Sexually transmitted infections, bacterial vaginosis, and candidiasis in women of reproductive age in rural Northeast Brazil: a population-based study

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Population-based data on sexually transmitted infections (STI), bacterial vaginosis (BV), and candidiasis reflect the epidemiological situation more accurately than studies performed in specific populations, but such data are scarce. To determine the prevalence of STI, BV, and candidiasis among women of reproductive age from a resource-poor community in Northeast Brazil, a population-based cross sectional study was undertaken. All women from seven hamlets and the centre of Pacoti municipality in the state of Ceará, aged 12 to 49 years, were invited to participate. The women were asked about socio-demographic characteristics and genital symptoms, and thereafter examined gynaecologically. Laboratory testing included polymerase chain reaction (PCR) for human papillomavirus (HPV), ligase chain reaction (LCR) for Chlamydia trachomatis *and* Neisseria gonorrhoeae, *ELISA for human immunodeficiency virus (HIV), venereal disease research laboratory (VDRL) and fluorescent treponema antibody absorption test (FTA-ABS) for syphilis, and analysis of wet mounts, gram stains and Pap smears for trichomoniasis, candidiasis, and BV. Only women who had initiated sexual life were included in the analysis (n = 592). The prevalences of STI were: HPV 11.7% (95% confidence interval: 9.3-14.7), chlamydia 4.5% (3.0-6.6), trichomoniasis 4.1% (2.7-6.1), gonorrhoea 1.2% (0.5-2.6), syphilis 0.2% (0.0-1.1), and HIV 0%. The prevalence of BV and candidiasis was 20% (16.9-23.6) and 12.5% (10.0-15.5), respectively. The most common gynaecological complaint was lower abdominal pain. STI are common in women in rural Brazil and represent an important health threat in view of the HIV pandemic.*

Key words: sexually transmitted infections - reproductive tract infections - prevalence - epidemiology - Brazil

Sexually transmitted infections (STI) are a major cause of morbidity throughout the world, particularly in developing countries (Gerbase et al. 1998). In women, STI are often chronic and present with little or no symptoms, but eventually may lead to severe sequels, such as chronic pelvic inflammatory disease, ectopic pregnancy, and infertility (WHO 2000). The impact of STI on the health of women tends to be more severe in resource-poor settings where diagnostic and treatment facilities are inappropriate. Here, women often are not aware of STI as health problems, and health care seeking behaviour is poor (Giffin & Lowndes 1999). Relatively high prevalences of STI have been documented in such settings e.g. from Brazil, Papua New Guinea, and The Gambia (Walraven et al. 2001, Mgone et al. 2002, Soares et al. 2003). STI, as well as bacterial vaginosis (BV), are considered to increase the risk of acquiring human immunodefiency virus (HIV) (Sewankambo et al. 1997, Rottingen et al. 2001).

In Brazil, the HIV epidemic is characterized by changing dynamics, currently reaching new population groups, namely women, underprivileged individuals, and communities outside the great urban centres (Fonseca et al. 2000, 2003, Brazilian Ministry of Health 2006). Reliable epidemiological data from Brazilian women on STI and other reproductive tract infections (RTI), such as BV and candidiasis, are scanty. Syphilis and HIV in pregnant women, AIDS, and congenital syphilis are notifiable infections, but the epidemiologic situation of other RTI is rather enigmatic. Studies have addressed the issue of STI in certain specific groups, such as patients attending STI clinics, gynaecology and obstetric outpatient departments, female prisoners or commercial sex workers (Miranda et al. 2000, 2001, Benzaken et al. 2002, Codes et al. 2002, Cook et al. 2004). However, these studies do not allow to conclude on the burden of disease on the community level.

To increase further the knowledge on the epidemiology of STI, BV, and candidiasis in rural women, we performed a population-based study in a small municipality in the hinterland of the state of Ceará, Northeast Brazil.

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METHODOLOGY

Study area - This study was conducted in the municipality of Pacoti, situated 100 km southwest of Fortaleza, the capital of the state of Ceará (Northeast Brazil). Pacoti has a total of 11,500 inhabitants, 77% living in small hamlets (Fundação Instituto de Pesquisa e Informação do Ceará 2000). The area is covered with Atlantic rainforest. Around the hamlets and the town centre, fruit and vegetables are cultivated, which are transported to the markets of Fortaleza.

In adults, illiteracy is in the order of 30%; 58% of the households have sewage disposal, and the garbage is collected in 30% of the households (Instituto Brasileiro de Geografia e Estatística 2004). Health service facilities are restricted to a small hospital (Padre Quiliano Hospital) for clinical emergencies and obstetric assistance, a physiotherapy clinic, and a gynaecology clinic for collection of Pap smears once a week. The population is assisted by four teams of the Family Health Program. There is no public transport between the hamlets and the centre of Pacoti.

Study population - All women from seven hamlets and the centre of Pacoti aged 12 to 49 years (910 individuals) were invited to participate in the study. Up to date information on the number of women of reproductive age were obtained from the local community health agents who assist the government Family Health Program. In order to maintain confidentiality on sexual life, all women, independently from their sexual history, were asked to come to the gynaecological examination, so that bystanders could not infer the sexual status of a woman from non-participation. However, only women who had initiated sexual life were eligible for the study.

Study design - The study was designed as a cross-sectional survey. Prior to data collection community meetings were held to explain the objectives of the study, to introduce the investigators, and to discuss possible concerns. Socio-demographic characteristics and gynaecological complaints were obtained in privacy using structured questionnaires applied by one investigator (KL), followed by gynaecological examination and specimen collection. The questionnaire was elaborated in collaboration with local clinical staff and based on focus group discussions realized by the investigators before data collection. They were pre-tested on 10 individuals. If during the interview a woman disclosed that she had not initiated sexual life, only vaginal swabs were collected to identify BV or candidiasis. All clinical investigators were female. The team was led by an experienced gynaecologist, who performed all gynaecological examinations (FAO). To minimize non-participation bias from the hamlets, free transport to the Padre Quiliano Hospital at the centre of Pacoti, where the interviews and gynaecological examinations were done, was offered for these women.

Gynaecological examination and specimen collection - The gynaecological examination consisted of: colposcopic inspection of the vulva and perianal area; determination of the vaginal pH (pH indicator strips; Merck Laboratories, Darmstadt, Germany); whiff test with 10% KOH (if positive, indicating bacterial vaginosis), collection of vaginal fluid after instillation of 2 ml saline in the vagina; collection of smears for bacteriological and cytological examination; and vaginal and cervical colposcopy. In the presence of suspicious cervical lesions, biopsies were taken for histopathological examination. Vaginal wet mounts were examined for the presence of *Candida* spp., clue cells (part of the Nugent criteria for bacterial vaginosis), and *Trichomonas vaginalis.*

Peripheral blood was obtained from each woman using disposable needles and vacutainer tubes, and sera were separated on the same day and stored at –20°C. Once per week sera samples were transported to the Central Laboratory of Ceará State (Lacen) in Fortaleza for further investigation. Anti-cardiolipin antibodies were detected by venereal disease research laboratory testing (VDRL) (Laborclin, Brazil). If positive, the screening test was counter-checked by fluorescent treponema antibody absorption test (FTA-ABS) (WAMA Laboratories, Brazil). An ELISA test was performed to detect HIV-1 and HIV-2 antibodies (Abbott Laboratories, US and Sanofi-Pasteur Diagnostics, France).

For the detection of BV, slides were Gram stained and examined at Lacen. Cytology smears were Papanicolaou stained and read at the Cancer Prevention Institute in Fortaleza (IPCC). BV was diagnosed according to the Nugent criteria (Nugent et al. 1991). Trichomoniasis was defined as the presence of trophozoites in either wet mount or Pap smear; candidiasis was diagnosed if fungal hyphae or budding yeasts were present in wet mounts, Pap smears or Gram stains.

Chlamydia trachomatis and *Neisseria gonorrhoeae* were detected by ligase chain reaction (LCR) (Buimer et al. 1996). LCR was performed with 1 ml aliquots of vaginal lavage fluid using commercially available kits (Abbott Laboratories) and following the manufacturer's instructions.

Screening of vaginal lavage fluid for human papillomavirus (HPV) was done by polymerase chain reaction (PCR) using the Digene SHARP Signal System (Digene, Beitsville, US) according to the manufacturer's instructions. Positive probes were confirmed with the INNO-LipA HPV Genotyping test kit (Innogenetics, Belgium).

Statistical analysis - Data were entered twice using the EPI-INFO software package version 6.04d (Centers for Disease Control and Prevention, Atlanta, US), checked for entry-related errors and analyzed using the STATA version 7.0 (Stata Corporation, College Station, TX, US). Fisher's exact test was performed to compare relative frequencies.

Ethical aspects - Ethical clearance was obtained from the Ethical Committee of the Federal University of Ceará (COMEPE/UFC). Prior to data collection, the objectives of the study were explained in community meetings. Informed written consent was obtained from all study participants or, in case of minors, from their carers. All women received their laboratory results in written form, and results were explained during a consultation. If any RTI was diagnosed, women were treated according to national guidelines and free of charge. In the presence of a STI, partners were also treated. Women with cervi-

cal abnormalities diagnosed by histopathology were referred to IPCC in Fortaleza for appropriate treatment.

RESULTS

Of the 550 women from the urban centre, 412 (75%), and of the 360 women from the hamlets, 322 (90%) participated in the clinical study. Of these 734 women, 592 (80.7%) had initiated sexual life and were included in the analysis. The median age of the study participants was 31 (interquartile range: 25-38) in women from the urban area and 32 years (interquartile range: 23-39) in women from the hamlets (p = 0.7). Women from the hamlets were significantly younger, had a lower level of education and a lower socio-economic status (Table I).

At least one STI was diagnosed in 20% of the study participants, whereas the overall prevalence of at least one RTI (STI, BV, and/or candidiasis) was 45% (Table II). The most prevalent STI was HPV infection, followed by *C. trachomatis* and *T. vaginalis*. Serum of only one woman was VDRL-reactive, and active infection was indicated by a positive FTA-ABS test. HIV results were negative for all women. Almost 10% of the women had co-infetions, the association of HPV and BV being the most common. There was no statistical significant difference between the hamlets and the centre of Pacoti in the prevalences of any STI with exception of chlamydia infection (2.6% in the centre vs 7.3% in the hamlets; p = 0.01). Hence, for further analysis the data of the two subgroups were combined.

The point prevalences of the most common RTI stratified by age are shown in the Figure. The prevalence of at least one STI, at least one RTI, HPV, and chlamydia decreased significantly with increasing age of the participants (all p < 0.005). BV was more common in the older age group, while trichomoniasis and candidiasis were more common in the intermediate age groups. However, these differences were not statistically significant (all p > 0.1).

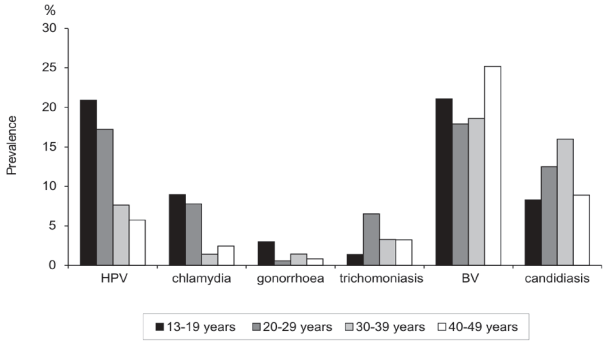
The frequency of gynaecological complaints are depicted in Table III. Only 17% of all women stated the absence of complaints during history taking.

TABLE II

Prevalence of sexually transmitted infections (STI), bacterial vaginosis, and candidiasis in women of reproductive age

	Examined [a]	Positive	% (95% confidence interval)
HPV	579	68	11.7 (9.3-14.7)
Chlamydia trachomatis	579	26	4.5 (3.0-6.6)
Trichomonas vaginalis	592	24	4.1 (2.7-6.1)
Neisseria gonorrhoeae	574	7	1.2 (0.5-2.6)
Treponema pallidum	584	1	0.2 (0.0-1.1)
HIV	584	0	-
At least one STI	570	112	19.6 (16.5-23.2)
At least two STI	567	12	2.1 (1.2-3.8)
Bacterial vaginosis	579	116	20.0 (16.9-23.6)
Candidiasis	592	74	12.5 (10.0-15.5)
At least one RTI	569	255	44.8 (40.7-49.0)
At least two RTI	557	53	9.5 (7.3-12-3)

a: results of laboratory tests not available for all women; HPV: human papillomavirus; HIV: human immunodeficiency virus; RTI: reproductive tract infection.



Prevalence of sexually transmitted infections, bacterial vaginosis, and candidiasis in the study population, stratified by age

TABLE I

Socio-demographic characteristics of the study population, stratified by urban and rural area (n = 592)

	n	Urban centre n (%)	Hamlets n (%)	p value
Age				
13-19 years	72	33 (9.5)	39 (16.1)	0.02
19 years and older	520	316 (90.5)	204 (83.9)	
Marital status				
Single/widow/divorced	151	89 (25.5)	62 (25.5)	1.0
Married/living together	441	260 (74.5)	181 (74.5)	
Level of education				
None/some primary school	317	125 (36.9)	192 (79.3)	< 0.001
Completed primary school or more	264	214 (63.1)	50 (20.7)	
Monthly family income [a]				
< 2 minimum wages	446	238 (70.2)	208 (86.0)	< 0.001
2 minimum wages or more	135	101 (29.8)	34 (14.0)	
Number of family members				
< 6	399	247 (72.9)	152 (62.8)	0.01
6 or more	182	92 (27.1)	90 (37.2)	

a: one official minimum wage corresponds to ca. US$160.

TABLE III
Frequency of complaints associated with reproductive tract infections (RTI)

	Trichomonas vaginalis n (%)	Bacterial vaginosis n (%)	*Chlamydia trachomatis* n (%)	*Neisseria gonorrhoeae* n (%)	HPV n (%)	*Candida* spp. n (%)	No RTI n (%)
Abnormal vaginal discharge	12 (50)	47 (40.5)	10 (40)	6 (85.7)	33 (49.3)	42 (56.8)	125 (39.8)
Lower abdominal pain	18 (75)	48 (41.4)	14 (53.9)	4 (57.1)	29 (42.7)	37 (50)	152 (48.4)
Vulvovaginal itching	11 (45.8)	28 (24.1)	3 (11.5)	2 (28.6)	24 (35.3)	35 (47.3)	101 (32.2)
Dyspareunia [a]	8 (44.4)	24 (23.8)	3 (12.5)	3 (50)	17 (27)	21 (30.4)	89 (30.7)
Abnormal vaginal bleeding	5 (20.1)	31 (26.7)	6 (23.1)	4 (57.1)	17 (25)	13 (17.6)	70 (22.3)
No complaints	1 (4.2)	25 (21.6)	8 (30.8)	0	14 (20.6)	12 (16.2)	51 (16.2)

a: only women with at least one partner in the last 6 months (n = 532); HPV: human papillomavirus.

DISCUSSION

In developing countries, most of the current STI burden estimates are based on women attending antenatal clinics, family planning services, and specialized STI clinics: population groups which are usually not representative of the female population in general (WHO 2002). It is widely agreed that there is an urgent need for community-based assessment of STI in different socio-cultural settings, as such infections have been found to be highly prevalent even in women considered to be at low risk (Duncan et al. 1994, Behets et al. 2001). Unfortunately, population-based data on STI prevalence and patterns are scanty in developing countries and in Brazil only two studies have been published (Soares et al. 2003, Miranda et al. 2004).

The present study shows that STI, BV, and candidiasis are endemic in a small community in Northeast Brazil, and that 45% of the women are affected by at least one genital infection.

In another study conducted in the rural area of the state of Alagoas, also in the Brazilian Northeast, the overall prevalence of RTI was higher than in Pacoti: 51% of those women had at least one RTI, while 6.4% had gonorrhoea, 6.4% chlamydia infection, 2.6% syphilis, 26.6% HPV, 10.3% trichomoniasis, 15.3% BV, and 5.8% candidiasis (Soares et al. 2003). Among teenagers from Vitória, a main city in the Southeast region of the country, the prevalences of chlamydia infection and gonorrhoea were 12.2 and 1.9%, respectively (Miranda et al. 2004). Among 155 women living in the slums from Salvador, a main city in the Northeast, 12.9% had chlamydia infection, 3.2% gonorrhoea, 5.1% syphilis, and 0.6% HIV infection (Codes et al. 2006). However, the authors discuss that the participants may not be representative of the general population from the slums. Irrespective of the setting, the age distribution in the present study was similar to the ones from Alagoas and Vitória, indicating that young Brazilian women are most vulnerable to HPV and chlamydia infection, whereas older women are at higher risk for candidiasis and BV.

In a comprehensive study in 18 rural districts in Peru higher frequencies of BV (44%) and trichomoniasis (17%) were found, whereas vulval candidiasis and HPV infection were less frequent when compared to our data (Garcia et al. 2004). Among rural women in Papua New Guinea the prevalence of *T. vaginalis*, *C. trachomatis*, and *N. gonorrhoeae* infections were even higher (Mgone et al. 2002). This indicates that, on the population level, the pattern and the frequency of STI/RTI depend heavily on the socio-cultural setting in which the women live.

In a study with more than 1000 pregnant women attending public antenatal clinics in Ceará, the prevalences of RTI, including the low prevalences of HIV infection and syphilis, were similar to the ones found in our investigation (Martins 2002). These findings support the notion that, at least in Northeast Brazil, data from RTI surveillance in pregnant women may be used as a proxy of the disease burden in the general female population.

The observation of higher prevalences of STI, particularly of HPV, gonorrhoea, and chlamydia in adolescents, when compared to older age groups, is in accordance with other studies (Soares et al. 2003, Miranda et al. 2004). The decreasing prevalence of HPV and chlamydia with increasing age might be due to a higher susceptibility of younger women (e.g. due to cervical ectopy and lower vaginal pH), the development of protective immune responses (particularly against HPV), and riskier behaviour (e.g. earlier sexual debut, more sexual partners per unit of time, unprotected sex) (Berman & Hein 1999, McIlhaney 2000). Riskier behaviour of young women may also be responsible for increased prevalence of gonorrhoea in this age group. The causes for the age distribution patterns of the other RTI are difficult to disentangle, as probably various behavioural, physiological, and immunological variables interact.

Our study is subject to some limitations. The different proportion of young women from the town centre and the hamlets included in the study may have biased prevalence estimates, as the frequency of some RTI was clearly age-dependent. In general, participation was high, however, non-participation was higher in the urban than in the rural area. This observation may have several reasons. In the urban area, there was a higher proportion of eligible women who were employed and could not attend gynaecological examination during working hours. Free transport from the hamlets may also have minimized non-participation bias of women from the rural area; the availability of the transport may have also induced a peer

pressure, mainly in the adolescents, towards participation. In addition, coverage of the area by community health workers, who considerably collaborated in the recruitment of volunteers, was higher in the rural than in the urban areas.

Inter-observer bias can be ruled out because the examinations were done in all cases by the responsible gynaecologist (FAO).

Interestingly, many women without any diagnosed RTI complained of gynaecological symptoms during history taking, being the most common lower abdominal pain and abnormal vaginal discharge. The high frequency of complaints not associated with RTI may be attributed to the false interpretation of physiological signs and symptoms as abnormal. In addition, less than 25% of the women who complained of abnormal vaginal discharge actually had an abnormal discharge detected during the gynaecological examination (data not shown). In Bangladesh, it was found that 70% of women complaining of abnormal vaginal discharge in fact had no STI, BV or candidiasis detected (Hawkes et al. 1999).

Although no case of HIV infection was observed, the important burden of STI indicates that women in Pacoti, and presumably elsewhere in rural Brazil, are at high risk along with the current spread of the AIDS epidemic towards smaller municipalities, lower income groups and women (Fonseca et al. 2000, 2003). Therefore, our study calls for urgent control measures against STI in rural areas, including improved diagnostic and treatment facilities, and appropriate case management. Health education should be directed particularly to adolescents, and should also empower women to recognize symptoms and signs related to STI/RTI as early as possible.

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