Culture confirmation of gonococcal infection by recall of subjects found to be positive by nucleic acid amplification tests in general practice

Jens Kjølseth Møller

Culture confirmation of gonococcal infection by recall of subjects found to be positive by nucleic acid amplification tests in general practice

Jens Kjølseth Møller [1,2]

[1]Department of Clinical Microbiology, Lillebælt Hospital, Vejle, Denmark

[2]Department of Clinical Microbiology, Aarhus University Hospital, Skejby, Denmark

Keywords: NAAT, *Neisseria gonorrhoeae*, culture, recall samples, general practice

Revised version

Correspondence to

Dr J.K. Møller, Department of Clinical Microbiology, Institute of Regional Health Services Research, University of Southern Denmark, Lillebælt Hospital, Kabbeltoft 25, DK-7100 Vejle, Denmark; jkm@dadlnet.dk

Abstract

Objectives: To evaluate a routine notification of general practitioners to recall nucleic acid amplification test (NAAT) positive subjects for culture of *Neisseria gonorrhoeae* to confirm gonococcal infection in the community.

Methods: A retrospective observational study of the routine testing for *N. gonorrhoeae* by analysis of test results compiled from the laboratory information system in two departments of clinical microbiology.

Results: Altogether, 158 male and female subjects with NAAT positive results for *N. gonorrhoeae* were included in the study. Samples for culture of *N. gonorrhoeae* were collected from 102/158 (64.6%) subjects recalled after a NAAT assay was found positive. Growth of *N. gonorrhoeae* was seen in the samples from 54/102 (52.9%) of the re-examined NAAT positive subjects. Among subjects with samples collected within the first week after the positive NAAT test, 34/44 (77%) were confirmed positive by culture.

Conclusion: This study shows that it is possible for the general practitioner to recall a substantial number of NAAT positive subjects to collect swabs for culture of *N. gonorrhoeae* to confirm gonococcal infection in the community. The majority of recall samples is culture positive if collected within a week of the NAAT positive test, and may provide a sufficient monitoring of the drug susceptibility of *N. gonorrhoeae* strains in the community.

INTRODUCTION: The gold standard of laboratory diagnosis of gonorrhoea has been the culture of the causative agent, *Neisseria gonorrhoeae* (1). An increasing number of patients in our area are primarily diagnosed with gonorrhoea by a nucleic acid amplification test (NAAT) only. The aim of this study was to evaluate the recall of NAAT positive subjects to collect swabs for culture confirmation of gonococcal infection in the community.

METHODS: A retrospective observational study was performed from March 2009 to March 2010 including all male and female subjects diagnosed by a general practitioner (GP) with a NAAT positive sample for *N. gonorrhoeae* (NG) only. Samples were received and examined in two department of clinical microbiology from their combined catchments area of 1,000,000 people. The Gen-Probe APTIMA COMBO 2® (AC2) and the APTIMA® GC (AGC) NAAT assays (Gen-Probe, San Diego, USA) were used. Positive AC2 assays were repeated and furthermore examined with the AGC assay to confirm the initial positive result. The final laboratory report routinely notified the GP that the patient should be re-examined by culture to obtain an antimicrobial susceptibility pattern.

Swabs for culture of NG (charcoal imbibed cotton swab in a Stuart medium) were transported to the laboratory on the same day or mailed by ordinary postal service, and cultured on a chocolate agar plate supplemented with Polymyxin B, Lincomycin, and Amphotericin B at 35°C and 10% CO_2 for 48 hours. *N. gonorrhoeae* was identified by Gram stain and conventional biochemical tests.

The unique Danish civil registry number (CPR) was used to cross link the laboratory results of all samples for NG testing of an individual, and to establish a chronological order of the samples.

RESULTS: A total of 158 subjects, 52 from Vejle and 106 from Aarhus, were included. All initially positive AC2 assays were confirmed by repeat testing and the single analyte assay AGC. Table 1 shows the distribution of female and male samples. Altogether, 102/158 (64.6%) NAAT positive subjects had swabs collected for culture by a recall examination. A positiv culture of *N. gonorrhoeae* was found in 54/102 (52.9%). Twenty subjects were not included in the study because they had a culture for *N. gonorrhoeae* collected together with the positive NAAT sample, 16/20 (80%) were culture positive. Corresponding figures during a two-year period at the clinic of Genito-Urinary Medicine using similar NAAT and culture assays were 40/45 = 89%.

Growth of *N. gonorrhoeae* was seen in 34 of 44 (77%) subjects recalled within the 1st week after their positive NAAT test. Corresponding figures for the 2nd week, and the 3rd week or later were 13/32 (41%) and 7/21 (33%), respectively (χ^2=15.1, degrees of freedom=2, P<0.001).

DISCUSSION:

Recall samples for culture of *N. gonorrhoeae* were collected from two thirds of NAAT positive subjects. Among subjects with samples collected within the first week after the positive NAAT test, three quarters were confirmed positive by culture. The weakness of the retrospective observational design of the present study is the lack of data on antibiotic treatment prior to or after the initial NAAT screening assay, which may preclude the growth of *N. gonorrhoeae* in a recall culture. Furthermore that information was not available to show whether failure to recall was due to subjects not attending or to GPs not routinely requesting culture samples. Culture confirmation of the NAAT positive results is also challenging because of the rapid loss of viability of *N. gonorrhoeae* strains during the transportation of the samples from the community to the laboratory (1). Previous studies also found that about two

thirds of the NAAT results could be confirmed by culture (2, 3). However, urgent transportation and culture of samples can increase the percentage of samples with growth of *N. gonorrhoeae* to more than 85% (3). The present study shows that it is possible for the general practitioner to recall a substantial number of NAAT positive subjects to collect swabs for culture of *N. gonorrhoeae* to confirm gonococcal infection in the community. Provided that random selections of NAAT positive subjects are re-examined by culture, recall cultures may provide an accurate picture of antibiotic resistance in *N. gonorrhoeae* strains in the community. To obtain additional follow-up samples, GPs may be requested to recall subjects from whom samples were not received for culture within a week after the initial positive NAAT assay.

In conclusion, the majority of recall samples is culture positive if collected within a week of the NAAT positive test, and may provide a sufficient monitoring of the drug susceptibility of *N. gonorrhoeae* strains in the community.

ACKNOWLEDGEMENTS, COMPETING INTERESTS, FUNDING

There are no competing interests.

Word count: Abstract (208), Text (749).

REFERENCES

1. **Iwen PC, Walker RA, Warren KL, Kelly DM, Linder J, and Hinrichs SH.** 1996. Effect of off-site transportation on detection of Neisseria gonorrhoeae in endocervical specimens. Arch. Pathol. Lab. Med. **120:**1019-1022.

2. **Gopal Rao G, Bacon L, Evans J, Dejahang Y, Hardwick R, Michalczyk P, Wong J, and Donaldson A.** 2009. Can culture confirmation of gonococcal infection be improved in female subjects found to be positive by nucleic acid amplification tests in community clinics? Sex. Transm. Infect. **85:**531-533. doi: 10.1136/sti.2009.036525.

3. **Rao GG, Bacon L, Evans J, Dejahang Y, Michalczyk P, Donaldson N, and Lewisham Chlamydia and Gonoccoccus Screening Programme.** 2008. Prevalence of Neisseria gonorrhoeae infection in young subjects attending community clinics in South London. Sex. Transm. Infect. **84:**117-121. doi: 10.1136/sti.2007.026914.

Tables

Table 1. Recall of NAAT positive subjects to collect swabs for culture of *N. gonorrhoeae*

	NAAT positive	Swabs for culture			Culture positive		
	No.	No.	%	(95%CI)	No.	%	(95%CI)
Female subjects	**69**	51	**74**	(62 - 83)	22	**43**	(30 - 57)
Urogenital swab[a]	65	49			21		
Urine	4	2			1		
Male subjects	**89**	51	**57**	(47 - 67)	32	**63**	(49 – 75)
Urine	70	42			28		
Urethral swab	19	9			4		

[a] Samples from urethra, cervix and vagina