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Evaluation of clinical algorithms for the diagnosis of gonococcal and chlamydial infections among men with urethral discharge or dysuria and women with vaginal discharge in Benin

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Objective: To evaluate the validity of clinical algorithms proposed in Benin for the diagnosis of gonococcal or chlamydial infections among men with urethral discharge or dysuria and women with vaginal discharge consulting health services in Benin. These algorithms were adapted from those proposed by the World Health Organisation.
Methods: Consecutive patients with these symptoms were enrolled at three primary healthcare centres. The reference test for gonorrhoea was a combination of results from culture and polymerase chain reaction and chlamydial infection was ascertained by enzyme linked immunosorbent assay and PCR. In women, two algorithms were evaluated, one based on symptoms and risk assessment (algorithm A), the other relying also on speculum examination (algorithm B). The first algorithm evaluated in men relied on clinical examination only (algorithm C) whereas the other used microscopic examination of urethral smears (algorithm D). Sensitivity, specificity, and positive and negative predictive values of these algorithms were assessed using standard methods.
Results: In 192 women, the prevalence of gonococcal and chlamydial infections was 5.7% and 2.1% respectively (combined prevalence of 7.8%). The sensitivity, specificity, positive and negative predictive values of algorithm A (algorithm B) were respectively 86.7% (93.3%), 41.8% (34.5%), 11.2% (10.8%), and 97.4% (98.4%). In 105 men, the corresponding prevalences were 39.0% and 7.6% respectively (for a combined prevalence of 44.8%). The sensitivity, specificity, positive and negative predictive values of algorithm C (algorithm D) were respectively 91.5% (87.2%), 60.3% (67.2%), 65.2% (68.3%), and 89.7% (86.7%).
Conclusion: A syndromic approach for the diagnosis of urethritis in men appears appropriate. In women, the diagnosis of gonococcal or chlamydial infection without specific laboratory tests, which are not easily affordable in developing countries, remains highly problematic.
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Introduction

Sexually transmitted diseases (STDs) have been shown to increase the risk of HIV transmission.[1–3] In the particular case of developing countries, effective programmes that would include affordable strategies for screening, diagnosis, and treatment of those diseases are urgently required.

The World Health Organisation (WHO) has recently developed diagnostic algorithms for STDs to be used in developing countries.[4,5] These algorithms rely on elements from the medical and sexual history, simple clinical data, and readily available laboratory screening tests. However, the validity of these algorithms has not been extensively evaluated. Existing data suggest that for the diagnosis of gonococcal or chlamydial infections in women, algorithms perform poorly,[6,7] whereas in men those algorithms have an acceptable sensitivity and specificity.[8,9] For women in general, the use of diagnostic algorithms is complicated by the frequent absence of symptoms related to genital infection with *Neisseria gonorrhoeae* and *Chlamydia trachomatis*.[6,7]

We studied the validity of diagnostic algorithms among men with urethral discharge or dysuria, and women with vaginal discharge, consulting healthcare facilities in Benin, comparing their performance with standard laboratory diagnostic procedures. Infection with *N gonorrhoeae* was determined by culture and polymerase chain reaction (PCR), and *C trachomatis* infection was assessed by enzyme immunoassay (EIA) and PCR.

Patients and methods

Men and women consulting for STD symptoms were recruited from two clinics, one in Cotonou (Guézo camp) and the other at Dangbo. We also recruited non-pregnant women consulting for STD symptoms at the Lagune maternal clinic in Cotonou. All men consulting for urethral discharge or dysuria and all women consulting for vaginal discharge were

invited to participate. A total of 192 women and 105 men was recruited over a period of 12 months, starting in March 1993.

At each participating clinic, a physician was responsible for administering the questionnaire and examining the study participants. Information regarding various demographic and personal characteristics, including sexual behaviours and previous history of STDs was collected. For women, questions about use of contraception, gynaecological, and obstetric problems were also asked. The physician performed a genital examination, with direct visualisation of the cervix using a speculum. He noted the presence of ulcers, inguinal adenopathies, warts, vaginal and cervical discharge, and cervical motion tenderness. For men, he noted the presence of ulcers, warts, inguinal adenopathies, and urethral discharge.

Urethral samples were obtained from men whereas, for women, vaginal and cervical samples were taken, and the presence of pus on a cervical swab was noted (swab test). Blood and first void urine samples were taken from each patient.

Urethral, vaginal, and endocervical smears were prepared for direct microscopic examination and for Gram stain. Unstained vaginal smears were examined for *Candida albicans* and *Trichomonas vaginalis*. The presence of white blood cells and Gram negative diplococci was ascertained on Gram stained slides. A leucoesterase dipstick (LED, Nephur test, Boehringer Mannheim, Mannheim, Germany) test was performed by immersion in urine. Finally, urine was centrifuged and white blood cells counted. All these tests were performed on site at the clinics.

Modified Thayer–Martin media were seeded on site at the clinics, placed in candle extinction jars, and carried at ambient temperature to the National Public Health Laboratory in Cotonou, Benin, whereas other specimens were carried in iced containers. At the National Laboratory, the following tasks were done: incubation of the Thayer–Martin media and identification of *N gonorrhoeae* by oxidase testing and Gram stain; a rapid plasma reagin test (RPR test, Becton–Dickinson, Cockeysville, MD, USA) and a *Treponema pallidum* haemagglutination test (TPHA test, Fujirrbio, Tokyo, Japan) for syphilis serology; an enzyme immunoassay (EIA test, Vironostica HIV mixt, Organon Teknika, Boxtel, Netherlands), followed by a rapid confirmatory test (Recombigen HIV–1/2, Cambridge Biotec, Galway, Ireland) for positive sera by EIA test and a Pepti LAV VIH–1/2 test (Diagnostics Pasteur, Marne La Coquette, France) for HIV serology; finally, *C trachomatis* was diagnosed by an enzyme immunoassay (Microtrak EIA, Syva, Palo Alto, CA, USA) and confirmed by a blocking assay using reagents from the same manufacturer.

For each participant, a genital specimen was stored at −20°C and shipped to the microbiology research laboratory at the Hôpital Saint-Sacrement (Quebec, Canada), for detection of *C trachomatis* and *N gonorrhoeae* by polymerase chain reaction (PCR test, Amplicor CT/NG, Roche, Branchburg, NJ, USA).

For *N gonorrhoeae*, when there was discordance between the culture and the PCR results, an aliquot of the sample was sent to the St Joseph's Hospital Regional Virology and Chlamydiology Laboratory (Hamilton, Canada) where it was tested blindly with a PCR for *N gonorrhoeae* using different primers from those used in the Amplicor test (cppB gene derived primers[10]). A patient was considered to be infected when the culture was positive or, in case of negative culture, when both PCRs were positive.

A patient was considered to be infected with *C trachomatis* when both EIA and Amplicor PCR tests were positive. In case of discordance, two additional PCRs were performed blindly before making a decision on the infection status. Firstly, in Quebec, samples were tested with a PCR using major outer membrane protein (MOMP) primers with 1024 base pairs.[11] Secondly, in Hamilton, they were tested with a PCR using the KL1–KL2 plasmid primers.[12] Samples had to be positive by at least two of the tests (the EIA and one PCR or two different PCRs) to be considered positive for *C trachomatis*.

Different diagnostic algorithms have been developed by WHO for the care of STD patients in developing countries.[4,5] In women, we evaluated the performance of two algorithms presented in figure 1 (algorithms A and B). These algorithms were only slightly modified from those recently proposed by the WHO[5] to take into account the fact that the presence of lower abdominal pain may also be indicative of pelvic inflammatory disease (PID), a condition where treatment for gonorrhoea and chlamydial infection is required. Algorithm A corresponds to the situation where no gynaecological examination is possible whereas algorithm B may be used when available facilities allow such an examination. Algorithms in figure 2 (algorithms C and D) were applied to men consulting for STD symptoms. These algorithms differ slightly from those proposed by the WHO in that they include both dysuria and urethral discharge as a starting point. In addition, algorithm D (to be used when microscopy is available) do not distinguish gonorrhoea from chlamydial infection and do not require clinical ascertainment of the urethral discharge. This latter algorithm was chosen with the purpose of comparing two different methods of documenting urethritis: one based on clinical examination (algorithm C) and the other on microscopy (algorithm D). Finally, all the algorithms we evaluated were being considered at that time for field use in Benin.

The gold standard to which the diagnosis made with those algorithms was compared was the presence of either gonococcal or chlamydial infection according to the reference tests as defined above. These two infections were considered together because they are difficult to distinguish clinically and because the outcome of the algorithms imply that they are treated simultaneously. Based on these comparisons, we determined the sensitivity, specificity, and

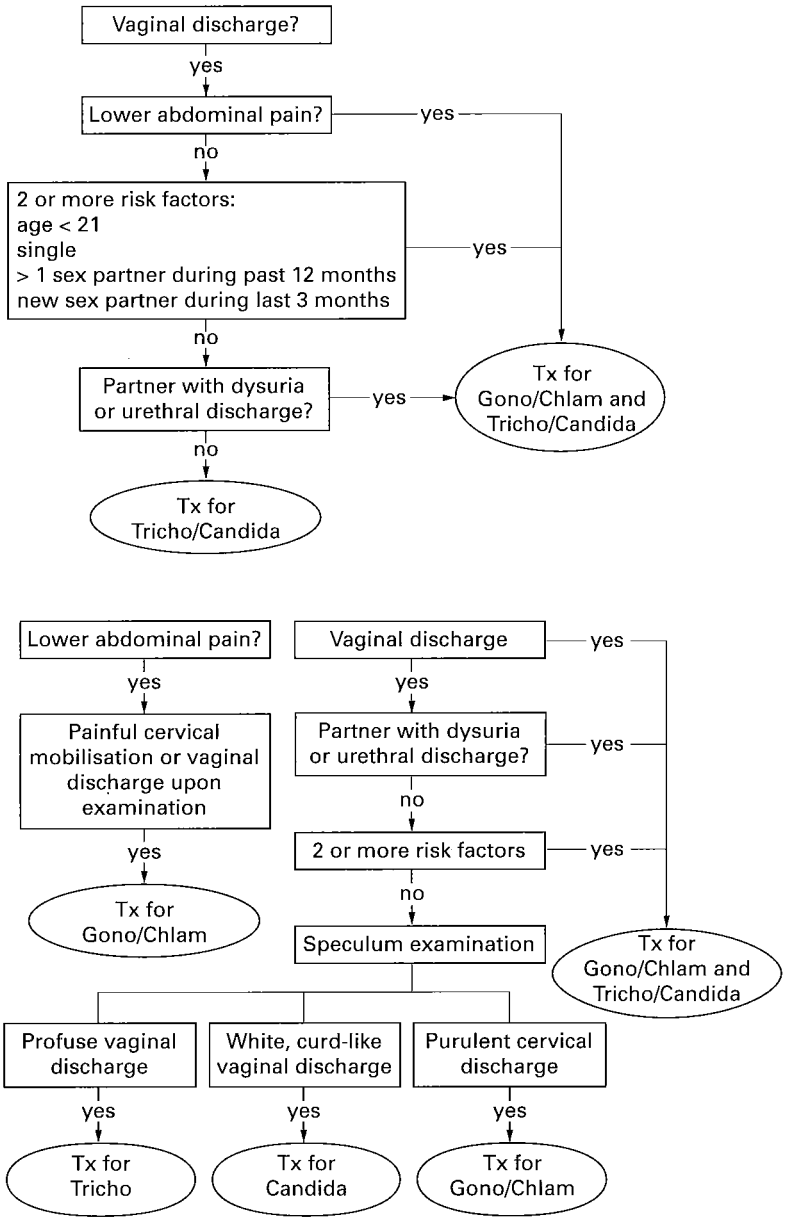

Figure 1 Algorithms evaluated in women. The algorithm at the top of the figure is algorithm A. The algorithm at the bottom of the figure is algorithm B. In algorithm B, the "2 or more risk factors" are the same as in algorithm A. In algorithm B, vaginal discharge and lower abdominal pain are evaluated in parallel. Women with suspected pelvic inflammatory infection (PID) according to this algorithm receive a more aggressive treatment than the others but this treatment also includes drugs for gonorrhoea and chlamydial infection. Tx = treatment. Gono/Chlam = gonorrhoea and chlamydia. Tricho = trichomonas. Tricho/Candida = trichomonas and candida.

positive and negative predictive values of the algorithms. In statistical analyses, proportions were compared using the Yates's corrected χ^2 statistic or the Fisher's exact test.

Results

We first compared the results of gonococcal culture and chlamydial EIA test with those of PCRs after resolution of discrepant samples, using data from both men (n = 105) and women (n = 192). Overall, 52 of the 297 samples (17.5%) were considered positive for *N gonorrhoeae*. Of these, 14 were positive by both culture and at least one PCR, 35 were negative by culture but positive by both PCRs and three were positive by culture but negative by both PCRs. The latter three samples were all from women. Among the 14 samples positive by culture and by at least one PCR, four (28.6%) were Amplicor PCR negative and cppB PCR positive. Thus, among the 52 samples considered positive for *N gonorrhoeae*, 45 were positive by the Amplicor PCR, for a sensitivity of 86.5%. Among the 245 samples resolved as negative, three (1.2%) were positive by this PCR for a specificity of 98.8%. In this study, the sensitivity of gonococcal culture was very low (28.6%). By definition, its specificity was 100%. It has to be noted that intracellular Gram negative diplococci were detected on the urethral smears of 36 of the 41 men with gonococcal infection for a much better sensitivity (87.8%) than that of culture. The specificity of this examination of Gram stained smears was 87.5% (56/64).

Overall, 12 samples (4.0%) were considered positive for *C trachomatis*. Of these, six were positive by both EIA and Amplicor PCR (they were thus not submitted to additional tests) and six were negative by EIA but positive by at least two PCRs (three were positive by all the PCRs, two were positive by Amplicor and KL1–KL2 plasmid PCRs but negative by the MOMP PCR, and one was positive by Amplicor and MOMP PCRs but negative by the KL1–KL2 plasmid PCR). In addition, two samples were positive by EIA but negative by the three PCRs, resulting in a specificity of 99.3% for EIA (283/285). However, the sensitivity of this test was only 50% (6/12). One of the 285 samples considered as negative (in-

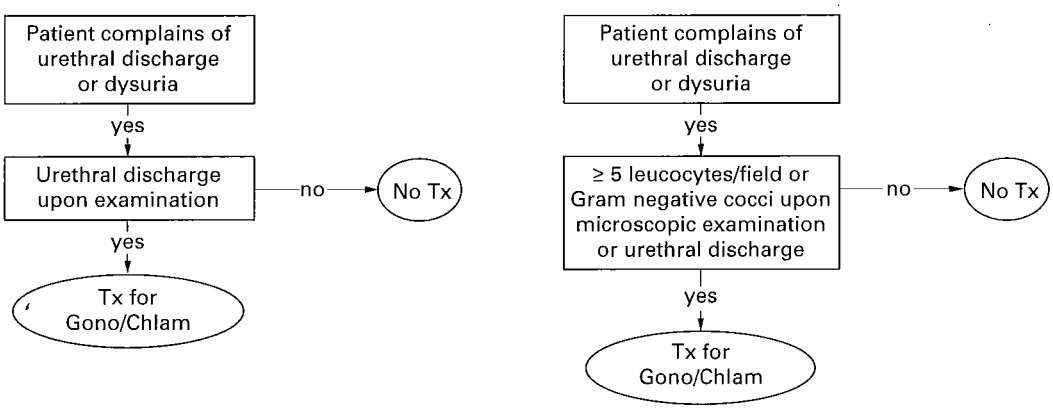

Figure 2 Algorithms evaluated in men. The algorithm on the left is algorithm C. The algorithm on the right is algorithm D. Tx = treatment. Gono/Chlam = gonorrhoea and chlamydia.

Table 1 Prevalence of different STDs among men consulting for dysuria or urethral discharge and women consulting for vaginal discharge in Benin

	Men (n=105)	*Women (n=192)*
Gonorrhoea	39.0%	5.7%
Chlamydia	7.6%	2.1%
Gonorrhoea or chlamydia	44.8%	7.8%
Trichomoniasis	—	11.5%
Candidiasis	—	32.3%
Genital ulcers	3.7%	9.9%
Positive syphilis serology*	4.8%	2.1%
HIV-1†	1.0%	2.1%

* RPR+ and TPHA+.
† No subject was positive for HIV-2.

Table 2 Prevalence of infection by N gonorrhoeae or C trachomatis according to selected characteristics among 192 women complaining of vaginal discharge

	Characteristic present?			
	Yes (n)	*No (n)*	*OR*	*p Value**
Age <25 years	14.3% (49)	5.6% (143)	2.8	0.065
Single	7.1% (42)	8.0% (150)	0.9	1.000
>1 partner, last year	8.7% (23)	7.7% (169)	1.1	0.697
New sexual partner, last 3 months	15.4% (13)	7.3% (179)	2.3	0.269
Symptoms				
Dysuria	12.9% (62)	5.4% (130)	2.6	0.086
Lower abdominal pain	11.1% (117)	2.7% (75)	4.6	0.051
Clinical signs				
Vaginal discharge	8.9% (169)	0.0% (23)	—	0.223
Cervical mucopus	14.3% (49)	5.6% (143)	2.8	0.065
Cervical motion tenderness	11.0% (73)	5.9% (119)	2.0	0.268
Simple laboratory tests				
Positive swab test	15.9% (44)	5.4% (148)	3.3	0.048
>10 leucocytes/field (cervix)	13.8% (29)	6.7% (163)	2.2	0.250
>10 leucocytes/field (urine)	11.8% (17)	7.4% (175)	1.7	0.627
Gram negative diplococci (cervix)	11.1% (18)	7.5% (174)	1.5	0.637

* Two tailed Fisher's exact test.

Table 3 Prevalence of infection by N gonorrhoeae or C trachomatis according to selected characteristics among 105 women complaining of urethral discharge or dysuria

	Characteristic present?			
	Yes (n)	*No (n)*	*OR*	*p Value**
Age <25 years	38.7% (31)	47.3% (74)	0.5	0.214
Single	33.3% (48)	54.4% (57)	0.4	0.049
>1 partner, last year	50.0% (72)	33.3% (33)	2.0	0.167
No condom use during last year	50.7% (75)	30.0% (30)	2.4	0.088
Symptoms				
Dysuria	41.4% (87)	61.1% (18)	0.5	0.126
Urethral discharge	63.8% (69)	8.3% (36)	19.4	<0.001
Clinical signs				
Urethral discharge	65.2% (66)	10.3% (39)	16.4	<0.001
Simple laboratory tests				
Positive LED†	60.0% (70)	12.1% (33)	12.1	<0.001
>5 leucocytes/field‡ (urethral sample)	68.3% (60)	13.3% (45)	14.0	<0.001
>10 leucocytes/field (urethral sample)	75.0% (36)	29.0% (69)	7.4	<0.001
>10 leucytes/field (urine)	70.2% (47)	23.2% (56)	7.8	<0.001
Gram negative diplococci (urethra)	81.8% (44)	18.0% (61)	20.5	<0.001

* Two tailed Yates's corrected χ^2.
† Urine samples were not obtained from two men.
‡ Criteria used in algorithm D, figure 2.

Table 4 Validity of different screening algorithms (figs 1 and 2) for the diagnosis of gonococcal and chlamydial infections among men and women with STD symptoms in Benin

	Women		*Men*	
	Algorithm A (fig 1)	*Algorithm B (fig 1)*	*Algorithm C (fig 2)*	*Algorithm D (fig 2)*
Number tested	192		105	
Number infected*	15		47	
Number of cases diagnosed†	116	130	66	60
Sensitivity (%)	86.7	93.3	91.5	87.2
Specificity (%)	41.8	34.5	60.3	67.2
Positive predictive value (%)	11.2	10.8	65.2	68.3
Negative predictive value (%)	97.4	98.4	89.7	86.7

* According to the reference test combining culture for *N gonorrhoeae*, EIA for *C trachomatis* and resolved PCR for both pathogens.
† According to the algorithm.

cluding the two samples with only a positive EIA) was positive by Amplicor but negative by the other PCRs, for a specificity of 99.6% for this test. According to our diagnostic criteria, its sensitivity was 100%, but it has to be noted that chlamydial culture was not performed in this study.

Table 1 shows the prevalence of the different STDs by sex. Among men, the most prevalent pathogen associated with urethral discharge or dysuria was *N gonorrhoeae*. Only three men with gonorrhoea had concomitant chlamydial infection. Among women, the most frequent cause of vaginal discharge was *C albicans*, followed by *T vaginalis*. The prevalence of both gonococcal and chlamydial infections was much lower in symptomatic women than in symptomatic men. Only five subjects (one man and four women) were HIV infected. Genital ulcers, according to clinical examination, were relatively frequent, especially among women. However, the prevalence of active syphilis (both RPR and TPHA positive) was relatively low.

Table 2 shows the association between selected characteristics and gonococcal or chlamydial infection in women. The characteristics most strongly related to infection among women were the presence of lower abdominal pain and a positive swab test on speculum examination. In addition, the prevalence of either disease tended to be higher among women of less than 25 years of age ($p=0.065$), among those complaining of dysuria ($p=0.086$), and among those with endocervical mucopus at clinical examination ($p=0.065$). With the exception of age, variables used in risk assessment were not at all associated with gonococcal or chlamydial infection (being single, reporting more than one sexual partner during the past year) or were reported by a very small proportion of women. Indeed, only 13 women (6.8%) reported a new sexual partner in the last 3 months whereas no woman knew about the presence of dysuria or urethral discharge in her partner. Concerning age, the cut off value used in the algorithm is not optimal as the prevalence of either disease was similar in women aged less than 21 years of age (15.4%) and those aged 21–24 (13.9%).

The mean age of men with gonococcal or chlamydial infection (30.7 years) was comparable with that of uninfected men (29.8 years). Urethral discharge as a symptom or on clinical examination as well as all indicators of urethral inflammation and the presence of intracellular Gram negative diplococci on the urethral smear were strongly associated with infection (all p values <0.001, table 3). Living as a couple with a woman ($p=0.049$) and lack of condom use ($p=0.088$) were also marginally associated with the presence of either disease. All the other studied characteristics were not significantly associated with gonococcal or chlamydial infection among men.

Table 4 shows results of the performance of the different algorithms applied to the two populations under study. Algorithms A and B (fig 1) applied in women had comparable performances, with a high sensitivity and a low specificity. Because of the low prevalence of

infection in this population group, the positive predictive value was only slightly higher than 10%. Among those women, algorithm B also allowed us to test for the presence of infections with *T vaginalis* and *C albicans*. When taken together (using the presence of candida and/or trichomonas on microscopic examination of vaginal discharge), the algorithm had a sensitivity and a specificity of 52.1% and 58.0% respectively and positive and negative predictive values of 46.8% and 63.0%, respectively. By definition, algorithm A had a sensitivity of 100% and a specificity of 0% for the detection of *C albicans* or *T vaginalis*, with a positive predictive value equivalent to the prevalence of either of these infections (42.2%).

Among men, the algorithm based on clinical examination alone (algorithm C, fig 2) had a sensitivity of more than 90% and a specificity of approximately 60%. The algorithm based on microscopic examination of urethral smears among all subjects complaining of urethral discharge or dysuria (algorithm D, fig 2) only slightly increased specificity at the price of a small decrease in sensitivity. In addition, 13 of the 19 subjects positive for algorithm D but without confirmed gonococcal or chlamydial infections had more than five leucocytes per field at physical examination and could be considered as having urethritis. Finally, if evidence of urethral discharge at clinical examination would have been required before performing microscopic examination, specificity would have been increased to 82.8% with still a relatively high sensitivity (83.0%). The positive and negative predictive values of this modified algorithm would have been 79.6% and 85.7% respectively.

Discussion

In the initial study design, the intention was to rely on gonococcal culture and *C trachomatis* EIA for defining the presence or absence of infection. However, owing to logistic problems with specimen transportation, it became obvious that, at least for gonococcal culture, sensitivity was suboptimal. Subsequent analysis of clinical specimens by PCR confirmed our impression. Firstly, the results of the Amplicor PCR showed a much higher prevalence than that measured by culture and EIA. Secondly, the difference between the prevalence estimates of chlamydial infection obtained by EIA and PCR was less pronounced than for gonorrhoea, which probably reflects the lower susceptibility of the EIA technique to adverse transport conditions of genital specimens. Thirdly, after resolution of discrepant samples using additional PCRs, the Amplicor PCR appeared to be highly sensitive and specific both for *N gonorrhoeae* and *C trachomatis*, in concordance with previously published results.[13 14] Finally, among men, the presence of Gram negative intracellular diplococci, a generally reliable test for the diagnosis of gonococcal urethritis,[15 16] was much more strongly correlated with PCR results than with culture.

Some of the samples positive for *N gonorrhoeae* by culture were negative by both PCRs. This may be related to the presence of unidentified inhibitors that could have contributed to decrease the sensitivity of the PCR assays.[17 18] In the case of chlamydial infection, since culture was not used, the samples positive by EIA but negative by all three PCRs were possibly related to polymerase inhibition or were false positives, as this technique is not absolutely specific even when a blocking assay is used for confirmation.[19] Overall, the results obtained with traditional laboratory methods underscore their limitations (especially for gonococcal culture) when applied to field research in developing countries.

In this study, few personal and clinical characteristics were associated with gonococcal or chlamydial infection, particularly among women. This may be due, to some extent, to the relatively small sample sizes and even more to the low prevalence of either disease among women. In addition, STD symptoms and clinical signs are usually less sensitive and specific in women than in men. Furthermore, the factors used in the risk assessment system proposed by the WHO for the diagnosis of cervicitis were not very good indicators of this condition in the Beninoise context. Indeed, the prevalence of gonococcal or chlamydial infection was similar in all women aged less than 25 whereas the other factors either were not associated with these infections or were reported by a very low proportion of women. Both algorithms used in women were highly sensitive to detect gonococcal or chlamydial infection but their specificity was very low. Algorithm A has the advantage of relying only on elements from the medical and sexual history without the necessity to proceed to genital examination, thus simplifying patient care. Finally, algorithm B had a poor performance with regard to the diagnosis of trichomoniasis and candidiasis. However, this performance could have been better if bacterial vaginosis, a condition not specifically assessed in this study, had been considered.

Among men, it is interesting to see that the algorithm that takes into account microscopic examination of Gram stained urethral smears (algorithm D) did not perform much better than algorithm C which relies only on the presence of symptoms and signs of urethritis. Even though this laboratory test is relatively simple, its use in most situations encountered in developing countries is thus questionable, because it may complicate patient care and increase costs. Because of this, algorithm C is now in use in most primary healthcare facilities in Benin. However, in settings where microscopy is readily available (generally in hospitals or large urban health centres), algorithm D modified to take into account the presence of urethral discharge on clinical examination and to distinguish gonorrhoea from chlamydia based on the presence of Gram negative intracellular diplococci is currently recommended.

In conclusion, this study shows that, in men, simple algorithms for urethral discharge or dysuria, relying on elements from the questionnaire and physical examination, have

acceptable sensitivity and specificity that can greatly simplify STD patients' care. However, the low prevalence of gonococcal and chlamydial infections among women consulting for vaginal discharge in Benin correlates with a low positive predictive value with regard to these infections. In this population, the diagnosis of gonococcal and chlamydial infection without specific laboratory test remains highly problematic. Thus, there is an urgent need for the development of cheap, reliable, and simple laboratory tests for the diagnosis of gonococcal and chlamydial infections to be used among women from developing countries.

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