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Comparison of Ligase Chain Reaction and Culture for Detection of *Neisseria gonorrhoeae* in Genital and Extragenital Specimens

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In addition to the urogenital tract, *Neisseria gonorrhoeae* infects extragenital sites such as the pharynx and anorectal canal. Culture and a ligase chain reaction (LCR)-based assay were compared for their performance for the diagnosis of *N. gonorrhoeae* infection with specimens from various urogenital and extragenital sites of 200 men and 125 women. The sensitivity and specificity of the LCR assay with male urethral swabs were both 100%, compared to values of 95.9 and 100%, respectively, for culture of urethral swabs or 98.0 and 100%, respectively, for LCR with first-void urine (FVU). For women, LCR with FVU showed the highest sensitivity (94.7%), and culture of urethral samples showed the lowest sensitivity (63.2%) ($P < 0.05$). In a selected subgroup of 47 men and 22 women at increased risk, the rates of pharyngeal infection were 15 and 18%, respectively, and those of anorectal infection were 13 and 45%, respectively. The sensitivity of LCR was greater than that of culture for both pharyngeal and anorectal specimens. Thus, the overall performance of LCR testing with swabs or FVU was better than that of culture for the diagnosis of genital or extragenital gonorrhea.

Nucleic acid amplification methods, such as those based on PCR or the ligase chain reaction (LCR), detect *Neisseria gonorrhoeae* in urogenital samples with high sensitivity and specificity compared with those of culture, the current diagnostic "gold standard" (1–3). As has been shown for *Chlamydia trachomatis* (8), LCR also effectively detects *N. gonorrhoeae* (12) in first-void urine (FVU) of infected women and thus provides a practical alternative to culture for screening high-risk populations. Preliminary studies also indicate that the sensitivity of detection of chlamydial infection by PCR is similar for both vaginal swabs collected by the patients themselves and endocervical swabs taken by physicians (18) and that vulval samples are highly effective for the detection of *C. trachomatis* infection by LCR (13). These observations indicate that a complete evaluation of the performance of nucleic acid amplification-based diagnostic tests requires thorough analysis of the various possible sites and modes of sampling, taking into account not only sensitivity and specificity but also aspects such as convenience and simplicity of the assay procedure.

We have compared the performance of LCR and culture for the detection of *N. gonorrhoeae* in 325 individuals, both men and women, attending an outpatient clinic in Vienna, Austria. Endocervical and urethral specimens from women and urethral specimens from men were tested by both techniques, and results were compared with those obtained by LCR testing of FVU. In addition, anorectal and pharyngeal samples from a subpopulation of 47 men and 22 women were tested to examine whether extragenital gonorrhea can be detected by LCR.

MATERIALS AND METHODS

Patient population. The total study population comprised 200 men and 125 women who either were referred to an outpatient sexually transmitted disease center in Vienna or attended the center because of clinical symptoms or contact tracing. Most (87.5%) of the men showed symptoms, such as discharge, whereas 44.5% of women attended the center because of discharge, 11.2% attended because of pelvic inflammatory disease, and 28.8% attended because of partner control.

Specimen collection. Urethral specimens from men as well as urethral and endocervical specimens from women were collected with swabs or a sterile bacteriologic loop. Consistency in the collection procedure was achieved by restricting the performance of specimen collection to two physicians. For cervical sampling, excess mucous was removed with a cotton swab before specimen collection. The specimens to be cultured were inoculated directly onto duplicate agar plates. In addition, smears from the endocervical and urethral specimens was subjected to Gram staining. For those individuals who were at the highest risk of infection (those with a positive Gram stain or urethral discharge or sexual partners of individuals infected with gonorrhea), anorectal and pharyngeal specimens were collected during the same visit. Anorectal sampling was performed by inserting a swab 2 to 3 cm into the anal canal without the aid of an anoscope. Pharyngeal specimens were collected by swabbing the posterior pharynx, including the tonsillar area. Patients also received instructions to collect the first 15 to 20 ml of urine in a sterile plastic collection cup; urine specimens were also collected at the clinic, immediately after the medical examination and urogenital sampling.

Culture. Both selective medium (Thayer-Martin agar) and nonselective medium (modified New York agar) were used for the cultivation of *N. gonorrhoeae* by standard techniques (11, 15). The testing laboratory and the clinic were at the same site, allowing immediate inoculation of agar plates in a candle extinction jar and incubation for up to 48 h at 37°C. Colonies were confirmed by a positive oxidase reaction. To distinguish *N. gonorrhoeae* from other *Neisseria* species in extragenital samples, we tested oxidase-positive colonies with a monoclonal antibody-based colorimetric identification kit (GonoGen II; Becton Dickinson, Cockeysville, Md.).

LCR. LCR was performed with a commercial test under development by Abbott Laboratories (Chicago, Ill.). The test is based on a target sequence in the *opa1* gene. Sample processing and testing procedures were performed according to the manufacturer's instructions. Confirmation testing was performed by an LCR assay with probes derived from the pilin gene. Both tests have been described previously (1, 2, 12).

Interpretation of test results. All individuals with positive culture results were considered to be infected with *N. gonorrhoeae*. Culture-negative, LCR-positive specimens were retested by LCR, coded so that investigators conducting confirmatory testing would be blinded to the results, and shipped to Abbott Laboratories for confirmation by pilin-based LCR. Identification of infected persons and calculation of sensitivity and specificity were based on an expanded gold standard that included all culture-positive and all confirmed LCR-positive specimens.

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TABLE 1. Detection of *N. gonorrhoeae* in women by culture and LCR assay of urogenital and urine specimens

No. of specimens	Endocervix		Urethra		Urine LCR	Patient status (no. of patients)	
	Culture result	LCR result	Culture result	LCR result		Infected	Not infected
105	−	−	−	−	−	0	105
11	+	+	+	+	+	11	0
3	+	+	−	+	+	3	0
2	+	+	−	−	+	2	0
2	−	+[a]	−	−	−	1	1
1	−	−	+	+	+	1	0
1	−	−	−	+	+	1	0
Total no. (n = 125)	16	18	12	16	18	19	106

[a] One specimen was positive by repeat LCR testing and was confirmed to be positive by pilin LCR; one specimen was not positive by repeat testing and was not confirmed to be positive.

RESULTS

Detection of *N. gonorrhoeae* in urogenital specimens. The results obtained by culture were compared with those obtained by LCR with probes designed from the *opa1* gene sequence. The results for all *opa1* LCR-positive and culture-negative samples were confirmed by pilin LCR testing, with the technician(s) who carried out the confirmation test being blinded to the initial screening results. Of the 200 men who provided urogenital specimens, concordant results with regard to both assay and sample types were obtained for 197 individuals: 151 were negative and 46 were positive by both culture and LCR with both the urethral and FVU specimens. One individual tested positive by both culture and LCR of the urethral specimen, but negative by LCR assay of FVU. Urethral specimens from two men tested positive by LCR but negative by culture; one of the two discrepant urethral samples was positive by Gram staining, and FVU specimens from both men tested positive by LCR. The repeatedly LCR-positive urethral specimens from these two culture-negative men were confirmed to be positive by pilin LCR. Thus, a total of 49 men (24.5%) were classified as true positives, of whom 47 (95.9%) were identified by culture, 49 (100%) were identified by LCR assay of urethral specimens, and 48 (98%) were identified by LCR assay of FVU.

A more complex pattern of results was obtained with the female population because of the greater diversity of collection sites. Of 125 women tested, 105 were negative and 11 were positive by both culture and LCR assay of all sample types (Table 1). Specimens from nine women showed discrepant results between assays and sample sites. Overall, no women tested positive only by culture, and two women who tested negative by culture of samples from both urogenital sites were confirmed to be positive by LCR.

Performance of LCR and culture with urogenital specimens. The performance of culture and LCR relative to the expanded gold standard was assessed according to patient gender and site of urogenital sample collection (Table 2). For men, culture or LCR performed with urethral specimens and LCR performed with FVU were approximately equally effective in detecting *N. gonorrhoeae* infection. In contrast, although the number of subjects was relatively small, the sensitivity of LCR was greater than that of culture for both urethral and endocervical specimens from women. However, one false-positive result reduced the specificity and positive predictive value of LCR with endocervical samples. The overall highest performance for women was obtained by LCR applied to FVU. In particular, the sensitivity of LCR performed with FVU was significantly greater than that of culture with urethral specimens (18 of 19 versus 12 of 19; $P < 0.05$ by the Wilcoxon nonparametric test).

Detection of *N. gonorrhoeae* infection at extragenital sites. Pharyngeal and anorectal specimens from 47 men and 22 women who were at the highest risk of infection were collected and tested by LCR and culture. Six male pharyngeal specimens were confirmed to be positive by LCR (both *opa1* and pilin LCR positive), whereas two were confirmed to be positive by culture (Table 3). Among the male anorectal specimens, six tested positive by LCR and none tested positive by culture.

More pharyngeal specimens from women also tested positive by LCR than by culture (4 of 22 versus 1 of 22). Although the overall rate of pharyngeal infection was similar in men (7 of 47; 14.9%) and women (4 of 22; 18.2%), the rate of rectal gonorrhea was higher in women than in men (45.5 versus 12.8%). Furthermore, all 10 positive anorectal specimens were detected by LCR alone.

TABLE 2. Performance characteristics of culture and LCR for the detection of *N. gonorrhoeae* in urogenital specimens

Gender, specimen, and test	Sensitivity (%)	Specificity (%)	Positive predictive value (%)	Negative predictive value (%)
Men				
Urethra				
Culture	95.9	100.0	100.0	98.7
LCR	100.0	100.0	100.0	100.0
Urine LCR	98.0	100.0	100.0	99.3
Women				
Urethra				
Culture	63.2	100.0	100.0	93.8
LCR	84.2	100.0	100.0	97.2
Endocervix				
Culture	84.2	100.0	100.0	97.2
LCR	89.5	99.1	94.4	98.1
Urine LCR	94.7	100.0	100.0	99.1

TABLE 3. Detection of extragenital gonococcal infection in high-risk individuals

Gender and no. of individuals tested	Urogenital test result	Pharynx		Anus-rectum	
		Culture result	LCR result	Culture result	LCR result
Men					
37	+	−	−	−	−
3	+	−	−	−	+
2	+	−	+	−	+
1	+	+	+	−	+
1	+	+	−	−	−
3	+	−	+	−	−
Total no. (n = 47)	47	2	6	0	6
Women					
5	−	−	−	−	−
2	−	−	−	−	+
6	+	−	−	−	−
5	+	−	−	−	+
2	+	−	+	−	+
1	+	−	+	−	−
1	+	+	+	−	+
Total no. (n = 22)	15	1	4	0	10

DISCUSSION

Our data confirm previous results suggesting that nucleic acid amplification-based assays for *N. gonorrhoeae* show a specificity equivalent to that of culture, but a sensitivity only slightly higher than that of culture for the diagnosis of infection with this organism in men (2, 5, 10). The sensitivity of LCR applied to FVU from men was only slightly less than that of the same assay applied to urethral swabs, suggesting that gonococcal infection can reliably be detected by LCR assay of this specimen that is obtained by noninvasive means.

The benefit of the improved sensitivity provided by LCR was more apparent with samples from women, although the interpretation of the data was complicated by the greater number of sampling sites. Only 58% of infected women tested positive by both culture and LCR and by testing with all three urogenital specimens (endocervical and urethral swabs and FVU). Culture results alone indicated that 5 (26%) of the 19 women with confirmed infections were infected only in the endocervix; however, LCR revealed that these individuals were also infected in the urethra, as indicated by the positive results obtained with urethral swabs and FVU (three women) or with FVU alone (two women). Only one case of endocervical infection was not confirmed by LCR testing of FVU. Unexpectedly, two women showed gonococcal infections apparently restricted to the urethra. Although such cases have been described in women who have undergone hysterectomy (6), this situation did not apply to our two patients, from whom endocervical swabs were obtained. The negative results for the endocervix could have been attributable to inadequate sampling; however, this explanation is not likely because two separate endocervical specimens were collected for culture and LCR, and both tested negative. It has been proposed that the detection of gonococcal organisms in the anorectal canal does not necessarily indicate an independent site of infection but may result from contamination from the urogenital tract (7). It is also possible that infection apparently restricted to the urethra is attributable to contamination from a bona fide anorectal infection; anorectal swabs from both women tested positive by LCR.

The overall performance of LCR was better than that of culture, although the sensitivity reached 100% only with male urethral specimens. The advantage of LCR over culture has been demonstrated previously (2, 12). In the present study, the overall performance of culture was better than that in other studies, probably, in part, because of the absence of a delay between collection and processing of the samples. For women, the best overall performance was provided by LCR with FVU. This somewhat unexpected result supports the concept that *N. gonorrhoeae* infection is urogenital and not restricted to the endocervix. The efficacy of FVU testing may also be attributable to contamination of urine by cervical discharge that contains the infectious organism; in addition, urine collection circumvents the variability and technique-dependent nature of swab sampling. Given that up to 50% of women infected with *N. gonorrhoeae* are asymptomatic, the noninvasive nature of FVU collection provides a substantial advantage over the labor-intensive and time-consuming procedures for obtaining urogenital swabs. Although in this study we did not find any culture-positive, LCR negative samples among paired samples, it has been shown that up to 5% false-negative results by PCR or LCR can occur due to sample inhibitors (2, 8). Thus, the performance of LCR or PCR testing with FVU can be further improved by the removal or neutralization of endogenous inhibitors present in urine that can interfere with nucleic acid amplification. However, until antibiotic resistance can be tested by nucleic acid technologies, LCR or PCR is not likely to replace culture for routine testing in Europe, although many clinical laboratories in the United States have replaced culture for *N. gonorrhoeae* with probe testing.

The frequency of anorectal gonococcal infection has been reported to range between 5 and 50% for certain selected populations (4, 16). Such infection can originate from receptive anal sexual practices by both sexes or from urogenital contamination in women. Our results indicate a relatively high frequency of anorectal infection in both the men (13%) and women (45%) who were at the highest risk of infection in our study population. The large discrepancy between culture and LCR testing for anorectal swabs may, in part, reflect the growth of large numbers of other bacteria in some culture plates, even though both nonselective and selective media were used. However, the more likely explanation lies in the difference in sensitivity between DNA amplification-based methods and culture for specimens with a low organism load. The sensitivity of a nonamplified DNA test was also shown to be markedly higher than that of culture for the detection of *N. gonorrhoeae* in anorectal samples (4). If these preliminary observations are confirmed with a larger sample size, it would also imply that the extent of extragenital infections by *N. gonorrhoeae* may have been underestimated.

The frequency of pharyngeal infection (15 to 18%) in our study subpopulation was similar to that obtained in previous studies based on the use of culture or DNA probes for diagnosis (9, 17). As for anorectal specimens, the sensitivity of LCR was markedly greater than that of culture for the detection of infection in the pharynx.

Although neither culture nor LCR provided a perfect diagnostic tool, the overall performance of LCR with female FVU was the most efficient single test for the diagnosis of urogenital gonorrhea in women. Our data emphasize that, even with a sensitive technique such as LCR that is able to detect a low organism load, the overall rate of detection of *N. gonorrhoeae* infection in women is lower than that in men because of the complexity associated with multiple sites of infection and the

occurrence in some women of infection at only one of these sites.

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