Rapid identification of \textit{Corynebacterium vaginale} in non-purulent vaginitis

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SUMMARY A simple set of tests is proposed to give excellent probability for the identification of \textit{Corynebacterium vaginale} from clinical material. Using these tests, 380 \textit{C vaginale} were isolated from genital tract specimens from 1402 patients. Of these isolates 70 were from symptomatic patients. These 70 isolates were subjected to a further set of tests to confirm their identity. The advantage of these primary tests is that they can be completed on the day of isolation of the organism. Of these 70 isolates 66 were confirmed as \textit{C vaginale} thus giving the primary set of tests a 94\% rate of accurate identification. However this rate may be increased beyond 97\% by the promotion of one of the key secondary tests to the primary set.

Many reports published over the last decade have attached significance to \textit{C vaginale} as a pathogen in vaginitis. Although some doubt still exists, there is a growing acceptance of its role in gynaecological disease. The taxonomic status of \textit{C vaginale} continues to be unsettled, hampered largely by laboratory difficulties in its isolation and identification. Nevertheless some workers have recently proposed a new genus, Gardnerella, for the organism.\textsuperscript{1} Procedures, proposed from time to time,\textsuperscript{2,3} are often beyond acceptance to routine service laboratories, either because of their complexity or the length of time required for adequate identification.

As the prevalence of non-purulent vaginitis (NPV) \textsuperscript{vide infra}, is high, being second only to vaginal candidiasis in frequency of occurrence, there is a need for a rapid method of identification to assist treatment of the disorder. A report recommending tests for the presumptive identification of \textit{C vaginale}, suffers from the disadvantage of requiring more incubation time after isolation to complete identification.\textsuperscript{3} Our paper records a set of simple tests that can be completed on the day of isolation without a need for further subculture. This recommended battery of rapid, simple tests have an accuracy of at least 97\% from our study.

Material and methods

\textbf{STUDY GROUP}

A total of 1402 consecutive, unselected women attending the gynaecological clinics of King Edward Memorial Hospital for Women in Perth between May, 1979 and March, 1980 inclusive were studied. Using an unlubricated vaginal speculum to expose the vaginal vault, three swabs were collected from each patient. The clinical history was obtained at interviews or by postal questionnaire if the patient changed address.

\textbf{CULTURAL METHODS}

Specimens collected from these patients were distributed as 66\% high vaginal and 34\% endocervical swabs. The materials from each patient was apportioned as follows:

1 A buffered charcoal swab in Stuart’s transport medium was plated on to Thayer-Martin agar (T-M); chocolate agar (Choc agar) which is Thayer-Martin agar without antibiotics; prereduced lysed blood agar containing Columbia agar, palladium chloride, gentamicin, menadione, cysteine and lysed horse blood (BA + GPC); modified peptone starch dextrose agar (PSD) of Dunkelberg\textsuperscript{4} and then was inoculated into Feinberg-Wittington (FW) broth. The T-M Agar and Choc agar were incubated at 36°C for 48 h under 5\% CO\textsubscript{2} for the isolation of \textit{Neisseria gonorrhoeae}. The BA + GPC agar was incubated at 36°C for 48 h anaerobically (BBL Gas-Pak system) for the isolation of anaerobic pathogens.

The PSD agar was incubated at 36°C for 48 h anaerobically (BBL Gas-Pak system) for the isolation of \textit{C vaginale}. The F-W broth was incubated at 36°C for 48 h for the isolation of \textit{Trichomonas vaginalis}.

2 Two smears, on microscope slides, were made...
from a buffered plain cotton wool swab for a Gram-stain with methanol fixation and acetone
decolourisation. Methanol fixation is important for the cytological preservation of smears from the
gene tract.

3 The third plain cotton swab was inoculated on to horse blood agar, plated out and incubated at 36°C
for 24 h aerobically. This plate permitted the isolation of candida and other aerobic pathogens. Candida
can also be isolated from the T-M and Choc agar plates.

**MEDIA USED**

The T-M Choc, BA + GPC, F-W and horse blood
media were all standard preparations. The modified
PSD agar had the following formula: Proteose peptone No 3 (DIFCO) 2%; maize starch (AJAX)
1%; dextrose 0.2%; Na₂HPO₄ 0.2%; NaH₂PO₄.
2H₂O 0.2%; agar 1.5%; and pH to 6.8. Insoluble
maize starch was used to show the clear zones of starch hydrolysis around the colonies and the
concentration of sodium phosphate salts was doubled to enhance metaphosphate (volutin) granule
formation.

**IDENTIFICATION OF ISOLATES**

Accepted techniques and criteria were used to identify *Neisseria gonorrhoeae, Trichomonas vagi-
alis, Candida albicans* and candida—related yeasts.

Identification of *C. vaginale* was carried out in two
steps. From the PSD agar plate a primary set of
tests were carried out on the day of isolation.

Cultures which display appropriate colonial morphol-
y and starch hydrolysis were Gram-stained,
Albert-stained, and tested for catalase production.

Isolates with white colonies showing slightly
darkened centres and starch hydrolysis, that were
Gram-variable diphtheroid bacilli, that produced
metaphosphate granules, that were catalase-negative,
were recorded as *C. vaginale*. Two reference strains of
*C. vaginale*, ATCC 14018 (Gardner & Dukes strain
594) and ATCC 14019 serve as control organisms
and were subjected to these primary tests as required
(Table 1).

From the 1402 patients, *C. vaginale* was isolated on
380 occasions. Of these 380 patients, 70 were
symptomatic, with vaginal discharge and these
isolates were reported as pathogens. Arbitrarily
these 70 isolates were subcultured on to PSD again
and then lyophilised before further testing.

The secondary set of tests were used to confirm the
presumptive identification of *C. vaginale* from the
primary set of tests. The secondary tests include
hippurate hydrolysis, which uses the rapid method
of Hwang, β-haemolysis on human blood agar
(HBA), lack of haemolysis on sheep blood agar

Sensitivity to metronidazole at 50 µg/disc,
resistance to sulphonamides at 1.5 mg/disc,
growth inhibition by weak bacitracin (TAXO A discs,
DIFCO), and growth inhibition by *Streptococcus
gangius*. The two reference strains were used as
controls.

A comparison was also made for growth under
anaerobic conditions as against 5% CO₂ on PSD
agar plates.

**Results**

When specimens from 1402 patients were tested by
the primary set of tests, *C. vaginale* was isolated on
380 occasions, which is a prevalence rate of 27%.

Of these 380 patients with positive cultures, 70 had
symptoms and no other vaginal pathogens isolated.
The symptoms of these 70 patients were suggestive of
a *C. vaginale* infection, and they also had discharge
smears showing the typical appearance of a *C
vaginale* infection.

These 70 isolates were then subjected to the secondary set of tests to confirm the preliminary
identification. The results are shown in Table 2.

**Table 1 Primary tests results of *C. vaginale* reference
strains (controls)**

<table>
<thead>
<tr>
<th>Test</th>
<th>ATCC 14018</th>
<th>ATCC 14019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colonial morphology</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Starch hydrolysis</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Gram-stain</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Granules</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Catalase test</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

+ = characteristic present.
- = characteristic absent.

(SBA), sensitivity to metronidazole at 50 µg/disc,
resistance to sulphonamides at 1.5 mg/disc,
growth inhibition by weak bacitracin (TAXO A discs,
DIFCO), and growth inhibition by *Streptococcus
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**Table 2 Results of the secondary tests on the 70
isolates identified as *C. vaginale* by the primary tests**

<table>
<thead>
<tr>
<th>Test</th>
<th>Positive</th>
<th>Negative</th>
<th>ATCC 14018</th>
<th>ATCC 14019</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hippurate hydrolysis</td>
<td>64</td>
<td>6</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>β-haemolysis on HBA</td>
<td>69</td>
<td>1</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Haemolysis on SBA</td>
<td>3</td>
<td>67</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Inhibition by Bacitracin</td>
<td>66</td>
<td>4</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Inhibition by <em>Strep sanguis</em></td>
<td>70</td>
<td>0</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Metronidazole 50 µg/disc</td>
<td>51S</td>
<td>19R</td>
<td>S</td>
<td>S</td>
</tr>
<tr>
<td>Sulphamamide 1.5 mg/disc</td>
<td>0S</td>
<td>70R</td>
<td>R</td>
<td>R</td>
</tr>
</tbody>
</table>

S = sensitive.
R = resistant.

Four of the 70 isolates identified by the primary
tests were not confirmed as *C. vaginale*. This error of
four instances in 70 identifications corresponds to an
accuracy of 94%.

These four isolates may be the *C. vaginale*-like
bacteria reported by Bailey. All four of these isolates did not hydrolyse hippurate. In reviewing the primary and secondary sets of tests for presumptive identification of C. vaginale, the hippurate hydrolysis test is considered to be quite specific for C. vaginale. We found 64 of 66 isolates positive (97%). This test can be utilised as a primary test on the day of isolation to increase the accuracy of the primary tests and it would give excellent probability of correct presumptive identification of suspected isolates.

When the growth on PSD agar of these 70 isolates incubated anaerobically and under CO2, were compared, only 49 (70%) isolates grew under CO2, whereas all isolates grew under anaerobic conditions. Clearly anaerobiosis is preferred for the isolation of C. vaginale.

Gram-stains of the isolates from PSD agar again invariably show Gram-variable to Gram-negative diphtheroid bacilli. Confusion with other Gram-variable to Gram-positive bacilli isolated from the genital tract, can be eliminated by reference to the other primary test results.

The presence of "clue" cells in the discharge smears was not a constant finding. Clue cells, which are vaginal squames covered with numerous adherent coccobacilli, were present in approximately one in five cases and we do not consider their presence a requisite in the microscopic diagnosis of a typical C. vaginale infection. The appearance of the Gram-stained discharge smears were consistent in all of the 70 cases with discharge. They all showed a few or no leucocytes, many epithelial cells and masses of Gram-variable coccobacilli giving the "pepper and salt" appearance described by Balsdon et al. The consistent absence or scantiness of pus cells in an uncomplicated vaginitis due to C. vaginale, is the reason for our preference of non-purulent vaginitis (NPV) for the clinical description of the condition. Hence, a Gram-stain of the discharge is essential in the laboratory diagnosis of NPV.

Of the 310 C. vaginale isolated from the asymptomatic patients, 127 (41%) were classified as having heavy growth, 107 (34%) moderate, and 76 (25%) light. However of the 70 isolates from symptomatic patients, 66 (94%) had heavy growth which was virtually pure. Relative purity was acceptable because pure cultures of any bacteria from the female genital tract is an unlikely finding. When C. vaginale is incidentally isolated concurrently with a heavy growth of mixed anaerobes, the C. vaginale is not thought to be the significant organism in a symptomatic patient. In all cases of uncomplicated NPV, the constant finding is the predominance of the organism.

In considering the isolation rates of genital tract pathogens from the symptomatic cases of 1402 patients, the results clearly show that C. vaginale infections are second only in frequency to candidal infections as causative agents of vaginal discharge. These results are shown in Table 3.

<table>
<thead>
<tr>
<th>Organism</th>
<th>No of isolations</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Candida albicans (93)</td>
<td>98</td>
<td>7.0</td>
</tr>
<tr>
<td>Candida tropicalis (1)</td>
<td>4</td>
<td>0.3</td>
</tr>
<tr>
<td>Torulopsis glabrata (4)</td>
<td>98</td>
<td>7.0</td>
</tr>
<tr>
<td>C. vaginale</td>
<td>66</td>
<td>4.7</td>
</tr>
<tr>
<td>Trichomonas vaginalis</td>
<td>33</td>
<td>2.4</td>
</tr>
<tr>
<td>Neisseria gonorrhoeae</td>
<td>2</td>
<td>0.1</td>
</tr>
</tbody>
</table>

We have not found a wet preparation of vaginal discharge any more informative than the Gram-stain of a methanol-fixed smear. Besides, the wet preparation is time-consuming with large numbers of specimens.

Discussion

With increasing acceptance of the pathogenic role of C. vaginale in NPV, comes the requirement for rapid and reliable identification procedures. To the routine diagnostic laboratory, identification of isolates that require lengthy investigations is impractical. "Short set" identification procedures that can be completed on the day of isolation are most desirable. This is important to both the large hospital laboratories as well as the small non-specialised public or private laboratories.

For the identification of C. vaginale a "short set" of steps is recommended that gives near 100% probability of correct identification. These recommended tests are performed on isolates from PSD agar plates incubated anaerobically for 48 h, and include: colonial morphology, starch hydrolysis, Gram-stain, Albert's stain, Catalase test, and hippurate hydrolysis.

In our experience, these tests are not difficult, and more important, they do not require further sub-culture. These rapid tests are readily adapted to any routine laboratory procedure. Other bacterial flora of the genital tract that may be cultured from these patients, are easily distinguished by the primary set of tests and should not be confused with C. vaginale as a causative agent in NPV. In assessing the significance of C. vaginale in NPV, three factors constantly recur:

1. The symptoms are uniformly similar.
2. The Gram-stain appearance of the discharge is highly characteristic and readily recognised.
3. The discharge produces a heavy growth of C. vaginale.
The clinical details, and results of treatment of NPV form the subjects of another report elsewhere.

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References


7 Smith RF, Dunkelberg WE. Inhibition of Corynebacterium vaginale by metronidazole. Sex Transm Dis 1977;4:20-1.


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