Gentamicin compared with Ceftriaxone for the treatment of gonorrhoea: a randomised trial (G-ToG Trial)

Short title: Gentamicin for the Treatment of Gonorrhoea: G-ToG Trial

Authors and affiliations:

Authors: Jonathan DC Ross[1], Clare Brittain[2], Michelle Cole[3], Claire Dewsnap[4], Jan Harding[1], Trish Hepburn[2], Louise Jackson[5], Matthew Keogh, Tessa Lawrence[1], Alan A Montgomery[2], Tracy E Roberts[5], Kirsty Sprange[2], Wei Tan[2], Sukhwinder Thandi[2], John White[6], Janet Wilson[7], Lelia Duley[1] , on behalf of the G-ToG Trial Team

[1]Birmingham University Hospitals, Birmingham, UK (Prof. JDC Ross (MD), J Harding (PhD), T Lawrence (PhD); [2]**Nottingham Clinical Trials Unit, University of Nottingham, Queen's Medical Centre, Nottingham, UK** (W Tan (MSc), Prof AA Montgomery (PhD), C Brittain (BMedSc), K Sprange (MSc) S Thandi (PhD), Prof L Duley (MD), T Hepburn (BSc),); **[3]Antimicrobial Resistance and Healthcare Associated Infections (AMRHAI) Reference Unit**, Public Health England, London, UK (Michelle Cole (DBMS);[4] **Sheffield Royal Hallamshire Hospital**, Sheffield, UK (Claire Dewsnap (MD)); [5]**Health Economics Unit, Institute of Applied Health Research, University of Birmingham, UK** (L Jackson (PhD), Prof TE Roberts (PhD)); **[6]Guy's and St Thomas' NHS Foundation Trust,** London, UK (Dr J White (FRCP); **[7]Leeds Teaching Hospitals NHS Trust, Leeds**, UK (Dr J Wilson (FRCP))**; Patient and Public Involvement representative, UK** (M Keogh)

Corresponding author: Professor Jonathan Ross, University Hospitals Birmingham NHS Foundation Trust, Whittall Street Clinic, Birmingham, UK, B4 6DH

Tel: +44 (0)121 237 5721

e-mail: jonathan.ross@uhb.nhs.uk

Research in context

Evidence before this study

Two systematic reviews have evaluated the efficacy of gentamicin for the treatment of gonorrhoea. They included randomised trials, quasi randomised trials and prospective studies with concurrent controls published between 1950 and June 2014. A subsequent search of Medline and Embase using the terms "gonorrhoea/gonorrhea/Neisseria gonorrhoeae" and "gentamicin" was conducted for studies published from 2013 to 2017. In total, six studies evaluated single dose gentamicin treatment of which three were randomised trials, one was quasi-randomised and two were non-randomised. Cure rates of 62% to 100% were reported with gentamicin treatment. Methodology was poorly described and there was a high risk of bias within most studies. The largest and best quality study was a non-comparative evaluation of 157 patients, which reported that gentamicin cured 100% of infections. This study used a relatively less sensitive culture technique to diagnose gonorrhoea and assess cure, and included few extra-genital infections (ten pharyngeal, one rectal). Gentamicin was administered with a 2g dose of azithromycin. The combination regimen was poorly tolerated causing nausea in 26% of patients and vomiting in 10%.

Added value of this study

Due to antibiotic resistance, treatment options for gonorrhoea are diminishing. G-ToG is the first randomised trial to compare gentamicin with the current first line treatment, ceftriaxone, for gonorrhoea. The trial provides insufficient evidence to conclude the non-inferiority of gentamicin compared with ceftriaxone, as gentamicin was associated with a relatively high failure rate in patients with extra-genital infection. Cure rates for genital infection were similar between the two groups, however, so for these patients gentamicin may be useful second line therapy. Single dose gentamicin was safe and well tolerated.

Implications of all the available evidence

Ceftriaxone should remain the first line treatment for gonorrhoea, with gentamicin as an alternative particularly for patients with genital infection, and those who are allergic or intolerant to ceftriaxone, or

harbour ceftriaxone resistant gonococci. Further research is required to identify and test new alternatives to ceftriaxone for the treatment of gonorrhoea.

Abstract

Background: Gonorrhoea is a common sexually transmitted infection for which ceftriaxone is the current first line treatment, but antimicrobial resistance is emerging. The objective of this study was to assess the effectiveness of gentamicin as an alternative to ceftriaxone (both combined with azithromycin) for treatment of gonorrhoea.

Methods: We performed a blinded, non-inferiority randomised trial in 14 sexual health clinics in England. Adults with a diagnosis of uncomplicated genital, pharyngeal or rectal gonorrhoea were randomised using a secure web based system, stratified by clinic. Allocation was either to gentamicin 240 mg or to ceftriaxone 500 mg, both administered as a single intramuscular injection. All participants also received 1 g oral azithromycin. The primary outcome was clearance of *Neisseria gonorrhoeae* at all infected sites.

Findings: We enrolled 720 participants and randomly assigned them to gentamicin (n=358) or ceftriaxone (n=362), with 292 and 306, respectively, included in the primary analysis. Non-inferiority of gentamicin to ceftriaxone was not demonstrated (adjusted risk difference for microbiological clearance -6.4%, 95% CI -10.4%, -2.4%, NI margin -5%). Clearance of genital infection was similar in the two groups: 94% gentamicin versus 98% ceftriaxone: but for pharyngeal and rectal infections were lower in the gentamicin group (80% vs 96% and 90% vs 98%, respectively). The side effect profiles were comparable between the allocated groups, other than reported pain at the injection site, which was higher for gentamicin.

Interpretation: Gentamicin is not an appropriate alternative first line treatment for gonorrhoea, but remains potentially useful for patients with isolated genital infection, or who are allergic/intolerant to ceftriaxone, or harbour a ceftriaxone resistant isolate. Further research is required to identify and test new alternatives to ceftriaxone for the treatment of gonorrhoea.

Funding: NIHR Health Technology Assessment Programme

Introduction

Each year gonorrhoea accounts for over 40,000 infections annually in the UK and around 78 million infections globally[1], with a disproportionate burden in young adults, men who have sex with men (MSM), and specific ethnic groups. Infection leads to local inflammation causing genital pain and discomfort, and localized immune activation that facilitates the acquisition and transmission of HIV. For women, infection can spread to the fallopian tubes and ovaries causing pelvic inflammatory disease with resultant tubal scarring, infertility, chronic pelvic pain and an increased risk of ectopic pregnancy. In men, it can spread to the testicles leading to epididymo-orchitis, and for MSM it may involve the rectum causing proctitis that can lead to abscess and fistula formation.

The causative organism, *Neisseria gonorrhoeae*, readily develops resistance to antibiotics. High-level resistance to penicillins, sulphonamides, tetracyclines and quinolones has led to these no longer being recommended as treatment. Current guidance is to treat with intramuscular ceftriaxone, given as dual therapy with azithromycin.[2-4] Surveillance data in the UK show a reduction in susceptibility to ceftriaxone over time, with an upward drift in the minimum inhibitory concentration [MIC].[1] A similar reduction in susceptibility for other antimicrobials used for gonorrhoea was followed within a few years by widespread treatment failure, and sporadic clinical failure of cephalosporins has been reported.[5, 6] If ceftriaxone becomes ineffective, options for treatment are limited. With the exception of gentamicin, alternative agents have either not been assessed *in vivo* (e.g. ertapenem, piperacillin/tazobactam), are still in early development before licensing (e.g. zoliflodacin, gepotidicin), are reserved for other infections (e.g. rifampicin for tuberculosis), or have the potential for resistance to develop rapidly (e.g. azithromycin, spectinomycin). Untreatable, multi-drug resistant gonorrhoea is a real possibility, and new clinical trial data are needed to inform treatment guidelines.[7] The World Health Organisation and European Centre for Disease Prevention and Control have called for urgent research into the efficacy of new regimens to treat gonorrhoea, including combination regimens and the assessment of antimicrobial efficacy at extra-genital sites.[8, 9] There is a particular need for effective, safe and low cost treatment in low and middle income countries, many of which have a high burden of gonorrhoea infection.

Gentamicin is an aminoglycoside antibiotic that inhibits protein synthesis by irreversibly binding to 30S ribosomal subunits. Studies in the 1970s/80s assessed gentamicin for gonorrhoea, but all were small and had a high risk of bias.[10, 11] The dose used in these studies was usually 240 mg (range 160 mg-5mg/kg) with no apparent dose response effect across studies, and no gentamicin associated adverse events were reported. *In vitro* susceptibility testing suggests that *N. gonorrhoeae* remains susceptible to gentamicin[12] although the *in vivo* response and associated susceptibility breakpoints have been poorly characterised. Gentamicin can cause ototoxicity and nephrotoxicity[13], but their frequency and severity following single dose therapy is not known.

Recent systematic reviews report the clinical and microbiological cure rate with gentamicin for the treatment of gonorrhoea (mostly urogenital) to be 62-98%.[10, 11] Data on its efficacy when treating pharyngeal or rectal gonorrhoea are scarce, although antibiotics for gonorrhoea are sometimes less effective at these sites.[14] A recent large randomised non-comparative trial reported a cure rate of 100% when gentamicin was combined with 2 g oral azithromycin, but a high incidence of gastrointestinal adverse effects limited the tolerability of this regimen.[15]

The aim of our study was to assess whether single dose gentamicin therapy is an acceptable alternative to (i.e non-inferior to) ceftriaxone for the treatment of gonorrhoea, when both antibiotics are combined with azithromycin.

Methods

Study design

The G-ToG trial was a multi-centre, parallel group, blinded, pragmatic randomised trial comparing treatment with gentamicin versus ceftriaxone for patients with gonorrhoea. All participants also received 1 g oral azithromicin. The trial was conducted in 14 sexual health clinics in the UK. The protocol has been published,[16] and is summarised here. The study was approved by Health Research Authority South Central – Oxford C Research Ethics Committee (14/SC/1030). The trial was registered prior to start of recruitment (ISRCTN51783227).

Participants

Adults aged 16-70 years were eligible for participation if they had a diagnosis of untreated genital, pharyngeal and/or rectal gonorrhoea (i.e. had not received any antibiotic in the previous 28 days that could have treated gonorrhoea, either partially or completely). To reflect normal practice, all patients who had an initial positive test for gonorrhoea and presented for treatment were eligible for inclusion. Diagnosis was based on detection of intracellular Gram-negative diplococci by microscopy (urethral, cervical, vaginal or rectal specimens), or positive Nucleic Acid Amplification Test (NAAT) from first void urine, urethral, endocervical, vulvovaginal, pharyngeal or rectal swabs. Any licensed NAAT test platform result was accepted for assessing eligibility for inclusion into the trial. Exclusion criteria were known concurrent bacterial sexually transmitted infection (apart from chlamydia); known bacterial vaginosis and/or *Trichomonas vaginalis* infection; known contra-indications or allergy to gentamicin, ceftriaxone, azithromycin or lidocaine; complicated gonorrhoea infection, for example pelvic inflammatory disease or epididymo-orchitis; and patient weight being less than 40 kg. Women who were pregnant or breast-feeding were also excluded. Participants were only eligible to participate in the trial once. They provided written informed consent at their initial consultation.

Randomisation and blinding

Participants were randomised to receive either intramuscular gentamicin 240 mg (intervention) or intramuscular ceftriaxone 500 mg (control). All participants also received 1 g oral azithromycin.

Randomisation was in a 1:1 ratio, stratified by clinic and performed using a secure web-based system. We used a computer generated pseudo-random code with permuted blocks of randomly varying size created by the Nottingham Clinical Trials Unit (NCTU) in accordance with their standard operating procedure. The allocated treatment was administered from routine clinic stock. To maintain blinding, when a member of the research team randomised a participant, the system confirmed that randomisation had been successful but did not reveal the allocated treatment. An unblinded 'injecting nurse' who was trained only in the trial's treatment administration procedure and not involved with any other trial procedures then logged onto the randomisation system to determine which treatment had

been allocated, and administered the injection and oral azithromycin. The 'injecting nurse' did not reveal the treatment allocation to participants, research staff or investigators, who all remained blinded. The allocation sequence remained concealed until the database was locked at the end of the trial.

Procedures

Ceftriaxone 500 mg in powder formulation was dissolved in 1% lidocaine and administered as a single 2 ml intramuscular injection. Gentamicin 240 mg (3 x 80 mg in 2 ml vials) was administered as a single 6 ml intramuscular injection. In addition, all participants received a single oral dose of 1 g azithromycin. All participants were asked to avoid sexual contact until review after 2 weeks.

Participants provided samples for *N. gonorrhoeae* testing before treatment. This varied by gender and sexual orientation: for heterosexual men – NAAT and culture testing from urethra (a first pass urine sample could be taken as an alternative to the urethra for NAAT); for men who have sex with men – NAAT and culture testing from urethra, pharynx and rectum (a urine sample could be taken as an alternative to the urethra for NAAT); for women – NAAT and culture testing from cervix, pharynx and rectum (a vaginal sample could be taken as an alternative to the cervix for NAAT). Follow up was two weeks after treatment, when NAAT and culture testing for *N. gonorrhoeae* was repeated for sites that had been positive at baseline. All baseline and post-treatment samples were required to be tested using NAAT (Hologic, Aptima Combo 2®). If the local laboratory did not use Aptima Combo 2® NAAT, additional samples were tested at Public Health England (PHE). Culture specimens were processed according to local laboratory procedures and pure viable cultures confirmed to be *N. gonorrhoeae* were frozen (≤-70°C) and shipped to PHE for antimicrobial sensitivity testing.

Blood samples for creatinine measurement (allowing calculation of the estimated glomerular filtration rate) were taken at baseline and two week follow up attendances.

Outcomes

The primary outcome was clearance of *N. gonorrhoeae* at all initially infected sites defined as a negative NAAT (Hologic, Aptima Combo 2®) two weeks post treatment.[17] Analyses were performed without knowing the treatment allocation. Secondary outcomes were clinical resolution of symptoms, change in renal function (estimated glomerular filtration rate [eGFR]) and comparative cost effectiveness at two weeks. The relationship between clearance of *N. gonorrhoeae* and *in vitro* measurement of antibiotic minimum inhibitory concentration (MIC) using Etests (BioMérieux) on GC base agar (Becton Dickinson) with 1% Vitox (Oxoid) was also investigated. Safety outcomes were the frequency of known side effects (nausea/vomiting, hearing loss, dizziness and rash), frequency of any other adverse events reported by participants, and tolerability of the treatment injection measured on a visual analogue scale (VAS) where 0 represented no pain and 100 worst imaginable pain. The results of the cost effectiveness analyses will not be presented in this paper.

Statistical Analyses

Based on 96% clearance for the ceftriaxone regimen, a total sample size of 646 (323 in each group) was required to detect non-inferiority with a lower confidence limit of 5% for the risk difference, with 90% power and 0.025 one-sided significance. To allow for loss to follow-up of 10%, the trial had a target recruitment of 720 participants.

All analyses were carried out using Stata/SE 13·1. The primary approach to between-group comparisons was to analyse participants according to randomised allocation without imputation of missing outcome data. Planned analysis of the primary outcome was modified, before the database was locked and treatment codes revealed. The initial analysis plan in the protocol was to use a general linear model for binary outcome adjusted by clinic site, with the primary efficacy parameter comparing gentamicin with ceftriaxone being the risk difference in the proportion of participants clear of infection at follow up along with the 95% confidence interval. However, additional clinics joined the trial, some of which recruited small numbers of participants. This meant that there was the chance that some clinics would have no participants whose infection had not cleared, making the inclusion of clinic as a

fixed effect inappropriate. Therefore, we modified the between-group comparative analyses to use generalised estimating equations (GEE) for binary outcomes adjusted by recruiting clinic as a random effect with robust standard errors. The GEE model used an identity link function to enable estimation of adjusted risk difference. Gentamicin was to be regarded as non-inferior if the lower 95% confidence limit for the risk difference (gentamicin group versus ceftriaxone group) in confirmed clearance was -5 percentage points or greater (i.e. closer to zero). Analysis of the primary outcome included only participants who had follow up data and, since this was a pragmatic trial, included all participants meeting the trial entry criteria, irrespective of the baseline visit *Neisseria gonorrhoeae* test result. Sensitivity analyses were performed to assess the robustness of the primary outcome analysis including multiple imputation using chained equations; assuming all missing data were cleared and not cleared, excluding participants who did not have any positive baseline samples, excluding those who had not received their allocated treatment and excluding those who did not have a full set of baseline samples.

Secondary outcomes were similarly analysed using appropriate regression models dependent on data type, adjusted for clinic site and baseline value of the outcome variable if collected. Clearance at each site was investigated separately for each infection site. MIC data were summarised on a per participant basis. The relationship between clinical effectiveness and MIC was examined by plotting the distribution of the highest MIC detected per participant categorised by clearance at all sites at two weeks.

Safety and tolerability analyses were descriptive. Frequency counts and percentages of the pre-specified main categories of side-effects were presented by treatment arm. Adverse events were coded using MedDRA version 17.1 and summarised by System Organ Class.

Full details of the analysis are documented in the Statistical Analysis Plan, which was finalized prior to database lock and release of treatment allocation codes for analysis. An independent data monitoring committee oversaw the trial and were provided with unblinded data by treatment group, prepared by a statistician who was independent of the trial team.

Role of the funder

The study was funded by the UK National Institute for Health Research (NIHR) Health Technology Assessment programme. The NIHR had input into trial design through peer review of the funding proposal. The funders did not have a role in data collection, data analysis, data interpretation or writing of the report but had sight of the paper prior to publication. The corresponding author had full access to all the data and had final responsibility for the decision to submit.

Results

Of 1762 patients approached at 14 sexual health clinics in England, 720 were randomised between 7 October 2014 and 14 November 2016; 362 to receive ceftriaxone and 358 to receive gentamicin. The main reasons for non-randomisation were participants not being interested, the trial taking too much time, a belief that the standard treatment would be successful and the trial taking too many extra/intrusive samples. Fourteen participants did not receive their allocated medication; four in the ceftriaxone group and ten in the gentamicin group (Figure 1). 96 participants did not return for their follow up visit – the reasons for 83 of these participants were unknown, and reasons for the others included withdrawal of consent, participants being found to be ineligible after randomisation, medication being out of stock and injecting nurses not being available. 26 participants who returned for their follow up visit did not have primary outcome data due to incorrect sampling. Primary outcome data were therefore available for 306 participants randomised to ceftriaxone (85%) and 292 participants randomised to gentamicin (82%). (Figure 1).

The baseline characteristics of participants were well balanced across treatment groups (Table 1). Treatment groups appeared to be balanced with respect to participants' history of sexually transmitted infections (41% had at least one previous diagnosis of gonorrhoea, 34% chlamydia, 14% syphilis, and for 3% of women pelvic inflammatory disease).

Protocol deviations were reported in 121/362 (33%) of participants receiving ceftriaxone and 124/358 35%) of those receiving gentamicin, the majority being considered minor. Two major protocol deviations were identified - Not receiving treatment according to randomisation (14 participants – 4

randomised to ceftriaxone and 10 randomised to gentamicin) and not fulfilling eligibility criteria (18 participants – 5 randomised to ceftriaxone and 13 randomised to gentamicin). (Supplementary Table 3). It was considered unlikely that the imbalance in the proportion of major protocol deviations was due to selection bias or knowledge of treatment allocation and that these violations did not impact on the validity of the trial.

Overall 322/362 (89%) participants allocated to ceftriaxone and 302/358 (84%) allocated gentamicin attended their follow up visit. The median number of days from randomisation to follow up was 16 (Q1-Q3 14-20) and 15 (Q1-Q3 14-20) in the ceftriaxone and gentamicin groups respectively. Most (82%) participants in both groups returned within 21 days.

At two weeks post-treatment infection had cleared, as defined by a negative NAAT, for 299/306 (98%) participants allocated ceftriaxone compared to 267/292 (91%) allocated gentamicin; adjusted risk difference -6.4%, 95% CI -10.4% to -2.4% (Table 2). Sensitivity analyses were consistent with the primary analysis (Figure 2).

Of the participants who had a genital infection, infection had cleared at follow up for 98% and 94% in the ceftriaxone and gentamicin groups respectively (Table 3). For participants with pharyngeal infection, a greater proportion receiving ceftriaxone had clearance at their follow up visit (96%) compared with gentamicin (80%). Similarly, a greater proportion of participants with rectal infection in the ceftriaxone group showed clearance (98%) compared with gentamicin (90%). There was no evidence of a clear difference between treatment groups in resolution of symptoms (Table 4).

A similar proportion of participants experienced nausea in the ceftriaxone and gentamicin groups (12% vs 14%). Few participants experienced vomiting, reduction in hearing, dizziness/unsteadiness or skin rash, and proportions were similar in the two treatment groups. (Table 5). The majority reported injection site pain, 98% and 99% allocated ceftriaxone/azithromycin and gentamicin/azithromycin respectively, with the mean pain score measured using a visual analogue scale higher in the gentamicin

group (36/100 vs 21/100). The median time to resolution of injection pain was one hour for ceftriaxone and 1.5 hours for gentamicin. At least one adverse event (AE) was reported by 15% of participants allocated ceftriaxone and 13% allocated gentamicin, the majority of these were mild (45/54 ceftriaxone and 35/43 gentamicin). One serious adverse event (SAE) (grade 4 dizziness) was reported, and was not considered to be related to the trial medication. Changes in eGFR between baseline and follow up were similar in both arms (mean difference (-1.4 ml/min [IQR -6.9, 3.8]). In addition to the side effects participants were specifically asked about, 86 participants reported at least one other AE (48 receiving ceftriaxone and 38 gentamicin), most commonly gastrointestinal disorders (14 in the ceftriaxone group and 22 in the gentamicin group). More than 80% of AEs in each treatment arm were considered mild. Three AEs were considered severe (Table 5).

There were no differences between treatment groups with respect to additional medications (including antibiotics) taken during the trial, reported sexual behaviour or condom use during the trial.

We did not find a clear relationship between *in vitro* gentamicin, ceftriaxone or azithromycin MICs and the response to treatment, with the majority of treatment failures occurring in isolates expected to be susceptible according to EUCAST resistance breakpoints[18] (Figure 3).

Discussion

Our study was unable to demonstrate that a single dose of gentamicin (240 mg) is non-inferior to single dose ceftriaxone (500 mg) for the treatment of gonorrhoea, when both drugs are combined with a 1g dose of oral azithromycin. The trial was not designed to assess superiority, but the 6.4% greater clearance of infection in the ceftriaxone group (lower 95% confidence limit 2.4%), and the consistency of the findings on sensitivity analyses, suggest that ceftriaxone is better than gentamicin for the microbiological cure of gonorrhoea. The failure to show non-inferiority appeared to be because gentamicin had markedly lower clearance rates in pharyngeal and rectal gonorrhoea, (-15.3% and -

7.8% respectively). Gentamicin performed better for genital gonorrhoea, achieving microbiological cure in 94% of infections compared to 98% for ceftriaxone.

Two systematic reviews [10, 11] have reported a wide variation in the efficacy of gentamicin for the treatment of gonorrhoea and noted a significant risk of bias in previous studies. A more recent study evaluating intramuscular gentamicin (240 mg) combined with oral azithromycin (2 g) reported a cure rate of 100% (lower 95% CI 97.6%).[15] This study differed from GToG by using culture to diagnose gonorrhoea, using a 2g dose of azithromycin, involving few women, and including only a small number of participants with pharyngeal and rectal infections. The large number of extra-genital infections included in G-ToG, with their associated lower cure rate, provides a partial explanation for the difference in treatment efficacy reported in the two studies.

Azithromycin as a component of dual therapy

The use of dual therapy with azithromycin 1g did not prevent treatment failure occurring in a significant proportion of participants receiving gentamicin. Azithromycin monotherapy has previously been shown to be effective treatment for gonorrhoea using a single dose of either 1 g or 2 g[19] when culture is used to diagnose infection and assess cure. A reduction in *in vitro* sensitivity to azithromycin has been reported in many geographical locations[20, 21] and occurs in 5% of infections in England and Wales,[1] including a recent outbreak of high level resistance.[22] The large majority of gonococcal isolates from participants in GToG (262/274[96%]) had azithromycin MICs of ≤ 0.5 mg/L. Of the 12 azithromycin resistant isolates (MIC > 0.5 mg/L), 2 (17%) were from patients who had treatment failure, but the majority of failures (11/16[69%]) occurred in participants who had isolates with an MIC ≤ 0.25 mg/L, with the remaining 3 isolates harbouring azithromycin MICs of 0.5 mg/L (intermediate susceptibility). Thus, we found *in vitro* azithromycin resistance did not reliably predict treatment failure with the 1 g azithromycin dose if we assume gentamicin failed to treat. However, further investigation into the relationship between azithromycin MICs and treatment failure is warranted. A poor association between pre-treatment azithromycin MIC and cure has been reported by others, with emergence of *in vivo* resistance.[23, 24] A higher dose of azithromycin than that used in GToG (e.g. 2 g)[15] might be more effective but without a direct comparative study this is speculative, and a 2 g dose is

also poorly tolerated leading to nausea in 26% and vomiting in 10% of patients.[15] An extended-release formulation of azithromycin which has lower peak drug levels might reduce the incidence of side-effects and improve tolerability but there are limited data comparing it directly with the immediate-release formulation.

Current UK, CDC and WHO treatment guidelines for gonorrhoea recommend dual therapy incorporating azithromycin (1g) to reduce the development of resistance in *N. gonorrhoeae* by providing additional microbiological cover should resistance develop.[25] [3, 4] In G-ToG we found significant rates of microbiological failure when using a 1 g dose of azithromycin as part of dual therapy, suggesting that the azithromycin component may not achieve this aim, particularly for patients with extra-genital infection.

Safety of gentamicin and ceftriaxone

Both ceftriaxone and gentamicin, when combined with azithromycin, were well tolerated. Nausea was the most common side effect occurring in 12% of participants receiving ceftriaxone/azithromycin and 14% for gentamicin/ azithromycin. Nausea and vomiting are uncommon side effects of ceftriaxone (incidence ≥ 1/1000 - < 1/100) and have been reported in association with gentamicin, but are common following use of oral azithromycin (≥1/100 to <1/10).[26] It is therefore likely that the gastrointestinal side effects reported were principally caused by azithromycin although the higher reported rate of vomiting in those receiving gentamicin (4% cf. 1% for ceftriaxone) suggests that it may also have been a contributing factor. Gentamicin was associated with more injection site pain than ceftriaxone (mean VAS 36 compared to 21) which took longer to resolve (median 1.5 hours compared to 1 hour), and was probably related to the larger volume of injection (6mls for gentamicin vs 2mls for ceftriaxone) and the local anaesthetic effect of lidocaine as the dissolving agent for ceftriaxone.

Gentamicin is potentially vestibulotoxic, and can cause dizziness, ataxia and nystagmus. Most previous gentamicin studies have evaluated a prolonged course of treatment and the safety of a single dose is less well characterized, but a recent systematic review of single dose therapy found vestibulotoxicity to be rare[27] which is consistent with our findings. Gentamicin can also cause renal impairment following

re-uptake of the drug in the proximal renal tubule where it is concentrated. A transient rise in creatinine is common when single dose gentamicin is used as antibiotic prophylaxis in elderly, surgical patients[27] but this is less likely to occur in younger, healthier individuals, and little change in the eGFR was found in G-ToG participants (-1.4 ml/min [IQR -6.9, 3.8]).

Gentamicin Resistance

The mechanisms for development of gentamicin resistance are not fully understood but may include decreased cell membrane permeability and modification of the drug by cellular enzymes.[28] *In vitro* measurement of the MIC provides a phenotypic assessment of antimicrobial susceptibility but the 'breakpoint' MIC value, below which clinical cure occurs and above which gentamicin is ineffective, has not been established. The penetration of gentamicin into rectal and pharyngeal tissue is not known but has been reported to be suboptimal in the pharynx for spectinomycin, which belongs to a similar antibiotic class.[25] It has been tentatively suggested that an isolate with an MIC < 8 mg/L is susceptible, MIC 8-16 mg/L is intermediate and MIC > 16 mg/L is resistant.[29] The European Network for Sexually Transmitted Infection Surveillance found that 95% of isolates had gentamicin MICs in the range 4-8 mg/L[12] which is similar, after accounting for differences in testing methodology, to G-ToG participants where 74% of isolates had an MIC of 4-8 mg/L. The MIC in GToG participants was not, however, predictive of treatment failure with only three isolates having an MIC > 4 mg/L, all of which were cleared. Of those isolates with an MIC of 4 mg/L treated with gentamicin, 12(13%) failed therapy compared to 81(87%) which were cleared. It is possible that a higher dose of gentamicin would be more effective although the limited association between the gentamicin MIC and clinical response does not directly support this.

Drug Interactions

An antagonistic interaction between gentamicin and azithromycin could potentially reduce the efficacy of the combination. However, *in vitro* testing does not suggest either antagonism or synergy,[30] and the potential for a high cure rate using this regimen in genital infections diagnosed by culture has been demonstrated[15]. A clinically important interaction between the two agents is therefore unlikely.

Trial Design

The G-ToG trial had a robust design resulting in well-balanced treatment arms and a low risk of bias. The trial was appropriately powered, pragmatic in design and likely to be relevant to clinical practice in the UK and other countries with similar health care systems. It included symptomatic and asymptomatic patients, a wide age range, HIV-positive and negative individuals, men and women, heterosexual men and MSM, both genital and extra-genital infections, and a wide variety of ethnic groups. The distribution of age, gender, ethnicity and sites of infection for participants in GToG were comparable to those in the UK Gonoccocal Resistance to Antimicrobials Surveillance Programme (https://www.gov.uk/government/publications/gonococcal-resistance-to-antimicrobials-surveillance-programme-grasp-report) suggesting that our results are widely applicable.

Unexpectedly, a number of patients who were recruited to the trial were found to have a negative NAAT at their baseline visit (ceftriaxone arm n=46 and gentamicin arm n=39) despite having been tested previously and found to be positive, and being recalled to the clinic to be given antibiotic treatment. In routine clinical practice a repeat NAAT would not be performed prior to treatment. The apparent spontaneous reversion from positive to negative NAAT observed in these trial participants could have resulted from an initial false positive NAAT prior to trial entry, a false negative NAAT at the baseline trial visit, or natural clearance of gonorrhoea without antibiotic therapy. A previous large study has reported spontaneous clearance of pharyngeal gonorrhoea in 6% (139/2204) of patients which would be consistent with our findings.[31] However, even though NAATs for *N. gonorrhoeae* have high sensitivities and specificities we cannot exclude the possibility of some false positive/negative results especially when testing a low prevalence population. The occurrence of negative tests in some patients at their baseline visit does not bias our results since they were equally distributed between the treatment arms, and a secondary sensitivity analysis excluding these participants (risk difference -7.1%, [-11.4%, -2.8%]) was consistent with the primary ITT analysis. The NAAT test can remain positive for several days following effective treatment of gonorrhoea but the test of cure was taken at least 14 days after receiving antibiotics in accordance with UK national guidance, to minimise this possibility. In addition, due to the randomised trial design, a false positive test of cure would not bias our results.

In conclusion we found that gentamicin plus azithromycin cannot be considered to be non-inferior to ceftriaxone plus azithromycin, with a relatively high treatment failure rate occurring in patients with extra-genital gonorrhoea. Gentamicin cannot therefore be recommended to replace ceftriaxone as first line therapy for gonorrhoea. However, gentamicin combined with 1g azithromycin achieved a cure rate of 94% for genital gonorrhoea and its use may be appropriate in patients who are allergic, intolerant or harbour a ceftriaxone resistant infection. A 1 g dose of azithromycin as a component of dual therapy for gonorrhoea had limited efficacy in treating gentamicin-resistant infections and this suggests that its widespread use in other combination regimens to prevent the development of resistance requires review.

Author contributions

JR was the Chief Investigator and conceived the study. JR, AAM, LD, TH, LJ, TR contributed to the design of the study. JR, CD, JW, JW were Principal Investigators at recruiting sites. CB, ST and KS were responsible for managing the trial. WT, TH and AAM were responsible for the statistical analysis plan and carried out the statistical analyses. LJ and TR were responsible for the health economic component. MC was responsible for the microbiological component of the trial at PHE. All authors assisted with interpretation of the data. JR, WT, KS, TH, LJ and MC drafted the manuscript. All authors reviewed and approved the final manuscript.

Declarations of interests:

Jonathan Ross reports personal fees from GSK Pharma, Hologic Diagnostics, Tallis plc and Janssen Pharma, outside the submitted work 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) and is an NIHR Journals Editor. Alan A Montgomery is a member of

the NIHR Health Technology Assessment Clinical Evaluation and Trials Board. Janet Wilson reports non-financial support from Hologic/GenProbe and personal fees from BD Diagnostics outside the submitted work. John White reports personal fees from Hologic, GlaxoSmithKline and Becton Dickinson UK Pty Ltd, outside the submitted work; as well as personal fees from SAGE publishing and is Editor in Chief of the International Journal of STDs & AIDS. Trish Hepburn reports ownership of shares in AstraZeneca. During the trial Lelia Duley was the Director of the Nottingham Clinical Trials Unit, a unit with CTU Support funding.

Acknowledgements

We wish to thank the study investigators, Nottingham Clinical Trials Unit staff, site staff, and the participants who took part in this study. We would particularly like to thank our Co-applicants and acknowledge the contributions of the members of the Trial Steering Committee: Prof Judith Stevenson (Chair) Professor of Reproductive and Sexual Health, University College London; Prof John McLeod Professor in Clinical Epidemiology and Primary Care, University of Bristol; Dr Andy Winter, Consultant in Sexual Health and HIV, NHS Greater Glasgow and Clyde; Mr David Roberts-Jones, Patient Representative. And the Data Monitoring Committee Prof Chris Butler, (Chair) Professor of Primary Care, University of Oxford; Dr Mike Bradburn, Senior Statistician, University of Sheffield; Prof Charles Lacey, University of York. We would also like to thank all the study sites (Presented in alphabetical order): Barts Health NHS Trust, Burrell Street Clinic, Chelsea and Westminster Hospital NHS Foundation Trust, Coventry and Warwickshire Partnership Trust, John Hunter Clinic, Chelsea and Westminster Hospital NHS Trust, Manchester Royal Infirmary, Central Manchester University Hospitals NHS Foundation Trust, Royal Berkshire NHS Foundation Trust, Royal Free London NHS Foundation Trust, Sheffield Teaching Hospitals NHS Foundation Trust, St James University Hospital, The Leeds Teaching Hospitals NHS Trust, St Mary's Hospital, Imperial College Healthcare NHS Trust, University Hospitals Birmingham NHS Foundation Trust, Whittall Street Clinic, Western Community Hospital, Solent NHS Trust, West Middlesex, West Middlesex University Hospital, Chelsea and Westminster Hospital NHS Trust.

This project was funded by the National Institute for Health Research Health Technology Assessment Programme (project number 12/127/10). The views and opinions expressed therein are those of the authors and do not necessarily reflect those of the Health Technology Assessment Programme, NIHR, NHS or the Department of Health.

The trial was sponsored by the University of Nottingham, was coordinated from the Nottingham Clinical Trials Unit, and was supported by the NIHR Clinical Research Network.

References

1. Public Health England. Surveillance of antimicrobial resistance in Neisseria gonorrhoeae in England and Wales Key findings from the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP). Data to June 2017. 2017. URL: https://assets.publishing.service.gov.uk/government/uploads/system/uploads/attachment_data/file/651636/GRASP_Report_2017.pdf (accessed 27Apr2018).

2. British Association for Sexual Health and HIV. UK National Guideline for the Management of Gonorrhoea in Adults. URL: https://www.bashh.org/documents/3920.pdf (accessed 05June2017).

3. Centers for Disease Control and Prevention. Sexually Transmitted Diseases Treatment Guidelines. 2015. URL: http://www.cdc.gov/std/tg2015/default.htm. (accessed 14February2018).

4. World Health Organisation. WHO Guidelines for the Treatment of Neisseria gonorrhoeae. 2016. URL: http://apps.who.int/iris/bitstream/10665/246114/1/9789241549691-eng.pdf?ua=1 (accessed 11June2017).

5. Fifer H, Natarajan U, Jones L, Alexander S, Hughes G, Golparian D, et al. Failure of Dual Antimicrobial Therapy in Treatment of Gonorrhea. N Engl J Med 2016;374:2504-6. https://doi.org/10.1056/NEJMc1512757

6. Golparian D, Ohlsson A, Janson H, Lidbrink P, Richtner T, Ekelund O, et al. Four treatment failures of pharyngeal gonorrhoea with ceftriaxone (500 mg) or cefotaxime (500 mg), Sweden, 2013 and 2014. Euro Surveill 2014;19.

7. Alirol E, Wi TE, Bala M, Bazzo ML, Chen XS, Deal C, et al. Multidrug-resistant gonorrhea: A research and development roadmap to discover new medicines. PLoS medicine 2017;14:e1002366. https://doi.org/10.1371/journal.pmed.1002366

8. European Centre for Disease Prevention and Control. Response plan to control and manage the threat of multidrug-resistant gonorrhoea in Europe. 2012. URL: https://ecdc.europa.eu/sites/portal/files/media/en/publications/Publications/1206-ECDC-MDR-gonorrhoea-response-plan.pdf (accessed 13February2018).

9. World Health Organisation. Global action plan to control the spread and impact of antimicrobial resistance in Neisseria gonorrhoeae. 2012. URL: http://www.who.int/reproductivehealth/publications/rtis/9789241503501/en/ (accessed 14February2018).

10. Dowell D, Kirkcaldy RD. Effectiveness of gentamicin for gonorrhoea treatment: systematic review and meta-analysis. Sex Transm Infect 2012;88:589-94. https://doi.org/10.1136/sextrans-2012-050604

11. Hathorn E, Dhasmana D, Duley L, Ross JD. The effectiveness of gentamicin in the treatment of Neisseria gonorrhoeae: a systematic review. Syst Rev 2014;3. https://doi.org/10.1186/2046-4053-3-104

12. Chisholm SA, Quaye N, Cole MJ. An evaluation of gentamicin susceptibility of Neisseria gonorrhoeae isolates in Europe. J Antimicrob Chemother 2011;66. https://doi.org/10.1093/jac/dkq476

13. Kahlmeter G, Dahlager JI. Aminoglycoside toxicity - a review of clinical studies published between 1975 and 1982. J Antimicrob Chemother 1984;13 Suppl A:9-22.

14. Judson FN, Ehret JM, Handsfield HH. Comparative study of ceftriaxone and spectinomycin for treatment of pharyngeal and anorectal gonorrhea. Jama 1985;253:1417-9.

15. Kirkcaldy RD, Weinstock HS, Moore PC, Philip SS, Wiesenfeld HC, Papp JR, et al. The efficacy and safety of gentamicin plus azithromycin and gemifloxacin plus azithromycin as treatment of uncomplicated gonorrhea. Clin Infect Dis 2014;59. https://doi.org/10.1093/cid/ciu521

16. Brittain C, Childs M, Duley L, Harding J, Hepburn T, Meakin G, et al. Gentamicin versus ceftriaxone for the treatment of gonorrhoea (G-TOG trial): study protocol for a randomised trial. Trials 2016;17 p. 558.

17. British Association for Sexual Health and HIV (BASHH). Clinical Effectiveness Group Guidelines. URL: https://www.bashh.org/guidelines (accessed 20June2017).

18. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for interpretation of MICs and zone diameters 2017. URL: http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf. (accessed 05June2017).

19. Bignell C, Garley J. Azithromycin in the treatment of infection with Neisseria gonorrhoeae. Sexually Transmitted Infections 2010;86:422.

20. Barbee LA, Soge OO, Dombrowski JC, Katz DA, Holmes KK, Golden MR. Azithromycin-resistant neisseria gonorrhoeae in men who have sex with men (MSM) in Seattle, Washington: 2014-2015. Sex Transm Infect 2015;91:A25.

21. Ni C, Xue J, Zhang C, Zhou H, van der Veen S. High prevalence of Neisseria gonorrhoeae with high-level resistance to azithromycin in Hangzhou, China. J Antimicrob Chemother 2016;71:2355-7. https://doi.org/10.1093/jac/dkw131

22. Fifer H, Cole M, Hughes G, Padfield S, Smolarchuk C, Woodford N, et al. Sustained transmission of high-level azithromycin-resistant Neisseria gonorrhoeae in England: an observational study. The Lancet Infectious Diseases 2018;18:573-81. https://doi.org/10.1016/S1473-3099(18)30122-1

23. Ison CA, Hussey J, Sankar KN, Evans J, Alexander S. Gonorrhoea treatment failures to cefixime and azithromycin in England, 2010. Eurosurveillance 2011;16:19833. https://doi.org/doi:https://doi.org/10.2807/ese.16.14.19833-en

24. Soge OO, Harger D, Schafer S, Toevs K, Raisler KA, Venator K, et al. Emergence of increased azithromycin resistance during unsuccessful treatment of Neisseria gonorrhoeae infection with azithromycin (Portland, OR, 2011). Sex Transm Dis 2012;39:877-9. https://doi.org/10.1097/OLQ.0b013e3182685d2b

25. Moran JS, Levine WC. Drugs of choice for the treatment of uncomplicated gonococcal infections. Clin Infect Dis 1995;20 Suppl 1:S47-65.

26. Electronic Medicine Compendium URL: https://www.medicines.org.uk/emc/ (accessed 04Apr2018).

27. Hayward RS, Harding J, Molloy R, Land L, Longcroft-Neal K, Moore D, et al. Adverse effects of a single dose of gentamicin in adults: a systematic review. British Journal of Clinical Pharmacology 2018;84:223-38. https://doi.org/10.1111/bcp.13439

28. Mingeot-Leclercq MP, Glupczynski Y, Tulkens PM. Aminoglycosides: activity and resistance. Antimicrob Agents Chemother 1999;43:727-37.

29. Brown LB, Krysiak R, Kamanga G, Mapanje C, Kanyamula H, Banda B, et al. Neisseria gonorrhoeae antimicrobial susceptibility in Lilongwe, Malawi, 2007. Sex Transm Dis 2010;37:169-72. https://doi.org/10.1097/OLQ.0b013e3181bf575c

30. Wind CM, de Vries HJC, van Dam AP. Determination of in vitro synergy for dual antimicrobial therapy against resistant Neisseria gonorrhoeae using Etest and agar dilution. International Journal of Antimicrobial Agents 2015;45:305-8. https://doi.org/https://doi.org/10.1016/j.ijantimicag.2014.10.020

31. Hananta IPY, De Vries HJC, van Dam AP, van Rooijen MS, Soebono H, Schim van der Loeff MF. Persistence after treatment of pharyngeal gonococcal infections in patients of the STI clinic, Amsterdam, the Netherlands, 2012-2015: a retrospective cohort study. Sex Transm Infect 2017;93:467-71. https://doi.org/10.1136/sextrans-2017-053147