

Title: Is previous azithromycin treatment associated with azithromycin resistance in *Neisseria gonorrhoeae*? A cross-sectional study using national surveillance data in England

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Abstract:

Objectives: It has been suggested that treatment of STIs with azithromycin may facilitate development of azithromycin resistance in *Neisseria gonorrhoeae* (NG) by exposing the organism to sub-optimal doses. We investigated whether treatment history for non-rectal *Chlamydia trachomatis* (CT), non-gonococcal urethritis (NGU) or NG (as proxies for azithromycin exposure) in sexual health (GUM) services was associated with susceptibility of NG to azithromycin.

Methods: Azithromycin susceptibility data from the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP 2013-15, n=4606) and additional high-level azithromycin resistant isolates (HL-AziR) identified by the Public Health England reference laboratory (2013-2016, n=54) were matched to electronic patient records in the national GUMCAD STI surveillance dataset (2012-2016). Descriptive and regression analyses were conducted to examine associations between history of previous CT/NGU/NG and subsequent susceptibility of NG to azithromycin.

Results: Modal azithromycin Minimum Inhibitory Concentration (MIC) was 0.25 mg/L (1 dilution below the resistance breakpoint) in those with and without history of previous CT/NGU/NG (previous 1 month/6 months). There were no differences in MIC distribution by experience of CT/NGU/NG in the previous 6 months (CT/NGU: p=0.98; NG: p=0.81), nor in the odds of having an elevated azithromycin MIC (>0.25 mg/L) (Adjusted Odds Ratio for CT/NGU 0.97 (95% CI 0.76-1.25); AOR for NG 0.82 (95% CI: 0.65-1.04)) compared to those with no CT/NGU/NG in the previous 6 months. Among patients with HL-AziR NG, 3 (4%) were treated for CT/NGU and 2 (3%) for NG in the previous 6 months, compared with 6% and 8% respectively for all GRASP patients.

Conclusions: We found no evidence of an association between previous azithromycin treatment in GUM services and subsequent presentation with an azithromycin-resistant strain. As many CT

diagnoses occur in non-GUM settings, further research is needed to determine whether azithromycin resistant NG is associated with azithromycin exposure in other settings and for other conditions.

Key Messages

1. There was no evidence that previous treatment with azithromycin in GUM services was associated with subsequent presentation with an azithromycin-resistant strain of *Neisseria gonorrhoeae* (NG).
2. Azithromycin treatment for STIs in GUM is unlikely to be a major driver for azithromycin resistance in NG.
3. Further research should explore whether azithromycin exposure in other settings and for other conditions facilitates azithromycin resistance in NG.

INTRODUCTION

Recommended first-line treatment for *Neisseria gonorrhoeae* (NG) in the UK is with 500 mg intramuscularly-injectable ceftriaxone plus 1 g oral azithromycin; this dual therapy approach aims to effectively treat patients while preventing the emergence of resistance to ceftriaxone[1]. Increasing prevalence of azithromycin resistance in NG, including high-level resistance, raises questions about the effectiveness of dual therapy in meeting this aim[2–5]. Given evidence that NG is becoming less susceptible to ceftriaxone over time and that there are few new antimicrobial agents in the pipeline, the prospect of untreatable gonorrhoea has become a major public health concern[5–8].

Azithromycin has a long half-life[9] and repeat infection with bacterial STIs is common[10], therefore it is plausible that treatment of NG and other STIs with azithromycin may facilitate the development of resistance by exposing NG to sub-therapeutic levels. Indeed, recent analysis of data from attendees at a sexual health clinic in Amsterdam found some evidence of a linear association between azithromycin MICs in NG and number of days since previous azithromycin treatment[11]. We investigated whether treatment for chlamydia (CT), non-gonococcal urethritis (NGU), or NG in GUM, as proxies for azithromycin exposure, was associated with subsequent NG azithromycin susceptibility using data from the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP), surveillance of high-level azithromycin-resistant NG, and the GUMCAD STI Surveillance System in England.

We hypothesised mechanisms by which treatment for STIs with azithromycin could lead to decreased azithromycin susceptibility in NG (panel 1). Firstly, treatment for CT may occur while concurrent NG infection goes undetected (hypothesis 1a), or a patient could be infected with NG shortly after receiving treatment for CT or NGU (hypothesis 1b), both of which could exert selection pressure for azithromycin resistance, which may then go undetected due to lack of follow up for test

of cure. Secondly, a patient could be treated for NG but reinfected while sub-therapeutic levels of azithromycin are present (hypothesis 2a), or unsuccessfully treated for NG with azithromycin monotherapy (hypothesis 2b), again exerting selection pressure for resistance.

{Panel 1 submitted as a separate high resolution image}

METHODS

Data sources

A detailed description of the GRASP methodology has been published elsewhere[2]. Briefly, isolates from individuals with gonorrhoea attending 25 sentinel specialist sexual health clinics in England during a three month period each summer 2013-15 were cultured and submitted to Public Health England (PHE) for susceptibility testing. An agar dilution method was used to determine minimum inhibitory concentrations (MICs) for azithromycin. All patients diagnosed with gonorrhoea during this period were eligible for inclusion, however approximately half of episodes do not have susceptibility data due to culture not being attempted or successful[12]. Deterministic matching was used to link susceptibility data to national electronic STI surveillance data (GUMCAD) on previous attendances at that clinic (2012-2015)[13], using patients' clinic-specific ID numbers.

The GRASP 2013-15 data included 8 high-level azithromycin resistant (HL-aziR) isolates. An additional 54 HL-aziR isolates referred to the National Reference Laboratory at PHE between 2013 and September 2016 were matched to GUMCAD (2012-2016) and included in analyses of HL-aziR isolates.

DST Medium

The Diagnostic Sensitivity Test (DST) medium used for susceptibility testing for GRASP isolates was changed in 2015, whereupon MICs for azithromycin increased[2]. An internal validation study comparing the new and old media indicated that the old medium underestimated azithromycin MICs in recent years by approximately one dilution. Azithromycin MIC data for 2013 and 2014 (the years in which the problems with growth on the old medium were seen) were therefore adjusted upwards by a factor of one dilution to enable more accurate description of trends over time.

Definition of exposures and outcomes

To identify any differences in susceptibility, not just those resulting in clinically-relevant resistance, the outcomes of interest were modal and mean azithromycin MIC and a binary variable indicating elevated azithromycin MIC (>0.25 mg/L). Exposure variables were attendance with CT, NGU, or NG in the previous 6 months (binary variable), time since previous attendance with CT, NGU, or NG (categorical variable: <1 month, 1-<6 months, ≥6 months/never), and days since CT, NGU, or NG diagnosis (continuous variable). This range of exposures was chosen as, according to our hypotheses, we might expect associations with very recent treatment with azithromycin, yet it is also plausible that an asymptomatic azithromycin-resistant infection could remain undetected for some time. Although NG is likely to spontaneously resolve within weeks or months, the maximum duration of infection is unclear[14]. We have assumed that an infection would not remain undetected for more than 6 months, and therefore grouped infections diagnosed >6 months previously with never.

GUMCAD does not capture information on prescribing, therefore diagnosis with CT, NGU, or NG was used as a proxy for azithromycin treatment. In all analyses, previous rectal CT was excluded as treatment guidelines recommend treatment with doxycycline rather than azithromycin, and this is

thought to reflect clinical practice for some time before the change to treatment guidelines in 2015[15]. Those attending as partners of chlamydia contacts were assumed to have been given epidemiological treatment with azithromycin and were therefore included as 'previous chlamydia'. Previous NGU was combined with previous CT, given the small numbers with NGU and similar hypothesised mechanisms. As NGU is not always treated with azithromycin[16], sensitivity analyses were also performed using previous CT only. Previous CT/NGU were analysed separately to NG due to the different hypothesised pathways by which resistance could arise. All hypotheses rely on NG being undetected and untreated between the previous CT/NGU/NG attendance and the GRASP NG episode, therefore we used GUMCAD records to exclude any previous attendances which had been followed by a negative NG test more than 20 days after the most recent CT/NGU/NG episode. Tests of cure within 20 days were not counted as hypotheses 1b) and 2b) propose reinfection shortly after treatment as a mechanism for the development of resistance. The half-life of azithromycin is 1-4 days in tissues, therefore azithromycin would be expected to have cleared from tissues in under 20 days following treatment[9].

Descriptive and statistical analyses

Descriptive analyses of MIC distribution in NG (histograms) by history of previous attendance with CT/NGU (hypotheses 1a and 1b) and NG (hypotheses 2a and 2b) were performed for all GRASP isolates. Due to the dispersed nature of MIC data, negative binomial regression was used to assess differences in azithromycin MIC distribution by history of CT/NG, with resulting Incidence Rate Ratios (IRRs) indicating the relative difference in predicted (mean) MIC compared with the reference category (previous episode >6 months ago or no previous episodes). Multivariable analyses were adjusted for year, age, and gender/sexual orientation (groupings: MSM, heterosexual men, all women). Crude and multivariable logistic regression were used to determine associations between previous attendances and elevated azithromycin MIC (>0.25 mg/L). Linear regression of azithromycin MIC (transformed on the natural logarithm scale due to over-dispersion and skewed distribution) by

days since previous CT/NGU or NG was performed to assess evidence of a time-dependent association with previous azithromycin treatment (previous month). Clustering of azithromycin resistance within clinics was accounted for in all regression analyses using generalised estimating equations under the assumption of an exchangeable correlation matrix[17]. Data for the 62 HL-AziR isolates were analysed descriptively due to the lack of a comparison group. Analyses were carried out using Stata version 13 (Stata Statistical Software: Release 13. College Station, Texas, USA: StataCorp, 2013).

Ethics and governance

In its role providing infectious disease surveillance Public Health England has permission to handle data obtained by GRASP, GUMCAD, and the reference laboratory under Regulation 3 of the Health Service (Control of Patient Information) Regulations 2002.

RESULTS

Among the 4606 GRASP isolates, 6% (n=263) had attended that clinic in the previous 6 months with CT, 1% (n=49) with NGU, and 8% (n=369) with NG (appendix table 1). Modal azithromycin MIC was 0.25 mg/L (one dilution below the resistance breakpoint). This did not vary by treatment for CT/NGU or NG in the previous 6 months (appendix figure 1). Similarly, there was no evidence of a difference in predicted azithromycin MICs by experience of previous CT/NGU or NG in the previous 6 months (p-values 0.97 and 0.71 respectively, data not shown), and no differences by recency of previous CT/NGU or NG episode (p-values 0.98 and 0.85 respectively) in the negative binomial regression model adjusted for year, age, and gender/sexual orientation (table 1).

According to the logistic regression model, there was no evidence of an association between elevated azithromycin MIC (>0.25 mg/L) and treatment for CT/NGU (Adjusted Odds Ratio 0.97 [95%

Confidence Interval 0.76-1.25]) or NG (AOR 0.82 [95% CI: 0.65-1.04]) in the previous 6 months (Table 2). A sensitivity analysis replicating this logistic regression but using the outcome of azithromycin resistance (MIC >0.5 mg/L) also found no evidence of an association with previous CT/NG ($p=0.49$) or NG ($p=0.81$). Furthermore, linear regression of the natural logarithm (ln) of azithromycin MIC on days since previous CT/NGU or NG found no evidence that azithromycin MIC was higher among those who had been most recently treated (figure 1). Sensitivity analyses excluding previous attendances with NGU from the previous CT exposure variables found similar results for all regression analyses (data not shown).

Among the 62 high-level azithromycin-resistant (HL-AziR) NG isolates identified either in GRASP or referred to the national reference laboratory at PHE, 3 (4%) were treated for CT/NGU and 2 (3%) were treated for NG in the previous 6 months (table 3). There was therefore no indication that this was more common in HL-AziR isolates than among all GRASP isolates (appendix table 1).

DISCUSSION

We found no evidence of an association between azithromycin susceptibility in NG and previous treatment for chlamydia, non-gonococcal urethritis, or gonorrhoea in these national GUM clinic surveillance data. These findings suggest that the use of azithromycin to treat STIs in British sexual health clinics is not a major driver for azithromycin resistance in NG. Other potential drivers, such as the use of azithromycin to treat infections in other settings and importation of resistant strains of NG, may therefore warrant investigation. It should also be borne in mind that evidence from whole genome sequencing of the high-level azithromycin resistant NG outbreak indicated that most of these cases were clonal, suggesting that in most cases resistance was sexually acquired rather than

de novo development[18,19]. Thus, in a sentinel surveillance programme such as GRASP it may be difficult to detect the case where the mutation leading to azithromycin resistance first arose.

A strength of this analysis was the ability to combine azithromycin MIC data with complete episode-level information on previous clinic attendances. However, as patient data cannot be linked across different clinics or with other health services, this is likely lead to some underestimation of previous azithromycin exposure for the treatment of STIs. As information about prescribing is not captured in GUMCAD, we made assumptions about treatment of STIs with azithromycin which would not always be correct, however we would expect these to be accurate in the majority of cases. For example audit data from 2015 found 83% of chlamydia cases in specialist sexual health clinics were treated with azithromycin (English National Chlamydia Screening Programme (NCSP) audit team, personal communication, 24th August 2017). It is also possible that our data may underestimate recent treatment of NGU prior to gonorrhoea episodes, in circumstances where a patient is initially treated for NGU which is subsequently confirmed as gonorrhoea by laboratory tests. In these cases, clinics may not retain the original record of NGU in the surveillance dataset thus in some cases leading to under-reporting. Despite these limitations, the lack of any indication of an association found in these data suggests the relative contribution of previous azithromycin treatment for STIs on azithromycin resistance in NG is likely to be small.

Other authors have raised concerns about the impact of widespread use of azithromycin on azithromycin resistance in STIs including NG[5,20,21]. Our findings contrast partly with the only comparable epidemiological study on this topic, which found evidence of a linear association between days since azithromycin treatment and azithromycin MIC, among a sub-analysis of 14 patients treated with azithromycin in the previous 30 days in an STI clinic in Amsterdam[11].

Azithromycin MICs in that study were tested using the e-test method, which generates more MIC categories than agar dilution (as used in our study), thus it is possible that our data on azithromycin

MIC were not fine-grained enough to identify a linear trend. However, even with our less detailed MIC data, we would expect to see some indication of a trend if an association it existed in this setting. Analysis of other time frames in the Amsterdam study did not yield any associations, in line with our findings. Previously, GRASP and the European Gonococcal Antimicrobial Surveillance Programme have found associations between azithromycin resistance and previous NG infection[22,23], however neither study was designed to test this specific hypothesis and lacked detailed information on exposure intervals. An ecological study in the USA found no association between regional outpatient prescribing of specific antibiotics, including azithromycin, and NG susceptibility[24], leading the authors to propose that the importation of resistant strains may play a greater role than domestic prescribing practice, which may also be relevant in the UK context.

We found no evidence from national surveillance data that treatment of STIs with azithromycin in GUM is driving azithromycin resistance in NG. However, as we could not explore prior exposure in general practice or other settings our findings may have limited generalizability to other settings, and only go part way towards answering questions about the impact of widespread azithromycin use, including by the NCSP, on the emergence of resistance in NG. In these settings patients are less likely to be tested for gonorrhoea, making our hypothesis of undiagnosed gonorrhoea being exposed to azithromycin (without appropriate follow-up/test of cure) more likely. However, a 2013 survey of NCSP commissioners found over half of commissioners were using dual CT/NG testing[25], and this figure is likely to have increased since then. Further data on the extent to which testing and management of NG diagnosed outside specialist sexual health clinics is carried out in line with national guidelines[1,26] would provide better understanding of the generalizability of these results to other settings. Although overall data on GP azithromycin prescribing exists via the Open Prescribing tool[27], a more detailed breakdown by age and other risk factors for NG would shed light on the extent to which the group at risk of NG are exposed to azithromycin more generally outside GUM. Despite the limitations of the available data, the findings presented here call into

question assumptions that azithromycin use for the treatment of STIs is a major factor in the development of resistance, although further research into the mechanisms of emergence of azithromycin resistance in NG including exposure to azithromycin in other settings and for other conditions, is needed.

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Competing interests:

HF is on the Scientific Advisory Board for Discuva Ltd. The other authors have no competing interests to declare.

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Table and figures

Table 1: Association between previous treatment for chlamydia/NGU or gonorrhoea and subsequent azithromycin MIC in *Neisseria gonorrhoeae* (negative binomial regression) (GRASP 2013-15, England)

		N	Modal MIC (mg/L)	crude IRR	95% CI	p-value	adjusted IRR	95% CI	p-value
Previous attendance for NGU or chlamydia^{a,b}	never/>6 months ago	4299	0.25	1.00	-	0.98	1.00	-	0.98
	1-<6 months	269	0.25	1.01	(0.88-1.16)		1.00	(0.87-1.15)	
	<1 month	38	0.25	1.01	(0.71-1.43)		1.03	(0.72-1.49)	
Previous attendance for gonorrhoea^a	never/>6 months ago	4237	0.25	1.00	-	0.81	1.00	-	0.85
	1-<6 months	322	0.25	1.00	(0.88-1.14)		0.99	(0.87-1.12)	
	<1 month	47	0.06	0.90	(0.66-1.24)		0.91	(0.65-1.28)	

^a Only previous episodes at that clinic are known; excludes those with a subsequent negative gonorrhoea test

^b Excludes rectal chlamydia. Includes epidemiological treatment for chlamydia.

NGU=Non-gonococcal urethritis

IRR=incidence rate ratio for increasing MIC dilutions calculated using negative binomial regression. Adjusted IRR adjusted for year, age, gender/sexual orientation. Clinic-level clustering accounted for using Generalised Estimating Equations.

Azithromycin data for 2013-14 adjusted to account for problem with the DST medium (see methods for further details)

Table 2: Association between treatment for CT/NGU or NG in the previous 6 months and elevated azithromycin MIC (>0.25 mg/L) (GRASP 2013-15, England)

		N	%	crude OR	95% CI	p-value	adjusted OR	95% CI	p-value
Previous attendance for NGU or chlamydia^{a,b}	never/>6 months ago	4299	32.5	1.00	-	0.91	1.00	-	0.81
	<6 months	307	33.9	1.01	(0.79-1.30)		0.97	(0.76-1.25)	
Previous attendance for gonorrhoea^a	never/>6 months ago	4237	32.7	1.00	-	0.26	1.00	-	0.10
	<6 months	369	31.2	0.87	(0.69-1.11)		0.82	(0.65-1.04)	

^a Only previous episodes at that clinic are known; excludes those with a subsequent negative gonorrhoea test

^b Excludes rectal chlamydia. Includes epidemiological treatment for chlamydia.

OR=Odds Ratio calculated using logistic regression. Adjusted OR adjusted for year, age, gender/sexual orientation. Clinic-level clustering accounted for using Generalised Estimating Equations.

Azithromycin data for 2013-14 adjusted to account for problem with the DST medium (see methods for further details)

Figure 1: Linear regression of ln(azithromycin MIC) (GRASP 2013-15, England) by days since previous:

- a) chlamydia or non-gonococcal urethritis^{a,b}
- b) gonorrhoea^a

{Figure 1 submitted separately as a high resolution image}

Figure 1 footnotes:

^a Excludes rectal CT. Includes epidemiological treatment for chlamydia.

^b Only previous episodes at that clinic are known; excludes cases with subsequent negative NG test >20 days after previous CT episode

Linear regression p-values:

- a) CT/NGU: i) <1 month p=0.71, ii) 1-3 months p= 0.72, iii) 3-6 months p=0.04
- b) NG: i) <1 month p=0.77, ii) 1-3 months p=0.09, iii) 3-6 months p=0.74

Table 3: descriptive analysis of previous attendances for CT/NGU or NG among n=XX high-level azithromycin-resistant *Neisseria gonorrhoeae* isolates in England (n=8 from GRASP 2013-2015, n=54 cases referred to the National Reference Laboratory at Public Health England 2013-16)

		n	%
Previous attendance for NGU or chlamydia^{a,b}	never/ >6 months ago	59	88%
	1 - <6 months	3	4%
	<1 month	0	0%
Previous attendance for gonorrhoea^a	never/ >6 months ago	60	90%
	1 - <6 months	2	3%
	<1 month	0	0%

^a Only previous episodes at that clinic are known; excludes those with a subsequent negative gonorrhoea test

^b Excludes rectal chlamydia. Includes epidemiological treatment for chlamydia.

n=54 Cases HL-AziR cases identified via the Bacterial Reference Laboratory, n=8 identified via GRASP