

# Homology of cryptic plasmid of *Neisseria gonorrhoeae* with plasmids from *Neisseria meningitidis* and *Neisseria lactamica*

CATHERINE A ISON,\* C M BELLINGER,\* J WALKER†

From the \*Department of Medical Microbiology, St Mary's Hospital Medical School, London, and †Diagnostics, Wellcome Research Laboratories, Beckenham, Kent

**SUMMARY** DNA probe hybridisation was used to examine the relation between the cryptic plasmid from *Neisseria gonorrhoeae* and plasmids carried by pharyngeal isolates of *Neisseria meningitidis* and *Neisseria lactamica*. The complete gonococcal cryptic plasmid and *Hinf*I derived digestion fragments subcloned into *Escherichia coli* were used to probe Southern blots of plasmid extracts. Homology was found to a plasmid of approximate molecular weight 4.5 kilobase pairs (Kb) but not to plasmids of < 3.2 Kb or 6.5 Kb. Eleven of 16 strains of *N meningitidis* and two of six strains of *N lactamica* carried plasmids that showed strong hybridisation with the 4.2 Kb gonococcal plasmid. Hybridisation of plasmids from non-gonococcal species of neisseria with the gonococcal cryptic plasmid indicates that caution should be taken when using the cryptic plasmid as a diagnostic probe for gonorrhoea.

The genus *Neisseria* contains two pathogenic species, *Neisseria meningitidis* and *Neisseria gonorrhoeae*. *N meningitidis* colonises the upper respiratory tract and invades to produce systemic disease, whereas *N gonorrhoeae* infects the columnar epithelium of the genital tract and rectum and only rarely causes systemic disease. Other species of neisseria have occasionally been associated with infection but are generally regarded as commensals.<sup>1</sup> Within the genus there is strong DNA homology between the species,<sup>2</sup> which is highest between *N meningitidis* and *N gonorrhoeae* (> 80%). Despite their close genetic association pathogenic neisseria can be distinguished from commensal neisseria by their ability to produce IgAI protease,<sup>3</sup> adhere to columnar epithelium,<sup>4</sup> and produce an outer membrane antigen designated H8.<sup>5</sup> *N meningitidis* and *N gonorrhoeae* differ from each other in their ability to utilise carbohydrates, requirement for cysteine and cystine, and their aminopeptidase profiles.<sup>6</sup> In most clinical laboratories only the ability of *N meningitidis* to produce acid from maltose in addition to glucose is routinely used for differentiation.

A major distinguishing factor is the carriage of plasmids by strains of *N gonorrhoeae*.<sup>7</sup> Several plas-

mids have been isolated; a cryptic plasmid, molecular weight 4.2 Kb; a conjugal plasmid (37 Kb); and two penicillinase coding plasmids (7.1 Kb and 5.6 Kb). In contrast, there have been only a few reports of plasmids in meningococci.<sup>8-13</sup> Commensal neisseria have also been reported to carry plasmids.<sup>7</sup>

DNA probes are being introduced for the detection and identification of micro-organisms. The specificity of any such probe depends on the choice of a suitable piece of DNA present in the organism to be detected. The cryptic gonococcal plasmid, which is carried by at least 90% of strains,<sup>14</sup> has the potential to fulfil this criterion. Totten *et al*<sup>15</sup> described the use of a cryptic plasmid probe to detect *N gonorrhoeae* in urethral exudate from men and it has also been used in combination with a penicillinase coding plasmid probe on clinical samples.<sup>16</sup>

We used subcloned fragments, as well as the entire plasmid, to determine the homology between the cryptic gonococcal plasmid and low molecular weight plasmids carried by throat isolates of *N meningitidis*<sup>12,13</sup> and *N lactamica*.

## Material and methods

### BACTERIAL STRAINS

Sixteen strains of *N meningitidis* and six strains of *N*

*lactamica* known to carry plasmids were studied. All strains were originally isolated from the throat of patients attending a sexually transmitted disease clinic. They were collected during studies for screening throat isolates of neisseria for plasmid carrying strains.<sup>13</sup>

*N gonorrhoeae*, strain 38/2, was used to prepare the probes and was kindly provided by Dr P Novotny, Wellcome Research Laboratories. Strain HI, a laboratory strain from St Mary's, which carries the 4.2 Kb cryptic plasmid, was used as a reference on Southern blots.

*Escherichia coli*, strain TG1, was used in subcloning experiments.

#### MEDIA

Strains were initially isolated on GC agar base (Difco), 36 g/l, with the addition of 1% IsoVitalax (BBL) and vancomycin, colistin, trimethoprim, and amphotericin after incubation at 36°C in 7% carbon dioxide for 48 hours. All further subcultures were made on GC agar base with the addition of Kellogg's supplements.<sup>17</sup>

#### IDENTIFICATION

Strains were identified as either *N meningitidis* or *N lactamica*, if they were Gram negative cocci, oxidase positive, and produced acid from glucose and maltose but not sucrose. Carbohydrate utilisation tests were performed on serum free medium containing glucose, maltose, and sucrose poured into petri dishes.<sup>18</sup>

*N lactamica* strains were distinguished from those of *N meningitidis* by their ability to split o-nitrophenyl-galactoside, as previously described.<sup>12</sup> All strains were examined for their aminopeptidase profiles<sup>19</sup> and their ability to grow in the absence of cysteine and cystine.<sup>20</sup> The Phadebact GC monoclonal test (Pharmacia) was used to detect the presence of gonococcal antigens. This reagent contains antibodies that are directed against two epitopes of the gonococcal outer membrane protein (OMP-PI).

#### PLASMID EXTRACTION

Plasmids were isolated from strains of neisseria by the alkali extraction method of Birnboim and Doly.<sup>21</sup> A modification of this technique<sup>22</sup> was used for large scale preparation of the cryptic plasmid from *N gonorrhoeae* and of its subclones grown in *E coli* strain TG1. For large scale plasmid extraction, cells of *N gonorrhoeae* (38/3) were obtained by scraping growth from GC medium, while *E coli* strain TG1 was pelleted after growth in shake flasks of LB broth.<sup>22</sup>

Plasmids used for subcloning and as probes were further purified by centrifugation through caesium chloride-ethidium bromide density gradients.

#### SUBCLONING

The methods for the manipulation of DNA were as described by Maniatis *et al.*<sup>22</sup> Based on a previously published restriction enzyme map of the cryptic plasmid of *N gonorrhoeae*,<sup>23</sup> the density gradient purified plasmid was digested with *Hinf*I, yielding two fragments of 2.6 (large) and 1.7 (small) kilobase pairs. The fragments were blunt ended using the Klenow fragment of polymerase I, then ligated with the plasmid vector pUC-8, which had been *SMA*I digested.

The ligation mixture was used to transform competent cells of *E coli* strain TG1. Transformants selected on LB plates containing ampicillin, were screened by colony blots with nick translated large and small fragment probes.

Clones of pUC-8 containing the small fragment (pGC1) and large fragment (pGC3) of the cryptic plasmid from *N gonorrhoeae* were used for the preparation of plasmid probes.

#### PLASMID ANALYSIS

Plasmids were electrophoresed in 1% agarose, as previously described.<sup>12</sup> Triplicate gels were prepared, and, following staining by ethidium bromide and photography, the DNA was transferred to nitrocellulose membranes by Southern blotting.<sup>24</sup> DNA was fixed to the membranes by baking for two hours at 80°C in a vacuum oven.

#### NICK TRANSLATION

The cryptic plasmid of *N gonorrhoeae* and its subclones pGC1 and pGC3 were labelled with  $\alpha$ -<sup>32</sup>P-dATP by nick translation, using a commercially available kit (Amersham International) to a specific activity of  $4 \times 10^7 - 2 \times 10^8$  dpm/ $\mu$ g DNA.

#### HYBRIDISATION

Membranes were prehybridised for three hours at 37°C in a solution containing 50% (v/v) deionised formamide; 4  $\times$  SSC (1  $\times$  SSC = 0.15M sodium chloride, 0.015M sodium citrate, pH 7.0); 0.1% sodium dodecyl sulphate; 1  $\times$  Denhardt's solution (0.1% Ficoll, 0.1% polyvinylpyrrolidone, and 0.1% bovine serum albumin); and 100  $\mu$ g/ml denatured salmon sperm DNA. About 1  $\times$  10<sup>6</sup> dpm of heat denatured probe was added to each membrane in 1 ml of the above hybridisation solution and incubated at 37°C overnight. Triplicate membranes were probed with either the cryptic plasmid, pGC1, or pGC3. Membranes were then washed in four changes of 0.2  $\times$  SSC at room temperature before autoradiography at -70°C using x ray film (X-OmatS, Kodak) with an intensifier screen. Usually four to five hours' exposure was required.

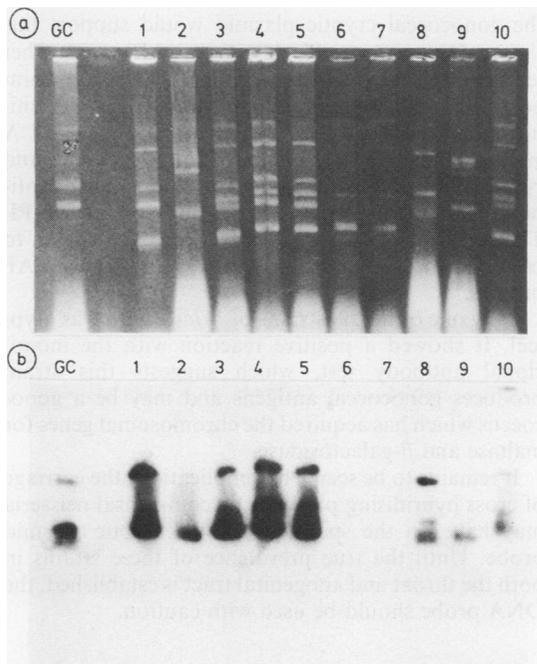


Fig 1 (a) agarose gel electrophoresis; (b) autoradiograph of Southern blot of (a) probed with complete cryptic plasmid probe. GC represents *N gonorrhoeae*, strain H10. Tracks 1-5, 8 and 10 are strains of *N meningitidis*; tracks 6, 7, and 9 are strains of *N lactamica*.

## Results

All strains of *N meningitidis* and *N lactamica* were identified by carbohydrate utilisation and ability to split ONPG. The aminopeptidase profile, ability to

Table 1 Characteristics of plasmid carrying strains of *N meningitidis* and *N lactamica*

	<i>N meningitidis</i>		<i>N lactamica</i>
	No of strains showing positive reaction		
Total	13	3	6
Aminopeptidase activity			
Glutamyl	13	0	0
Hydroxyproline	0	3	6
GC monoclonal reagent	0	0	1
Requirement for cysteine and cystine	0	0	0

Table 2 Hybridisation between plasmids carried by *N meningitidis* and *N lactamica* and cryptic plasmid probe\*

Plasmid (Kb) present	<i>N meningitidis</i>		<i>N lactamica</i>	
	Total	No showing homology	Total	No showing homology
< 3.2 alone	4	0	4	0
4.5 alone	4	4	1	1
< 3.2 + 4.5	7	7	1	1
6.5	1	0	0	0

\*Concordant results were obtained with the subclones of the cryptic plasmid.

grow in the absence of cysteine and cystine, and Phadebact GC monoclonal test were used to detect any unusual features in these strains. Thirteen of the 16 meningococci showed a typical aminopeptidase profile—that is, glutamyl aminopeptidase activity but not hydroxyproline aminopeptidase activity (table 1). The remaining three strains showed the converse, which is typical of *N gonorrhoeae* or *N lactamica*. None of the meningococci showed a positive reaction with the Phadebact GC monoclonal reagent or required cysteine and cystine for growth.

All strains of *N lactamica* had a typical aminopeptidase profile and did not require cysteine and cystine for growth. One of the six strains, however, gave a strong positive reaction with the Phadebact GC monoclonal test.

Several plasmids were isolated from these neisseriae, of approximate molecular weight < 3.2, 4.5, and 6.5 Kb, either alone or in combination. The ability of these plasmids to hybridise with radiolabelled probes of the whole cryptic plasmid and the large (pGC3) and small (pGC1) fragment subclones was tested by Southern blotting. Only 4.5 Kb plasmids showed homology (figure) whether present alone or in combination with lower molecular weight plasmids (table 2). All strains carrying 4.5 Kb plasmids, however, showed hybridisation. Strains that showed hybridisation with the complete cryptic plasmid also showed strong hybridisation with both the pGC1 and pGC3 subclones. Higher stringency washes of  $0.2 \times$  SSC at 55°C did not remove the probes. Cross hybridisation was not observed between the pUC-8 vector and strains of either gonococci or other species of neisseria. This indicates that observed hybridisation was the result of homology between the subcloned fragments and plasmids from neisseria.

There was no homology between the group of plasmids of < 3.2 Kb or 6.5 Kb and the cryptic plasmid probe in any of the extractions (figure).

## Discussion

We showed the presence of plasmids in 11 strains of *N meningitidis* and two strains of *N lactamica* that show homology with the gonococcal cryptic plasmid. The plasmids also hybridise strongly with the two sub-

cloned fragments created by *Hinf*I digestion of the cryptic plasmid. Higher stringency washes did not remove probes from Southern blots. We suggest that this strongly indicates a large degree of homology rather than cross hybridisation with a small portion of DNA. Preliminary experiments, in which Southern blots of restriction fragments from the cryptic plasmid have been probed with plasmids from commensal strains, have also shown a large degree of homology. Further work, however, is required to determine accurately the extent of homology. The new plasmids are usually slightly larger, about 4.5 Kb, than the gonococcal cryptic plasmid. This may be due to extra or inverted repeat segments.<sup>23</sup>

Attempts to size accurately the plasmid from one particular strain have been hampered because the plasmid does not seem to be readily digested by a selection of restriction endonucleases.<sup>13</sup>

There was no homology between any of the probes used and the plasmids of molecular weight < 3.2 Kb. These plasmids seem to vary in size, without any particular size being restricted to strains of either *N meningitidis* or *N lactamica*.

We experienced difficulty in maintaining plasmids during storage of some of these strains at both -70°C and in liquid nitrogen. Their function is unknown and therefore we were unable to maintain by selection. This differs from the gonococcal cryptic plasmid, which is stable on storage and subculture.

The use of the cryptic plasmid as a DNA probe to detect gonococci in clinical samples has two major problems. Firstly, the sensitivity depends on the presence of the plasmid in all strains of *N gonorrhoeae*. Isolates that require proline, citrulline, and uracil for growth are known not to carry the cryptic plasmid, although the numbers of these isolates are usually low.<sup>14</sup> Secondly, the specificity depends on the DNA sequence on the cryptic plasmid being unique to gonococci. Totten *et al*<sup>15</sup> showed homology between 124 of 134 strains of *N gonorrhoeae*, one of 11 strains of *N mucosa*, and one of seven strains of *N cinerea* tested. We show homology between plasmids carried by meningococci and *N lactamica* isolated from the throat of patients attending a sexually transmitted disease clinic. In unpublished observations (CA Ison and CM Bellinger) the prevalence of plasmid carrying strains was 5% of throat meningococci and 4% of anogenital meningococci tested. Unfortunately, plasmids from anogenital strains were not available for testing with the probes.

We have previously suggested that gonococci may accept chromosomal DNA from mouth flora by transformation *in vivo*, and consequently the ability to utilise maltose. They may then become misidentified as meningococci. Our finding in this study of a plasmid in meningococci with homology to

the gonococcal cryptic plasmid would support this theory. It was impossible, however, to show any other features common to gonococci in 13 of the 16 meningococci. The three remaining meningococci had aminopeptidase profiles that were more typical of *N gonorrhoeae*, but they did not require cysteine and cystine for growth or react with monoclonal antibodies to the gonococcal outer membrane protein-PI. Throat meningococci have previously been shown to be a heterogeneous group and to have atypical AP profiles.<sup>12</sup>

Only one of the six strains of *N lactamica* was atypical. It showed a positive reaction with the monoclonal antibody test, which suggests this strain produces gonococcal antigens and may be a gonococcus which has acquired the chromosomal genes for maltase and  $\beta$ -galactosidase.

It remains to be seen what implications the carriage of cross hybridising plasmids by commensal neisseria may have on the specificity of the cryptic plasmid probe. Until the true prevalence of these strains in both the throat and anogenital tract is established, the DNA probe should be used with caution.

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Requests for reprints to: Dr Catherine A Ison, Department of Medical Microbiology, Wright Fleming Institute, St Mary's Hospital Medical School, London W2 1PG, England.