Original Article

Aetiology of sexually transmitted infections in Maputo, Mozambique

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Abstract

Introduction: The study sought to ascertain the prevalence of the aetiological agents of genital discharge and genital ulcer diseases in Maputo, Mozambique.

Methodology: Consecutive consenting patients presenting to the Centro de Saúde do Porto in Maputo between March and April 2005 with genital discharge syndrome and/or genital ulcer diseases were recruited. Specimens were collected for the identification of STI pathogens.

Results: Of 346 recruited patients, 164 were male and 182 female. The prevalence of confirmed single aetiological agents for male urethritis was as follows: *N. gonorrhoeae*, 35%; *C. trachomatis*, 10%; *and M. genitalium* 4%. For vaginal discharge, *N. gonorrhoeae* was found in11% of the women tested, followed by *C. trachomatis* (6.5%), bacterial vaginosis (34%), and *T. vaginalis* (2%). The prevalence of genital ulcers was as follows: Herpes simplex virus type 2, 62%; *H. ducreyi* 4 %; and *C. trachomatis* biovar LGV, 4%. Five percent of patients with genital ulcers had a positive syphilis serology (RPR ≥ 1:8 and confirmed by TPHA) and 35% of all tested patients were HIV-1/2 infected.

Cases of mixed infections were present in 5%, 11% and 3% of patients with male urethritis, vaginal discharge, and genital ulcers respectively.

Conclusion: The classic sexually transmitted infection aetiologies are still prevalent in Maputo. The study highlights the need for a periodic surveillance to inform syndromic management protocols.

Key words: sexually transmitted infections; syndrome aetiologies; Maputo

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Introduction

Sexually transmitted infections (STIs) are a major health care problem in many parts of the world, and they have been associated with increased human immunodeficiency virus (HIV) transmission [1,2]. The prevalence of HIV infection has reached astronomical proportions in many areas of sub-Saharan Africa [3]. Control of STIs is hampered by non-specific presentation of the different infections, leading to inaccurate clinical diagnosis, the absence of adequate laboratory facilities and a large proportion of asymptomatic infections [2,4]. In 1994 the World Health Organization (WHO) proposed the syndromic approach for the management of STIs to reduce their prevalence in developing countries [4]. Gilson *et al.* (1997) and White *et al.* (2008) pointed out that the management of curable STIs could be the most cost-effective intervention to decrease HIV transmission (despite a small relative impact on HIV incidence) in African countries with higher HIV prevalence rates [2,5].

Studies from different African countries show aetiological variability between regions and countries [6-10]. Therefore, syndromic management (SM) guidelines need to be area specific and based on knowledge of the prevalence of the aetiological agents of the different syndromes and their drug susceptibility. In Mozambique, the syndromic approach for patients with genital discharge syndrome includes a single dose of kanamycin (2 grams intramuscularly) for the treatment of potential infection with *Neisseria gonorrhoeae*, or doxycycline (100 mg twice daily for seven days) for treatment of potential infection with *Chlamydia trachomatis* in males. This regimen is supplemented with metronidazole as a single 2 gram dose in non-pregnant women for the treatment of potential infection with *Trichomonas vaginalis* and to a lesser extent bacterial vaginosis. In pregnant women, doxycycline is replaced by erythromycin 500 mg four times daily for five days. The syndromic approach for patients with genital ulcer diseases (GUD) includes a single dose of intramuscular bezanthine penicillin 2.4

MU for *Treponema pallidum* that cause syphilis, and erythromycin 500 mg four times daily for seven days which is included in the syndrome regimen to treat possible infection with *C. trachomatis, Haemophilus ducreyi* and *Calymmatobacterium granulomatis*.

Apart from the reports by Vuylsteke *et al.* [11] and Mbofana *et al*., no other studies have been published recently reporting the aetiology of STIs in Mozambique. In addition to the paucity of research reports, there is no surveillance program in place to regularly monitor changes in the aetiology of STIs and the susceptibility profiles of causative agents. This information is essential to adjust the syndromic management guidelines. Hence the aim of this study was to establish the aetiology of sexually transmitted infections in Maputo, Mozambique.

Methodology

This cross-sectional study was conducted in Maputo, which has a population of 1,094,315 people. Consecutive consenting patients attending Centro de Saúde do Porto in this city between March and April 2005 with symptoms and signs of male urethritis syndrome (MUS), vaginal discharge syndrome (VDS) and genital ulcer disease (GUD) were enrolled. The study was approved by the Ethics Committee of the Ministry of Health in Maputo, Mozambique (Ref: 05/CNBS/2005).

Current symptoms were obtained through a standard questionnaire. A physical examination was performed by the attending medical practitioner and signs of genital tract infection were noted. All patients were treated using the standard of care treatment in Mozambique for the presenting syndrome.

Two Probetec (Becton Dickinson, Sparks, Maryland, USA) swabs were used to collect genital specimens from male patients who presented with urethritis syndrome. The first swab was used to inoculate New York City (NYC) medium (GC agar base supplemented with yeast autolysate, lincomycin, colistin, Amphotericin B, trimethoprim (Oxoid Ltd, Basingstoke, UK) and lysed horse blood) immediately after sample collection, for the isolation of *N. gonorrhoeae*. The second swab was placed back into its original container and stored in a cooler box with ice before being transported to the laboratory.

One cervical and two vaginal Probetec swabs were obtained from patients with VDS. The cervical specimens were used to inoculate NYC plates. The first vaginal swab, which collected material from the posterior fornix, was used to make a smear onto a glass slide for Gram staining. The second vaginal swab was used to collect specimen for polymerase chain reaction (PCR). This was stored in a dry container and stored in a cooler box with ice awaiting transport to the laboratory.

All inoculated NYC plates were immediately placed in a candle extinction jar, at room temperature, awaiting transport to the laboratory.

Specimen collection from patients with GUD was done as follows: impression smears were made onto glass slides for Rapidiff (Clinical Sciences Diagnostics Ltd, Booysens, South Africa) staining and material for PCR was collected from the ulcer base and edges with a sterile Dacron swab (Pro-Gen Diagnostics, Magaliesview, South Africa). This was then suspended in 1.0 mL PBS, pH 7.6 and stored in a cooler box with ice awaiting transport to the laboratory.

Venous blood was collected for the serologic diagnosis of syphilis, HIV infection, and herpes.

All plates and specimens were transported to the laboratory at Maputo Central Hospital within six hours. Swabs and suspensions for PCR were stored until transported to Durban, South Africa, at -70^{o}C.

Culture for *N. gonorrhoeae* was performed in the laboratory at Maputo Central Hospital. NYC plates were incubated for 48 hours at 37°C in a CO_2 incubator. Plates were only examined after 48 hours. Gram-negative and oxidase-positive colonies were subcultured onto NYC plates and incubated overnight. Overnight cultures were suspended in Tryptic soy broth (Difco laboratories, Detroit, Michigan) containing 20% glycerol and transported to Durban at -70˚C, where identification tests were confirmed by means of carbohydrate utilization tests performed in the Department of Medical Microbiology, Nelson R Mandela School of Medicine.

BD nucleic acid amplification (Becton Dickinson Probetec Assays, Sparks, Maryland, USA) tests were performed on all urethral and cervical swabs for the detection of *C. trachomatis* and for *N. gonorrhoeae* on all culture negative specimens.

In-house PCRs were performed for the detection of *Mycoplama genitalium* on DNA isolated from the urethral and vaginal swabs and for *Trichomonas vaginalis* on vaginal material only. DNA was extracted from a 20-µL aliquot of genital material using a QIAamp DNA purification kit (Qiagen, Chastsworth, CA) per the manufacturer's instructions, and eluted in 200 µl of elution buffer

Table 1. HIV rates (in %) by STI syndromes

	n =	HIV positive	HIV negative	p value
Male Urethritis Syndrome	116	32 (28%)	84 (72%)	0.04
Vaginal Discharge Syndrome	154	43 (28%)	111 (72%)	0.01
Genital Ulcer Diseases	76	47 (62%)	29 (38%)	< 0.0001
Total	346	122 (35%)	224 (65%)	

(Qiagen). Overall, in-house PCR assay was performed following the method described by Tabrizi *et al*. [12] and Jensen *et al*. [13]. The PCR products were hybridized and detected using an automated analyzer (Enzymun-Test DNA Detection Assay, Boehringer Mannheim ES System, Germany) following the manufacturer's protocol. Biotin-labelled oligonucleotides capture probes NGP (5'-ACAGCCCTGCTATGACTATCAA-3', CT3, MG16S-240 Bio and TVB (5'-GACCTCTAGAAGAAGACTCAG-3') (Roche Diagnostics, Basel, Switzerland) were used for the detection of *N. gonorrhoae*, *C. trachomatis*, *M. genitalium* and *T. vaginalis* respectively.

Bacterial vaginosis (BV) was diagnosed by means of Gram stain microscopy using Nugent's score. In-house PCR for the detection of Herpes simplex virus, *H. ducreyi*, *C. trachomatis* biovar-LGV, and *T. pallidum* was performed on the ulcer specimens. Multiplex PCR was performed for the diagnosis of *H. ducreyi*, HSV2, and *T. pallidum* following the protocol by Orle *et al*. [14]. The following primers were used for *T. pallidum*: KO3A (5'-biotinyl-GAAGTTTGTCCCAGTTGCGGTT) and KO4 (5'-biotinyl-CAGAGCCATCAGCCCTTTTCA); HSV2: KS30 (5'-biotinyl-TTCAAGGCCACCATGTACTACAAAGACGT) and KS31 (5'-biotinyl-GCCGTAAAACGGGGACATGTACACAAAGT); *H.ducreyi*: KO7A (5'-biotinyl-CAAGTCGAACGGTAGCACGAAG) and KO8A (5'-biotinyl-TTCTGTGACTAACGTCAATCAATTTTG) (Roche Diagnostics, Basel, Switzerland). Detection and identification of amplification products was performed by means of a colorimetric detection system in the AMPLICOR (Roche Molecular Systems) using microwells containing immobilized oligonucleotide capture probes KS54 (5'-BSA-GGTCTCGTGGTCGTCCCGGTGAAA), KO17 (5'-BSA-CGGGCTCTCCATGCTGCTTACCTTA), KO15 (5'-BSA-CCGAAGGTCCCACCCTTTAATCCGA) respectively for HSV2, *T. pallidum* and *H. ducreyi*.

For the diagnosis of LGV, PCR-restriction fragment length polymorphism was conducted as described by Sturm *et al.* [15]. The Rapidiff stained smear was screened for the presence of *C. granulomatis*.

The rapid plasma reagin (RPR) test was used to screen *for* syphilis antibodies. All reactive RPR results (RPR $\geq$ 1:8) were confirmed by *Treponema pallidum* haemaglutination assay (TPHA).

HIV-1/2 antibodies were detected by means of two separate HIV rapid test kits, namely the Determine HIV-1/2 test (Abbott Laboratories, Illinois, USA) and the SmartCheck test (World Diagnostics Inc, Miami, USA). Antibodies against HSV-2 were detected by means of the Herpeselect-2 test (Focus Diagnostics Inc, Cypress, Ca, USA).

Chi-square test was used to determine whether there was any significant difference between observed proportions, and a p value of < 0.05 was considered significant.

Results

The median age of the 346 enrolled patients was 35.5 years. The level of education ranged from 7.5% with no education, 17.7% with primary school education, 58.3% with secondary school education, 14.7% with matriculation, and 1.8% with a tertiary level education. More than one sexual partner in the previous three months was reported by 218 patients.

Of the enrolled patients, 116 (33.5%) presented with MUS and 154 (44.5%) with VDS. The remaining 76 (22%) presented with GUD, of whom 28 (37%) were female and 48 (63%) male (Table 1). Genital ulcers and discharge syndrome were concurrently present in 29 (8.4%) patients, 16 female and 13 male.

Table 2. Aetiology of male urethritis syndrome (n = 116)

Causative agents	Number	Percentage
Single pathogen		
Neisseria gonorrhoeae	40	35%
Chlamydia trachomatis	11	10%
Mycoplasma genitalium	5	4%
Mixed infection		
N. gonorrhoeae and *C. trachomatis*	4	3%
C. trachomatis and *M. genitalium*	2	2%
No confirmed laboratory diagnosis	48	41%

Table 3. Aetiology of vaginal discharge syndrome (n = 154)

Causative agents	Number	Percentage
Single pathogen		
Neisseria gonorrhoeae	17	11%
Chlamydia trachomatis	10	7%
Trichomonas vaginalis	3	2%
Bacterial vaginosis (BV)	53	34%
Mixed infection		
BV and *T. vaginalis*	10	7%
BV and *T. vaginalis* and *C. trachomatis*	3	2%
T. vaginalis and *C. trachomatis*	2	1%
C. trachomatis and *N. gonorrhoeae*	2	1%
No confirmed laboratory diagnosis	34	22%

The overall prevalence of HIV-1/2 infection was 35%. Genital ulcer disease was associated with a significantly higher HIV prevalence ($p < 0.0001$) (Table 1).

Five percent of patients with GUD had a positive syphilis serology (RPR ≥ 1:8, confirmed by TPHA); additionally, the prevalence of HSV-2 infection was 85% using serologic tests.

Aetiology of male urethritis syndrome

N. gonorrhoeae as a single pathogen was detected in 40 (35%) patients (38 by culture and two by BD Probetec Assay). *C. trachomatis* was detected in 11 (10%) patients and *Mycoplasma genitalium* in five (4%) patients (Table 2). Six (5%) patients had

mixed infection: four (3%) with *N. gonorrhoeae* and *C. trachomatis* and two (2%) with *C. trachomatis* and *M. genitalium*. The remaining 48 (41%) patients with MUS had no confirmed laboratory diagnosis.

Table 4. Aetiology of genital ulcer diseases (n = 76) using PCR assays

Aetiology by PCR	Number	Percentage
HSV-2	47	62%
Chlamydia trachomatis LGV serovar	3	4%
Haemophilus ducreyi	3	4%
Calymmatobacterium granulomatis	0	0%
Treponema palllidum	0	0%
No confirmed diagnosis by PCR	23	30%

Aetiology of vaginal discharge syndrome

Of the 154 patients with VDS, *N. gonorrhoeae* was diagnosed as a single pathogen in 17 (11%) cases (cultured in 15 patients and detected in a further two by the BD Probetec Assay). *C. trachomatis* was detected in 10 (7%) patients, *T. vaginalis* in three (2%) and BV in 53 (34%) patients (Table 3). Mixed infections were present in 17 (11%) patients (Table 3). The remaining 34 (22.1%) cases of VDS had no definitive laboratory diagnosis.

Aetiology of genital ulcer disease

PCR analyses showed that a total of 47 (62%) patients had a confirmed diagnosis of HSV-2 infection from the ulcer lesions, 29 (62%) of whom were male and 18 (38%) were female. *C. trachomatis* biovar LGV as well as *H. ducreyi* were each detected in three (4%) cases, two males and one female. Mixed infections with HSV-2 and LGV were diagnosed in one male and one female (Table 4).

T. pallidum was not detected in the ulcer specimens of any of the patients (Table 4) even though 5% of patients with GUD had a positive syphilis serology (RPR ≥ 1:8 confirmed by TPHA). *C. granulomatis*, the pathogenic agent of donovanosis, was also not detected in patients with GUD.

Discussion

This study showed that 38% of cases of MUS had laboratory confirmation of gonococcal infection in total. Mbofana *et al*. reported from a study in Mozambique that 19% of MUS cases were due to *N. gonorrhoeae* [7]. This percentage, however, was an underestimation of the situation probably due to the methodology used that consisted of a modified WHO recommended protocol for observing STD case management in health facilities [7,16]. In addition, the prevalence of STI cases could have changed over time.

N. gonorrhoeae as a causative agent of VDS accounted for 12%. This observation supports the results of studies from the neighbouring KwaZulu-Natal in South Africa which showed that the prevalence of gonococci among women attending STD clinics was approximately 12.4% [8]. In most African countries, the prevalence of gonococcal infection ranges from 2.9% in family planning clinics to over 38% among STD clinic attendees [11,17-19]. Cases of MUS and VDS due to *C. trachomatis* accounted for 15% and 11% respectively. Vuylsteke *et al.* reported the prevalence of *C. trachomatis* infection to be 7.1% and 12% respectively for males and females during a survey in rural Mozambique [11]. However, our data correspond with results from other studies in Africa [6,8,20-24].

M. genitalium was found in 6% of cases in our study. This observation is similar to those of reports from Durban, South Africa [15,25].

The prevalence of *T. vaginalis* among those with VDS was 12%, similar to the report by Mbofana *et al*. [7]. Bacterial vaginosis with a prevalence of 43% was the main cause of VDS in this study.

T. pallidum was not detected in any of the ulcer specimens in patients with GUD while Mbofana *et al.* [7] reported a prevalence of 13% in the cases they studied. However, 5% of our GUD patients had a positive syphilis serology (RPR ≥ 1:8 confirmed by TPHA). Sturm *et al.* determined that the seropositivity for syphilis with negative PCR is strongly associated with HIV-1 infection (OR 2.76; 95% CI: 1.60 - 4.82, $p < 0.0001$), suggesting that at the muco-cutaneous level, HIV-1 infection decreases the chances for infection with *T. Pallidum* [26]. This finding could offer a possible explanation for the decline of syphilis in HIV-1 prevalent areas without a decline in other curable STIs. However, the decline of the prevalence of syphilis in numerous African countries could also be due to a positive outcome resulting from the WHO promotion of universal testing for syphilis in antenatal clinics, a population-based advertising campaign as an adopted strategy by health-care institutions of these numerous countries, as well as an improved treatment coverage with the wide use of benzyl penicillin as part of the syndromic management package for GUDs [4,27,28].

Cases of GUD caused by *H. ducreyi* have increased from 2% in 2002 [7] to 4% in this study (2005) while *C. granulomatis* was not detected in any of our GUD patients. Donovanosis, caused by *C. granulomatis*, has been known to have an unusual geographical distribution, predominantly in tropical

regions. However, additional foci of infection have been reported in South Africa and Zambia [29,30]. In addition, the clinical features of lesions caused by *C. granulomatis* are sufficiently suggestive of the diagnosis in most of cases [29,30]; and none of the GUD patients in this study had such typical clinical lesions.

Cases of HSV-2 infection were found to be the most common among all patients with GUD, accounting for 62% using PCR. Forty-three percent of all patients with HSV-2 infection also had a positive HIV-1/2 serology result. Numerous published studies have reported that HSV-2 increases acquisition and transmission of HIV infection while HIV increases susceptibility to and shedding of HSV-2 infection [31,32]. However, Watson-Jones *et al.* reported a lack of effect of HSV-2 suppressive therapy (using acyclovir 400mg twice daily) on HIV acquisition [33]. The World Health Organization recommends the use of an antiviral treatment for herpes in high HSV-2 prevalent areas [34]. Although acyclovir, a synthetic analogue of 2'-deoxyguanosine, is the recommended drug of choice for widespread treatment of herpes, resistance development is currently a growing concern [35,36].

Rates of GUD caused by *T. pallidum, H. ducreyi and C. granulomatis* were identified in Africa in the mid-1980s as increasing the risks for HIV transmission. However, these are currently in decline with respect to HSV-2 infection. Wald *et al.* reported from a meta-analysis study that a population-attributable risk percentage of HIV-1 transmission rose up to 47% in a population with 80% HSV-2 serology positive [37, 38].

C. trachomatis biovar LGV infections were found in 4% of all patients with GUD. A previous study reported prevalences of 2% and 6% respectively in female and male patients [7].

To our knowledge, this is the first report of a comprehensive analysis of STI syndromes in Maputo. The limitation of this study is that data have been collected more than four years ago, and the numbers are small. However, the study underscores the need for periodic surveillance to adjust the existing treatment guidelines.

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