Evaluation of self-collected rectal swabs for the detection of bacteria responsible for sexually transmitted infections in a cohort of HIV-1-infected patients

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Abstract

Purpose. The standard approach to screening sexually transmitted infections (STIs) has often been restricted to urogenital specimens. Most current guidelines, however, also recommend testing extra-genital sites, including rectal locations, because asymptomatic rectal carriage of pathogens has often been reported. The aim of our study was to evaluate self-collected rectal swabs to screen bacterial STIs in HIV-infected patients in Marseille, France.

Methodology. Between January 2014 and December 2015, 118 HIV-infected patients (93 males and 25 females) agreed to self-sample anal swabs for detection of bacterial STI. Detection of Neisseria gonorrhoeae, Chlamydia trachomatis, Treponema pallidum, Mycoplasma genitalium and Haemophilus ducreyi was performed using in-house qPCR assay.

Results/Key findings. Bacterial STIs were found in 8 % (9/118) of the patients. C. trachomatis was the most commonly detected bacterium (4.2 %) followed by N. gonorrhoeae (2.5 %), M. genitalium (1.7 %) and T. pallidum (0.8 %). All the positive patients were males. The rectal carriage of pathogenic bacteria was fortuitously discovered for seven men (78 %) who did not present rectal signs of STIs and was suspected for two men who presented proctitis (22 %).

Conclusion. In conclusion, testing extra-genital sites is crucial for the diagnosis of STIs in men and women presenting or not concomitant urogenital infections in order to detect asymptomatic carriage with the aim of controlling and preventing transmission to their sexual partners.

INTRODUCTION

In recent years, a significant global resurgence in sexually transmitted infections (STIs) has been observed, including syphilis, gonorrhoea, Chlamydia trachomatis, lymphogranuloma venereum and human immunodeficiency virus (HIV) seroconversion [1, 2]. HIV infection continues to be a major health concern around the world, particularly for men who have sex with men (MSM), for whom the rate of seroconversion increased by 12 % between 2009 and 2013 [3]. Neisseria gonorrhoeae, C. trachomatis and Treponema pallidum were the three most commonly reported bacteria, but around 30 other micro-organisms, including bacteria, viruses, parasites and fungi, have been listed as possible agents of STIs.

The standard approach for the screening of STIs has been often restricted to urogenital specimens, but some recent studies using sensitive nucleic acid amplification tests showed a high prevalence of bacteria responsible for STIs in extra-genital specimens, including rectal and pharyngeal sites [4]. Therefore, most current international guidelines also recommend testing extra-genital sites, particularly in high-risk populations [5]. A routine annual screening of extra-genital sites in MSM is recommended [5] but few recommendations exist regarding the screening of extra-genital sites for women [4]. Indeed, most studies focus on the high prevalence of extra-genital C. trachomatis and N. gonorrhoeae in MSM, although rectal and oropharyngeal infections in women may be significant [4, 6]. The vast majority of extra-genital STIs are asymptomatic and may go undiagnosed, which promotes their spread [3, 6].

The prevalence of rectal bacterial STIs has rarely been studied in HIV-infected patients in France. The aim of our study...
was to evaluate self-collected rectal swabs to screen for rectal bacterial STIs in this population.

METHODS

Patients

Participants were enrolled on a voluntary basis at Marseille University Hospital between January 2014 and December 2015. All patients were being monitored for their HIV-1 infection in a clinical outpatient unit. During the consultation for their routine HIV care, they were asked to self-sample anal swabs to detect carriage of bacteria responsible for STIs. The inclusion criteria were adults over 18 years being monitored in our unit for their HIV infection who agreed to participate to the study and gave their informed consent. Patients were included regardless of their HIV stage, HIV RNA load, CD4 cell count, sexual behaviour and clinical symptoms. We excluded patients under 18 years old and those who refused to perform self-collected rectal swabs. Demographic and epidemiologic characteristics, clinical and highly active antiretroviral therapy (HAART) data, CD4 cell count, and HIV RNA load were obtained from the clinical records.

A total of 118 participants were enrolled. The median age was 47 (ranging from 21 to 77 years); 79 % (93/118) were men and 21 % (25/118) were women. Of the participants, 53 % (62/118) were MSM, 24 % (28/118) were heterosexual and information was unavailable for 28 patients. Among them, 11 % (13/118) were intravenous drug users and data on the mode of HIV transmission was unavailable for 25 patients. Ninety-two per cent (108/118) of the patients were treated by HAART. The mean CD4 cell count was 673 mm$^{-3}$ ± 311 mm$^{-3}$ (range 83–1578) and 17 % (20/118) presented a detectable HIV RNA load with a mean of 3.5±1.5 log$_{10}$ copies ml$^{-1}$ (range 1.7–6.4). Twelve patients (10 %) presented rectal STI signs, including six patients with human papillomavirus (HPV)-associated lesions, two with granulomatous anal lesions, one with rectal ulceration, two with rectal discharge and one with anal pruritus.

A sterile Dacron swab (Mast Diagnostic, Amiens, France) was given to each participant with a transport medium for the self-collected anal specimens. In seven patients presenting evident clinical signs of STIs, concurrent urogenital samples (urine and/or urethral swabs) were collected for routine diagnosis of STI including qPCR detecting bacterial pathogens. The study was approved by our IHU ethics committee (no. 2016-015) and written informed consent was obtained from all participants.

qPCR assay

DNA was extracted from the rectal swab using the QIAamp tissue kit according to the manufacturer’s instructions (Qiagen, Courtabœuf, France). DNA was eluted in 100 µl elution buffer. We used an in-house qPCR system to detect N. gonorrhoeae, C. trachomatis, Mycoplasma genitalia, T. pallidum and Haemophilus ducreyi [7] (Table 1). The specificity of the primers and probes was verified in silico by conducting a BLAST search in GenBank and performing qPCR on purified genomic DNA from a panel of 100 bacterial strains isolated from human clinical samples (Tables S1 and S2, available in the online Supplementary Material). qPCR was performed using a CFX96 thermocycler (Biorad, Marne la Coquette, France) with the QuantiTect Probe PCR Kit (Qiagen) and 5 µl of DNA per reaction. The initial denaturation step at 95°C for 15 min was followed by 40 PCR cycles at 95°C for 30 s and 60°C for 1 min each. The cutoff $C_t$ value for positive results was ≤35. Synthetic positive controls produced with a pUC57 plasmid and negative controls (distilled water) were used in each run. DNA extraction quality and adequacy of sampling were checked by qPCR detecting the human albumin gene [7, 8].

Serological tests

In our routine serological testing for syphilis, T. pallidum hemagglutination (TPHA) was performed using Architect Syphilis TP, an automated chemiluminescent microparticle immunoassay (Abbott Diagnostics, Wiesbaden, Germany) and a nontreponemal test was performed using a rapid plasma reagin (RPR) latex test kit (Cortez Diagnostics, Los Angeles, USA) following the manufacturer’s instructions.

Statistical analysis

For data comparison and statistical analysis, the $\chi^2$ and Mann–Whitney test, and standard statistical software (GraphPad Prism 5) were used. A $P$-value of <0.05 was considered to be significant.

RESULTS

One hundred and eighteen patients were enrolled. Rectal STI was found in 8 % (9/118) of patients with at least one bacterium. All patients harbouring bacteria responsible for STIs were male and the median age was 35 years (range 23–60). The mean CD4 cell count was 580±336 mm$^{-3}$ (range 75–1305). The HIV RNA load was only detectable for two patients who had not yet been treated by HAART because their HIV infection had been diagnosed very recently (Table 2). The seven other patients were being treated with HAART at the time of the sampling and the HIV RNA load was undetectable. Of the nine positive patients, seven were MSM, one was presumed to be a heterosexual male and data was unavailable for one patient. Four of them were totally asymptomatic, two presented anal HPV-associated lesions and one patient presented a syphilitic cutaneous eruption but without rectal lesions. Two patients presented rectal symptoms of STI with rectal discharge and anal granulomatous lesions, respectively.

The most commonly detected bacteria were C. trachomatis, which was detected in five patients (4.2 %), followed by N. gonorrhoeae in three patients (2.5 %), M. genitalia in two patients (1.7 %) and T. pallidum in one patient (0.8 %) (Table 2). No patients were positive for H. ducreyi. Multiple infections were observed in two patients presenting a positive qPCR for both N. gonorrhoeae and C. trachomatis. No correlation between the presence of symptoms and the $C_t$...
value of qPCR was found. C. trachomatis was also detected in urogenital samples for one patient who presented with anal granulomatous lesion associated with C. trachomatis.

The median age of patients presenting with qPCR-positive rectal STIs (median=35) was lower compared to the patients without STIs (median=48) but the difference was not significant (P=0.05 using Mann–Whitney test). The mean CD4 cell count was 572/mm$^3$ in patients with rectal STIs and 646/mm$^3$ in negative patients (P=0.2912 using Mann–Whitney test). No significant difference was found for the HIV RNA load between these two groups.

Among the 109 negative patients, eight presented rectal STI symptoms, including four patients with anal HPV-associated lesions, two patients with anal pruritus and two patients with rectal discharge. Concomitant urogenital samples were performed for five of the negative patients and all were negative by qPCR. Serological syphilis testing was performed for 28 patients and it was negative for 20 of them, four presented a past infection and four presented a serological profile compatible with active syphilis.

**DISCUSSION**

This study demonstrated that self-collected rectal swabs can contribute to the diagnosis of bacterial STIs. We found a prevalence of 8% (9/118) in the studied population. The rectal carriage of bacteria responsible for STIs was discovered fortuitously for seven patients (78%) who presented no rectal signs of STIs and was suspected for two patients who presented with proctitis (22%). Because rectal swabs are minimally invasive and easy to self-administer, they are readily accepted by patients [8]. Recent studies reported that anal self-sampling showed similar results for STI diagnosis compared to clinician-collected samples [9, 10].

N. gonorrhoeae and C. trachomatis were the two most common bacteria detected by qPCR in rectal STIs [4, 6]. In the literature, the observed prevalence of rectal N. gonorrhoeae and C. trachomatis varied between 6–21% and 1–18%, respectively, in MSM and 0–3% and 7–17% in women [4, 6]. The prevalence of N. gonorrhoeae and C. trachomatis could reach 19.2 and 77.5%, respectively, in women in very high risk groups [6, 11]. Most women with a urogenital infection presented a concomitant rectal STI; 33–83% of women presenting with a C. trachomatis infection at the genital site were found to have a concomitant rectal location of the bacteria [4, 12], despite most of them not reporting receptive anal intercourse [4, 6, 12]. In contrast, MSM with anorectal infections do not usually present a concomitant urogenital infection [4].

The rectal prevalence of M. genitalium was estimated at 4–5% in MSM [13] and in women [14]. For the diagnosis of syphilis, most studies used serology, which remains the gold standard. We did not find any study reporting the prevalence of the rectal carriage of T. pallidum using qPCR, with the exception of a few cases reporting symptomatic anorectal infection [15]. H. ducreyi, the causative agent of chancroid, was not detected in any of the patients tested, which is not surprising because asymptomatic carriage is very rare [16].

The prevalence of rectal STIs detected in our group of HIV-infected patients was low compared to that reported in the literature. No women were found to be positive in our study but the sample size was small and none presented urogenital or rectal signs of STIs. The lower prevalence of rectal STIs could be explained by several factors. A selection bias was introduced because we only included patients who agreed to self-collect specimens and perhaps more high-risk patients were less likely to participate. The median patient age (46 years) was high compared to other studies, as rectal STIs were more prevalent in younger patients [17]. A limit of our study is that samples from other sites (urine, vaginal/urethral swab) were not collected, so we could not compare the prevalence of

**Table 1. Primers and probes used in this study**

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Table 2. Positive patients characteristics, qPCR and serology results

| Age (yr) | Gender | Sexual behaviour | C34 cell count (cell/mm^3) | HIV RNA load (log10 copies/ml) | qPCR results on swab | qPCR results on rectal swab | Rectal signs of STI | T. pallidum* carriage | Serology | HAART | TPHA | TPNA | RPR titer | C trachomatis (conjunctivitis) | C. trachomatis (urogenital) | C. trachomatis (anal) | M. genitalium | T. pallidum | H. ducreyi |
|----------|--------|------------------|-----------------------------|-------------------------------|-------------------------|-------------------------|--------------------------|---------------------|-------------------|---------|-------|------|------|---------|-----------------------------|-----------------------------|---------------------------|-------------|-----------|---------|
| 1        | 46     | Male             | 390                         | Undetectable                  | Positive               | Negative                | Yes                       | No                  | Negative          | Negative | No     | No    | No   | 1/323 | Reaction                        | Negative                  | Negative                  | Negative                | No         | No      |
| 2        | 60     | Male             | 376                         | Undetectable                  | Positive               | Negative                | Yes                       | No                  | Negative          | Negative | No     | No    | No   | 1/32  | Reactive                         | Negative                  | Positive                  | Negative                | No         | No      |
| 3        | 35     | Male             | 625                         | Under detectable              | Positive               | Negative                | Yes                       | No                  | Negative          | Negative | No     | No    | No   | 1/32  | Reactive                         | Negative                  | Positive                  | Negative                | No         | No      |
| 4        | 29     | Male             | 572                         | Undetectable                  | Positive               | Negative                | Yes                       | No                  | Negative          | Negative | No     | No    | No   | 1/32  | Reactive                         | Positive                  | Positive                  | Negative                | No         | No      |
| 5        | 40     | Male             | 480                         | Undetectable                  | Positive               | Negative                | Yes                       | No                  | Negative          | Negative | No     | No    | No   | 1/32  | Reaction                        | Positive                  | Negative                  | Negative                | No         | No      |
| 6        | 23     | Male             | 1305                        | 1.9                           | Under detectable       | No                      | No                       | No                  | Negative          | Negative | No     | No    | No   | 1/32  | Reactive                         | Negative                  | Positive                  | Negative                | No         | No      |
| 7        | 33     | Male             | 640                         | Under detectable              | Positive               | Negative                | Yes                       | No                  | Negative          | Negative | No     | No    | No   | 1/32  | Reactive                         | Positive                  | Positive                  | Negative                | No         | No      |
| 8        | 45     | Male             | 75                          | No                            | Under detectable       | No                      | No                       | No                  | Negative          | Negative | No     | No    | No   | 1/32  | Reactive                         | Negative                  | Positive                  | Negative                | No         | No      |
| 9        | 31     | Male             | 754                         | Under detectable              | Positive               | Negative                | Yes                       | No                  | Negative          | Negative | Yes    | No    | No   | 1/32  | Reactive                         | Positive                  | Positive                  | Negative                | No         | No      |

Table 3.

Characteristics, qPCR and serology results

sexually transmitted pathogens at other body sites. The quality of sampling and DNA extraction was checked with albumin gene detection. The lower prevalence in our study may have been linked to the major focus on STI prevention at our HIV centre. However, one study in France on the rectal carriage of N. gonorrhoeae, C. trachomatis and M. genitalium reported a prevalence of 7, 8 and 1%, respectively, in asymptomatic MSM HIV-infected patients reporting recent sexual intercourse, which is in line with our results [18]. The prevalence of C. trachomatis, N. gonorrhoeae, M. genitalium and T. pallidum among MSM in our study was 8, 5, 3 and 2%, respectively. We found one man presenting with M. genitalium anal carriage; however he reported himself as heterosexual. This patient is likely denying his homo/bisexuality. However, prevalence rates of 0–5.7% for rectal N. gonorrhoeae and 0–11.8% for rectal C. trachomatis have been reported in heterosexual men [6].

Only testing urogenital specimens will result in non-diagnosis for many patients with rectal STIs. In addition, testing for STIs should be carried out at extra-genital sites in order to screen for asymptomatic carriers and to treat and prevent transmission in order to limit these infections. In fact, most extra-genital infections are asymptomatic, with rates estimated to be 36–100% in women and 25–100% in MSM presenting with rectal STIs [6, 19–21]. Moreover, C. trachomatis and N. gonorrhoeae infections may persist for a long time at rectal sites, with a persistence of 579 and 346 days, respectively, in MSM [22]. The implementation of extra-genital testing in the STI diagnosis strategy has led to increased case detection and improved patient management [23, 24].

The increase in pathogens and the triple-site testing process leads to additional costs for STI diagnosis. Using new diagnostic tools such as multiplexed tests could reduce this cost [25]. Recent data showed that pooling samples from the three different sites for the same individual is an effective and cost-saving method, using nucleic acid amplification testing [9].

C. trachomatis, N. gonorrhoeae and M. genitalium are commonly detected in rectal sites in HIV-MSM. Testing extra-genital sites is critical for the diagnosis of STIs and for the detection of asymptomatic carriage with the aim of controlling and preventing transmission to sexual partners, particularly in MSM but also in women [6]. The anal self-sampling was well accepted by patients [8]. In this study, we found a 10% (9/93) carriage rate of rectal bacterial STIs in HIV-infected men. The rectal carriage of bacteria responsible for STIs was unsuspected for 78% of them, who were asymptomatic. Our data supports the claim that screening of extra-genital sites should be performed systematically, particularly in populations at a high risk of STIs, including MSM and HIV-infected patients, but also female sex workers and women reporting unprotected anal intercourse.

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Conflicts of interest
The authors declare that there are no conflicts of interest.

References