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The tip of the iceberg: opportunistic screening for *Chlamydia trachomatis* in asymptomatic patients attending a young people's health clinic reveals a high prevalence — a pilot study

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ABSTRACT. ***Objectives:*** We implemented an opportunistic screening programme for *Chlamydia trachomatis* amongst patients presenting to a young peoples' health service in the city of Geelong, Australia, to define the prevalence of infection and to identify specific risk factors. ***Methods:*** Over a 7-month period sexually active patients attending the young peoples' clinic were offered *C. trachomatis* screening by nucleic acid amplification test. There was 100% acceptance rate among those offered the test. Patient demographics, reason for presentation at the clinic and reported symptoms were documented by the clinicians and correlated with laboratory findings. ***Results:*** 163 patients between the ages of 12–25 were tested, nine males and 154 females. The prevalence of chlamydia infection was 5.8% and was highest (16.0%) among patients presenting for the morning after pill. Inhibition of the nucleic acid amplification test occurred in 11.0% of urine samples. All patients with inhibited tests were asked to provide a repeat sample for retesting, but only 50% complied with this request. The majority of repeat samples (88.9%) had no inhibitors present and yielded a negative result. There was no correlation between symptoms and a positive chlamydia result. ***Conclusions:*** Chlamydia infection is common in young people engaging in unsafe sexual practice and cannot be predicted by the presence of symptoms. The high prevalence of infection in Geelong would make screening cost effective in this age group. Ongoing population screening of sexually active young people should be encouraged in community health centres. Inhibition of the nucleic acid amplification test was common but repeat testing of urine a few days later usually gave satisfactory results.

Introduction

Chlamydia trachomatis is the most common cause of notifiable sexually transmitted infections (STIs) in Australia and annual notifications have increased three-fold in the last decade.[1] Chlamydia infection is particularly prevalent in adolescents and young adults, with age under 25 as the strongest risk factor and up to 70% of cases occurring in women between the ages of 15 and 24.[2] Recent Australian data showed that 94.4% of positive results from an inner city clinic in Melbourne were in women less than 25 years of age with a prevalence of 6.2% within that population.[3] The official notification data reports only 50.5% of cases occurring in men and women under 25; 61.5% of notifications in female patients are in the under 25 age group. In total, 72.0% of the Victorian chlamydia burden occurs in patients under 30.[4]

Chlamydia may cause cervicitis and urethritis acutely, however up to 80% of infections in women are asymptomatic.[5] The most significant complications of chlamydia infection are related to development of pelvic inflammatory disease, ectopic pregnancy, chronic pelvic pain in women and infertility in both men and women at a cost of $A90 to $A160 million annually.[6] Complications in pregnancy such as premature rupture of membranes and small for gestational dates infants can occur, and vertical transmission during parturition may result in conjunctivitis and neonatal pneumonia.[7,8] Many cases of pelvic inflammatory disease (PID) are asymptomatic[9] and the majority of women with chlamydia-related tubal infertility have no history of PID.[10] The absence of symptoms means that most people will not present for investigation and treatment. Screening is the only way that a diagnosis can be

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made, complications avoided and population prevalence established. Bacterial STIs are also a marker of high-risk sexual behaviour and a risk factor for acquisition of HIV.[1,11,12]

Until recently, diagnosis of *Chlamydia trachomatis* required the use of invasive cervical/urethral swabs that were unacceptable for the screening of young asymptomatic individuals. Nucleic acid amplification tests, performed on a first pass urine sample, are acceptable to both sexes. These tests have a specificity approaching 100% and sensitivity greater than 85% in symptomatic and asymptomatic patients.[5,13,14] Treatment for chlamydia is both simple and effective, either with single dose azithromycin or doxycycline for 7 days. Chlamydia infection fulfils the World Health Organisation criteria for amenability to a screening programme.[6] There is currently no such programme in place in Australia, despite evidence showing significant decreases in rates of acute cases and complications when screening has been implemented.[1] The Victorian Department of Human Services and The Australasian College of Sexual Health Physicians have made recommendations regarding screening but no consensus has been reached regarding which patient group should be targeted.[1] Current strategies include targeting high-risk patients (the characteristics of which have not been defined) or testing on suspicion or symptoms alone. Overseas studies and economic modelling have suggested that a wide screening strategy of opportunistically testing all sexually active women under the age of 25 is cost effective when the population prevalence of chlamydia infection exceeds 3.1%.[1,13] In Victoria, Australia, it has been suggested that universal testing would be cost-effective when the prevalence is greater that 2.1%.[3]

Nucleic acid amplification tests such as ligase chain reaction (LCR), polymerase chain reaction (PCR) and strand displacement amplification (SDA) are recognised as the diagnostic tests of choice for chlamydia infection,[5] although LCR is no longer available as a diagnostic test. Nucleic amplification tests can be performed on endocervical swabs, urine or self-inserted tampons and are significantly more sensitive than culture.[14] Inhibition of the test, presumed to be due to substances that interfere with amplification by the polymerase enzyme, can give an inconclusive result. Inhibition has been reported from 3.9 to 18.0% of samples and is more common when testing female urine.[5,15] Nitrites, BHCG, haemoglobin and crystals have been implicated as potential inhibitors. Inhibition may be removed by manoeuvres such as cold storage, freezing, heating or diluting samples in the laboratory.[5,15] These manoeuvres are not standardised and require significant time and resources that are not available in non-research, service laboratories.

The purpose of this investigation was to perform nucleic acid amplification tests to detect *C. trachomatis* on sexually active patients presenting for any reason to a young peoples' health service in Geelong. We sought to identify specific risk factors for *C. trachomatis* infection that could then be used later to target high-risk patients. We also sought to determine the prevalence of infection in the wider Geelong community and compared this with the index clinic.

Methods

Setting

The city of Geelong is a provincial centre in south-eastern Australia with a population of ~220000 people. It has comprehensive primary, secondary and tertiary health care. The index clinic ('Clockwork') is a general practitioner (GP) focused health service for young people. It includes allied health support such as drug and alcohol workers, psychologists and community health nurses. Clockwork provides services for young people aged from 12 years of age (attending high school) to 25 years. These services include medical, allied health services and peer support groups. The annual consultation rate is ~5000 patients. Consultations are for a variety of problems such as drug and alcohol abuse, mental illness, sexual health and general medical complaints. A number of part-time practitioners work at Clockwork. Three of the GPs actively enrolled patients in this study. The remaining GPs tested according to their usual practice.

Patient inclusion, index clinic

Over a 7-month period young people between the ages of 12 and 25 seen by participating GPs at Clockwork were offered chlamydia testing if they were identified as being sexually active. It was not possible to opportunistically test all patients presenting to Clockwork during the study period as not all practitioners were involved in the study and because many of the consultations were crisis situations where it was not appropriate to obtain consent for unrelated testing. Those patients presenting for the morning after pill (MAP) or contraception were readily identified as being sexually active. Other patients were discretely questioned about their sexual behaviour and if sexually active were offered testing for chlamydia.

Patients were informed of the nature of the test and the significance of and potential complications associated with untreated chlamydia infection prior to informed consent being obtained. Pamphlets about chlamydia were available for patients to take away. Consent was given verbally and recorded in the patient file. No ethics approval was required for this study as there was no alteration to normal standard of care and no identifying patient characteristics were recorded.

A confidential record was kept of all tests ordered, patient data such as age, attending doctor, previous STI history and the reason for attending the health clinic were documented.

Patients were classified according to reason for presentation into three categories: (i) presentation for morning after pill (MAP); (ii) sexually active, but not symptomatic nor seeking MAP; and (iii) symptoms that prompted specific screening for STIs.

Patient inclusion, community

PathCare Consulting Pathologists Pty Ltd was the only pathology service provider in the Geelong region at the time of the study. It processed all index clinic and community specimens. Overall, ~350 specimens were processed each month for *Chlamydia trachomatis*. Total requests for chlamydia testing including positive and inhibited results over the study period were extracted from the PathCare computerised pathology record system. Breakdown by sex and age was achieved by analysing the non-identifying data but no clinical information was available. Comparison was made between the prevalence of infection at the index clinic and the local community (including the index clinic data).

Table 1. Results of *C. trachomatis* testing according to reason for attendance at Clockwork young peoples clinic

Reason for attendance	Result of nucleic acid amplification test for *C. trachomatis*			
	Positive (%)	Negative (%)	Inhibited (%)	Total
Morning after pill	5 (16.0)*	21 (68.0)*	5 (16.0)	31
Sexually active (other)	4 (3.4)*	102 (87.9)*	10 (8.6)	116
Symptomatic	0 (0.0)	13 (81.3)	3 (18.7)	16

* $P < 0.02$.

Pathology

Samples were transported from the index clinic to the laboratory on a daily basis and were refrigerated at 4°C until testing was performed. Samples were tested three times a week, without pooling, ensuring that the maximum time from taking the sample to its testing would be less than 72 h. Testing for *C. trachomatis* was performed on first pass urine, cervical or urethral swabs using the BDProbeTec ET *Chlamydia trachomatis* Amplified DNA Assay (Becton, Dickinson and Co., Sparks, MD, USA) using strand-displacement amplification by diagnostic microbiology scientists. Inhibited results were reported to the clinicians as 'inhibited' and a repeat sample was requested. It was not routine practice in the laboratory to perform manouvres to overcome inhibition, thus for the purposes of the study only the routine testing protocol was used.

Urinalysis or serum BHCG was not performed on any of the patients in this study unless otherwise indicated.

Statistics

Fisher's exact probability test was used to compare groups within the index clinic population and a Chi-square test was used to compare the index clinic population to the local community. A *P*-value of <0.05 was considered significant.

Results

Index clinic.

All of the patients offered the chlamydia test agreed to have it performed. Over the 7-month period from 1 October 2002 to 1 May 2003 there were 163 requests for *C. trachomatis* tests from Clockwork Young Peoples Health Clinic. There were nine positive results (5.8%) and 18 inhibited results (11.0%).

The age range of patients tested was from 12 to 25 years with a median of 17 years. Of the nine positive results, seven were in patients between the ages of 15 and 20, two were in patients older than 20. These numbers were too small to allow statistical analysis of age as a predictor of disease.

Twenty-three patients had more than one test done during the study period; nine of these were repeated because of inhibition on a previous sample; four were repeated after treatment for a positive result and all were subsequently negative. The remaining 10 repeated tests were done for high-risk exposure or symptoms.

Thirteen tests were performed on endocervical swabs, the remaining 150 tests were performed on first pass urine tests. All 13 cervical swabs gave negative results for *C. trachomatis*. There were no inhibited results, even in the two swabs taken to investigate vaginal bleeding.

Only nine males (all asymptomatic) were tested and all were negative.

Nine tests were performed for both *C. trachomatis* and *N. gonorrhoeae,* and these were negative for both pathogens.

Table 1 shows the results for each group of patients according to their reason for presentation to Clockwork.

Thirty-one patients attended Clockwork for the morning after pill, five (16%) of these women had a positive *C. trachomatis* test and five (16%) had inhibited samples. No pregnancies occurred after administration of MAP, within 72 h of unprotected intercourse BHCG would not be detectable. When the inhibited tests are excluded from the analysis the proportion of positive results is 19% in women attending for the morning after pill compared to 3% presenting for other reasons (Fisher exact probability *P*-value <0.02).

Sixteen patients presented with symptoms that prompted STI screening such as dyspareunia, dysuria, pelvic or abdominal pain. None of these patients was positive for *C. trachomatis*; there was no significant association between symptoms and detection of *C. trachomatis* ($P = 0.8$). A high percentage (18.7%) of these women also had inhibition of the test, but sample size was too small to allow statistical analysis.

Of the 18 patients who had inhibitors in their urine nine (50%) were retested ~30 days after the inhibited test and 8/9 (88.9%) were negative on repeat testing. One patient had ongoing inhibition of the test. None of those retested had received any specific anti-chlamydial treatment in the interim period. Those patients who had an inhibited test and did not return for repeat testing were lost to follow-up.

All patients that tested positive for *C. trachomatis* were treated with oral azithromycin. Patient-initiated contact tracing was encouraged by the clinic. There was no formal testing for other STIs in patients who returned a positive chlamydia test, directed testing was performed at the practitioner's discretion.

Community

Over the same 7-month period there were 2268 requests for *C. trachomatis* testing processed by PathCare Consulting Pathologists from the City of Greater Geelong and Bellarine Peninsula.

Demographic information was available for 2129 of these. Clockwork patients were not excluded from this analysis due

to de-identification of patient data. The age range tested was from <10 years of age up to 86 years of age. The median age range was from 25 to 30 years. Only 1004 (47.2%) of tests were performed on patients under 25 years; 1344 (63.1%) were performed on patients under 30 years of age

Overall, there were 142 positive (6.3%) and 86 inhibited results (3.8%). Males made up 18.0% (384/2129) of all patients tested and 9.4% (36/384) of males were positive for *C. trachomatis*. There was a higher prevalence of inhibited results in the index clinic patients than in the community patients (11.0% *v.* 3.8%; $P < 0.0001$). During the study period there was a total of 309 requests for PCR testing for *Neisseria gonorrhoea* (index clinic and community). There were three positive PCR results for *N. gonorrhoea*, none of which occurred in patients with *C. trachomatis* infection.

Discussion

Evidence supports the view that screening for and treatment of *C. trachomatis* in asymptomatic persons under the age of 25 is acceptable, effective and cost-effective in preventing the sequelae of infertility and neonatal disease.[13,16] Modelling studies have demonstrated cost effectiveness when the population prevalence is greater that 3.1%.[1,13] We opportunistically tested all sexually active patients presenting to a young peoples health clinic over a 7-month period and showed a prevalence of 5.8% among this population. There was a similar prevalence (6.3%) of *C. trachomatis* infection among patients tested according to clinician discretion in the Geelong community.

Notifications of *C. trachomatis* to the Health Department of Victoria have increased markedly over the last decade, possibly reflecting improvement in testing methods and attitudes to testing. Use of education, promotion and funding of screening programmes in communities should encourage wider screening of sexually active young people under the age of 25.

Our study showed for the first time a higher rate of *C. trachomatis* infection among young women presenting for the morning after pill (emergency contraception), perhaps reflecting higher risk sexual behaviour. Termination of pregnancy and lack of antenatal care have been associated with a higher prevalence of chlamydia infection.[17] Our finding makes a strong case for all practitioners seeing patients for MAP to specifically test for chlamydia infection. This result also has implications given the current debate over change of availability of MAP. If MAP is made available as an over the counter medication the opportunity to counsel, educate and screen these women for common STIs is lost.

Our study also confirms the lack of correlation between presence of *C. trachomatis* and symptoms, emphasising the need to consider testing for infection in all sexually active young people. It is also important to educate and inform young people that sexually transmitted infections are common and that they may be asymptomatic.

A positive correlation between chlamydia infection and gonorrhoea infection was reported among Indigenous Australian women[18] and it is understood that the presence of one STI increases the likelihood of concurrent infections. In Geelong, the community prevalence of *N. gonorrhoea* was 1.0%. However, the rate of testing for gonorrhoea was much lower than for chlamydia. We did not describe any co-infections in the index clinic or community population, but few patients were tested for both infections. Ideally we would advise clinicians seeing young adults engaging in unsafe sexual practice to test for both pathogens. In our population *C. trachomatis* appears to be a more significant infective agent, and if a positive chlamydia test is returned further STI investigations may be needed.

There were only nine males tested at the index clinic. This is not unexpected, firstly as the practitioners actively enrolling for the study saw mostly female patients and second, young men infrequently attend medical practitioners. There were higher numbers of men tested in the community and this may have been in men presenting with symptoms. Most research into chlamydia infection has concentrated on women because the sequelae of infection are more significant than in males. It is also often assumed that men will be identified by contact tracing of infected female partners. This may not be a valid assumption given that many sexually active young people have multiple sequential or simultaneous partners, contact tracing is not structured in any way and relies on the patients' involvement. The burden of chlamydia infection among young men may be higher than expected and make up an unrecognised reservoir for continued transmission. The incidence of 9.4% in the community patients tested in this study is higher than we would have expected and should prompt clinicians to test asymptomatic sexually active males presenting for medical review. A study in the UK that tested all male military recruits in one intake found a prevalence of 9.9%, which was higher than predicted, and of which 88.0% were asymptomatic.[19] This raises two issues, first the importance of testing sexually active young men when they present to a medical practitioner, and second to encourage and facilitate contact tracing of infected female partners. This may need to take the form of pamphlets, support and assistance from practitioners and nursing staff and funding should be directed at these measures.

We also described a significantly higher rate of inhibition of urine nucleic acid amplification tests especially among women presenting for the morning after pill. The majority of repeated tests then yielded a negative result on retesting. Blood, BHCG, leukocytes and nitrites have previously been reported to cause inhibition of the amplification process.[15] One possible explanation for the higher rate of inhibition in women presenting for the morning after pill may be presence of semen in the genitourinary tract. Physiologically, this may be explained by the high levels of magnesium and zinc in

human semen as magnesium at high concentrations is known to inhibit the *Taq* polymerase enzyme.[20] We also considered whether alcohol or other drugs, which may be associated with high-risk sexual behaviour, could inhibit the test. Further investigation of substances that cause inhibition of nucleic acid amplification is warranted. This may better define the causes of inhibition and suggest techniques that can be used in the laboratory to overcome inhibitory substances and to provide immediate results to practitioners and patients. Our study suggests that if an inhibited result is obtained and the laboratory is not able to perform manoeuvres to remove inhibitory substances repeat testing should be requested, as the majority of patients retested will not have ongoing inhibition of the test.

Our study was limited by small numbers and further study of a larger population over a longer period of time may be able to better define prevalence, risk factors, and specific groups where targeted screening and education would be most useful.

Summary

We conducted a pilot study to determine the prevalence of and risk factors for *Chlamydia trachomatis* infection in sexually active young people by opportunistically testing such patients presenting to the index health clinic.

We found a prevalence of 5.8%. Infection was entirely among asymptomatic young people. Women presenting for the morning after pill had a significantly higher prevalence of chlamydia infection (16%) and these women should be targeted for testing at time of presentation. Our study shows a burden of disease among asymptomatic sexually active young people and supports the need for further education and funding of a structured screening and contact tracing programme in Australia.

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