A preliminary survey of *Atopobium vaginae* in women attending the Dunedin gynaecology out-patients clinic: Is the contribution of the hard-to-culture microbiota overlooked in gynaecological disorders?

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**Abstract**

Preliminary studies have indicated that the recently described bacterium *Atopobium vaginae* may have an association with bacterial vaginosis (BV). Fifty-five women attending the gynaecology out-patient’s clinic were tested for the presence of this micro-organism, *Gardnerella vaginalis*, *Mobiluncus* and *Bacteroides* species by polymerase chain reaction (PCR)-based assays. The frequency of detection was 40%. PCR detection of *Gardnerella vaginalis* with *A. vaginae*, occurred in 50% of *A. vaginae*-positive cases. Due to the high detection rate of *A. vaginae* we believe that it is important to determine whether this and other hard-to-culture microorganisms have a role in gynaecological disorders.

**Key words:** *Atopobium vaginae*, bacterial vaginosis.

**Introduction**

Bacterial vaginosis has been associated with higher acquisition rates of HIV and other sexually transmitted diseases and is also possibly implicated in preterm labour and other gynaecological disorders.1,2 Recent studies using molecular analysis of the vaginal microbiota have shown that the composition of this microecosystem differs substantially from that derived by culture analysis. This may have major implications for women’s health.3–10 Indeed, certain microorganisms, such as the Gram-positive anaerobe *Atopobium vaginae*, are rarely detected by culture but appear to be frequently present in the vagina.11 Preliminary studies indicate that *A. vaginae* may be commonly associated with bacterial vaginosis in women of child-bearing age and post menopausal women.5–7,12

Interestingly, one study5 has reported that isolates are highly resistant to metronidazole, a therapeutic agent widely used for the treatment of various vaginal infections. Little is known about this micro-organism with regard to its contribution to the polymicrobial consortium implicated in vaginosis and other gynaecological diseases. However, one ponders the potential importance of this micro-organism given that the recurrence rate of vaginosis is up to 20% after therapy with metronidazole or up to 50% with other agents,13 whilst longer term recurrence rates may be as high as 80%.2

The aim of the current study was to determine the incidence of *A. vaginae* amongst women attending the Gynaecology Out-Patient’s Clinic at Dunedin Hospital and whether other organisms associated with vaginosis are also present, as preliminary North American5,12 and European studies have shown.6,7

**Methods**

The study was approved by the Otago Ethics Committee, and all participants provided written consent after having received explanation of the procedures involved. Fifty-five women were recruited from the Gynaecology Out-Patient’s Clinic at Dunedin Hospital, after referral for various reasons including, colposcopy, postpartum check, peri-operative visit, endometriosis, peri-menopausal consultation and post-operative surgery check. Subjects ranged in age from 16 to 73 years, though the average age was 33.43 years. Subjects were sexually active, but not pregnant and did not have a history of recent antibiotic or steroid use. Swabs from subjects that had bleeding were excluded to prevent possible interference with the PCR-based assay. High vaginal swabs were collected by a gynaecologist using a speculum. Extraction of bacterial
DNA from the swab contents has been described previously.\(^3\) The PCR primers and conditions for detection of \textit{A. vaginae} were as outlined by Verhelst \textit{et al.},\(^4\) whilst those for \textit{Gardnerella vaginalis}, \textit{Bacteroides} and \textit{Mobiluncus} species were from the study of Obata \textit{et al.}\(^5\) PCR amplicons were resolved on 2% agarose gels. Random amplicons were purified by using a Qiagen PCR purification kit (Qiagen, Valencia, CA), DNA sequenced and BLAST searched to confirm sequence homology. DNA sequencing was conducted by the Allan Wilson Centre Genome Service, Massey University. Fisher’s exact test was used to determine \(P\)-values.

## Results

The PCR results using the specific primers are presented in Table 1. Three amplicons generated using \textit{Atopobium vaginae}-specific primers were DNA sequenced and BLAST searched, and these had high homology (99%, 363/364 base pairs) to an \textit{Atopobium vaginae} sequence (AF325325) in the NCBI database. This confirmed that the PCR primers were specific for this micro-organism. \textit{Gardnerella vaginalis} was significantly more often present in \textit{A. vaginae}-positive compared to negative swabs (\(P = 0.001\)). There did not appear to be an association between any particular reason for attending the clinic, condition and \textit{Atopobium vaginae} in this study. The most common reason for patients to attend the clinic was for colposcopy (\(n = 33\)), \textit{A. vaginae} was detected in 12 of these patients. There were seven patients attending the clinic for a well woman routine check up, three of which were positive for \textit{A. vaginae}. Whilst there were many other reasons for attending the clinic, those that yielded positive samples were; consultations for tubal ligation (2/2), hysterectomy (1/2) and infertility (1/1), postoperative check (1/1), pregnancy test (1/1) or attending for other reasons (1/4).

## Discussion

Whilst the cohort of women tested in this study would not necessarily be considered representative of the normal Dunedin population because they were attending a gynaecology clinic, the detection rate of \textit{A. vaginae} was high amongst the group (40%), suggesting that this microorganism may be prevalent amongst women in the Dunedin region. There also appeared to be an association between the presence of \textit{A. vaginae}, \textit{G. vaginalis} and to a lesser extent \textit{Mobiluncus} spp., all organisms implicated in vaginosis. This adds further support for the contention that \textit{A. vaginae} may be an important part of the polymicrobial microbiota associated with vaginosis and that current treatment options might require some modification.

Metronidazole is the agent of choice for many vaginal infections. Whilst it is generally considered effective, there are a high number of relapses in subjects on this therapy. This may in part be due to the high incidence of metronidazole resistance observed in clinical isolates of \textit{G. vaginalis}.\(^1\) Metronidazole is also effective against some of the gram-negative bacteria that are thought to act synergistically with \textit{G. vaginalis} in vaginosis.\(^1\)\(^6\) It may also be the case that metronidazole resistant \textit{A. vaginae} have a synergistic relationship with \textit{G. vaginalis} due to its frequent codetection. Thus in order to eradicate the infection it may be necessary to concurrently treat with other antimicrobial agents that will reduce other implicated bacteria such as \textit{A. vaginae}. Which treatment regimens are likely to be effective is unclear because the antibiotic susceptibilities of \textit{A. vaginae} are not well defined as relatively few strains have been studied. However, those that have seem quite susceptible to clindamycin,\(^5\) although this agent is currently not publically funded in New Zealand for vaginosis. It is likely that additional antimicrobial treatments that reduce \textit{A. vaginae} are also likely to reduce the beneficial microbiota such as \textit{Lactobacillus} species and may cause further complications by allowing unregulated growth of other micro-organisms such as yeast and perhaps leading to vaginitis. Treatment may involve replenishment of the ‘normal’ vaginal lactobacilli by supplementation of the vagina with clinically proven probiotics.\(^1\)\(^7\)

In conclusion, \textit{A. vaginae} is present in the vaginal microbiota of a large proportion of women who attend the Dunedin gynaecology clinic. The bacterium remains largely unstudied with regards to its possible role in gynaecological disorders. This is only one of several micro-organisms recently shown to be present in the vagina by non-culture techniques. Further studies are required to establish the way in which the presence of this and other such microbes impact on women’s health. Studies are currently being conducted at Dunedin hospital to determine whether women who undergo preterm labour have a higher incidence of \textit{A. vaginae} and vaginosis compared to age-matched controls who deliver full-term.

## Acknowledgements

Author’s JB and CC are employed by BLIST Technologies Ltd. Author’s JB, CC, JT and CD were involved in the planning of the study. CD with input from DW conducted the sampling. JB and MA-Q performed the experimental analysis. HB

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**Table 1** Detection of \textit{Atopobium vaginae} and other vaginosis-associated bacteria by PCR on high vaginal swabs

<table>
<thead>
<tr>
<th></th>
<th>Subjects ((n))</th>
<th>\textit{G. vaginalis}</th>
<th>\textit{Mobiluncus} spp.</th>
<th>\textit{Bacteroides} spp.</th>
</tr>
</thead>
<tbody>
<tr>
<td>\textit{Atopobium vaginae}-positive</td>
<td>22</td>
<td>11</td>
<td>3</td>
<td>0</td>
</tr>
<tr>
<td>\textit{Atopobium vaginae}-negative</td>
<td>33</td>
<td>3</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

PCR, polymerase chain reaction.

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Australian and New Zealand Journal of Obstetrics and Gynaecology 2005; 45: 450–452
undertook the statistical analysis. All authors had an intellectual input into the analysis of the data and in the drafting of the manuscript.

References