Comparison of a Ligase Chain Reaction-Based Assay and Cell Culture for Detection of Pharyngeal Carriage of Chlamydia trachomatis

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In 264 genitourinary medicine clinic attenders reporting recent fellatio, the prevalence of pharyngeal Chlamydia trachomatis determined by an expanded standard including cell culture and two in-house PCR tests was 1.5% in 194 women and zero in 70 men. The ligase chain reaction (Abbott LCx) had a specificity of 99.2% and a positive predictive value of 60%.

Chlamydia trachomatis is the commonest bacterial sexually transmitted pathogen in most developed countries. Infection with the genital serovars of C. trachomatis is usually acquired through unprotected vaginal or anal sex, or vertically at birth. The orogenital route has not previously been considered an important mode of transmission of C. trachomatis, and testing for pharyngeal C. trachomatis infection as part of a routine sexual infection screen is not advised in recent United States (2) or United Kingdom (3) guidelines. However, the practice of fellatio by women has increased (5), and among the sexually inexperienced, fellatio can be a substitute for vaginal intercourse, preserving "technical virginity" (6). Condom use during fellatio is rare, and so it is possible that orogenital transmission of C. trachomatis could occur in spite of "safer sex" precautions for genital sex or where the participants do not believe they have actually "had sex" (12). We now report the first evaluation of a ligase chain reaction (LCR)-based assay (Abbott LCx) compared with cell culture (including a single blind passage step) in detecting pharyngeal infection with C. trachomatis.

We recruited 264 subjects (194 women and 70 men) aged over 18 years who presented to a large urban genitourinary medicine clinic over a 9-month period with a new problem. Three women (1.5%) had confirmed pharyngeal C. trachomatis by LCR, plating of cervical, urethral, and rectal swabs (as indicated) for gonococcal isolation as above, and syphilis serology.

Detection of a target sequence of C. trachomatis DNA from the cryptic plasmid by commercial LCR (Abbott Laboratories, North Chicago, Ill.) was conducted according to the manufacturer’s directions (4). C. trachomatis was cultivated in McCoy cells by standard methods, with the addition of a single blind passage step. Samples from subjects with a positive test for pharyngeal chlamydia by LCR or cell culture or both were further investigated with two PCR-based assays targeting sequences in both the major outer membrane protein gene and the cryptic plasmid. Pharyngeal swabs frozen in Chlamydia transport medium were transported on dry ice to an independent reference laboratory and were tested blind along with a matching number of pharyngeal swabs taken from subjects free of genital or pharyngeal Chlamydia. Nucleic acid was extracted using the QIAamp viral RNA kit (Qiagen) (13). PCR was performed using the LightCycler (Idaho Technology, Idaho Falls, Idaho), a glass capillaryycler with real-time fluorescent detection of PCR product and the ability to analyze reaction products by melting-point analysis. The major outer membrane protein-based PCR amplifies a 155-bp region between base pairs −53 and +51 of the sequence using primers MOMPFP2 (5′ CCA GAA AAA GAT AGC GAG CAC AAA 3′) and MOMPRI (5′ AGC AGA ACT CAA AGC GGC AAA T 3′). The plasmid-based PCR was adapted from Loeffelholz et al. (9) and targets a 207-bp fragment of the cryptic plasmid located 195 bp downstream of the BamHI restriction site. The detection limit of both assays is approximately 10 gene copies. Subjects with two or more positive results by different techniques (LCR, PCR, cell culture) and, in the case of LCR and PCR, with different targets (plasmid or major outer membrane protein [MOMP]) were considered true positives.

Female and male subjects had a mean age of 26.6 years (standard deviation, 7.5 years) and 33.5 years (standard deviation, 10.9 years), respectively. Unprotected fellatio was very common: no men and only two women (1%) had consistently used condoms for receptive oral sex in the 3 months before attendance. Sixty-seven women (35%) and 21 men (30%) were found to have at least one sexually transmitted infection, including 36 (19%) women and 3 (4.3%) men with genital C. trachomatis infection detected by LCR.

Three women (1.5%) had confirmed pharyngeal C. trachomatis according to the expanded standard (see Table 1). In
TABLE 1. Detection of C. trachomatis by LCR, cell culture, MOMP-based PCR, and plasmid-based PCR in genital and pharyngeal swabs

<table>
<thead>
<tr>
<th>No. of subjects</th>
<th>Genital swabs by LCR</th>
<th>Pharyngeal swabs by:</th>
<th>PCR</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Culture</td>
<td>LCR</td>
<td>MOMP</td>
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<tr>
<td>Women (n = 194)</td>
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</tr>
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<td>152</td>
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<td>ND</td>
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<td>33</td>
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<td>4</td>
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<td>1&lt;sup&gt;b&lt;/sup&gt;</td>
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<tr>
<td>3</td>
<td>–</td>
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<tr>
<td>Men (n = 70)</td>
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<td></td>
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<tr>
<td>66</td>
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<td>3</td>
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<td>1&lt;sup&gt;c&lt;/sup&gt;</td>
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</table>

<sup>a</sup> Cervical swab (women) or urethral swab (men).
<sup>b</sup> ND, not done.
<sup>c</sup> Positive LightCycler plasmid-based PCR could not be confirmed. Subject not aware of any exposure to C. trachomatis.
<sup>d</sup> Positive LightCycler plasmid-based PCR but not MOMP-based PCR.
<sup>e</sup> Patient recently exposed to C. trachomatis.
<sup>f</sup> LCR equivocal on first run and positive at low level on second run but could not be confirmed.

these women all four assays were positive and all had genital chlamydial infection, giving a prevalence of pharyngeal chlamydial infection among those with genital chlamydia of 8.3%. None of the positive samples gave a high reaction rate in the LCR assay, with the highest being 700 (sample-to-cutoff ratio [S/CO] reading, 1.5), compared to a usual positive reaction rate for cervical samples in our hands of >2,000 (S/CO, 4.0). A fourth woman tested positive by LCR and LightCycler plasmid-based PCR but was negative by cell culture and LightCycler MOMP-based PCR. No women were found to have pharyngeal infection with Neisseria gonorrhoeae.

No male subjects had pharyngeal C. trachomatis according to the expanded standard (see Table 1). One man had a positive pharyngeal test by LCR alone; this sample yielded an equivocal result on the first LCR run and was weakly positive on retesting, but all other nucleic acid amplification tests were negative. Two men (2.9%) were diagnosed with pharyngeal gonorrhoea.

Three of the four definite or possible cases of pharyngeal chlamydial infection reattended for repeat testing after treatment with either oxytetracycline or doxycycline. No follow-up pharyngeal swabs were positive by either cell culture or LCR.

The small number of true positives precludes a formal comparison of the sensitivity and negative predictive values of LCR and culture. Compared to a strict expanded standard, LCR had an overall specificity of 99.2% (259/261), giving a positive predictive value of 60% (3/5) in this low prevalence population.

The previously reported prevalence of C. trachomatis detected by cell culture among attenders at sexually transmitted disease clinics ranges from zero in a group of 118 unselected women (1) or 160 men who have sex with men (MSM) (11) to 4.3% in another group of 51 MSM (15). When the sensitivity of cell culture was optimized by adding a passage step, pharyngeal C. trachomatis was found in 3.2% of 626 women and 3.7% of 706 heterosexual men (8). Detection of C. trachomatis DNA in pharyngeal swabs by Amplicor plasmid-based PCR has been assessed in two groups of genitourinary medicine clinic attenders. In 124 unselected women and 13 MSM, no cases were detected by cell culture but three cases (2.2%) were positive by both Amplicor and an in-house PCR (7). A second study demonstrated pharyngeal C. trachomatis by unconfirmed Amplicor PCR in three of 193 women (1.5%) but failed to find pharyngeal C. trachomatis in any of 208 men (including 31 who had had sex with men) (10). Finally, in preliminary work Stary et al. found pharyngeal C. trachomatis DNA in 5 of 487 women (1%) using plasmid-based LCR confirmed with MOMP-based LCR, but they did not compare the performance of LCR with an accepted, expanded gold standard including cell culture (14).

In conclusion, pharyngeal carriage of C. trachomatis appears to be uncommon in a variety of settings and when detected by a variety of techniques, occurring in no more than 3 to 4% of subjects overall and in less than 10% of subjects with genital chlamydia infection. In our study, LCR failed to detect any additional true positives by a strict expanded standard, but was more convenient than cell culture. In spite of a specificity over 99%, the very low prevalence of pharyngeal chlamydia resulted in a low positive predictive value. In our study, all individuals with possible cases of pharyngeal C. trachomatis also had genital infection or were known chlamydia contacts and so would have received appropriate antibiotics. Thus we do not recommend separate routine testing of the pharynx, even in those who have recently performed fellatio. Because it is conceivable that pharyngeal chlamydia could be transmitted by oral sex, we suggest advising patients infected with genital C. trachomatis to avoid all types of penetrative sexual activity including oral sex until appropriate treatment and partner notification is complete. Our limited data on three follow-up tests suggest that standard antibiotic regimens appear to be effective in eradicating pharyngeal C. trachomatis infection.

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REFERENCES


