***Treponema pallidum* in female sex workers from the Brazilian Marajó Archipelago: prevalence, risk factors, drug-resistant mutations and coinfections**

Evelen C. Coelho ^a, Samara B. Souza ^b, Camila Carla S. Costa ^c, Luana M. Costa ^c, Luiz Marcelo L. Pinheiro ^d,
Luiz Fernando A. Machado ^e, Gláucia C. Silva-Oliveira ^f, Luísa Caricio Martins ^{c,g}, Paula Cristina R. Frade ^c,
and Aldemir B. Oliveira-Filho ^{b,c,f,*}

^aResidência Multiprofissional em Saúde da Mulher e da Criança, Hospital Santo Antônio Maria Zaccaria, Bragança PA, Brazil; ^bPrograma de Pós-Graduação em Linguagens e Saberes na Amazônia, Universidade Federal do Pará, Bragança PA, Brazil; ^cPrograma de Pós-Graduação em Doenças Tropicais, Universidade Federal do Pará, Belém PA, Brazil; ^dFaculdade de Ciências Biológicas, Campus do Marajó, Universidade Federal do Pará, Soure PA, Brazil; ^eLaboratório de Virologia, Instituto de Ciências Biológicas, Universidade Federal do Pará, Belém PA, Brazil; ^fLaboratório de Células e Patógenos, Instituto de Estudos Costeiros, Universidade Federal do Pará, Bragança PA, Brazil; ^gLaboratório de Patologia Clínica de Doenças Tropicais, Núcleo de Medicina Tropical, Universidade Federal do Pará, Belém PA, Brazil

*Corresponding author: Instituto de Estudos Costeiros, Universidade Federal do Pará. Alameda Leandro Ribeiro, s/n. Aldeia. CEP 68600-000. Bragança PA, Brazil. Tel: +55 91 3425 1209 (Branch 227); E-mail: olivfilho@ufpa.br

Received 6 July 2020; revised 15 October 2020; editorial decision 17 October 2020; accepted 20 October 2020

Background: Female sex workers (FSWs) are an especially vulnerable group for syphilis and other sexually transmitted infection (STIs). This study determined the prevalence of syphilis in FSWs and factors associated with this disease in the Marajó Archipelago (northern Brazil), as well as the frequency of point mutations (A2058G and A2059G) in the 23S rRNA gene of *Treponema pallidum* and coinfections with hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis D virus (HDV).

Methods: FSWs were diagnosed using a rapid qualitative test and the isolates were evaluated for the presence of point mutations by real-time PCR. Blood samples with *T. pallidum* were tested for the presence of HBV, HCV and HDV by ELISA and confirmed by real-time PCR. The factors associated with syphilis were identified using Poisson regression models.

Results: Overall, 41.1% FSWs tested positive for syphilis and 23.5% were infected with strains having A2058G/A2059G point mutations. HBV (23.0%) and HCV (8.1%) were detected among FSWs with syphilis. Six factors were associated with syphilis: low levels of education, reduced income, drug use, unprotected sex, a lengthy career in prostitution and a lack of regular medical check-ups.

Conclusions: These findings indicate an urgent need for implementation of effective strategies to diagnose, prevent and treat syphilis, as well as other STIs, in this Brazilian region.

Keywords: Brazil, drug resistance, epidemiology, prostitution, sexually transmitted infections, syphilis

Introduction

Syphilis is a sexually transmitted infection (STI) caused by the bacterium *Treponema pallidum*. It is curable, exclusive to humans and, when not detected and treated early, can progress to a chronic condition, with irreversible long-term sequelae.¹ *T. pallidum* is transmitted primarily through sexual contact or by vertical transmission during pregnancy.² Annually, an esti-

mated 6 million new cases of syphilis are registered in people aged 15–49 y³ and the disease is endemic in many cities and regions that have populations with reduced socioeconomic status.⁴

Syphilis is most frequently treated using penicillin, a low-cost antibiotic with proven efficacy against *T. pallidum*. However, due to the global scarcity of this drug and cases of allergy to penicillin in many patients, alternative types of medication,

such as macrolide antibiotics (which include azithromycin and erythromycin), have increasingly been used in recent years.⁵ Although macrolides have been shown to be effective for the treatment of syphilis, an increasing incidence of drug-resistant strains has been reported in recent years. Drug resistance is associated with the point mutations A2058G and A2059G in the 23S rRNA gene, which have been detected in some strains of *T. pallidum*. The widespread and indiscriminate use of antibiotics in many populations, often without a prescription, has resulted in the selection and spread of drug-resistant bacterial strains, which hampers the treatment of both syphilis and other types of bacterial infection.^{6,7}

Although syphilis is easy to diagnose and treat, *T. pallidum* infection rates remain high. In 2016, the WHO launched a new strategy to combat STIs, in particular syphilis. The strategy prioritizes the elimination of congenital syphilis and control of the disease in key populations, in particular sex workers.³ Female sex workers (FSWs) are an especially vulnerable group for syphilis and other STIs, not only because of the large numbers of sexual partners and the occurrence of unprotected sex, but also because of the individual, social and programmatic vulnerabilities of these individuals, which facilitate the acquisition of *T. pallidum*. The key aspects of these individuals are their high geographic mobility, low levels of education and economic income, societal barriers related to their gender, social stigma and their restricted access to public health services.^{8–10}

In northern Brazil, epidemiological studies of syphilis and coinfections in FSWs are still scarce. Two studies have revealed a high prevalence of syphilis in FSWs operating along the highway system (36.9%) and in three towns in the interior of the state of Pará (14.1%). A number of factors are associated with the prevalence of syphilis in this key group, including the use of illicit drugs, unprotected sex and anal sex, the length of career in prostitution and the provision of highly priced sexual services.^{11,12}

The Marajó Archipelago, in northern Brazil, is one of the least developed areas of South America.¹³ This archipelago consists of ~2500 islands and islets, with a population of almost 487 000 inhabitants, distributed in 16 municipalities. Most of these municipalities have very low human development indices and a range of socioeconomic problems, including malnutrition, illiteracy, drugs trafficking and addiction, sexual abuse, as well as exploitation of minors and prostitution.^{13–15} In this archipelago, epidemiological studies with multiple infectious diseases have indicated a prevalence of antibodies to *T. pallidum* (8.9%), HBV (12.4%) and HCV (1.3%)¹⁶ in the general population, as well as higher rates of HBV (13.7%)¹⁴ and HCV (10.7%)¹⁷ in FSWs.

In this epidemiological scenario, the present study estimated the prevalence of syphilis and the factors associated with this disease in FSWs from the Marajó Archipelago, as well as assessing the occurrence of the A2058G and A2059G point mutations in the 23S rRNA gene of *T. pallidum* and identifying coinfections with HBV, HCV and hepatitis D virus (HDV). The findings of the study will be essential for the development of effective strategies of control and assistance and the prevention of new infections in this key social group, and in the general population of the study area in this underdeveloped region of northern Brazil.

Materials and Methods

Study design and sampling

During the present, cross-sectional study, clinical samples and personal data were collected from women providing sexual services in 7 towns and 18 riverside communities in the Marajó Archipelago, in northern Brazil (Figure 1). Three sampling approaches—respondent-driven sampling, time location sampling and take-all sampling—were employed to guarantee the collection of a representative sample (Tables S1 and S2). These complementary approaches were used to assess the largest possible number of local FSWs. Initially, 153 FSWs were assessed from April 2015 to December 2017 in 5 towns and 18 riverside communities,¹⁴ and a further 27 FSWs were assessed in two other municipalities from July 2018 to January 2019 (Table 1).

The criteria for the inclusion of sex workers in the sample were (1) being female, (2) having sex in exchange for money in the Marajó Archipelago for at least 3 mo, (3) being aged ≥ 18 y and (4) agreeing to provide clinical samples and personal information. FSWs were excluded from the sample whenever (1) they were under the influence of psychotropic drugs during an interview or (2) they represented a risk to the physical integrity of the researcher. The identification of all study participants was kept confidential to preserve privacy and not cause harm. All researchers who accessed the participants signed an information confidentiality agreement and expressed their professional commitment to maintain the confidentiality of the information and the privacy of the participants.

Laboratory tests

At all sites, the blood samples collected from the FSWs were examined using the rapid qualitative test (RQT) for syphilis (SD Bioline Syphilis 3.0, Standard Diagnostics Inc., Yongin-si, Kyonggi, South Korea), following the manufacturer's instructions. Blood samples (5 mL) were also collected by venipuncture with disposable syringes and tubes containing anticoagulants to assess the presence of viruses in FSWs with syphilis. When the RQT indicated a reaction, a sample of exudate was obtained by swabbing each FSW that reported the presence of ulcerations or abrasions of the oral and/or genital mucosa. In the case of the FSWs that reacted to the RQT and did not have mucosal lesions, but presented skin lesions, such as macules or papules, these lesions were swabbed to obtain a sample of serous exudate. A sample of DNA was isolated from each swab using the Nucleospin Blood kit (Macherey-Nagel Eurl, France) and syphilis was diagnosed based on the detection of a specific fragment of the *polA* gene of *T. pallidum* using real-time PCR.¹¹ The isolates that tested positive were also analyzed to detect the presence of point mutations (A2058G or A2059G) in the *T. pallidum* 23S rRNA gene, also by real-time PCR.⁶ The success of the isolation of the DNA of the samples was assessed by amplifying a fragment of the albumin gene.¹⁸

In addition, blood samples from FSWs with reactive results for syphilis were also tested for the presence of hepatitis B surface antigen (HBsAg—AxSYM HBsAg, Abbott, Chicago, Illinois, USA) antibodies to the HBV core antigen (anti-HBc—Total Murex Anti-HBc, DiaSorin, Saluggia, Vercelli, Italy) and antibodies to HCV

Figure 1. Location of the 25 sites from which biological samples and personal data were collected from female sex workers in the Marajó Archipelago, northern Brazil, for analysis in the present study. Points 1 to 5, 24 and 25 are towns: Breves (1), Bagre (2), Curralinho (3), Melgaço (4), São Sebastião da Boa Vista (5), Soure (24) and Salvaterra (25). Points 6 to 23 are small riverside communities: Antônio Lemos (6), Capinal (7), São Francisco (8), Ramex (9), Sebastião (10), Nossa Senhora de Fátima (11), Mainard (12), Intel (13), Campo Beija Flor (14), Zé Gama (15), Nova Cana (16), Santa Cruz (17), Monte Tabú (18), São José (19), Corcovado (20), Magebras (21), Bom Jesus (22) and Jupatituba (23).

(Murex anti-HCV 4.0, DiaSorin, Saluggia, Vercelli, Italy). All samples of FSWs with positive results for HBsAg were evaluated for the presence of antibodies against HDV (Anti-HDV—ETIAB-DELTAK-2, DiaSorin, Saluggia, Vercelli, Italy). Positive results from HBV and HCV by ELISA were confirmed using real-time PCR.^{14,15} In all amplification reactions, positive and negative samples were used as controls to assess the quality of the assays.

Statistical analysis

The data on the demographic, socioeconomic and professional characteristics of the participants were obtained using epidemio-

logical forms. This research tool has been used successfully in previous epidemiological studies of FSWs.^{11,14,19} These data were fed into an Microsoft Excel database and converted to SPSS format for all procedures and statistical analyses. The 95% CIs were determined to estimate the prevalence of syphilis and point mutations in the *T. pallidum* 23S rRNA gene. χ^2 was used to evaluate the possible association between variables and the result of the RQT for syphilis (outcome). Poisson regression models were used to estimate the reasons of prevalence (RPs) for each category of exploratory variable, with the lowest expected risk category being used as a reference. This modeling procedure estimated the raw prevalence ratio for each exploratory variable, using Wald's

Table 1. Information on the sampling sites and the collection of personal data from female sex workers in the present study in the Marajó Archipelago, northern Brazil (clinical samples were collected from all individuals).

Location	Type of site	Principal access	Method ^a	Sample size (individuals)
Breves	Town	Parauau River	TLS, RDS	30
Bagre	Town	Jacundá River	TLS	11
Curralinho	Town	Pará River	RDS	13
Melgaço	Town	Tajapurú River	RDS	13
São Sebastião da Boa Vista	Town	Boa Vista River	RDS	12
Antônio Lemos	Community	Tajapurú River	TAS	7
Capinal	Community	Tajapurú River	TAS	5
São Francisco	Community	Tajapurú River	TAS	5
Ramex	Community	Tajapurú River	TAS	5
São Sebastião	Community	Tajapurú River	TLS	4
Nossa Senhora de Fátima	Community	Tajapurú River	TLS	3
Mainard	Community	Jaburú River	TLS	3
Intel	Community	Mearin River	TLS	3
Campo Beija Flor	Community	Tajapurú River	TLS	3
Zé Gama	Community	Pracaxi River	TLS	2
Nova Canaã	Community	Pracaxi River	TLS	3
Santa Cruz	Community	Pracaxi River	TLS	3
Monte Tabú	Community	Parauau River	TLS	4
São José	Community	Parauau River	TLS	3
Corcovado	Community	Parauau River	TLS	4
Magebras	Community	Parauau River	TAS	5
Bom Jesus	Community	Aturiá River	TAS	6
Jupatituba	Community	Parauau River	TAS	6
Soure	Town	Paracauari River	TAS	15
Salvaterra	Town	Paracauari River	TAS	12

^aSampling methods used to collect personal data: respondent-driven sampling (RDS), time location sampling (TLS) and take-all sampling (TAS). For more details on the sampling methods, see Table S1.

statistic test, with a significance level of $p=0.05$. The adjusted RP values were then calculated using a regression model, which simultaneously included all the variables that returned $p<0.05$ for Wald's statistic test. The data were analyzed using SPSS Statistics 20.0 (IBM, Armonk, NY, USA).

Results

Population sample

This study initially assessed 229 FSWs in the Marajó Archipelago of northern Brazil, 49 of whom were excluded from the study because they were aged <18 y ($n=25/49$; 51.0%), showed signs of intoxication during interview ($n=10/49$; 20.4%) or requested payment to provide clinical samples or personal information ($n=14/49$; 28.6%). This reduced the total sample to 180 FSWs. Most of these women ($n=106/180$; 58.9%) offered their sexual services in bars, restaurants, town squares, ports and gas stations in the towns of Bagre, Curralinho, Melgaço, Salvaterra, São Sebastião da Boa Vista, Salvaterra and Soure (Table 1). The other FSWs ($n=74/180$; 41.1%) worked in bars and gas stations located on the river margins of small riverside communities in the Marajó

archipelago (Table 1). These two groups were not compared in the analyses because the majority of the women assessed in riverside communities ($n=59/74$; 79.7%) reported that they would often travel to larger towns to work.

Characteristics of the FSWs

Most of the study participants ($n=163/180$; 90.6%) reported having been born in the Marajó Archipelago. The mean age of the FSWs was 23.5 y and the majority identified themselves as single, heterosexual and non-white, with low levels of education and a low monthly income (Table 2). The mean monthly income was ~R\$550.00 (~US\$145.00), with the mean price of a sexual encounter being R\$20.00 (equivalent to US\$5.00). Most of the women ($n=109/180$; 60.6%) reported having been a sex worker for >7 y. Some of the FSWs also reported engaging in unprotected sex (without a condom), in particular with clients who paid more for the sexual encounter ($n=42/180$; 23.3%) and regular clients ($n=20/180$; 11.1%). Almost two-thirds ($n=116/180$; 64.4%) of the FSWs reported having had a wound, ulcer or itching in the genitalia in the previous 12 mo and 47.2% ($n=85/180$) reported having used medication to care for their health, but without any

Table 2. Demographic and socioeconomic characteristics of the female sex workers surveyed in the present study in the Marajó Archipelago, northern Brazil and their respective prevalence of syphilis

Characteristic	N	Syphilis + (%)	Syphilis - (%)	p
All participants	180	74 (41.1)	106 (58.9)	-
Age (y)				
18–24	103	43 (41.7)	60 (58.3)	0.24
25–30	69	30 (43.5)	39 (56.5)	
≥31	8	1 (12.5)	7 (87.5)	
Color/race (self-declared)				
White	42	15 (35.7)	27 (64.3)	0.24
Brown (mixed race)	84	35 (41.7)	49 (58.3)	
Black	54	24 (44.4)	30 (55.6)	
Sexual orientation				
Heterosexual	171	72 (42.1)	99 (57.9)	0.49
Homosexual	5	1 (20.0)	4 (80.0)	
Bisexual	4	1 (25.0)	3 (75.0)	
Education				
Illiterate	30	19 (63.3)	11 (36.7)	< 0.01
Incomplete/complete elementary school	130	54 (41.5)	76 (58.5)	
Incomplete/complete high school	18	1 (5.6)	17 (94.4)	
Incomplete college	2	0 (0.0)	2 (100.0)	
Marital status*				
Single	168	72 (42.9)	96 (57.1)	0.13
Married + stable union	7	2 (28.6)	5 (71.4)	
Separated + widow	5	0 (0.0)	5 (100.0)	
Monthly income in terms of minimum wage ^a				
≤1	145	71 (49.0)	74 (51.0)	<0.01
2–3	29	3 (10.3)	26 (89.7)	
>3	6	0 (0.0)	6 (100.0)	

*Past 12 months; ^aMinimum wage=R\$890 (equivalent to US\$220).

clinical evaluation or medical guidance. Antibiotics and supplies for the health of these women, such as condoms and lubricating gel, were obtained from pharmacies and small commercial establishments in municipalities and riverside communities. According to the FSWs, health services in the municipalities are precarious and do not adequately serve the population. However, 35.6% of FSWs (n=64/180) reported having undergone medical/gynecological screening in the last 12 mo at basic health units located in the municipalities of the Marajó Archipelago because there are no health services available in riverside communities.

Prevalence of syphilis, coinfections and resistant mutations

Overall, 74 (41.1%) of the 180 FSWs presented reactive results for syphilis in the RQT, of whom 68 (37.8%) reported the presence of lesions on the skin (n=15) or oral/genital mucosa (n=53) and provided samples of exudate. The isolation of DNA from these samples was confirmed by the detection of a fragment of the albumin gene using real-time PCR. The A2058G and A2059G mutations of the *T. pallidum* 23S rRNA gene were detected in 16 (23.5%) of the FSWs with active syphilis and 5 (7.4%) of these women

had both mutations (Table 3). All the FSWs diagnosed with *T. pallidum* strains containing mutations in the 23S rRNA gene reported having self-medicated. Among the FSWs with reactive results for syphilis, 23 (31.1%) FSWs had coinfections (Table 3). In this sample, 17 (23.0%) FSWs were exposed to HBV, of whom 2 tested positive for HBsAg and 15 had anti-HBc. Three FSWs exposed to HBV had virus nucleic acid. HDV was not detected among FSWs with reactive results for syphilis and those positive for HBsAg. Regarding HCV, six (8.1%) FSWs tested positive for anti-HCV antibodies and two of them had HCV RNA. Finally, two (2.7%) FSWs showed positive results for the presence of *T. pallidum*, HBV and HCV.

Factors associated with syphilis

Eight factors were associated with syphilis based on the Poisson regression models: (1) reduced schooling (illiterate or elementary school education), (2) a monthly income of less than the minimum wage in the past 12 mo, (3) the use of illicit drugs (inhaled/injectable) at some time, (4) having unprotected sex in the previous 7 d, (5) having unprotected sex with a regular client in the previous 7 d, (6) having unprotected sex with a client who paid more for the encounter in the previous 7 d, (7) >7 y as a sex

Table 3. Prevalence of syphilis, coinfections and point mutations in the *Treponema pallidum* 23S rRNA gene in female sex workers from the Marajó Archipelago, in northern Brazil

Diagnosis	Prevalence		
	Positive/Total	%	95% CI
Syphilis	74/180	41.1	38.3 to 44.8%
Wild strain*	50/68	77.0	73.2 to 81.4%
A2058G or A2059G mutant strain	16/68	23.5	20.3 to 26.4%
A2058G mutant strain	5/68	7.4	3.2 to 12.2%
A2059G mutant strain	6/68	8.8	4.9 to 13.0%
A2058G+A2059G mutant strains	5/68	7.4	3.2 to 12.2%
Coinfections	23/74	31.1	28.2 to 33.5%
HBV-TP	17/74	23.0	19.8 to 25.7%
HCV-TP	6/74	8.1	4.6 to 10.9%
HBV-HCV-TP	2/74	2.7	0.0 to 6.6%

HBV: hepatitis B virus; HCV: hepatitis C virus; TP: *Treponema pallidum*.
*No mutations (A2058G or A2059G).

worker and (8) not having a medical/gynecological examination in the previous 12 mo (Table 4). Spending >7 y as a sex worker was identified as the principal risk factor for syphilis in the FSWs (RP>17.0). No significant association with syphilis was found in any of the other 11 factors analyzed here (Table S3).

Discussion

More than half of the Amazon rainforest is located in northern Brazil. This region has enormous biological diversity, but is underdeveloped, in general, with high levels of poverty, limited transportation infrastructure and inadequate public healthcare services.¹⁴ The characteristics of the FSWs assessed in the Marajó Archipelago are typical of the socioeconomic problems of this Brazilian region. Most of the participants in this study were young, non-white, with a low income, low schooling level and engaged in unprotected sex to attract clients or charge more for the encounter. These findings are consistent with those of previous studies in both Brazil and other countries, such as Rwanda, Zambia and Argentina, which have described similar FSW profiles and emphasize the vulnerability of this group to *T. pallidum* and other pathogens.^{11,12,20–23}

A high prevalence of syphilis was recorded in the present study. This prevalence was much higher than that recorded in three towns in the interior of the state of Pará (14.1%), based on samples collected in 2007 and 2008,¹² although it was similar to the rate recorded in FSWs in 10 municipalities in Pará (36.94%).¹¹ The prevalence of syphilis detected in the present study was fivefold higher than that recorded by Ferreira-Junior et al.,²⁴ that is, 8.5%

in other Brazilian regions, although it was similar to that observed in FSWs in Argentina, where a rate of 45.5% was recorded.²² Much lower rates have been recorded in FSWs in other countries, however, such as Iran and Mexico, with infection rates of 0.4% and 7.8%, respectively.^{25,26} The prevalence of syphilis detected in the current study in FSWs from the Marajó Archipelago was the highest for any survey conducted on FSWs in Brazil and should serve as an urgent warning to the local public health authorities.

A high prevalence of *T. pallidum* strains with the A2058G and A2059G point mutations in the rRNA gene was also observed in the present study, further aggravating the epidemiological scenario. These mutations have already been identified in a number of countries, including China, the UK, the USA and Ireland.^{27–30} The present study is the first scientific report of the occurrence of these drug-resistant mutations in Brazil. This finding contributes to the understanding of the geographical distribution of drug-resistant *T. pallidum* strains in the world and, in particular, serves as a wake-up call for the public health surveillance service in Brazil.

The occurrence and spread of drug-resistant strains have a major impact on public health, given that these mutations can hinder the treatment of syphilis, requiring the use of macrolides, which may be in limited supply in some underdeveloped regions.^{6,27,29} The high prevalence of drug-resistant *T. pallidum* strains in the Marajó Archipelago may be related directly to the local level of self-medication, which was reported frequently by FSWs and is a very common practice in Brazil. The extensive and indiscriminate use of antibiotics, such as azithromycin, to treat miscellaneous infections of organs such as the skin, respiratory tract and genitals, contributes to the increase in prevalence of drug-resistant strains.⁷ The indiscriminate use of antibiotics by FSWs, in the absence of medical advice on the dosage of medication and duration of the treatment, may be selecting specific drug-resistant *T. pallidum* strains acquired during sex work. Given this, macrolides (azithromycin and erythromycin) should be used with extreme caution for the treatment of syphilis, and only when penicillin and doxycycline are unavailable, in particular in areas where the prevalence of macrolide-resistant strains is unknown. Chen et al.⁶ emphasized the need for strict monitoring and the controlled use of medicines by healthcare workers, although in the Marajó Archipelago public health services are rudimentary, and it may be nearly impossible to halt the selection and spread of drug-resistant *T. pallidum* strains in this Brazilian region.

Of the risk factors associated with syphilis, unprotected sex and drug use were prominent, and reflect the failure of educational programs and preventive measures to combat STIs. Other factors, such as low income and education levels, a long career in prostitution and a lack of regular medical examinations, contributed to the vulnerability of FSWs and the risk of acquiring and spreading *T. pallidum*. These factors have already been reported in other epidemiological studies of syphilis and other STIs in FSWs in Brazil and other South American countries.^{11,12,19,22,23}

Epidemiological information on coinfections in FSWs is still limited in northern Brazil.²⁴ Studies have reported high rates of HBV infection (13.7%)¹⁴ and HCV (10.7%)¹⁷ among FSWs in the Marajó Archipelago. Like *T. pallidum*, other pathogens such as HIV, HBV and HCV are being acquired and transmitted by the same route

Table 4. Factors associated with syphilis in female sex workers from the Marajó Archipelago, in northern Brazil

Factors	Syphilis + / Total (%)	Raw RP (95% CI)	Adjusted RP CI
Low educational level*	73/160 (45.6)	13.7 (1.8 - 28.7)	11.2 (2.2 - 24.5)
Low income (less than minimum wage) ^a	71/145 (49.0)	10.2 (2.9 - 33.8)	10.6 (3.0 - 29.3)
Drug use (injectable/inhaled) ^b	36/47 (76.6)	8.1 (3.7 - 17.6)	7.7 (3.5 - 15.2)
Unprotected sex ^c	54/71 (76.1)	14.1 (6.7 - 28.4)	14.8 (6.5 - 26.6)
Did not use a condom with a regular client ^c	14/20 (70.0)	3.9 (1.4 - 10.7)	4.2 (1.5 - 9.4)
Did not use a condom when the client paid extra ^c	35/42 (83.3)	12.5 (4.9 - 27.5)	12.8 (5.1 - 23.6)
More than seven years working as a sex worker	68/109 (62.4)	17.6 (7.1 - 40.2)	18.2 (7.3 - 35.8)
No regular medical/gynecological screening ^a	69/116 (59.5)	17.3 (6.3 - 41.0)	17.6 (6.2 - 36.5)

RP: reason of prevalence; 95% CI: 95% confidence interval; *Low educational level = illiterate or incomplete/complete elementary school.

^aPast 12 months.

^bDuring lifetime.

^cPast 7 days.

and mechanism of transmission: unprotected sex.^{17,31} This study identified 23 cases of syphilis with hepatotropic viruses (HBV and HCV) in this key population. Thus, public health action is necessary for the early detection of syphilis in FSWs, which also includes screening testing for other pathogens, so it will be possible to identify and treat women with STIs.

Overall, then, the epidemiological findings of the present study highlight the urgent need for strategies to control, prevent and support FSWs in the Marajó Archipelago and, in turn, throughout northern Brazil.^{14,17,32} One fundamental strategy should be the active identification of FSWs by public health institutions, followed by the intervention of community healthcare workers in the towns and riverside communities, advising FSWs to seek diagnosis, treatment, preventive measures and other clinical assessments in local public clinics. Education programs are also necessary, not only to guarantee the health of the individual, but also with the aim of valuing and promoting self-esteem, self-worth, self-confidence, self-care, women's rights and social inclusion, as well as combating violence against women, along the lines of the initiatives implemented among FSWs in the Brazilian municipality of Bragança.³² These activities can modify the perception of the risk of acquiring STIs by these vulnerable women and, in particular, guarantee their access to healthcare services.

The present study has a number of limitations that should be considered with caution. Despite the use of a number of complementary methods, sampling was not necessarily representative, given the difficulty of accessing participants. Similarly, as the personal data were self-reported, some information, such as drug use or the practice of unprotected sex, may have been underestimated. The findings of the present study nevertheless provide important insights into the epidemiological scenario of syphilis in northern Brazil, and should be considered by the authorities when developing strategies for the control and prevention of this disease in this key social group, and also in the general population of the Marajó region, as well as in other regions with similar socioeconomic conditions and limited public healthcare services.

Conclusions

This study provided important insights into the epidemiology of syphilis in FSWs in a poorly developed region of Brazil. A high prevalence of *T. pallidum* infections and a high frequency of strains resistant to macrolides were detected in FSWs from the Brazilian Marajó Archipelago. The acquisition and spread of *T. pallidum* was associated with the socioeconomic status of the FSWs and their high-risk behaviors. In addition, several cases of HBV and HCV infections among FSWs with syphilis have been identified, clearly indicating the importance of screening for other STIs in this key population. Adequate measures for the control and prevention of syphilis must be planned and implemented urgently to prevent the further spread of *T. pallidum* and antibiotic-resistant strains in the general population of this Brazilian region. The general scenario observed in the present study highlights the need for initiatives to limit the vulnerability of these women, including social assistance and healthcare practices, which should hopefully guarantee a reduction in the number of new cases of syphilis and other STIs in the region.

Supplementary data

Supplementary data are available at [Transactions](#) online.

Authors' contributions: All authors contributed to the development of research. Study design: ABOF. Investigation and methodology: ECC, SBS, CCSC, LMC, LMLP, LFAM, GCSO, LCM, PCRF and ABOF. Writing—original draft: ECC, PCRF and ABOF. Writing—review and editing: SBS, CCSC, LMC, LMLP, LFAM, GCSO and LCM. Project administration: LMLP, LCM and ABOF. Funding acquisition: LMLP, GCSO and ABOF. All authors read and approved the final manuscript.

Acknowledgements: The authors are grateful for the generous assistance of the local facilitators (community health workers, business own-

ers and members of local communities) who helped us to make contact with the local sex workers, especially in the riverside communities.

Funding: This work was supported by Fundação Amazônia de Amparo a Estudos e Pesquisas, Brazil [FAPESPA—ICAAF 154/2014]. LMLP, LFAM, LCM and ABOF acknowledge the support of the Universidade Federal do Pará (UFPA), Brazil.

Competing interests: None declared.

Ethical approval: All the participants of the present study provided informed and written consent, and also received the results of their laboratory tests. Participants with syphilis were referred to public clinics for counseling and treatment. This study was approved by the Research Ethics Committee of the Núcleo de Medicina Tropical at the Universidade Federal do Pará in Belém, Brazil (CAAE: 37536314.4.0000.6287).

Data availability: The data underlying this article will be shared on reasonable request to the corresponding author.

References

- Ministério da Saúde, Brazil. Protocolo Clínico e Diretrizes Terapêuticas para Atenção Integral às Pessoas com Infecções Sexualmente Transmissíveis (IST)/Relatório de recomendação. 2015. Available at <http://www.aids.gov.br/pt-br/pub/2015/protocolo-clinico-e-diretrizes-terapeuticas-para-atencao-integral-pessoas-com-infecoes> [accessed January 20, 2020].
- Avelaira JCR, Bottino G. Syphilis: diagnosis, treatment and control. *An Bras Dermatol*. 2006;81(2):111–26.
- World Health Organization. Global Health Sector Strategy on Sexually Transmitted Infections 2016–2021. Towards Ending STIs. 2016. Available at <https://apps.who.int/iris/bitstream/handle/10665/246296/WHO-RHR-16.09-eng.pdf?sequence=1> [accessed January 19, 2020].
- Kojima G, Klausner JD. An update on the global epidemiology of syphilis. *Curr Epidemiol Rep*. 2018;5:1–12.
- Gultom DA, Rosana I, Efendi I, et al. Detection and identification of azithromycin resistance mutations on *Treponema pallidum* 23S Rna gene by nested multiplex polymerase chain reaction. *Med J Indones*. 2017;26(2):90–6.
- Chen CY, Chi KH, Ballard RC, et al. Detection of the A2058G and A2059G 23S rRNA gene point mutations associated with azithromycin resistance in *Treponema pallidum* by use of a TaqMan Real-Time Multiplex PCR assay. *J Clin Microbiol*. 2013;51(3):908–13.
- Marra CM, Godornes RP, Tantalo LC, et al. Antibiotic selection may contribute to increases in macrolide-resistant *Treponema pallidum*. *J Infect Dis*. 2006;194(12):1771–3.
- Ayres JRCM, Calazans GJ, Saletti HCF, et al. Risco, vulnerabilidade e práticas de prevenção e promoção da saúde. In: Campos GWS (ed). *Tratado de Saúde Coletiva*. 2nd edn. São Paulo: Hucitec: Fiocruz; 375–417.
- Figueiredo P, Peixoto M. Sex professionals and vulnerability. *Bol Inst Saúde*. 2010;12(2):1518–812.
- Nurlan SN, Davies SCSC, Kaldor JJ, et al. Prevalence over time and risk factors for sexually transmissible infections among newly-arrived female sex workers in Timika, Indonesia. *Sex Health*. 2011;8(1):61–4.
- Cavalcante NS, Barbosa ESS, Costa NPS, et al. Syphilis in female sex workers: an epidemiological study of the highway system of the state of Pará, northern Brazil. *Rev Soc Bras Med Trop*. 2019;52:1–5.
- Souza RL, Madeira LPS, Pereira MVS, et al. Prevalence of syphilis in female sex workers in three countryside cities of the states of Pará, Brazilian Amazon. *BMC Infect Dis*. 2020;20:129.
- Andrade AP, Pacheco SD, Silva FQ, et al. Characterization of hepatitis B virus infection in illicit drug users in the Marajó Archipelago, northern Brazil. *Arch Virol*. 2017;162(1):227–33.
- Frade PCR, Costa LM, Lemos JAR, et al. Prevalence and genotyping of hepatitis B virus: a cross-sectional study conducted with female sex workers in the Marajó Archipelago, Brasil. *J STD AIDS*. 2019;30(9):902–10.
- Silva FQ, Santos FJA, Andrade AP, et al. Hepatitis C virus infection among illicit drug users in an archipelago of the Amazon. *Arch Virol*. 2018;163:617–22.
- Ferreira GRON, Freitas FB, Queiroz MAF, et al. Epidemiology and risk factors for Chlamydia trachomatis, Treponema pallidum, Hepatitis B virus and Hepatitis C virus in the Marajo. *J Community Med Health Educ*. 2019;9:1–11.
- Oliveira-Filho AB, Aires DW, Cavalcante NS, et al. Hepatitis C virus among female sex workers: a cross-sectional study conducted along rivers and highways in the Amazon region. *Pathogens*. 2019;8(4):236.
- Tamegão-Lopes BP, Rezende PR, Maradei-Pereira LMC, et al. Carga proviral do HTLV-1 e HTLV-2: um método simples através da PCR quantitativa em tempo real. *Rev Soc Bras Med Trop*. 2006;39(6):548–52.
- Costa LM, Raiol NC, Lisboa BLA, et al. Prevalence and risk factors for HIV infection among female sex workers: distinct offers of sexual services in a municipality of the Brazilian Amazon. *AIDS Res Hum Retroviruses*. 2019;35(9):826–32.
- Ingabire R, Parker RL, Nyombayire J, et al. Female sex workers in Kigali, Rwanda: a key population at risk of HIV, sexually transmitted infections, and unplanned pregnancy. *Int J STD AIDS*. 2019;30(6):557–68.
- Kilembe W, Inambao M, Sharkey T, et al. Single mothers and female sex workers in Zambia have similar risk profiles. *AIDS Res Hum Retroviruses*. 2019;35(9):814–25.
- Pando MA, Berini C, Bibini M, et al. Prevalence of HIV and other sexually transmitted infections among female commercial sex workers in Argentina. *Am J Trop Med Hyg*. 2006;74(2):233–6.
- Pinto VM, Tancredi MV, Alencar HDR, et al. Prevalence of syphilis and associated factors in homeless people of São Paulo, Brazil, using a Rapid Test. *Rev Bras Epidemiol*. 2014;17(2):341–54.
- Ferreira-Junior OC, Guimarães MDC, Damascena GN, et al. Prevalence estimates of HIV, syphilis, hepatitis B and C among female sex workers (FSW) in Brazil, 2016. *J Medicine*. 2018;97(1S):S3–8.
- Mirzazadeh A, Shokoohi M, Karamouzian M, et al. Declining trends in HIV and other sexually transmitted infections among female sex workers in Iran could be attributable to reduced drug injection: a cross-sectional study. *Sex Transm Infect*. 2019;96(1):68–75.
- Patterson TL, Strathdee SA, Semple SJ, et al. Prevalence of HIV/STIs and correlates with municipal characteristics among female sex workers in 13 Mexican cities. *Salud Pub Mex*. 2019;62(2):116–24.
- Li Y, Li J, Hu W, et al. Gene subtype analysis of *Treponema pallidum* for drug resistance to azithromycin. *Exp The Med*. 2018;16(2):1009–13.
- Lukehart SA, Godornes C, Molini BJ, et al. Macrolide resistance in *Treponema pallidum* in the United States and Ireland. *N Engl J Med*. 2004;351(2):154–8.
- Šmajš D, Grillová L, Paštěková L. Macrolide resistance in the syphilis spirochete, *Treponema pallidum* ssp. *pallidum*: Can we also expect

- macrolide-resistant yaws strains? *Amj Trop Med Hyg.* 2015;34(9):678–83.
- 30 Tipple C, McClure MO, Taylor GP. High prevalence of macrolide resistant *Treponema pallidum* strains in a London centre. *Sex Transm Infect.* 2011;87(6):486–8.
- 31 Gibney L, Macaluso M, Kirk K, et al. Prevalence of infectious diseases in Bangladeshi women living adjacent to a truck stand: HIV/STD/ hepatitis/genital tract infections. *Sex Transm Inf.* 2001;77: 344–50.
- 32 Frade PCR, Da Costa LM, Lisboa BLA, et al. Strategies and actions to access and assist in the health promotion of female sex workers. In Silva Neto BR (ed) *Public Health and Collective Health: Dialogue on Thematic Interfaces*, 1st edn. Ponta Grossa, Brazil: Atena; 2019;4: 214–24.