BACTERIAL STI

The prevalence of proline iminopeptidase negative *Neisseria gonorrhoeae* throughout England and Wales

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Background: The accurate laboratory identification of *Neisseria gonorrhoeae* is an essential element of the diagnosis of gonorrhoea and is particularly important for medicolegal cases. The detection of proline iminopeptidase (Pip) activity is widely used as a marker for gonococci, although Pip negative *N gonorrhoeae* isolates have been shown to generate false negative identifications when using biochemical kits. This study aimed to determine the frequency of Pip negative gonococci in England and Wales.

Methods: A total of 2055 isolates were collected from consecutive patients attending 26 genitourinary medicine centres as part of the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP). Upon receipt the identity of all isolates was confirmed using *N gonorrhoeae* specific monoclonal antibodies and the Pip status was determined using the Gonocheck II kit.

Results: The overall prevalence of Pip negative isolates was found to be 4.33%. Significant geographical variation was observed between isolates from centres outside London ($p \leq 0.001$). Variation was also observed within London between the nine different clinics submitting isolates ($p = 0.025$). There was also a higher frequency of these isolates among men who have sex with men ($p \leq 0.001$), which may account for geographical variations.

Conclusion: Pip negative *N gonorrhoeae* isolates are a very serious cause for concern as currently all biochemical test kits available within the United Kingdom require the presence of the Pip enzyme for an unambiguous identification of this pathogen. Raising awareness of the current prevalence of Pip negative *N gonorrhoeae* isolates is critical for the successful control of gonorrhoea.

Gonorrhoea is the second most common bacterial sexually transmitted infection (STI) in the United Kingdom and control of this disease is dependent on the successful, rapid, and accurate identification of the pathogen *Neisseria gonorrhoeae*. One of the most common methods routinely used in clinical microbiology settings for the identification of this pathogen, is the detection of preformed enzymes. The use of preformed enzymes as a tool for the differentiation of *Neisseria* spp was first described by D'Amato *et al*, who reported the production of the enzymes gamma glutamyl transferase (GGT) by *N meningitidis* and proline iminopeptidase (Pip) by *N gonorrhoeae*.¹ Following this, several commercial kits were developed which exploit the presence of these enzymes for the purposes of discriminating between *N gonorrhoeae* and other Gram negative diplococci.

Molecular cloning and characterisation of the *N gonorrhoeae* *pip* gene was performed in 1993 where it was confirmed that *pip* was a single copy gene and, while non-essential for growth in vitro was present in virtually all isolates of *N gonorrhoeae*.² However since this time the widespread distribution of Pip negative *N gonorrhoeae* throughout England has been reported.³ Currently all biochemical kits available within the United Kingdom for the identification of *Neisseria* spp, including the *Neisseria* preformed enzyme test (PET), Gonocheck II, RapID NH, and the API NH monitor the presence of this enzyme. Consequently Pip negative gonococcal isolates generate ambiguous identifications ranging from "doubtful *N gonorrhoeae*" to "presumptive *Kingella kingae*" when examined using these kits.⁴ This problem becomes more significant when using test kits such as the *Neisseria* PET test and the Gonocheck II where a presumptive *N gonorrhoeae* identification is based solely on the presence of this enzyme. False positive *N gonorrhoeae* identifications can also be generated when using these kits

as many non-pathogenic *Neisseria* spp are Pip positive and some have been reported to grow well on selective media.^{4, 5} However despite these reports the use of preformed enzyme based tests kits remains popular and they are widely used for the routine identification of *N gonorrhoeae*.

While it is acknowledged that commercially available biochemical kits generate ambiguous results when used to examine isolates of *N gonorrhoeae* which lack Pip, the incidence of isolates lacking this enzyme is currently unknown. This study aimed to determine the frequency of Pip negative *N gonorrhoeae* within a representative sample from surveillance isolates in England and Wales.

METHODS

A total of 2055 isolates of *N gonorrhoeae* from consecutive patients attending 26 genitourinary medicine (GUM) clinics throughout England and Wales collected over a 3 month period during 2004 as part of the Gonococcal Resistance to Antimicrobials Surveillance Programme (GRASP) were tested. All isolates were positively identified as *N gonorrhoeae* at the local laboratory using a range of methods and then forwarded to the Sexually Transmitted Bacteria Reference Laboratory for testing. Upon receipt, isolates were subcultured on GC agar base (Beckton Dickinson) supplemented with Vitox and purity was determined by a Gram stain. The identity of all isolates was further confirmed using in-house monoclonal antibodies specific to *N gonorrhoeae*. In a few instances where in house monoclonal antibodies failed to react, identity was confirmed using the API NH kit

Abbreviations: GGT, gamma glutamyl transferase; GRASP, Gonococcal Resistance to Antimicrobials Susceptibility Programme; GUM, genitourinary medicine; MSM, men who have sex with men; PET, preformed enzyme test; Pip, proline iminopeptidase; STI, sexually transmitted infections

Table 1 The proline iminopeptidase (Pip) status of *Neisseria gonorrhoeae* isolates, broken down by submitting centres

Hospital (no of isolates tested)	Frequency of Pip negative <i>N gonorrhoeae</i> isolates (no of Pip negative isolates)
Total Outer London (1078)	4.27 (46)
New Cross Hospital, Wolverhampton (29)	0.00 (0)
Gloucestershire Royal Hospital (19)	0.00 (0)
Northern General Hospital Sheffield (101)	1.98 (2)
Newcastle (35)	0.00(0)
Manchester Royal Infirmary (112)	5.36 (6)
Royal Liverpool Hospital (103)	1.94 (2)
Birmingham Queen Elizabeth Hospital (107)	2.80 (3)
Nottingham City Hospital (101)	2.97 (3)
The Royal Berkshire Hospital, Reading (37)	0.00 (0)
Addenbrooke's Hospital, Cambridge (14)	21.43 (3)
Bristol Royal Infirmary (129)	10.08 (13)
Luton and Dunstable Hospital (33)	0.00 (0)
Cardiff Royal Infirmary (72)	0.00 (0)
The Royal Sussex County Hospital Brighton (56)	17.86 (10)
Leeds General Infirmary (86)	4.65 (4)
The Royal Gwent Hospital, Newport (25)	0.00 (0)
Northampton General Hospital (19)	0.00 (0)
Total London (977)	4.40 (43)
Kings College Hospital (239)	2.51 (6)
Homerton University Hospital (176)	2.32 (4)
University College London Hospital (140)	9.29 (13)
St Mary's Hospital (129)	6.20 (8)
St George's Hospital (96)	3.13 (3)
Chelsea and Westminster healthcare NHS Trust, Victoria Clinic (60)	8.33 (5)
Chelsea and Westminster, The John Hunter Clinic (59)	5.08 (3)
Charing Cross Hospital (43)	0.00 (0)
Queen Elizabeth Hospital Woolwich (35)	2.86 (1)

(bioMerieux). Following identification, the Pip status of all isolates was established using the Gonocheck II test (E-Y Laboratories Inc).

Bivariate analysis using the χ^2 test was applied to the whole data set where possible. Extended patient information such as sexual orientation was only available for isolates of *N gonorrhoeae* from patients attending GUM clinics. Therefore isolates originating from patients attending GP practices or other hospital departments were not included in some of the final analysis.

RESULTS

The overall prevalence of Pip negative *N gonorrhoeae* was found to be 4.33% (89 of 2055). There was geographical variation observed in the frequency of Pip negative *N gonorrhoeae* isolates submitted from centres outside London ($p \leq 0.001$; table 1). Variation in prevalence was also

observed within London, between the nine different clinics submitting isolates ($p = 0.025$; table 1).

The reason for the difference in the prevalence of Pip negative isolates throughout different cities in England and Wales and even between different clinics within London is unknown. Differences in geographical prevalence can not be explained by some of the primary sites using only preformed enzyme kits (for example, Gonocheck or the Neisseria PET Test) for *N gonorrhoeae* identification, which would lead to an under-representation of Pip negative isolates. All clinics or centres submitting isolates used either adequate or multiple methods for the identification of all gonococcal isolates.⁶ There was however a clear association between prevalence and sexual orientation (table 2), with a higher prevalence of Pip negative isolates being significantly associated with infection in men who have sex with men (MSM) ($p \leq 0.001$). As expected the number of isolates sourced from MSM submitted from the 26 different centres was not equal and this is clearly a reflection of the MSM population being unevenly distributed throughout England and Wales, with centres such as Brighton and Manchester contributing the highest number of isolates sourced from this patient group.

DISCUSSION

This study has highlighted that 4.33% of *N gonorrhoeae* isolated in England and Wales lack the Pip enzyme, using a representative collection of isolates from a sentinel surveillance programme. This is a significant problem given that there are several kits, which monitor solely the presence of this enzyme for the detection of this pathogen. Pip negative *N gonorrhoeae* isolates have previously been shown to generate a false negative identification using biochemical tests.^{4,7} However confusion over which kits detect the presence of this enzyme may be attributed to incorrect terminology used in the manufacturers' kit instructions. The enzyme proline iminopeptidase is a hydrolase, which selectively removes the N-terminal proline residue from peptides. However names used for this enzyme in biochemical kit inserts include proline aminopeptidase, prolylaminopeptidase, and proline arylamidase. It is the responsibility of manufacturers to adopt consistent, up to date, and scientifically correct names for the enzymes that their kits are monitoring. This would ensure that users of particular tests are able to make informed judgments as to the limitations of particular test systems in response to published literature.

Until relatively recently Pip negative isolates of *N gonorrhoeae* had not been widely reported. This is in contrast with *N lactamica* and *N meningitidis* where both Pip negative and Pip positive isolates have both been documented.^{8,9} In a method evaluation carried out in 1991, only two out of 398 isolates of *N gonorrhoeae* were found to be Pip negative.¹⁰ Interestingly a report from the Gonococcal Reference Unit in Bristol during 2001, raised awareness of this as 17 Pip negative *N gonorrhoeae* isolates had been forwarded to them over a 5 week period for confirmatory identification.³ The emergence and apparent increase in the prevalence of these isolates suggests that they may have a selective advantage. It is possible to speculate that such isolates are more likely to be misidentified and therefore not treated. Alternatively it is also plausible that the mutation which renders the Pip enzyme inactive does not have any effect on the fitness of the gonococcal isolate and that the only reason for their apparent increase in prevalence and widespread geographical dissemination could be that they have found a host in a highly sexually active community. Alternatively this apparent increase in prevalence could just be an artefact of increasing awareness of Pip negative gonococcal isolates, with laboratories which have previously been using inadequate methods for gonococcal identification

Table 2 The relation between the frequency of proline iminopeptidase negative *N gonorrhoeae* and gender and/or sexual orientation of patient ($p \leq 0.001$).

	Frequency of Pip negative <i>N gonorrhoeae</i> isolates (no of Pip negative isolates)
MSM (460)	8.47 (39)
Male (575)	2.43 (14)
Female (541)	2.40 (13)

Total = 1576, excludes 479 isolates where the sexual orientation and/or gender of the patient was unknown.

Key messages

- Proline iminopeptidase (Pip) negative *Neisseria gonorrhoeae* isolates generate anomalous results when examined by commercially available biochemical test kits
- The overall prevalence of Pip negative *N gonorrhoeae* isolates in England and Wales is 4.33%
- There is a higher prevalence of Pip negative *N gonorrhoeae* in men who have sex with men

having adopted the national standard guidelines which has resulted in an increase in reporting.⁶

This study suggests that all clinical isolates of *N gonorrhoeae* be identified using two methods, preferably both biochemical and immunological, especially if the biochemical method relies totally on the detection of preformed enzymes.⁶ This is of particular importance when testing isolates taken from children and victims of sexual abuse where medicolegal issues must be addressed and in these cases the use of carbohydrate tests is particularly recommended.

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CONTRIBUTORS

SA was responsible for the isolate screening methods and data analysis; IM was responsible for supervision on the practical stage of the project; KF designed the isolate and patient data, collection protocol and was GRASP lead investigator; CI was responsible for initiation and supervision of the project. All authors have read and approved the final version of this manuscript.

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REFERENCES

- 1 **D'mato RF**, Erique LA, Tomfohrde KM, *et al*. Rapid identification of *Neisseria gonorrhoeae* and *Neisseria meningitidis* by using enzymatic profiles. *J Clin Microbiol* 1978;**77**:77–81.
- 2 **Albertson NH**, Koomey M. Molecular cloning and characterization of a proline iminopeptidase gene from *Neisseria gonorrhoeae*. *Mol Microbiol* 1993;**9**:1203–11.
- 3 **Health Protection Agency**. Prolyliminopeptidase-negative isolates of *Neisseria gonorrhoeae*. *Commun Dis Rep CDR Wkly* [serial online] 11 (49). 2001. Available at <http://www.hpa.org.uk/cdr/archives/2001/cdr4901.pdf>.
- 4 **Alexander S**, Ison C. An evaluation of commercial kits for the identification of *Neisseria gonorrhoeae*. *J Med Microbiol* 2005;**54**:827–31.
- 5 **Knapp JS**, Totten PA, Mulks MH, *et al*. Characterisation of *Neisseria cinerea*, a non-pathogenic species isolated on Martin-Lewis Medium selective for pathogenic *Neisseria* spp. *J Clin Microbiol* 1984;**19**:63–7.
- 6 **Health Protection Agency**. Identification of *Neisseria* species. National Standard Method. 2005. Available at <http://www.hpa-standardmethods.org.uk/documents/bsopid/pdf/bsopid6.pdf>.
- 7 **Blackmore T**, Herrera G, Shi S, *et al*. Characterization of prolyl iminopeptidase-deficient *Neisseria gonorrhoeae*. *J Clin Microbiol* 2005;**43**:4189–90.
- 8 **Sperry JF**, Cohenford MA, Campagnone P, *et al*. Increased detection of prolylaminopeptidase in *Neisseria meningitidis* by Identicult-*Neisseria*. *J Clin Microbiol* 1986;**24**:145–5.
- 9 **Yajiko DM**, Chu A, Hadley WK. Rapid confirmatory identification of *N gonorrhoeae* with lectins and chromogenic substrates. *J Clin Microbiol* 1984;**19**:145.
- 10 **Dealler SF**, Gough KR, Campbell L, *et al*. Identification of *Neisseria gonorrhoeae* using the Neisstrip rapid enzyme detection test. *J Clin Path* 1991;**44**:376–9.