Using pooled urogenital, anorectal and oropharyngeal specimens to detect Chlamydia trachomatis and Neisseria gonorrhoeae among men who have sex with men in China: a multisite diagnostic accuracy study

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ABSTRACT

Objectives Screening for Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) at both urogenital and extragenital sites has been recommended in many countries. Testing of the infections using pooled specimens from urogenital and extragenital sites offers the opportunity to shorten the testing time and reduce the testing cost. Ex-ante pooling is placing the original single-site specimens in a tube with transport media, while ex-post pooling is making a pool of the transport media from both anorectal and oropharyngeal specimens and the urine. This study aimed to conduct a multisite performance evaluation of two pool-specimen approaches (ex-ante and ex-post) in detection of CT and NG using the Cobas 4800 platform among men who have sex with men (MSM) in China.

Design Diagnostic accuracy study.

Setting, participants and outcome measures Participants were recruited from MSM communities at six cities in China. Two oropharyngeal and anorectal swabs collected by clinical staff and 20 mL first-void urine collected by the participant himself were used for evaluating sensitivity and specificity.

Results A total of 1311 specimens were collected from 437 participants in six cities. The sensitivities of ex-ante pooling approach as compared with single-specimen approach (reference standard) were 98.7% (95% CI, 92.7% to 100.0%) for detection of CT and 89.7% (95% CI, 75.8% to 97.1%) for NG, and the specificities were 100.0% (95% CI, 98.0% to 99.9%) and 98.7% (95% CI, 97.1% to 99.6%), respectively. The sensitivities of ex-post pooling approach were 98.7% (95% CI, 92.7% to 100.0%) for CT and 100.0% (95% CI, 91.0% to 100.0%) for NG, and the specificities were 100.0% (95% CI, 99.0% to 100.0%) and 100.0% (95% CI, 99.1% to 100.0%), respectively.

Conclusions The ex-ante and ex-post pooling approaches show good sensitivity and specificity in detecting urogenital and extragenital CT and/or NG, indicating that these approaches can be used in epidemiological surveillance and clinical management of CT and NG infections, particularly among MSM population.

STRENGTHS AND LIMITATIONS OF THIS STUDY

⇒ The study was the first investigation to validate the diagnostic accuracy of the Cobas 4800 Chlamydia trachomatis/Neisseria gonorrhoeae (CT/NG) platform on pooled urine, anorectal and oropharyngeal specimens for detection of CT and NG among men who have sex with men.

⇒ The study was conducted in multiple sites including six cities across China with a statistically sufficient sample size.

⇒ The study simultaneously evaluated and compared performance of the two pooling specimen approaches (ex-ante and ex-post) in detection of the infections.

⇒ A randomised approach was not applied in specimen collection order when the anorectal or oropharyngeal specimens were allocated for specimen pooling or as the reference standard.

INTRODUCTION

Globally, Chlamydia trachomatis (CT) and Neisseria gonorrhoeae (NG) infections are the most common bacterial sexually transmitted infections (STIs), accounting for more than 55% of the global estimates of four curable STIs among people aged 15–49 years in 2021.1 If left untreated, CT and NG infections cannot only cause urethritis, epididymitis and infertility in men and pelvic inflammatory disease, ectopic pregnancy, infertility and chronic pelvic pain in women but also result in further transmission of these infections in community.2 High prevalence of CT and/or NG infections at anorectal and oropharyngeal sites among men who have sex with men (MSM) has called for the need to screen for these infections at these anatomical sites.3-5 Studies from the North America, Europe, Australia, Asia and
Africa have reported the prevalence of anorectal CT and NG infections ranging 2.1–23.0% and 0.2–24.0%, respectively, and the prevalence of oropharyngeal CT and NG infections ranging 0–3.6% and 0.5–16.5%, respectively. A study conducted by us in China has indicated a higher CT infection at anorectal site (15.6%) than urogenital site (3.2%) and a higher NG infection at anorectal site (5.0%) than oropharyngeal site (2.1%) or urogenital site (0.8%) among MSM. If screened only at urogenital site for CT and NG, 14–85% of the infections would be missed for intervention. While testing multiple anatomical sites separately is ideal, increase of cost and/or workload related to this testing strategy would be one of the major concerns. To respond to this concern, several studies have investigated the diagnostic accuracy of pooled specimens from the three anatomical sites (urethra, pharynx and rectum) to detect CT and NG. Among these studies, the ex-ante pooling approach or the ex-post were used. Ex-ante pooling is placing the original single-site specimens in a tube with transport media, while ex-post pooling is making a pool of the transport media from both anorectal and oropharyngeal specimens and the urine. However, up to date there has not been any published study to evaluate performance of the pooled specimens using the Cobas 4800 CT/NG platform which is one of most widely available systems for detection of CT and NG globally. This study aimed to conduct a multisite performance evaluation of two pool-specimen approaches (ex-ante and ex-post) in detection of CT and NG with the Cobas 4800 CT/NG platform among MSM in China.

MATERIALS AND METHODS

Study population and setting

Participants were recruited from MSM communities in six cities (Nanjing, Wuxi, Zhenjiang, Huaiian, Tianjin and Maanshan) in China between January and July 2022. MSM aged 16 years or above were eligible to participate in the study and those who had received antibiotic treatment in the past month were excluded. All eligible participants were invited to participate in the study through providing them a study introduction. After giving an informed consent, the participant was asked to complete a simple questionnaire in electronic format to collect socio-demographic and behavioural information as well as previous history of any STI (HIV/AIDS, syphilis, gonorrhoea, chlamydia, condyloma acuminatum or genital herpes).

Specimen collection

For the participant who agreed to provide the specimens, two oropharyngeal and two anorectal swabs were collected by the trained study staff, one for the ex-ante pooling approach (ie, ex-ante swab) and the other for the single-specimen and ex-post pooling approach (ie, ex-post swab). The sampling order was arbitrarily done for the ex-ante and ex-post swab. The standard operating procedures were followed for collection of these specimens. Briefly, oropharyngeal swabs were taken by swabbing the tonsils and the posterior pharynx, and anorectal swabs were taken by inserting a cotton swab 1.5 inches into the anus and gently rotating it three times. For first-void urine (FVU) collection, participants were instructed to collect around 20mL (half a container) of the initial urine stream into a sterile urine collection container. The Cobas swab specimen collection kit (Roche P/N 07958021190) was used to collect the oropharyngeal and anorectal swab specimen and the Cobas urine specimen collection kit (Roche P/N 05170486190) was used for the urine specimen.

Specimen preparation

Single-site specimens

One anorectal swab (hereafter called ANO-specimen) and one oropharyngeal swab (hereafter called ORO-specimen) were separately placed into a tube containing 4.3mL Cobas PCR Media (stabilising reagent) and 5–8mL FVU (hereafter called FVU-specimen) was transferred into a separate tube containing the Media (keeping the volume within the recommended fill lines). These single-site specimens (ANO-specimens, ORO-specimens and FVU-specimens) were temporarily stored at 4°C for a maximum of 2 weeks in the local laboratories and then transported under ‘cold chain’ condition to the laboratory centre (the Guangzhou Kingmed Diagnostics Group Medical Laboratory Center).

Multisite pooled specimens

For preparing ex-ante pooled specimens, we placed an oropharyngeal swab and an anorectal swab together with 5–8mL FVU into a tube containing 4.3mL Cobas PCR Media immediately after collection of these specimens. The ex-ante pooled specimens were temporarily stored at 4°C for a maximum of 2 weeks in the local laboratories and then transported under ‘cold chain’ condition to the laboratory centre. For preparing the ex-post pooled specimens, we transferred 2mL ANO-specimens and ORO-specimens each and 5mL FVU-specimen into a dry tube.

In the Cobas 4800 CT/NG platform, we selected the ‘Swab’ as sample type for the single-site ANO-specimens and ORO-specimens and the ‘Urine’ for the FVU-specimens, ex-ante and ex-post pooled specimens and then all the specimens were automatically tested in the same run. Because the swabs in the tubes could interfere with the movement of the probe, the swabs were removed from the tubes before the specimen was tested.

Specimen storage and transportation

The single-site ANO-specimens, ORO-specimens, FVU-specimens and ex-ante pooled specimens were temporarily stored at 4°C for a maximum of 2 weeks at the local laboratories. They were then transported under ‘cold chain’ condition to the Guangzhou Kingmed Diagnostics Group Medical Laboratory Center.
Infection definition and treatment

Participant was defined as being uninfected with CT or NG if his specimens from the three anatomical sites were negative for CT or NG. Participant was defined as being infected with CT or NG if any specimen from the three anatomical sites was positive for CT or NG. Those participants infected with CT or NG were provided with a free treatment according to the Guidelines of Clinical Management of Sexually Transmitted Diseases in China (doxycycline 100mg orally two times per day for at least 10 days for CT and ceftriaxone 1 g as a single dose for NG).20

Statistical analysis

The sample size needed for the evaluation was calculated assuming that the prevalence of CT in the study population was 20% and the sensitivity and specificity of either ex-ante or ex-post pooling approach were 95% and 95%, respectively. The target sample size would be 365 (73 participants positive for CT) to provide precision around exact binomial 95% CIs of 88.3% to 97.5% for sensitivity, and 95% CI, 92.3% to 96.6% for specificity. The sensitivity and specificity of the pooling approaches and their 95% CIs were calculated by comparing the results (positivity or negativity) detected using the pooled specimens with the single-site specimens (reference standards). Concordance was also evaluated using Cohen’s kappa coefficient by comparing the results detected by ex-ante and ex-post pooling approaches, respectively, with each other against the reference standards. For continuous variables, we calculated the median and the 25–75% IQR. For categorical variables, we calculated frequencies or proportions and identified the differences between groups using $\chi^2$ or Fisher’s exact test. A p value was considered statistically significant if it is 0.05 or less. All statistical analyses were conducted with R program V.4.1.3 (R Development Core Team) and GraphPad Prism software V.6.0 for Windows (GraphPad Software, San Diego, California, USA).

Patient and public involvement

Patient and public involvement (PPI) representatives worked with us to discuss the on-site implementation process of the study and refine the questions in the proposed questionnaire as well as the content of the informed consent form. In addition, PPI representatives were involved in the dissemination plans of this study.

RESULTS

A total of 444 participants were enrolled into the study and 437 (98.4%) provided the specimens from all three anatomical sites. The participants’ socio-demographic and behavioural characteristics and the information related to their previous STI history are summarised in table 1.

CT and NG prevalence

The prevalence of CT and NG in the study population based on detection using the single-site specimens are shown in figure 1. The prevalence of CT (16.9%; 95% CI, 13.4% to 20.5%) was significantly higher than NG (8.9%; 95% CI, 6.2% to 11.6%; p=0.001). Anorectal site had significantly higher prevalence of CT (12.8%; 95% CI, 9.7% to 16.0%) than urogenital site (3.4%; 95% CI, 1.7% to 5.1%; p<0.001) or oropharyngeal site (2.7%; 95% CI, 1.2% to 4.3%; p<0.001). Anorectal and oropharyngeal sites had the highest prevalence rates of NG (5.3%; 95% CI, 3.2% to 7.4% and 4.8%; 95% CI, 2.8% to 6.8%) than urogenital site (1.1%; 95% CI, 0.1% to 2.1%). Co-infection of CT and NG was more frequently found in anorectal site (2.3%) than oropharyngeal site (0.5%) or urogenital site (0.5%). Majority of the participants (88.3%) reported to have anal or oral sex with other man in the last 6 months and the prevalence of CT infection was higher among those who had multiple sexual partners during the past 6 months (table 1).

Performance of pooling approaches

As comparison of the CT and NG results determined in the multisite pooled specimens with the results of the single-site specimens, the overall sensitivities were 98.7% (95% CI, 92.7% to 100.0%) and 89.7% (95% CI, 75.8% to 97.1%) for ex-ante pooled specimens, 98.7% (95% CI, 92.7% to 100.0%) and 100.0% (95% CI, 91.0% to 100.0%) for ex-post pooled specimens; their corresponding specificities were 99.5% (95% CI, 98.0% to 99.9%), 98.7% (95% CI, 97.1% to 99.6%), 100.0% (95% CI, 99.0% to 100.0%) and 100.0% (95% CI, 99.1% to 100.0%). The pooled specimens in either ex-ante or ex-post pooling approach had good agreement rate in detection of CT or NG with the single-site specimens, indicating a high Cohen’s kappa coefficient (table 2). However, the ex-ante approach showed discordant CT results in three specimens (one positive in single anorectal swab but negative in the pooled specimen, and two positives only in the pooled specimens) and discordant NG results in nine specimens (two positives only in single anorectal swabs, two positives only in single oropharyngeal swabs and five positives only in the pooled specimens). The discordant result in ex-post approach was only found in one specimen (one positive only in single oropharyngeal swab) for CT and not found for NG.

Cycle threshold value

The ex-ante pooling approach missed one anorectal CT infection, two oropharyngeal NG infections and two anorectal NG infections. For the case missing to detect CT infection, the cycle threshold (Ct) value of the specimen was significantly higher than that of the overall positive specimens (37 vs 33.8, p<0.001). For the four cases missing to detect NG infection, the mean Ct value of the specimen was slightly higher than that for the overall oropharyngeal positive specimens (36.1 vs 34.2, p=0.42) or the overall anorectal positive specimens (31.5 vs 30.2,
The mean Ct value of the ex-ante pooled specimens missing detection of the infections in the single-site specimens was higher than that of the overall CT-positive specimens (37.1 vs 33.8, p=0.19) but the significant difference was only found in comparison with the overall NG-positive specimens (36.3 vs 32.1, p=0.04). In addition, one ex-post pooled specimen missing detection of oropharyngeal CT infection also had a significantly higher Ct value than the overall oropharyngeal CT-positive specimens (39.8 vs 35.5, p=0.001).

**DISCUSSION**

To our knowledge, our evaluation on multisite pooling specimen approach is the first study to investigate the diagnostic accuracy of the Cobas 4800 CT/NG platform on the pooled specimens of urine, and anorectal and oropharyngeal swabs among MSM. In addition, our study simultaneously evaluated the two pooling specimen approaches (ex-ante and ex-post) in the same study for the first time. The sensitivity of our ex-ante pooling approach in detection of NG (89.7%) was similar to Sultan’s findings (89.9%) in the largest study in the UK using self-collected specimens and the Aptima Combo 2 assay. This similarity is probably because of the comparability in sensitivity of the Cobas 4800 platform and the Aptima Combo 2 assay in detection of NG in the single-site specimens. For the ex-post pooling approach, most of the previous evaluations were based on the GeneXpert platform, reporting the sensitivities ranging between 90% and 98% for detection of CT and 89% and 96% for NG. Our ex-post pooling approach using the Cobas 4800 platform had relatively higher sensitivities for CT (98.7%) and NG (100%) than these studies. The GeneXpert assay has showed a good concordance with the Cobas 4800 platform for the detection of CT and NG using urine specimens, but the data on direct comparison between the GeneXpert assay and the Cobas 4800 platform for detection of CT and NG in extragenital specimens are limited. It is noted from a meta-analysis that the GeneXpert assay had relatively low sensitivity (less than 90%) for detection of extragenital CT infections, which may compromise the sensitivity of the GeneXpert assay in detection of CT when extragenital specimens were pooled with urine.

Among the two pooling approaches using the Cobas 4800 platform, the ex-post pooling approach had a better agreement with the single-site specimens than the ex-ante pooling approach while the ex-ante approach, which avoided a procedure to pool the specimens before testing, was easier to operate than the ex-post approach. However, miss-detection of infections in ex-ante pooling
Figure 1  Prevalence of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* at different anatomical sites.

Table 2  Concordance in detection of *Chlamydia trachomatis* and *Neisseria gonorrhoeae* between the pooling-specimen and the single-specimen strategies

<table>
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<tr>
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<td>Total</td>
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*Single-specimen strategy to detect *C. trachomatis* or *N. gonorrhoeae* was considered as the reference standard.
approach may be a public health and clinical concern. In our study, the ex-ante pooling approach missed four infected cases, including one with anorectal CT and NG infection, two oropharyngeal NG infections and one anorectal NG infection. For the case missing anorectal CT and NG infection, faecal remains were seen in the ex-ante pooled specimen, indicating the PCR inhibitors were responsible. For the remaining three cases missing NG infection, the low pathogen load and the dilution effect of the urine in the ex-ante pooled specimens may be the cause of false-negative results. In addition, the ex-ante pooling approach found seven (two CT and five NG) positive results to be negative in single-specimen testing. A low pathogen load of these specimens may result in this discordance although further study is needed. Although the increased workload is a major concern, the ex-post pooling approach demonstrates high sensitivity and specificity for detection of CT and NG and also makes the site-specific CT/NG infection possible if required. In our study, the ex-post pooling approach only missed one oropharyngeal CT infection. The difference in Ct values may suggest that a low pathogen load was responsible for the false-negative result.

Successful control of CT and NG is still mainly based on behavioural interventions and medical care. Active screening for these infections and timely treatment of infected cases are key elements in medical care. Although both urogenital and extragenital screening for CT and NG have been widely implemented in clinical practice particularly for people who have anal and/or oral sex, the use of pooled specimens collected from urogenital, anorectal and oropharyngeal sites for the screening has not yet recommended by the available laboratory guidelines.23-24 Treatment of patients with the pooled specimen positive for CT or NG may be a concern.8 12 14 17 Previous studies have shown that a 7-day course of doxycycline is superior to single-dose azithromycin in the treatment of anorectal CT infection25 and ceftriaxone is superior to spectinomycin in treatment of oropharyngeal NG infection.26 However, doxycycline is also recommended by many guidelines for treatment of urogenital and oropharyngeal CT infection (except during pregnancy) and ceftriaxone for urogenital and anorectal NG infection.3 20 27 28 Therefore, from the perspective of the clinical intervention, further identification of the site-specific infection is not indicative if the standard treatment (doxycycline for CT or ceftriaxone for NG infection) is applied.

One of the important implications to introduce the pooling specimen approaches is cost saving. However, the cost-effectiveness of these approaches is substantially influenced by the background prevalence of CT/NG. In the population with a CT/NG prevalence of 22.2%, as indicated in our study, the pooling approach could roughly demonstrate a cost reduction by 44% even if the single-site specimens are retested when the pooled specimen is positive.

In summary, our study provides additional evidence based on the evaluation of the Cobas 4800CT/NG platform to indicate a good performance of the molecular diagnostics using the pooled specimens from anorectal, oropharyngeal and urogenital sites in detection of CT or NG among MSM. These findings have important implications for developing an affordable CT and NG screening programme for the target populations. However, for introduction of the pooling specimen approaches into clinic-based or community-based practice of screening for CT and NG, further studies on feasibility from health facility (such as testing result reports), and patients’ perspectives (such as self-collection of oropharyngeal and anorectal specimens), and evaluation of intervention impact and cost-effectiveness in different scenarios are needed.

Contributors T-TJ and X-SC conceptualised and designed the study. T-TJ and N-XC coordinated the site study. T-TJ, N-XC, T-JJ, OZ, J-WL, JZ and YZ conducted the data and specimen collection in study sites. M-QS and YPY coordinated the laboratory testing. T-TJ and X-SC did the data analysis and explanation. T-TJ drafted the manuscript. X-SC made a critical revision of the manuscript. All authors reviewed the manuscript. All authors approved this final version. X-SC is the guarantor of the study.

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Competing interests None declared.

Patient and public involvement Patients and/or the public were involved in the design, or conduct, or reporting, or dissemination plans of this research. Refer to the Methods section for further details.

Patient consent for publication Not applicable.

Ethics approval This study was approved by the Medical Ethics Committee of Chinese Academy of Medical Sciences Institute of Dermatology and the National Center for STD Control in China on 9 October 2021 (approval number 2021-KY-037).

Provenance and peer review Not commissioned; externally peer reviewed.

Data availability statement Data are available upon reasonable request. Data requests can be made to X-SC, chenxs@ncifdc.org.

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