

## ORIGINAL ARTICLE

# Sexually transmitted infections in male clients of female sex workers in Benin: risk factors and reassessment of the leucocyte esterase dipstick for screening of urethral infections

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**Objectives:** (1) To assess risk factors for urethral infections with *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and *Trichomonas vaginalis* among male clients of female sex workers (FSWs) in Benin; (2) to study the validity of LED testing of male urine samples compared to a highly sensitive gold standard (PCR) for the diagnosis of urethral infections with the organisms cited above.

**Methods:** Male clients of FSWs (n = 404) were recruited on site at prostitution venues in Cotonou, Benin, between 28 May and 18 August 1998. A urine sample was obtained from each participant just before he visited the FSW, and tested immediately using a leucocyte esterase dipstick (LED) test. It was then tested for HIV using the Calypte EIA with western blot confirmation, and for *C trachomatis*, *N gonorrhoeae*, and *T vaginalis* by PCR. After leaving the FSW's room, participants were interviewed about demographics, sexual behaviour, STI history and current symptoms and signs, and were examined for urethral discharge, genital ulcers, and inguinal lymphadenopathies.

**Results:** STI prevalences were: *C trachomatis*, 2.7%; *N gonorrhoeae*, 5.4%; either chlamydia or gonorrhoea 7.7%; *T vaginalis* 2.7%; HIV, 8.4%. Lack of condom use with FSWs and a history of STI were independently associated with *C trachomatis* and/or *N gonorrhoeae* infection. Over 80% of these infections were in asymptomatic subjects. The overall sensitivity, specificity, positive and negative predictive values of the LED test for detection of either *C trachomatis* or *N gonorrhoeae* were 48.4%, 94.9%, 44.1%, and 95.7%, respectively. In symptomatic participants (n = 22), all these parameters were 100% while they were 47.4%, 94.7%, 37.5%, and 96.4% in asymptomatic men (n = 304).

**Conclusions:** Since most STIs are asymptomatic in this population, case finding programmes for gonorrhoea and chlamydia could be useful. The performance characteristics of the LED test in this study suggest that it could be useful to detect asymptomatic infection by either *C trachomatis* or *N gonorrhoeae* in high risk men.

In developing countries, preventive interventions for HIV and sexually transmitted infections (STIs) directed at high risk populations often target female sex workers (FSWs), even though the male clients of these women also represent a significant source of STIs and HIV, as well as acting as a bridge for disease transmission between FSWs and women in the general population. In fact, reports from sub-Saharan Africa have shown high prevalence of HIV in clients of FSWs<sup>1</sup> and in populations of men frequently using prostitution services, ranging from 8.4% to 56%.<sup>2–4</sup> Despite these dramatic figures, these men are rarely specific targets for intervention because they are considered very hard to reach.<sup>5–8</sup>

A better understanding of sociodemographic and behavioural characteristics associated with STIs and HIV in male clients of FSWs in Africa could aid in the formulation of effective interventions for their prevention. STI control and HIV prevention strategies in men should also focus on the diagnosis and treatment of curable STIs. Such a combined strategy, including promotion of condom use as well as detection and treatment of STIs, has been shown to reduce the prevalence of these diseases and of HIV infection among FSWs in sub-Saharan Africa.<sup>9–12</sup>

In the developing world, diagnosis and treatment of STIs are generally based on the syndromic approach, a strategy that has been shown to be effective for male urethritis.<sup>13 14</sup>

However, results from several studies suggest that a significant proportion of men with positive laboratory tests for *Chlamydia trachomatis* or *Neisseria gonorrhoeae* in their urethra are asymptomatic.<sup>15–18</sup> *Trichomonas vaginalis* has also been shown to be an important cause of urethral infection in some African settings, including in asymptomatic men.<sup>16 17</sup> The leucocyte esterase dipstick (LED) test has been proposed as a cheap non-specific test that could allow the detection of polymorphonuclear cells in asymptomatic men and also be used to confirm symptomatic urethritis.<sup>19–21</sup> However, most studies evaluating the LED test were performed in the era preceding the wider use of nucleic acid amplification tests (NAAT) and showed sensitivities varying from 50% to 100% and specificities between 50% and 80%.<sup>15–23</sup> With such results, in most instances, LED was not recommended as a screening test because of its low positive predictive value, nor was it considered particularly useful in symptomatic patients. More recently, Bowden carried out a study in Australia on the validity of the LED compared to NAAT (in this case, polymerase chain reaction, PCR) for the detection of *C trachomatis* or *N gonorrhoeae* in urine among a population of largely asymptomatic men.<sup>24</sup> He used a cut-off value of trace for the LED test to maximise the negative predictive value with the objective of selecting men for whom it would be useful to perform the PCR assay. The use of this cut off

yielded a sensitivity and specificity of 77.8% and 80.8%, respectively. From the detailed results of the study, it can be observed that a 1+ cut off for the LED test would result in a sensitivity of 50% and a specificity of 93.6%, this latter figure being higher than any previously reported.

The objectives of this study were: (1) to evaluate risk factors for urethral infections by *C trachomatis*, *N gonorrhoeae*, and *T vaginalis* among male clients of FSWs in Benin; (2) to study the validity of LED testing of male urine samples compared to a highly sensitive gold standard (PCR) for the diagnosis of urethral infections caused by the agents cited above.

## METHODS

### Background

This study was undertaken within a larger project whose overall aim was to document the STI/HIV epidemiological situation among clients of FSWs in Cotonou, Benin, the sociodemographic characteristics of these men and their sexual behaviour with FSWs and other female partners.<sup>1</sup> Health seeking behaviour for STIs was also investigated, as was the feasibility of interventions targeting these men. The study was conducted in Cotonou, the largest city of Benin with approximately 800 000 inhabitants. In this city, an ongoing (started in 1993) HIV/STI intervention project funded by the Canadian International Development Agency targets FSWs for HIV and STI prevention. The project strategies involve promotion of correct condom use and free STI treatment according to local syndromic approach guidelines, including a screening algorithm for FSWs.<sup>25</sup>

### Study population and study procedures

The data collection period for this study was between 28 May and 18 August 1998. The study population consisted of male clients of FSWs recruited directly at 13 different prostitution sites (not selected randomly, but chosen to ensure a diversity of types of prostitution venues and adequate geographical distribution in the city) between 8 pm and 1 am. The study was proposed to the men by a field worker in collaboration with the FSWs themselves and the bar and brothel owners. The study was entirely anonymous and there was no payment made for participation. However, free condoms were given to participants and, in case of STI symptoms or signs or when the LED test was positive (see below for procedures), STI treatment was provided at no charge. The study procedures were performed on site in a room or a curtained off space outside provided by the bar or brothel owner. After verbal informed consent, a first void urine sample was obtained from each participant just before he went into a room with the FSW to have sex. After the client came out of the FSW's room, he was interviewed for a duration of 15–30 minutes on demographics, sexual behaviour, STI history, and current symptoms as well as on health seeking behaviour for STIs. Thereafter, the genital area was examined for the presence of urethral discharge, ulcers, and inguinal lymphadenopathies. Ethical approval for this study was given by the ethics committee of the Centre hospitalier affilié universitaire de Québec and by the Ministry of Health, Benin.

### Laboratory procedures

A LED (Chemstrip 10A, Boehringer Mannheim, Québec, Canada) test was performed on the urine sample immediately after collection on site and aliquots were stored at 4°C for HIV testing and at –20°C for PCR testing for STIs. HIV testing was performed locally in Cotonou and in Montreal, Canada, with the Calypte EIA (Calypte Biomedical Corporation, Berkeley, CA, USA) followed by western blot confirmation (Cambridge Biotech/Calypte Biomedical

Corporation).<sup>26</sup> The Amplicor CT/NG detection kit from Roche Diagnostics was performed in Montreal for detection of chlamydial and gonococcal infections. Finally, an in-house PCR for *Trichomonas vaginalis* targeting the 650 base pair repetitive sequence was performed in the Department of Microbiology of the Université de Sherbrooke, using an adaptation<sup>27</sup> of the procedure of Shaio and collaborators.<sup>28</sup>

### Data analysis

The data were analysed using Epi-Info (Center for Diseases Control and Prevention, USA, World Health Organization, Geneva, Switzerland) and SAS (SAS Institute, Cary, NC, USA). For the analysis of STI risk factors, the prevalence odds ratio (POR) was used as the measure of association. The  $\chi^2$  and Fisher's exact tests were used for univariate analysis whereas logistic regression was used for multivariate analysis. We calculated the sensitivity and specificity (with 95% confidence intervals) as well as the positive and negative predictive values of the LED test, using a cut off of 1+, meaning that samples with a result greater than trace were considered LED positive (the possible values for the test were: negative, trace, 1+, and 2+), in comparison with a gold standard based on PCR results.

## RESULTS

A total of 404 male clients of FSWs provided a urine sample over 47 nights of data collection between 28 May and 18 August, 1998. From 17 June to 18 August, the numbers of clients refusing to participate in the study were recorded. Of 486 clients approached, 329 (67.7%) accepted and 157 (32.3%) declined participation. Of the 404 subjects who provided a urine sample, 330 (81.7%) answered the questionnaire and 298 (73.8%) had a physical examination. Among those answering the questionnaire, median age was 25.5 years (range 17–53). Overall, 34 (8.4%) of the participants were infected with HIV; 22 (5.4%) with *N gonorrhoeae*; 11 (2.7%) with *C trachomatis*; 11 (2.7%) with *T vaginalis*; 31 (7.7%) with either *N gonorrhoeae* or *C trachomatis*; and 41 (10.1%) with any of the latter three pathogens.

Among the 326 men who provided information on STI symptoms, seven (2.1%) reported urethral discharge while 17 (5.2%) complained of dysuria. Physical examination revealed that six clients out of 298 (2.0%) had genital ulcers while presence of urethral discharge was confirmed in three men (1.0%). Overall, 22 men reported either dysuria or urethral discharge or had discharge upon clinical examination. Table 1 shows the association between symptoms/signs of urethritis and STIs.

Table 1 also shows the risk factors other than symptoms/signs associated with the different STIs studied. Factors that did not show any significant association with STIs include age, ethnicity, country of origin, city of residence, marital status, and frequency of visits to FSWs. Whereas price paid to FSW, STI history, and current urethritis signs/symptoms were significantly associated with infection by either *N gonorrhoeae* or *C trachomatis* (all  $p < 0.05$ ), none of these variables were significantly related to *T vaginalis* infection. Even though current urethritis signs/symptoms were associated with gonococcal or chlamydial infection, the majority of the men infected with either pathogen (81.3%) were asymptomatic.

For the multivariate analysis (table 2), we entered all the variables identified as risk factors in the univariate analysis in a multiple logistic regression model (for condom use, since there was a strong correlation between the two condom use variables shown in table 1, we chose to enter in the model only the variable on condom use with FSWs in general). In this analysis, history of previous STI and condom use were significantly associated with chlamydial or gonococcal

**Table 1** Univariate analysis of clinical signs/symptoms and risk factors for *N gonorrhoeae*, *C trachomatis*, and *T vaginalis* infection among male clients of FSWs in Cotonou, Benin

Symptoms/signs or risk factor	No	No (%) with NG* or CT†	Prevalence odds ratio	p Value‡	No (%) with TV§	Prevalence odds ratio	p Value‡
<b>Current symptoms and/or signs<sup>¶</sup></b>							
Yes	22	5 (22.7)	4.4	0.02	2 (9.1)	4.2	0.12
No	304	19 (6.3)			7 (2.3)		
<b>Price paid to FSW</b>							
≤ 1 US \$	202	20 (9.9)	3.3	0.03	6 (3.3)	1.3	0.76
> 1 US \$	124	4 (3.2)			3 (2.4)		
<b>Condom use with FSW just seen</b>							
No	144	17 (11.8)	3.4	<0.01	5 (3.5)	1.6	0.49
Yes	182	7 (3.9)			4 (2.2)		
<b>Condom use with FSW in general</b>							
Always/mostly	173	7 (4.1)	1.0		8 (4.7)	1.0	
Sometimes	67	4 (6.0)	1.5	<0.01**	0 (0.0)	0.0	0.11**
Never	83	13 (15.7)	4.4		1 (1.2)	0.3	
<b>STI history</b>							
Yes	161	20 (12.4)	5.8	<0.01	6 (3.7)	2.1	0.29
No	166	4 (2.4)			3 (1.8)		
<b>Education</b>							
Primary/none	137	15 (11.0)	2.5	0.03	2 (1.5)	0.4	0.8
≥ high school	193	9 (4.7%)			7 (3.6%)		

\**N gonorrhoeae*; †*C trachomatis*; ‡According to  $\chi^2$  unless otherwise specified; §*Trichomonas vaginalis*; ¶reported urethral discharge or dysuria or urethral discharge on physical examination; \*\* $\chi^2$  for linear trend.

infection, whereas urethritis signs/symptoms were only of borderline significance.

The LED test was positive in 34 of the 404 subjects (8.4%). The overall sensitivity, specificity, positive and negative predictive values of the LED test, in comparison with the gold standard PCR results, for the detection of either *C trachomatis* or *N gonorrhoeae*, are shown in table 3. Whereas among symptomatic men the concordance between LED and PCR results was perfect, among asymptomatic men the sensitivity was just under 50% and the specificity approximately 95%, resulting in a positive predictive value of 37.5%. In contrast with the very strong association between LED positivity and an infection with either *N gonorrhoeae* or *C trachomatis* ( $p < 0.0001$ ), there was no significant association between a positive LED test and the presence of *T vaginalis* ( $p = 0.24$ ), irrespective of symptoms. The performance of the LED test to detect *T vaginalis* was thus very poor, with a sensitivity of 18.2% (95% CI: 2.8 to 51.8), a specificity of 91.9% (95% CI: 88.7 to 94.4), a positive predictive value of 5.9%, and a negative predictive value of 97.6%.

## DISCUSSION

Our data show that about 8% of male clients of FSWs in Cotonou were infected with either *N gonorrhoeae* or *C trachomatis*. Similar results were found by Steen and colleagues, who reported a prevalence of 10.9% for *N gonorrhoeae* or *C trachomatis* among male clients of FSWs in South Africa.<sup>29</sup> The prevalence of *T vaginalis* infection was relatively low in our study population and was not associated

with known STI risk factors in our data. In studies carried out in Kenya<sup>17</sup> and Tanzania,<sup>16</sup> *T vaginalis* was much more frequent than either *N gonorrhoeae* or *C trachomatis* in truck drivers and men in the general population, respectively, independently of the presence of symptoms. In a study on aetiology of urethritis in five west African countries, the prevalence of *T vaginalis* varied considerably from one country to the other, from 2.5% in Côte d'Ivoire to 24.5% in Senegal.<sup>30</sup> In the latter study, the prevalence of *T vaginalis* among men consulting for urethral discharge in Benin was 8.1%. These results are compatible with ours for the subgroup of symptomatic men (prevalence of 9.1%, table 1), and suggest important geographical variations in the prevalence of *T vaginalis*.

Among symptomatic subjects, the prevalence of gonorrhoea or chlamydial infection was low at only 22.7%. One reason for this could be that we did not screen the participants for *Mycoplasma genitalium*. This organism was found to be the most common cause of non-gonococcal urethritis in a study by Morency and colleagues in Bangui, Central African Republic,<sup>27</sup> and one important aetiological agent of urethral discharge in west Africa.<sup>30</sup> Furthermore, studies on symptomatic men such as those mentioned above<sup>27-30</sup> generally enrol subjects consulting spontaneously at health centres for their symptoms. In our study, enrolment

**Table 2** Multivariate analysis of risk factors for infection by either *N gonorrhoeae* or *C trachomatis* among male clients of FSWs in Cotonou, Benin

Factor	POR*	95% CI	p Value
<b>Condom use with FSW in general</b>			
Always/mostly	1.0		
Sometimes	1.1	0.3-3.9	< 0.01†
Never	4.2	1.5-11.3	
<b>STI history</b>			
STI history	6.0	1.9-18.5	<0.01
Current urethritis symptoms/signs	3.0	0.9-9.8	0.06

\*Prevalence odds ratio; †test for trend.

**Table 3** Performance of the LED test in the diagnosis of *C trachomatis* and/or *N gonorrhoeae* among male clients of FSWs in Cotonou, Benin

	All men (n = 404)	Symptomatic men* (n = 22)	Asymptomatic men (n = 304)
No (%) with NG or CT	31 (7.7%)	5 (22.7%)	19 (6.3%)
Sensitivity (%) (95% CI)	48.4 (31.3 to 65.7)	100 (47.3 to 100)	47.4 (26.1 to 69.4)
Specificity (%) (95% CI)	94.9 (92.3 to 96.8)	100 (84.7 to 100)	94.7 (91.7 to 96.9)
PPV (%)	44.1	100	37.5
NPV (%)	95.7	100	96.4

\*Men with urethritis signs/symptoms.

NG = *Neisseria gonorrhoeae*; CT = *Chlamydia trachomatis*;

PPV = positive predictive value; NPV = negative predictive value.

was not based on spontaneous complaints, but symptoms were rather actively elicited from all participants. This could well lead to an overestimation of the frequency of symptoms and, consequently, to a lower STI prevalence than that observed in studies on men with spontaneous complaints of dysuria or urethral discharge. This is very likely given that urethral discharge was confirmed in only three men on clinical examination. Finally, PCR on urine samples is less sensitive than on urethral swabs,<sup>31</sup> although it is still considered an acceptable screening test,<sup>31</sup> whereas the use of PCR on female urine samples can be much more problematic with reported sensitivities as low as 54%.<sup>32</sup>

In Cotonou, a study carried out among FSWs, at the same time as this one, demonstrated high prevalence of *N gonorrhoeae* or *C trachomatis* among these women (24.5%).<sup>10</sup> Similar results were reported from the study by Steen *et al* (24.9% among FSWs).<sup>29</sup> The observed difference in STI prevalence among FSWs and their male clients may be attributed to the fact that duration of infection is generally longer in women than in men (women being more often asymptomatic than men) and that women are more susceptible than men to acquiring these infections, as well as much more frequently exposed to STIs. In fact, while the median number of visits to FSWs by the clients was 24 per year in this study,<sup>1</sup> the corresponding figure for FSWs was 17 male clients per week.<sup>10</sup>

In multivariate analysis, gonorrhoea or chlamydial infection was associated with lack of condom use with FSW and STI history. Lower price paid to the FSW, as well as lower educational level, which were also associated with these infections in univariate analysis, did not remain significantly associated in the final multivariate model, probably because they were found to be predictors of condom use in this population.<sup>1</sup> Although condom use rates by clients with the FSW just seen were suboptimal at 56%,<sup>1</sup> our data nevertheless provide further evidence for the protective effect of condom use on STIs. The association with a history of STI, however, underlines the need for intensive preventive counselling of men when an STI is diagnosed. The recurrence of STIs in these men indicates that this opportunity is being lost in current clinical practice in Benin.

The fact that the vast majority (>80%) of infections with *N gonorrhoeae* or *C trachomatis* were asymptomatic in this population suggests that case finding programmes in this high risk male population could be very useful. Such programmes could include regular sessions of LED testing at prostitution venues, inspired from the methodology used in this study, as well as the development and promotion of specific clinical services for high risk men where LED testing would be available.

### Key points

- About 10% of the clients of female sex workers in Cotonou, Benin, had a urethral infection caused by *Neisseria gonorrhoeae*, *Chlamydia trachomatis*, or *Trichomonas vaginalis*.
- Lack of condom use and previous history of STI were associated with infection by *N gonorrhoeae* or *C trachomatis*, whereas no specific factor was associated with *T vaginalis*.
- The leucocyte esterase dipstick (LED) test performed relatively well for the detection of *N gonorrhoeae* and *C trachomatis*, but not for the detection of *T vaginalis*.
- STI case finding among high risk men in developing countries is a priority and the LED test could be useful for this purpose.

In our study, the performance of the LED test was very similar to that derived from the data presented by Bowden,<sup>24</sup> with a sensitivity of around 50% and a specificity of around 95%. In another recent study, comparing LED to ligase chain reaction (LCR) for chlamydial infection only, the results were even better, with a sensitivity of 87.5% and a specificity of 92.4%.<sup>33</sup> These results suggest that the LED test has a much better specificity (and thus higher positive predictive value) than when previously evaluated in comparison with non-NAAT technologies.<sup>15–17–23</sup> Although schistosomiasis has been associated with a positive LED test<sup>16–22</sup> and could have resulted in a poor specificity in studies carried out in east Africa where the prevalence of this parasitic infection is relatively high, it could not have affected results of studies conducted in developed countries<sup>18–21</sup> where poor specificity of the LED test has also been reported. It thus appears that some true cases were detected by the LED test but not by non-NAAT technologies, resulting in an apparent lack of specificity of the former.

Such results suggest that LED could be a useful case finding tool in high risk men in developing countries. Indeed, in our study, the positive predictive value of the LED test among asymptomatic men was 37.5%, a figure which, although lower than is generally found for the syndromic approach in the management of men presenting with symptoms of urethral discharge, is much higher than that of another standard of care for STI management in developing countries, the syndromic approach for the diagnosis of cervical infections among symptomatic women.<sup>34</sup> In addition, the fact that the LED test is rapid and inexpensive and can be administered at the point of care makes it a particularly useful test in a context where high rates of non-attendance for return visits occur, as shown for example in FSWs in Cotonou.<sup>35</sup> It has been shown that even in the industrialised world, non-attendance for follow up visits can have a negative impact on STI treatment rates.<sup>36</sup>

In our study, the LED test had perfect performance parameters in symptomatic men. However, these findings are based on a very small number of men and need to be confirmed by other studies.

In conclusion, the performance of the LED test in our study suggests that it could be a useful case finding tool for STIs in high risk asymptomatic men in developing countries. Given the fact that the prevalence of STIs in clients of FSWs was found to be much higher than that of the general population of men in Cotonou (1% for gonorrhoea and 2% for chlamydial infection<sup>37</sup>), and that these men constitute a bridge between FSWs and the general population of women in terms of STI and HIV transmission,<sup>38</sup> interventions targeted towards this population, involving both STI screening and treatment and outreach prevention activities, are urgently needed.

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### CONTRIBUTORS

MA was a co-principal investigator, was responsible for the data analyses related to this article and was the main writer of the manuscript; CML was a co-principal investigator and contributed to data analysis and to the writing of the manuscript; LMT contributed to data analysis and to the writing of the manuscript; CABG was responsible for data collection in the field and contributed to data analyses; EB, NG, and PJ contributed to the data collection; EF was

responsible for the laboratory tests carried out in Cotonou; FB and JRJ were responsible for the laboratory tests for chlamydial and gonococcal infections; EF was responsible for the PCR testing for trichomonas. EB and SA were the local co-investigators and contributed to the organisation and implementation of the study. All authors contributed to the interpretation of the data and reviewed and commented the manuscript.

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