

BMJ Open Rapid spread of *Neisseria gonorrhoeae* ciprofloxacin resistance due to a newly introduced resistant strain in Nuuk, Greenland, 2012–2015: a community-based prospective cohort study

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ABSTRACT

Objectives: To determine the antimicrobial susceptibility and genotype distribution of *Neisseria gonorrhoeae* strains isolated from a cohort of patients in Nuuk, Greenland in order to assess the risk of rapid spread in the event of introduction of new strains.

Methods: Gonococcal isolates (n=102) obtained from a prospective cohort study of ciprofloxacin resistance were collected between March 2012 and February 2013. Etest minimal inhibitory concentrations (MICs) were determined for ciprofloxacin, azithromycin, ceftriaxone, penicillin, tetracycline, spectinomycin and gentamicin. All isolates were subjected to molecular typing using *N. gonorrhoeae* multiantigen sequence typing (NG-MAST). After the introduction of a ciprofloxacin-resistant strain in early 2014, an additional 18 isolates were characterised.

Results: During the study period, all 102 isolates were fully susceptible to ciprofloxacin (≤ 0.03 mg/L), azithromycin, spectinomycin, gentamicin and ceftriaxone. 10 different NG-MAST types circulated in Nuuk but 7 were found as single isolates, and 3 of the 7 belonged to 1 of the 3 major genogroups (G210, G9816 and G9817) together comprising 96% of the 102 isolates. ST210 accounted for 55% of the 102 strains. The newly introduced ciprofloxacin resistant strain belonged to ST2400 and dominated the population with 59% resistant strains within 6 months after its introduction. All G2400 strains had MICs ≥ 2 mg/L.

Conclusions: Introduction of a ciprofloxacin-resistant strain into a very homogeneous *N. gonorrhoeae* population led to an explosive spread of the resistant clone, probably as a result of large sexual networks suggested by the strain homogeneity. Careful surveillance of antimicrobial susceptibility is essential to avoid widespread treatment failure in closed populations.

INTRODUCTION

Sexually transmitted infections (STIs) including infections with *Neisseria gonorrhoeae* are

Strengths and limitations of this study

- The study comprised all *Neisseria gonorrhoeae* infections diagnosed by culture in Nuuk, the capital of Greenland, and provided information about the rapid spread of a new ciprofloxacin-resistant clone.
- *N. gonorrhoeae* multiantigen sequence typing (NG-MAST) with subsequent genogroup assignment was useful to understand the molecular epidemiology.
- As a limitation, a relatively small proportion of diagnosed gonorrhoea cases were studied by culture for *N. gonorrhoeae*.
- Owing to logistic constraints in this arctic setting, strains from other towns and settlements could not be analysed.

highly prevalent in Greenland.^{1–3} Thus, in 2012, a total of 1435 cases of gonorrhoea were reported corresponding to 2529 cases per 100 000 inhabitants³ compared with only 12.3 cases per 100 000 inhabitants in Denmark in 2012.⁴ The reason for the high prevalence of STIs in the community is complex and not obvious. The most dramatic increase in prevalence of STIs was observed in the 1960s and 1970s and has been linked to social problems caused by dramatic and rapid changes towards a modern industrial society.¹ In general, urbanisation has been related to an increased risk of STIs among many indigenous populations.⁵

Until 2005, the diagnosis of gonorrhoea in Greenland was based on microscopy and culture of *N. gonorrhoeae*. At that time, the diagnostic procedure was replaced by a nucleic acid amplification test (NAAT) (strand displacement amplification, Becton Dickinson ProbeTec) and no systematic

antimicrobial susceptibility testing has been performed. Testing for gonorrhoea has been performed on urine samples since January 2011 and has been offered to all attendees at the healthcare centre. The test was made available to all inhabitants in Nuuk, irrespective of symptoms. Patients with a *N. gonorrhoeae* positive urine test were offered treatment with a single 500 mg oral dose of ciprofloxacin.

In many parts of the world, ciprofloxacin-resistant *N. gonorrhoeae* strains have been observed, and in a 2009 European survey of gonococcal resistance among 1366 strains collected from 17 countries, 63% of all *N. gonorrhoeae* strains were resistant to ciprofloxacin.⁶ Therefore, both American and European treatment guidelines have discouraged treatment with ciprofloxacin without isolation and antimicrobial susceptibility testing.^{7 8} Instead, treatment with an intramuscular injection of ceftriaxone is recommended. However, even this treatment is challenged since cephalosporin-resistant *N. gonorrhoeae* strains have emerged⁹ and gonorrhoea may become difficult to treat in the future.¹⁰ As a consequence, the most updated guidelines now suggest a combination of ceftriaxone and azithromycin.⁷

An older study documented penicillin resistance among 60% of *N. gonorrhoeae* strains isolated in Nuuk, Greenland in 1998 and 1999 and found a single imported strain with ciprofloxacin resistance.¹¹ However, even in the absence of systematic antimicrobial-resistance surveillance, this import did not lead to a spread in the community as suggested by a small recent study which found no ciprofloxacin-resistant *N. gonorrhoeae* among 32 strains isolated in Nuuk in 2011.¹² However, the sample size was small and ciprofloxacin resistance could have been overlooked. Consequently, a larger study was designed with an aim to detect susceptibility to other antibiotics, as well as to determine the genotype distribution of the *N. gonorrhoeae* strains in Nuuk in order to assess the risk of rapid spread in case of introduction of new strains in a homogeneous strain population.

PATIENTS AND METHODS

Greenland has a total population of ~56 000 inhabitants. Nuuk is the largest city and the Queen Ingrid Primary Health Care Centre is the only primary health clinic in Nuuk serving all 16 500 inhabitants with free healthcare service.

Patients attending the Healthcare Centre who had an *N. gonorrhoeae* NAAT-positive urine sample (ProbeTec ET analysed on a Viper automated platform, Becton, Dickinson and Company, Sparks, Maryland, USA) in the study period (March 2012–February 2013) were invited to participate. On return after a positive NAAT test, they were offered an *N. gonorrhoeae* culture test with subsequent ciprofloxacin susceptibility testing in addition to immediate treatment with ciprofloxacin 500 mg given as a single oral dose. Initially, only males were included

due to limited resources, while both males and females were included in the final 4-month period. After completion of the study, monitoring of ciprofloxacin resistance using the established scheme was continued locally in Nuuk. In January 2014, a single ciprofloxacin-resistant isolate was obtained from a patient living in a small settlement on the west coast south of Nuuk who had failed ciprofloxacin treatment but it was initially believed that this introduction was contained as no other resistant strains were isolated in the early phase. However, in September/October 2014, 59% of 44 isolates were ciprofloxacin resistant.¹³ The initial ciprofloxacin-resistant isolate and 17 randomly selected isolates from June 2014 to January 2015 were subsequently included in the present study.

Samples for the *N. gonorrhoeae* culture were taken from the urethra in men and from the cervix in women using a liquid transport medium (ESwab, Copan, Brescia, Italy) for transport to the Central Laboratory at Queen Ingrid Hospital. Culture for *N. gonorrhoeae* was performed on chocolate-agar plates containing selective antibiotics (lincomycin, polymyxin B, trimethoprim and amphotericin B). Identification of *N. gonorrhoeae* strains was performed on Gram-negative, oxidase-positive diplococci using Minibact-N (Statens Serum Institut (SSI), Copenhagen, Denmark). Ciprofloxacin susceptibility testing was performed with Etest (bioMérieux S.A., Marcy l'Etoile, France) on chocolate-agar plates without antibiotics. The WHO reference strains G, K, M, O and P¹⁴ were used for quality control and showed ciprofloxacin minimal inhibitory concentration (MIC) within accepted ranges. Strains with a MIC for ciprofloxacin ≤ 0.032 mg/L were considered susceptible. No test of cure was performed, as this is not routine practice in Greenland.

After isolation and identification, all the *N. gonorrhoeae* strains were frozen at -80°C and transported by air to SSI in Copenhagen, Denmark. At SSI, MICs were determined with Etest for azithromycin, ceftriaxone, benzylpenicillin, tetracycline, spectinomycin and gentamicin. The WHO MIC breakpoints were used where available; the European Committee on Antimicrobial Susceptibility Testing (EUCAST) azithromycin and tetracycline and the US Gonococcal Isolate Surveillance Project (GISP) gentamicin tentative breakpoints were used.¹⁵ In addition, the *N. gonorrhoeae* strains were tested for production of β -lactamase using the nitrocefin test.¹⁵

N. gonorrhoeae multiantigen sequence typing (NG-MAST) DNA strain typing¹⁶ was performed according to the standard protocol with minor modifications (use of a Hot-start Taq-DNA polymerase and a touch-down PCR), and sequence types (STs) were assigned using the <http://www.ng-mast.net> website. Sequences were analysed in BioNumerics V.6.6 (Applied Maths, Sint-Martens-Latem, Belgium). In order to study the relationship between strains with different STs, the *porB* and *tbpB* sequences were concatenated and trees were constructed. Closely related STs were clustered using a previously described genogroup definition.¹⁷ Thus, STs

that shared one identical allele, with the other allele showing $\geq 99\%$ identity, were clustered together in a genogroup, named after the predominant ST.

Variables were described using medians and quartiles (Q1–Q3). Medians were compared using the Mann–Whitney U-test. The p value at 0.05 was used as the level of significance. Estimates were calculated with 95% CIs.

RESULTS

Antimicrobial susceptibility

A total of 102 *N. gonorrhoeae* strains were isolated in Nuuk during the study period. The *N. gonorrhoeae* strains were mainly isolated from males (81 males and 21 females) since females were only included in the last 4 months of the study period. The median age for the whole group was 29 (22–41) years. All 102 *N. gonorrhoeae* strains were transferred to SSI, and were susceptible to ciprofloxacin with MICs below 0.005 mg/L (range <0.002–0.004 mg/L) indicating a low prevalence of ciprofloxacin-resistant *N. gonorrhoeae* strains in Greenland (95% CI 0% to 3.6%). The result of the antimicrobial susceptibility tests are shown in table 1. All strains were fully susceptible to ceftriaxone, spectinomycin, azithromycin and gentamicin while only 8% of the strains were fully susceptible to benzylpenicillin (MIC ≤ 0.06 mg/L). However, only two strains were resistant with MICs of 1.5 mg/L, and none of the strains were β -lactamase producing. No strain was tetracycline resistant, but 21 had intermediate susceptibility. Among the 18 strains collected in June 2014–January 2015 after the introduction of ciprofloxacin resistance (including the index strain from January 2014), 4 (22%) were ciprofloxacin susceptible, whereas 14 (78%) were resistant to ciprofloxacin. The index ciprofloxacin-resistant ST 2400 strain was susceptible to ceftriaxone, tetracycline, gentamicin and spectinomycin, and had intermediate susceptibility to benzylpenicillin and azithromycin.

Molecular typing

Strain typing by NG-MAST revealed that 10 different NG-MAST types circulated in Nuuk in the study period. Of these, seven were found as single isolates, and three of the seven were in the same genogroup as one of the

major clusters. Seven new NG-MAST types that had not been previously described were found. A major cluster comprising 56 strains (55% of all examined strains) belonged to ST 210, and the second largest cluster comprised 31 strains (30% of all examined strains) belonging to ST 9816, a newly discovered ST with two additional strains (STs 9975 and 9977, respectively) belonging to the same genogroup. The third cluster comprised eight ST 9817 with an additional ST 9976 belonging to the same genogroup 9817. When a tree was constructed with the concatenated sequences (figure 1), it was shown that 98 (96%) of the 102 strains from the study period belonged to one of the three major genogroups. All three clusters were apparently present in equal proportions throughout the study period, suggesting ongoing transmission in three different networks. However, the number of strains were limited. Four unrelated *N. gonorrhoeae* strains were also identified, suggesting infections contracted outside Nuuk in other cities in Greenland or even outside of Greenland.

The ciprofloxacin-resistant strain introduced in January 2014 belonged to a unique ST 2400 not previously found in Greenland. Apparently, this ST further developed with two isolates belonging to ST11783, which differed in the *porB* allele in only two nucleotide positions from the ST2400 *porB* allele (allele type 1489), thus, belonging to genogroup 2400. The four ciprofloxacin-susceptible strains isolated in 2014 all belonged to ST210, present as the largest cluster during the study period.

DISCUSSION

This study documented absence of ciprofloxacin-resistant *N. gonorrhoeae* during the study period (95% CI <3.6%) in Nuuk, Greenland. In addition, the *N. gonorrhoeae* strains were highly susceptible to all the examined antibiotics with the exception of benzylpenicillin to which most strains had intermediate susceptibility and probably could be treated with high doses.

The study also demonstrated that monitoring of antimicrobial resistance among *N. gonorrhoeae* cases in Nuuk was feasible. Fortunately, the monitoring was continued after the study ended. This enabled early detection of the introduction of a new ciprofloxacin-resistant strain and

Table 1 MIC in mg/L and susceptibility according to the EUCAST (http://www.eucast.org/clinical_breakpoints) for selected antimicrobial agents among 102 ciprofloxacin susceptible *Neisseria gonorrhoeae* strains isolated in Nuuk, Greenland

Antimicrobial	MIC ₅₀	MIC ₉₀	MIC range	Susceptible N	Breakpoint mg/L	Intermediate N	Resistant N	Breakpoint (mg/L)
Ceftriaxone	0.003	0.004	<0.002–0.006	102 (100%)	≤ 0.125	0	0	>0.125
Benzylpenicillin	0.125	0.19	0.064–1.5	8 (8%)	≤ 0.06	92 (90%)	2 (2%)	>1
Spectinomycin	12.0	12	4–12	102 (100%)	≤ 64	0	0	>64
Azithromycin	0.064	0.125	0.023–0.125	102 (100%)	≤ 0.25	0	0	>0.5
Tetracycline	0.38	0.75	0.064–0.75	81 (79%)	≤ 0.5	21 (21%)	0	>1
Gentamicin*	2.0	3.0	1.5–4.0	102 (100%)	≤ 4	0	0	>4

*US GISP MIC breakpoint.

EUCAST, European Committee on Antimicrobial Susceptibility Testing; MIC, minimal inhibitory concentration.

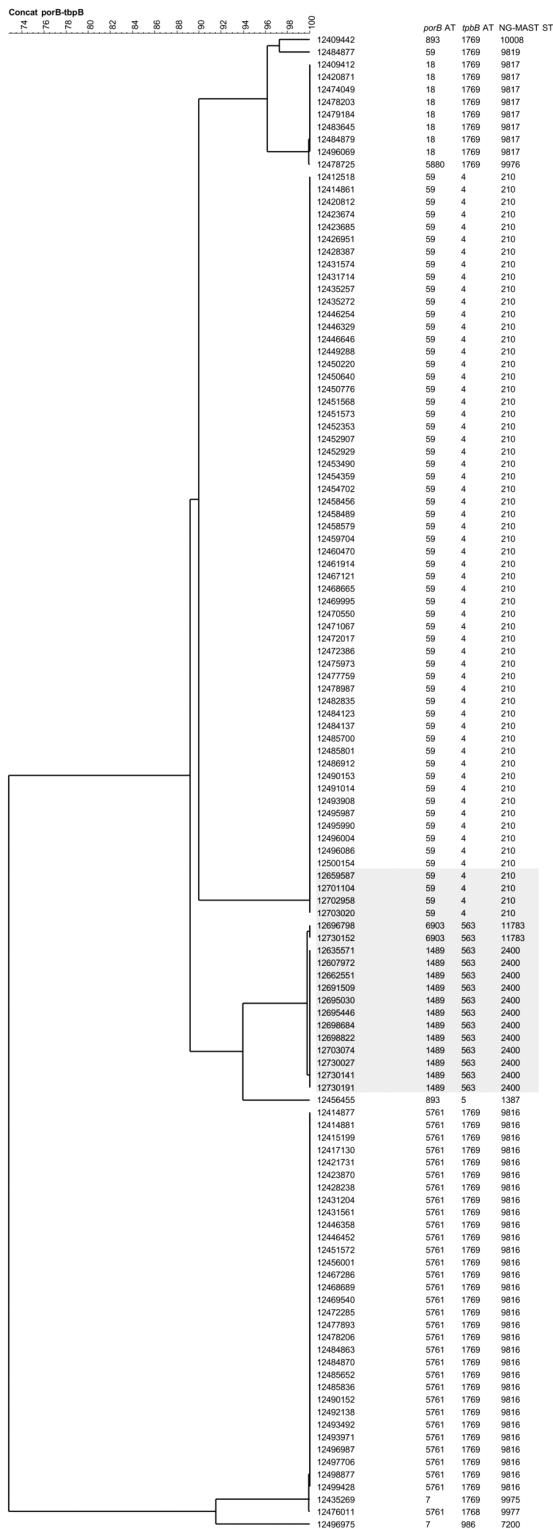


Figure 1 Dendrogram constructed by pairwise alignment and Unweighted Pair Group Method with Arithmetic Mean (UPGMA) clustering showing concatemerised *tbpB* and *porB* sequences of 120 *Neisseria gonorrhoeae* strains. The 18 strains isolated during the outbreak of ciprofloxacin-resistant *N. gonorrhoeae* are marked with grey shading. NG-MAST, *N. gonorrhoeae* multiantigen sequence typing; AT, allele type; ST, sequence type.

led to a subsequent rapid change in the treatment guidelines for Greenland. Unfortunately, antimicrobial susceptibility testing was not possible outside of Nuuk since culture can only be performed in Nuuk, and transport of samples for culture within Greenland is not realistic as a daily routine due to logistic and economic constraints.

Surprisingly, all *N. gonorrhoeae* strains were fully susceptible to azithromycin, which is almost universally used for treatment of *Chlamydia trachomatis* infections as a single 1 g dose. *C. trachomatis* infections are extremely common in Greenland with an incidence in 2012 of 6358 cases per 100 000 inhabitants³ compared with 473 cases per 100 000 inhabitants in Denmark in the same year.¹⁸ The high use of azithromycin for chlamydia is believed to be the explanation for the unusual 100% prevalence of macrolide resistance in *Mycoplasma genitalium* in Greenland¹⁹ and since *C. trachomatis* and *N. gonorrhoeae* are commonly found as co-infections, selection of macrolide resistance in *N. gonorrhoeae* would have been expected. On the other hand, undetected *N. gonorrhoeae* infections may have been occasionally treated together with the chlamydial infection keeping the gonorrhoea prevalence lower.

A remarkable homogeneity in the *N. gonorrhoeae* population structure was found. Although seven STs with only one representative was found, several of the rare types were very similar to the types in the major clusters with only a few base changes in one of the two alleles, suggesting that they were variants of the predominant types (figure 1). This was particularly clear when a tree was constructed with the concatemerised sequences. Here, it was shown that 98 (96%) of the 102 strains from the study period belonged to one of the three major clusters. Thus, only three sequence clusters were responsible for 96% of the cases throughout the study with no apparent temporary change in the ST distribution. This could indicate few but widespread sexual networks in Nuuk, although behavioural data and possible links between the cases were not collected. Such large networks are at high risk of a rapid spread of newly introduced clones, and this was indeed the case in the Nuuk population with the introduction of the ciprofloxacin-resistant ST2400 strain. The first case was found due to treatment failure in a small settlement south of Nuuk in early 2014, intensive partner tracing was carried out and the spread of the strain was believed to be contained. However, in March 2014, the first ciprofloxacin-resistant isolate was detected in Nuuk as a result of the ongoing surveillance. By careful partner tracing, additionally four resistant isolates were found until August 2014, but despite this effort the proportion of ciprofloxacin-resistant strains increased dramatically to 59% in September/October.¹³ Consequently, in October 2014, it was decided to change therapy from the previously used ciprofloxacin regimen to the internationally recommended dual therapy with ceftriaxone 500 mg

intramuscular supplemented with 2 g of azithromycin orally, both as a single dose.⁸

The ST210 clone dominating before the introduction of ciprofloxacin resistance is quite common worldwide and was found in Denmark as part of an outbreak of prolyliminopeptidase (PIP) negative *N. gonorrhoeae* strains isolated in 2002 and 2003.^{20–21} It has also been previously reported in Canada.^{22–23} Whether the strain was introduced from Denmark or Canada cannot be concluded, but it has shown a strong tendency to spread and persist also in other populations.²¹ The ciprofloxacin ST2400 clone spreading rapidly has become very common in Europe (M Unemo, personal communication, 2016), and its introduction in Greenland may well be from a European source. The finding that it rapidly branched out to the very closely related novel ST11783 is interesting and it could be speculated that the shift in environment led to the change.

In conclusion, the homogeneous population structure of the *N. gonorrhoeae* strains in Nuuk, Greenland suggested that introduction of new resistant strains could spread rapidly due to the presence of large sexual networks, and this was clearly demonstrated by the introduction of the ciprofloxacin-resistant ST2400 strain, which became dominant within a very short period of time. Monitoring of antimicrobial susceptibility was shown to be crucial for rapid intervention with a change in treatment, but improvement of patient management with better partner tracing is also warranted.

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Data sharing statement MIC values for the individual strains included in the study are available by contacting the corresponding author.

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