Aetiological molecular identification of sexually transmitted infections that cause urethral discharge syndrome and genital ulcer disease in Brazilian men: a nationwide study

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ABSTRACT

Background Little is known about the aetiology of urethral discharge syndrome (UDS) and genital ulcer disease (GUD) in Brazil due to limited access to laboratory tests and treatment based mainly on the syndromic approach.

Objectives To update Brazilian treatment guidelines according to the current scenario, the first nationwide aetiological study for UDS and GUD was performed.

Methods Male participants with urethral discharge (UD) and/or genital ulcer (GU) reports were enrolled. Sample collection was performed by 12 sentinel sites located in the five Brazilian regions. Between 2018 and 2020, 1141 UD and 208 GU samples were collected in a Universal Transport Medium-RT (Copan). A multiplex quantitative PCR kit (Seegene) was used to detect UD: *Chlamydia trachomatis* (CT), *Mycoplasma genitalium* (MG), *M. hominis* (MH), *Neisseria gonorrhoeae* (NG), *Trichomonas vaginalis* (TV), *Ureaplasma parvum* (UP), *U. urealyticum* (UU) and another kit to detect GU: cytomegalovirus (CMV), *Haemophilus ducreyi* (HD), herpes simplex virus type 1 (HSV1), herpes simplex virus type 2 (HSV2), lymphogranuloma venereum (LGV), *Treponema pallidum* (TP) and varicella-zoster virus (VZV).

Results In UD samples, the frequency of pathogen detection was NG: 78.38%, CT: 25.6%, MG: 8.3%, UU: 10.4%, UP: 3.5%, MH: 3.5% and TV: 0.9%. Coinfection was assessed in 30.9% of samples, with 14.3% of NG/CT coinfection. The most frequent pathogen identified in GU was HSV2, present in 40.8% of the samples, followed by TP at 24.8%, LGV and CMV at 1%, and HSV1 at 0.4%. Coinfection of TP/HSV2 was detected in 4.4% of samples. VZV and HD were not detected. In 27.7% of the GU samples, no pathogen was detected.

Conclusion This study provided the acquisition of unprecedented data on the aetiology of UDS and GUD in Brazil, demonstrated the presence of a variety of pathogens in both sample types and reaffirmed the aetiologies known to be most prevalent globally.

WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ One million people are estimated to acquire a sexually transmitted infection (STI) every day around the globe, and the spread of antimicrobial resistance is a threat to the syndromic approach as a treatment guide.

WHAT THIS STUDY ADDS

⇒ This study provided national information on the aetiologies of urethral discharge syndrome and genital ulcer disease in Brazil.

HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ The data acquired will allow for more effective STI management and control strategies by the Brazilian Ministry of Health.

BACKGROUND

People acquire more than 1 million curable sexually transmitted infections (STIs) every day worldwide. The majority of STI cases are asymptomatic. When presented, the most frequent symptoms are genital ulcers (GUs) and urethral and vaginal discharge.[1 2] They can cause adverse outcomes, such as infertility, increased risk of cancer and HIV infection. Also, some STIs directly affect reproductive and child health by causing adverse pregnancy outcomes, fetal deaths and abnormalities, and general ill health.[3 4]

STIs are caused by various bacteria, viruses and parasites transmitted from one human being to another, primarily by vaginal, anal or oral sexual contact. Hence, more than one STI can be present or be transmitted simultaneously, and any such infection increases the risk of contracting other STIs, such as HIV. The absence of treatment leads to chronic illness and the spread of antimicrobial resistance.[5]

STI cases disproportionately affect low-income and middle-income countries. The previous prevalence study by the WHO, in 2020, estimated 129 million new infections with chlamydia, 82 million cases of gonorrhoea, and 7.1 million syphilis and trichomoniasis cases. In 2016, more than 490 million people were living with genital herpes.[6] In Brazil, infections that cause urethral discharge (UD) follow high worldwide rates. The last epidemiology study performed in 2015 estimated an incidence in the general population of around 500 000 new cases per year, and the prevalence of gonorrhoea in the population between 15 years and 49 years of age of approximately 1.4%.[7 8]

UD is a common symptom in men with *Neisseria gonorrhoeae* (NG), *Chlamydia trachomatis* (CT) and *Mycoplasma genitalium* (MG). *Trichomonas vaginalis* (TV), *M. hominis* (MH), *Ureaplasma urealyticum* (UU) and *U. parvum* (UP) are known to cause the minority of UD syndrome (UDS).[9] The most common agents of GU disease (GUD) caused by STIs are herpes simplex virus (HSV) and *Treponema pallidum* (TP). Less frequent are *Haemophilus ducreyi* (HD), *Klebsiella granulomatis* and CT (serovar L1–L3).[10]

The diagnosis of STIs remains a challenge in view of the syndromic approach instituted since the 1980s and recommended worldwide by the WHO. In low-income countries, it remains the most used practice, given the limited access to laboratory facilities and tests for aetiological identification, which often requires a complex laboratory structure and specialised professionals. Furthermore, even when testing is available, results can be delayed by several days.[8 11]

The treatment of male UD and GU is based on data published by international institutions and local studies.[8 11] A larger number of samples, to increase the representativity, is needed to verify which pathogens are more frequent in cases of UDS and GUD samples in men in Brazil. This study performed a nationwide aetiological study for UDS and GUD in Brazil, in order to adapt and update national treatment protocols according to this reality.

METHODS

A descriptive study was performed in 2018–2020, including 12 health services from the healthcare network for individuals with STIs or the primary care network of the Unified Health System. All Brazilian geographical regions were represented in the study (North (Alfredo da Mata Tropical Dermatology and Venereology Foundation, Manaus, Amazonas); Northeast (Specialized State Center in Diagnosis, Care and Research, Salvador, Bahia; AIDS Health Foundation and Central Laboratory, Recife, Pernambuco; Giselda Trigueiro Hospital and Federal University of Rio Grande do Norte, Natal, Rio Grande do Norte); Central-West (Asa Sul Polyclinic, Brasília, Distrito Federal); Southeast (STI/AIDS Reference and Training Center, São Paulo, São Paulo; Belo Horizonte Municipal Health Secretariat, Belo Horizonte, Minas Gerais; Reference Center for Infectious Diseases, São José dos Campos; Adolfo Lutz Institute and Ribeirão Preto Municipal Health Secretariat, Ribeirão Preto, São Paulo); and South (Curitiba Municipal Health Secretariat and Clinic Hospital Complex of Federal University of Paraná, Curitiba, Paraná; Clinical Analysis Department, University Hospital, Federal University of Santa Catarina; Florianópolis Municipal Health Secretariat, Florianópolis, Santa Catarina; and Sanitary Dermatology Outpatient Clinic, Porto Alegre, Rio Grande do Sul). These institutions are part of the Brazilian Gonococcal Antimicrobial Surveillance Group and have participated in the former Brazilian NG resistance surveillance study[12 13] in which the samples of both studies were collected simultaneously for NG culture and identification, and for molecular aetiological identification of STIs from UD and/or GU.

A sample of penile UD and/or GU was collected from symptomatic individuals with the following inclusion criteria: men over 18 years of age who agreed to participate by signing the informed consent form. Participants were invited to answer questions about sexual orientation and gender. UDS and GUD definitions were performed through clinical evaluation of health professionals who performed the sample collection. Participants who had not yet started sexual activity, with suspected sexual abuse, on antibiotic therapy within 7 days of collection and using topical medications in the genital region during this same period were not enrolled in this study. It was not defined a period of GU onset to include the sample.

Sites were encouraged to collect approximately 100 UD samples and 30 GU exudate samples during the study period. Collections were performed using the Universal Transport Medium-RT transport (Copan, Italy), which was refrigerated and sent to the national reference laboratory Molecular Biology, Microbiology and Serology Laboratory for molecular assays and participants were treated according to national STI guidelines.[8]

Molecular assay

DNA purification from GU and UD samples was performed using the PureLink Genomic DNA Mini Kit (Invitrogen, USA) following the manufacturer's instructions. After DNA purification, the multiplex real-time PCR kit Allplex STI Essential Assay (Seegene, Seoul)[14] was used to detect in UD: CT, MG, MH, NG, TV, UP, UU and Allplex Genital Ulcer Assay (Seegene, Seoul)[14] kit to detect: cytomegalovirus (CMV), HD, HSV type 1, HSV type 2 (HSV2), lymphogranuloma venereum, TP and varicella-zoster virus (VZV) in GU samples. The tests were performed according to the manufacturer's instructions, and amplification was performed in CFX96 real-time thermocycler (Bio-Rad, Hercules, California, USA).

RESULTS

A total of 1349 samples were included in the study, 208 for GUD and 1141 for UDS. The mean age of men who participated in the study was 28.1 years (median=26 years) and 1113 participants answered the questions about gender identity (100% cis male) and sexual orientation (68% reported to be heterosexual, 25.9% homosexual and 6.1% bisexual). Table 1 shows the distribution of the GU and UD samples by sentinel site.

A total of 208 GU samples were collected. Of these, two had an invalid result, which may represent a failure at the time of collection, as the internal control (human gene) was not detected during the amplification reaction. Given the characteristic of forming a healing crust in the ulcer, this condition may have influenced obtaining the sample, avoiding the absorption of exudate and contact with the injured epidermis. Two hundred six samples of GUs were analysed, and the results are illustrated in table 2. No positive samples were found for HD and VZV among the seven pathogens analysed. The most frequently identified pathogen was HSV2, representing 40.8% of the samples, followed by TP, with 24.8%. In 27.7% of samples, no pathogen was detected (ND).

Urethral discharge

For UD samples, 1141 were collected and analysed. The results obtained in the molecular analysis of UD showed that 50% (n=571) of the samples detected only NG, and this pathogen

Table 1 Genital ulcer (GU) and urethral discharge (UD) samples' distribution by site in Brazil, 2018–2020

Site	Region	GU, n	UD, n
Brasília/DF	Central-west	36	113
Porto Alegre/RS	South	31	90
Florianópolis/SC		9	55
Curitiba/PR		5	91
Manaus/AM	North	31	100
Salvador/BA	Northeast	8	109
Recife/PE		19	135
Natal/RN		0	35
Ribeirão Preto/SP		18	138
São Paulo/SP	Southeast	25	105
Belo Horizonte/MG		7	138
São José dos Campos/SP		19	32
Total		208*	1141

*Two invalid samples excluded from GU sampling.
AM, Amazonas; BA, Bahia; DF, Distrito Federal; MG, Minas Gerais; PE, Pernambuco; PR, Paraná; RN, Rio Grande do Norte; RS, Rio Grande do Sul; SC, Santa Catarina; SP, São Paulo.

was present in 78.4% (n=895) of the samples. Since this study included only participants with UD, a predominance of NG and/or CT was expected. Still, the second most frequent detection was NG+CT coinfection, with 14.4% of cases, followed by the result 'ND' with 8% of cases, and finally, detection of only CT with 6% of cases. CT was detected in 25.6% (n=292), MG 8.2% (n=94) of the samples, UU 10.2% (n=116), UP 3.2% (n=37), MH 3.4% (n=39) and TV 0.9% (n=10), as presented in table 3. The most frequent detections alone or in combination are shown in table 4, the percentage/number of samples with respective pathogens detected alone or in combination. Other combinations/pathogen detection represented 8% (n=92) of the sampling.

DISCUSSION

These results show NG and CT as the major causes of UD among Brazilian men, and a similar study conducted only in Brazil northern region has found these two pathogens as the most frequent STI causatives in UD.[15] Recent studies performed in South Africa and Zimbabwe have shown identical aetiological agent distribution both in UD and GU samples.[16–19] Genital herpes type 2 was the main cause of GUs. The causative agents of UDS and GUD in Brazil are little known; and therefore, Brazilian treatment protocols are based on international guidelines and regional studies due to a lack of a Brazilian representative study.[8 12] STIs that cause GU and UD are easily treated with antimicrobials. However, severe complications of these infections are observed due to the absence of treatment and further complications. STIs in heterosexual populations are seen to strongly affect the epidemic outbreak and lead to an increase of the epidemic threshold, and men-to-women transmission can impact the adverse outcomes in reproductive health.[4 5] Syphilis and gonorrhoea can be transmitted during pregnancy and childbirth and potentially cause severe comorbidities, increasing the social impact related to STIs.[11] A positivity greater than 50% of the samples for NG was expected, given the project's syndromic nature of sample collection. NG alone was responsible for causing 50.0% of the UD, and in 70.5% of the samples, NG and/or CT were responsible for the infection. Studies carried out in Uganda[20] and Kuwait[21] also found the predominance of these pathogens in UD. Non-gonococcal urethritis is known to be mainly caused by CT.[1 5]

The detection of MG in our study showed a higher number (4.7%) than the global estimates for men (1–4%). It is a concern considering that this pathogen can cause urogenital complications and may increase the risk of HIV infection.[22] Furthermore,

Table 2 Genital ulcer samples distributed by pathogen detection in Brazilian men (n=206), 2018–2020

Identification	%	n
HSV2	40.8	84
ND	27.7	57
TP	24.8	51
TP+HSV2	4.4	9
LGV	1.0	2
CMV+HSV2	1.0	2
HSV1	0.5	1

CMV, cytomegalovirus; HSV1, herpes simplex virus type 1; HSV2, herpes simplex virus type 2; LGV, lymphogranuloma venereum; ND, no pathogen detected; TP, *Treponema pallidum*.

Table 3 Percentage of pathogens (alone or in combination) detected in all samples in Brazilian men (n=1141)

Identification	%*	n†
NG	78.4	895
CT	25.6	292
UU	10.2	116
MG	4.7	94
MH	3.4	39
UP	3.2	37
TV	0.9	10

*The total is greater than 100% because, in the same sample, more than one pathogen can be detected.
†The total is greater than 1141 because, in the same sample, more than one pathogen can be detected.
CT, *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; MH, *Mycoplasma hominis*; NG, *Neisseria gonorrhoeae*; TV, *Trichomonas vaginalis*; UP, *Ureaplasma parvum*; UU, *Ureaplasma urealyticum*.

Table 4 Most frequent detections in urethral discharge samples (n=1141)

Identification	%*	n†
NG	50.0	571
CT+NG	14.4	164
ND	8.0	91
CT	6.0	69
NG+UU	3.9	45
MG	3.2	37
MG+NG	2.5	28
CT+NG+UU	2.0	23
NG+UP	1.1	12
UU	0.8	9

*Total percentage is lower than 100% because other combinations/pathogen detection represented 8%.
†Total number of samples is lower than 1141 because other combinations/pathogen detection represented 92 samples.
CT, *Chlamydia trachomatis*; MG, *Mycoplasma genitalium*; ND, no pathogen detected; NG, *Neisseria gonorrhoeae*; UP, *Ureaplasma parvum*; UU, *Ureaplasma urealyticum*.

isolates resistant to macrolides have been more frequent, increasing cases of treatment failure and consequent continuity of transmission of the infection.[3 23]

The evident circulation of these pathogens reinforces the need for rational use of antimicrobials and increased efforts to reduce the transmission of these agents to interrupt the transmission chain considering the potential for infections caused by NG to become untreatable.[24–26] Data from the most recent Brazilian national study showed increased resistance to ciprofloxacin, azithromycin and cefixime.[19] Despite this, current treatment with azithromycin and ceftriaxone dual therapy remains effective for both gonorrhoea and chlamydia infections in Brazil.[8 12 13]

To carry out this study, we chose to use a kit identifying seven different microorganisms, including MH, UU and UP, which are known to be part of the genital microbiota, and are defined as causing urethritis in a few cases, considering that its presence is often accompanied by another microorganism characterised as causing STIs such as CT or NG.[8 9 11] In only 19 samples (1.7%), MH, UU and UP were identified as single microorganism and, therefore, as potential causes of urethritis.

We highlight the great variety of pathogen combinations, considering that cases of coinfection were responsible for 38.8% of our findings. Detection of MG as a single pathogen or the coinfection of MG/CT or MG/NG is an important alert as the treatment for most people in the country is based on a syndromic approach. MG is not often considered in UD, and it is resistant to ceftriaxone and frequently resistant to azithromycin which leads to a potential lack of effective treatment in syndromic approach.[23 27]

There is an international consensus that the most frequent aetiological agents causing GUD are HSV2 and TP. This study found that Brazilian data follow international data and that the current treatment guideline efficiently treats those conditions. Brazilian protocol recommends chancroid and TP treatment in the syndromic approach of anogenital ulcers. Because of the absence of HD detection, it would be important to review this recommendation. The WHO already only recommends treatment for chancroid for people with anogenital ulcers if local surveillance shows reported or emerging cases.[1 3 6 8 11]

More than 25% of the GU samples did not have a pathogen detected, highlighting a possible limitation of the sample collection technique due to a shortage of genetic material of the pathogen (which depends on the stage of the disease–late lesion) or due to some skipped step of sample collection procedures. Hence, this finding may be related to ulcers not caused by pathogens, such as autoimmune conditions (Behcet's disease, inflammatory bowel disease, lichen planus), drug reactions, premalignant lesions and others.[28]

The limitation of the study was to have collected only male and symptomatic samples. However, it was possible to observe that the most frequent pathogens corresponded to international data on HSV2 and TP as the principal responsible for GU aetiology.[3 19 29] We found a variety of aetiological agents in the GUs, and coinfection cases were verified in 5.3% of the samples. However, the pathogens HD and VZV were not detected.

Extragenital infections are frequently asymptomatic, and regular testing with treatment infection remains a main role in preventing the spread of infection and the development of sequelae in individuals. The extragenital sites correspond to the rectum and oropharynx. They are considered reservoirs of infections and, consequently, bacterial gene exchange pathways and the acquisition of resistance to antimicrobials, especially the oropharynx for NG.[30] In the case of men who have sex with men, for example, if only a urogenital test is done, approximately 70% of gonococcal and chlamydial infections might be missed.[24] Thus, there is an urgent need to screen for extragenital infections to interrupt the chain of transmission and reduce the impact of antimicrobial resistance.[25]

This study provided the acquisition of unprecedented national data on the aetiology of UDS and GUD in Brazil, demonstrated the presence of a variety of pathogens in both sample types and reaffirmed the aetiologies known to be most prevalent globally. Local epidemiological data are essential to update the syndromic approach guidelines where aetiological diagnosis and treatment are unavailable for everyone. Beyond that, many STIs are asymptomatic, and the epidemiological knowledge acquired by studies like this provides essential information on STI control strategies. Given the worldwide concern about resistance and the health consequences of STIs, it is crucial to raise the quality of STI management, seeking to cure and break the chain of transmission.

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Contributors MLB designed and conducted the study and data analysis, wrote and revised the manuscript and is guarantor for this manuscript. HdMM conducted the study, performed sample and data analysis, and wrote and revised the manuscript. JMM and MAS conducted the study, performed sample tests and data analysis, and revised the manuscript. KB and FHB performed sample analysis. PCG, AEM and ABi conducted the study, and wrote and revised the manuscript. ABe designed the study and revised the manuscript. LdFA, FF, MFdCRdS, RJCS, LFAJ, LAdSN, RMF and WAF coordinated the sites of sample collection as well as performed sample collection. Brazilian-GASP Network collaborators performed sample collection and/or laboratory assessments.

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