Comparison of culture and microscopy in the diagnosis of *Gardnerella vaginalis* infection

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**SUMMARY** A comparison was made between human blood agar containing amphotericin B, nalidixic acid and either gentamicin or colistin for the isolation of *Gardnerella vaginalis* from cases of non-specific vaginitis seen in a clinic for sexually transmitted diseases. The medium containing gentamicin was more inhibitory for non-*Gardnerella* species, but not sufficiently inhibitory to allow direct plating in the clinic without spreading for single colonies. The diffuse beta haemolysis produced by *G vaginalis* on human, but not on horse blood agar, proved very useful in differentiating it from other vaginal organisms and was not affected by the antibiotics used. This characteristic, together with Gram stain morphology, oxidase and catalase, provides a simple, reliable method of identifying *G vaginalis*. Sixty women with symptoms of vaginitis, in whom no other pathogen was isolated, were examined by culture and microscopy. *Gardnerella vaginalis* was grown from 45 whereas only 31 had positive microscopy (clue cells or Gram-variable bacilli). There was no significant difference between the rate of isolation of *G vaginalis* in the group with positive microscopy (25/31) and that with negative microscopy (20/31).

Non-specific vaginitis is a common condition characterised by an increased vaginal discharge, often foul smelling, which is not attributable to uterine infection, *Trichomonas vaginalis* or candidal infection. *Gardnerella vaginalis* has been closely associated with this condition. The isolation and identification of *G vaginalis* in the routine laboratory can be both time-consuming and difficult. Diagnosis is therefore most usually made on the basis of the foul smelling discharge, on the liberation of amines with a fishy odour when 10% KOH is added to it, and on the characteristic microscopical appearance of the wet preparation and of Gram-stained smears of the discharge. Some workers have, however, found a poor correlation between the isolation of *G vaginalis* and the microscopical appearance of the discharge and any detailed study of the aetiology and epidemiology of the condition needs a good, preferably simple, cultural basis.

*Gardnerella vaginalis* is a slow-growing organism which, on ordinary media, is difficult to differentiate from other vaginal bacteria. Its taxonomy has been unsatisfactory, having been previously classified both as a Haemophilus and a Corynebacterium. *Gardnerella vaginalis* produces a characteristic morphology on Dunkelberg's medium, but the differential haemolysis described by Greenwood et al. (beta haemolysis on human blood, but not on sheep blood) and the development of a selective medium using this principle probably gives a better basis for large scale clinical work.

Our aim was to design a medium with increased selectivity and to use this medium to compare the isolation of *G vaginalis* with the presence of "clue cells" and/or Gram-variable rods by microscopy, in patients with non-specific vaginitis. This was achieved, firstly, by determining the minimal inhibitory concentration (MIC) of the antibiotics vancomycin, colistin, gentamicin, sulphonamide, trimethoprim and nalidixic acid for five strains of *G vaginalis*. Subsequently, four combinations of antibiotics were tested against strains of *G vaginalis* isolated in our laboratory and some closely related bacteria. Two antibiotic combinations were particularly good at selecting *G vaginalis*: amphotericin, nalidixic acid and colistin; amphotericin, nalidixic acid and gentamicin. These were compared using samples from women attending the Praed Street Clinic for sexually transmitted diseases (STD) using both direct plating in the clinic and swabs sent to the laboratory in Amies' transport medium.
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Material and methods

MEDIA
Peptone starch dextrose agar⁵ or double layer human blood agar⁸ was used. Antibiotics were added to agar, which had been cooled to 50°C, at the appropriate concentration. After inoculation, both media were incubated in 7% CO₂ at 36°C for 48 hours.

BACTERIA
Gardnerella vaginalis, NCTC 10287 and NCTC 10915 were used as control strains. Other strains were isolated from clinical specimens received either from the gynaecological outpatients or the STD clinic. Corynebacterium xerosis, NCTC 8481, Corynebacterium cervicis, NCTC 10604, Corynebacterium hofmanii, NCTC 231, and Lactobacillus acidophilus, NCTC 2949 were chosen as closely related bacteria which may be present in the vagina.

ANTIBIOTIC SENSITIVITY TESTING
The MIC was determined using vancomycin in the range 0-5-8-0 mg/l; trimethoprim 0-06-4-00 mg/l; sulphonamide 2-0-256-0 mg/l; gentamicin 2-0-32-0 mg/l; colistin 0-12-8-0 mg/l; nalidixic acid 1-0-256-0 mg/l. All antibiotics were tested in the presence and absence of amphotericin at 2-0 mg/l. Bacteria were grown on human blood agar, without antibiotics, in 7% CO₂ at 36°C for 48 h. The growth was scraped from the plates and resuspended in saline. The suspension was adjusted to an absorbance of 1.0 (λ = 540 nm) and then further diluted 1/100. This represents approximately 10⁹ organisms/cm³ and 10⁷ organisms/cm³ respectively. Bacterial suspensions were inoculated on to duplicate plates using a multipoint inoculator (Denley) delivering 1 µl volumes. Both suspensions were tested with each antibiotic, this resulting in a final inoculum of 10⁶ and 10⁴ organisms.

SPECIMENS
Two vaginal swabs were taken from patients attending the STD clinic with symptomatic vaginitis of no known microbial cause, including Candida spp, Trichomonas vaginalis, and Neisseria gonorrhoeae. None of the patients had received any antimicrobial therapy within the last month.

One swab was transported in Amies’ medium to the laboratory and cultured on the day of collection on human blood agar containing two combinations of antibiotics. This swab was inoculated on to the plate and then spread, using a loop, for discrete colonies.

The remaining swab was cultured directly on to the plate in the clinic without subsequent spreading and stored at 36°C in 7% CO₂ until transported to the laboratory.

MICROSCOPY
A separate swab was taken, from which a Gram stain and a wet mount in saline was made. This was examined by a number of clinic staff as part of their normal routine. No special microscopical examination was undertaken as part of the study. The Gram stain was examined for large numbers of Gram-variable rods, and the wet mount for the presence of “clue” cells. If either was positive this was taken as an indication that G vaginalis was present.

IDENTIFICATION
Any Gram-variable rods, which showed beta haemolysis on human but not horse blood agar were screened. All G vaginalis isolates were confirmed by being oxidase- and catalase-negative, and by their ability to ferment starch, maltose and dextrose. The fermentation media were prepared as described by Bailey et al., but were poured into Petri dishes rather than bottles or tubes. Two representative clinical isolates were confirmed as G vaginalis by the National Collection of Type Cultures, Colindale.

Results

ANTIBIOTIC SUSCEPTIBILITY OF G VAGINALIS
The MIC of six antibiotics for five strains of G vaginalis is shown in Table 1. Each strain was tested and the results are summarized in Table 2.

Table 1 Minimal inhibitory concentration (mg/l) for five strains of G vaginalis

<table>
<thead>
<tr>
<th>Antibiotics (mg/l)</th>
<th>G vaginalis strains</th>
<th>Clinical isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>NCTC 10287</td>
<td>NCTC 10915</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>ND</td>
<td>4.0</td>
</tr>
<tr>
<td>Sulphonamide</td>
<td>&gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>4.0</td>
<td>8.0</td>
</tr>
<tr>
<td>Colistin</td>
<td>&gt; 8.0</td>
<td>&gt; 8.0</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>128</td>
<td>128</td>
</tr>
</tbody>
</table>

ND = not tested.

in the presence and absence of amphotericin B (2-0 mg/l). All strains of G vaginalis were resistant to this concentration of amphotericin B, and its presence in the medium had no effect on the MIC of the other agents. From these results the effect of gentamicin, colistin, sulphonamide and nalidixic acid used in varying combinations on the growth of G vaginalis, C xerosis, C cervicis, C hofmanii and L acidophilus, was assessed (Table 2). The presence of the sul-
Table 2 Growth on human blood agar (10^6 organisms) containing different combinations of antibiotics (for concentrations see Table 1)

<table>
<thead>
<tr>
<th>Bacterial strains</th>
<th>Combinations of antibiotics</th>
</tr>
</thead>
<tbody>
<tr>
<td>G vaginalis</td>
<td>+ + +</td>
</tr>
<tr>
<td>NCTC 10287</td>
<td>+ + +</td>
</tr>
<tr>
<td>NCTC 10915</td>
<td>+ + +</td>
</tr>
<tr>
<td>Clinical isolates</td>
<td>+ + +</td>
</tr>
<tr>
<td>19907</td>
<td>+ + +</td>
</tr>
<tr>
<td>19812</td>
<td>+ + +</td>
</tr>
<tr>
<td>H65128</td>
<td>+ + +</td>
</tr>
<tr>
<td>C albicans</td>
<td>+ + +</td>
</tr>
<tr>
<td>C hofmanii</td>
<td>+ + +</td>
</tr>
<tr>
<td>L acidophilus</td>
<td>+ + +</td>
</tr>
<tr>
<td>C xerosis</td>
<td>+</td>
</tr>
</tbody>
</table>

| C | colistin 10-0 mg/l. | N | nalidixic acid 30-0 mg/l. | S | sulphonamide 500 mg/l. | A | amphotericin 2-0 mg/l. | G | gentamicin 2-0 mg/l. |

Phenamid showed no discriminatory effect, possibly due to its inactivation in the presence of blood. Gentamicin at a concentration of 2-0 mg/l appeared to be more inhibitory than colistin for organisms other than G vaginalis. Despite the MIC results, raising the concentration of gentamicin to 4-0 mg/l had no effect on the isolation of G vaginalis from clinical material. Despite the sensitivity of some strains of G vaginalis to > 2-0 mg/l colistin it was decided to use this antibiotic at the higher concentration of 10-0 mg/l as described by Spiegel et al.8

Comparison of Selective Media

Based on these studies two selective human blood agars were chosen for comparative studies on the isolation of G vaginalis from clinical samples. Both contained amphotericin B (2-0 mg/l) and nalidixic acid (30-0 mg/l). One contained, in addition, gentamicin (4-0 mg/l) the other, colistin (10-0 mg/l). Fifty-five vaginal swabs were cultured on both media, both directly in the clinic without further plating, and in the laboratory, plating out for single colonies. Neither medium was sufficiently inhibitory to enable satisfactory subcultures of G vaginalis to be made easily from the plates inoculated in the clinic, although a presumptive identification of G vaginalis could be made from these plates. The isolation rate of G vaginalis was the same on both media, but the presence of gentamicin inhibited the non-Gardnerella species to a greater extent. The characteristic diffuse beta haemolysis of G vaginalis was not affected by either gentamicin or colistin, although successive subculturing improved both the size of the colonies and the degree of haemolysis on both media. Both media inhibited the growth of Proteus sp. All catalase- and oxidase-negative Gram-variable bacilli which showed differential beta haemolysis on human and horse blood were identified biochemically as G vaginalis.

Correlation Between Microscopy and Isolation of G vaginalis

Vaginal swabs from 62 women with non-specific vaginitis were examined by microscopy and culture. Gardnerella vaginalis was isolated from 45 (74%). In only 31 women, however, were there positive microscopical findings (clue cells, Gram-variable bacilli, or both). Twenty-five of this group of 31 were culture-positive as compared with 20 of the group of 31 with negative microscopy (Table 3). The correlation between microscopy and culture was not statistically significant. Although no strict quantitative count of G vaginalis was made there was no gross difference between the growth of G vaginalis in these two groups.

Table 3 Correlation of isolation of G vaginalis with presence of "clue cells," Gram-variable rods, or both in 62 patients with vaginitis $\chi^2 = 2-0 - NS$

<table>
<thead>
<tr>
<th>Microscopy</th>
<th>Isolation of G vaginalis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive</td>
<td>%</td>
</tr>
<tr>
<td>Positive</td>
<td>25</td>
</tr>
<tr>
<td>Negative</td>
<td>20</td>
</tr>
</tbody>
</table>

Discussion

The role of G vaginalis in non-specific vaginitis has been a matter of some controversy. Gardner and Dukes1 isolated this organism, often in pure culture, from cases of vaginitis which were associated with a
foul-smelling discharge and the presence of "clue" cells. They found further evidence for its casual role in this condition by inoculating volunteers with material from the vaginas of women with this infection and reproducing the signs and symptoms. Others have also found a strong relation between the isolation of \textit{G} \textit{vaginalis} and vaginitis, where other recognised pathogens (\textit{Trichomonas vaginalis}, \textit{Candida} \textit{spp}, \textit{Neisseria gonorrhoeae}) were not found. An association between \textit{G} \textit{vaginalis} and both \textit{Mycoplasma hominis} and anaerobes was noted by Pfeifer \textit{et al.} although a clinical response to therapy was unrelated to the persistent isolation of \textit{M} \textit{hominis}. Against this is the fact that \textit{G} \textit{vaginalis} can be a normal inhabitant of the vagina and neither Levison \textit{et al.}, nor McCormack \textit{et al.} found any association between \textit{G} \textit{vaginalis} and non-specific vaginitis.

These apparent discrepancies may be the result of different clinical definitions of vaginitis and bacteriological techniques. \textit{Gardnerella vaginalis} is a slow-growing organism which, on ordinary media, is difficult to differentiate from other vaginal organisms, particularly coryneforms. Dunkelberg has described a protocol for its isolation and identification, but this is time-consuming. Bailey \textit{et al.} explored the possibility of using API-20A, Minitek and rapid-buffered substrate fermentation (RFT) systems and compared them to conventional tests. Results, using 32 strains, showed API and Minitek to give variable and unreliable results, although RFT was comparable with conventional tests. Greenwood confirmed this, using 63 strains and also noted that \textit{G} \textit{vaginalis} produced a characteristic diffuse, beta haemolysis on human, but not sheep blood agar. This was confirmed by two other studies. This haemolysis together with catalase and oxidase tests differentiates \textit{G} \textit{vaginalis}-like organisms from \textit{G} \textit{vaginalis}, the latter being catalase- and oxidase-negative. The fermentation of starch, maltose and dextrose has been used to differentiate \textit{G} \textit{vaginalis} from coryneforms which ferment starch alone or are non-fermentative. However, different groups have found dextrose fermentation to be variable.

We have confirmed the unusual differential beta haemolysis of \textit{G} \textit{vaginalis} substituting horse for sheep blood, and have used this in combination with oxidase, catalase and carbohydrate fermentation to identify our strains. The human blood agar medium has also been used in selective form. In our study, although human blood agar with amphotericin B, nalidixic acid and colistin worked well as a primary isolation medium, gentamicin was more effective than colistin in suppressing other vaginal organisms. This was not surprising considering the broader spectrum of activity of this aminoglycoside, particularly against Gram-positive organisms. More importantly, the use of this antibiotic combination did not affect the production of beta haemolysis by \textit{G} \textit{vaginalis}. The quality of the haemolysis was, however, dependent on the age of the blood, that which was just time-expired being best.

The antibiotic sensitivity pattern of the corynebacteria and lactobacilli which can be confused with \textit{G} \textit{vaginalis} in vaginal discharges, suggested that the development of a fully selective medium, which would allow the subculture of \textit{G} \textit{vaginalis} from plates inoculated in the clinic without any attempt to plate out for single colonies, would not be possible. This turned out to be so, although the presumptive identification of \textit{G} \textit{vaginalis}, based on colonial morphology, haemolysis and Gram staining, could be made from these plates. However, the use of swabs and transport media, rather than direct plating, did not result in any failure to isolate \textit{G} \textit{vaginalis}.

Because of the relative difficulty of isolating \textit{G} \textit{vaginalis}, a presumptive diagnosis is often made on the microscopy of wet and Gram-stained preparations of vaginal discharge, looking for "clue" cells and short Gram-negative bacilli. Gardner and Dukes found a close correlation between microscopy and culture as did Balsdon \textit{et al.} and Pfeifer \textit{et al.} Several groups have, however, failed to find this association. Although 25/31 (80\%) of patients with symptoms and positive microscopy had \textit{G} \textit{vaginalis} on culture, so did 20/31 (65\%) of symptomatic patients with negative microscopy. If microscopy had been the sole diagnostic laboratory feature 14 of the 62 symptomatic cases would have been missed. The standard of microscopy in the clinic was not evaluated and it is possible that if special attention had been paid to this, more women would have been found to be positive. It is unlikely, however, that the standard of microscopy in this clinic differs markedly from that in any similar large busy clinic. This is the standard against which any new diagnostic test for \textit{G} \textit{vaginalis} must be assessed.

Even allowing for the fact that microscopy might be made more sensitive, our overall isolation of \textit{G} \textit{vaginalis} from these 62 patients was, at 74\%, much higher than the 50\% in whom we found positive microscopy. Without a clinical and microbiological survey of the response of the patients to antimicrobial treatment it is impossible to compare the significance of \textit{G} \textit{vaginalis} when isolated from symptomatic patients with and without significant microscopy. The amine test was not evaluated and this, taken in conjunction with microscopy, may have increased the numbers in whom findings suggestive of \textit{G} \textit{vaginalis} infection were associated.
with the actual isolation of this organism. A strict quantitative comparison of the numbers of *G. vaginalis* from the two groups of patients was not made, but there was no obvious difference between the growth of *G. vaginalis* in patients with and without positive microscopy.

Non-specific vaginitis may result from the combination of *G. vaginalis* and other organisms, notably anaerobes. If this is the case the isolation of *G. vaginalis* alone might not correspond to the presence of either symptoms or significant microscopical findings. We are now extending our study to see whether the isolation of *G. vaginalis* together with obligate anaerobes, both assessed quantitatively, gives a closer correlation with significant microscopy than the isolation of *G. vaginalis* alone.

We thank Dr JRW Harris for permission to study patients under his care.

References


Requests for reprints to: Dr CSF Easmon, Department of Bacteriology, St Mary's Hospital Medical School, London W2 1PG, England.
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doi: 10.1136/jcp.35.5.550

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