Oct. 2011, Volume 8, No. 10 (Serial No. 83), pp. 577-580
Journal of US-China Medical Science, ISSN 1548-6648, USA

Low Sensitivity of Pooled Chlamydia Testing in a Sample of the Young German General Population

Karin Haar[1], Thomas Meyer[2], Sarika Desai[1], Michael Thamm[3], Matthias an der Heiden[1], Viviane Bremer[4] and Osamah Hamouda[1]

1. Department for Infectious Disease Epidemiology, HIV/AIDS, STI and Bloodborne InfectionsUnit, Robert Koch-Institute, Berlin, Germany

2. Department of Medical Microbiology, Virology and Hygiene, Universitätsklinikum Hamburg-Eppendorf, Hamburg, Germany

3. Department of Epidemiology and Health Reporting, Central Epidemiological Laboratory Unit, Robert Koch-Institute, Berlin, Germany

4. European Centre for Disease Prevention and Control, Stockholm, Sweden

Abstract: Objective: To compare sensitivity of pooled urine testing with single specimen testing using NAAT (nucleic acid amplification test). To determine Chlamydia prevalence among adolescents in Germany. **Methods:** Urine samples from 15-17 year old female and 16-17 year old male participants of a nationwide representative study in children and adolescents sampled between 2003 and 2006 were tested individually and in pools of 4 using BDProbeTec ET System (strand displacement amplification). Specificity, sensitivity, positive and negative predictive values (PPV, NPV) for pooled testing with 95%-confidence-intervals (95% CI) was calculated. Chlamydia prevalence with 95% CI was calculated for individual testing. **Results:** Among individually tested 1925 specimens, 27 were positive for chlamydia, resulting in a prevalence of 1.4%. Chlamydia prevalence ranged from 0.2% in 16-year old males to 3.2% in 17-year old females. Using individual testing as the standard, a specificity of 99.6% and a sensitivity of 56.0% was found for pooled testing. The PPV was 87.5% and the NPV 97.6%. Inhibition was higher in individual testing compared to pooled testing. **Conclusion:** In this setting of a national health survey the very low sensitivity of 56.0% for pooled testing is concerning and differs from previously reported results. Pooled chlamydia test results should be interpreted with caution, especially if urine samples were not collected or stored under optimal conditions. Possible causes such as dilution effects, lack of prior DNA purification, long-term-storage and the use of urines that may not be first-void should be ruled out. Recommendations for population screening to use pooled testing might need to be re-evaluated should larger studies on more recent samples confirm our findings.

Key words: Chlamydia, pooled testing, sensitivity, KiGGS.

1. Introduction

Worldwide, infections with urogenital Chlamydia trachomatis (CT) are common [1-3].

In Germany, chlamydia is not a notifiable sexually transmitted infection (STI), however data from the national STD-sentinel surveillance system suggested chlamydia to be the most frequent laboratory-confirmed STI, with a 6% positivity rate [4]. In an age-stratified random sample of 1815 urines from a total of 5755 participants aged 12-17 years, infections with chlamydia were found only in girls aged 15-17 and boys aged 17 years [5]. KiGGS (The German Health Interview and Examination Survey for Children and Adolescents) was a nationwide representative study conducted among 0-17 year olds between 2003 and 2006.

In 2008, an opportunistic national chlamydia screening programme using NAAT was introduced for all women under the age of 25 years in Germany. Pooling up to 5 urine samples is permitted in countries with estimated prevalences of up to 4% to be

Corresponding author: Karin Haar, MD, research fields: infectious disease epidemiology, public health. E-mail: HaarK@rki.de.

cost-effective [6]. Depending on chlamydia prevalence, pooling has found to be cost-saving with only minor losses in sensitivity and specificity [6-11].

In our study we compared pooling versus individual testing of urines from participants of the KiGGS-study to establish the impact of pooling on the sensitivity and specificity in the setting of a national health survey.

2. Methods

2.1 Sample

All urines from females aged 15-17 years and males 16-17 years who participated in the KiGGS-study and gave a urine sample were tested for chlamydia.

2.2 Specimens and Assays

Informed consent for AUT (anonymous unlinked testing) was given by each participant or their parents or legal guardians in the context of the KIGGS. Specimens were collected between 2003 and 2006, stored at -40°C and transported frozen to the analysing laboratory. Between February 2009 and February 2010 urine samples were tested for chlamydia using BDProbeTec ET System, a strand displacement amplification (SDA) system that detects cryptic plasmid of CT. Assays were performed according to the manufacturer's guidelines for individual testing, except using 2 ml urine instead of 4 ml. For pooled testing, 500 µl urine-portions of 4 patients were combined and tested like individual samples. If positive or inhibitory, all 4 specimens were tested individually.

Each sample was analyzed in a second reaction which contained external CT DNA to control for amplification efficiency and inhibitors. Results were considered negative only when amplification control signals were positive. DNA amplification was performed under routine diagnostic PCR conditions with standard precautions to prevent contamination. No transport medium was used for urine samples.

2.3 Statistics

Statistical analyses were performed using SPSS 17.0.3 and STATA 10. Specificity, sensitivity, positive and negative predictive values (PPV/NPV) with 95% confidence intervals (95% CI) were calculated for pooled testing using individual testing as the standard. Only pools of four were included in these analyses. Changes in sensitivity over time were calculated using logistic regression.

2.4 Ethical Approval

Ethical approval was obtained from the Ethics Commission of the Charité University Medicine, Berlin.

3. Results

In individual testing, 27/1925 specimens were positive for chlamydia (1.4%, 95% CI: 0.97-2.03%). Age- and gender-specific prevalences are shown in Table 1.

29 incorrectly pooled specimens were excluded from analyses.

Table 1 Results from individual specimen testing: Chlamydia prevalence by age and gender.

Age in years	Gender	Negative	Positive	Total	Prevalence
15	♀	375	6	381	1.6 %
16	♀	372	6	378	1.6 %
	♂	407	1	408	0.2 %
17	♀	365	12	377	3.2 %
	♂	379	2	381	0.5 %
Total		1898	27	1925	1.4 %

Table 2 Results from individual specimen testing compared to pooled testing in pools of 4 specimens

		Individual testing		
		Positive	Negative	Total
Pooled testing	Positive	14	2	16
	Negative	11	447	458
	Total	25*	449	474

*27 individual specimens tested positive; 2 pools contained 2 positives each.

Sensitivity = 14/25 = 56.0%

Specificity = 447/449 = 99.6%

PPV = 14/16 = 87.5%

NPV = 447/458 = 97.6%

In total, 16 pools were positive, 14 of them correctly, accounting for a PPV of 87.5% (95% CI: 61.7-98.4%). 458 pools were negative, compared to 449 by individual testing, constituting a NPV of 97.6% (95% CI: 95.7-98.8%).

In total, 27 individual specimens tested positive; one negative and one positive tested pool contained two positive specimens each. The specificity for pooled testing was 99.6% (95% CI: 98.4-99.9%) and the sensitivity 56.0% (95% CI: 34.9-75.6%).

No inhibition or invalid testing occurred in pooled testing whereas 21 individual tests showed inhibition (1.1%) and 132 were invalid (6.9%). These extracts were all negative.

Sensitivity of pooled testing increased with time, although not significantly (2003: 57%, 2004: 43%, 2005: 67%, 2006: 100%; p = 0.095).

4. Discussion

Our low sensitivity of pooled testing is worrying, particularly, as we tested pools of four compared to the national recommendation of testing in pools of five [6]. Potential reasons for low sensitivity include dilution and lack of prior DNA purification [12]. Additionally, KiGGS-participants were not explicitly requested to collect first-void urine, which could result in lower DNA-loads per sample. In first-void urine, defined as 20 to 30 ml of the initial flush of urine, the highest concentrations of chlamydia can be found and collection of the first 4 to 5 ml of urine has even shown to contain up to a sixfold higher organism load [13, 14].

As no specific transport medium was used, it cannot be attributed to 2-sucrose phosphate buffer which previously reduced sensitivity of pooled SDA-testing to 86.5% [15].

Sensitivity did not significantly vary over time, however, numbers were small. Long-term storage could however have influenced CT prevalence by increasing false negative results in individual testing.

Consistent with Low et al., the number of inhibitory tests in pooled testing was lower than in individual testing, namely 0 versus 21 in individual testing in our study [16].

Pooling of specimens in low-prevalence countries is less costly due to reduction of test-kits but the labour-intense lab methods should be taken into consideration.

As confidence intervals for sensitivity were relatively wide, larger studies are needed in low-prevalence communities to provide more precise estimates and to advocate changes in German screening strategy.

Until these data are available, results of pooled urine samples should be interpreted cautiously.

For research purposes specimens stored longer than recommended should be individually tested.

5. Key Messages

• Specificity of 4xpooled NAAT testing was 99.6% compared to individual NAAT testing

• Sensitivity of 4xpooled NAAT testing was as low as 56.0% compared to individual NAAT testing

• Although pooling of urine sample might be more cost-effective in low-chlamydia prevalence countries, reduction of sensitivity with NAAT should be kept in mind

6. Competing Interests

The authors declare that they have no competing interests.

7. Authors' Contributions

KH was Principal Investigator on the project, analyzed data, conceived this paper and took primary responsibility in writing it. TM was Field Director on the project, organized adequate specimen analyses and took part in the writing and editing of the paper. SD conceptualized the preliminary study, obtained ethic

committee approval and contacted various counterparts. She further edited the paper. MT contributed to specimen and patient data acquisition, as well as correct storage and transport facilities and took part in editing the paper. MadH helped conducting the statistical analyses reported here, as well as taking part in editing the paper. VB helped conceptualize and write the proposal for the project, served as its consultant on previous developments, and took part in editing the paper. OH was Project Director, oversaw data collection and analysis, and took part in editing the paper.

Acknowledgements

The authors would like to acknowledge Dr. Gerrit Mohrmann (Labor Lademannbogen, Hamburg) and his team for performing the analyses. The authors would further like to thank Dr. Heribert Stolzenberg for data matching support.

References

[1] ECDC, Review of chlamydia control activities in EU countries, in Project SCREen, Final Report, ECDC: Stockholm, 2008.

[2] World Health Organization, Global strategy for the prevention and control of sexually transmitted infections: 2006-2015: Breaking the chain of transmission, Department of Reproductive Health and Research, WHO Press: Geneva, 2007, pp. 1-61.

[3] J. S. Wilson et al., A systematic review of the prevalence of Chlamydia trachomatis among European women, Hum Reprod Update 8 (4) (2002) 385-394.

[4] Robert-Koch-Institut, Sechs Jahre STD-Sentinel-Surveillance in Deutschland – Zahlen und Fakten, Epidemiologisches Bulletin 3 (2010) 20-27.

[5] S. Desai et al., Prevalence of Chlamydia trachomatis among young German adolescents, 2005-06, Sexual Health 8 (2011) 120-122.

[6] GBA, Screening auf genitale Chlamydia trachomatis-Infektionen bei Frauen 2008, Gemeinsamer Bundesausschuss: Siegburg.

[7] M. Mund et al., Introduction of chlamydia trachomatis screening for young women in Germany, JDDG - Journal of the German Society of Dermatology 6 (12) (2008) 1032-1037.

[8] M. J. Currie et al., Pooling of clinical specimens prior to testing for Chlamydia trachomatis by PCR is accurate and cost saving, J Clin Microbiol 42 (10) (2004) 4866-4867.

[9] R. W. Peeling et al., Pooling of urine specimens for PCR testing: A cost saving strategy for Chlamydia trachomatis control programmes, Sex Transm Infect 74 (1) (1998) 66-70.

[10] S. A. Morré, R. Welte and M. J. Postma, Major improvements in cost effectiveness of screening women for Chlamydia trachomatis using pooled urine specimens and high performance testing, Sexually Transmitted Infections 78 (1) (2002) 74-75.

[11] J. Krepel et al., The impact on accuracy and cost of ligase chain reaction testing by pooling urine specimens for the diagnosis of Chlamydia trachomatis infections, Sex Transm Dis. 26 (9) (1999) 504-507.

[12] G. I. Rours et al., Use of pooled urine samples and automated DNA isolation to achieve improved sensitivity and cost-effectiveness of large-scale testing for Chlamydia trachomatis in pregnant women, J Clin Microbiol 43 (9) (2005) 4684-4690.

[13] C. A. Wisniewski et al., Optimal method of collection of first-void urine for diagnosis of Chlamydia trachomatis infection in men, Journal of Clinical Microbiology 46 (4) (2008) 1466-1469.

[14] M. Chernesky et al., Impact of urine collection order on the ability of assays to identify Chlamydia trachomatis infections in men, Sex Transm Dis. 30 (4) (2003) 345-347.

[15] D. Bang et al., Comparison of the Becton Dickinson strand displacement amplification and Cobas Amplicor Roche PCR for the detection of Chlamydia trachomatis: Pooling versus individual tests, Clinical Microbiology and Infection 9 (10) (2003) 1020-1023.

[16] N. Low et al., Epidemiological, social, diagnostic and economic evaluation of population screening for genital chlamydial infection, Health Technol Assess 11 (8) (2007) 1-165.