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► **To cite this version:**

Imali Fernando, Helen Palmer, Hugh Young. Characteristics of patients infected with common *Neisseria gonorrhoeae* NG-MAST sequence type strains presenting at the Edinburgh GUM clinic.. *Sexually Transmitted Infections*, 2009, 85 (6), pp.443. 10.1136/sti.2008.034538 . hal-00552803

**HAL Id: hal-00552803**

**<https://hal.science/hal-00552803>**

Submitted on 6 Jan 2011

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**Title: Characteristics of patients infected with common *Neisseria gonorrhoeae* NG-MAST sequence type strains presenting at the Edinburgh GUM clinic.**

**Short title: Common *N. gonorrhoeae* NG-MAST STs at a GUM clinic**

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**Summary (160 words):**

**Objective:** Characterisation of population groups infected with common *Neisseria gonorrhoeae* NG-MAST sequence types (ST), presenting at Edinburgh GUM.

**Methods:** All patients with gonococcal infection attending over a two-year period were reviewed. Patients infected with unique, paired and clustered gonococcal STs were compared. Characteristics of patients infected with common STs were analysed. Concordance of gonococcal strains between sexual partners was examined.

**Results:** There were 78 unique, 17 paired, and 34 clustered STs: the three groups varied significantly in relation to patient gender and origin/ location of recent sexual contacts. There were nine large ST clusters (containing 11-24 isolates each), and these varied in terms of patient gender, sexual orientation and HIV prevalence. There was high concordance (94%) of sequence types between sexual contacts.

**Conclusion:** There was a trend towards significance when comparing the risks of carriage/ contact with HIV between different ST clusters. Further research is therefore warranted to determine if NG-MAST data can be used to help identify high-risk sexual networks.

**Keywords (MESH HEADINGS – MAX 5):**

NG-MAST sequence type, *Neisseria gonorrhoeae*, HIV, sexual networks

**Key messages:**

- There is a diversity of gonococcal strains circulating in Edinburgh, with infection episodes involving unique, paired and clustered NG-MAST sequence types.
- There was high concordance of sequence types between identified sexual contacts.
- The gonococcal strain clusters were distinct from one another, **indicating that several separate transmission networks are present in Lothian.**
- Patients contracting gonococcal infection of certain sequence types may be at an elevated risk of carriage/ contact with HIV.

## **Introduction:**

Genital *Neisseria gonorrhoeae* is the second commonest bacterial STI in the UK, with over 18,000 infections diagnosed in Genitourinary Medicine clinics during 2007.<sup>1</sup> It carries the risk of long-term complications in women and can cause ophthalmic disease in neonates following perinatal transmission.<sup>2</sup> In addition, in common with other bacterial STIs, genital *N. gonorrhoeae* directly facilitates HIV transmission by increasing both donor infectivity<sup>3,4</sup> and recipient susceptibility.<sup>5-7</sup>

Importantly, infection with *Neisseria gonorrhoeae* has also been demonstrated to be a marker of high-risk sexual activity.<sup>8,9</sup> Thus, elucidation of local *N. gonorrhoeae* transmission chains can be helpful in the delineation of high-risk community sexual networks, and in targeting public health interventions.<sup>10</sup> Over the last decade, the introduction of molecular techniques for typing *N. gonorrhoeae* (e.g. opa typing,<sup>11-13</sup> por gene sequencing<sup>9,14</sup>) has greatly augmented information obtained by routine contact tracing methods.

The *Neisseria gonorrhoeae* Multi Antigen Sequence Typing (NG-MAST) system<sup>15</sup> is the latest innovation in molecular typing and has been in use at the Scottish Bacterial Sexually Transmitted Infections Reference Laboratory (SBSTIRL) since April 2004. The NG-MAST system consists of sequencing internal fragments of two highly polymorphic gene loci: *por* and *tbpB* genes. These genes code for bacterial outer membrane proteins, requiring ongoing rapid evolution to evade host immunity. Research has validated the use of NG-MAST in deciphering local *N. gonorrhoeae* transmission patterns in large urban centres, demonstrating common demographic and behavioural characteristics in groups of individuals infected with the same gonococcal sequence type (ST) strain.<sup>16</sup>

The Edinburgh GUM clinic is situated in the city centre, and serves the Lothian Health Board catchment area. In 2006, it managed a total of 23,972 patient visits: 14,266 new and rebook and 9706 return patients.<sup>17</sup> Data released by Health Protection Scotland (HPS) for 2006 showed a total of 900 *Neisseria gonorrhoeae* infections reported from Scotland overall; a significant proportion (205, 23%) were from the Lothian Health Board.<sup>18</sup> The gonococcal incidence rate in Lothian for 2006 was 29 per 100000, the second highest in Scotland.<sup>18</sup>

Thus, studying characteristics of population groups infected with common ST strains presenting at the Edinburgh GUM clinic may grant valuable insight into Scottish urban high-risk sexual networks. With this in mind, we undertook a retrospective study to review all gonococcal infections managed at the Edinburgh GUM clinic over a two-year period.

## **Methods:**

All culture confirmed *N. gonorrhoeae* positive patients who attended the Edinburgh GUM Clinic in the period 01/04/04 to 31/03/06 were included in the study. Case records of the patients were reviewed and the following data recorded: patient clinic number, age, gender, sexual orientation, recent sexual history including origin and location of sexual contacts, date positive sample obtained, concurrent STIs diagnosed at visit and HIV sero-status at time of visit. The NG-MAST sequence type (ST) assigned to each patient isolate at diagnosis, by the SBSTIRL, was also ascertained.

Patient HIV positive sero-status was defined as follows: patients were considered HIV positive if diagnosed prior to or at time of incident *N. gonorrhoeae* infection. Patients were considered HIV negative if there was a documented HIV negative test result within the 3-months prior to the

incident infection (or at any point following it). Patients were deemed of unknown HIV status if there was no record of a HIV test within 3-months of the incident *N. gonorrhoeae* infection or at a point following this.

Patients were considered to have repeat infections if they had repeat gonococcal diagnoses at attendances more than 4 weeks apart. (A single patient was however considered to have repeat infections following gonococcal diagnoses 3-weeks apart. This was due to him having a negative test of cure between these two repeat attendances, and also having different strains of gonococcal infection at these attendances.)

Health advisor records of partner notification for the gonococcal infections were reviewed in detail and all positive partners were identified. Clinic protocol for partner notification 'look-back' periods was compatible with national guidelines.<sup>19</sup>

SPSS 14.0 software (manufacturer SPSS incorporated, 233 South Wacker Drive, 11<sup>th</sup> Floor, Chicago, USA) was used for data analysis. Patient characteristics were compared using the Chi-squared test, results considered statistically significant when  $p < 0.05$ . Where the small size of some patient groups prevented the use of the Chi-squared test, the Fischer's test was used instead (these instances are specifically mentioned in the text).

## **Results:**

### ***Overview of all patients with a *N. gonorrhoeae* diagnosis –***

Over the study period, 370 culture positive *N. gonorrhoeae* infections were diagnosed at the Edinburgh GUM clinic. Three hundred and thirty seven patients were infected (298 men and 39 women). Twenty seven patients (8%) had repeat infections during the study period: 22 patients suffering two separate infections, four patients suffering three infections and one patient contracting four infection episodes over the study period.

Of the 337 individuals diagnosed with gonococcal infection on one or more occasions, 33 (10%) were HIV positive at clinic attendance, 213 (63%) were negative and 92 (27%) had unknown sero-status. Twenty nine of the 33 HIV positive individuals in the study were already identified HIV positive at the time of the incident *N. gonorrhoeae* infection, while the remaining four received simultaneous diagnoses of gonococcal infection and positive HIV sero-status. One of these four patients presented with two episodes of gonococcal infection over the course of the study period (20-months apart) and had a negative HIV test at his first attendance.

### ***Comparison of patients with single and repeat gonococcal infections during the study –***

There was no significant difference (using Fisher's test) in gender distribution between patients presenting with single and repeated gonococcal infections over the study period: 273 of the 310 patients presenting with a single infection were male, comparable with 25 of the 27 patients with repeat infections.

Patients with repeat gonococcal infections were however significantly more likely to be of gay/ bisexual orientation ( $p=0.04$ ) and positive HIV sero-status ( $p=0.0003$ ). Thus, of the 27 patients with repeat *N. gonorrhoeae* infections, 21 were gay/ bisexual and 8 were HIV sero-positive. In comparison, only 179 of the 310 patients with a single *N. gonorrhoeae* infection were gay/ bisexual and only 25 were HIV sero-positive.

The 27 patients with repeat infections had a total of 60 gonococcal infection episodes over the study period, in 23 of these an additional concurrent STI was diagnosed. Of the 310 patients presenting with single gonococcal infection, a concurrent STI was discovered in 93 of the infection incidents. Concurrent STI diagnosis was not statistically different between the two groups.

**Comparison of patients with unique and repeat NG-MAST gonococcal sequence types –**

From the total 370 *N. gonorrhoeae* incident infections over the study period, 129 NG-MAST distinct sequence types (STs) were identified. Seventy-eight sequence types occurred only once during the study period, 17 sequence types were represented by pairs of isolates and 34 sequence types were represented by clusters of 3-24 isolates over the study period.

	Unique sequence types	Paired sequence types	Clustered sequence types	p value
Total isolates	78 (21%)	34 (9%)	258 (70%)	
Age (years)	Mean 32 Median 30	Mean 30 Median 28	Mean 31 Median 29	
Gender	Male 75 (96%) Female 3 (4%)	Male 27 (79%) Female 7 (21%)	Male 227 (88%) Female 31 (12%)	<b><u>0.0236</u></b>
Sexual orientation	Heterosexual 34 (44%) Homosexual/ bisexual 44 (56%)	Heterosexual 19 (56%) Homosexual/ bisexual 15 (44%)	Heterosexual 92 (36%) Homosexual/ bisexual 166 (64%)	0.0508
HIV sero-status	Positive 12 (15%) Negative/ did not test 66 (85%)	Positive 3 (9%) Negative/ did not test 31 (91%)	Positive 29 (11%) Negative/ did not test 229 (89%)	0.5174
Location of recent sexual contacts	Within the clinic catchment area only 22 (28%) Outside the clinic	Within the clinic catchment area only 22 (65%) Outside the clinic	Within the clinic catchment area only 132 (51%) Outside the clinic	<b><u>0.0005</u></b>

	catchment area 47 (60%)  Not known 9 (12%)	catchment area 8 (23%)  Not known 4 (12%)	catchment area 94 (37%)  Not known 32 (12%)	
Other STI at visit*	Yes 18 (23%)  No 60 (77%)	Yes 11 (32%)  No 23 (68%)	Yes 87 (34%)  No 171 (66%)	0.2049

**Table 1. Characteristics of patients with unique, paired and cluster NG-MAST gonococcal sequence types**

(\*Other STIs: Chlamydia, Syphilis, Trichomoniasis, genital warts or genital herpes)

As shown in Table 1, there was no significant differences between the three groups in relation to HIV sero-status or the presence of a concurrent STI. Male gender was predominant in all three groups, but was least pronounced among patients with paired NG-MAST strains and most pronounced among patients with unique NG-MAST strains. Similarly, there was some variation in the distribution of sexual orientation between the patient categories, though this difference did not reach full statistical significance.

There was however a very statistically significant difference between the location of recent sexual contacts between the three groups; patients infected with gonococcal strains with unique sequence types were significantly more likely to have had contacts from outside the clinic catchment.

**Analysis of the large NG-MAST sequence type clusters –**

There were nine large ST clusters over the study period involving 10 or more isolates each, and 151 isolates in total (41%). The characteristics of patients in these clusters are detailed in Table 2. Six of the nine large clusters contained both identified HIV infected and non-infected patients and seven affected a predominantly (>75%) homosexual/bisexual population.

	Cluster ST 4	Cluster ST 26	Cluster ST 249	Cluster ST 5	Cluster ST 64	Cluster ST 210	Cluster ST 147	Cluster ST 225	Cluster ST 40
Total isolates	11	11	12	13	17	20	21	22	24
Mean (Median) age in years	35 (32)	25 (24)	24 (21)	32 (30)	32(29)	36 (36.5)	35 (30)	29 (29)	30 (26.5)
Gender	Males 11 (100%)	Males 11 (100%)	Males 6 (50%) Females 6 (50%)	Males 11 (85%) Females 2 (15%)	Males 17 (100%)	Males 20 (100%)	Males 17 (81%) Females 4 (19%)	Males 21(95%) Females 1 (5%)	Males 24 (100%)
Sexual orientation	Heterosexual 1(9%) Homo/bisexual 10 (91%)	Homo/bisexual 11 (100%)	Heterosexual 11 (92%) Homo/bisexual 1 (8%)	Heterosexual 3 (23%) Homo/bisexual 10 (77%)	Heterosexual 4 (24%) Homo/bisexual 13 (76%)	Heterosexual 1 (5%) Homo/bisexual 19 (95%)	Heterosexual 12 (57%) Homo/bisexual 9 (43%)	Heterosexual 2 (9%) Homo/bisexual 20 (91%)	Homo/bisexual 24 (100%)
HIV sero-status	Negative 8 (73%) Not tested 3 (27%)	Positive 2 (18%) Negative 6 (55%) Not tested 3 (27%)	Negative 10 (83%) Not tested 2 (17%)	Negative 9 (69%) Not tested 4 (31%)	Positive 1 (6%) Negative 8 (47%) Not tested 8 (47%)	Positive 2 (10%) Negative 15 (75%) Not tested 3 (15%)	Positive 3 (14%) Negative 10 (48%) Not tested 8 (38%)	Positive 6 (27%) Negative 16 (73%)	Positive 3 (12%) Negative 17 (71%) Not tested 4 (17%)
Other STI diagnosed at visit	Chlamydia 2 (18%) Nil 9 (82%)	Chlamydia 3 (27%) Nil 8 (73%)	Chlamydia 7 (50%) Other STI (warts) 1 (8%) Nil 5 (42%)	Chlamydia 2 (15%) Nil 11 (85%)	Chlamydia 2(12%) Nil 15 (88%)	Chlamydia 3 (15%) Nil 17 (85%)	Chlamydia 7 (33%) Other STI (syphilis) 1 (5%) Nil 13 (62%)	Chlamydia 6 (27%) Other STI (syphilis) 2 (9%) Nil 14 (64%)	Chlamydia 4 (17%) Nil 20 (83%)

**Table 2. Characteristics of patients in the various large NG-MAST sequence type cluster patient populations**

In our study, the persistence of STs over time was variable, with some occurring over the entire study period (e.g. ST 147), while others were restricted to a period of about a year (e.g. ST 4).

As shown in Table 2, some of the large sequence type clusters also exhibited markedly divergent patient characteristics: for example, the two most disparate clusters involving ST 225 and ST 249, diverged widely in distribution of patient gender, sexual orientation and HIV positive prevalence. Comparing these two very distinctive sequence type clusters, this discrepancy in gender and sexual orientation was statistically significant by the Fischer's test ( $p < 0.05$ ). The difference in HIV positivity rates between the two ST clusters however failed to reach full statistical significance, though there was a trend towards this ( $p = 0.07$ ).

#### ***Concordance of sequence types in identified sexual contacts –***

Of the 370 gonococcal infection episodes in the study, 96 occurred in sexual partners identified by routine contact tracing. There was extremely high concordance of *N. gonorrhoeae* sequence types between these sexual contacts, with identical sequence patterns occurring in all cases except 6 (6%).

Of the three discordant sexual partners in the study, one pair's *N. gonorrhoeae* isolate sequence types only differed by a single nucleotide in the *por* alleles (349 versus 930), implying evolution of the gonococcal strain rather than a true mismatch. It is noteworthy that the gonococcal cultures from these sexual partners were performed more than 3 weeks apart. In the remaining two sexual contact pairs, the sequence types differed widely between partners. In both cases, at least one partner admitted to multiple sexual contacts in the period preceding the gonococcal diagnosis.

#### **Discussion:**

These data demonstrate that the patient population attending the Edinburgh GUM clinic suffered both single and repeat episodes of gonococcal infection over the study period. Patients presenting with repeat gonococcal infections more commonly had differing *N. gonorrhoeae* sequence types isolated at repeat attendances (67%) – suggesting these individuals were part of more than one network of gonococcal infections. The remainder, with repeated infections of the same ST, were most likely to signify re-infection rather than treatment failure, since all patients were treated with cefixime<sup>19</sup> and there have been no records of resistant isolates in Scotland.<sup>20</sup>

Our research also confirmed the previously identified high concordance of *N. gonorrhoeae* sequence types between identified sexual contacts,<sup>21</sup> with identical STs found in 94% of known sexual partners.

The primary aim of our study was characterisation of patient groups affected with common sequence types of *N. gonorrhoeae* attending the Edinburgh GUM clinic. Our results indicate a diversity of gonococcal strains circulating in Edinburgh, with 21% (78/370) of infection episodes involving a unique ST, 9% (34) a paired ST and 70% (258) a clustered ST. These three patient groups varied in terms of gender distribution and the location/ origin of sexual: as may be expected, patients with unique gonococcal STs were significantly more likely to have had sexual contacts from outside the clinic catchment area.

Of the sequence type clusters identified in the study, the nine largest included over 10 isolates each, indicating that several distinct transmission networks were present in Lothian. Interestingly,

the six largest study ST clusters (ST 5, 64, 210, 147, 225 and 40) were among the ten most frequent sequence types identified Scotland-wide in the same time period.<sup>22</sup> In addition, the gonococcal strains associated with the five largest clusters in our study (STs 40, 225, 147, 210 and 64) were duplicated among the 21 commonest cluster strains identified in Choudhury et al's 2004 London study (which examined *N. gonorrhoeae* infections presenting at 13 London GUM clinics).<sup>16</sup> This suggests that gonococcal transmission networks commonly extend beyond clinic catchments, and both local and national contexts should be included in any epidemiological deliberations.

In our research, there was marked divergence of patient characteristics between some sequence type clusters, in terms of spread of gender, sexual orientation and HIV positive sero-status. These discrepancies, except HIV sero-status, reached full statistical significance when comparing the very distinctive sequence type clusters of ST 225 and ST 229. There was also a trend towards significance in the HIV positive prevalence between the two clusters, though this failed to reach full significance within the small patient groups evaluated.

This implies that, even within this already high risk population with incident *N. gonorrhoeae* infections, contracting a gonococcal infection with certain sequence types (e.g. ST 225) may confer a further elevated chance of carriage/ contact with HIV. This interesting finding therefore warrants further investigation with larger studies, evaluating bigger sequence type clusters. It must also however be acknowledged that, even if certain STs were shown to associated with an increased HIV risk, this risk relationship may vary temporally and geographically, and thus requires ascertainment on a local basis and continuous ongoing monitoring. With these caveats, our research suggests a possible novel mechanism for future identification of members of high-risk sexual networks from clinic populations, enabling more specific targeting of health promotion intervention efforts.

(following inclusion of extra data as requested by the reviewers)

**Acknowledgements:**

We thank Dr K Welch for his help with statistical analysis for the study.

**Contributions of each author:**

- IF gathered data from case note review and wrote the paper.
- HP supplied the NG-MAST sequence typing analysis and reviewed the paper.
- HY supplied the original research concept.

**Statement:**

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