

# Persistence of *Neisseria gonorrhoeae* DNA Following Treatment for Pharyngeal and Rectal Gonorrhea Is Influenced by Antibiotic Susceptibility and Reinfection

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(See the Editorial Commentary by Zenilman on pages 564–5.)

**Background.** To guide interpretation of gonorrhea tests of cure using nucleic acid amplification testing, this study examined the persistence of *Neisseria gonorrhoeae* DNA following treatment for pharyngeal and rectal gonorrhea.

**Methods.** Men who had sex with men diagnosed with pharyngeal or rectal gonorrhea underwent swabbing from the pharynx or rectum 7 and 14 days following treatment. Repeat testing for *N. gonorrhoeae* was undertaken using real-time polymerase chain reaction (PCR) assays targeting the *opa* gene and *porA* pseudogene.

**Results.** One hundred pharyngeal and 100 rectal gonorrhea infections in 190 men were included. For pharyngeal gonorrhea, positivity of *N. gonorrhoeae* DNA on both PCR assays was present at days 7 or 14 in 13% (95% confidence interval [CI], 6.4%–19.6%) and 8% (95% CI, 2.7%–13.3%), respectively. For rectal gonorrhea, DNA positivity was present in 6% (95% CI, 1.4%–10.7%) and 8% (95% CI, 2.7%–13.3%), respectively. Among 200 baseline pharyngeal and rectal isolates, there were 10 with ceftriaxone minimum inhibitory concentration (MIC)  $\geq 0.06$  mg/L and azithromycin MIC  $\geq 0.5$  mg/L, of which 3 (30%) had DNA detected at day 14; among the 190 isolates with lower ceftriaxone and azithromycin MICs, only 13 (7%) had persistent DNA (odds ratio, 5.8 [95% CI, 1.3–25.4];  $P = .019$ ). One man initially infected with *N. gonorrhoeae* multiantigen sequence type 2400 had type 4244 infection at day 14, indicating reinfection.

**Conclusions.** Pharyngeal and rectal gonorrhea DNA persisted in 8% of men 14 days after treatment. Persistence was associated with elevated ceftriaxone and azithromycin MICs. Persistence can also reflect reinfection.

**Keywords.** gonorrhea; minimum inhibitory concentration of antibiotics; reinfection; persistent DNA on NAAT testing; test of cure.

Rising gonorrhea prevalence, growing antimicrobial resistance, and increasingly limited antimicrobial options globally have made gonorrhoea a major public health challenge. Gonorrhea causes urethral infections in

men and cervical infections and pelvic inflammatory disease in women. Pharyngeal and rectal infections, which are usually asymptomatic, are a reservoir for further transmission [1–5]. Furthermore, the pharynx is thought to be an important site in the development of gonococcal resistance [6, 7].

Pharyngeal and rectal gonorrhea are common among men who have sex with men (MSM), and rectal gonorrhea enhances human immunodeficiency virus (HIV) transmission [8, 9]. Screening of MSM for pharyngeal and rectal gonorrhea is recommended using nucleic acid amplification testing (NAAT) [10–12]. Culture at

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these extragenital sites is highly specific for gonorrhoea but insensitive compared to NAAT [13].

Currently, a number of guidelines recommend treatment for gonorrhoea using ceftriaxone plus azithromycin [5, 10]. Ceftriaxone is the last fully effective antibiotic against *Neisseria gonorrhoeae*; however, there have been steady increases in the minimum inhibitory concentration (MIC) for extended-spectrum cephalosporins and the appearance of azithromycin-resistant gonorrhoea [14–16]. The recommended dose of ceftriaxone for gonorrhoea in Australia and the United Kingdom is 500 mg [5]; in the United States it is 250 mg [10]. Although there have been no reported cases of genital gonorrhoea failing ceftriaxone, verified cases of pharyngeal gonorrhoea failing ceftriaxone 500 mg have been reported [17–20].

Repeat testing for gonorrhoea following treatment is essential for identifying treatment failures [5, 21]. UK guidelines recommend a gonorrhoea test of cure using NAAT 2 weeks after completion of antibiotic therapy, followed by culture if NAAT positive [5]. The US Centers for Disease Control and Prevention (CDC) recommends that a test of cure be performed only where there is suspected gonorrhoea treatment failure [12], rather than routinely. For pharyngeal and rectal infections, the CDC recommends that tests of cure be undertaken using culture in patients who have received recommended treatment, had a subsequent positive *N. gonorrhoeae* NAAT  $\geq 7$  days after treatment, and who did not engage in sexual activity after treatment [12]. There are few data to inform the appropriate timing for repeat testing for gonorrhoea using NAATs. In 2 studies, *N. gonorrhoeae* DNA from genital infections were negative within 2 weeks [22, 23]. Few data exist to guide interpretation of repeat NAAT following treatment of pharyngeal or rectal gonorrhoea, even though this is increasingly undertaken [24, 25]. The aim of this study was to examine the persistence of *N. gonorrhoeae* DNA following treatment for pharyngeal and rectal gonorrhoea and factors associated with DNA persistence.

## MATERIALS AND METHODS

### Recruitment

This study was undertaken at the Melbourne Sexual Health Centre between April 2012 and March 2013. Men eligible were those who reported sex with a man within 12 months and who were culture positive for gonorrhoea from the pharynx or rectum. MSM attending the clinic were offered screening for pharyngeal and rectal gonorrhoea using pharyngeal and anal swabs. Men were also tested for chlamydia from first-void urine and an anal swab by strand displacement assay, and for syphilis and HIV by serology [11].

Men from whom *N. gonorrhoeae* was isolated from the pharynx or rectum and who had not already been treated were recalled for treatment with ceftriaxone 500 mg by intramuscular

injection combined with oral azithromycin 1 g. Men were asked to abstain from sex until a test of cure was obtained using culture 7 days following treatment.

### Study Procedures

Men who consented to the study underwent additional testing from the infected site on the day they returned for treatment (day 0), prior to treatment being administered. On day 0, men with pharyngeal gonorrhoea had a swab taken from the posterior oropharynx, then a separate swab taken from both tonsils. Swabs obtained from the pharynx were collected carefully with coverage of the posterior oropharynx and both tonsils [26, 27].

The posterior oropharyngeal and tonsillar swabs were plated onto separate plates for gonorrhoea culture, then each swab was placed in separate vials for *N. gonorrhoeae* NAAT. On day 0, men with rectal gonorrhoea had an anal swab taken using a swab inserted 5 cm into the anus.

Men were required to return 7 and 14 days following treatment. Men treated for pharyngeal gonorrhoea had a single pharyngeal swab taken from both tonsils together with the posterior oropharynx on days 7 and 14. Men treated for rectal gonorrhoea had an anal swab taken on days 7 and 14.

### Laboratory Methods for *N. gonorrhoeae*

All study swabs from days 0, 7, and 14 were first plated onto modified Thayer–Martin media for gonorrhoea culture, then placed into a vial with phosphate-buffered solution for storage at  $-80^{\circ}\text{C}$  for NAAT testing. Vials were posted on dry ice to the Royal Children’s Hospital, Brisbane, for *N. gonorrhoeae* NAAT. Inoculated culture plates, including those obtained by clinician testing prior to day 0, were immediately taken to the clinic’s on-site laboratory and incubated at  $36^{\circ}\text{C}$  in 5% carbon dioxide for 48 hours. Presumptive *N. gonorrhoeae* colonies were selected and a smear was prepared using Gram staining. Colonies were oxidase tested and speciation was confirmed by carbohydrate reaction tests.

Antimicrobial susceptibility testing was performed on gonococcal isolates obtained prior to day 0 using agar plate dilution MIC as per the method of the Australian Gonococcal Surveillance Programme (AGSP) [28]. By the AGSP method, the following MICs are categorized: ceftriaxone  $\geq 0.06$  mg/L, decreased susceptible; azithromycin  $>0.5$  mg/L or  $\geq 1$  mg/L, resistant; penicillin  $\geq 1$  mg/L, resistant; and ciprofloxacin  $\geq 1$  mg/L, resistant.

Detection of *N. gonorrhoeae* DNA by NAAT was performed using 2 individual real-time polymerase chain reaction (PCR) methods, 1 targeting the gonococcal *porA* pseudogene and a second NAAT targeting multicopy *opa* genes (*porA*-monoplex and *opa*-monoplex) as previously described [29]. The specificities of the methods have previously been examined using panels of commensal *Neisseria* species and found to be highly specific.

Likewise, the methods have been validated for use on pharyngeal samples [30, 31]. A NAAT result was reported as positive only where both assays were positive. *Neisseria gonorrhoeae* multiantigen sequence type (NG-MAST) testing was performed in selected DNA-positive cases as previously described [32].

### Sampling Adequacy

To assess sampling adequacy, DNA extracts were tested using a real-time PCR assay targeting human endogenous retrovirus 3 (ERV3) [33, 34]. The cycle threshold (Ct) values obtained using the ERV3 real-time PCR were used as a semiquantitative measure of human DNA as previously described [34].

### Organism Load Estimation

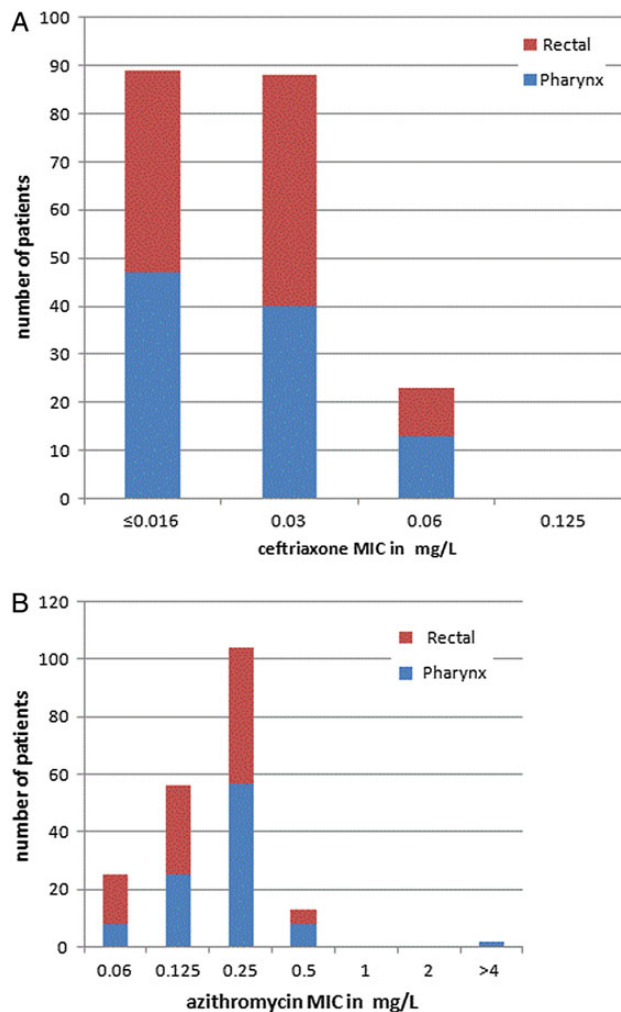
An estimate of the load of *N. gonorrhoeae* in NAAT-positive specimens was obtained using the Ct values gathered using the *N. gonorrhoeae* porA-monoplex and opa-monoplex real-time PCR methods. In a real-time PCR, a Ct value is the cycle of the PCR at which an amplification curve of a positive sample crosses above background fluorescent signal. Ct values are inversely proportional to the bacterial DNA load. For this reason, we are able to use the Ct values obtained from the porA and opa assays as semiquantitative markers of *N. gonorrhoeae* load. We considered a sample to have a high bacterial load if the Ct values were  $\leq 28$  cycles, which was the median of all the load values.

### Assessment for Sexual Reexposure

Men self-completed a questionnaire that focused on sexual behaviors between treatment at day 0 and retesting at days 7 and 14. Potential reexposure to gonorrhea was regarded as any receptive oral sex reported by men treated for pharyngeal gonorrhea or any receptive anal sex reported by men treated for rectal gonorrhea.

### Sample Size and Statistical Analyses

A sample size of 100 pharyngeal and 100 rectal infections was obtained based on 95% confidence intervals (CIs) around an assumed prevalence of 10% at each anatomical site (4.9%–17.6%). The  $\chi^2$  or Fisher exact test was used to compare categorical data and McNemar test for paired proportions using SPSS version 21. Logistic regression was used to calculate odds ratios investigating the associations of factors such as sexual reexposure and isolate MIC with persistence of *N. gonorrhoeae* DNA at day 14. Only unadjusted odds ratios and 95% CIs were calculated because of the small number of cases of persistent DNA at day 14, but all analyses accounted for any intracluster correlation within individuals providing specimens from multiple sites. Analyses were conducted using the logistic regression commands with the cluster option in Stata software, version 12.0. Reimbursement of AUD\$30 was offered to participants. Ethical



**Figure 1.** Distribution of ceftriaxone (A) and azithromycin (B) minimum inhibitory concentration (MIC) values for pharyngeal (n = 100) and rectal (n = 100) *Neisseria gonorrhoeae* isolates.

approval was granted by the Alfred Hospital Research Ethics Committee (453/11).

## RESULTS

### Recruitment and Participation

During the study period, there were 108 cases of pharyngeal gonorrhea and 115 cases of rectal gonorrhea diagnosed among MSM attending the clinic. Of these, 190 men were recruited, with 100 cases of pharyngeal gonorrhea and 100 cases of rectal gonorrhea. Ten men had gonorrhea at both the pharynx and rectum. Men were excluded if they received treatment for gonorrhea on the day of initial clinic presentation either as contacts of gonorrhea (n = 8) or because of symptomatic urethral gonorrhea (n = 10). The remainder were not available for follow-up (n = 5).

**Table 1. Laboratory Results for Men With Persistent *Neisseria gonorrhoeae* DNA 14 Days Following Treatment for Pharyngeal or Rectal Gonorrhea**

Case	MIC at Diagnosis		Day 0 Results			Day 14 Results			Sexual Reexposure <sup>b</sup>
	Ceftriaxone	Azithromycin	porA Assay <sup>a</sup>	opa Assay <sup>a</sup>	NAAT	porA Assay <sup>a</sup>	opa Assay <sup>a</sup>	NAAT	
Pharyngeal									
1	0.06	0.5	29	29	Positive	36	42	Positive	No
2	<0.008	0.125	32	29	Positive	36	42	Positive	No
3	<0.008	0.25	34	34	Positive	36	37	Positive	No
4	0.016	0.25	28	26	Positive	36	42	Positive	No
5	0.06	0.5	26	25	Positive <sup>c</sup>	29	27	Positive <sup>c</sup>	Yes
6	0.03	0.125	28	26	Positive	36	42	Positive	No
7	0.06	0.25	29	26	Positive	38	34	Positive	No
8	0.03	0.25	29	25	Positive	38	38	Positive	Yes
Rectal									
9	<0.008	0.25	30	29	Positive	37	38	Positive	Yes
10	<0.008	0.25	23	22	Positive	36	37	Positive	No
11	0.016	<0.06	24	23	Positive	37	35	Positive	Yes
12	0.03	0.125	29	26	Positive	35	33	Positive	No
13	0.03	0.125	37	36	Positive	38	38	Positive	No
14	0.06	0.5	25	24	Positive	37	40	Positive	No
15	0.03	0.125	20	18	Positive	33	32	Positive	Yes
16	0.03	0.25	26	25	Positive <sup>d</sup>	24	23	Positive <sup>d</sup>	Yes

Abbreviations: MIC, minimum inhibitory concentration; NAAT, nucleic acid amplification testing.

<sup>a</sup> Cycles of  $\leq 28$  considered high load infections; cycles of  $> 28$  considered low load.

<sup>b</sup> Refers to possible reexposure to gonorrhea (ie, receptive oral sex for men for pharyngeal gonorrhea or receptive anal sex for men with rectal gonorrhea any time between treatment and day 14).

<sup>c</sup> *Neisseria gonorrhoeae* multiantigen sequence type (NG-MAST) was 5533 at both day 0 and day 14.

<sup>d</sup> NG-MAST was type 2400 at day 0 and type 4244 at day 14.

There was a median interval of 7 days (range, 1–11 days) between initial clinic presentation and treatment (day 0). The median intervals between the day 0 and the day 7 and day 14 posttreatment visits were 7 days (range, 6–9) and 14 days (range, 13–18), respectively. All pharyngeal and rectal samples among the 190 men from days 0, 7, and 14 were available for analysis.

### Demographic and Behavioral Characteristics

The median age among men was 31 years (range, 19–59); 26 men (13%) were HIV positive. Six percent of men were concurrently infected with rectal chlamydia and 7% with urethral chlamydia.

Among men with pharyngeal gonorrhea, 9% reported engaging in receptive oral sex during the first week following treatment; 22% reported receptive oral sex during the second week. Among men with rectal gonorrhea, 10% reported engaging in receptive anal sex during the first week following treatment; 36% reported receptive anal sex during the second week. Eight men reported sex with a partner with gonorrhea, but none of these men had persistent *N. gonorrhoeae* DNA.

### Laboratory Results

The distributions of ceftriaxone and azithromycin MIC values for the pharyngeal and rectal specimens are shown in Figure 1A and 1B.

All pharyngeal and rectal gonorrhea cultures obtained at days 7 and 14 were negative, except for a single rectal sample that was positive for gonorrhea culture at day 14. Among men treated for pharyngeal gonorrhea, persistent DNA was detected by NAAT at days 7 or 14 in 13% (95% CI, 6.4%–19.6%) and 8% (95% CI, 2.7%–13.3%), respectively ( $P = .24$ ). Among men treated for rectal gonorrhea, persistent DNA was detected by NAAT at days 7 or 14 in 6% (95% CI, 1.4%–10.7%) and 8% (95% CI, 2.7%–13.3%), respectively ( $P = .77$ ). Results for the 16 men who were still positive by NAAT at day 14 at the pharynx or rectum are shown in Table 1.

### Organism Load

The mean load of *N. gonorrhoeae* at the pharynx at day 14 was significantly lower than the load at day 0 for both the porA assay ( $P = .001$ ) and the opa assay ( $P = .001$ ). Similarly, the mean load of *N. gonorrhoeae* in the rectum at day 14 was significantly

**Table 2. Factors Potentially Associated With Persistent *Neisseria gonorrhoeae* DNA 14 Days Following Treatment for Pharyngeal and Rectal Gonorrhoea**

Factor	DNA Negative at Day 14, No. (%)	DNA Positive at Day 14, No. (%)	Unadjusted OR (95% CI) <sup>a</sup>	<i>P</i> Value
<b>Sexual reexposure</b>				
No	128 (70)	10 (63)	1.4 (.5–4.0)	.560
Yes	56 (30)	6 (37)		
<b>Sampling at day 14<sup>b</sup></b>				
ERV >28	79 (43)	9 (56)	1.7 (.6–4.8)	.312
ERV ≤28	105 (57)	7 (44)		
<b><i>N. gonorrhoeae</i> load at day 0<sup>c</sup></b>				
Lower load	122 (66)	9 (56)	0.7 (.2–1.8)	.417
Higher load	62 (34)	7 (44)		
<b>HIV status</b>				
Negative	160 (87)	14 (88)	1.0 (.2–4.5)	.951
Positive	24 (13)	2 (12)		
<b>Ceftriaxone MIC</b>				
<0.06 mg/L	165 (90)	12 (75)	2.9 (.8–10.0)	.094
≥0.06 mg/L	19 (10)	4 (25)		
<b>Ceftriaxone and azithromycin MIC</b>				
<0.06 mg/L and <0.5 mg/L	177 (95)	13 (81)	5.8 (1.3–25.4)	.019
≥0.06 mg/L and ≥0.5 mg/L	7 (5)	3 (19)		

Abbreviations: CI, confidence interval; ERV, endogenous retrovirus 3; HIV, human immunodeficiency virus; MIC, minimum inhibitory concentration; OR, odds ratio.

<sup>a</sup> Accounting for any intracluster correlation within individuals providing specimens from multiple sites.

<sup>b</sup> ERV cycles >28 suggests lower human DNA; ERV cycles ≤28 suggests higher human DNA.

<sup>c</sup> Cycles ≤28 considered higher load infections; cycles >28 considered lower load.

lower than the load at day 0 for both the porA assay ( $P = .014$ ) and the opa assay ( $P = .016$ ; Table 1).

### Factors Associated With DNA Positivity at Day 14

Factors potentially associated with persistence of DNA at day 14 are shown in Table 2. There was no association between sexual reexposure during the 2 weeks following treatment and persistent DNA at day 14 ( $P = .560$ ).

When all 200 pharyngeal and rectal isolates were combined, there were 10 isolates with a ceftriaxone MIC ≥0.06 mg/L together with an azithromycin MIC ≥0.5 mg/L, of which 3 (30%) were associated with persistent DNA detection at day 14. Among the remaining 190 isolates with lower ceftriaxone and azithromycin MICs, significantly fewer ( $n = 13$  [7%]) had persistent DNA detected at day 14; thus, isolates that were less susceptible to ceftriaxone and also resistant to azithromycin

were more likely to be associated with persistent DNA at day 14 (odds ratio, 5.8 [95% CI, 1.3–25.4];  $P = .019$ ).

Two cases with persistent DNA positivity at day 14 are worth noting (Table 1). Case 5 was a man with pharyngeal gonorrhoea who was treated with azithromycin 2 g only, as he had prior anaphylaxis to ceftriaxone. He was the only participant not to receive ceftriaxone and azithromycin. His isolate had an azithromycin MIC of 0.5 mg/L. His NG-MAST types at day 0 and day 14 were identical (5333) with a relatively high bacterial load at day 14. Case 16 was a man with rectal gonorrhoea who was reinfected with a different strain of *N. gonorrhoeae*. He was originally infected with NG-MAST type 2400 and reported receptive anal sex during week 2. The bacterial load at day 14 was relatively high, with NG-MAST type 4244 detected and positive rectal gonorrhoea culture.

## DISCUSSION

Among men treated for pharyngeal and rectal gonorrhoea, 8% had *N. gonorrhoeae* DNA detected 14 days following treatment with ceftriaxone and azithromycin. Infections involving gonorrhoea isolates with elevated MICs to ceftriaxone and azithromycin were more likely to have persistent DNA detected 2 weeks following treatment. We also verified that reinfection with *N. gonorrhoeae* can also account for persistent DNA as early as 14 days following treatment. Together, these results indicate that persistently positive pharyngeal and rectal gonorrhoea NAAT results may be caused by reinfection or reflect reduced antimicrobial susceptibility.

It is uncertain whether the DNA detected 14 days following treatment represented viable organism, that is, continued infection with ongoing transmission potential or successfully treated infection with residual nonviable organism. It is possible that the greater persistence of DNA seen with infections involving higher MICs reflected a slower kill of *N. gonorrhoeae*. Although gonorrhoea culture establishes the viability of *N. gonorrhoeae*, the sensitivity of culture for pharyngeal and rectal gonorrhoea is poor [13, 35, 36]. Culture at these sites may be negative with lower load infections [36], and PCR loads in this study were significantly lower at day 14 compared with loads prior to treatment.

The strengths of this study are that all infections were diagnosed by culture, which has 100% specificity for gonorrhoea, as opposed to NAAT, where false-positive *N. gonorrhoeae* results can occur. Furthermore, culture permitted MIC testing. However, there are a number of study limitations. First, it is possible that the use of culture caused bias toward higher load infections being included [36]. Second, some of the persistent DNA may reflect reinfection due to the high rate of sexual reexposure, and the rate of persistent DNA could be higher where reinfection is more common. Third, the association between DNA



persistence and raised MICs suggests that persistent DNA could be more common in settings with more gonococcal resistance and lower where resistance is less common.

To date, all verified gonococcal treatment failures following treatment with ceftriaxone have been pharyngeal infections [17–20]. The reasons for this are unclear; however, suboptimal antibiotic penetration of the pharynx may contribute [37]. With eventual treatment failures to ceftriaxone at other sites seemingly inevitable, tests of cure following treatment for gonorrhoea need to be considered together with careful surveillance. How tests of cure for extragenital gonorrhoea should be undertaken in the era of NAAT requires further work. NAAT-positive tests of cure may reflect treatment failure, nonviable DNA, or reinfection, and this cannot be distinguished without additional testing. New algorithms that allow tests of cure for extragenital gonorrhoea to be undertaken and interpreted using NAAT should be investigated. In the meantime, the capacity for gonorrhoea culture should be maintained.

## Notes

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