Diagnostic Performance of the Roche AMPLICOR PCR in Detecting *Neisseria gonorrhoeae* in Genitourinary Specimens from Female Sex Workers in Cotonou, Benin

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The objective of this study was to evaluate the diagnostic performance of the Roche multiplex AMPLICOR *Chlamydia trachomatis*/*Neisseria gonorrhoeae* PCR test for the detection of *Neisseria gonorrhoeae* infection in female urine specimens and wet and dry endocervical swabs. Endocervical swabs and urine specimens were collected from 342 female sex workers from Cotonou, Benin, and were tested using the AMPLICOR *C. trachomatis*/*N. gonorrhoeae* test (Roche Diagnostic Systems, Inc., Branchburg, N.J.) with internal control detection. Endocervical swabs were also cultured on Thayer-Martin medium. A series of alternate standards that included a combination of all the tests but not the test being evaluated was used to assess the performance of the test with each type of specimen. The sensitivity, specificity, and positive and negative predictive values for the urine were 53.8, 98.9, 93.5, and 87.5%, respectively. Corresponding figures for the wet swab were 91.5, 100, 100, and 97.4%, respectively. Those for the dry swab were 96.3, 96.2, 88.5, and 98.8%, respectively. Based on this study, the AMPLICOR PCR assay showed a low sensitivity for detection of *N. gonorrhoeae* infection in urine specimens, whereas the test was found to be highly sensitive and specific with endocervical specimens.

Neisseria gonorrhoeae infection has been recognized as a major public health problem in developing countries for decades, particularly in sub-Saharan Africa (6, 13). The emergence of human immunodeficiency virus (HIV) and AIDS has contributed to an increasing awareness of the importance of *N. gonorrhoeae* since this pathogen was identified as a cofactor for the transmission and acquisition of HIV both in epidemiological studies and in studies on genital shedding (4, 10).

While reliable laboratory facilities for the diagnosis of gonococcal infection are not usually available in many developing countries, sophisticated DNA technologies, especially nucleic acid amplification (PCR and ligase chain reaction), have been developed and evaluated in industrialized countries over the past 10 years (2, 3, 9, 11). To date, PCR has been the most widely used amplification method. The diagnostic performance of this test for detection of gonococcal infection has been evaluated with usual endocervical and urethral swab specimens, and more recently with urine specimens (2, 11). Several studies have shown that PCR assays perform well in detecting gonococcal infection on genital specimens (9, 11). However, more recently, a study by Farell suggested that the AMPLICOR *Chlamydia trachomatis*/*N. gonorrhoeae* test (Roche Diagnostic Systems, Inc., Branchburg, N.J.) may have a poorer specificity than initially reported (5). Consequently, Roche

now recommends the confirmation of positive specimens by a 16S rRNA assay.

To our knowledge, no report from sub-Saharan Africa evaluating the validity of the PCR technique for the detection of *N. gonorrhoeae* in urine specimens has been published. The high prevalence of gonococcal infection (37.0% in 1993) (7) in the female sex worker (FSW) population in Cotonou offers an excellent opportunity to evaluate this newer technique. However, in the context of developing countries, it could be difficult to implement systematic confirmatory assays. Thus, we were interested in the evaluation of the AMPLICOR PCR (without 16S rRNA confirmation); we examined its performance in the diagnosis of gonococcal infection in urine samples from FSWs in a developing country, Benin. We also evaluated its validity for endocervical swabs kept dry or inserted in a 2-SP transport medium.

MATERIALS AND METHODS

Study population. From November 1997 to August 1999, 342 FSWs living in Cotonou, Benin, were, after written informed consent, screened for participation in a UNAIDS-sponsored trial on the effectiveness of the N-9-based microbicide COL-1492 (Advantage S; Columbia Laboratories, Paris, France) in the prevention of male-to-female HIV transmission through heterosexual intercourse.

Collection and storage of samples. Three endocervical swabs were collected by a physician during pelvic examination with a speculum. The first cotton-tipped swab was inserted into the endocervix, rotated for 15 to 30 s, and used to culture gonococcus in Cotonou. The other two swabs served to collect material for PCR testing. The first of these swabs was inserted into a dry Nalgene cryogenic vial and the second one was inserted into a 2-SP transport medium made in-house with 68.46 g of sucrose–2.01 g of K_2HPO_4–1.01 g of KH_2PO_4/liter and immediately frozen at −20°C. All the participants also provided a urine sample in a clean 50-ml plastic container. Urine specimens, collected alternatively before or after pelvic examination, were aliquoted into two 2-ml Nalgene tubes and frozen at −20°C. Endocervical wet and dry swabs and urine specimens were shipped together, frozen in a container with dry ice, to the Institute of Tropical Medicine in Antwerp, Belgium. Endocervical wet swabs and urine specimens were repacked on dry ice and sent to the laboratory at the University of Montreal in Canada.

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TABLE 1. Diagnostic performance of AMPLICOR PCR in detection of urogenital *N. gonorrhoeae* infection among 342 FSWs from Cotonou, Benin[a]

Specimen	% of women with positive test ($n = 342$)	Sensitivity[b]	Specificity[b]	Positive predictive value	Negative predictive value
Urine	13.5	43/80 (53.8)	259/262 (98.9)	43/46 (93.5)	259/296 (87.5)
2-SP swab	21.9	75/82 (91.5)	260/260 (100)	75/75 (100)	260/267 (97.4)
Dry swab	25.4	77/80 (96.3)	252/262 (96.2)	77/87 (88.5)	252/255 (98.8)

[a] The validity parameters for each type of specimen were calculated using a rotating independent standard that included all the other tests but not the test being evaluated. Data for sensitivity, specificity, and positive and negative predictive value are numbers of correctly identified specimens/total number tested, with percentages in parentheses.
[b] Using the latent-class model, the sensitivity and specificity of the Amplicor PCR with urine were 56.0 and 99.0%. Corresponding figures for the wet swab were 98.0 and 100%, respectively, and for the dry swab were 100 and 96.0%, respectively.

The dry swabs were tested in Antwerp with the AMPLICOR kit within 2 to 3 months after the sampling in Cotonou, but the 16S rRNA confirmatory assay was performed between 6 and 15 months later. In Montreal, the wet swabs and urine specimens were kept frozen and were tested by AMPLICOR PCR between 5 and 14 months after sampling. All the AMPLICOR-positive samples were then retested with the 16S rRNA assay within 2 weeks.

N. gonorrhoeae culture. Endocervical swabs collected for *N. gonorrhoeae* culture were immediately inoculated onto modified Thayer-Martin media in the clinic, stored in candle extinction jars, and then transported daily to the laboratory of the Centre National Hospitalier Universitaire in Cotonou. Plates were incubated at 36°C and inspected at 24 and 48 h. Isolates were presumptively identified on the basis of typical colony morphology, Gram stain result, and oxidase testing. The strains were stored in skimmed milk at −20°C and sent every 2 months to the Institute of Tropical Medicine for formal identification using a panel of sugars (glucose, maltose, lactose, and sucrose) as well as monoclonal antibodies (18).

PCR testing. The PCR tests were performed with the multiplex AMPLICOR *C. trachomatis*/*N. gonorrhoeae* kit (Roche Diagnostic Systems, Inc.) as instructed by the manufacturer. Specimens positive for *N. gonorrhoeae* were retested in duplicate at the Institute of Tropical Medicine (for dry swabs) and at the University of Montreal (for 2-SP swabs and urine specimens). If two of the three tests were positive, the specimen was confirmed by the *N. gonorrhoeae* 16S rRNA assay provided by Roche. In Antwerp, the confirmation was done locally, while positive specimens in Montreal were sent for confirmation at the Centre Hospitalier Régional de Trois-Rivières, Québec, Canada, the only center in Québec to which Roche provided this assay at the time of the study. Culture and PCR specimens were processed blindly in the different laboratories involved.

Definition of standards. The sensitivity, specificity, and positive and negative predictive values of the AMPLICOR PCR for each type of specimen (urine and wet and dry swabs) were calculated using an independent standard that included a combination of all the other tests but not the test under evaluation (for example, positive culture or positive AMPLICOR PCR confirmed positive by 16S rRNA assay of wet or dry swabs was used as the standard to evaluate AMPLICOR PCR with urine). We also compared Roche AMPLICOR PCR results for the three types of specimens using a latent-class model without assuming any "gold standard" as explained by Walter and Irwigh (19). We estimated the prevalence of gonococcal infection in the study population using both the latent-class model and a standard taking into account all the tests performed in this study (either positive culture or positive AMPLICOR test confirmed by 16S rRNA with any type of sample).

The culture was not evaluated since it was considered by definition 100% specific. However, the presumptive identification of *N. gonorrhoeae* by culture was considered for the clinical management of the participants since formal identification of this pathogen with sugars, monoclonal antibodies, and PCR are not available in Cotonou.

A formal discrepant analysis of the samples that were culture negative and positive by AMPLICOR PCR with the wet or dry swab was not carried out since this kind of analysis has been criticized because of the bias introduced by the selective nature of the confirmatory testing (8, 12). However, discordant results are presented and discussed.

RESULTS

The overall prevalence of *N. gonorrhoeae* infection in the study population according to the standard defined above was 24.0%. The latent-class approach resulted in an estimated prevalence of 22.4%. Table 1 presents the diagnostic performance of the different tests in comparison with the independent standards we used as well as the estimates of sensitivity and specificity obtained with the latent-class model for each type of specimen. Out of a total of 60 isolates with positive

presumptive identification in Cotonou, 50 were sent to the Institute of Tropical Medicine in Antwerp. The 46 isolates surviving transportation were all formally identified as *N. gonorrhoeae*.

The *N. gonorrhoeae* 16S rRNA assay with urine specimens confirmed only 28 of 41 (68.3%) cases positive by the AMPLICOR kit. The 16S rRNA test was not performed for 5 of the 46 urine samples positive with the AMPLICOR kit, because of insufficient volume of urine. Among the 13 nonconfirmed cases, only one was a true negative since it tested negative by culture and by PCR with both wet and dry swabs. All the other 12 specimens were positive by the presumptive identification in Cotonou. Of these 12 specimens, 9 survived transportation to the laboratory in Antwerp and were all formally identified as *N. gonorrhoeae*. The three specimens that did not survive transportation were all confirmed positive by 16S rRNA PCR with both wet and dry swabs.

The *N. gonorrhoeae* 16S rRNA assay confirmed 65 of the 72 (90.3%) results that were positive by the AMPLICOR kit for wet swabs. This test was not performed for three AMPLICOR PCR-positive samples. All the seven unconfirmed results were AMPLICOR PCR positive when dry swabs were tested, and six specimens were positive by culture. The only culture-negative specimen was confirmed positive with urine by the 16S rRNA assay.

The *N. gonorrhoeae* 16S rRNA test with dry swabs confirmed only 65 of the 87 (74.7%) samples positive by AMPLICOR PCR. Only 8 of the 22 unconfirmed results could be considered truly negative taking into account culture and PCRs with wet-swab and urine specimens.

Culture was negative for 18 of the 75 (24.0%) samples for which PCR with wet swabs was positive. Seventeen of these specimens were confirmed by the *N. gonorrhoeae* 16S rRNA assay. The only unconfirmed specimen was confirmed positive (positive by both AMPLICOR and 16S rRNA PCR) with urine.

The sensitivity of the AMPLICOR PCR with urine did not decrease with increasing delays of storage before testing (41.7% [$n = 12$] for delays of <6 months; 46.7% [$n = 30$] for delays of 6 to 9 months; 63.2% [$n = 38$] for delays of >9 months; $P = 0.12$ by chi-square test for trend). Similar results were found with the wet swab (data not shown).

DISCUSSION

In this study, the Roche AMPLICOR PCR kit had a very low sensitivity for the detection of *N. gonorrhoeae* in urine specimens according to the rotating independent reference we used (53.8%). However, since the choice of the best standard to use in the evaluation of newer tests has been a topic of disagreement among authors (8, 12, 16), we also evaluated the

performance of the AMPLICOR PCR with different specimens using the latent-class model, which does not assume any "gold standard" (19). The results obtained with this model were quite similar to those from the series of independent standards we used.

Given this poor performance of the AMPLICOR kit in diagnosing gonococcal infection in urine specimens, one may speculate on the possible endogenous amplification inhibitors in urine that may produce false-negative results in this population with a very high prevalence (53.3%) (1) of HIV infection. However, only six of all urine specimens showed inhibitory activity, which was removed after dilution. On the other hand, one may question the effect on sensitivity of long-term storage (median of 10 months) of urine specimens prior to testing, although 2-SP swab samples have been kept for an equivalent period without apparent effect on sensitivity. Indeed, in a previous study, Puolakkainen et al. (15) retested frozen (at −20°C) first-void urine after 6 months for detection of *C. trachomatis*. The authors observed that 10 of the 78 results initially positive by the Roche Cobas AMPLICOR PCR *C. trachomatis/N. gonorrhoeae* test became negative. Could this phenomenon apply to *N. gonorrhoeae*? In this study, the sensitivity of AMPLICOR PCR with urine taking into account different delays of storage prior to testing did not decrease over time. Freezing and unfreezing to perform the different laboratory manipulations could also have a negative impact on the sensitivity of the test with urine specimens. In contrast with our results, the AMPLICOR kit has been found to be highly sensitive and specific (sensitivity and specificity of 100%) in detecting *N. gonorrhoeae* infection in urine specimens from males (14). One may speculate that the urethra is not the main site for *N. gonorrhoeae* infection in females to explain the poor results obtained in our study. In fact, although data on this topic are not available from sub-Saharan Africa, some reports from the developed world have shown that female urine could be a suitable specimen for detection of gonococcal infection in women, at least when the ligase chain reaction is used (3, 17, 20).

Our data showed a relatively high level of discordance between the *N. gonorrhoeae* 16S rRNA confirmatory assay and the AMPLICOR test for urine specimens. Similar results were observed in a previous study by Bassiri et al. (2), who reported that all the nine urine specimens from women which tested positive by the AMPLICOR PCR proved to be negative with the 16S rRNA-based PCR. The lower frequency of positive results of the 16S rRNA test in comparison to the AMPLICOR has been attributed to lack of specificity of the latter, which may produce false-positive results in the presence of certain strains of *Neisseria subflava* and *Neisseria cinerea* (5; Roche Diagnostic Systems, Inc., AMPLICOR *Chlamydia trachomatis/Neisseria gonorrhoeae* (CT/NG) test package insert, 1996). Indeed, Farell (5) reported that 15.6% (15 of 96) of the results positive by the AMPLICOR kit were false positives since they were not confirmed either by in-house nested PCR for the *cppB* gene or by the 16S rRNA PCR assay. In our study, discrepancy between the AMPLICOR and the 16S rRNA PCRs may be attributed to lack of sensitivity of the 16S rRNA test, especially for urine samples. Indeed, of 13 urine specimens which tested positive by the AMPLICOR kit but negative by the 16S rRNA PCR, 12 corresponded to culture-positive endocervical swabs. The 16S rRNA assay also resulted in a nonnegligeable false-negative rate for both wet and dry swabs. The poor performance of this test with all types of specimens could be attributed to its lack of sensitivity or to the long storage of specimens before testing. However, our results do not suggest that a long delay due to storage affects the results of the AMPLICOR test, at least for cervical samples.

Based on our results, the Roche AMPLICOR PCR showed a low sensitivity for detection of *N. gonorrhoeae* infection in urine specimens from women, whereas the test was found to be highly sensitive and specific with endocervical specimens.

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