Bacterial flora in abnormalities of the female genital tract

A. M. Gordon, H. E. Hughes, and G. T. D. Barr

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SYNOPSIS The bacterial flora associated with certain common abnormalities of the female genital tract were studied. The abnormalities included were trichomonal infestation of the vagina, the epithelial inflammation and cellular atypia associated with protozoal infestation, and erosions of the cervix.

*Trichomonas vaginalis* infestation and marked epithelial inflammation were associated with a very varied bacterial flora in which Mycoplasma species, streptococci, and *'Haemophilus vaginalis'* (Gardner and Dukes, 1955) were often prominent. No cases of vaginitis attributable to *Haemophilus vaginalis* were detected. An essentially normal bacterial flora accompanied erosions of the cervix.

This paper describes the results of an investigation of the bacterial flora accompanying inflammation of the female genital tract. The principal purpose of this study was to establish the microbial floral variations in the presence of *Trichomonas vaginalis* infestation, with special reference to the group of patients showing cytological evidence of the severe epithelial cellular reactions to be described elsewhere (Hughes, Gordon, and Barr, 1966). An investigation of the vaginal flora associated with cervical erosions was also included, and in view of their equivocal status as pathogens of the female genital tract, it was considered of particular interest to undertake special cultural examinations for organisms of the Mycoplasma (P.P.L.O.) group, and for *'Haemophilus vaginalis'* as described by Gardner and Dukes (1955).

MATERIAL AND METHODS

The clinical material utilized in this investigation, the methods of clinical assessment and classification of the patients, and the specimen collection procedures were as described elsewhere (Hughes et al., 1966).

PREPARATION, EXAMINATION, AND GRADING OF SMEARS

Each cervical smear was air-dried, heat-fixed, and stained by Gram's method, using the modification of technique described by Preston and Morrell (1962). The Gram-stained films were examined by light microscopy, under oil immersion, and were assigned to one of three morphological floral grades (I, II, or III) based upon the following criteria: Smears showing a morphological flora exclusively of Döderlein type, were assigned to grade I, smears showing a mixed morphological flora, but which included organisms morphologically resembling lactobacilli (Döderlein's bacilli) were classified as grade II; a grade III smear was defined as one showing a mixed bacterial flora, totally devoid of organisms morphologically resembling lactobacilli.

CULTURAL TECHNIQUES For the cultivation of the aerobic and anaerobic flora of the genital tract, freshly prepared moist plates of Casman's medium enriched with 10% defibrinated horse blood (Burroughs Wellcome) were inoculated, in duplicate, from each cervical swab specimen. To satisfy the microaerophilic and frankly anaerobic requirements of many of the constituents of the vaginal flora, one plate was incubated anaerobically in a McIntosh and Fildes jar, while the second plate was incubated under increased CO₂ tension. All cultures were maintained at 37°C. for 72 hours, after which the anaerobic and CO₂ plates were examined in parallel, and the bacterial flora of the specimens fully elucidated by standard bacteriological methods. In particular, the identification of *Haemophilus vaginalis* was based upon the criteria set by Dukes and Gardner (1961).

The solid medium employed for the cultivation of organisms of the Mycoplasma (P.P.L.O.) group was essentially that of Chanock, Hayflick, and Barile (1962), comprising Difco P.P.L.O. agar, 7 parts, horse serum, 2 parts, and yeast extract, 1 part.

The P.P.L.O. agar base was prepared in 70-ml. aliquots, and sterilized by autoclaving. After cooling to 50°C., the base medium was enriched with 20 ml. sterile (Seitz filtered) horse serum (Burroughs Wellcome no. 3) and 10 ml. of a freshly prepared 25% extract of baker's yeast. The medium was further supplemented with 20 μg/ml. of a sterile aqueous solution of D.N.A. (sodium salt of D.N.A. from calf thymus gland, B.D.H. Ltd.).

Penicillin in a concentration of 500 units per ml., and thallium acetate in a final concentration of 1:2,000 were included as bacterial inhibitors and the medium was used at a final pH of 7.9. Following inoculation, plates were sealed with adhesive, and were incubated aerobically in a moist canister at 37°C. for four days, after which they were examined in obliquely transmitted light, using a...
low-power objective, for ‘fried-egg’ colonies suggestive of
mycoplasmas. A more precise morphological examination
of such colonies was made by the agar-block fixation and
Giemsa staining technique of Klinebeerger-Nobel (1950).
In order to avoid confusion with possible L-forms of
bacteria, final identification of these organisms as myco-
plasmas was dependent upon their successful and stable
subculture on P.P.L.O. medium devoid of penicillin.

RESULTS

Table I summarizes the bacteriological findings in
each of three clinical categories, ‘trichomonal dis-
charge’, ‘non-trichomonal discharge’, and ‘discharge
absent’, based on symptoms and on the clinical
appearances of the vaginal discharge. Table II shows
the variations of microbical flora in the presence of
Trichomonas vaginalis infestation.

**TABLE I**

<table>
<thead>
<tr>
<th>Microbial Flora</th>
<th>Clinical Category</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Discharge Absent (%)</td>
</tr>
<tr>
<td>Grade I</td>
<td>43:0</td>
</tr>
<tr>
<td>Grade II</td>
<td>33:4</td>
</tr>
<tr>
<td>Grade III</td>
<td>23:6</td>
</tr>
<tr>
<td>Mycoplasma</td>
<td>15:3</td>
</tr>
<tr>
<td><em>Haemophilus vaginalis</em></td>
<td>9:8</td>
</tr>
<tr>
<td>Aerobic streptococci</td>
<td>24:2</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>81:0</td>
</tr>
<tr>
<td>Corynebacteria</td>
<td>39:0</td>
</tr>
<tr>
<td>Candida species</td>
<td>9:7</td>
</tr>
<tr>
<td>Acinetobacter species</td>
<td>0:0</td>
</tr>
<tr>
<td>Neisseria species</td>
<td>0:0</td>
</tr>
<tr>
<td><em>Micrococcus</em></td>
<td>9:4</td>
</tr>
<tr>
<td>†Enterobacteria</td>
<td>12:2</td>
</tr>
<tr>
<td>†Anaerobic flora</td>
<td>21:0</td>
</tr>
</tbody>
</table>

*Includes staphylococcus and micrococcus.
†Includes Escherichia, Proteus-Providencia, Klebsiella-Aerobacter groups.
‡Includes anaerobic streptococci, Veillonella, Bacteroides, Clostridium.

Specimens from the two negative clinical cat-
egories showed no significant difference in the dis-
tribution of floral gradings, nor in the frequency of
occurrence of individual members of the vaginal
bacterial flora. In particular, the incidence of
P.P.L.O. and of *Haemophilus vaginalis* was very
similar. The bacterial flora of specimens associated
with clinically positive cases showed wide differences
from the two preceding groups. The striking morpho-
logical differences were reflected in the cultural
findings. Thus, lactobacilli were cultured from only
52% of these specimens, while the frequency of iso-
lation of *Haemophilus vaginalis* (28-0%), and of
P.P.L.O. (32-6%) was significantly higher than in the
two non-trichomonal clinical categories.

When the microbical flora were considered in rela-
tion to the degree of protozoal infestation, marked
differences of incidence were noted. The frequency
of grade I bacterial flora declined steeply from 57% in
specimens devoid of protozoa to only 6-4% in
specimens showing numerous protozoa. On culture,
the most impressive variations of incidence were
shown by the aerobic streptococci and the myco-
plasmas. A good correlation was noted between the
frequency of streptococci (predominantly non-
haemolytic strains) and increasing degrees of pro-
protozoal infestation, rising from 12-8% in specimens
devoid of trichomonads, to 64-5% in heavily
infested specimens. The incidence of mycoplasmas rose
progressively from 10-5% in trichomonad-negative
specimens to 59-0% in specimens containing many
protozoa. Less striking differences were noted in the
frequency of isolation of other micro-organisms. The
incidence of *Haemophilus vaginalis* was higher
(25-8%) in heavily infested vaginal exudates than in
uninfested exudates. The strictly anaerobic vaginal
species and members of the Enterobacteriaceae were
also present in an appreciably higher percentage of
the *Trichomonas vaginalis*-infested specimens. Can-
dida species were infrequently detected in heavily
infested exudates.

A comparison of trichomonal infestation and of
the microbical flora of specimens with cytological
evidence of mild or moderate inflammation with that
of specimens showing a severe inflammatory reaction
(with or without nuclear atypia) is shown in Table
III. A comparison of the two categories revealed
significant differences in the distribution both of
*Trichomonas vaginalis* and of bacterial floral grades.

On culture, a significantly higher incidence of
mycoplasmas, non-haemolytic aerobic streptococci,
and of *Haemophilus vaginalis* was found in the severe
inflammatory group. The frequency of occurrence of
**TABLE III**

**MICROBIAL FLORA ASSOCIATED WITH VARYING DEGREES OF VAGINAL EPITHELIAL INFLAMMATION**

<table>
<thead>
<tr>
<th>Microbial Flora</th>
<th>Papanicolaou Smear</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mild or Moderate Inflammation (%)</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>47.8</td>
</tr>
<tr>
<td>Grade I</td>
<td>43.2</td>
</tr>
<tr>
<td>Grade II</td>
<td>36.5</td>
</tr>
<tr>
<td>Grade III</td>
<td>20.3</td>
</tr>
<tr>
<td>Mycoplasma species</td>
<td>15.5</td>
</tr>
<tr>
<td>Haemophilus vaginalis</td>
<td>10.2</td>
</tr>
<tr>
<td>Aerobic streptococci</td>
<td>20.2</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>80.4</td>
</tr>
<tr>
<td>Corynebacteria</td>
<td>46.7</td>
</tr>
<tr>
<td>Candida species</td>
<td>9.4</td>
</tr>
<tr>
<td>Acinetobacter species</td>
<td>1.0</td>
</tr>
<tr>
<td>Neisseria species</td>
<td>0.3</td>
</tr>
<tr>
<td>Micrococi</td>
<td>7.1</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>12.7</td>
</tr>
<tr>
<td>Anaerobic flora</td>
<td>21.6</td>
</tr>
</tbody>
</table>

*Candida albicans* was low in both categories, but particularly insignificant (4.2%) in the specimens showing severe inflammation.

The incidence of trichomonal infestation and the bacterial flora of eroded and non-eroded cervices is shown in Table IV. The distribution both of *Trichomonas vaginalis* and of the floral gradings was very similar in both groups. Although mycoplasmas and *Haemophilus vaginalis* were isolated from a higher percentage of eroded cervices than those not eroded, these differences are not statistically significant. No significant differences were observed between the two categories with respect to the remainder of the vaginal bacterial flora.

**TABLE IV**

**MICROBIAL FLORA ASSOCIATED WITH EROSIONS OF THE CERVIX**

<table>
<thead>
<tr>
<th>Microbial Flora</th>
<th>Erosions + (%)</th>
<th>Erosions - (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trichomonas vaginalis</td>
<td>50.3</td>
<td>58.0</td>
</tr>
<tr>
<td>Grade I</td>
<td>32.3</td>
<td>38.8</td>
</tr>
<tr>
<td>Grade II</td>
<td>43.4</td>
<td>33.5</td>
</tr>
<tr>
<td>Grade III</td>
<td>24.3</td>
<td>27.7</td>
</tr>
<tr>
<td>Mycoplasma species</td>
<td>26.2</td>
<td>17.7</td>
</tr>
<tr>
<td><em>Haemophilus vaginalis</em></td>
<td>17.2</td>
<td>12.5</td>
</tr>
<tr>
<td>Aerobic streptococci</td>
<td>27.3</td>
<td>24.0</td>
</tr>
<tr>
<td>Lactobacilli</td>
<td>75.7</td>
<td>72.3</td>
</tr>
<tr>
<td>Corynebacteria</td>
<td>41.3</td>
<td>30.9</td>
</tr>
<tr>
<td>Candida species</td>
<td>7.1</td>
<td>10.1</td>
</tr>
<tr>
<td>Acinetobacter species</td>
<td>2.9</td>
<td>2.1</td>
</tr>
<tr>
<td>Neisseria species</td>
<td>0.0</td>
<td>0.0</td>
</tr>
<tr>
<td>Micrococi</td>
<td>10.4</td>
<td>12.8</td>
</tr>
<tr>
<td>Enterobacteria</td>
<td>7.1</td>
<td>11.8</td>
</tr>
<tr>
<td>Anaerobic flora</td>
<td>27.1</td>
<td>24.3</td>
</tr>
</tbody>
</table>

**DISCUSSION**

In this study, the varied nature of the vaginal bacterial flora in the presence of protozoal infestation and of severe vaginal epithelial inflammation is in good agreement with generally accepted views that the almost exclusively aciduric Döderlein flora of the healthy vagina is largely replaced by a mixed flora in the presence of vaginitis. The strikingly low incidence of grade I (Döderlein) flora in smears showing cytological evidence of severe inflammation was associated, on culture, with a much more varied aerobic and anaerobic flora. In particular, significantly higher incidences of mycoplasma, aerobic streptococci, and of *Haemophilus vaginalis* were seen in the severe inflammatory category, while obligatory anaerobic streptococci, bacteroides spp., and members of the Enterobacteriaceae were isolated more frequently in the presence of severe inflammation. A similar pattern of variation was observed if the frequency of occurrence of these micro-organisms was correlated with the degree of *Trichomonas vaginalis* infestation. It is, of course, well known that vaginal lactobacilli bear a more or less inverse relationship to the degree of trichomonal infestation of the vagina, and this is well illustrated by the results of the morphological floral gradings, only 6.4% of smears containing numerous trichomonads being classified as grade I. The most impressive variations were seen in the incidence of the mycoplasma group and of the aerobic streptococci. Fifty-nine per cent of heavily infested exudates yielded mycoplasmas, but only 10.5% of non-infested exudates yielded these organisms. The incidence of the aerobic streptococci in specimens containing large numbers of protozoa was approximately five times that observed in non-infested specimens.

It is tempting to attach some special significance to the association of these organisms with trichomonal infestation, and to suggest some contributory role in the production of severe inflammation, but it seems likely that their more frequent occurrence in these circumstances merely reflects a more favourable environment for their proliferation. It is well documented that the growth of mycoplasmas in the vagina is greatly favoured by a shift of vaginal pH from the normal acid range of 4.0 to 4.5 towards alkalinity (Freundt, 1958; Bercovici, Persky, Rozansky, and Razin, 1962), while streptococci and 'coliform' organisms grow well above pH 6. Such conditions pertain in trichomonal infection, and, furthermore, tissue necrosis associated with the inflammatory reaction favours the growth of most of these bacteria, especially the microaerophilic and anaerobic species. It is probable, therefore, that the presence of these organisms, normally commensals of the female genital tract, is entirely secondary to the protozoal infestation. Notwithstanding, the mycoplasma group and *Haemophilus vaginalis* merit special discussion in view of the conflicting views expressed regarding their pathogenic role in genital tract pathology.
Although the first isolation of a P.P.L.O. from the human genito-urinary tract was from a Bartholin's abscess (Dienes and Edsall, 1937), it is still not clear whether these organisms play a significant pathogenic role in genito-urinary diseases. An extensive and often conflicting literature has developed during the last 20 years, and the subject has been widely reviewed by Edward (1954), Klieieberger-Nobel (1962), and recently by Hayflick and Chanock (1965). Occasionally P.P.L.O. have been found in abundance in inflammatory and pyogenic lesions of the genital tract. Among these have been Bartholin’s gland abscesses, pelvic abscesses associated with purpural infection or with salpingitis (Dienes, Ropes, Smith, Madoff, and Bauer, 1948), and tubo-ovarian abscesses (Randall, Stein, and Ayres, 1950). Slingerland and Morgan (1952) isolated P.P.L.O. from the blood and cervix of a woman with a post-partum febrile illness, and Stokes (1955) has reported a similar case of purpural sepsis. Certainly, the increased frequency of P.P.L.O. in association with inflammatory diseases of the genital tract, especially trichomonal and gonococcal infections, is well documented (Randall et al., 1950; Freundt, 1953; Nicol and Edward, 1953; Somerson, Rubin, Smith, and Morton, 1955; Klieieberger-Nobel, 1959; Bercovici et al., 1962), the incidence often reaching 70% or 80% in these circumstances. These findings have been amply confirmed in the present investigation, almost 60% of the heavily infested exudates yielding a mycoplasma species.

The evidence regarding the pathogenicity of the small Gram-negative bacillus designated 'Haemophilus vaginalis' by Gardner and Dukes (1955) is equally conflicting. Furthermore, the taxonomic status of this organism is very doubtful. Thus Zinnemann and Turner (1963) have proposed that this organism more properly belongs to the genus Corynebacterium, although Amies and Garabedian (1963) believe that Haemophilus vaginalis is a dissociated form of certain lactobacillus strains. Several authors, notably Gardner and Dukes (1955, 1959), and Gardner, Damper, and Dukes (1957), Ray and Maughan (1956), and Brewer, Halpern, and Thomas (1957), have contended that this organism is the principal aetiological agent in cases of 'non-specific' bacterial vaginitis. Edmunds (1959) found an association between Haemophilus vaginalis and the presence of leucorrhea or of a post-partum pyrexia, and suggested that this was the principal organism concerned in cases of so-called ‘mixed bacterial’ infections of the vagina. In contrast, several other authors (Heltai and Taleghany, 1959; Frampton and Lee, 1964; Robinson and Mirchandani, 1965) have not found any definite association between Haemophilus vaginalis and lower genital tract pathology.

Although, in the present investigation, Haemophilus vaginalis was more frequently observed in the presence of Trichomonas vaginalis infestation, a low incidence of the organism was found in the clinically non-trichomonal categories, and in no case was the organism present as a predominant constituent of the vaginal flora. Thus, no evidence was obtained that Haemophilus vaginalis itself was responsible for the non-trichomonal vaginal discharges.

With regard to cervical erosions, no significant differences in the distribution of bacterial flora of specimens from eroded and non-eroded cervixes was found, though it is of interest to note the slightly higher incidence of mycoplasmas in the eroded group. This difference is not, however, statistically significant. These essentially normal bacteriological findings are quite consistent with the view that erosions of the cervix per se are not associated with gross disturbances of vaginal pH nor with marked inflammation in the vagina.

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REFERENCES

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