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CLINICAL REPORT

Appropriate Time for Test-of-Cure when Diagnosing Gonorrhoea with a Nucleic Acid Amplification Test

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Culture is commonly regarded as the gold standard for diagnosis of *Neisseria gonorrhoeae*. However, nucleic acid amplification tests (NAATs) have rapidly replaced culture for diagnostics in many settings. The aim of the present study was to investigate the appropriate time for test-of-cure (TOC) when NAATs are used for diagnosis of gonorrhoea. In total, 30 patients (28 men and 2 women) provided urethral, cervical, rectal or pharyngeal specimens for TOC. All included patients, except one who did not return for second TOC before day 19, tested negative within 2 weeks after treatment with cefixime 400 mg × 1. Antimicrobial susceptibility testing showed that 68% of the culture-positive strains were resistant to ciprofloxacin. Thus, the recommended empirical treatment with ciprofloxacin in Norway should be changed immediately. TOC can be performed 2 weeks after treatment when NAATs are used for diagnosis of gonorrhoea. *Key words: TOC; test-of-cure; N. gonorrhoeae; PCR; NAAT.*

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Neisseria gonorrhoeae is the aetiological agent of gonorrhoea, which is the second most prevalent bacterial sexually transmitted infection (STI) globally. Microscopy of urethral smears (Gram- or methylene-blue-stained) has a high sensitivity (≥95%) for symptomatic men. However, the sensitivity of microscopy in asymptomatic men or cervical samples is too low for reliable diagnostics (≤55%) (1). Culture has been regarded as the gold standard for diagnosis of gonorrhoea, but nucleic acid amplification tests (NAATs) have rapidly replaced culture due to, among other advantages, their improved sensitivity (2).

However, NAATs do not allow antimicrobial susceptibility testing. This is a major disadvantage, especially when the level of antimicrobial resistance in *N. gonorrhoeae* to all antimicrobials previously recommended as first-line treatment options is high (3–6). Furthermore, susceptibility to the currently recommended first-line treatment, extended-spectrum cephalosporins (ESCs) is declining globally (3, 4, 6). These ESCs, i.e. ceftriaxone (injectable) and cefixime (oral), are the last remaining treatment options in several settings. Using ceftriaxone, no treatment failure of urogenital gonorrhoea has yet been reported. However, verified treatment failures with cefixime, which is the standard treatment in many countries, have been reported in Japan since 2007 (7). Worryingly, the first 2 cases of failure outside Japan were reported recently in Norway (8). Test-of-cure (TOC) after provision of gonorrhoea therapy may soon be crucial in many settings.

Appropriate evidence-based recommendations for the appropriate time for TOC using different NAATs for diagnosis of gonorrhoea is lacking. In contrast to some international guidelines (1, 9), which do not recommend TOC for uncomplicated gonorrhoea, if a recommended treatment has been given, in Norway TOC is recommended for all cases of gonorrhoea. This recommendation has been fortunate considering the reluctance to abandon ciprofloxacin as the recommended empirical treatment for gonorrhoea in Norway (10, 11). Currently a strictly validated in-house *porA* pseudogene polymerase chain reaction (PCR) (12, 13) is used extensively for diagnosis of gonorrhoea in Norway; nevertheless, the national recommendations for TOC are based on the time for TOC using culture, i.e. 7 days.

The aim of the present study was to investigate the appropriate time for TOC when detection of *N. gonorrhoeae* is performed using an in-house *porA* pseudogene PCR.

MATERIALS AND METHODS

Study population

A total of 257 consecutive patients with suspected genital and/or extra-genital gonorrhoea attending an STI outpatient clinic (Olafiaklinikken) in Oslo, Norway were recruited from June 2006 through January 2007. The 257 patients comprised 23 women (mean age 31 years, range 15–46 years) and 234 men (mean age 33.7 years, range 15–73 years), where the majority (66.7%) was men having sex with men (MSM).

The study was approved by the Regional Committee for Medical and Health Research Ethics (REK NORD) and each included participant provided written consent.

Clinical samples

Samples for PCR were collected using either a urethral flocked swab (Copan, Brescia, Italy) or an endocervical flocked swab (Copan). The urethral swab was used for sampling the urethra. The endocervical swab was used for sampling the cervix, rectum and pharynx. Each sample was collected with individual swabs that were placed and transported in universal transport media – room temperature (UTM-RT; Copan) for PCR analysis. The samples for culture were consistently taken before the samples for PCR to avoid reducing the quality of the routine culture diagnostics. The sampling sites were chosen based on the medical history of each patient. Patients were asked to return after one week for a follow-up examination and TOC, and subsequently every week until two negative samples were deposited. In total, 669 clinical samples were collected from the 257 patients. Patients with positive samples who did not return for any TOC within 2 weeks were excluded from the study.

Culture diagnostics

Culture diagnostics were performed at Oslo University Hospital Ullevål, Oslo, Norway as part of their routine diagnostics by identification of characteristic colonies on selective culture medium according to standard laboratory procedures. For thorough species verification, oxidase test, identification of Gram-negative diplococci in microscopy, sugar utilization test, and Phadebact GC Monoclonal test (Bactus AB, Huddinge, Sweden) were used.

Antimicrobial susceptibility testing

Determination of the minimum inhibitory concentrations (MICs, mg/l) of ceftriaxone, ciprofloxacin and spectinomycin was performed using the Etest method according to the instructions of the manufacturer (bioMérieux, Solna, Sweden). Interpretative criteria from the European Committee on Antimicrobial Susceptibility testing (EUCAST, www.eucast.org) were used.

DNA preparation

All the UTM-RT samples were vortexed for 10 s and 200 µl sample were subsequently used for DNA preparation with the infectious disease protocol on the Biorobot M48 workstation (Qiagen, Hilden, Germany), with an elution volume of 100 µl.

Real-time PCR

A real-time TaqMan FAST *porA* pseudogene PCR was performed as previously described (12, 13), using 11.5 µl DNA template and 13.5 µl mastermix. All positive specimens were confirmed by repeated testing from a new DNA isolation.

Antimicrobial treatment

All patients except one were treated with cefixime 400 mg × 1 (oral dose), despite the Norwegian recommendations of using ciprofloxacin for empirical treatment. The remaining patient was administered spectinomycin 2 g × 1 intramuscularly.

RESULTS

A total of 50 gonorrhoea patients was identified. Eight of these patients were excluded because they did not return for any TOC, and 12 were excluded for returning later than 2 weeks after treatment for the first TOC. Accordingly, 30 patients who were *N. gonorrhoeae* positive, diagnosed by culture ($n = 27$) and/or NAAT ($n = 30$), in at least one clinical specimen were further examined. These 30 positive patients comprised two women (mean age 24.5 years, range 21–28) and 28 men (mean age 37.4 years, range 22–58), of whom 50% ($n = 14$) were MSM. Seven clinical specimens (representing different anatomical sites) from 7 patients were positive using NAAT, but negative with culture. Three of these patients (10% of all included patients) did not have any positive culture sample and, accordingly, would have been reported falsely negative if not also NAAT was used for diagnostics. No patients were positive by culture only. Twenty-five patients were diagnosed with gonorrhoea at a single urogenital site, two patients had only extra-genital gonorrhoea (pharyngeal and rectal), and three patients had multiple infected sites (Table SI; available from http://www.medicaljournals.se/acta/content/?doi=10.2340/00015555-1275). All patients diagnosed with urogenital gonorrhoea reported symptoms, such as discharge and dysuria, while all the extra-genital infections were asymptomatic.

Nineteen patients (63%) returned for TOC within 7 days (days 4 to 7) after treatment, and 16 (84%) of these were negative using NAAT. Two of the patients who remained positive (positive TOC on days 4 and 6) provided a negative sample within 14 days (day 11 for both patients). The remaining patient (positive TOC on day 7) did not return before day 19, but then had a negative TOC (Table SI; Fig. 1A).

There were 11 (37%) patients who did not return for their initial TOC before day 8 to day 14 after treatment; however, they were then all negative (Table SI; Fig. 1B).

Antimicrobial resistance testing was performed on *N. gonorrhoeae* isolates from 25 of the 30 included patients. Isolates from 17 (68%) of these patients were ciprofloxacin-resistant; however, no isolate was resistant to ceftriaxone (Table SI), or spectinomycin.

DISCUSSION

The high level of antimicrobial resistance in *N. gonorrhoeae* is a public health problem worldwide. The internationally recommended first-line ESCs are the only remaining options for effective treatment of gonorrhoea in several settings (8, 14). However, the susceptibility to all the ESCs is decreasing and treatment failures of urogenital gonorrhoea have been identified using the oral ESC cefixime (6–8). Furthermore, Ohnishi et al. (15) recently described the first strain with high-level resistance to ceftriaxone, which most likely was related to a treatment failure of pharyngeal gonorrhoea.

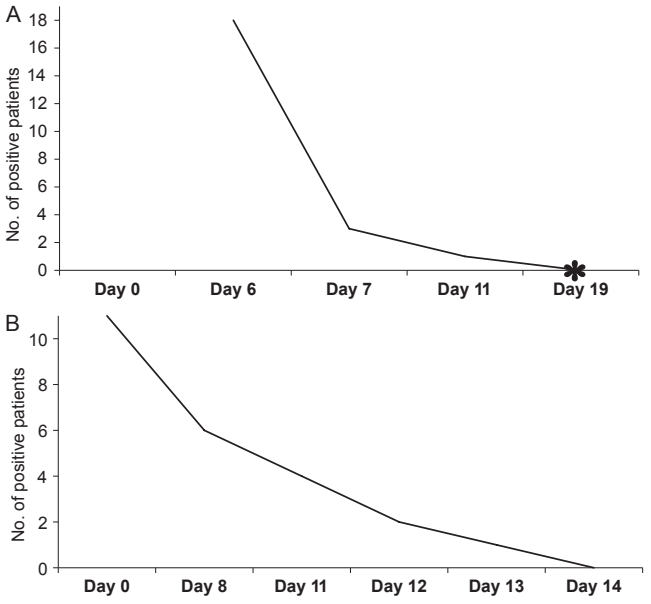

Fig. 1. (A) Number of positive patients, of the ones that returned within 7 days for their first test-of-cure (TOC), following treatment of gonorrhoea. *Did not return before day 19 for the second TOC. (B) Number of positive patients, of the ones that returned after 8 or more days for their first TOC, following treatment of gonorrhoea.

Accordingly, gonorrhoea may become untreatable in certain circumstances and especially in some settings. Appropriate verification/falsification of presumed clinical treatment failures needs to be emphasized worldwide.

A more extensive use of TOC to assess treatment outcome is accordingly warranted. However, the use of TOC for *N. gonorrhoeae* is debated and the recommendations vary for uncomplicated gonorrhoea. Centers for Disease Control and Prevention (CDC) (16) recommend re-testing within 3 months rather than TOC for uncomplicated gonococcal infection, as a high number of positive cases after treatment may be re-infections (17, 18). The British Association of Sexual Health and HIV (BASHH; www.bashh.org/documents/3611) and The International Union against Sexually Transmitted Infections (IUSTI) (1) do not recommend the routine use of TOC for anogenital infections if a recommended treatment has been given. Indications for TOC are: persistence of symptoms, re-exposure to infection, possible antimicrobial resistance, stipulated by national practice or in case of pharyngeal infections. Manavi et al. (19) also recommends TOC for pharyngeal infections because of higher rate of treatment failure. Accordingly, pharyngeal gonococcal infections, in particular, pose an additional problem because these can be difficult to treat (20–22), and are often asymptomatic, resulting in potential reservoirs for further transmission.

NAATs are rapidly replacing culture for detection of *N. gonorrhoeae*, and for non-culture-based diagnostics, such as NAATs, adequate evidence-based recommendations for appropriate time for TOC are lacking. One study has previously been published regarding appropriate time for TOC using NAAT for detection of *N. gonorrhoeae*. This study, by Bachmann et al. (23), examined urine and patient-obtained vaginal swab specimens using ligase chain reaction (LCR). They found that all gonococcal DNA was absent from urine samples by day 6 (regardless of sex) and vaginal swabs by day 9, and concluded that TOC could be taken within 14 days after appropriate treatment regardless of specimen. In the present study all individuals, except one, were negative within 2 weeks after treatment using an in-house *porA* pseudogene PCR. The remaining patient did not return before day 19 for his second TOC, but then had a negative TOC. Furthermore, 84% of the patients returning for their first TOC (within one week) were already negative. The present study using an in-house *porA* pseudogene PCR (12, 13) fully supports the findings by Bachmann et al. (23). Accordingly, an appropriate time for TOC using NAATs for diagnosis of gonorrhoea seems to be 14 days after treatment. It is advantageous to avoid having a longer time before TOC, i.e. to reduce the risk of a positive TOC due to re-infection instead of treatment failure.

This study also showed that 24% of the initial 50 gonorrhoea patients did not return within 2 weeks for TOC despite strict instructions to return already after one week, and 16% never returned for TOC. Failure to return for TOC is a greater concern in cases where only molecular results are available and treatment outcome cannot be assessed due to lack of antimicrobial resistance testing. Partner notification and close follow-up of the index patient was performed vigilantly in this study.

The main limitations of the present study included that it was a low number of examined gonorrhoea patients, several patients did not return for TOC at requested time-points, and additional TOCs were not performed with short intervals (not possible in the routine diagnostics), as performed in the study by Bachman et al. (23).

In Norway, the national recommendation for empirical first-line treatment of gonorrhoea remains ciprofloxacin (10). In the present study, 68% of the culture-positive patients were infected by a ciprofloxacin-resistant *N. gonorrhoeae* strain. Thus, the guidelines for empirical treatment should be changed immediately. This conclusion is further supported by a few previous Norwegian publications (13, 24) as well as numerous international reports (25–29).

In conclusion, an appropriate time for TOC seems to be 14 days after appropriate treatment when an in-house *porA* pseudogene PCR is used for detection of *N. gonorrhoeae*. Despite differences in sensitivity, this time for TOC is most probably similar using most NAATs (at least the DNA-based NAATs). TOC and re-testing should be performed more frequently, as part of a vi-

gilant follow-up of partners and index patients. Facing the threat of untreatable gonorrhoea, the selection and use of antimicrobials should be evidence-based and warrants timely surveillance programmes nationally and internationally. Accordingly, the Norwegian gonorrhoea treatment guidelines should be modified immediately based on the high prevalence of ciprofloxacin-resistant isolates.

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