


# Impact of time of culture specimen collection on the recovery of *Neisseria gonorrhoeae* after a positive nucleic acid amplification test

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## ABSTRACT

**Objectives** Culture of *Neisseria gonorrhoeae* remains essential for antimicrobial resistance (AMR) surveillance. We evaluated the effect of time of specimen collection on culture yield following a positive nucleic acid amplification test (NAAT).

**Methods** We retrospectively assessed *N. gonorrhoeae* culture yield among asymptomatic individuals (largely men who have sex with men) who attended for sexual health screening and had a positive NAAT. Participants underwent either same-day testing and notification (Drassanes Exprés) or standard screening with deferred testing.

**Results** Among 10 423 screened individuals, 809 (7.7%) tested positive for *N. gonorrhoeae*. A total of 995 different anatomical sites of infection culture was performed in 583 of 995 (58.6%) of anatomical sites (Drassanes Exprés 278 of 347, 80.1%; standard screening 305 of 648, 47.1%;  $p < 0.001$ ). Recovery was highest when culture specimens were collected within 3–7 days of screening with only a slight drop in recovery when the interval extended to 7 days. Recovery from pharynx was 38 of 149 (25.5%) within 3 days, 19 of 81 (23.4%) after 4–7 days ( $p = 0.7245$ ), 11 of 102 (10.7%) after 8–14 days ( $p < 0.0036$ ) and 1 of 22 (4.5%) with longer delays ( $p = 0.00287$ ). Recovery from rectum was 49 of 75 (65.3%) within 3 days, 28 of 45 (62.2%) after 4–7 days ( $p = 0.7318$ ), 41 of 69 (59.4%) after 8–14 days ( $p = 0.4651$ ) and 6 of 18 (33.3%) with longer delays ( $p = 0.0131$ ). Median culture specimen collection time was 1 day within Drassanes Exprés vs 8 days within standard screening. Consequently, the overall culture yield was slightly higher within Drassanes Exprés (102/278, 36.6% vs 99/305, 32.5%;  $p = 0.2934$ ).

**Conclusion** Reducing the interval between screening and collection of culture specimens increased *N. gonorrhoeae* recovery in extragenital samples. Implementing a same-day testing and notification programme increased collection of culture samples and culture yield in our setting, which may help AMR surveillance.

## INTRODUCTION

*Neisseria gonorrhoeae* has progressively developed antimicrobial to different antimicrobials, hindering

## WHAT IS ALREADY KNOWN ON THIS TOPIC

⇒ Culture for *Neisseria gonorrhoeae* in asymptomatic, nucleic acid amplification test (NAAT)-positive extragenital infections usually has a low yield and is not frequently performed.

## WHAT THIS STUDY ADDS

⇒ Decreasing time between the screening specimen and the culture specimen increases culture recovery of *N. gonorrhoeae* in NAAT-positive asymptomatic extragenital infections.

## HOW THIS STUDY MIGHT AFFECT RESEARCH, PRACTICE OR POLICY

⇒ Same-day result notification programmes may help increase the prompt collection of culture specimens and culture yield.

treatment.<sup>1 2</sup> Global emergence of multidrug-resistant (MDR-NG) strains, especially against extended-spectrum cephalosporins (ESCs), highlights the need for vigilant surveillance against MDR-NG.<sup>2</sup>

At present, nucleic acid amplification test (NAAT) is the first-line method for detection of *N. gonorrhoeae* due to high sensitivity and specificity, rapid turnaround time, ease of specimen transport and greater automation compared with culture.<sup>3</sup> Currently, there are commercial resistance assays for ciprofloxacin<sup>4</sup> and azithromycin<sup>5</sup> but not for ESCs, which have more complex and heterogeneous mechanisms of resistance.<sup>6</sup>

Culture remains essential for antimicrobial susceptibility testing (AST). Most symptomatic infections (80–95%) undergo AST. In contrast, AST is only performed in 40–50% of asymptomatic infections, dropping to 20–30% for asymptomatic extragenital infections.<sup>7–9</sup> Additionally, growth recovery rates of *N. gonorrhoeae* from extragenital samples range from 10% to 45%.<sup>7 10 11</sup> Obtaining culture only from genital samples collected from symptomatic patients could lead to incomplete antimicrobial surveillance data.

Data about factors that impact on *N. gonorrhoeae* growth recovery in culture rate are scarce

and come, mainly, from retrospective studies with heterogeneous populations and management and laboratory protocols. Presence of symptoms, genital infection and shortening time between NAAT screening and culture collection specimens seem to boost recovery rate.<sup>7 12</sup>

Here, we evaluate the impact of the interval between collection of the initial screening sample and subsequent collection of culture specimens on the growth recovery of *N. gonorrhoeae* among asymptomatic individuals who underwent screening of pharynx, etc. We also assessed the impact of any delay between culture sample collection and culture inoculation.

## METHODS

### Study population

All individuals over 18 years old diagnosed with asymptomatic gonorrhoea at the Drassanes Vall d'Hebron Centre for International Health and Transmissible Diseases (Barcelona, Spain) in 2019 were included in this study. In this centre, two asymptomatic gonorrhoea screening programmes are available: the Drassanes Exprés Programme<sup>13</sup> and a standard screening programme. The Drassanes Exprés Programme offers walk-in attendance, on-site testing and same-day results to asymptomatic individuals at risk of sexually transmitted infections (STIs); we previously reported a median time of 2.4 hours from screening to result notification.<sup>13</sup> Standard screening is mainly for individuals receiving HIV pre-exposure prophylaxis (PrEP) and sexual contacts of individuals diagnosed with an STI; sampling takes place locally and NAAT is performed at the reference laboratory located in the Microbiology and Parasitology Department of Hospital Universitari Vall d'Hebron with a turnaround time for results of 4 days. In both programmes, specimens for NAAT are self-collected and individuals complete an anonymous epidemiological questionnaire including: age, sex and sex of sex partners.

### Laboratory screening tests

Screening samples comprised first-void urine and rectal swabs, pharyngeal and/or vaginal swabs depending on self-reported sexual practices. No urethral swab was collected. At the Drassanes Exprés Short Turn Around Testing Laboratory, *N. gonorrhoeae* detection was performed by Xpert CT/NG (Cepheid, California, USA) with the GeneXpert Infinity-48s instrument. The test is approved by the Food and Drug Administration (FDA) for both genital and extragenital specimens. The test has sequence-specific probes that correspond to two different *N. gonorrhoeae* targets, thus conforming with guidelines from the British Association for Sexual Health and HIV (BASHH) and International Union against Sexually Transmitted Infections (IUSTI) on *N. gonorrhoeae* testing in low-prevalence settings.<sup>14 15</sup>

For specimens processed through the standard screening programme, detection at the reference laboratory was performed with Allplex STI-7 Essential Assay (Seegene, Seoul, Korea) using Seegene STARlet automated Extraction and PCR Setup System and CFX96 Touch Real-Time PCR Detection System (BioRad, California, USA). *N. gonorrhoeae* positive results in oropharyngeal specimens were confirmed with Xpert CT/NG. Specimens with cycle thresholds (Ct) superior to 35 were always retested.

### Reporting of results and follow-up

Within the Drassanes Exprés Programme, STI detection was notified via short message service (SMS)<sup>13</sup> with a recommendation to attend the STI Unit Vall d'Hebron-Drassanes in order to establish appropriate treatment. Within the standard screening programme, a follow-up appointment was made for 7 days

after screening; in case results were not available, the follow-up appointment was rescheduled. At the STI Unit, all individuals who had tested positive for *N. gonorrhoeae*, independently of the screening workflow, were treated with single-dose ceftriaxone 1g intramuscularly. Prior to treatment, the clinician collected culture specimens from the site of the identified infection (pharynx, rectum, vagina and/or urethra).

### Culture

Urethral, rectal, vaginal and/or pharyngeal specimens (DeltaSwab, flocked swab in 1 mL of liquid Amies medium, Deltalab, Spain) were stored at room temperature during transport. Once at the reference laboratory (Microbiology Department, Hospital Universitari Vall d'Hebron), specimens were plated onto selective chocolate agar PolyViteX-VCAT3 (bioMérieux, France), a commercial assay based on modified Thayer Martin media<sup>16</sup> and incubated at 35–37°C in a 5% CO<sub>2</sub> atmosphere for 24–48 hours. *N. gonorrhoeae* strains were identified by oxidase reaction (oxidase-positive) and mass spectrometry (Vitek-MS system, bioMérieux).

### Data analysis

We described the study population calculating frequencies and percentages for dichotomous and categorical variables and the median for continuous variables. Time of initial *N. gonorrhoeae* screening by NAAT, specimen collection for *N. gonorrhoeae* culture and inoculation in culture were systematically registered in the laboratory information system. The culture specimen collection time for each patient was defined as the number of days between the initial screening visit and the follow-up visit when the culture specimen was collected. The inoculation time for each sample was defined as the number of hours from culture sample collection to inoculation and streaking on culture media. We calculated 95% CIs by normal or Wilson method as appropriate. Medians (including median times) and proportions were compared by the Mann-Whitney U test and X<sup>2</sup> test, as appropriate. All statistical analyses were performed using Stata/IC V.16.1 (Stata Corp).

## RESULTS

### Demographic characteristics and sexual behaviour of the study population

Between 1 January 2019 and 31 December 2019, 10 423 asymptomatic individuals (18–75 years) were screened for *N. gonorrhoeae*: 49.7% (n=5185) within Drassanes Exprés and 50.2% (n=5238) within the standard programme. Within standard screening, most individuals (4586 of 5238, 87.5%) were men who have sex with men (MSM), followed by cisgender women (401 of 5238, 7.6%), transgender women (162 of 5238, 3.1%) and men who have sex with women (MSW, 89 of 5238, 1.7%). Within Drassanes Exprés, 4168 of 5185 (80.4%) were MSM, 927 of 5185 (17.9%) cisgender women, 60 of 5185 (1.2%) transgender women and 30 of 5185 (0.6%) MSW. Prevalence of *N. gonorrhoeae* infection was higher within the standard programme (529 of 5238, 10.1% (95% CI 9.2% to 10.9%)) than within Drassanes Exprés (280 of 5185, 5.4% (95% CI 4.8% to 6.0%)) (p<0.0001). Among participants with positive *N. gonorrhoeae* NAAT results, most (Drassanes Exprés 230 of 280, 80.4%; standard programme 493 of 529, 93.2%) were MSM. Age distribution and self-reported sexual orientation of individuals who tested positive for *N. gonorrhoeae* in each programme are shown in table 1.

**Table 1** Age distribution and self-reported sexual orientation of asymptomatic participants who tested positive for *Neisseria gonorrhoeae* in the Drassanes Exprés and standard screening programmes

Age (years)	Drassanes Exprés									
	Standard screening					Drassanes Exprés				
	MSM	Cisgender women	Transgender women	MSW	Total	MSM	Cisgender women	Transgender women	MSW	Total
	n=493	n=25	n=9	n=2	n=529	n=230	n=44	n=4	n=2	n=280
18–25 years	107 (21.7%) (18.1%–25.6%)	7 (28.0%) (14.3%–47.6%)	4 (44.4%) NA	2 (22.6%) NA	120 (22.7%) (19.3%–26.4%)	42 (18.3) (13.5%–23.9%)	11 (20.7%) (13.2%–40.3%)	0 (0%) NA	0 (0%) NA	53 (18.9%) (14.7%–23.9%)
25–45 years	367 (74.4%) (70.3%–78.2%)	18 (72.0%) (52.4%–85.7%)	5 (55.5%) NA	0 (0%) NA	390 (73.8%) (69.8%–77.3%)	163 (70.9%) (64.5%–76.6%)	30 (68.2%) (52.4%–81.4%)	3 (75.0%) NA	1 (50.0%) NA	197 (70.4%) (64.8%–75.4%)
45–75 years	55 (11.1%) (8.5%–14.3%)	0 (0%) NA	0 (0.0%) NA	0 (0%) NA	55 (10.4%) (8.1%–13.2%)	25 (10.9%) (7.1%–15.6%)	3 (6.8%) (2.3%–18.2%)	1 (25.0%) NA	1 (50.0%) NA	30 (10.7%) (7.6%–14.8%)

MSM, men who have sex with men; MSW, men who have sex with women; NA, not applicable.

***N. gonorrhoeae* infections by anatomical site**

The distribution of *N. gonorrhoeae* infection by anatomical sites within each screening programme is shown in figure 1. Pharyngeal infections were more frequent within Drassanes Exprés (201 of 347, 57.9%; 95% CI 52.5% to 63.2%) than within the standard programme (329 of 648, 50.7%; 95% CI 46.8% to 54.7%) ( $p < 0.01$ ). In the latter, rectal infections were more frequent (277 of 648, 42.8%; 95% CI 39.0% to 46.6%) than within Drassanes Exprés (118 of 347, 34.0%; 95% CI 29.0% to 38.9%) ( $p = 0.01$ ). In Drassanes Exprés, there were 28 of 347 (8.0%, 95% CI 5.1% to 10.8%) of genital infections (vaginal 4.9%, 17 of 347; urethral 3.1%, 11 of 347) and in standard screening, 42 of 648 (6.5%, 95% CI 4.6% to 8.3%) infections were genital (vaginal 2.2%, 14 of 648; urethral 4.3%, 28 of 648). No differences were observed in the proportion of genital infections ( $p = 0.3115$ ) (figure 1).

**Impact of time of culture specimen collection on the recovery of *N. gonorrhoeae***

Within the standard programme, culture was performed in 305 of 648 (47.1%) NAAT-positive infections, comprising 173 of 329 (52.6%) of pharynx infections, 127 of 277 (45.8%) of rectal infections and 5 of 42 (11.9%) of genital infections (vaginal 14.2%, 2 of 14; urethral 10.7%, 3 of 28). Culture was more frequently performed within Drassanes Exprés: 278 of 347 (80.1%) of NAAT-positive participants, comprising 181 of 201 (90.0%) of pharynx infections, 80 of 118 (67.8%) of rectal infections and 18 of 28 (64.2%) of genital infections (vaginal 82.3%, 14 of 17; urethral 36.3%, 4 of 11) ( $p < 0.001$  for all comparisons).

Culture recovery was highest for all anatomical sites when specimens were collected within 3 days of the initial screening, with only a slight drop in recovery when the interval extended to 7 days (table 2).

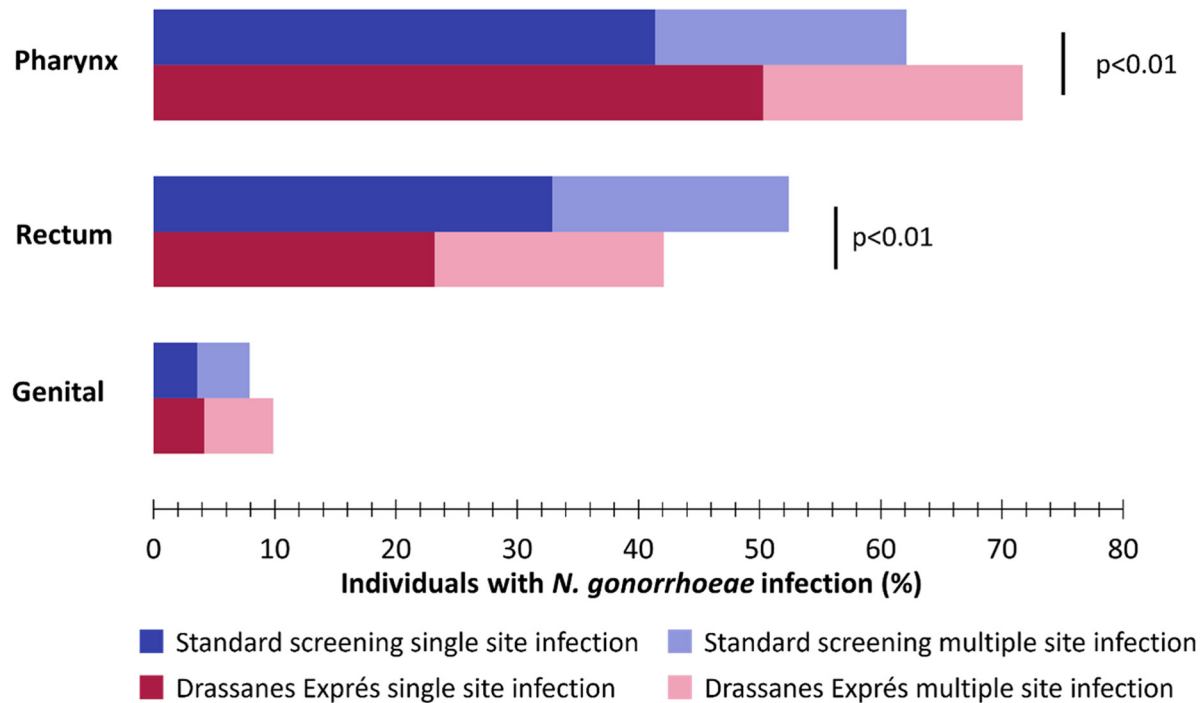
The median time from the screening visit to the collection of the culture specimen was 8 days (range: 7–11) within standard screening, and most cultures (164 of 305, 53.7%) were performed 8–14 days after the screening visit. Within Drassanes Exprés, the median time was 1 day (range: 0–3); most cultures (227 of 278, 81.7%) were performed within 3 days. Table 3 shows growth recovery rates by anatomical site and screening programme.

**Influence of time of inoculation into culture on recovery rate**

Within the standard programme, the median interval between collection of culture specimens and inoculation was 8 hours (IQR 6–15), and no difference was observed between negative (8 hours (IQR 6–14)) and positive (8 hours (IQR 5–16)) cultures ( $p = 0.7670$ ). Likewise, within Drassanes Exprés, the median interval was 6 hours (IQR 5–8) and similar in positive cultures (6 hours (IQR 5–7)) and negative cultures (5 hours (IQR 5–9)) ( $p = 0.534$ ). No difference was observed between the screening programmes ( $p = 0.787$ ).

**DISCUSSION**

Pursuing culture in asymptomatic *N. gonorrhoeae* infections is not widespread, at around 40% of total infections, and when performed, culture recovery is low.<sup>7</sup> We detected a high proportion of asymptomatic extragenital *N. gonorrhoeae* infections in our study, which makes it worthwhile to continue *N. gonorrhoeae* culture with the aim of also informing antimicrobial resistance (AMR) surveillance.<sup>1 17</sup> This study confirms our limited ability to culture *N. gonorrhoeae* in asymptomatic extragenital



**Figure 1** Distribution of *Neisseria gonorrhoeae* infection by anatomical site and screening programme. Percentages refer to number of patients in each programme.

infections. At the same time, we demonstrate that reducing the interval between the initial screening by NAAT and the collection of culture specimens increases both the number of cultures performed and culture recovery.

Our Drassanes Exprés Programme increased the number of cultures performed by 40% compared with standard screening. Shorter intervals between screening and follow-up visit, 1 day in Drassanes Exprés vs 8 days in standard screening, were

**Table 2** *Neisseria gonorrhoeae* culture recovery by time of culture collection and anatomical location

Interval from screening day to day of culture specimen collection	Anatomical site	Number of culture specimens	Culture specimens positive for <i>N. gonorrhoeae</i>			
			Number	%	P value	
≤3 days	Pharynx	149	38	25.5	Ref	
	Rectum	75	49	65.3		
	Vaginal	12	4	33.3		
	Urine	4	3	75		
	Total	240	94	39.2		
4–7 days	Pharynx	81	19	23.4	0.7245	≤3 days vs 4–7 days
	Rectum	45	28	62.2	0.7318	
	Vaginal	2	0	0	ND	
	Urine	2	0	0	ND	
	Total	130	47	36.1	0.5578	
8–14 days	Pharynx	102	11	10.7	0.0036	≤3 days vs 8–14 days
	Rectum	69	41	59.4	0.4651	
	Vaginal	1	0	0	ND	
	Urine	0	0	NA	NA	
	Total	173	52	30.0	0.1933	
≥15 days	Pharynx	22	1	4.5	0.0287	≤3 days vs ≥15 days*
	Rectum	18	6	33.3	0.0131	
	Vaginal	0	0	NA		
	Urine	1	1	100		
	Total	41	8	19.5	0.0154	

Due to the lower number of specimens (n=22), statistical analysis of the effect of time on culture yield was not performed in genital specimens.

\*Longest delay by sample type: pharynx (32 days), rectum (29 days), vaginal (12 days) urine (15 days).

NA, not applicable; ND, not determined.

**Table 3** *Neisseria gonorrhoeae* culture recovery rate by site and screening programme

Site	Screening programme	Total cultures, N	<i>N. gonorrhoeae</i> positive culture, n	<i>N. gonorrhoeae</i> recovery rate, % (95% CI)	P value
Pharynx	DrassExp*	181	43	23.7 (23.5 to 39.6)	0.023
	Standard	173	26	15.0 (10.1 to 21.2)	
Rectum	DrassExp	80	53	66.3 (54.8 to 76.4)	0.0955
	Standard	127	71	55.9 (46.8 to 64.7)	
Genital	DrassExp	17	6	35.3 (17.3 to 58.7)	0.8477
	Standard	5	2	40.0 (11.7 to 76.9)	
Total	DrassExp	278	102	36.6 (30.9 to 42.2)	0.2934
	Standard	305	99	32.5 (27.2 to 37.7)	

\*In DrassExp, culture was conducted on 4 of 11 urine specimens and 13 of 17 vaginal specimens, compared with the standard screening where 3 of 28 urine specimens and 2 of 14 vaginal specimens were cultured.

DrassExp, Drassanes Exprés Programme screening; Standard, standard screening programme.

associated with an increased collection of culture specimens. We did not analyse the reasons why culture was not performed in many cases. Clinicians' preference, history of antimicrobial treatment, absence of symptoms or signs of infection, and the issue of collecting culture specimens from one versus multiple anatomical sites have been previously proposed.<sup>18</sup> Education towards the importance of performing cultures even in asymptomatic patients seems crucial to improve AMR surveillance. Even in our centre, where we adhere to BASHH and IUSTI guidelines, the number of cultures performed was low in the standard programme. We cannot rule out that composition of the population in the standard programme (quarterly STI screening for PreP and sexual contacts screening) could potentially account for the difference in the number of cultures with Drassanes Exprés Programme (walk-in attendance).

In our study, culture recovery rates were highest when culture specimens were collected within 3 days of the initial screening, with only modest reduction when the interval extended to 7 days. Shortening the interval between asymptomatic screening and collection of culture specimens increased recovery rates in previous studies.<sup>7 12 18</sup> Vyth *et al* reported that waiting for more than 7 days increased the likelihood of negative cultures by 2.6.<sup>12</sup> However, in their study, most patients (63%) were symptomatic and it was focused on genital infections; site of infection impacts culture recovery. With extragenital specimens, Nash *et al* demonstrated a 20% reduction in recovery when collection was delayed by 1–7 days compared with same-day collection,<sup>7</sup> aligning with our results. Yet, their data included symptomatic/asymptomatic individuals and different centres, NAAT protocols, collection tubes and policies for culture of *N. gonorrhoeae* infections. Our Drassanes Exprés Programme had a median interval between screening visit and culture specimen collection of 1 day, compared with 8 days in standard screening. This led to higher recovery rates in extragenital specimens, notably the pharynx, where recovery increased from 15.0% within the standard programme to 23.7%. Spontaneous clearance of *N. gonorrhoeae* has been reported to occur over a median of 10 days (IQR 7–15) after an initial NAAT diagnosis and more frequently in pharynx infections where bacterial load is lower<sup>19 20</sup>; this may have increased the difference in growth recovery rates relative to observations made with rectum specimens, with a significant drop in recovery after more than 14 days.

Ideally, same-day culture collection is the best option but currently, in our setting, it seems difficult to achieve; even when the STI same-day test and result notification for Drassanes Exprés was launched, median time to follow-up visit was 1 day. Another option is culturing specimens in all individuals undergoing screening, and performing the culture only when a

positive NAAT result for *N. gonorrhoeae* is obtained; however, many commercial NAATs use specific specimen collection kits inappropriate for bacterial culture.<sup>3</sup> Thus, this strategy would imply taking an additional specimen for culture which would only be used if NAAT is positive, unnecessarily overloading the laboratory and increasing costs.

Despite the impact of the Drassanes Exprés Programme on shortening time to culture specimen collection, culture positivity rates were still low (25.5%) in pharynx infections; similar rates are reported for this anatomical location, ranging from 10% to 30% in other studies.<sup>7</sup> Missing AMR data due to the poor yield of culturing pharynx infections should not be accepted as the pharynx is the most frequent site of asymptomatic *N. gonorrhoeae* infections<sup>7 14 21</sup>; in our study, it was found in 71.9% of individuals within Drassanes Exprés and 62.2% of individuals with standard screening. Additionally, the oropharyngeal saprophytic bacteria are a potential reservoir of AMR genes, which through horizontal gene transfer, could lead to treatment failure.<sup>2</sup> Ceftriaxone failure treatment has been described in oropharyngeal infections in Japan, some European countries and Australia.<sup>2</sup> Therefore, additional strategies are needed to increase culture recovery in pharynx infections. Direct inoculation onto culture media at collection<sup>3</sup> could be effective, although in our setting, the interval between collection and inoculation did not impact culture results. Reflex molecular assays for detecting AMR genes may be a fast and promising tool in pharyngeal infections for resistance-guided therapy. However, they are only reliable for detection of ciprofloxacin resistance and high (minimum inhibitory concentration (MIC): >256 mg/L) and moderate-level (MIC: 4–128 mg/L) azithromycin resistance; therefore, detection of resistance to ESCs should be improved for the purpose of AMR surveillance.<sup>4 6</sup> In the absence of improved resistance detection through NAAT for *N. gonorrhoeae*, culture and susceptibility testing are crucial, in accordance with the guidelines of BASHH and IUSTI, which advocate for the performance of susceptibility testing in all individuals diagnosed via NAAT, including asymptomatic extragenital sites.

This study has limitations. First, we conducted a retrospective study based on our standard practice; thus, it may contain inherent bias. We could not perform a comparison of our approach with simultaneous sampling for molecular testing and culture, as in our centre, the collection tube used for NAAT screening is not suitable for culture. Further research is needed on the use of collection tubes that allow both techniques and preserve the viability of *N. gonorrhoeae* to facilitate same-day culture specimen collection. Second, our findings could only be applied to asymptomatic extragenital infections as the number of asymptomatic genital infections was very low. This prevented us

from obtaining sufficient data on the impact of time on culture recovery in genital infections. Third, although none of the patients received treatment against *N. gonorrhoeae* before culture specimen collection, information about antibiotic consumption for other infections was not systematically registered, and this could affect culture recovery rate. Another limitation is that clinicians performed the specimen collection for culture, which may have impacted culture recovery due to different sampling techniques.<sup>22</sup> We believe that this was not a source of significant bias in our study as the STI Unit has permanent staff who work following the same protocols and guidelines. Finally, AMR data were not included in the findings of this study, given that they did not constitute the primary objective of the analysis. Nevertheless, it is noteworthy that our centre does undertake AMR surveillance.<sup>23 24</sup>

There could be concerns about false-positive results especially when testing extragenital specimens by NAAT. Due to the high prevalence of *N. gonorrhoeae* infection in our population,<sup>13</sup> the positive predictive value of NAAT exceeds 90% and therefore, confirmation is not needed.<sup>14</sup> In the Drassanes Exprés Programme, the test used for screening (Xpert CT/NG) is FDA cleared for extragenital specimens and the test employs two different targets, both of which must be detected to obtain a positive result. In the standard screening programme, positive oropharyngeal specimens by the Allplex STI-7 Essential Assay were confirmed using Xpert CT/NG as the first assay is not cleared for oropharyngeal specimens. Additionally, specimens with Ct superior to 35 were always retested. Therefore, the probability of false-positive results biasing our results was minimised.

The main strength of this study is that it was performed in a setting with a consistent approach at testing, following national and international guidelines.<sup>14 15 25</sup> Only asymptomatic individuals were included and they all attended the same STI centre. All cultures were processed at the same reference laboratory and culture methods did not vary during the study period, which reduced variability.

## CONCLUSION

There is a need to increase the collection of specimens for culture following a positive NAAT for *N. gonorrhoeae*. This will provide valuable data for AMR surveillance. Reducing the interval between initial screening and collection of culture specimens should be an aim to increase *N. gonorrhoeae* culture recovery from extragenital samples. Even if same-day NAAT and culture specimen collection is not available, it is crucial to work towards reducing the interval to less than 7 days to improve *N. gonorrhoeae* culture recovery. Same-day testing and result notification programmes such as Drassanes Exprés increase the prompt collection of culture specimens and boost the success of *N. gonorrhoeae* culture recovery in extragenital specimens.

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## REFERENCES

- Fifer H, Natarajan U, Jones L, *et al.* Failure of dual antimicrobial therapy in treatment of Gonorrhoea. *N Engl J Med* 2016;374:2504–6.
- Unemo M, Shafer WM. Antimicrobial resistance in *Neisseria Gonorrhoeae* in the 21st century: past, evolution, and future. *Clin Microbiol Rev* 2014;27:587–613.
- Meyer T, Buder S. The laboratory diagnosis of *Neisseria Gonorrhoeae*: current testing and future demands. *Pathogens* 2020;9:91.
- Lee DYJ, Ashcroft MM, Chow EPF, *et al.* Reflex detection of ciprofloxacin resistance in *Neisseria Gonorrhoeae* by use of the speedx resistanceplus GC assay. *J Clin Microbiol* 2021;59:e00089–21.
- Hadad R, Cole MJ, Ebeyan S, *et al.* Evaluation of the speedx resistanceplus GC and speedx GC 23S 2611 (beta) molecular assays for prediction of antimicrobial resistance/susceptibility to ciprofloxacin and azithromycin in *Neisseria Gonorrhoeae*. *J Antimicrob Chemother* 2021;76:84–90.
- Allan-Blitz LT, Adamson PC, Klausner JD. Resistance-guided therapy for *Neisseria Gonorrhoeae*. *Clin Infect Dis* 2022;75:1655–60.
- Nash EE, Pham CD, Raphael B, *et al.* Impact of anatomic site, specimen collection timing, and patient symptom status on *Neisseria Gonorrhoeae* culture recovery. *Sexual Trans Dis* 2021;48:S151–6.
- Serra-Pladevall J, Caballero E, Roig G, *et al.* Comparison between conventional culture and Naats for the microbiological diagnosis in Gonococcal infection. *Diagn Microbiol Infect Dis* 2015;83:341–3.
- Davidson KW, Barry MJ, Mangione CM, *et al.* Screening for Chlamydia and Gonorrhoea: US preventive services task force recommendation statement. *JAMA* 2021;326:949–56.
- Ota KV, Fisman DN, Tamari IE, *et al.* Incidence and treatment outcomes of pharyngeal *Neisseria Gonorrhoeae* and Chlamydia Trachomatis infections in men who have sex with men: a 13-year retrospective cohort study. *CLIN INFECT DIS* 2009;48:1237–43.
- Schachter J, Moncada J, Liska S, *et al.* Nucleic acid amplification tests in the diagnosis of Chlamydial and Gonococcal infections of the oropharynx and rectum in men who have sex with men. *Sex Transm Dis* 2008;35:637–42.
- Vyth R, Leval A, Eriksson B, *et al.* Gonorrhoea diagnostic and treatment uncertainties: risk factors for culture negative confirmation after positive nucleic acid amplification tests. *PLoS ONE* 2016;11:e0155017.
- Hoyos-Mallecot Y, García JN, Sulleiro E, *et al.* Drassanes exprés: a public and confidential testing service for asymptomatic STIs with same-day result notification. *Sex Transm Infect* 2022;98:166–72.
- Unemo M, Ross J, Serwin AB, *et al.* European guideline for the diagnosis and treatment of Gonorrhoea in adults. *Int J STD AIDS* 2020;956462420949126.
- Fifer H, Saunders J, Soni S, *et al.* 2018 UK national guideline for the management of infection with *Neisseria Gonorrhoeae*. *Int J STD AIDS* 2020;31:4–15.
- Boiko I, Stepas Y, Krynytska I. Comparison of deferred and bedside culture of *Neisseria Gonorrhoeae*: a study to improve the isolation of Gonococci for antimicrobial susceptibility testing. *Iran J Microbiol* 2020;12:216–22.
- Unemo M, Golparian D, Hestner A. Ceftriaxone treatment failure of Pharyngeal Gonorrhoea verified by international recommendations, Sweden, July 2010. *Euro Surveill* 2011;16:19792.
- Nadal-Baron P, Salmerón P, García JN, *et al.* *Neisseria Gonorrhoeae* culture growth rates from asymptomatic individuals with a positive nucleic acid amplification test. *Lett Appl Microbiol* 2022;75:1215–24.
- Mensforth S, Ayinde OC, Ross J. Spontaneous clearance of genital and extragenital *Neisseria Gonorrhoeae*: data from Gtog. *Sex Transm Infect* 2020;96:556–61.

- 20 van der Veer BMJW, Hoebe CJPA, Dukers-Muijters NHTM, *et al.* Men and women have similar *Neisseria Gonorrhoeae* bacterial loads: a comparison of three anatomical sites. *J Clin Microbiol* 2020;58:e011171–20.
- 21 Harryman L, Scofield S, Macleod J, *et al.* Comparative performance of culture using Swabs transported in amies medium and the Aptima combo 2 nucleic acid amplification test in detection of *Neisseria Gonorrhoeae* from genital and extra-genital sites: a retrospective study: table 1. *Sex Transm Infect* 2012;88:27–31.
- 22 Razali MF, Fairley CK, Hocking J, *et al.* Sampling technique and detection rates for Pharyngeal Gonorrhoea using culture. *Sex Transm Dis* 2010;37:522–4.
- 23 Salmerón P, Viñado B, El Ouazzani R, *et al.* Antimicrobial susceptibility of *Neisseria Gonorrhoeae* in Barcelona during a five-year period, 2013 to 2017. *Euro Surveill* 2020;25:1900576.
- 24 Salmerón P, Viñado B, Arando M, *et al.* *Neisseria Gonorrhoeae* antimicrobial resistance in Spain: a prospective multicentre study. *J Antimicrob Chemother* 2021;76:1523–31.
- 25 Grupo de expertos del grupo de estudio de SIDA de la SEIMC (GESIDA, Secretaria del Plan Nacional sobre el SIDA (SPNS), Grupo de estudio de ITS de la SEIMC (GEITS). Documento de Consenso Sobre Diagnóstico Y Tratamiento de Las Infecciones de Transmisión sexual en Adultos. 2017. Available: <https://gesida-seimc.org/wp-content/uploads/2017/05/gesida-guiasclinicas-ITS-201703.pdf> [Accessed 16 Feb 2023].