SHORT ARTICLES

ANAEROBIC PERIURETHRAL FLORA OF HEALTHY WOMEN AND WOMEN SUSCEPTIBLE TO URINARY-TRACT INFECTION

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SUMMARY. The anaerobic periurethral microbial flora of 25 healthy women was compared with that of 29 women attending the urinary-tract-infection clinic at the Royal Free Hospital. The latter group consisted of 19 patients receiving long-term prophylactic antimicrobial therapy and 10 with proven recurrent urinary-tract infection not receiving prophylactic treatment. The numbers and species of anaerobes isolated from each group were similar. Lactobacillus spp. were the most frequently isolated organisms in each group and the most numerous. Bacteroides spp. were the next most frequently isolated. In any one subject, the anaerobic flora varied considerably during the study period of approximately 6 months. Thus, the anaerobic flora is not affected by recurrent urinary-tract infection in the past nor by the use of prophylactic chemotherapy. It does not appear to exert a protective role against the initiation of urinary-tract infection.

INTRODUCTION

Stamey et al. (1971) stressed that the single most important factor leading to urinary-tract infection in women is preliminary colonisation of the periurethral area by the infecting organism. Hence the normal flora of this area—oxygenic and anaerobic—in the healthy female is suggested to have a protective role in inhibiting this colonisation (Marrie, Harding and Ronald, 1978). However, our studies (Cooper et al., 1980) of the aerobic flora of women with recurrent urinary-tract infections have failed to confirm Stamey’s findings concerning colonisation by aerobic bacteria. In this present study we have tried to assess the place of the normal anaerobic periurethral flora by comparing the flora of healthy women without urinary-tract infection at the time of examination with that of women with a positive history of such infection. The effect of long-term prophylactic therapy on the anaerobic flora has also been investigated.

MATERIALS AND METHODS

Subjects. Three groups of women were studied: group A—25 patients (mean age 30 years) attending the Well-Woman Screening Clinic at the Royal Free Hospital. One periurethral specimen for anaerobic culture was taken from each woman. Any previous history of a urinary-tract infection was noted and details of any laboratory investigation were recorded; group B—19 patients (mean age 34 years) attending the Urinary Infection Clinic at the Royal Free Hospital. All had previously documented urinary-tract infections, as judged by symptoms and urine culture, and were receiving nitrofurantoin or trimethoprim as prophylactic long-term antimicrobial therapy. Some of them had more than one periurethral specimen taken; group C—10 patients (mean age 42 years) with documented urinary-tract infections, attending the clinic but not receiving long-term antimicrobial therapy.

Sampling technique. A quantitative method of sampling the periurethral flora was based on

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the method of Bollgren et al. (1979). The needle end of a 30-ml syringe was cut off to form an open tube. The plunger was retracted and about 5 ml of a 30% (w/v) gelatin solution were poured in. When this had set, the gelatin cylinder was advanced up the syringe and pressed against the urethral orifice. The gelatin cylinder was immediately expressed into 9 ml of diluting fluid (sterile distilled water containing yeast extract 0-05% (w/v) and 1 mM dithiothreitol). This solution was incubated at 37°C for c. 15 min to dissolve the gelatin. Serial dilutions, 10¹ and 10², were made in diluting fluid and 0·1-ml portions were inoculated onto the following selective anaerobic culture media: Anaerobic Base (Oxoid) + whole blood 5% + vancomycin 7·5 µg/ml + kanamycin 100 µg/ml for the isolation of Bacteroides spp.; Fusobacterium medium (Ohtani, 1970) modified by the addition of crystal violet 0·01g/L; Veillonella Agar (Difco) + vancomycin 7·5 µg/ml for the isolation of Veillonella spp.; Rogosa Agar (Difco) + glacial acetic acid 1·32 ml/L for the isolation of Lactobacillus spp.; Anaerobic Base (Oxoid) + neomycin 10 µg/ml + egg yolk 10% for the isolation of Clostridium spp.; Phenylethylalcohol Agar (BBL) containing phenylethyl alcohol 2·5 ml/L for the isolation of anaerobic gram-positive cocci.

Plates were read after incubation for 6 days in an anaerobic chamber containing nitrogen 80%, carbon dioxide 10%, and hydrogen 10%.

Identification and enumeration of anaerobic bacteria. Different colonial types were counted and recorded. They were then inoculated onto lysed-blood medium and incubated in air and anaerobically for 3 days. All obligate anaerobes were identified by gram staining followed by antibiograms when necessary (Leigh and Simmons, 1977; Wren, Eldon and Dakin, 1977). The density of bacteria on the periurethral surface was calculated in terms of number of bacteria per cm² by counting the number of colonies transferred from the gelatin cylinder and multiplying by a factor of 14 (Cooper et al., 1980).

**RESULTS**

Anaerobic bacteria were isolated from every patient. Those most commonly isolated were lactobacilli, (see the table) followed by Bacteroides spp. There was no difference between the types of organism carried by the three groups of patients, nor in the average number of species isolated from patients in the different groups. An average of three anaerobic species was isolated from each patient in each of the three groups. There was no significant difference

<table>
<thead>
<tr>
<th>Species</th>
<th>Specimen Group A (25 specimens)</th>
<th>Specimen Group B (29 specimens)</th>
<th>Specimen Group C (10 specimens)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lactobacillus spp.</td>
<td>92</td>
<td>82</td>
<td>70</td>
</tr>
<tr>
<td>Bacteroides spp.</td>
<td>40</td>
<td>41</td>
<td>20</td>
</tr>
<tr>
<td>B. fragilis</td>
<td>44</td>
<td>27</td>
<td>30</td>
</tr>
<tr>
<td>B. melaninogenicus</td>
<td>12</td>
<td>6</td>
<td>0</td>
</tr>
<tr>
<td>Veillonella spp.</td>
<td>36</td>
<td>34</td>
<td>40</td>
</tr>
<tr>
<td>Clostridium spp.</td>
<td>32</td>
<td>48</td>
<td>30</td>
</tr>
<tr>
<td>Peptococcus spp.</td>
<td>28</td>
<td>41</td>
<td>40</td>
</tr>
<tr>
<td>Peptostreptococcus spp.</td>
<td>4</td>
<td>10</td>
<td>0</td>
</tr>
<tr>
<td>Anaerobic gram-positive cocci</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>not identified</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table**

Frequency of occurrence of anaerobic species isolated from periurethral samples in three groups of patients

Patient group A = 25 patients attending the Well Woman Screening Clinic; patient group B = 19 patients attending the Urinary Infection Clinic with previously documented urinary-tract infection and receiving prophylactic long-term antimicrobial therapy; patient group C = 10 patients with previously documented urinary-tract infection but not receiving long-term antimicrobial therapy.
between the numbers of organisms carried by each patient in each group. Lactobacilli and
clostridia were isolated in largest numbers (>1400/cm²); the other species were isolated in
numbers ranging from 14 to 1400/cm².

Only three of the patients attending the Urinary Infection Clinic had an infection at the time
that their periurethral flora was determined and this did not affect their anaerobic flora.

The healthy control group (A) was divided into 10 patients with a previously documented
symptomatic urinary-tract infection and 15 with no such history. There was no difference
between the type of organisms carried, the number of different species and the number of
organisms carried by patients in these two subgroups. There was, however, considerable
variation in the anaerobic flora of any individual patient depending upon the time of sampling.
For example, from one to four different species, in varying numbers, could be isolated from the
periurethral area at different times. The specimens were generally taken at monthly intervals.

DISCUSSION

Various groups of workers have isolated different predominating anaerobes in the
periurethral flora. Bartlett et al. (1977) studied vaginal secretions in healthy women and found
Peptococcus spp. to be the predominating organisms. Marrie et al. (1978) isolated Bacteroides
spp. most often from the periurethral area of healthy females. Bollgren et al. (1979) showed
that the anaerobic periurethral flora of healthy girls was mainly gram-positive rods and
gram-positive cocci; gram-negative rods were rarely isolated. Another study by Bollgren et al.
(1981) showed that pre-menarchal girls prone to urinary-tract infection had an increased
colonisation with anaerobic gram-negative rods, particularly Bacteroides spp. Marrie, Swantee
and Hartlen (1980) have shown that the anaerobic flora is distinct in women of different age
groups—prepubertal, of child-bearing age and postmenopausal. Our results suggest that the
periurethral flora is so variable that predominating organisms cannot be predicted for a
particular patient or group of patients. We have shown that the periurethral flora is not affected
by long-term antimicrobial therapy with trimethoprim or nitrofurantoin. This is not
unexpected because trimethoprim is only weakly active against anaerobic bacteria (Then and
Angehrn, 1979) and nitrofurantoin, although active against some Bacteroides strains in vitro, did
not change the anaerobic periurethral flora in Bollgren’s study (Bollgren et al., 1981). We found
that the anaerobic flora of patients with a history of urinary-tract infection was not different
from that of healthy women. This situation differs from that of the aerobic periurethral flora.
Healthy women have been shown to be lightly colonised but women with a history of
urinary-tract infection are very heavily colonised although generally with organisms such as
Staphylococcus epidermidis and Micrococcus spp. and not, as might be expected, with coliforms
(Cooper et al., 1980). After the urine is infected, the periurethral area becomes heavily colonised
with the infecting organism as the urine washes over this area. The role of the anaerobic
periurethral flora is not completely understood. However, because there is no difference
between the nature of the flora found in different groups of patients in our study, we conclude
that the anaerobic flora does not exert a protective role against ascending infection of the bladder
urine by a pathogenic organism.

We thank Dr M. Pollock and staff of the Family Planning and Well Woman Clinic at the Royal
Free Hospital for co-operation in the taking of the periurethral specimens.

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