SHORT REPORT

Detection of mixed infection of *Neisseria gonorrhoeae*

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We report the first published evidence, to our knowledge, of the demonstration of the potential existence of mixed infections of *Neisseria gonorrhoeae* in vivo through the use of a highly discriminatory genotypic technique.

Mixed gonococcal infection, where an individual is colonised simultaneously with more than one strain in the same anatomical site, is reported to occur frequently, particularly in highly sexually active populations[1–4]; however, there is not a strong evidence base. When more than one strain occurs together, in vivo, it is hypothesised that lysis of the bacteria will occur, releasing DNA, which acts as the source for recombination resulting in a highly genetically variable population.[2 4] *Neisseria gonorrhoeae* also undergoes mutation and intragenic recombination contributing to the genetic variation.[2] There is substantial evidence that *N gonorrhoeae* is genetically diverse and is a non-clonal or panmictic population.[5 6] However, the true prevalence of mixed infections, in vivo, is less clear and the evidence is largely anecdotal.[7]

The difficulty arises in providing direct evidence that mixed infections occur in vivo. After isolation of the bacteria from the patient, in vitro, it is possible to use typing methods. *N gonorrhoeae* has been characterised using phenotypic, auxotyping and serotyping, and genotypic methods.[8] Phenotypic techniques characterise strains by the products of gene expression, but show limited discrimination. Variation in the auxotype of individual colonies has been detected, but as recombination between gonococci can occur in culture this does not necessarily indicate the presence of more than one strain.[1] The detection of an unknown reaction pattern with the panel of monoclonal antibodies used for serotyping may indicate the strain was not from a single population, or the emergence of a new serovar. Genotypic methods offer a higher level of discrimination, where techniques such as pulsed field gel electrophoresis (PFGE), *opa* typing, or DNA sequencing, can distinguish between strains from different sources[7 8] However, while evidence of more than one strain on a primary isolation may indicate mixed infection, if only one strain is detected this may not indicate the lack of a mixed infection but may reflect the selection of a single strain on isolation media.

In this study we addressed the question of whether mixed gonococcal infections can be detected in vitro using the molecular technique *opa* typing.[2] Individual primary isolation plates were obtained from 10 separate male patients attending the GUM clinic at St Mary's Hospital, London, with a urethral infection. Seven individual colonies were picked off the primary isolation plate, containing vancomycin, colistin, amphotericin, and trimethoprim,[7] with a small metal loop and subcultured onto a fresh agar plate without any antibiotics.[7] A subculture of a sweep of at least 10 colonies was also made. All plates were incubated overnight at 36°C in 6% carbon dioxide and the resulting growth resuspended in phosphate buffered saline (PBS) to an optical density of 1.0 at 540 nm and used as the DNA source for *opa* typing.[2] Briefly, *opa* genes were amplified by polymerase chain reaction (PCR), the product was purified using Geneclean III kit (Anachem) before digestion, initially with *Taq*I and also with *Hin*PI if profiles were indistinguishable. Digested products were end labelled with P-32 and separated on a non-denaturing polyacrylamide gel. On exposure to an autoradiograph the resulting profiles were compared visually. Profiles were only considered to be the same when all bands, regardless of intensity, were identical. One band difference in profiles was classed as being different.

In each of the 10 cases of gonorrhoea, the *opa* profiles from the individual colonies and the sweep culture showed no variation (an example from one patient is shown in fig 1). The *opa* profile from each infected patient was different.

Variation in *opa* profile was not detected between individual colonies from the same primary isolation plates, suggesting that either the patients tested were infected with a single

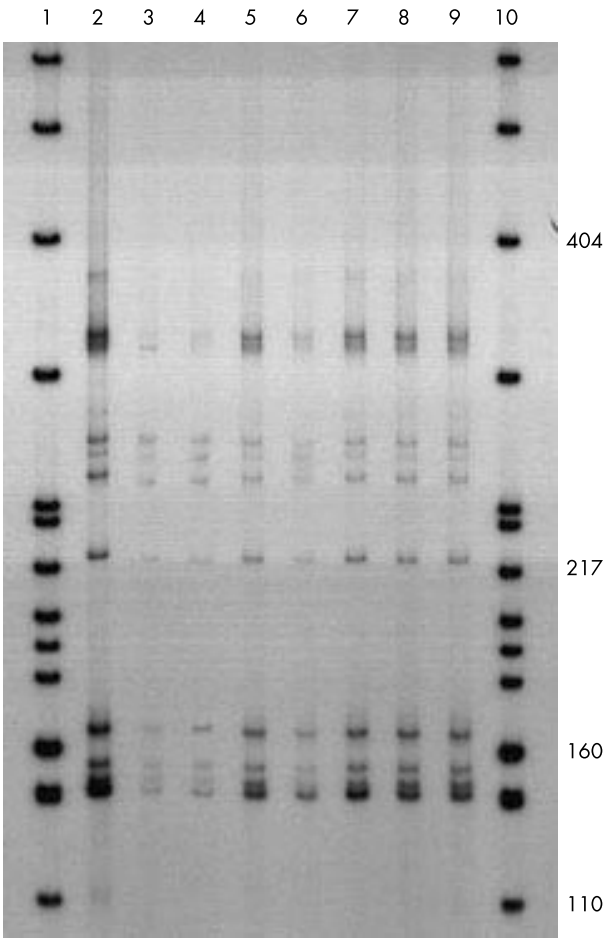

Figure 1 *Opa* profiles of individual colonies from a primary isolation plate from a male patient. Lanes 1 and 10, PBR322, molecular weight marker (bp). Lane 2, *opa* profile of a sweep subculture. Lanes 3–9, *opa* profile of individual colonies.

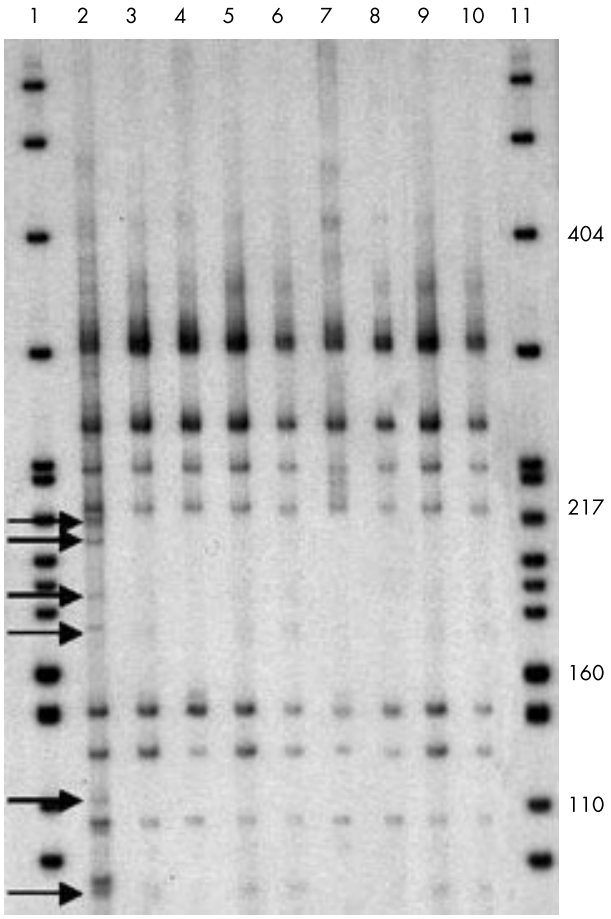

Figure 2 *Opa* profiles of a non-cultured specimen and colonies from the primary isolation plate from the same male patient. Lane 2 *opa* profile from a non-cultured specimen. Note the extra bands of the profile compared to lane 3 from a sweep subculture and lanes 4–10 from individual colonies from the primary isolation plate. Lanes 1 and 11, molecular weight marker (bp). Arrows indicate the presence of extra bands.

strain of *N gonorrhoeae*, or that a single strain had been selected by culture. Different growth rates exist between gonococcal strains and it is possible that if two strains from a mixed infection were inoculated onto an isolation plate that one strain could overgrow the other, or even inhibit its growth preventing its detection.[9 10] Strains with an arginine, hypoxanthine, and uracil requiring auxotype are known to grow more slowly in vitro.[9 10] To address these questions direct patient specimens that were non-cultured were obtained to continue the investigation into mixed infections.

Non-cultured specimens were obtained from patients who were known to be culture positive for *N gonorrhoeae* where a primary isolation plate was also available. The samples were obtained from the patient at the same clinic visit. The swabs taken from the urethra of male patients and the cervix of female patients were put into a sterile buffer solution (LCX, Abbott Laboratories Diagnostics), which was being used for testing for *Chlamydia trachomatis*. The sample was used as the DNA source to amplify the *opa* genes of *N gonorrhoeae*. Colonies from the primary isolation plate were sampled as described above.

Nineteen non-cultured specimens and colonies from their matched primary isolation plate were *opa* typed, 14 from men and five from women. No variation in the *opa* profile between the individual colonies and the non-cultured specimen was detected in 15 samples. In four samples, from male patients, the non-cultured specimen had a different profile from the individual colonies and sweep subculture (an example from one patient is shown in fig 2). An additional restriction digest using *HinP*I confirmed the different *opa* profile result in the four samples. The *opa* profiles of two of the four non-cultured specimens compared to those from the individual colonies showed multiple band differences, the other two non-cultured samples shared some common bands, with some extra or fewer bands compared to the individual colonies.

To determine the limit of detection of the PCR of mixed samples of *N gonorrhoeae*, suspensions of two different clinical gonococcal strains were made in PBS with an optical density of 1.0 at 540 nm. The suspensions were mixed together in 1:2, 1:4, 1:6, 1:8, and 1:10 proportions for both strains and used as the DNA source to amplify the *opa* genes. The *opa* profile at ratios of 1:2 and 1:4 showed bands found in the profile of both strains, differing only in intensity, while at ratios of 1:6, 1:8, and 1:10 the profile was of the majority strain, indicating that a strain was only detected when present at 25% or more. This is a similar finding to the amplification of gonococcal pilin genes from two isolates.[11]

The detection of different *opa* profiles in some non-cultured specimens compared to the primary culture indicates that strains of *N gonorrhoeae* with different genotypes in vivo, a mixed gonococcal infection, may exist. From the known limit of detection of the *opa* PCR the strains must have been present in the site tested at 25% or more.

Mixed infections of gonorrhoea are likely to occur in focal points of transmission chains where individuals have multiple sexual partners and where the incidence of gonorrhoea is high, such as in core groups. However, it may be possible for a mixed infection to be transmitted simultaneously by one sexual partner. In vivo a mixed gonococcal infection would provide an environment where intergenic recombination between gonococci could occur creating new genetic variants, contributing to the diversity of *N gonorrhoeae*. Sexual contacts with *N gonorrhoeae* would be expected to have strains that are concordant in phenotype and genotype; however, discordance between contacts within a transmission chain could occur due to mixed infections.[12] The risk of contracting a mixed infection will depend on the sexual activity of an individual and that of their partners.

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CONTRIBUTORS

IMCM carried out the laboratory work; CAI contributed towards the methodological design of the project; IMCM and CAI both prepared this article.

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Prevalence of and risk factors for hepatitis C virus infection among sexually transmitted disease clinic clientele in Miami, Florida

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Objectives: Hepatitis C virus (HCV) is the most common chronic blood borne viral infection in the United States. We assessed the HCV prevalence, risk factors, and sensitivity of the Centers for Disease Control and Prevention's (CDC) routine screening criteria among clients of a large urban sexually transmitted disease (STD) clinic.
Methods: Participants were recruited from a public STD clinic in Miami, Florida, and were interviewed regarding known and potential risk factors. The survey assessed CDC screening criteria, as well as other risk factors (for example, intranasal drug use, history of incarceration, exchanging sex for money, number of lifetime sex partners, and history of an STD). Testing was done by enzyme immunoassay (EIA) and confirmed by recombinant immunoblot assay (RIBA).
Results: The prevalence of anti-HCV positivity was 4.7%. Four variables were significantly associated with being anti-HCV positive, independent of confounding factors. These included injection drug use (odds ratio (OR) = 31.6; 95% confidence intervals (CI) 11.0 to 90.5); history of incarceration (OR = 3.0; 95% CI 1.1 to 8.1); sexual contact with an HCV positive person (OR 12.7; 95% CI 2.5 to 64.7); and older age (OR 1.4; 95% CI 1.2, 1.6). The sensitivity of CDC's routine screening criteria was 69% and specificity was 91%.
Conclusions: The prevalence of anti-HCV in this clinic was similar to that determined in studies of comparable populations. Having sexual contact with an HCV positive person and history of incarceration were independently associated with being anti-HCV positive. CDC's screening criteria identified approximately two thirds of the anti-HCV positive participants.

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