

Syndromic Versus Laboratory-Based Diagnosis of Cervical Infections Among Female Sex Workers in Benin

Implications of Nonattendance for Return Visits

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Background: The syndromic diagnostic approach is the most realistic and cost-effective strategy for controlling sexually transmitted infections (STIs) in the developing world. Its potential advantages should be evaluated.

Goal: The goal of the current study was to examine whether the syndromic approach might diagnose more cases of cervicitis due to *Neisseria gonorrhoeae* or *Chlamydia trachomatis* than laboratory tests.

Study Design: The participants were 481 female sex workers in Benin, screened for STIs and treated on the basis of the clinical findings. They were asked to return to the clinic within 10 days for laboratory test results and appropriate treatment when necessary.

Results: The prevalence of cervical infections was 24.5%. In comparison to the gold standard, the sensitivity of the syndromic diagnosis approach for the detection of *N gonorrhoeae/C trachomatis* infections was 48.3%; that of the locally performed laboratory tests was 74.6%. However, the sensitivity of the laboratory tests dropped to 28.8% when it was taken into consideration that 57.6% of the infected women did not return to the clinic within 10 days.

Conclusions: The syndromic diagnosis approach should continue to be used for female sex workers in Benin because returning for treatment is problematic. Presumptive treatment at their initial visit could be a complement to this approach, given the high prevalence of cervicitis in this population.

THE TWO MAJOR KNOWN CAUSES of cervical infec-

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tions in women are *Neisseria gonorrhoeae* and *Chlamydia trachomatis*. These pathogens are common in countries of sub-Saharan Africa¹ and particularly in female sex workers (FSWs).²⁻⁸

Complications and severe sequelae attributed to *N gonorrhoeae* or *C trachomatis* infection in women and newborns have been documented,⁹⁻¹² as well as the negative impact of these pathogens on fertility¹³ and, concomitantly, on net population growth in sub-Saharan Africa.^{14,15} Furthermore, it has been demonstrated that cervical infections may act as cofactors for the acquisition and transmission of HIV infection.¹⁶⁻²⁰ Therefore, it is critical to control these often asymptomatic diseases, particularly in FSW populations, which, with their clients, contribute substantially to the propagation of HIV and sexually transmitted infections (STIs).

The most realistic and cost-effective strategy for controlling STIs in the developing world is the syndromic diagnostic approach, as recommended by the World Health Organization (WHO).²¹ However, the algorithms proposed by WHO should be validated under field conditions and periodically evaluated, and the susceptibility of the causative agents of STIs to available drugs should be monitored.

Two studies have evaluated the performance of screening algorithms and a score system, in comparison with etiologic laboratory diagnosis, for the detection of genital infections with *N gonorrhoeae* or *C trachomatis* in FSWs in Benin.^{4,5} These and other studies in sub-Saharan Africa demonstrate that the sensitivity and specificity of the syndromic approach for detection of *N gonorrhoeae/C trachomatis* infections are suboptimal in comparison with those of specific laboratory tests.^{2,6–8}

However, these studies did not take into account the potential practical advantages of the syndromic approach, namely, that immediate treatment can be given without having to wait for laboratory results, with the consequent risk of losing infected women to follow-up. Moreover, even though the practical difficulty of achieving high rates of patients returning for laboratory test results (and treatment, if appropriate) is underlined in the rationale for treatment based on syndromic diagnosis, the extent to which this issue affects the effective performance of specific laboratory tests in comparison with syndromic diagnostic approaches, either in Benin or elsewhere in sub-Saharan Africa, has not been explored.

We therefore carried out this study to evaluate the performance of syndromic diagnosis of cervical *N gonorrhoeae/C trachomatis* infections and to assess its practical utility, in comparison with a strategy based on locally available laboratory test results, for FSWs in Benin.

Methods

Background

The Cotonou 1 STI clinic in Benin was one of the four sites at which a multicenter randomized clinical trial was conducted on the effectiveness of the N-9-based microbicide COL-1492 (Advantage 24) in the prevention of male-to-female sexual transmission of HIV and other STIs. The current study was based on the screening visit of FSWs in Cotonou, when eligibility to participate in the trial was assessed.

Study Population and Study Design

The participants were FSWs from Cotonou, recruited over a period of 2 years, from November 1997 to October 1999. After explanation of the objectives and procedures of the study to the potential participants, 481 women were recruited, at which point written informed consent was obtained. A physician recorded data on sociodemographic characteristics, sexual behavior, and condom use by administering a structured questionnaire. All participants took part in HIV pretest counseling and intensive STI education. Finally, the physician performed a physical examination that included abdominal palpation, speculum examination, and bimanual palpation of the pelvis. Particular attention

was given to signs of inflammation of the vulva and vagina, friability of the cervix, characteristics of vaginal and cervical discharge, and presence of ulcers. During the examination, vaginal and cervical swab samples were taken for STI diagnosis (by testing for *N gonorrhoeae*, *C trachomatis*, *Trichomonas vaginalis*, and *Candida albicans*). The FSWs also provided 10 ml of venous blood for *Treponema pallidum* and HIV screening. When an STI was diagnosed according to the syndromic findings, treatment was prescribed in accordance with the Benin Ministry of Health guidelines. The results of direct microscopy for *T vaginalis* and *C albicans* were available within 15 to 20 minutes, but the women were asked to come back to the clinic within 10 days for the other laboratory results and to be given appropriate treatment when necessary. At the return visit, only the participants who met the inclusion criteria for the randomized trial and consented to participate in it were physically examined and screened once again for HIV and the other STIs mentioned above.

Laboratory Procedures

During speculum examination, the physician used a cotton-tipped applicator to swab the posterior fornix and the lateral vaginal walls for *T vaginalis* and *C albicans*. Three endocervical swab specimens were collected. The first swab was immediately inoculated onto modified Thayer–Martin medium in the clinic, stored in a candle extinction jar, and then transported the same day to the laboratory of the Centre National Hospitalier Universitaire, in Cotonou, where plates were incubated at 36 °C and inspected at 24 and 48 hours. *N gonorrhoeae* isolates were identified on the basis of typical colony morphology, oxidase reaction, and Gram stain result. The second endocervical swab was used to diagnose *C trachomatis* infection by means of an enzyme immunoassay (MicroTrak EIA; Syva, Palo Alto, CA, USA). Positive samples were confirmed by a blocking assay from the same manufacturer. The last endocervical swab was inserted into a Nalgene cryogenic vial and immediately frozen at –20 °C. This swab was shipped, frozen on dry ice, to the laboratory of the ITM for PCR processing with the multiplex Amplicor *C trachomatis/N gonorrhoeae* kit (Roche Diagnostic Systems, Branchburg, NJ) as instructed by the manufacturer. Samples positive for *N gonorrhoeae* with the Amplicor test were retested for confirmation with a 16S rRNA prototype PCR (Roche Diagnostic Systems).

For HIV testing, an EIA (Vironostika HIV mixt; Organon Teknika, Boxtel, Netherlands) was used. All positive samples were confirmed by a second EIA (ICE HIV-1.0.2; Murex Diagnostics S.A., France). Confirmed positive samples were assayed with the Multispot HIV1/HIV2 test (Sanofi Diagnostics Pasteur S.A., France) to distinguish HIV-1/HIV-2 profiles. Syphilis serology involved the use of a rapid plasma reagin (RPR) test (Becton-Dickinson, Cock-

eysville, MD), and all positive samples were confirmed by *Treponema pallidum* hemagglutination test, a procedure replaced during the study by a *T pallidum* particle agglutination test (TPPA; Fujirebio, Tokyo, Japan).

Definitions of Syndromic Diagnosis for Cervical Infections

In Benin, current syndromic diagnosis for cervicitis in FSWs is based on the presence of one of the clinical signs of cervicitis (mucopus, a positive swab test, or the observation of blood on the endocervical swab), a diagnosis of pelvic inflammatory disease (PID) (based on cervical motion tenderness and/or lower abdominal pain during the bimanual palpation of the pelvis), or a count of more than 10 polymorphonuclear leukocytes (PMNs) per field in a vaginal smear.

Data Analysis

We used SAS system 6.12 software (SAS Institute, Cary, NC) to analyze the data. In univariate analysis, proportions were compared with the chi-square or Fisher exact test when necessary. Multivariate analysis was performed by means of logistic regression. Using the coefficients of the model, we calculated a score as an alternative to the current syndromic diagnosis approach. Each coefficient was multiplied by 10 and then rounded.² The sum of the rounded coefficients defined the score. The cutoff value for a positive score was chosen so that the sum of sensitivity and specificity was maximal. The syndromic approach, the laboratory tests performed locally in Cotonou to detect *N gonorrhoeae/C trachomatis*, and the score system were evaluated according to a "gold standard," defined as follows: isolation of *N gonorrhoeae* in culture, a positive Amplicor PCR (confirmed by an *N gonorrhoeae* 16S rRNA assay), or an Amplicor PCR positive for *C trachomatis* was considered evidence of cervical infection with *N gonorrhoeae* or *C trachomatis*.

Results

Sociodemographic and Behavioral Data

The mean age of the 481 women included in this study was 28.8 years (median, 28). Among these women, approximately 80% were temporary immigrants from surrounding African countries. Only 22.2% of the women were Beninese. Of all the participants, 17.3% had never gone to school, whereas 49.2% had at least a secondary level education. The mean duration of prostitution among the participants was 2.7 years (median, 2). The women reported having sexual intercourse with an average of four clients per working day. The median percentage of condom use with clients in the previous week was 75%. Finally, 48.6% of the

TABLE 1. Prevalence of STIs and HIV Infection Among 481 Female Sex Workers in Benin

Finding	Number (%) Infected	95% CI
<i>Neisseria gonorrhoeae</i>	102 (21.2)	17.7–25.0
<i>Chlamydia trachomatis</i>	25 (5.2)	3.5–7.5
<i>N gonorrhoeae</i> or <i>C trachomatis</i>	118 (24.5)	20.8–28.5
<i>Trichomonas vaginalis</i>	39 (8.1)	5.9–10.8
<i>Candida albicans</i>	42 (8.7)	6.5–11.5
HIV*	199 (41.4)	37.0–45.8
<i>Treponema pallidum</i> [†]	7 (1.5)	0.6–3.0

*All these women were reactive to HIV-1; 2.2% had a dual HIV-1/HIV-2 serologic profile.

[†]Because of low prevalence, syphilis testing was stopped during the course of the study (n = 457).

STI = sexually transmitted infection.

women reported taking antibiotics (mainly ampicillin) as a method of STI prevention.

Prevalence of STIs and HIV Infection

Table 1 presents the prevalences of STIs and HIV infection in the study population. Overall, cervical infections with *N gonorrhoeae* or *C trachomatis* were detected in 24.5% of the women. *N gonorrhoeae* was the most prevalent pathogen, present in 86% (102) of all 118 cervical infections. One hundred ninety-nine participants (41.4%) had a confirmed positive HIV serology.

Risk Factors for Cervical Infections

As shown in Table 2 (univariate analysis), the risk of *N gonorrhoeae/C trachomatis* cervicitis was increased in women who had been in prostitution for less than 2 years, reported having four or more sexual partners per working day, had vaginal discharge on physical examination, had any clinical sign of cervicitis or more than 10 PMNs/field, or had a sign indicative of HIV infection. In the multivariate analysis, all these variables remained significantly correlated with cervicitis. Age, condom use with clients during the previous week, current use of antibiotics for STI prevention, and diagnosis of PID were not significantly associated with gonococcal/chlamydial infections in this population. Even though PID was not statistically associated with cervicitis, evidence of *N gonorrhoeae/C trachomatis* infection was noted in seven women with diagnosed PID. None of them had any cervical signs or more than 10 PMNs/field on the vaginal smear.

Return Visits and Adjustment of Treatment

At the return visit, if necessary, treatment was adjusted according to the Cotonou laboratory test results (positive *N gonorrhoeae* culture or positive *C trachomatis* EIA, con-

TABLE 2. Association of Selected Characteristics With Cervical *Neisseria gonorrhoeae* or *Chlamydia trachomatis* Infections Among 481 Female Sex Workers in Benin

Characteristic	Number Tested	% Infected	Crude OR (95% CI)	Adjusted OR (95% CI)*
Age (y)				NA [†]
<25	145	19.3	1	
25–34	229	26.2	1.48 (0.89–2.46)	
≥35	106	27.4	1.57 (0.87–2.85)	
Country of nationality				NA
Benin	107	18.7	1	
Nigeria	174	26.4	1.56 (0.87–2.82)	
Ghana	103	30.1	1.87 (0.99–3.56)	
Togo	79	25.3	1.48 (0.73–2.98)	
Condom use with clients in previous week				NA
Never (0%)	47	21.8	1	
Sometimes (1–49%)	65	32.3	1.77 (0.74–4.22)	
Often (50–99%)	95	24.2	1.18 (0.50–2.74)	
Always (100%)	136	22.8	1.09 (0.49–2.44)	
Prophylactic use of antibiotics for STI prevention				NA
No	247	24.3	1	
Yes	234	24.8	1.03 (0.68–1.56)	
Duration of prostitution (y)				
≥2	259	19.3	1	
<2	222	30.6	1.85 (1.22–2.80)	2.17 (1.36–3.44)
Number of clients per working day				
<4	212	15.0	1	
≥4	264	32.6	2.72 (1.72–4.29)	2.36 (1.45–3.85)
Vaginal discharge (on gynecologic examination)				
Absent	349	20.9	1	
Present	132	34.0	1.96 (1.25–3.03)	1.79 (1.09–2.95)
Any clinical sign of cervicitis [‡]				
Absent	376	20.7	1	
Present	105	38.1	2.35 (1.48–3.75)	2.28 (1.37–3.81)
Pelvic inflammatory disease				NA
Absent	441	23.8	1	
Present	40	32.5	1.54 (0.77–3.09)	
Polymorphonuclear lymphocytes				
≤10/field	435	22.3	1	
>10/field	46	45.7	2.93 (1.57–5.46)	2.05 (1.02–4.12)
HIV infection				
Negative	282	17.7	1	
Positive	199	34.2	2.40 (1.58–3.68)	2.58 (1.61–4.13)

*Only for variables that remained significantly associated with cervicitis in the multivariate analysis.

[†]NA = not applicable because not included in the final logistic regression model.

[‡]Positive swab test, mucopus, or cervical friability.

OR = odds ratio; STI = sexually transmitted infection.

firmed by the blocking assay). Within the 10-day period after the screening visit, a total of 215 participants (44.7%) returned to the clinic. Among these women, 50 (23.3%) had evidence of gonococcal/chlamydial infections (gold standard). Twenty-eight (56%) of the 50 infected women had infection diagnosed and treated after gynecologic examination (first visit), whereas 16 received treatment on the basis of the laboratory tests in Cotonou (return visit). As of April 30, 2000 (6 months after completion of enrollment), a total of 76 (15.8%) of 481 participants had not returned to the clinic for their laboratory results. Cervical infection by *N gonorrhoeae* or *C trachomatis* was detected in 25 (32.9%) of these women. Details for the different delays are shown in Table 3.

Performance of the Different Approaches for Diagnosis of Cervical Infections

Details of the performance characteristics of the different approaches are presented in Table 4. The current syndromic approach for the diagnosis of gonococcal/chlamydial infections yielded a sensitivity, specificity, and positive and negative predictive values of 48.3%, 74.7%, 38.3%, and 81.6%, respectively. Higher sensitivity would be achieved if vaginal discharge noted on gynecologic examination was considered in the current screening algorithm or with a score system including the duration of prostitution, the number of clients per working day, the presence of vaginal discharge on gynecologic examination, and the presence of any clinical sign of cervicitis in the model. This model

TABLE 3. Return Visits to the STI Clinic for 481 Female Sex Workers in Benin

Return Visit	No. (%) Who Returned (N = 481)	% With Cervical Infection (Gold Standard)*†	% Infected With HIV*
Within 10 days	215 (44.7)	23.3	36.3
Within 15 days	275 (57.2)	21.8	37.1
Within 30 days	347 (72.1)	22.6	37.5
Within 6 months of completion of enrollment	405 (84.2)	23.0	39.8
Never	76 (15.8)	32.9	50.0

*Among those who returned.

†Infection was with *Neisseria gonorrhoeae* or *Chlamydia trachomatis*. STI = sexually transmitted infection.

resulted in a sensitivity of 64.4% and specificity of 67.7%. Adding the PMN count did not significantly improve the performance of the score system. PID did not contribute at all to the score system.

Considered to be 100% specific by definition, culture for *N gonorrhoeae* was 81.4% sensitive (83/102) when compared with a reference defined as a positive *N gonorrhoeae* culture or positive Amplicor PCR (confirmed by an *N gonorrhoeae* 16S rRNA test). *C trachomatis* EIA showed a sensitivity of 28% (7/25) and a specificity of 99.3% (453/456) when the Amplicor *C trachomatis* PCR results were considered as the reference. Taken together, these tests were 74.6% sensitive and 99.5% specific in comparison with the laboratory "gold standard" for *N gonorrhoeae/C trachomatis* infection. Positive and negative predictive values were 97.8% and 92.3%, respectively. However, if the return rate of infected women within the fixed 10-day period is taken into account, the sensitivity of the local laboratory tests would be reduced from 74.6% to 28.8%. In fact, among the 118 participants with evidence of gonococcal/chlamydial

infections at screening (gold standard), only 34 of those with a positive result according to the local laboratory tests returned to the clinic within the agreed time. The sensitivity values for return visits within 15 and 30 days would be 33.9% and 49.2%, respectively.

Finally, the approach combining the screening algorithm and the laboratory tests in Cotonou resulted in a sensitivity of 61.9% and a specificity of 74.4%, when the 10-day delay was considered.

Discussion

Our findings demonstrate that the prevalence of cervical infections by *N gonorrhoeae* and/or *C trachomatis* still remains high in FSWs in Benin. However, the rate we found (24.5%) is lower than the previously reported prevalence among FSWs in that country (46% in 1993 and 33% in 1995–1996).^{4,5} The observed decline in the prevalence of cervical infections over time may be attributable to increased awareness by the women and their clients about the

TABLE 4. Performance of the Different Approaches for the Diagnosis of *Neisseria gonorrhoeae* or *Chlamydia trachomatis* Cervical Infections Among 481 Female Sex Workers (FSWs) in Benin

Diagnostic Approach	Sensitivity (%) (n = 118)	Specificity (%) (N = 363)	Positive Predictive Value (%)	% Diagnosed As Positive
Screening algorithm	48.3	74.7	38.3	31.0
Screening algorithm or vaginal discharge finding	66.1	58.7	34.2	47.8
Clinical findings only*	62.7	60.0	33.8	45.9
Score system† (>15)	64.4	67.7	39.8	40.1
Local laboratory tests in Cotonou				
Assuming all FSWs return‡	74.6	99.5	97.8	18.7
Within 10 days	28.8	99.7	97.1	7.2
Within 15 days	33.9	99.4	95.2	8.7
Within 30 days	49.2	99.4	96.7	12.5
Screening algorithm + local laboratory tests				
Assuming all FSWs return	89.0	74.1	52.8	41.3
Within 10 days	61.9	74.4	44.0	34.5
Within 15 days	63.6	74.1	44.4	35.6
Within 30 days	72.0	74.1	45.5	37.6

*Any cervical sign, pelvic inflammatory disease, or vaginal discharge.

†Risk scores for the variables in the model were as follows: <2 years in prostitution: 6; ≥4 clients/day: 10; any cervical sign: 8; and vaginal discharge: 7. The cutoff value for a positive score was >15.

‡Come back for the return visit.

importance of HIV infection and AIDS and the fact that STI counseling, diagnostic, and treatment services are available to them at the Cotonou 1 STI Clinic and have been since 1993. In the current study, approximately 40% of the women reported consistent condom use with their clients, versus 17% in the period of 1995 to 1996. In a study of male clients of FSWs in Cotonou in 1998, 39% of them acknowledged always using a condom for sexual intercourse with FSWs.²²

Despite the fact that cervical signs were not documented separately in this study design, the presence of any sign was significantly associated with gonococcal/chlamydial infections. Vaginal discharge on gynecologic examination was found to be related to cervical infections by either pathogen. Similar results were reported after previous studies of FSWs in sub-Saharan Africa.²⁻⁸ Thus, the inclusion of vaginal discharge in the current criteria for the syndromic approach would increase the sensitivity from 48.3% to 66.1%, but at the expense of specificity, as shown in Table 4.

The sensitivity of clinical diagnosis as currently applied in Cotonou is quite similar to that reported recently by Deceunick et al.⁸ for their algorithm based on clinical findings only. Even though our findings underline the poor positive predictive value of clinical diagnosis, resulting in a high proportion of overtreatment, the matter of false positives for FSWs in developing countries is not as important as in the industrialized world, since periodic presumptive treatment of these women is now being seriously considered. This strategy has recently been evaluated for FSWs in South Africa, where it resulted in a reduction in STI prevalence among both these women and their clients.²³ In addition, selective presumptive treatment of FSWs in the Philippines resulted in a significant decrease in the prevalence of gonorrhea.²⁴

A score system based on clinical findings (vaginal discharge, any cervical sign) and behavioral characteristics (duration of prostitution, number of clients per working day) performed better than the current syndromic approach. Although adding the PMN count to the above factors increased the sensitivity of the score system from 64.4% to 69.5%, it should be noted that in the context of African countries, even this basic laboratory test is not always available or may be problematic to perform. Apart from the technical difficulties related to the performance of laboratory tests in the developing world, such tests are generally not affordable for the health systems. When prescribed, they often have to be paid for by the patients, but most—including FSWs—cannot afford them. In addition, the extra costs of transportation to return to the clinic for the laboratory results and the asymptomatic nature of STIs in women do not encourage the attenders to pay for the tests.

In this study, *N gonorrhoeae* culture performed very well, in contrast with previous experiences in Cotonou and elsewhere in Africa, where it yielded sensitivities between

22.5% and 38%.^{3,8,25} The poor performance of the *C trachomatis* EIA did not dramatically affect the overall validity of the test-based diagnosis of *N gonorrhoeae/C trachomatis* in Cotonou because *N gonorrhoeae* was the most predominant pathogen causing cervical infections in our study population.

Even though the approach based on laboratory results showed an acceptable performance in this study, only 50 (34 with a positive result according to the locally available tests) of the 118 infected participants returned to the clinic within the agreed time of 10 days to receive their results. Some of these women may have come back because they were interested in receiving the study product of the COL-1492 microbicide trial and to collect the money given as compensation for transportation and time spent at the clinic. Despite these incentives, as well as special efforts made by the team of the clinic to convince the women to come back, the rate of return visits within the agreed time was low. We thus think that in the context of routine activities at the clinic, it would not be possible to improve this rate. Therefore, even when laboratory tests are available, the syndromic approach to diagnosis of cervical infections in FSWs in Benin should be applied concomitantly. Moreover, in addition to the high mobility of these women, the asymptomatic nature of genital infections with *N gonorrhoeae/C trachomatis* in women as well as the ease with which antibiotics are purchased in informal settings in Benin are negative factors for return visits. Thus, in the context of African countries, use of the syndromic approach may result in the correct treatment of a similar or even higher proportion of *N gonorrhoeae/C trachomatis* cervical infections than use of laboratory tests. Furthermore, in contrast with the disappointing rate for return visits some days after screening, all the participants agreed to wait approximately 15 to 20 minutes at the clinic for laboratory results from the direct microscopic tests. On the basis of this experience, we believe that the development of an inexpensive and reliable laboratory screening test for STIs in such populations should take into account the delay time before results are available. In fact, a study by Gift et al²⁶ has demonstrated that in settings in which return visits for treatment are problematic, a rapid test processed at the point of care could detect and treat more cases of *C trachomatis* in women than two more sophisticated and sensitive laboratory-based tests (culture and PCR). In their study, the rate of return visits was less than 65%. In our study, the rate of return visits for treatment within the agreed period of 10 days was 42.4%. Thus, when this return visit rate is taken into account, the screening algorithm resulted in the correct treatment of 48.3% of the infected women, in contrast to 28.8% for laboratory tests in Cotonou.

In summary, our data confirm the poor sensitivity of the syndromic approach in diagnosing cervical infections with

N gonorrhoeae/C trachomatis in FSWs and suggest that a score system could be a better alternative. To improve the sensitivity of the syndromic approach, we have suggested the inclusion of vaginal discharge in the current algorithm criteria in Benin. Despite its poor performance, when the low rate of return visits by FSWs to receive their results is taken into account, the sensitivity of the syndromic approach is actually higher than that of the local laboratory tests. The study thus demonstrates that the syndromic approach still remains very useful and should be applied in these populations, even when valid laboratory tests are available. Alternatively, especially when FSWs from a high-prevalence setting attend STI clinical services for the first time, presumptive treatment of cervical infection could be considered.

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