Original
articleMolecular epidemiology of *tetM* genes in *Neisseria gonorrhoeae*

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Objective: To examine the epidemiology of the *tetM* gene in *Neisseria gonorrhoeae* strains with high level resistance to tetracycline (TRNG) using a polymerase chain reaction (PCR) assay.

Methods: A single tube PCR was developed which distinguishes between the American and Dutch variants of the *tetM* gene. Between 1988 and 1995, 518 strains of TRNG (tetracycline MIC >8 mg/l) were referred to the Gonococcus Reference Unit by other laboratories or isolated from routine swabs taken at local clinics. The strains were analysed for plasmid content, auxotype, serovar, and the *tetM* gene type. Travel details of the patients were determined by a questionnaire.

Results: A PCR product was obtained from all TRNG examined. 387 TRNG strains produced a 778 bp PCR product (American type *tetM*) and 131 produced a 443 bp PCR product (Dutch type *tetM*). Infections acquired in the United Kingdom contributed 57% of the TRNG strains included in this study; 82% of these carried the American type of *tetM*. The number of UK acquired TRNG received by the GRU increased each year except 1993—from four strains received in 1990 to 92 in 1995. After the United Kingdom, Caribbean and African countries contributed most strains, with 56 and 60 TRNG acquired in each area respectively. All strains originating in Africa, except one from South Africa, contained the American type *tetM*. Infections caught in Nigeria and Kenya contributed most strains (15 and 14 respectively). The TRNG originating from Caribbean countries comprised 36% Dutch *tetM* type. Infections caught in Jamaica accounted for 82% of the Caribbean strains. All 35 TRNG strains originating in the Far East contained the Dutch type *tetM*. 25 of the Far East strains were also penicillinase producing (PPNG). Infections originating in Indonesia accounted for 49% of the Far East strains but these belonged to 12 different auxotype/serovar combinations. A geographical variation in the type of penicillinase coding plasmids found in PPNG/TRNG was also detected.

Conclusions: These data suggest that the Dutch type *tetM* may have originated in the Far East and the American type in the African continent. Subsequent spread has resulted in a heterogeneous distribution of TRNG types in other parts of the world. At completion of the survey the numbers of TRNG imported each year from the major overseas sources had reached a plateau while UK contracted TRNG continued to rise providing evidence for the establishment of endemic TRNG strains in the United Kingdom.

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Keywords: epidemiology; *Neisseria gonorrhoeae*; *tetM*; polymerase chain reaction

Introduction

Infections caused by gonococci with high level plasmid mediated resistance to tetracycline (TRNG) were first recognised in the United Kingdom in 1987.¹ Isolations of TRNG have also been reported from the Netherlands, France, Spain, Canada, Africa, and Uruguay,^{2–7} following the original recognition of TRNG in the United States in 1985.⁸ Subsequent epidemiological studies have suggested a worldwide distribution for TRNG strains.^{9 10}

High level tetracycline resistance (minimum inhibitory concentration (MIC) >8 mg/l) in gonococci is mediated by a *tetM* determinant carried on a 25.2 MDa conjugative plasmid.¹¹ The restriction endonuclease map of the 25.2 MDa conjugative plasmid from a TRNG strain imported from the United States has been found to differ from a map derived from a strain isolated in the Netherlands.¹² These two types of *tetM* carrying conjugative plasmids were designated “American” and “Dutch” respectively. The latter had a restriction map similar to the 24.5 MDa conjugative plasmid found in tetracycline sensitive strains of *N gonorrhoeae* whereas the map of the American type suggested a different origin. More recently the

nucleotide sequences of the *tetM* genes from the American and Dutch conjugative plasmids have been determined and found to differ significantly.¹³

As tetracycline is a cheap drug it may still be used in some parts of the world to treat both gonorrhoea and chlamydia infection.^{14 15} Laboratory tests to screen for resistance in gonococci have been described^{11 14 16}; however, a rapid test that simultaneously detects and types the gene responsible for tetracycline resistance may have a role in epidemiological studies of resistance. We have designed three oligonucleotide primers that will hybridise with the *tetM* gene in a polymerase chain reaction (PCR). The results presented here describe the use of these primers to detect and type the *tetM* gene in gonococci and, together with patient demographic data, describe the global distribution of the *tetM* gene.

Materials and methods

SOURCES OF *N GONORRHOEA* STRAINS

The gonococci used in the study were collected between 1988 and 1995. The isolates were received for confirmation and further tests from laboratories around the United Kingdom or were isolated by the Gonococcus Reference

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Unit (GRU) from routine diagnostic specimens taken at two local genitourinary medicine clinics. The identity of strains was confirmed by Gram film, oxidase reaction, and the production of acid from glucose but not maltose or sucrose in cysteine trypticase agar. The Phadebact Monoclonal GC Test (Boule Diagnostics AB, Huddinge, Sweden) was also used to confirm the identity of strains and to provide a serogroup result for strains that were not serotyped.

The World Health Organisation (WHO) set of strains designated A to E and a TRNG strain kindly provided by Dr C A Ison, St Mary's Hospital Medical School, Paddington, London, were used as controls for the antimicrobial sensitivity tests.

The PCR reaction was validated using 20 strains of *N. gonorrhoeae*, obtained from the culture collection of the GRU, carrying *tetM* conjugative plasmids with previously determined endonuclease restriction pattern type¹² and *tetM* type.¹⁷ Twenty two gonococci with tetracycline MICs that were borderline for high level resistance (MIC 8 mg/l) but did not contain the 25.2 MDa plasmid and 16 strains of gonococci known to be carrying 24.5 MDa conjugative plasmids in combination with penicillinase plasmids (tetracycline MIC range 1–4 mg/l) were also included. These two sets of strains were also obtained from the culture collection of the GRU. The borderline tetracycline resistant strains were originally isolated by laboratories in 12 cities in the United Kingdom and the conjugative plasmid carrying strains were isolated by laboratories in 10 cities.

ANTIBIOTIC SUSCEPTIBILITY TESTING

The agar dilution method was used. Appropriate dilutions of tetracycline were incorporated into Blood Agar Base No 2 (Difco) supplemented with 5% horse blood. A multipoint inoculator was used to deliver approximately 10⁴ colony forming units (cfu) of each strain onto the plates. Endpoints were read as complete inhibition of growth after 18 hours at 37°C in 5% carbon dioxide. WHO strains A–E and the control TRNG were included in each batch of organisms tested to ensure day to day reproducibility.

Penicillinase production was detected using a modification of the method of Hodge *et al.*¹⁸ essentially utilising protection of the "Oxford" strain of *Staphylococcus aureus* inoculated with the test strain onto heated blood agar containing 1 mg/l ampicillin.

PLASMID DETECTION

Gonococci with a MIC of tetracycline of ≥ 16 mg/l, and appropriate control strains were sub-cultured onto New York City agar. Growth was harvested from the plate after overnight incubation. Plasmid DNA was purified using a modification of the Birnboim and Doly method,¹⁹ separated by electrophoresis through 1% agarose gel and visualised by ultraviolet fluorescence after ethidium bromide staining.

POLYMERASE CHAIN REACTION

DNA was prepared by boiling a faintly turbid suspension (equivalent to McFarland No 1 standard) of gonococcal cells (grown on New York City agar) with 5% Chelex 100 resin (Bio-Rad) for 15 minutes; resin and cellular debris were removed by brief centrifugation. The PCRs were performed in a total volume of 50 μ l containing 0.25 U of Taq polymerase (Super Taq, HT Biotechnology Ltd), buffer (10 mM TRIS-HCl pH 9.0, 1.5 mM MgCl₂, 50 mM KCl, 0.1% v/v Triton X-100, 0.01% w/v gelatin), 20 μ M of each dNTP (Pharmacia), and 0.1 μ M of each of the three primers. A universal forward primer (UF) that hybridises with both variants was combined with reverse primers specific to each variant (AR (American) and DR (Dutch)). The primer sequences, derived from the sequences of pOZ100 (UF, AR) and pOZ101 (DR),¹³ were:

Primer UF:

5' ⁸²⁵CTCGAACAAGAGGAAAGC⁸⁴²

Primer AR:

5' ¹⁶⁰²GCATTCCACTTCCCAAC¹⁵⁸⁶

Primer DR:

5' ¹²⁶⁷TGCAGCAGAGGGAGG¹²⁵³

The GenBank accession number for the *tetM* gene sequence for pOZ100 is L12241 and that for pOZ101 is L12242. The prepared DNA (2 μ l) was added, the reaction mixture overlaid with two drops of paraffin oil and transferred to the thermal cycler (Omnigene, Hybaid, UK). The reaction mixture was incubated at 94°C for 3 minutes and then 30 cycles of PCR were performed consisting of denaturing for 20 seconds at 94°C, annealing for 1 minute at 55°C and extension for 1 minute 30 seconds at 72°C. In the final cycle there was a step of 7 minutes at 72°C to complete extension. The expected product sizes of this PCR assay were 778 and 443 base pairs from the American and Dutch types of *tetM* respectively.

A control "Dutch" *tetM* carrying strain (GR7698), a control "American" *tetM* strain (GR7646), and a negative control strain (SB5) carrying the 24.5 MDa conjugative plasmid were included in each PCR experiment.

PCR products were analysed by electrophoresis in 1% w/v agarose and visualised by ultraviolet fluorescence after ethidium bromide staining.

To confirm the identity of the PCR amplicons, the PCR products from 12 gonococcal isolates (three controls and three test strains of each *tetM* type) were digested with *Taq*-I endonuclease and the restriction products visualised after electrophoresis on 10% polyacrylamide gel. The restriction product sizes predicted were distinct for amplicons from the American and Dutch type *tetM* types even if a 443 bp (Dutch type) product were generated from an American type *tetM* by mispriming.

AUXOTYPING

The defined medium of Copley and Egglestone²⁰ was used to test for nutritional requirement for proline, arginine, hypoxanthine, and uracil. In addition, the ability of ornithine to replace a requirement for arginine was tested.

Figure 1 Ethidium bromide stained agarose gel electrophoresis of polymerase chain reaction products using *tetM* type specific reverse primers AR and DR combined with a universal forward primer (UF) with DNA from tetracycline resistant *N gonorrhoeae*. Lane 11: 100 base pair ladder molecular weight standard. Lane 1: Dutch type *tetM* control strain (GR7698), lane 2: negative (tetracycline sensitive) control strain (SB5), lane 3: American type *tetM* control strain (GR7646). Lanes 4, 5, 7, 8, 10, 12–18, and 20: American type *tetM* strains (778 base pair PCR product). Lanes 6, 9, and 19: Dutch type *tetM* strains (443 base pair PCR product).

SEROTYPING

Monoclonal antibodies raised against the major outer membrane protein I of *N gonorrhoeae* produced by Genetic Systems (GS) (supplied by Syva, Palo Alto, CA, USA) were used to serotype strains. Six monoclonal antibodies were specific for protein IA strains and six were specific for protein IB strains. Test strains were suspended in PBS and boiled for 10 minutes, after cooling a drop of this was added to an equal volume of each monoclonal antibody coated *Staphylococcus aureus* suspension on a glass slide. The co-agglutination reaction pattern with each panel was read after gentle rocking for 2 minutes and the nomenclature scheme of Knapp *et al.*²¹ was used to determine the serovar.

DEMOGRAPHIC DATA

A questionnaire was issued to the referring laboratory or clinic with the laboratory report of each tetracycline resistant gonococcus. This allowed information to be gathered on the travel history of the patient and other demographic information.

Results

The PCR assay gave a product consistent with the predicted size of 778 bp from the American type and 443 bp from the Dutch type of *tetM* (fig 1). The 778 bp product was obtained from all 12 strains of *N gonorrhoeae* known to be carrying American *tetM* type, and a 443 bp product was obtained from all eight strains of *N gonorrhoeae* carrying Dutch *tetM* type (table 1).

Restriction fragment analysis using *Taq*-1 was performed on the PCR amplicons from 12 gonococcal isolates (three controls and three test strains of each *tetM* type). Products of the predicted sizes (74, 130, and 572 bp for the 778 bp amplicons and 126, 130, and 185 bp for the 443 bp amplicons: predicted 2 bp products from both types were not visualised) were found in preparations made from all strains.

The specificity of the PCR assay was tested using DNA preparations from 16 tetracycline sensitive penicillinase producing strains carrying the 24.5 MDa conjugative plasmid (table 2) and 22 strains with tetracycline MICs of 8 mg/l. No product of any size was detected in the PCR assay from these two groups of strains.

In all, 518 strains of TRNG isolated in the United Kingdom but originating from infections acquired in 39 countries worldwide as well as the United Kingdom, were tested in the *tetM* PCR assay. A total of 387 TRNG strains produced 778 bp PCR products (American

Table 1 Phenotype and origin of 20 strains of *Neisseria gonorrhoeae* used to validate the *tetM* gene typing

Laboratory number	<i>TetM</i> gene type by PCR*	Published <i>TetM</i> gene type†	Plasmid Rf type‡	Tetracycline MIC (mg/l)	Plasmid profile (MDa)	Auxotype§/serovar	Country of acquisition
GR7418	A	A	A	32	2.6, 25.2	Arg/IB2	UK
GR7575	A	A	A	32	2.6, 25.2	NR/IB2	Unknown
GR7646	A	A	A	32	2.6, 25.2	NR/IB2	Unknown
GR7647	A	A	A	32	2.6, 25.2	NR/IB2	Unknown
GR7785	A	A	A	64	2.6, 3.2, 25.2	NR/IB5	Zaire
GR7891	A	A	A	32	2.6, 25.2	NR/IB16	UK
GR8122	A	A	A	32	2.6, 3.2, 25.2	NR/IB1	Jamaica
GR8224	A	A	A	32	2.6, 3.2, 25.2	Pro/IA6	Kenya
GR8258	A	A	A	32	2.6, 3.2, 25.2	Pro/IA6	Kenya
GR8260	A	A	A	32	2.6, 3.2, 25.2	Pro/IA6	UK
GR8958	A	A	A	32	2.6, 3.2, 25.2	Arg/IB2	W. Indies
GR8813	A	A	D	32	2.6, 4.4, 25.2	NR/IA6	Gabon
GR7272	D	D	D	32	2.6, 3.2, 25.2	NR/IB6	Unknown
GR7307	D	D	D	32	2.6, 3.2, 25.2	NR/IB6	UK
GR7698	D	D	D	16	2.6, 3.2, 25.2	Arg/IA2	Brazil
GR7926	D	D	D	32	2.6, 3.2, 25.2	Pro/IB4	Colombia
GR8104	D	D	D	16	2.6, 3.2, 25.2	NR/IB3	Jamaica
GR8136	D	D	D	16	2.6, 3.2, 25.2	NR/IB3	Jamaica
GR8219	D	D	D	32	2.6, 25.2	NR/IB3	Netherlands
GR8617	D	D	D	16	2.6, 3.2, 25.2	NR/IB6	UK

**tetM* type results obtained from the PCR assay described in the current study. A = American-type *tetM* gene or plasmid, and D = Dutch type.

†Data from Gascoyne-Binzi *et al.*¹⁷ and Professor PM Hawkey (personal communication).

‡Conjugative plasmid restriction endonuclease pattern data from Gascoyne *et al.*¹²

§Auxotypes are as follows: arginine requiring (Arg⁺); non-requiring (NR); proline requiring (Pro⁺).

Table 2 Results obtained from the PCR assay with 16 gonococci containing 24.5 MDa conjugative plasmids and 518 tetracycline resistant gonococci

Plasmid content (MDa)	No	PCR product		
		American	Dutch	None
2.6 + 3.0 + 24.5	5	0	0	5
2.6 + 3.2 + 24.5	10	0	0	10
2.6 + 4.4 + 24.5	1	0	0	1
2.6 + 25.2	153	111	42	0
2.6 + 3.0 + 25.2	1	0	1	0
2.6 + 3.2 + 25.2	318	257	61	0
2.6 + 4.4 + 25.2	46	19	27	0

type *tetM*) and 131 produced 443 bp PCR products (Dutch *tetM*) (table 2).

β Lactamase production was detected in 71% of TRNG strains carrying the American *tetM* and 68% of strains containing the Dutch *tetM*. The American type of *tetM* was found in 81% of TRNG strains carrying the 3.2 MDa β lactamase plasmid but was found in only 41% of strains carrying the 4.4 MDa plasmid.

The first TRNG strains were received by the GRU during 1988, one infection was acquired in Jamaica while three others had an unknown origin (table 3). One TRNG was received in 1989 and 30 in 1990 of which four were acquired in the United Kingdom. Overall, infections acquired in the United Kingdom contributed 57% (296 of 518) of the TRNG strains included in this study. Two hundred and forty two (82%) of these carried the American type of *tetM*.

After the United Kingdom, Caribbean and African countries contributed most strains with

56 and 60 TRNG acquired in each area respectively. All strains originating in Africa, except one from South Africa yielded PCR products characteristic of the American type *tetM*. Infections caught in Nigeria and Kenya contributed most strains, 15 and 14 respectively (table 3)—28 of these were also PPNG and 21 (75%) contained the 3.2 MDa β lactamase plasmid. Four auxotype/serovar (A/S) types were found among Kenyan strains, eight (57%) were proline requiring/IA6 and contained the 3.2 MDa β lactamase plasmid.

The TRNG originating from Caribbean countries comprised 36% (20/56) Dutch *tetM* type. Infections caught in Jamaica accounted for 46 (82%) of the strains in contrast with one from Barbados and two from Cuba (all three American type *tetM*) (table 3). American type *tetM* was detected in 29 (63%) of Jamaican TRNG, 20 of these 29 (69%) were PPNG. Penicillinase production was detected in 12 (71%) of the 17 Jamaican TRNG carrying the Dutch *tetM* type. All PPNG/TRNG acquired in Jamaica carried the 3.2MDa β lactamase plasmid. Jamaican TRNG carrying the American *tetM* type belonged to 10 different A/S types although 11 (38%) were non-requiring/IB2 and 10 of the 11 were PPNG. Jamaican TRNG carrying the Dutch *tetM* type belonged to nine different A/S types, the most frequent

Table 3 Reported geographical origin and *tetM* genotype of tetracycline resistant *Neisseria gonorrhoeae* strains received by the *Gonococcus* Reference Unit 1988–95

Geographical area/major country of acquisition	<i>tetM</i> Type	Year								Total
		1988	1989	1990	1991	1992	1993	1994	1995	
Africa										
Total	American			4	9	7	9	16	14	59
	Dutch							1		1
Kenya	American			2	4	2	4	1	1	14
	Dutch									0
Nigeria	American				2	4		6	3	15
	Dutch									0
South Africa	American								1	1
	Dutch							1		1
North, central, and South America										
Total	American					3		1		4
	Dutch			2	1	0		2	1	6
Caribbean										
Total	American			4	12	9	2	2	7	36
	Dutch	1		4	3	8	1	1	2	20
Barbados	American					1				1
	Dutch									0
Cuba	American								2	2
	Dutch									0
Jamaica	American			3	9	8	2	2	5	29
	Dutch	1		3	2	7	1	1	2	17
Europe										
Total	American			2	37	42	20	58	89	248
	Dutch			4	7	16	7	18	6	58
UK	American			2	36	41	20	56	87	242
	Dutch			2	7	15	7	18	5	54
Far East										
Total	American									0
	Dutch			1	8	4	9	5	8	35
Indonesia	American									0
	Dutch			1	7	2	3	3	1	17
Philippines	American									0
	Dutch								2	2
Thailand	American									0
	Dutch					1	2	1	4	8
Unknown	American	3	1	7	17	2		1	9	40
	Dutch			2	8			1		11
Total	American	3	1	17	75	63	31	78	119	387
	Dutch	1	0	13	27	28	17	28	17	131

was non-requiring/IB3 (five of 17, 29%) and three of these were PPNG.

All 35 TRNG strains originating in the Far East contained the Dutch type *tetM* (table 3). Penicillinase production was detected in 25 (71%) of the 35 Far East strains, 24 of these carried the 4.4 MDa β lactamase plasmid and the other was a 3.0 MDa β lactamase plasmid in a strain originating in the Philippines. Overall, a total of 27 TRNG/PPNG strains carrying the 4.4 MDa β lactamase plasmid and Dutch type *tetM* were detected from all sources (table 2). The three non-Far East strains with this plasmid pattern originated in Greece, Honduras, and the United Kingdom. Infections originating in Indonesia accounted for 17 (49%) of the 35 Far East strains with only Thailand (eight) providing more than three strains over the 8 year period (table 3). Of the 17 strains originating from Indonesia 14 (82%) were PPNG, these belonged to 12 different A/S combinations; however, eight were the non-requiring auxotype and of these four were the IA6 and three the IA4 serovar.

Discussion

In this study we have reported the development of a PCR technique which enables the simultaneous detection and typing of the *tetM* gene in gonococci. Complete agreement of *tetM* type was achieved with 20 strains of TRNG that had been examined using a restriction fragment polymorphism method reported by Gascoyne-Binzi *et al.*¹⁷ The PCR method reported here was applied to all 518 tetracycline resistant gonococci referred to the Gonococcus Reference Unit between 1988 and 1995. A PCR product was obtained from all strains and the product size always corresponded to that obtained from either the Dutch (443 bp) or the American (778 bp) type of *tetM* gene.

Two other PCR based methods for the detection and typing of the tetracycline resistance genotype in gonococci have been described. Ison *et al* described a PCR-RFLP assay in which the *tetM* gene is amplified by PCR but requires endonuclease digestion to discriminate between the American and Dutch types.¹⁴ Xia *et al* describe a differential PCR assay which does not require post-amplification restriction analysis.²² However the deletion responsible for the different sizes of products in this assay is downstream of the *tetM* gene and therefore the heterogeneity detected may not be reflected in the *tetM* nucleotide sequence. To facilitate this survey of the geographical distribution of *tetM* determinants we developed a simple single tube PCR assay which was specific to the *tetM* gene and did not require restriction of products. This assay could also be used to detect and characterise the *tetM* gene if it became integrated into the chromosome, as has been reported in non-pathogenic *Neisseria*²² but not, as yet, gonococci.

The *tetM* gene detection and typing data, together with collected travel and contact information, was used to describe the global distribution of TRNG as sampled by travellers diagnosed and treated for gonorrhoea in the United Kingdom. Although TRNG first ap-

peared in the United Kingdom in 1987¹ our data suggest that they did not become established until 1990. After this date there has been a steady rise in the numbers of TRNG infections contracted in the United Kingdom; four strains were received by the GRU in 1990 and by 1995 the number had risen to 92. Over the period of study 82% of UK acquired TRNG contained the American type *tetM* gene. Overall, 75% of TRNG received by the GRU contained the American type *tetM* gene. The rise in incidence in the United Kingdom provides evidence for persistence of TRNG in the United Kingdom as the numbers of TRNG imported each year from the major overseas areas (Africa, the Caribbean, and the Far East) have remained approximately constant from 1991 to 1995. The establishment of TRNG strains in the United Kingdom was predicted as early as 1990 as a likely result of the use of tetracyclines to treat chlamydia infections and pelvic inflammatory disease.²³

All TRNG strains isolated from patients infected in South East Asia were found to carry the Dutch *tetM* gene. All penicillinase producing TRNG originating in this region carried the 4.4 MDa plasmid except one previously reported²⁴ isolate from the Philippines which possessed a 3.0 MDa plasmid. This strain also demonstrated high level resistance to ciprofloxacin (MIC 16 mg/l). Other studies^{25, 26} have detected 3.2 MDa plasmids in PPNG isolated in the Far East but none was also TRNG. The type of *tetM* gene has been reported in one other study of Asian TRNG; of three strains isolated in Japan, one American type was found in an infection originating in Japan during 1985, and two Dutch *tetM* strains were found, one originating in Thailand in 1991 and the other was of unknown origin.²⁷ The WHO Western Pacific Region Gonococcal Antimicrobial Surveillance Programme (GASP) has revealed considerable differences in incidence in TRNG, isolated between 1992 and 1994, across this region, ranging from 0% in Japan to 100% in the Philippines.²⁸ Only Vietnam showed a significant rise in incidence during the period of study. The GASP study did not include Indonesia where most of the South East Asian TRNG originated from in the present study. However, a study of gonococci isolated during 1992–3 from female Indonesian sex workers revealed 98% of strains were TRNG.²⁹ Most of the TRNG isolated from these patients belonged to the proline requiring/IA8 A/S class in contrast with the strains in the present study where considerable heterogeneity was found. In a smaller study in the Philippines²⁶ most (5/6) TRNG or TRNG/PPNG were the arginine requiring/IA4 A/S class although there was heterogeneity among other gonococci in the study.

The emergence and spread of TRNG in west and central African countries is well documented.^{6, 15, 30, 31} In contrast, no TRNG were detected during 1990–3 in a study of isolates made from symptomatic men conducted in Durban, South Africa,³² or in Carltonville, South Africa between 1989 and 1990.³³ In most studies few data exist about the genetic

basis of the resistance other than plasmid size, although in isolates from Zaire the presence of *tetM* was confirmed by DNA hybridisation and the plasmid was demonstrated to be of the American type.⁶ In the present study, travellers visiting Kenya and Nigeria contributed most (29/60) of the TRNG infections originating in Africa. All TRNG strains from east, west, and central Africa contained the American type of *tetM*. All TRNG strains acquired in southern Africa except one from South Africa were also of the American type. A recent study of antibiotic resistant gonococci isolated in southern Africa did not detect TRNG in 55 isolates from South Africa but did find 11 TRNG among 19 isolates from Namibia and Botswana; all 11 carried the American type of *tetM* gene.³⁴ The high prevalence of proline requiring and IA6 serovar tetracycline resistant strains in African countries^{6 15 30} was confirmed in the present study. The TRNG originating from the two most common sources, Kenya and Nigeria, comprised 57% and 33%, respectively, of this A/S class although three other A/S classes were found in Kenyan strains and five in Nigerian strains.

The island of Jamaica provided the first high level tetracycline resistant isolate of *N. gonorrhoeae* in the present study, although three other isolates of unknown origin were also received in 1988. Over the period of the study Jamaica accounted for 82% of strains originating from the Caribbean region. In contrast with Africa and the Far East the TRNG originating from the Caribbean were of a mixed *tetM* type with 65% American type. A previous study has documented a high rate (69%) of TRNG among Jamaican gonococci isolated between October 1990 and March 1991.³⁵ As in the current study, all PPNG among the Jamaican study isolates carried the 3.2 MDa β lactamase plasmid although a previous survey of Jamaican strains isolated in 1983–4 had detected six strains out of 20 PPNG that carried the Asian (4.4 MDa) plasmid.³⁶ The results obtained from a survey of plasmid mediated antibiotic resistance in gonococci in Jamaica³⁵ indicated there was heterogeneity among tetracycline resistant strains. The TRNG and TRNG/PPNG in this study were found to belong to eight and 11 A/S classes respectively. This was confirmed by the present study where 10 A/S classes were found in strains carrying the American *tetM* type and nine A/S classes among the strains carrying the Dutch type.

The data presented here provide evidence for a variation in the global distribution of variants of the *tetM* gene. The distribution suggests that the Dutch type of *tetM* may have originated in the Far East, possibly in Indonesia, and that the American type may have originated in the equatorial regions of the African continent. International travel has since distributed both types of *tetM* to other parts of the world and heterogeneity of auxotype/serovar class would indicate that interstrain distribution of the *tetM* gene has also taken place. At completion of the survey the numbers of TRNG imported each year from the major overseas sources had reached a plateau while

UK contracted TRNG continued to rise, providing evidence for the establishment of endemic TRNG strains in the United Kingdom.

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Contributors: The project was initiated and designed by Drs Turner and Leeming. The laboratory work was carried out by Mrs Gough under the supervision of and with the aid of Dr Turner. The manuscript was written collaboratively by Dr Turner, Dr Leeming, and Mrs Gough.

- 1 Waugh MA, Lacey CJN, Hawkey PM, *et al.* Spread of *Neisseria gonorrhoeae* resistant to tetracycline outside the United States of America. *BMJ* 1988;298:898.
- 2 Roberts MC, Wagenvoort JHT, Van Klingeren B, *et al.* TetM and β -lactamase-containing *Neisseria gonorrhoeae* (tetracycline resistant and penicillinase producing) in The Netherlands. *Antimicrob Agents Chemother* 1988;32:158.
- 3 Casin I, Perene F, Isoire C, *et al.* High-level tetracycline resistance in penicillinase-producing *Neisseria gonorrhoeae* in France. *Eur J Clin Microbiol Infect Dis* 1989;8:929–31.
- 4 Vazquez JA, Berron S, Menéndez B. Isolate of TET M-containing *Neisseria gonorrhoeae* (TRNG) in Spain. *Genitourin Med* 1990;66:303–4.
- 5 Dillon JA, Carballo M. Molecular epidemiology and novel combinations of auxotype, serovar and plasmid content in tetracycline-resistant *Neisseria gonorrhoeae* isolated in Canada. *Can J Microbiol* 1990;36:64–7.
- 6 van Dyck E, Rossau R, Duhamel M, *et al.* Antimicrobial susceptibility of *Neisseria gonorrhoeae* in Zaire: high level plasmid-mediated tetracycline resistance in Central Africa. *Genitourin Med* 1992;68:111–6.
- 7 Marquez C, Xia M, Borthagaray G, *et al.* The first molecular characterization of tetracycline-resistant *Neisseria gonorrhoeae* from Uruguay. *J Antimicrob Chemother* 1996;37: 839–41.
- 8 Centers for Disease Control. Tetracycline-resistant *Neisseria gonorrhoeae*—Georgia, Pennsylvania, New Hampshire. *MMWR* 1985;34:563–70.
- 9 Gascoyne-Binzi DM, Hawkey PM, Heritage J, *et al.* World-wide distribution of high level tetracycline-resistant *Neisseria gonorrhoeae*. *Genitourin Med* 1992;68:277–8.
- 10 Turner A, Gough KR, Jephcott AE. Epidemiology of tetracycline-resistant *Neisseria gonorrhoeae* isolated in the UK, 1988–1991. In: Conde-Glez CJ, Morse S, Rice P, Sparling F, Calderon E, eds. *Pathobiology and immunobiology of Neisseriaceae*. Proceedings of the Eighth International Pathogenic *Neisseria* Conference. Cuernavaca, Mexico. INSP, 1994:305–12.
- 11 Morse SA, Johnson SR, Biddle JW, *et al.* High-level tetracycline resistance in *Neisseria gonorrhoeae* is result of acquisition of streptococcal *tetM* determinant. *Antimicrob Agents Chemother* 1986;30:664–70.
- 12 Gascoyne DM, Heritage J, Hawkey PM, *et al.* Molecular evolution of tetracycline-resistance plasmids carrying *tetM* found in *Neisseria gonorrhoeae* from different countries. *J Antimicrob Chemother* 1991;28:173–83.
- 13 Gascoyne-Binzi DM, Heritage J, Hawkey PM. Nucleotide sequences of the *tet(M)* genes from the American and Dutch type tetracycline resistance plasmids of *Neisseria gonorrhoeae*. *J Antimicrob Chemother* 1993;32:667–76.
- 14 Ison CA, Tekki N, Gill MJ. Detection of the *tetM* determinant in *Neisseria gonorrhoeae*. *Sex Transm Dis* 1993;20: 329–33.
- 15 Van Dyck E, Crabbe F, Nzila N, *et al.* Increasing resistance of *Neisseria gonorrhoeae* in West and Central Africa. Consequences on therapy of gonococcal infection. *Sex Transm Dis* 1997;24:32–7.
- 16 Communicable Disease Report. Tetracycline resistant *Neisseria gonorrhoeae* (TRNG). *CDR* 1987:47.
- 17 Gascoyne-Binzi DM, Hawkey PM, Heritage J. The distribution of variants of the TetM determinant in tetracycline-resistant *Neisseria gonorrhoeae*. *J Antimicrob Chemother* 1994;33:1011–16.
- 18 Hodge W, Ciak J, Tramont EC. Simple method for the detection of penicillinase producing *Neisseria gonorrhoeae*. *J Clin Microbiol* 1978;7:102–3.
- 19 Bennett PM, Heritage J, Hawkey PM. An ultra-rapid method for the study of antibiotic resistance plasmids. *J Antimicrob Chemother* 1986;18:421–4.
- 20 Copley CG, Egglestone SI. Auxotyping of *Neisseria gonorrhoeae* isolated in the United Kingdom. *J Med Microbiol* 1983;16:295–302.
- 21 Knapp JS, Tam MR, Nowinski RC, *et al.* Serological classification of *Neisseria gonorrhoeae* with use of monoclonal antibodies to gonococcal outer membrane protein I. *J Infect Dis* 1984;150:44–8.
- 22 Xia M, Pang Y, Roberts MC. Detection of two groups of 25.2MDa Tet M plasmids by polymerase chain reaction of the downstream region. *Mol Cellular Probes* 1995;9:327–2.
- 23 Easmon CSF. The changing pattern of antibiotic resistance of *Neisseria gonorrhoeae*. *Genitourin Med* 1990;66:55–6.
- 24 Turner A, Gough KR, Jephcott AE, *et al.* Importation into the UK of a strain of *Neisseria gonorrhoeae* resistant to

- penicillin, ciprofloxacin and tetracycline. *Genitourin Med* 1995;71:265–9.
- 25 Sarafian SK, Chu M-L, Jojima H, *et al.* Distribution of the 3.05 Mdal “Toronto” β -lactamase plasmid among penicillinase-producing isolates of *Neisseria gonorrhoeae* in the Far East. *Sex Transm Dis* 1991;18:201–4.
 - 26 Knapp JS, Mesola VP, Neal SW, *et al.* Molecular epidemiology, in 1994, of *Neisseria gonorrhoeae* in Manila and Cebu City, Republic of the Philippines. *Sex Transm Dis* 1997;24:2–7.
 - 27 Kuroki T, Murase T, Watanabe Y, *et al.* Characterisation of high level tetracycline resistant *Neisseria gonorrhoeae* isolates. *Genitourin Med* 1997;73:421–2.
 - 28 WHO Western Pacific Region Gonococcal Antimicrobial Surveillance Programme. Surveillance of antibiotic susceptibility of *Neisseria gonorrhoeae* in the WHO Western Pacific Region 1992–4. *Genitourin Med* 1997;73:355–61.
 - 29 Joesoef MR, Knapp JS, Idajadi A, *et al.* Antimicrobial susceptibilities of *Neisseria gonorrhoeae* strains isolated in Surabaya, Indonesia. *Antimicrob Agents Chemother* 1994;38:2530–3.
 - 30 West B, Changalucha J, Grosskurth H, *et al.* Antimicrobial susceptibility, auxotype and plasmid content of *Neisseria gonorrhoeae* in northern Tanzania: emergence of high level plasmid mediated tetracycline resistance. *Genitourin Med* 1995;71:9–12.
 - 31 Adegbola RA, Sabally S, Corrah T, *et al.* Increasing prevalence of penicillinase-producing *Neisseria gonorrhoeae* and the emergence of high-level, plasmid-mediated tetracycline resistance among gonococcal isolates in The Gambia. *Trop Med Int Health* 1997;2:428–32.
 - 32 Chenia HY, Pillay B, Hoosen AA, *et al.* Antibiotic susceptibility patterns and plasmid profiles of penicillinase-producing *Neisseria gonorrhoeae* strains in Durban, South Africa, 1990–1993. *Sex Transm Dis* 1997;24:18–22.
 - 33 Ison CA, Roope NS, Dangor Y, *et al.* Antimicrobial susceptibilities and serotyping of *Neisseria gonorrhoeae* in southern Africa: influence of geographical source of infection. *Epidemiol Infect* 1993;110:297–305.
 - 34 Chalkley LJ, van Vuuren S, Ballard RC, *et al.* Characterisation of penA and tetM resistance genes of *Neisseria gonorrhoeae* isolated in southern Africa—epidemiological monitoring and resistance development. *S Afr Med J* 1995;85:775–80.
 - 35 Knapp JS, Brathwaite AR, Hinds A, *et al.* Plasmid-mediated resistance in *Neisseria gonorrhoeae* in Kingston, Jamaica: 1990–1991. *Sex Transm Dis* 1995;22:155–9.
 - 36 Dillon JR, Carballo M, King SD, *et al.* Auxotypes, plasmid contents, and serovars of gonococcal strains (PPNG and non-PPNG) from Jamaica. *Genitourin Med* 1987;63:233–8.