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ORIGINAL ARTICLE

Conventional versus molecular detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* among males in a sexually transmitted infections clinic

Siti Fazilah SITU *MD*, Chuan Hun DING *DrPath*, Salbiah NAWI* *MPath*, Asmah JOHAR** *MMed* and Ramliza RAMLI *MPath*

*Department of Medical Microbiology & Immunology, Universiti Kebangsaan Malaysia Medical Centre, Kuala Lumpur, Malaysia, *Department of Pathology, Hospital Kuala Lumpur, Malaysia and **Department of Dermatology, Hospital Kuala Lumpur, Malaysia*

Abstract

Background: Chlamydia trachomatis and *Neisseria gonorrhoeae* are important bacterial pathogens of sexually transmitted infections (STIs) worldwide. This study sought to compare the analytical sensitivity and specificity of conventional methods against a rapid molecular method in detecting STIs caused by these bacteria. *Methods:* Ninety five first-time male attendees of the Genito-urinary Medicine Clinic in Hospital Kuala Lumpur were included in this cross-sectional study. The detection of *C. trachomatis* was achieved through direct fluorescence antibody (DFA) staining of urethral swabs and real-time polymerase chain reaction testing (Xpert® CT/NG assay) on urine specimens. *N. gonorrhoeae* was detected through Gram staining and culture of urethral swabs and Xpert® CT/NG assay on urine specimens. *Results:* From the Xpert® CT/NG results, 11 (11.6%) attendees had chlamydia, 23 (24.2%) had gonorrhoea and 8 (8.4%) had both STIs. The sensitivity and specificity of DFA in detecting chlamydia compared to Xpert® CT/NG were 5.3% (95% CI: 0-28) and 94.7% (95% CI: 86-98), respectively. For gonorrhoea, the sensitivity and specificity of Gram staining were 90.3% (95% CI: 73-98) and 95.3% (86-99), respectively, whereas the sensitivity and specificity of culture compared to Xpert® CT/NG were 32.2% (95% CI: 17-51) and 100% (95% CI: 93-100), respectively. *Conclusion:* Although Gram-stained urethral swab smears are sensitive enough to be retained as a screening tool for gonorrhoea, culture as well as DFA lack sensitivity and are poorly suited to screen for gonorrhoea and chlamydia, respectively. However, owing to their high specificity, conventional detection methods are still suitable as confirmatory tests for gonorrhoea and chlamydia.

Keywords: Chlamydia trachomatis, Neisseria gonorrhoeae, sexually transmitted infection, Xpert CT/NG

INTRODUCTION

Chlamydia trachomatis and *Neisseria gonorrhoeae* are among the most prevalent bacterial pathogens of sexually transmitted infections. STIs due to these bacteria are on the rise, with more and more cases being reported to the Centres for Disease Control and Prevention (CDC).[1] Worldwide, it is estimated that there will be 131 million new cases of chlamydia and 78 million new cases of gonorrhoea yearly.[2] Thus, it is essential to diagnose STIs accurately in order to curb their spread within the community.

Many individuals infected with *C. trachomatis* are asymptomatic and can unknowingly transmit the organism to others.[3] To detect *C. trachomatis*, cell culture was for a long time the reference standard against which other diagnostic tests were compared. However, cell culture is difficult to standardize, technically demanding, expensive and relatively insensitive. Thus, non-culture methods such as the direct fluorescent antibody (DFA) test for *C. trachomatis* antigen were developed. For DFA, depending on the kit used, cross-reactivity with non-chlamydial bacteria, as well as with *Chlamydophila pneumoniae* and *Chlamydia psittaci* have been reported.[4]

Gonococci cause a stronger inflammatory

Address for correspondence: Dr. Ding Chuan Hun, Department of Medical Microbiology & Immunology, Pre-clinical Building, UKM Medical Centre, Jalan Yaacob Latif, Bandar Tun Razak, 56000 Cheras, Kuala Lumpur, Malaysia. Tel: +603-9145 9531. Email: dingch@ppukm.ukm.edu.my

response than *C. trachomatis* and most patients present with urethritis.[5] The CDC recommends that a Gram-stained male urethral specimen showing polymorphonuclear leukocytes with intracellular gram-negative diplococci be interpreted as diagnostic for gonorrhoea in symptomatic men.[4] However, in asymptomatic men, a negative Gram stain is not sufficiently sensitive to rule out infection. Culturing specimens on agar media for *N. gonorrhoeae* is another inexpensive option but it is not ideal as a diagnostic tool because of stringent collection and transport requirements as well as the long period of time required for confirmation.[6]

Therefore, a rapid diagnostic test which is highly sensitive and highly specific for both *C. trachomatis* and *N. gonorrhoeae* is desirable. One such method is the Cepheid Xpert® CT/NG assay which is one of several FDA-cleared nucleic acid amplification tests (NAATs) recommended by the CDC.[4] Xpert® CT/NG is a self-contained and fully automated real-time polymerase chain reaction system which produces results within 120 minutes. For *C. trachomatis*, the assay's detection sensitivity and specificity using urine specimens from males were 97.5% and 99.9%, respectively.[7] Likewise, the assay was able to detect *N. gonorrhoeae* in urine specimens from male patients with a sensitivity of 98% and specificity of 99.8%.[7] In a recent systematic review of five point of care tests for *C. trachomatis* and *N. gonorrhoeae*, Xpert® CT/NG was found to have the best performance.[8] Unlike traditional culture or DFA methods which usually rely on urethral swabs, NAATs can utilize urine specimens as well.[4,8]

The objective of this study was to determine the analytical sensitivity and specificity of conventional laboratory methods in detecting *C. trachomatis* and *N. gonorrhoeae* by comparing them with the Xpert® CT/NG rapid molecular assay.

MATERIAL AND METHODS

Study design and population

This cross-sectional study was conducted over a period of two years among male patients at Hospital Kuala Lumpur (HKL)'s Genito-urinary Medicine (GUM) Clinic. First-time clinic attendees who were at least 18 years old and who had sexual activity in the preceding 6 months were interviewed for inclusion into the study. Attendees with a known medical history of gonorrhoea or chlamydia were excluded. Patients who have been prescribed antibiotics usually given for STIs (i.e. doxycycline, any macrolide, any β-lactam or any quinolone) within the preceding 3 months were also excluded from the study. Subjects were classified as symptomatic if they had at least one of the following symptoms: dysuria, urethral discharge, coital pain and scrotal pain. Subjects without any of these symptoms were classified as asymptomatic.

Specimen collection

From every male subject, two urethral swabs and one first-void urine specimen were collected. Swab specimens were collected using Dacron or rayon swabs by a trained laboratory technician stationed in the GUM clinic. First-void urine (i.e. the initial 10-30 mL of voided urine) specimens were self-collected by the patients in leak-proof sterile specimen containers.

Conventional detection of N. gonorrhoeae

Urethral swabs were inoculated onto selective agar media and smeared onto glass slides for Gram staining as soon as they were collected. The agar media used was the commercially sourced GC agar + LCAT (lincomycin, colistin, amphotericin B and trimethoprim) (Thermo Scientific Microbiology Sdn. Bhd., Malaysia). The inoculated agar plates were incubated at 35-37°C in an atmosphere supplemented with 3-7% CO_2 and examined at 24-hour intervals for up to 72 hours. The culture isolates were then used as the inoculum for additional tests. The biochemical identification of *N. gonorrhoeae* was achieved using the commercially available API NH kit (bioMerieux, France), as per the manufacturer's instructions. Each kit was inoculated with a 4 McFarland suspension of test organism prepared in sterile 0.9% saline. The inoculated kits were incubated aerobically for 2 hours at 35°C before their four-digit numerical profiles were recorded and entered in the api*web*™ software. An API NH percentage identity of at least 95% for *N. gonorrhoeae* was accepted as correct identification. For Gram staining, the swabs was rolled onto clean glass slides and smeared over an area of less than 1 cm^2. The smears were heat-fixed and Gram stained. The stained smears were then examined using a light microscope under oil immersion (1000x magnification) for gram-negative diplococci and their spatial relationship to polymorphonuclear leukocytes.

Conventional detection of C. trachomatis

DFA testing was performed using the

commercially available MicroTrak® *Chlamydia trachomatis* Direct Specimen Test kit (Trinity Biotech, USA). Monoclonal antibodies in this kit are labelled with fluorescein isothiocyanate (FITC) and react specifically with the major outer membrane protein of human serovars of *C. trachomatis*. Prior to specimen collection, patients were also asked if they had urinated within the past hour and sampling was only performed if a period of at least 1 hour had elapsed. During specimen collection, the technician inserted a swab 2-3 cm into the external urethral orifice and rotated it at least twice to ensure adequate sampling of epithelial cells.[4] The tests were performed by smearing the swabs directly onto glass slides as per the manufacturer's instructions. The slides were then examined using a fluorescence microscope under oil immersion (1000x magnification). Urethral swabs were positive for *C. trachomatis* antigens when the smeared cells had elementary bodies that fluoresced apple-green under ultraviolet light.

Molecular detection of N. gonorrhoeae and C. trachomatis

Urine samples were used for the molecular detection of both *C. trachomatis* and *N. gonorrhoeae*. The detection was performed on the Cepheid GeneXpert Instrument System using single-use disposable cartridges which contain the PCR reagents and host the PCR process, in accordance to the manufacturer's instructions. Retesting on the leftover urine was done using a new cartridge whenever the instrument flagged "error", "invalid" or "no result".

Data collection and analysis

A questionnaire was used to obtain sociodemographic data (i.e. age and ethnicity) and STI symptoms such as dysuria, urethral discharge, coital pain and scrotal pain. Patients' laboratory results were retrieved from HKL's laboratory information system. Data were analysed using Statistical Package for the Social Science (SPPS) software version 20.0 (IBM, USA). Exact 95% confidence intervals (CI) on sensitivities and specificities were calculated using an online calculator available from http://vassarstats.net/clin1.html.

RESULTS

Sociodemographic data and presenting symptoms of subjects with STIs

A total of 95 first-time male GUM clinic attendees were enrolled into the study. Based on Xpert® CT/NG results, 11 (11.6%) were diagnosed with chlamydia, 23 (24.2%) with gonorrhoea and 8 (8.4%) had both infections.

Table 1 shows the distribution of STIs according to the age and ethnicity of study subjects. For chlamydia and gonorrhoea, the Malay ethnic group recorded the highest number of cases, followed by the Chinese and Indian ethnic groups. Co-infection was only detected amongst the Malays. The age range of patients with chlamydia was from 22 to 49 years, with a median of 29 years while the age range of patients with gonorrhoea was from 18 to 43 years, with a median of 26 years. For patients with co-infection, the age range was from 18 to 35 years, with a median of 22 years.

TABLE 1: Sociodemographic data of study subjects

	All subjects (n=95)	Subjects with chlamydia (n=11)	Subjects with gonorrhoea (n=23)	Subjects with co-infection (n=8)
Ethnicity				
Malay	57 (60.0%)	6 (54.5%)	18 (78.3%)	8 (100%)
Chinese	14 (14.7%)	2 (18.2%)	2 (8.7%)	None
Indian	19 (20.0%)	2 (18.2%)	1 (4.3%)	None
Others	5 (5.3%)	1 (9.1%)	2 (8.7%)	None
Age range				
<20	7 (7.4%)	None	5 (21.8%)	1 (12.5%)
20-29	45 (47.4%)	7 (63.6%)	11 (47.8%)	5 (62.5%)
30-39	20 (21.0%)	2 (18.2%)	4 (17.4%)	2 (25.0%)
≥40	23 (24.2%)	2 (18.2%)	3 (13.0%)	None

TABLE 2: Presenting symptoms of subjects with STIs

Presentation	Chlamydia (n=11)	Gonorrhoea (n=23)	*p*-value*
Symptomatic			
Urethral discharge	7 (63.6%)	23 (100%)	**0.007**
Dysuria	6 (54.5%)	17 (73.9%)	0.434
Coital pain	0 (0%)	2 (8.7%)	1.000
Testicular pain	2 (18.1%)	4 (17.4%)	1.000
Asymptomatic	4 (36.6%)	0 (0%)	**0.007**

* two-tailed *p*-value derived from Fisher's exact test

As presented in Table 2, urethral discharge was the single most common presenting symptom for both chlamydia and gonorrhoea followed by dysuria. Some patients presented with more than one symptom. The eight patients with co-infection were excluded from analysis. Urethral discharge was a common complaint in all patients with gonorrhoea. Coital pain was the least common presenting symptom and was only present in patients with gonorrhoea. Some patients with chlamydia were categorized as asymptomatic. They either had symptoms not typical of chlamydia or gonorrhoea (e.g. genital ulcers or genital warts) or were referred to the GUM clinic for STI screening as part of sexual partner screening or due to a reactive rapid plasma regain (RPR) result.

Performance of conventional tests in detecting C. trachomatis and Neisseria gonorrhoeae

Urethral swabs were positive for *C. trachomatis* antigens when the smeared cells had elementary bodies that fluoresced apple-green under ultraviolet light. As presented in Table 3, when compared to Xpert® CT/NG, the sensitivity of DFA was extremely low due to its ability to capture only 5% of cases detected by NAAT. However, its specificity was reasonably high, as it managed to rule out the infection in approximately 95% of the NAAT-negative cases.

The visualization of intracellular gram-negative diplococci in a Gram-stained urethral swab smear was taken as evidence of *N. gonorrhoeae*. All 28 patients with smear-positive results were symptomatic. As presented in Table 4, direct microscopic examination of urethral swabs was found to be both highly sensitive and specific in detecting gonorrhoea. Urethral swabs were considered culture-positive if the colonies which grew on GC agar containing specific antimicrobial agents were identified as *N. gonorrhoeae* by API NH. All 10 culture-positive specimens were also direct smear-positive. As shown in Table 4, when compared to Xpert® CT/NG, conventional culture lacked sensitivity due to its inability to capture two thirds of NAAT-positive cases but had excellent specificity by not detecting any positive cases amongst the NAAT-negative cases.

DISCUSSION

Contrary to the World Health Organization's global estimates on the burden of STIs,[2] in

TABLE 3: Results of DFA staining for *C. trachomatis vs.* Xpert® CT/NG assay

		Xpert® CT/NG assay	
		Detected	Not detected
DFA staining for *C. trachomatis*	Positive	1	4
	Negative	18	72
		%	95% CI
Analytical sensitivity		5.3	0-28
Analytical specificity		94.7	86-98

CI: confidence interval

TABLE 4: Results of direct Gram staining and culture for *N. gonorrhoeae vs.* Xpert® CT/NG assay

		Xpert® CT/NG assay	
		Detected	Not detected
Direct Gram staining for *N. gonorrhoeae*	Positive	28	3
	Negative	3	61
		%	95% CI
Analytical sensitivity		90.3	73-98
Analytical specificity		95.3	86-99
		Detected	Not detected
Culture for *N. gonorrhoeae*	Positive	10	0
	Negative	21	64
		%	95% CI
Analytical sensitivity		32.2	17-51
Analytical specificity		100	93-100

CI: confidence interval

our cohort of patients, gonorrhoea appeared to be the more frequently diagnosed STI (either alone or as a mixed infection with chlamydia), accounting for almost a third of all cases while chlamydia (either alone or as a mixed infection with gonorrhoea) was detected in a fifth of all STI cases. This could be due to the exclusion of female attendees which could have potentially increased the number of chlamydial STIs detected due to the higher rates of chlamydia in females. In 2005, the World Health Organization reported that there were 4.01 million females but only 2.60 million males with new *C. trachomatis* STIs in Southeast Asia.[9] We excluded females from this study because at the time of writing there was no CDC recommendation on using urine for NAAT in females.[4] The unequal racial distribution of subjects with STIs in this study is merely a reflection of the fact that Malays are the predominant GUM clinic attendees. Regardless of the type of STI, the age group which recorded the highest number of cases was the 20-29 years age group, which could be attributed to the higher risk-taking behavior in youths. Our finding echoes that of the CDC which reported that youngsters (i.e. those aged up to 24 years) accounted for the highest rates of chlamydia and gonorrhoea in 2014.[1]

Although urethral discharge was the most common presenting complaint for both STIs, the association was statistically significant for gonorrhoea. It is the current practice of HKL's GUM clinic to empirically treat any direct smear-positive patient with this specific complaint for gonorrhoea. While our study found that urethral discharge was present in all patients with gonorrhoea, other investigators from the United Kingdom reported a much lower rate (81.9%) in their cohort of patients.[10] Dysuria was the second most common complaint but we found no statistically significant association between this symptom and the type of STI. While it has been reported that dysuria is the most common presenting complaint in men with symptomatic chlamydia,[11] it is also known that only certain serovars (e.g. serovar Ga) of *C. trachomatis* are significantly associated with dysuria.[12] Our study did not identify the locally prevalent chlamydial serovars although a high prevalence of non-G serovars could have helped explain the observation that dysuria was not the most common presenting symptom for chlamydia in our cohort of patients. Thus, in our local clinic setting, any male patient with dysuria is likely to have either gonorrhoea or chlamydia and should be investigated further.

With regards to utilizing direct Gram staining of urethral smears to diagnose gonorrhoea in symptomatic males, we found that owing to its higher specificity (95%), a positive result is valuable in diagnosing gonorrhoea but a negative

smear result should not be solely relied upon to rule out the infection due to is comparatively lower sensitivity (90%). The higher specificity but lower sensitivity of direct urethral smears in diagnosing gonorrhoea in our study are consistent with findings published by the CDC (i.e. >99% for specificity and 95% for sensitivity).[4] Urethral swab culture for *N. gonorrhoeae* was also found to have excellent specificity (100%) in our study but it lacked sensitivity (32%). This poor sensitivity is attributed to various factors such as delayed transportation and incubation of inoculated culture plates. In HKL's GUM clinic, once the urethral swab is inoculated onto agar media, there is often a delay before the plate can be subjected to the appropriate incubation conditions (35-37°C in a moist atmosphere enriched with 3-7% of CO_2) as the plate has to be delivered to the diagnostic microbiology laboratory in another building. Due to the inherent thermosensitive nature of *N. gonorrhoeae*, changes in temperature and long transportation times can adversely affect the isolation of *N. gonorrhoeae*.[13] Our study's findings on the performance of culture detection of *N. gonorrhoeae* are somewhat similar to those reported by another group of investigators in which the reported sensitivity and specificity of culture were 58.2% and 100%, respectively, when compared with a molecular method.[14]

The performance of DFA as a tool for detecting *C. trachomatis* among male patients in our setting was tarnished by a very low sensitivity of only 5.3% although its specificity was good at almost 95%. DFA requires laboratorians to be competent in fluorescent microscopic techniques and adequately trained to identify elementary bodies of *C. trachomatis*. DFA is also observer-dependent and thus the interpretation of findings can be subjective, similar to the issues with DFA testing on nasopharyngeal swabs to detect *Bordetella pertussis*.[15] The sequential collection of a urethral swab followed by a urine specimen may either result in the removal of antigen available for detection in the urine, or by disrupting the urethral epithelium, an increase in the amount of antigen released into the urine specimen.[16] The former may possibly explain why four of our DFA-positive cases were missed by the Xpert® CT/NG assay. The presence of blood, mucin or bilirubin has also been reported by the manufacturer to cause interference in the Xpert® CT/NG assay.

Our study was limited by the small sample size of 95 subjects. Although we did not evaluate the usage of clinical specimens other than urethral swabs for the conventional detection of genitourinary *C. trachomatis* and *N. gonorrhoeae* infections in males, it is unlikely that other specimen types will improve the sensitivity and specificity of conventional tests. With regards to DFA testing, the manufacturer's product insert does not recommend urine for the detection of genitourinary *C. trachomatis* infections. Likewise, for the diagnosis of genitourinary *N. gonorrhoeae* infections, performing Gram staining to visualise intracellular Gram-negative diplococci on specimens other than urethral swabs and culturing non-urethral swab specimens for *N. gonorrhoeae* are not recommended.[4] We also did not evaluate other conventional detection methods (e.g. cell culture for *C. trachomatis* and enzyme immunoassays for *C. trachomatis* and *N. gonorrhoeae*). Thus, our recommendations on the utility of conventional detection methods cannot be generalized to all tests.

In conclusion, gonorrhoea is the more common bacterial STI among male attendees of HKL's GUM Clinic. Although urethral discharge is the most common presenting complaint for both STIs, its association with gonorrhoea is stronger. When selecting a suitable detection method for chlamydia and gonorrhoea, the chosen method should ideally be a NAAT as it is both highly specific and highly sensitive, as recommended by the CDC.[4] With the exception of direct Gram staining of urethral smears to diagnose gonorrhoea, other conventional detection methods have very poor sensitivity and should not be utilized solely for screening purposes. However, due to their high specificity, conventional tests still have a role in the confirmation of gonorrhoea and chlamydia.

DISCLOSURE

Fifty Xpert® CT/NG cartridges were sponsored by Bio-Focus Saintifik Sdn. Bhd., the distributor for GeneXpert products in Malaysia. The rest of the cartridges used in the study were purchased by means of a university research grant.

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