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Prevalence of *Trichomonas vaginalis, Chlamydia trachomatis, Neisseria gonorrhoeae* and human papillomavirus in a sexual health clinic setting in urban Sri Lanka

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Summary

The prevalences of *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, and human papillomavirus (HPV) in Sri Lanka are not well reported; the objective of this study is to describe the prevalences of these four sexually transmitted infections among attendees of sexual health clinic in an urban setting. Vaginal swabs were collected from consenting women attending a sexual health clinic and tested for the presence of the above sexually transmitted infections using nucleic acid amplification techniques. Basic demographic details were sought from each participant (483 women of age range 14–61, median 30 years, IQR 12 years) via a research assistant–administered questionnaire. Overall, a prevalence of *T. vaginalis, C. trachomatis, N. gonorrhoeae* and HPV was 2.3%, (95% CI: 1.2–4.1%), 8.2% (95% CI: 5.6–11.4%), 7.6% (95% CI: 5.2–10.8%), and 44.4% (95% CI: 39.8–49.1%), respectively. Among the 197 positive for HPV, HPV6 accounted for 23.1%, HPV16 (12.5%), then HPV11, HPV66 and HPV58 were the commonest. Vaccine-related types (6/11/16/18) were detected in 59.9% of cases (95%CI: 52.7–66.8%). The high prevalence of sexually transmitted infections (45.2%) is a potential risk factor for an increase in HIV infections in the country and the high carriage of HPV supports the need for cervical cancer screening and prevention programmes.

Keywords

Sexually transmitted infections, nucleic acid amplification techniques, human papillomavirus, *Trichomonas vaginalis*, *Chlamydia trachomatis*, *Neisseria gonorrhoeae*, prevalence, screening, women, Sri Lanka

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Introduction

Sexually transmitted infections (STIs) such as human papillomavirus (HPV), trichomoniasis, chlamydial and gonorrhoeal infections are a major public health problem for men and women worldwide because they cause acute disease, as well as long-term sequelae if untreated.[1] Complications following chlamydia, gonorrhoea and trichomonas infections in women include pelvic inflammatory disease, chronic pelvic pain, tubo-ovarian abscesses, ectopic pregnancies and infertility.[2,3] In pregnancy these infections are associated with prematurity, neonatal morbidity and perinatal infections.[4,5] In men, these STIs may be complicated

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by epididymitis and result in urethral stricture and infertility (http://www.cdc.gov/std). Infection with STIs increases the risk of human immunodeficiency virus (HIV) infection and transmission[6] and plays a key role in the aetiology of malignancies in both men and women.[3,7,8] Infection with HPV can cause warts, whilst persistent oncogenic HPV infections can result in intraepithelial neoplasia, a proportion of which if untreated will result in cervical, vaginal, perianal, vulvar, penile and/or oropharyngeal cancers.[9] Of the oncogenic HPVs, 16 and 18 cause 70% of cervical cancers, the third most frequent cancer in women worldwide.[10]

Prevalence data for STIs are higher in developing countries where detection and treatment are less accessible.[1] While the trend for STIs in Sri Lanka may be lower than its neighbouring countries, the exact countrywide prevalence of various STIs is unavailable in the literature. However, data from the National STD/AIDS Control Programme (NSACP) of Sri Lanka shows an increasing trend of reported STI infections (http://www.aidscontrol.gov.lk).[11] The unavailability of more in-depth data primarily can be contributed to the unreliability in clinically diagnosing an STI,[12] utilisation of syndromic management which does not give definitive diagnoses, or limited laboratory diagnoses such as serology, bacterial culture and microscopic examination of smears which are not as sensitive as using molecular techniques. Moreover, the stigma associated with attending STI clinics makes patients reluctant to attend clinics and are therefore treated in private hospitals and thus unaccounted for.

Despite estimates of HIV being relatively low compared with its neighbours in south Asia, Sri Lanka has a large sexually active and potentially susceptible population aged between 15 and 49 years.[13] This age group accounts for 55% of the total population of the country's 20.5 million (http://www.statistics.gov.lk).[14] A study carried out on sexual behaviour of 3134 higher secondary school students aged 18–20 years in six geographically representative districts of Sri Lanka revealed that half of the men and approximately a third of the women were sexually active at the time, with only 26.5% of men and less than 10% of women reporting using condoms during intercourse.[15] Such risky sexual behaviour among youth in the country was further highlighted by a community-based study carried out on 812 youth aged between 18 and 24 years, in Southern Sri Lanka.[16] As this population becomes more sexually active, there could be more implications for the incidence of STIs in the country.[17]

In this study, we used sensitive nucleic acid amplification tests to more accurately define prevalence of STIs from vaginal swabs obtained from women attending an STI clinic in Sri Lanka.

Methods

Ethics

Ethics clearance was obtained to carry out this study from the Ethics Review Committee of the Faculty of Medicine, University of Kelaniya, Sri Lanka (P10/03/2007). Informed written consent was obtained from all those who participated in the study.

Study population and recruitment method

All women attending the STI Clinic (of the National STD/AIDS Control Programme or NSACP) located at the Colombo North Teaching Hospital, which is a 1387-bed hospital in Ragama,[18] a suburb of Colombo, were invited to the study by a research assistant. The women were attending the clinic (held daily from Monday to Saturday) for either a routine screen or for investigation of a symptomatic disease and were approached by the staff at the clinic over a 33-month period from August 2007 to May 2010 and invited to participate in the research study. Those approached and consented to participate were provided with an information sheet about the study. A medical officer made a provisional clinical diagnosis following a sexual health examination. A microscopic examination of a wet smear was prepared from a high vaginal swab, which was collected at the examination. No cervical cytology data were performed at this stage. All women diagnosed with STIs were provided with treatment. Any woman aged under 18 years who consented to participate in the study was evaluated for mature minor status and consented appropriately.

Sample and data collection

All participating women also completed a questionnaire with the assistance of a research assistant. The questionnaire explored personal demographics, sexual history and any previous history of STIs. The data were entered into an Excel spreadsheet for analysis.

In addition to the routine testing in place, the attending medical officer collected an additional high vaginal flocked swab (Copan Diagnostics, Brescia, Italy). This swab was rotated in 500 µl of sterile phosphate buffered saline (PBS) for 30 seconds, de-identified and stored at −20°C. Samples were transported on dry ice in batches of 100–150 to the Molecular Microbiology Laboratory, Department of Microbiology and Infectious Diseases, the Royal Women's Hospital, Melbourne, Australia, for further analysis.

Molecular analysis methodology

Total DNA was extracted from resuspended vaginal swabs using the MagNa Pure LC system (Roche

Diagnostics) with the DNA Isolation Kit I Protocol, eluting a final volume of 100 µl of DNA. Human A549 cells and DNA-free PBS were included in each DNA extraction run as controls. To determine the integrity of extracted DNA each sample was subjected to real-time PCR amplification and detection of human β-globin gene.[19,20]

Using a 20-µl aliquot of extracted DNA all samples were tested using a PGMY09/11-based HPV consensus PCR assay[21] in combination with a PCR-ELISA detection protocol.[22] Positive (SiHa DNA) and negative (water) controls were processed with each run. All samples tested positive for HPV DNA were genotyped using the PapType high-risk HPV detection and genotyping kits (Genera Biosystems Limited, Australia) according to the manufacturer's instructions and as described previously.[23,24] The PapType system detects 14 high-risk (HR types: 16, 18, 31, 33, 35, 39, 45, 51, 52, 56, 58, 59, 66, 68) and two low-risk (LR types: 6, 11) HPV genotypes.[23,24]

Chlamydia trachomatis and *Neisseria gonorrhoeae* DNA sequences were amplified using ROCHE COBAS Amplicor (Roche Diagnostics, NJ, USA) according to the manufacturer's instructions. All samples positive for *N. gonorrhoeae* were confirmed by PCR using primers and probes directed at a 90-base pair region of the *opa* gene.[25] With 5 µl of extracted DNA, samples were tested for the presence of *Trichomonas vaginalis* using a rapid real-time PCR (light Cycler, Roche Molecular Biochemicals) according to the manufacturer's instructions.

Statistical analysis

Descriptive statistics of completed questionnaires were assessed using counts, prevalence (percentage with 95% confidence interval) and mean (with standard deviation). The median age of sexual debut was assessed using Kaplan-Meier survival analysis. The effect of age on the incidence of STIs was assessed using logistic regression. All statistical analyses were conducted using SPSS 20.

Results

Over the study period, 483 women consented to participate in the study. The demographics of the study sample are summarised in Table 1. Participants' age ranged from 14 to 61, with the median age of the population being 30 years (IQR = 12 years). The attendees were mainly Sinhalese (97%), housewives (64%) and married (89%), with a mean of 2.3 children (95%CI: 1.8–2.8), whilst 8% of them were pregnant at the time of attending the clinic. The vast majority of participants reported having a current sexual partner (99%) and had their sexual debut at a median age of 20 years (95%CI: 19.4–20.6). The use of protection during sexual intercourse was low in all age groups and continued to decrease with age. The use of contraceptives was also low (20% in the 14–19 year age group), but increased to nearly 50% in the 35- to 39-year age group before declining with further increase in age.

Two samples were beta-globin negative and therefore, adequate samples for molecular diagnostic testing were available for 481 of the 483 participants (99.6%). Overall, results showed a prevalence of 2.3% (95% CI: 1.2–4.1%) for *T. vaginalis*, 8.2% (95% CI: 5.6–11.4%) for *C. trachomatis*, 7.6% (95% CI: 5.2–10.8%) *N. gonorrhoeae* and 44.4% (95% CI: 39.8–49.1%) for

Table 1. Demographics of the study population.

Variable	
Personal demographics	
Median age (years)	30 (IQR 12
Race:	
• Sinhalese	469 (97%)
• Tamil	7 (1.4%)
• Muslim	5 (1%)
• Burgher	2 (0.4%)
Marital status	
• Married	428 (89%)
• Never married	40 (8%)
Parity	
• 0	136 (27.2%)
• 1	118 (23.6%)
• 2	96 (19.2%)
• 3	59 (11.8%)
• 4 or more	31 (6%)
Pregnant	
• Yes	38 (7.9%)
• No	428 (88.8%)
Mean age when first pregnant (years)	23.03
Sexual history	
Median age of sexual debut (years)[a]	20 (95%CI: 19.4–20.6)
Currently in a relationship	
• Yes	471 (99%)
• No	5 (1%)
Lifetime number of sexual partners	
• 0	0 (0%)
• 1	342 (68.4%)
• 2	88 (17.6%)
• 3	18 (3.6%)
• >5	27 (5.4%)

[a]Kaplan-Meier analysis.

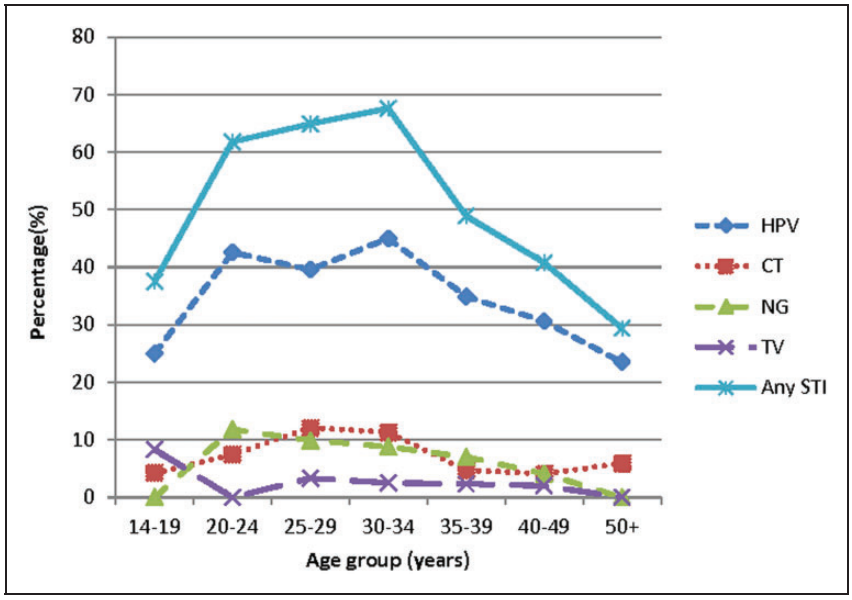

Figure 1. Comparison of age-specific prevalence of HPV, *C. trachomatis, N. gonorrhoeae and T. vaginalis* amongst the attendees of the STI clinic.

HPV. The age-specific data showed the prevalence of chlamydia peaked in the 25- to 29-year-olds (12.1%, 95%CI: 6.2–20.6%), whilst gonorrhoea peaked in the 20- to 24-year-olds (11.8%, 95%CI: 5.3–21.9%), and HPV demonstrated a bimodal distribution peaking in the 20- to 24-year-olds (42.6%, 95%CI: 30.7–55.2%), as well as in the 30- to 34-year-olds (45.0%, 95%CI: 33.8–56.5%) (Figure 1). Overall, the highest prevalence of STIs was observed in the 30–34 years age group (55%, 95%CI: 43.5–66.2%). There is a statistically significant decrease in the prevalence of all STIs with increase in age ($p = 0.029$), with the prevalence of STI presence decreasing by 2.4% per increase of 10 years of age. If we discounted the HPV data the prevalence of STIs was highest in the 25–29 years age group, but this difference was not statistically significant.

Overall, 46 of the 483 subjects (9.6%, 95%CI: 7.1–12.5%) demonstrated the presence of more than one STI, as diagnosed by molecular diagnostics (Table 2). Presence of multiple STIs peaked in the 25–29 years age group (14.7%, 95%CI: 8.8–22.4%). Of the mixed infections, there was one patient with conventional STIs (1 with *N. gonorrhoeae* and *C. trachomatis)* whilst all others were mixed with HPV i.e. 5 *T. vaginalis* and HPV, 16 *N. gonorrhoeae* and HPV, 20 *C. trachomatis* and HPV and 4 with HPV, *C. trachomatis* and *N. gonorrhoeae*. A trending decrease in mixed infections with increased age was observed, but was not statistically significant ($p = 0.331$).

Out of the 204 samples positive for HPV, seven samples were lacking in HPV genotyping test results and were thus excluded. Among the remaining 197 HPV-positive women, HPV6 was the most frequently detected genotype, accounting for 23.1% of genotypes detected, followed by HPV16 (12.5%), then 11, 66 and 58 (Figure 2).

Table 2. Diagnosis summary.

	Total ($n = 372$)
HPV	141 (37.9%)
Chlamydia	31 (8.3%)
Gonorrhoea	29 (7.8%)
Trichomonas	9 (2.4%)
Any STI	168 (45.2%)

HPV: human papillomavirus; STI: sexually transmitted infection.

HPV6 and/or 11 was identified in 49.7% (95%CI: 42.6–56.9%) of specimens, with HPV16 and/or 18 identified in 26.4% (95%CI: 20.4–33.1%) of cases; high-risk HPV genotypes were identified in 55.8% (95%CI: 48.6–62.9%) of cases. Vaccine-related types (6/11/16/18) were detected in 59.9% of cases (95%CI: 52.7–66.8%) (Table 3). Multiple HPV genotypes were detected in 39.1% (95%CI: 32.2–46.3%) of cases, with prevalence peaking in the over 50 years age group (71.4%, 95%CI: 29.0–96.3%) and the 14–19 years age group (66.7%, 95%CI: 34.9–90.1%). Overall, logistic regression found that age had no significant effect on the proportion of multiple infections ($p = 0.835$).

There were 38 pregnant women who participated in the study (two samples were excluded as above), from whom 16 were positive for HPV, two with

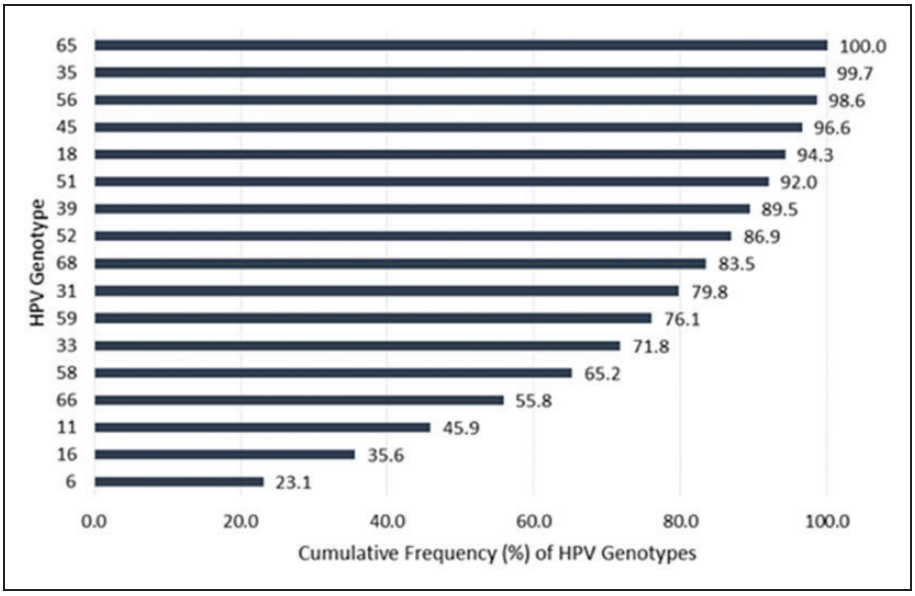

Figure 2. Cumulative graph showing frequencies of HPV genotypes.

Table 3. HPV genotype prevalence.

	Prevalence		
	N = 197	%	95% CI
6 and/or 11	98	49.7	42.6–56.9
16 and/or 18	52	26.4	20.4–33.1
6/11/16/18	118	59.9	52.7–66.8
Any HR-HPV[a]	110	55.8	48.6–62.9
16+ other HR	29	14.7	10.1–20.5
18+ other HR	7	3.5	1.4–7.2
Multiple infections (Other)[b]	77	39.1	32.2–46.3

HPV: human papillomavirus.
[a]As tested/typed by the PapType system.
[b]Excluding all HR and 6/11 types.

C. trachomatis, two with *N. gonorrhoeae* and two with *T. vaginalis*.

Of the 98 cases positive for HPV6 or 11, 16.3% (16) were diagnosed clinically with genital warts at the clinic visit. Moreover, of the 74 women clinically diagnosed with genital warts, only 13 (17.6 %) were positive for HPV6 and/or 11 in the vaginal swabs.

Discussion

This study provides the prevalence of four main STIs in a sexual health setting in urban Sri Lanka (Ragama) using NAAT and thereby an insight into the potential disease burden in the country which was previously unreported. Overall, 45.2% of attendees were infected with *N. gonorrhoeae, C. trachomatis, T. vaginalis* and/or HPV, of which 44.4% were positive for HPV, 8.2% for *C. trachomatis*, 7.6% for *N. gonorrhoeae* and 2.3% for *T. vaginalis*. There was no difference in correlation between percentage positivity and attendees' occupations (between a housewife and a sex worker), which may relate to either the unreliability of the classification of their occupation and/or participant hesitancy to divulge details about their occupation.

In a study consisting of 50 patients with non-gonococcal urethritis attending an STI clinic, Galagoda[26] showed a prevalence of 12% (three men and three women out of a sample of 50 patients) of *C. trachomatis* using culture methods, while Palihawadana et al.,[27] found a prevalence of 7.5% among a population of infertile couples using serology. It is accepted that NAAT-based methods are more sensitive in the detection of STIs and may be a reason for the higher prevalence in general obtained, apart from trichomoniasis. Our prevalence data of 2.1% for *T. vaginalis* are significantly less than that reported for the country. Iddawela et al.[28] showed a prevalence of 8.5% for *T. vaginalis* among a group of 82 women aged between 15 and 50 years attending the NSACP, STI Clinic (Kandy, Sri Lanka) using culture, wet smears and Giemsa staining techniques, while Fernando et al.[29] showed a prevalence of 7% by culture only.

Our data for HPV are significantly higher than previously reported for Sri Lankan women where 2000 ever married women aged between 20–59 years and using of a PCR-based testing of cervical samples carried out in the Gampaha district (north of Colombo district), showed an overall prevalence of HPV

infections of 3.3% (95%CI: 3.2–3.4%).[30] It is to be noted that these samples were obtained from a community-based sample whose mean age was 36 years, whereas ours was 30.9 years. The age-specific HPV prevalence data of our study showed a bimodal distribution (Figure 1), which is reflective of onset of sexual relations in early years and later higher rates of persistence of HPV infections in older women, a pattern that is suggestive of impact of a lack of an organised cytological screening, a pattern similar to that described in countries with no such programmes.[31]

This age demographic together with the fact that our samples were obtained from an STI clinic suggests differences in risk behaviours between the populations, apart from age. There is no other information on the prevalence of HPV genotypes reported for the country, apart from a pilot study carried out on archival cervical biopsies from cervical cancer cases from urban Sri Lanka.[32] In that study, 93% of the tumour samples were positive for HPV, with HPV types 16/18 collectively accounting for 83.4%. Our own data show 26% positive carriage rate for HPV types 16 and 18, which coupled with the presence of other high-risk HPV types (such as HPV types 31, 33, 39, 51, 58, 66) clearly shows the need for participation in cervical screening. The relatively high presence of HPV types 6 and particularly of type 11 is a surrogate for genital warts the prevalence of which is unknown. HPV11 positivity in the current study was higher than in other studies which may be due to a cohort effect.

The percentage of subjects testing positive for one or more STIs was lower amongst pregnant women as compared with the non-pregnant women. Since the sample size was small this was not considered significant.

There are several limitations in this study. Samples were obtained from an STI clinic and therefore the attendees could be classified as belonging to a 'high risk' group. There are no data on the number of clinic attendees who may have been approached but did not wish to participate in the study. Sex workers may be reluctant to divulge their occupation to the clinic due to the associated stigma, which may account for their statistic being inaccurate in the current study. As a result it is not possible to identify if more sex workers attended the particular clinic used in our study as opposed to other STI clinics. It is also not possible to know if the participants of the study were referrals from other sources or were self-referrals. The questionnaires were completed with the help of a research assistant and this could have hindered attendees divulging sensitive questions. Not being able to follow up patients who were positive on any of the STIs (particularly those detected with gonorrhoea and/or chlamydia infections) including those who were pregnant are further limitations.

The overall high prevalence of STIs could be a potential risk factor for an increase in HIV infections in the country given that there is already an increase in trend of STIs and HIV (http://www.aidscontrol.gov.lk). This, coupled with the high prevalence of unprotected intercourse noted amongst the participants of the current study, which is consistent with previous studies[15] and the increase in trends in risky sexual behaviour within the population,[16] are all risk factors, which highlights the need for more rigorous control for STIs in the country. Future studies will need to explore other low-risk populations in order to obtain accurate country-wide prevalence data.

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Declaration of Conflicting Interests

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