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# Gentamicin, azithromycin and ceftriaxone in the treatment of gonorrhoea: the relationship between antibiotic MIC and clinical outcome

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**Objectives:** To investigate the relationship between MIC and clinical outcome in a randomized controlled trial that compared gentamicin 240 mg plus azithromycin 1 g with ceftriaxone 500 mg plus azithromycin 1 g. MIC analysis was performed on *Neisseria gonorrhoeae* isolates from all participants who were culture positive before they received treatment.

**Methods:** Viable gonococcal cultures were available from 279 participants, of whom 145 received ceftriaxone/azithromycin and 134 received gentamicin/azithromycin. Four participants (6 isolates) and 14 participants (17 isolates) did not clear infection in the ceftriaxone/azithromycin and gentamicin/azithromycin arms, respectively. MICs were determined by Etest on GC agar base with 1% Vitox. The geometric mean MICs of azithromycin, ceftriaxone and gentamicin were compared using logistic and linear regression according to treatment received and *N. gonorrhoeae* clearance.

**Results:** As the azithromycin MIC increased, gentamicin/azithromycin treatment was less effective than ceftriaxone/azithromycin at clearing *N. gonorrhoeae*. There was a higher geometric mean MIC of azithromycin for isolates from participants who had received gentamicin/azithromycin and did not clear infection compared with those who did clear infection [ratio 1.95 (95% CI 1.28–2.97)], but the use of categorical MIC breakpoints did not accurately predict the treatment response. The geometric mean MIC of azithromycin was higher in isolates from the pharynx compared with genital isolates.

**Conclusions:** We found that categorical resistance to azithromycin or ceftriaxone *in vitro*, and higher gentamicin MICs in the absence of breakpoints, were poorly predictive of treatment failure.

## Introduction

Controlling gonorrhoea is challenging owing to the high number of cases,[1–3] patient compliance in avoiding unprotected sex while being treated[4,5] and the ability of *Neisseria gonorrhoeae* to continually develop antimicrobial resistance.[6] To preserve the last remaining clinically demonstrated monotherapy option, ceftriaxone, a dual-therapy regimen of ceftriaxone and azithromycin has been established. Intramuscular ceftriaxone 500 mg plus oral azithromycin 1 g was recommended in the UK until January 2019,[7] with similar dual-therapy regimens currently recommended in other countries.[8] Dual therapy was introduced primarily owing to increasing resistance to orally administered cefixime, especially in MSM. One of the main reasons to include azithromycin was to provide cover in the event of ceftriaxone failing, thereby delaying the emergence of resistance to ceftriaxone. Other reasons stated in the 2011 UK gonorrhoea management guidelines[7] included cover for chlamydia treatment, limited evidence of synergy between ceftriaxone and azithromycin and improved clearance of pharyngeal gonorrhoea when azithromycin is combined with a cephalosporin. Increasing azithromycin resistance and decreasing ceftriaxone susceptibility is being observed globally,[9,10] along with documented sustained transmission of isolates with very high-level azithromycin resistance (MIC $\geq$256 mg/L).[11] These concerning antimicrobial susceptibility profiles, along with verified

treatment failures using both ceftriaxone and azithromycin,[12,13] are clear evidence that trials to establish the effectiveness of alternative antibiotic regimens are needed.[9]

A recent randomized controlled trial in the UK, 'Gentamicin in the Treatment of Gonorrhoea (G-ToG)', compared gentamicin 240 mg plus azithromycin 1 g with the current first-line treatment of gonorrhoea.[5] The primary endpoint was clearance of *N. gonorrhoeae* using a nucleic acid amplification test (NAAT) 2 weeks post-treatment. The study revealed that gentamicin, in combination with azithromycin, had a lower cure rate (91%) than ceftriaxone in combination with azithromycin (98%).[5] For those administered gentamicin, clearance was better for those with a genital infection (94%), compared with pharyngeal (80%) and rectal (90%) gonorrhoea. Even though it is not possible to differentiate between the effectiveness of the individual agents in a two-component regimen, it is unlikely that azithromycin consistently provided sufficient microbiological cure when gentamicin failed to treat. These results cast doubt on whether the use of azithromycin in combination with ceftriaxone for the treatment of gonorrhoea is appropriate, particularly for extragenital infections.

Antimicrobial susceptibility testing is used to predict response to treatment, but just one other study has assessed the association between the laboratory measurement of MIC and the microbiological cure of *N. gonorrhoeae* infection when combination therapy was used.[14] Preliminary analysis of the G-ToG trial data did not reveal a clear relationship between gentamicin, ceftriaxone or azithromycin MICs *in vitro* and *N. gonorrhoeae* clearance, and most of the isolates cultured post-treatment were susceptible according to EUCAST breakpoints.[5] To investigate the relationship between MIC and clinical outcome further, MIC analysis was performed on *N. gonorrhoeae* isolates from all G-ToG trial participants who were culture positive before they received treatment, and also on post-treatment isolates from those for whom treatment failed. The aim of this analysis was to identify any relationship between response to treatment and the MICs of azithromycin, ceftriaxone and gentamicin, as well as to determine any differences in the MICs for isolates from patients in the two treatment arms and from different infection sites.

## Materials and methods

Methods for the G-ToG trial are described elsewhere.[5,15] Briefly, a blinded, non-inferiority randomized trial was performed in 14 sexual health clinics in England. Participants who had a diagnosis of uncomplicated gonorrhoea were randomized to receive gentamicin 240 mg or ceftriaxone 500 mg, both in combination with 1 g oral azithromycin. The primary outcome was demonstration of *N. gonorrhoeae* clearance by an NAAT at all infected sites 2 weeks post-treatment. Swabs were also taken for gonococcal culture from patients at the baseline and at a 2 week post-treatment visit.

A total of 720 participants were enrolled to G-ToG and primary outcome data were available for 598 randomized to receive ceftriaxone/azithromycin ($n$=306) or gentamicin/azithromycin ($n$=292). Overall, 333 viable gonococcal cultures were available from 279 participants, of whom 145 received ceftriaxone/azithromycin and 134 received gentamicin/azithromycin. The number of these participants who cleared infection was 261. Four participants (6 isolates) and 14 participants (17 isolates) did not clear infection in the ceftriaxone/azithromycin and gentamicin/azithromycin arms, respectively. The majority of isolates ($n$=329) were collected at the baseline visit, before treatment. Two isolates from the 2 week follow-up visit were included from two participants who did not have a pre-treatment isolate available, with the assumption that the isolates would be the same at the baseline and follow-up visit. A further two isolates from the follow-up visit were available; however, these were not included as baseline visit isolates were available from the two participants. All analyses were therefore based on 331 isolates.

### *N. gonorrhoeae* isolates

Frozen isolates were sent to PHE from the primary diagnostic laboratory associated with each sexual health clinic for antimicrobial susceptibility testing. Isolates were retrieved on GC agar base (BD Difco™; BD, Wokingham, UK) with 1% Vitox (Oxoid Ltd, Basingstoke, UK) and the identity of the isolates was confirmed using Gram staining, oxidase testing and MALDI-TOF (Bruker, Billerica, MA, USA). After an additional subculture, a 0.5 MacFarland suspension was created in saline, and azithromycin, ceftriaxone and gentamicin MICs were determined by Etest (bioMérieux UK Ltd, Basingstoke, UK) on GC agar base with 1% Vitox. Inoculated plates were incubated (36°C in 5% $CO_2$) and the MICs were recorded the following day. MICs were rounded up to the nearest doubling dilution and susceptibility categories for azithromycin (resistance MIC >0.5 mg/L) and ceftriaxone (resistance MIC >0.125 mg/L) were assigned using EUCAST 2018 breakpoints.[16] No EUCAST breakpoints were available for gentamicin.

### *Statistical analysis*

The number of isolates cleared/not cleared at each azithromycin, ceftriaxone and gentamicin MIC by treatment arm was established (Table 1).

The association between *in vitro* categorical azithromycin resistance (MIC >0.5 mg/L) and *N. gonorrhoeae* clearance (yes/no) was explored using Fisher's exact test. Linear regression was used to compare azithromycin, ceftriaxone and gentamicin continuous MICs with each treatment received and *N. gonorrhoeae* clearance. For the linear regression model, the MICs were log-transformed due to skewed data. Estimates from the linear regression on the log-transformed MICs correspond to a ratio of geometric mean MIC between two groups, such as those who received gentamicin/azithromycin and cleared infection and those who received gentamicin/azithromycin and did not clear infection.

To investigate whether baseline MIC modified the effect of the randomized treatment arm (gentamicin/azithromycin or ceftriaxone/azithromycin) on *N. gonorrhoeae* clearance (the outcome), we fitted separate logistic regression models for each of the three antimicrobials (azithromycin, ceftriaxone and gentamicin) that included an interaction term between baseline log-transformed MIC and the treatment arm. Evidence of an interaction term ($P$<0.05) will indicate a differential association (for clearance and baseline MIC) between the two treatment arms.

To investigate the effect of potential reinfection following treatment, a sensitivity analysis was performed by excluding 10 isolates from six participants who did not clear infection and who reported sex without consistent condom use before test of cure. Analyses were performed using Stata 13.1 (StataCorp, Texas, USA). Statistical analyses were not adjusted for multiple comparisons, therefore results should be interpreted with caution.

### *Ethics*

The trial was approved by Health Research Authority South Central – Oxford C Research Ethics Committee (14/SC/1030).

## Results

### *Participants administered ceftriaxone and azithromycin*

Within isolates from participants receiving ceftriaxone/azithromycin, there was little evidence of any difference in azithromycin ($P$=0.187) categorical resistance (Figure 1) (note: no ceftriaxone

**Table 1.** Percentage and number of isolates cleared/not cleared at each azithromycin, ceftriaxone and gentamicin MIC by treatment arm

| | | Ceftriaxone/azithromycin | | Gentamicin/azithromycin | |
|---|---|---|---|---|---|
| | MIC (mg/L) | % (*n*) cleared | % (*n*) not cleared | % (*n*) cleared | % (*n*) not cleared |
| Azithromycin | | | | | |
| | ≤0.016 | 100 (1) | 0 | | |
| | 0.032 | 100 (7) | 0 | 100 (7) | 0 |
| | 0.064 | 100 (30) | 0 | 95.8 (23) | 4.2 (1) |
| | 0.125 | 89.1 (41) | 10.9 (5) | 95.7 (44) | 4.3 (2) |
| | 0.25 | 97.9 (46) | 2.1 (1) | 85.4 (35) | 14.6 (6) |
| | 0.5 | 100 (37) | 0 | 82.8 (24) | 17.2 (5) |
| | 1 | 100 (7) | 0 | 62.5 (5) | 37.5 (3) |
| | 4 | 100 (1) | 0 | | |
| Ceftriaxone | | | | | |
| | ≤0.002 | 92.3 (24) | 7.7 (2) | 96.2 (25) | 3.8 (1) |
| | 0.004 | 98.8 (81) | 1.2 (1) | 93.2 (55) | 6.8 (4) |
| | 0.008 | 90.0 (27) | 10.0 (3) | 85.7 (24) | 14.3 (4) |
| | 0.016 | 100 (22) | 0 | 81.5 (22) | 18.5 (5) |
| | 0.032 | 100 (14) | 0 | 80.0 (12) | 20.0 (3) |
| | 0.064 | 100 (1) | 0 | | |
| | 0.125 | 100 (1) | 0 | | |
| Gentamicin | | | | | |
| | ≤1 | 100 (3) | 0 | 100 (3) | 0 |
| | 2 | 92.7 (51) | 7.3 (4) | 97.4 (38) | 2.6 (1) |
| | 4 | 98.2 (110) | 1.8 (2) | 85.5 (94) | 14.5 (16) |
| | 8 | 100 (5) | 0 | 100 (3) | 0 |
| | 16 | 100 (1) | 0 | | |

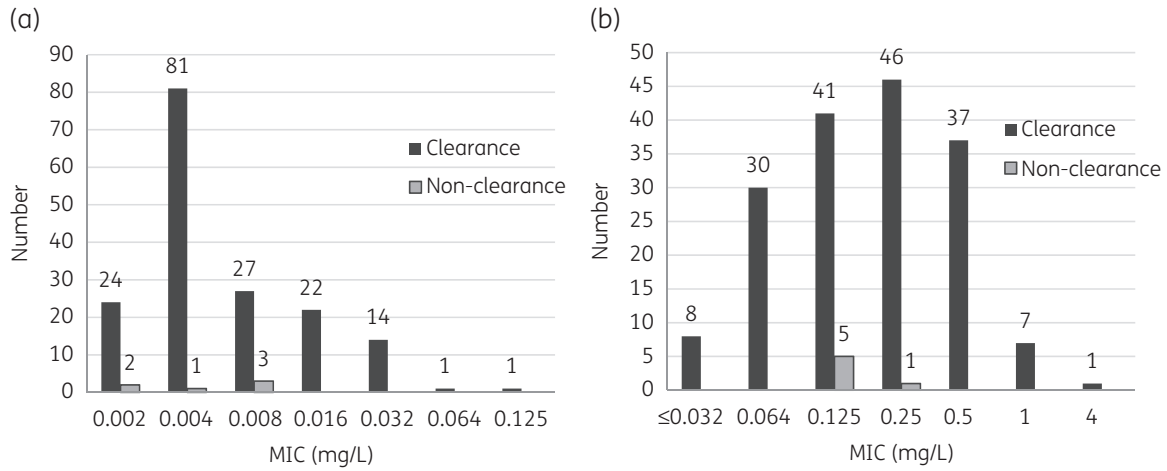


**Figure 1.** Distribution of (a) ceftriaxone or (b) azithromycin MIC (mg/L) by clearance/non-clearance of *N. gonorrhoeae* in those who received ceftriaxone and azithromycin.

resistance was identified). There was little evidence of any difference in the geometric mean MIC of azithromycin or ceftriaxone between those with cleared versus non-cleared infection (Table 2).

The six treatment failures in six participants who received ceftriaxone/azithromycin were from genital (three isolates), rectal (two isolates) and pharyngeal (one isolate) sites. Three of the six participants gave a history of unprotected sex following treatment, one of whom had sex with a new partner and two with a previous partner.

### *Participants administered gentamicin and azithromycin*

Infections in 14 participants (17 isolates; 6 genital, 6 pharyngeal and 5 rectal) were not cleared following treatment with

**Table 2.** Mode and geometric mean MICs of azithromycin, ceftriaxone and gentamicin for isolates from participants by treatment response, treatment arm and site of infection

| | | Azithromycin MICs (mg/L) | | | Ceftriaxone MICs (mg/L) | | | Gentamicin MICs (mg/L) | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | No. of isolates | mode | geometric mean | ratio[a] (95% CI), *P* value | mode | geometric mean | ratio[a] (95% CI), *P* value | mode | geometric mean | ratio[a] (95% CI), *P* value |
| All isolates | 331 | 0.25 | 0.185 | | 0.004 | 0.0061 | | 4 | 3.271 | |
| by treatment response | | | | | | | | | | |
| responder | 308 | 0.25 | 0.18 | 1 | 0.004 | 0.006 | 1 | 4 | 3.259 | 1 |
| non-responder | 23 | 0.25 | 0.266 | 1.479 (1.022–2.132), 0.038 | 0.008 | 0.008 | 1.343 (0.940–1.919), 0.105 | 4 | 3.441 | 1.056 (0.896–1.244), 0.517 |
| by treatment arm | | | | | | | | | | |
| ceftriaxone | 176 | 0.25 | 0.186 | 1 | 0.004 | 0.0059 | 1 | 4 | 3.234 | 1 |
| gentamicin | 155 | 0.25 | 0.184 | 0.986 (0.817–1.191), 0.883 | 0.004 | 0.0063 | 1.064 (0.886–1.277), 0.505 | 4 | 3.315 | 1.025 (0.943–1.115), 0.559 |
| by treatment arm and response | | | | | | | | | | |
| ceftriaxone and responder | 170 | 0.25 | 0.188 | 1 | 0.004 | 0.006 | 1 | 4 | 3.262 | 1 |
| ceftriaxone and non-responder | 6 | 0.125 | 0.14 | 0.746 (0.359–1.548), 0.429 | 0.004/0.008 | 0.0045 | 0.753 (0.379–1.496), 0.416 | 2 | 2.52 | 0.772 (0.555–1.074), 0.124 |
| gentamicin and responder | 138 | 0.125 | 0.171 | 1 | 0.004 | 0.006 | 1 | 4 | 3.256 | 1 |
| gentamicin and non-responder | 17 | 0.25 | 0.333 | 1.951 (1.284–2.966), 0.002 | 0.008 | 0.0098 | 1.649 (1.078–2.523), 0.021 | 4 | 3.84 | 1.180 (0.981–1.418), 0.078 |
| by site of infection | | | | | | | | | | |
| genital | 201 | 0.125 | 0.161 | 1 | 0.004 | 0.006 | 1 | 4 | 3.142 | 1 |
| pharyngeal | 50 | 0.25 | 0.288 | 1.783 (1.370–2.321), <0.001 | 0.004 | 0.0071 | 1.171 (0.904–1.518), 0.231 | 4 | 3.434 | 1.093 (0.970–1.232), 0.144 |
| rectal | 76 | 0.125/0.25 | 0.196 | 1.216 (0.971–1.521), 0.088 | 0.004 | 0.0055 | 0.913 (0.732–1.139), 0.418 | 4 | 3.521 | 1.120 (1.012–1.241), 0.029 |
| by site of infection and treatment arm | | | | | | | | | | |
| genital and ceftriaxone | 98 | 0.125 | 0.157 | 1 | 0.004 | 0.0061 | 1 | 4 | 3.123 | 1 |
| pharyngeal and ceftriaxone | 30 | 0.25 | 0.281 | 1.795 (1.255–2.568), 0.002 | 0.004 | 0.0058 | 0.954 (0.679–1.340), 0.784 | 4 | 3.325 | 1.065 (0.9–1.259), 0.461 |
| rectal and ceftriaxone | 45 | 0.125 | 0.196 | 1.252 (0.919–1.705), 0.154 | 0.004 | 0.0053 | 0.870 (0.648–1.167), 0.349 | 4 | 3.377 | 1.081 (0.936–1.250), 0.288 |
| genital and gentamicin | 103 | 0.125 | 0.166 | 1 | 0.004 | 0.006 | 1 | 4 | 3.161 | 1 |
| pharyngeal and gentamicin | 20 | 0.25 | 0.297 | 1.794 (1.202–2.677), 0.005 | 0.008 | 0.0095 | 1.589 (1.060–2.370), 0.025 | 4 | 3.605 | 1.141 (0.959–1.356), 0.135 |
| rectal and gentamicin | 31 | 0.25 | 0.196 | 1.182 (0.845–1.654), 0.326 | 0.004 | 0.0059 | 0.977 (0.696–1.371), 0.89 | 4 | 3.741 | 1.183 (1.024–1.368), 0.023 |
| by site of infection and response for those who received gentamicin[b] | | | | | | | | | | |
| genital and responder | 97 | 0.125 | 0.16 | 1 | 0.004 | 0.0058 | 1 | 4 | 3.115 | 1 |
| genital and non-responder | 6 | 0.25 | 0.282 | 1.757 (0.848–3.638), 0.128 | 0.004/0.016 | 0.0101 | 1.738 (0.856–3.529), 0.125 | 4 | 4 | 1.284 (0.916–1.800), 0.145 |
| pharyngeal and responder | 14 | 0.25 | 0.226 | 1 | 0.008 | 0.0076 | 1 | 4 | 3.623 | 1 |
| pharyngeal and non-responder | 6 | 0.5 | 0.561 | 2.479 (1.439–4.270), 0.003 | 0.004/0.008/ 0.016 | 0.016 | 2.102 (0.977–4.522), 0.057 | 4 | 3.564 | 0.984 (0.753–1.285), 0.898 |
| rectal and responder | 26 | 0.25 | 0.192 | 1 | 0.004/0.008 | 0.006 | 1 | 4 | 3.693 | 1 |
| rectal and non-responder | 5 | 0.25 | 0.218 | 1.133 (0.533–2.411), 0.737 | 0.004 | 0.0053 | 0.885 (0.394–1.985), 0.759 | 4 | 4 | 1.083 (0.879–1.336), 0.441 |

[a]Ratio of geometric mean MIC from the linear regression model.
[b]Little evidence of any geometric mean MIC difference from isolates from those who received ceftriaxone; results not shown. Ratios and *P* values from the linear regression model.

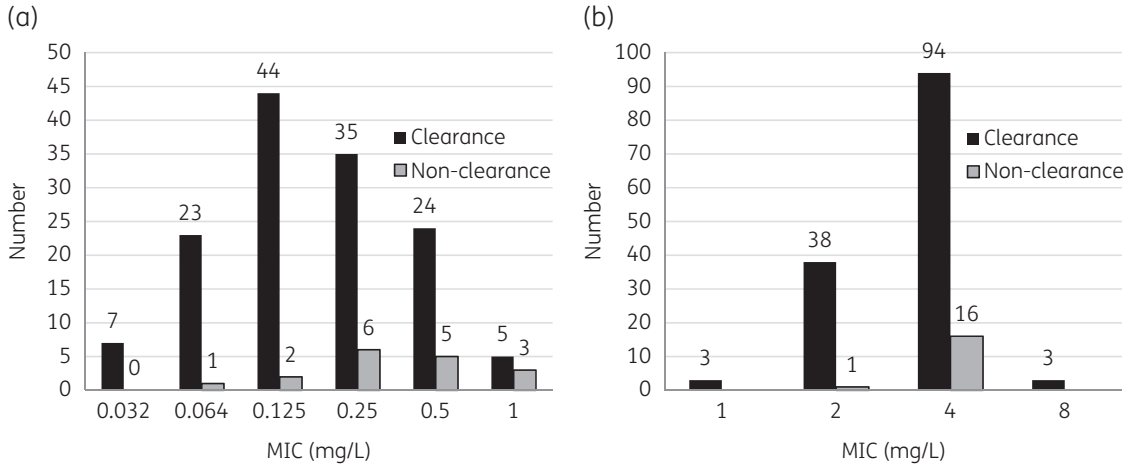


**Figure 2.** Distribution of (a) azithromycin or (b) gentamicin MIC (mg/L) by clearance/non-clearance of *N. gonorrhoeae* in those who received gentamicin and azithromycin.

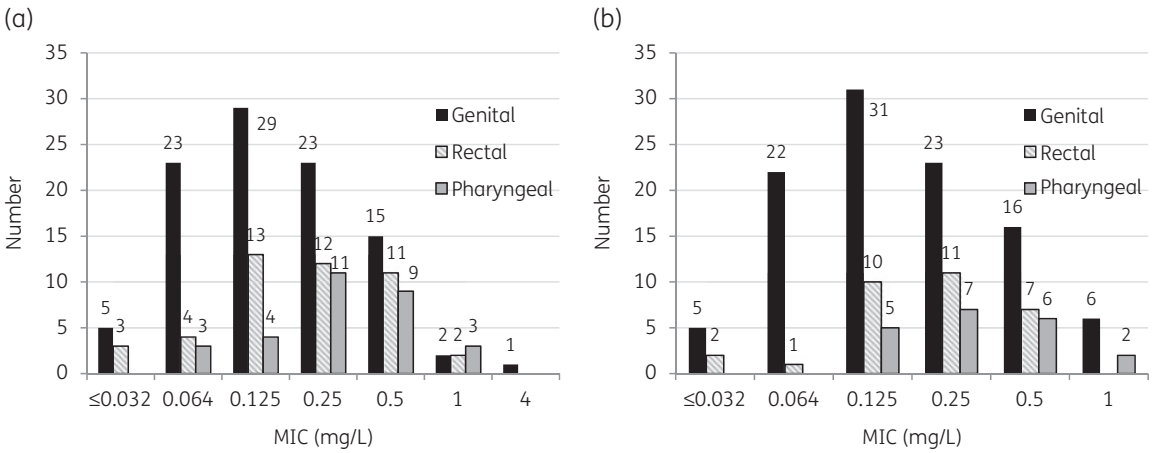


**Figure 3.** Distribution of azithromycin MIC (mg/L) by site of infection in those who received (a) ceftriaxone and azithromycin or (b) gentamicin and azithromycin.

gentamicin/azithromycin. Of these treatment failures, azithromycin resistance *in vitro* (MIC >0.5 mg/L) was found in three isolates from three participants (3/17, 18%). Five isolates from five participants (5/138, 4%) with cleared infection were resistant to azithromycin *in vitro* (Fisher's exact test, $P$=0.044) (Figure 2a). However, there was less evidence of an association between category of azithromycin resistance (MIC >0.5 mg/L) and treatment failure when the isolates from participants who had sex since entry into the trial were removed ($n$=2; azithromycin MIC of 0.5 and 1.0 mg/L, $P$=0.141).

There was strong evidence of a higher geometric mean MIC of azithromycin for isolates from participants who had received gentamicin/azithromycin and where infection was not cleared compared with those with cleared infection, with a ratio of 1.95 (95% CI 1.28–2.97, $P$=0.002) (Table 2). This higher geometric mean MIC of azithromycin remained when those who had unprotected sex following treatment were excluded (ratio 2.23, 95% CI 1.30–3.81, $P$=0.004).

For those who received gentamicin/azithromycin, the gentamicin MICs were mostly 4 mg/L [16/17 (94%, range 2–4 mg/L)] in the non-clearance group, with 94/138 (68%, range 1–8 mg/L) in the clearance group (Figure 2b). Azithromycin MICs were 0.125, 0.25 and 0.5 mg/L for three isolates with gentamicin MICs of 8 mg/L and infections in all three patients were cleared. There was little evidence of any difference between the geometric mean MIC of gentamicin and clearance of infection either overall or after excluding those with risk of reinfection (Table 2).

### *Site of infection*

The azithromycin MIC distribution and geometric mean MIC were assessed by anatomical site of infection independent of treatment and response (Figure 3, Table 2). The geometric mean MIC was higher in those with pharyngeal compared with genital infection, both in those who received ceftriaxone/azithromycin (ratio 1.80, 95% CI 1.26–2.57, $P$=0.002) and in those given gentamicin/azithromycin (ratio 1.79, 95% CI 1.20–2.68, $P$=0.005) (Table 2).

Even though the numbers are very small, the azithromycin MICs were higher in isolates from those who received gentamicin/azithromycin and failed treatment in the pharynx, with a mode of

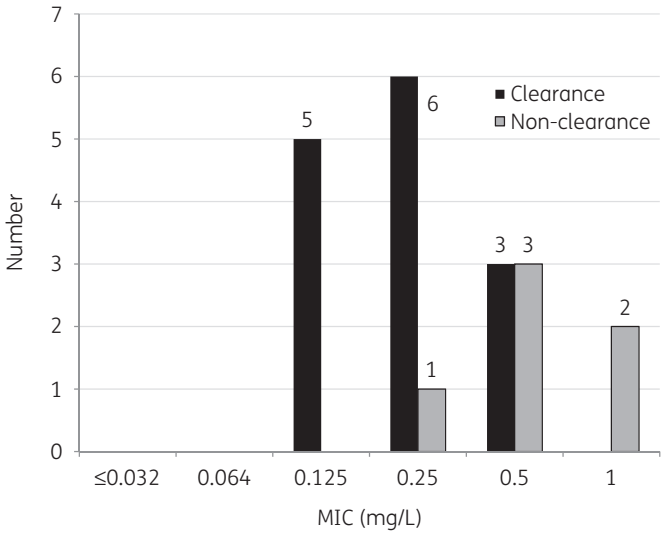


**Figure 4.** Distribution of azithromycin MIC (mg/L) by clearance/non-clearance of pharyngeal isolates from those who received gentamicin and azithromycin.

0.5 mg/L compared with 0.25 mg/L in the cleared pharyngeal infection group (Figure 4). A higher geometric mean MIC of azithromycin was also observed in those who did not clear pharyngeal infection compared with those with cleared pharyngeal infection, with a ratio of 2.48 (95% CI 1.44–4.27, *P*=0.003) (Table 2). Strong evidence of an association remained when the isolates from participants who reported unprotected sex following treatment were excluded (ratio 2.91, 95% CI 1.68–5.06, *P*=0.001).

In participants who received gentamicin/azithromycin, the ceftriaxone geometric mean MIC was also higher in the pharynx compared with genital isolates (ratio 1.59, 95% CI 1.06–2.37, *P*=0.025) (Table 2) and the geometric mean MICs of gentamicin were higher for rectal isolates compared with genital isolates (ratio 1.18, 95% 1.02–1.37, *P*=0.03) (Table 2). Higher geometric mean MICs of ceftriaxone were not identified in any infection site for isolates from the ceftriaxone/azithromycin arm (Table 2).

### *Interaction between treatment and baseline MIC*

We used the logistic regression model to investigate whether baseline azithromycin, ceftriaxone or gentamicin MIC modified the effect of randomized treatment on *N. gonorrhoeae* clearance. We identified evidence of interaction between treatment arm and baseline azithromycin MIC (OR 0.246, 95% CI 0.078–0.783, *P*=0.018); as the baseline azithromycin MIC increased, gentamicin/azithromycin became less effective at clearing *N. gonorrhoeae* compared with ceftriaxone/azithromycin. Evidence of this interaction remained in the sensitivity analysis that excluded those with possible reinfection (OR 0.169, 95% CI 0.029–0.034, *P*=0.029). Initial evidence of an interaction was identified between baseline gentamicin MIC and the gentamicin/azithromycin treatment arm (OR 0.045, 95% CI 0.003–0.634, *P*=0.022) but little evidence remained when the isolates from participants who had sex since entry into the trial were removed (*P*=0.120). There was little evidence of an interaction between baseline ceftriaxone MIC and the two treatment arms.

## Discussion

We found that as the azithromycin MIC increased the gentamicin/azithromycin treatment was less effective than ceftriaxone/azithromycin at clearing *N. gonorrhoeae*. This result is not unexpected when we consider the lower cure rate (91%) using gentamicin/azithromycin compared with ceftriaxone/azithromycin (98%) that was previously established from the large trial of gonorrhoea treatment.[5] What was unexpected was the poor predictive value of categorical resistance for azithromycin and ceftriaxone *in vitro*, and higher gentamicin MICs in the absence of breakpoints, in predicting treatment failure for participants. Of the 23 isolates recovered following treatment, only three (13%) would have been predicted using *in vitro* susceptibility testing. Reinfection (as opposed to antibiotic treatment failure) may have occurred in some individuals, but is unlikely to account for all participants who remained infected at follow-up. Specifically, excluding those with a history of post-treatment unprotected intercourse made little difference to the predictive accuracy of pre-treatment *in vitro* susceptibility testing. In addition, the overall higher treatment failure rate in individuals randomized to receive gentamicin/azithromycin (25/292; 9%) compared with ceftriaxone/azithromycin (7/306; 2%) also suggests that the majority of cases where infection failed to clear in those given gentamicin/azithromycin was due to lack of antibiotic efficacy rather than reinfection,[5] further supported by the evidence of an interaction between MIC and treatment arm. The geometric mean MIC of azithromycin was also significantly higher for isolates from the group failing therapy; however, this is of little practical value for clinicians in selecting therapy. Azithromycin MICs for 9 of 17 isolates from participants who failed treatment following gentamicin/azithromycin were categorized as susceptible (range 0.064–0.25 mg/L). This lack of correlation between clinical outcome and azithromycin MICs has been observed previously in Australia[17] where five patients who failed treatment with 1 g azithromycin had isolates with azithromycin MICs of 0.125–0.25 mg/L. Additionally, EUCAST have recently removed the clinical breakpoints for azithromycin from the 2019 breakpoint tables,[18] in part due to the poor correlation of azithromycin MICs and clinical outcome as well as a lack of pharmacokinetic and pharmacodynamic models, and because azithromycin is often used in conjunction with another effective agent. Both EUCAST[18] and CLSI[4] have established the epidemiological cut-off (ECOFF) at 1 mg/L; the point at which the WT distribution ends and the distribution of isolates with acquired azithromycin resistance mechanisms begins. It should be noted that while EUCAST has no breakpoints for azithromycin, CLSI regard ≤1 mg/L as the susceptible breakpoint.[4]

It is likely that azithromycin 1 g failed to provide microbiological cure when gentamicin treatment was unsuccessful despite previous studies of azithromycin demonstrating effectiveness.[19] This suggests that the use of 1 g azithromycin in dual therapy, which is in widespread use[8] to delay the emergence of resistance to ceftriaxone, needs to be reviewed. Consequently, the UK gonorrhoea management guidelines have recently been revised and recommend removal of azithromycin from the first-line therapy (https://www.bashhguidelines.org/media/1208/gc-2019.pdf). It should be noted that the EUCAST 2018 breakpoint for azithromycin was based on using the 2 g dose in monotherapy.[3] The 2 g dose for gonorrhoea treatment may be more effective and provide more

confidence in the predictive value of the MICs, albeit with a high rate of reported gastrointestinal side effects.[14,20] However, a Japanese study established an eradication rate of just 93.8% in men with gonococcal urethritis when a single 2 g dose of azithromycin extended-release formation was administered.[21] The widespread use of azithromycin for *N. gonorrhoeae* treatment has also been challenged due to a number of issues: (i) the ease with which azithromycin resistance can emerge both *in vivo*[22–24] and *in vitro*;[25] (ii) reports of increases in azithromycin resistance both nationally[26] and globally;[27–29] (iii) sustained transmission of isolates with high-level azithromycin resistance in England[11] and Hawaii;[30] and (iv) the recent emergence of isolates with both high-level ceftriaxone and azithromycin resistance in England[13] and Australia.[31]

Azithromycin's long half-life of 67 h[32] has been postulated to select for resistance.[22] For example, any surviving gonococci, or those acquired from infection some days after treatment with azithromycin, may be exposed to subMIC levels of azithromycin. The presence of prolonged low-level drug concentrations post-treatment also has a potential impact on the emergence of azithromycin resistance for other sexually transmitted infections (STIs) in patients who are, or become, coinfected.[33]

Resistance breakpoints for gentamicin have not been determined and our data highlight the difficulty in establishing these breakpoints, particularly as gentamicin was used as part of combination therapy. Most participants (68%, 94/138) with cleared infections harboured isolates with MICs $\geq$4 mg/L, whereas in isolates from participants who failed treatment the gentamicin MICs were 4 mg/L in all but one. Isolates with MICs above the tentative gentamicin resistance breakpoint of >16 mg/L[34] were not observed from participants experiencing treatment failure. Gentamicin is infrequently used for gonorrhoea treatment outside of Malawi[35] and Zambia,[36] so there is currently no selection pressure from gentamicin usage to induce resistance in gonococci isolated from patients in the UK. Treatment failure due to poor tissue penetration, rather than resistance, seems more plausible. The gentamicin MIC distribution in this study was similar to a 2016 European study[10] although it is difficult to determine potential breakpoints from this data when gentamicin was used in combination with azithromycin. Our data suggest that an increased dosage to achieve higher drug levels, especially at extragenital sites, needs further investigation. Pragmatically, however, the large volume of injection for gentamicin (6 mL for a 240 mg dose) would probably require a larger dose to be delivered via an additional injection or necessitate a reformulation of the drug into a smaller volume. A move away from single-dose, directly observed therapy to multiple-dose regimens to increase the effectiveness of antimicrobials in the pharynx could also be further explored.[37]

The proportion of patients who failed treatment despite having infections, particularly at the pharynx, caused by isolates 'susceptible' to gentamicin and azithromycin shows that MICs alone cannot predict outcome. As stated previously,[17] there may be pharmacokinetic properties of azithromycin in tissues, especially of the pharynx, that prevent the use of MICs to predict treatment outcome reliably. This may also apply to gentamicin. It is possible that alternative genetic markers identified through sequencing might better predict treatment outcome but this remains speculative. As well as differences in tissue penetration, there are a number of possible reasons why the gentamicin/azithromycin combination was less effective at the pharynx: 'protection' of *N. gonorrhoeae* by commensal organisms or within gonococcal biofilms, exposure to aminoglycoside-modifying enzymes from other organisms[38] and possibly some other inducible resistance such as mutations in genes within an intrinsic resistome similar to that observed with *Acinetobacter baumannii*;[39] results from ongoing molecular studies should help to resolve the mechanism(s) by which the gonococcal strains resisted clearance by gentamicin. Although ceftriaxone remained effective at the pharynx, some of these factors may also apply to other antimicrobials that have not been as effective when treating pharyngeal gonorrhoea.[40–42]

Interestingly we found higher geometric mean MICs of azithromycin in the pharynx compared with genital isolates in both treatment arms, and higher ceftriaxone MICs in the pharynx in the gentamicin/azithromycin arm. Higher MICs in the pharynx have been demonstrated in other studies[43–46] as it is postulated the throat is a hotspot for resistance due to the transfer of resistance determinants from commensal *Neisseria* and possibly selection of resistance in the pharynx is more likely due to reduced tissue penetration, which subsequently exposes the bacteria to suboptimal doses of antibiotic. The higher pharyngeal azithromycin MICs may have contributed to the reduced *N. gonorrhoeae* clearance in the gentamicin/azithromycin arm, suggesting that different breakpoints for different anatomical sites could be considered. It should, however, be noted that the clearance of the gonococci in the ceftriaxone/azithromycin treatment arm was not hindered by these higher MICs, and not all studies have detected differences in MIC according to anatomical site.[47,48]

As previously mentioned, a limitation of this study was not adjusting for multiplicity, so the results should be interpreted with caution. Another limitation is the small number of isolates available for MIC analysis; only 39% of participants had an isolate, so we should be wary that the culture dataset may not be representative of the whole study population, especially for extragenital infections. It is well established that culture is less sensitive than NAATs[49] and an analysis from the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) dataset revealed that only 46% of patients had culture-positive infections.[50] The culture-positive participants in our dataset may have had a higher bacterial load or more 'virulent' bacteria, so the distribution of MICs for pharyngeal isolates may be spurious. However, the characteristics of participants with and without any positive culture isolates at the baseline visit revealed that both groups were well matched; exceptions were the more frequent presence of symptoms in the culture-positive group (as expected), and the lower proportion of MSM in the culture-positive group (see Table S1, available as Supplementary data at *JAC* Online, describing the baseline characteristics for participants with and without any positive culture isolates at the first visit). The proportion of MSM in this group roughly corresponds with the epidemiological surveillance[1] data and overall the dataset is one of the largest available that directly evaluates MICs in relation to clinical outcome. The robust and standardized approach to data collection and laboratory testing is an additional strength of the study.

Decreasing azithromycin and ceftriaxone susceptibility is being observed globally, therefore alternative treatment options and strategies are urgently required. Even though gentamicin with azithromycin is a suitable alternative in uncomplicated genital infection, serious consideration of different dosages for different

sites or extended regimens to ensure *N. gonorrhoeae* is cleared at all sites is warranted. This is particularly relevant following recent reports of treatment failure with ceftriaxone in patients with pharyngeal gonorrhoea compared with successful treatment of their urogenital infection.[12,13] Clinicians also need to be aware that MICs are not always predictive of gonorrhoea clinical failure, especially for azithromycin and gentamicin, which may also impact the effectiveness of point-of-care tests for resistance-guided therapy.

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## Transparency declarations

Jonathan D. C. Ross reports personal fees from GSK Pharma, Hologic Diagnostics, Mycovia and Janssen Pharma as well as ownership of shares in GSK Pharma and Astrazeneca Pharma; and is author of the UK and European Guidelines on Pelvic Inflammatory Disease; is a Member of the European Sexually Transmitted Infections Guidelines Editorial Board; is a Member of the National Institute for Health Research HTA Commissioning Board; was previously a Member of the National Institute for Health Research HTA Primary Care, Community and Preventative Interventions Panel (2013–2016). He is an NIHR Journals Editor and associate editor of *Sexually Transmitted Infections* journal. He is an officer of the British Association for Sexual Health and HIV (vice-president), and the International Union against Sexually Transmitted Infections (treasurer). Neil Woodford and Michelle J. Cole have no personal conflicts; however PHE's AMRHAI Reference Unit has received financial support for conference attendance, lectures, research projects or contracted evaluations from numerous sources, including: Accelerate Diagnostics, Achaogen Inc., Allecra Therapeutics, Amplex, AstraZeneca UK Ltd, AusDiagnostics, Basilea Pharmaceutica, Becton Dickinson Diagnostics, bioMérieux, Bio-Rad Laboratories, BSAC, Cepheid, Check-Points B.V., Cubist Pharmaceuticals, Department of Health, Enigma Diagnostics, Food Standards Agency, GlaxoSmithKline Services Ltd, Helperby Therapeutics, Henry Stewart Talks, IHMA Ltd, Innovate UK, Kalidex Pharmaceuticals, Melinta Therapeutics, Merck Sharp & Dohme Corp., Meiji Seika Pharma Co. Ltd, Mobidiag, Momentum Biosciences Ltd, Neem Biotech, Nordic Pharma Ltd, Norgine Pharmaceuticals, Rempex Pharmaceuticals Ltd, Roche, Rokitan Ltd, Smith & Nephew UK Ltd, Shionogi & Co. Ltd, Trius Therapeutics, SpeeDx, VenatoRx Pharmaceuticals and Wockhardt Ltd. All other authors: none to declare.

### *Author contributions*

M.J.C., W.T., H.F. and J.D.C.R. initiated and designed the study described within the manuscript, and wrote the first draft of the manuscript. M.J.C., W.T., H.F., A.A.M., T.H., N.W. and J.D.C.R. performed the statistical analysis and/or interpreted the data. J.D.C.R. was the Chief Investigator of the G-ToG trial, with M.J.C., W.T., C.B., L.D., T.H., T.L., A.A.M., K.S. and S.T. contributing to the design, management and analysis of G-ToG. M.J.C., C.C. and F.T. carried out the laboratory work. All authors read, commented and approved the final manuscript.

## Disclaimer

The views and opinions expressed therein are those of the authors and do not necessarily reflect those of the Health Technology Assessment Programme, NIHR, the NHS or the Department of Health.

## Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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