediately for bacterial interference experiments (first subculture). This growth was also suspended in trypticase soy broth (BBL) containing 20% glycerol and stored at −70°C. Chocolate agar was prepared by adding 5% sheep blood to BBL blood agar base and heating in a boiling waterbath for 10 minutes.

BACTERIAL INTERFERENCE

Uniform suspensions were made from the first subculture of N gonorrhoeae in phosphate-buffered saline, pH 7.2, measuring turbidity visually against McFarlane tube 2. Lawns were prepared by swabbing the suspension on to the surface of chocolate agar.
plates. Isolates of \( \alpha \)-haemolytic streptococci were incubated overnight on Casman's blood agar and spotted directly on to the surface of the lawns; the plates were then incubated in CO\(_2\) jars at 37°C for 18-24 hours. Zones of inhibition of growth of \( N \) gonorrhoeae were measured from the edge of the streptococcal growth for the purpose of checking the consistency of the observations in repeated trials.

**Results**

Fig 1 shows zones of inhibition of growth of \( N \) gonorrhoeae around inoculation of \( \alpha \)-haemolytic streptococci, demonstrating varying degrees of inhibition; zones varied between 1 and 5 mm. The inoculum was not quantitated, but certain isolates of \( \alpha \)-haemolytic streptococci were found repeatedly to produce larger zones of inhibition than others.

**FIG 1** Four different isolates of \( \alpha \)-haemolytic streptococci spotted on lawns of \( N \) gonorrhoeae showing varying degrees of inhibition of growth.

Not all isolates of \( \alpha \)-haemolytic streptococci inhibited \( N \) gonorrhoeae; similarly, different isolates of \( N \) gonorrhoeae varied in their sensitivity to \( \alpha \)-haemolytic streptococci. This is shown in fig 2, in which 50 isolates of streptococci have been arranged along the ordinate in order of their decreasing inhibitory activity, measured by the number of strains of \( N \) gonorrhoeae they inhibit. Isolates of \( N \) gonorrhoeae are arranged along the abscissa in order of their decreasing sensitivity to \( \alpha \)-haemolytic streptococci. Only three of the 50 isolates of \( \alpha \)-haemolytic streptococci were without inhibitory activity to any of the 20 isolates of \( N \) gonorrhoeae tested; a further nine inhibited only one isolate of \( N \) gonorrhoeae (fig 2). On the other hand, 18 (36%)
isolates of α-haemolytic streptococci inhibited at
least 16 (80%) isolates of N gonorrhoeae, even
though only one isolate of streptococci inhibited all
20 isolates of N gonorrhoeae.

Isolates of N gonorrhoeae varied greatly in their
sensitivity to the inhibitory activity of α-haemolytic
streptococci; the strain designated Z was inhibited by
40 of the isolates of streptococci whereas strain W
was inhibited by only 14 (fig 2). It was interesting
that some of the isolates of streptococci which
inhibited the largest number of isolates of N gonor-
rhoeae (that is, 22) did not inhibit the growth of the
most sensitive strain (Z).

The distribution of species of the pharyngeal
isolates of α-haemolytic streptococci are shown in
Table I. S mitis was the predominant species, followed
by S sanguis II and S MG intermedius. Inhibitory
activity of streptococcal isolates toward N
gonorrhoeae was not associated with any single
species; both active and inactive strains of all species
listed in Table I were found. Table II shows the
frequency of isolation of different strains within
species of streptococci showing inhibition toward N
gonorrhoeae. Of S mitis strains isolated, 63·6%
inhibited more than 50% of strains of N gonorrhoeae
tested; 50% of S MG intermedius strains had similar
activity whereas only 22·2% of S sanguis II were
inhibitory.

**Table I** Distribution of species of 50 pharyngeal isolates
of α-haemolytic streptococcus

<table>
<thead>
<tr>
<th>Species</th>
<th>% Incidence</th>
</tr>
</thead>
<tbody>
<tr>
<td>Streptococcus mitis</td>
<td>59·10</td>
</tr>
<tr>
<td>Streptococcus sanguis II</td>
<td>20·50</td>
</tr>
<tr>
<td>Streptococcus MG intermedii</td>
<td>11·40</td>
</tr>
<tr>
<td>Streptococcus morbillorum</td>
<td>4·50</td>
</tr>
<tr>
<td>Streptococcus mutans</td>
<td>2·00</td>
</tr>
<tr>
<td>Streptococcus sanguis I</td>
<td>2·00</td>
</tr>
</tbody>
</table>

**Table II** Inhibitory activity of different species of strep-
tococci toward N gonorrhoeae

<table>
<thead>
<tr>
<th>Species</th>
<th>% of strains isolated showing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Low activity*</td>
</tr>
<tr>
<td>S mitis</td>
<td>36·4</td>
</tr>
<tr>
<td>S MG intermedius</td>
<td>50·0</td>
</tr>
<tr>
<td>S sanguis II</td>
<td>77·8</td>
</tr>
</tbody>
</table>

*Inhibitory to <50% of strains of N gonorrhoeae tested
†Inhibitory to >50% of strains of N gonorrhoeae tested

**Discussion**

Although the α-haemolytic streptococci used in these
experiments were a heterogeneous group, it appears
that a high percentage of pharyngeal streptococci are
able to inhibit N gonorrhoeae. It is interesting
to speculate whether or not this antagonism occurring
in vitro could provide protection against infection in
vivo. Although inhibition of N gonorrhoeae has
been demonstrated in vitro by a number of
organisms—Candida albicans,5 Staphylococcus
epidermidis,6 non-haemolytic streptococcus,7 and
various enteric Gram-negative bacteria8—attempts to
show that interference provides protection are few.
To have a protective effect, α-haemolytic strep-
tococci would need to occur frequently in the normal
flora and in large numbers. In a review of seven
studies on the normal flora of the vagina and cervix,9
the isolation rate for Streptococcus viridans or
α-haemolytic streptococci varied from 0 to 53%. In
more recent quantitative studies, α-haemolytic
streptococci have still occurred in varying frequencies
but in large numbers.10-12 The true incidence of
α-haemolytic streptococci may not yet be accurately
recorded, since the use of selective media for vaginal
cultures would inhibit its growth; furthermore, an
overgrowth of fast-growing bacteria, such as Proteus
spp or Escherichia coli, would mask its presence.

In our previous study,1 a protective effect by
α-haemolytic streptococci against gonococcal
colonisation was apparent in only one patient.
Recent attempts to relate the presence of certain
groups of organisms in the normal cervical flora to
protection against N gonorrhoeae have shown that
Staph epidermidis isolates were most active against N
gonorrhoeae in vitro,13 although no correlation was
looked for between the presence of Staph epider-
midis and infection with N gonorrhoeae. Saigh et al14
attempted to relate the presence of antagonistic
organisms, which were mostly non-haemolytic strep-
tococcal species, staphylococcal species, and
Lactobacillus spp, to infection with N gonorrhoeae.
The presence of Lactobacillus spp was found to correlate with lack of acquisition of N gonorrhoeae,
which was interpreted as a cause-and-effect
relationship. The differences in the isolation rate of
Lactobacillus spp were also noted in our study of
normal cervical flora in relation to gonorrhoea,1 but
this difference was most obvious between the two
population groups, public clinic and private patients.
Our interpretation was not necessarily one of cause
and effect; however, we believed that the lack of
Lactobacillus spp in the cervical flora of clinic
patients infected with N gonorrhoeae was either a
result of other factors, such as recurrent episodes of
gonorrhoea and treatment with antibiotics eliminat-
ing Lactobacillus spp, or due to the fact that N
gonorrhoeae itself might have an inhibitory effect on
Lactobacillus spp—an effect that we were able to
demonstrate in one instance.
While α-haemolytic streptococci may not occur in cervical flora sufficiently often or in great enough numbers to provide protection from gonorrhoea, it may play a role in the prevention of gonococcal pharyngitis. Pharyngeal colonisation by *N gonorrhoeae* usually affects less than 10% of populations attending venereal disease clinics.15 When certain specialised groups are selected, such as homosexual men who claim to have had oral contact with partners infected with *N gonorrhoeae*, the incidence is higher.16 17

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References

Bacterial interference of 
Neisseria gonorrhoeae by 
alpha-haemolytic streptococci.
M E McBride, W C Duncan and J M Knox

Br J Vener Dis 1980 56: 235-238
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