Gut 1994; **35:** 1562–1566

High prevalence of *Helicobacter pylori* metronidazole resistance in migrants to east London: relation with previous nitroimidazole exposure and gastroduodenal disease

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Abstract

A high prevalence of metronidazole resistance in *Helicobacter pylori* is reported in developing countries. This study examined whether migrants referred for diagnostic gastroscopy at a United Kingdom centre (n=54), had a higher prevalence of metronidazole resistance than subjects born in the United Kingdom attending endoscopy (n=46). Records of nitroimidazole treatment prescribed in the United Kingdom was obtained in 83 patients to find out if there was an association between *H pylori* metronidazole resistance and previous ingestion of either metronidazole or tinidazole. The prevalence of metronidazole resistant isolates varied according to country of birth: Bangladesh (90%, 27 of 30), other countries (67%, 16 of 24), and United Kingdom (37%, 17 of 46) ($p<0.001$). Among those born in the United Kingdom, women were more likely to harbour resistant *H pylori* than men (54% *v* 18% respectively, $p=0.01$) and more likely to have a history of previous nitroimidazole ingestion (41% *v* 11% respectively, $p=0.02$). Patients previously exposed to either metronidazole or tinidazole were more likely to harbour resistant strains (84% (27 of 32) *v* 41% (21 or 51), $p<0.0001$). The distribution of gastroduodenal disease, assessed endoscopically, was not affected by metronidazole resistance status.

(*Gut* 1994; **35:** 1562–1566)

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Accepted for publication 11 March 1994

Eradication of *Helicobacter pylori* is effective in the treatment of associated gastritis, gastroduodenal ulceration, and preventing duodenal ulcer relapse.[1 2] Regimens incorporating metronidazole have generally been the most effective for eradicating *H pylori*: such treatment is, however, less effective in patients harbouring metronidazole resistant strains.[3 4] A high prevalence of metronidazole resistance is found in developing countries.[4] It has not been established (a) whether migrants carry with them the *H pylori* metronidazole resistance rates from their country of origin and thus represent a population in whom *H pylori* is difficult to eradicate, and (b) whether *H pylori* resistance in itself is a marker of bacterial virulence. The population of The London Borough of Tower Hamlets, served by The Royal London Hospital Trust (RLH), provides an opportunity to test these hypotheses as 30% of patients referred for gastroscopy are immigrants from Bangladesh or India. We compared metronidazole resistance in *H pylori* isolates from the indigenous UK population and those born in other countries, correlating these results with previous nitroimidazole usage and the spectrum of *H pylori* associated gastroduodenal disease.

Methods

PATIENTS

Two hundred and seven consecutive patients attending for routine diagnostic upper gastrointestinal endoscopy at RLH were studied prospectively. Patients were asked to complete a questionnaire giving personal details including age, sex, and country of birth.

H pylori metronidazole resistance was determined in patients found to be *H pylori* gastric biopsy culture positive at endoscopy (n=100, 48%).

In those patients *H pylori* positive on gastric biopsy culture an attempt to record their previous use of metronidazole and tinidazole in the UK was made. The patient's present general practitioner was asked to obtain details of previous GP or hospital prescribed nitroimidazole treatment. The GPs were not aware whether the patients *H pylori* was metronidazole resistant or sensitive.

Approval for these studies was obtained from Tower Hamlets ethics committee.

ENDOSCOPIC CLASSIFICATION OF GASTRODUODENAL DISEASE

For the purposes of assessing *H pylori* related disease, gastric and duodenal mucosal appearances were classified separately as: (a) clinically significant damage: ulceration (≥5 mm, with depth), or inflammation with multiple (>3) erosions; (b) minor damage: mild inflammation with 0–3 erosions; (c) normal.

CULTURE OF *H PYLORI*

One antral gastric biopsy specimen from each patient was placed in 20% glucose transport medium, and within four hours of collection homogenised with sand (previously purified by acid) using a glass grinder. The resulting suspension was inoculated onto brain heart

infusion agar (BHIA: Oxoid CM375) with 5% horse defibrinated blood (Becton Dickinson, Cowley, Oxford, UK) and added *H pylori* selective supplement (Oxoid SR147E) containing vancomycin 10 mg/l, trimethoprim 5 mg/l, cefsulodin 5 mg/l, and amphotericin B 5 mg/l. Cultures were incubated for up to five days at 37°C in microaerobic gaseous conditions (5% O_2 and 10% CO_2) using a gas generating kit (Campylobacter system gas generating kit, Oxoid BR056A). Identity of *H pylori* was confirmed by Gram stain and production of urease, oxidase, and catalase.[6 7] Isolates were subcultured onto BHIA with 5% horse defibrinated blood, before metronidazole sensitivity was assessed.

DETECTION OF *H PYLORI* ON GASTRIC BIOPSY SPECIMENS BY CLO TEST

Rapid urease tests (CLO, Delta West, Australia) were performed on gastric biopsy specimens on 61 of 100 patients attending the clinic.

STANDARD METRONIDAZOLE DISC SENSITIVITY TESTING

Metronidazole sensitivity testing was performed by an investigator unaware of the clinical details.

The bacterial inoculum for sensitivity testing was prepared from a 48 hour old culture grown on BHIA with 5% horse defibrinated blood. Colonies were scraped from plates and suspended in brucella broth until a turbidity equivalent to 3 to 4 on the McFarland scale was obtained, corresponding with roughly 10^8 colony forming units/ml in our laboratory. A brucella agar plate with added 5% horse defibrinated blood was streaked with a cotton wool swab saturated with the suspension. A 5 μg metronidazole disc (Oxoid 039820) was placed in the centre of the plate and incubated at 37°C in a microaerobic atmosphere for 72 hours. A zone of ≥10 mm clear (diameter 25 mm) of *H pylori* around the disc was used to show metronidazole sensitivity. Control strains NCTC 12822 (sensitive) and NCTC 12823 (resistant) was included with all incubations (National Collection Type Cultures, London, UK).

MINIMUM INHIBITORY CONCENTRATION SUSCEPTIBILITY TESTING

Metronidazole minimum inhibitory concentrations were performed on 24 strains to ensure correlation between disc diffusion tests and plate dilution minimum inhibitory concentrations. The methods used have previously been described.[8] A minimum inhibitory concentration of <8 mg/l was used to indicate sensitivity.[4 9]

NITROIMIDAZOLE AVAILABILITY

An attempt was made to record the year of introduction and over the counter availability of metronidazole and tinidazole in the countries the 100 *H pylori* positive patients were born in. Details were obtained from Pfizer Pharmaceuticals, Sandwich, UK and Rhône Poulenc Rorer Ltd, Eastbourne, UK.

TABLE I H pylori *prevalence (defined by gastric biopsy specimen culture) and age group of patients attending endoscopy at The Royal London Hospital*

	Place of birth		
	UK	*Bangladesh*	*Other*
Number	115	57	35
Mean age (y)			
At endoscopy	55*	38	50
At migration	–	17	24
% Men	61	66	66
H pylori prevalence age group (y) (%)			
20–39	3/9 (33)	8/17 (47)	2/2 (100)
40–59	4/27 (15)†	13/27 (48)	9/14 (64)
60+	39/79 (50)	9/17 (53)	13/15 (87)
All	46/115 (40)‡	30/61 (49)	24/31 (80)§

*p<0·0001, mean age of UK *v* Bangladeshi patients (Student's *t* test); †p=0·007, proportion of *H pylori* stains isolated from UK *v* overseas born patients, aged 40–59 (χ^2); ‡p=0·001, proportion of *H pylori* strains isolated from UK *v* patients born overseas, for all age groups (χ^2); §Cyprus 3 of 5, India 4 of 4, Malta 3 of 4, West Indies 2 of 4, Somalia 3 of 3, Greece 0 of 2, Italy 2 of 2, South Africa 2 of 2, Turkey 2 of 2, China 1 of 1, Poland 1 of 1, and Spain 1 of 1.

STATISTICAL METHODS

Student's *t* test and χ^2 tests (including Fisher's two tailed exact tests where appropriate) were performed using EPI-INFO (CDC, Atlanta, Georgia, USA) software.

Results

Table I shows the frequencies of *H pylori* culture positivity by country of birth. Patients were divided into three groups; UK (n=115), Bangladeshi (n=61), and other (n=31). The last group consisted of patients born in: Cyprus (n=5), India (n=4), Malta (n=4), West Indies (n=4), Somalia (n=3), Greece (n=2), Italy (n=2), South Africa (n=2), Turkey (n=2), and China, Poland and Spain, one each. Results are stratified by age. UK patients were significantly older than Bangladeshis (p<0·001). Most patients born in Bangladesh migrated as children or young adults (mean 17 years, range 1–46 years). Overall, *H pylori* was less frequently cultured from patients born in the UK, compared with patients born overseas (p=0·007) (Table I).

TABLE II *Metronidazole resistance by country of birth and sex (n=100)*

	Women	*Men*	*Total*
*Isolates from patients born in the UK**			
Resistant	13	4	17†
Sensitive	11	18	29
Total	24	22	46
Isolates from patients born in Bangladesh			
Resistant	7	20	27
Sensitive	2	1	3
Total	9	21	30
Isolates from patients born in other countries			
Resistant	8	8	16‡
Sensitive	2	6	8
Total	10	14	24

*p<0·001, prevalence of *H pylori* resistant strains from UK *v* Bangladeshi patients (χ^2); †p=0·01, proportion of resistant *H pylori* in women *v* men (χ^2); ‡India 4 of 4, Malta 2 of 3, Somalia 3 of 3, Cyprus 2 of 3, West Indies 2 of 2, Italy 0 of 2, South Africa 1 of 2, Turkey 0 of 2, China 1 of 1, Poland 1 of 1, and Spain 0 of 1.

TABLE III *Nitroimidazole treatment before endoscopy (data obtained for 83 of 100* H pylori *culture positive patients) by sex*

	Women	*Men*	*Total*
Patients born in the UK			
Previous use	9*	2	11
No previous use	13	18	31
Total	22	20	42
Patients born in Bangladesh			
Previous use	3	8	11†
No previous use	4	7	11
Total	7	15	22
Patients born in other countries			
Previous use	6	4	10‡
No previous use	2	7	9
Total	8	11	19

*p=0·02, proportion of men *v* women born in the UK with a history of previous nitroimidazole use; †p=0·05, proportion of Bangladeshis *v* UK born with a history of nitroimidazole use before endoscopy; ‡p=0·04, proportion of others *v* UK born with a history of nitroimidazole use before endoscopy (χ^2 tests).

TABLE IV H pylori *metronidazole resistance according to previous nitroimidazole ingestion in the UK (data on previous ntroimidazole use obtained in 83 of 100* H pylori *culture positive patients)*

	Previous metronidazole use		
	No	*Yes*	*Total*
*Patients born in the UK**			
Resistant	7	8	15
Sensitive	24	3	27
Total	31	11	42
Patients born in Bangladesh†			
Resistant	10	10	20
Sensitive	1	1	2
Total	11	11	22
Patients born in other countries‡			
Resistant	4	9	13
Sensitive	5	1	6
Total	9	10	19

*p=0·003, †p=1·00, ‡p=0·06; proportion of *H pylori* metronidazole resistance in those with *v* those without a history of previous nitromidiazole treatment (for those born in the UK, Bangladesh and elsewhere), (χ^2 or Fisher's exact test as appropriate).

METRONIDAZOLE RESISTANCE AND COUNTRY OF BIRTH

Table II shows the relation between country of birth and metronidazole resistance in the 100 *H pylori* isolates. Ninety per cent (27 of 30) of isolates from patients born in Bangladesh were metronidazole resistant, compared with 37% (17 of 46) of isolates obtained from patients born in the UK (p<0·001). Of patients born in other countries 67% (16 of 24) of isolates were metronidazole resistant (India 4 of 4, Malta 2 of 3, Somalia 3 of 3, Cyprus 2 of 3, West Indies 2 of 2, Italy 0 of 2, South Africa 1 of 2, Turkey 0 of 2, China 1 of 1, Poland 1 of 1, and Spain 0 of 1).

There was no relation between gastric biopsy specimen CLO status and *H pylori* metronidazole status (37 of 41 resistant strains were CLO test positive and 18 of 20 sensitive strains were CLO test positive, p=1·0).

TABLE V *Distribution of gastroduodenal disease (assessed endoscopically) in patients harbouring* H pylori *metronidazole resistant or sensitive strains*

		Metronidazole resistant H pylori		*Metronidazole sensitive* H pylori	
*Endoscopic diagnosis**	*Total*	*No*	*%*	*No*	*%*
Gastric					
Clinically significant	8	3	37	5	63
Minor	47	29	62	18	38
Normal	45	28	63	17	37
Duodenal					
Clinical significant	26	17	65	9	35
Minor	16	9	56	7	44
Normal	58	34	59	24	41

*See methods for definition of endoscopic diagnoses.

METRONIDAZOLE RESISTANCE AND PREVIOUS NITROIMIDAZOLE TREATMENT

Records of previous metronidazole and tinidazole use in the UK were obtained in 83% (83 of 100) patients from GP records. Data were obtained from a similar proportion of each of the three ethnic subgroups: 91% (42 of 46) UK born; 73% (22 of 30) Bangladeshi born, and 82% (19 of 24) born in other countries. Nitroimidazole treatment was more frequently prescribed in the UK, in patients born in Bangladesh, 50% (11 of 22) than patients born in the UK, 26% (11 of 42) (p=0·05) (Table III). Nitroimidazole treatment was also more frequently prescribed in the UK, in patients born in other countries, 53% (10 of 19), than in patients born in the UK, 26% (11 of 42) (p=0·04) (Table III).

A history of previous nitroimidazole treatment was more frequent in women, 41% (9 of 22) than men, 11% (2 of 20) in those born in the UK (p=0·02) but not in those born outside the UK (Table III).

For *H pylori* positive patients born in the UK, a history of previous nitroimidazole treatment in the UK was associated with the presence of metronidazole resistant *H pylori* (p=0·003) (Table IV). There was no relation between previous nitroimidazole treatment in the UK and presence of metronidazole resistant *H pylori* in patients born in either Bangladesh (p=1·00) or other countries (p=0·06) (Table IV).

METRONIDAZOLE RESISTANCE AND SEX

In patients born in the UK, women were significantly more likely (p=0·01) to harbour resistant strains than men (Table II). Women were no more likely to harbour resistant strains than men in patients born in countries other than the UK (Bangladeshis, p=0·21; other countries, p=0·39) (Table II).

METRONIDAZOLE RESISTANCE AND GASTRODUODENAL DISEASE

There was no difference in the prevalence of significant *H pylori* associated gastric or duodenal abnormality (ulcer/multiple erosions) or minor gastritis or duodenitis between patients with *H pylori* metronidazole sensitive and resistant isolates in any of the ethnic subgroups or in the study group overall (Table V).

NITROIMIDAZOLE AVAILABILITY

Table VI shows the year of introduction and over the counter availability of metronidazole and tinidazole in the countries the patients were born in. Metronidazole was introduced into Bangladesh in 1957 and is available as an over the counter preparation. Metronidazole was introduced in Bangladesh before any of

TABLE VI *Year of introduction and availability of 'over the counter' preparations of metronidazole and tinidazole*

	Metronidazole		*Tinidazole*	
Country	*Year of introduction by Rhône Poulenc Rorer*	*Availability of 'over the counter' preparation*	*Year of introduction by Pfizer*	*Availability of 'over the counter' preparation*
Bangladesh	1957	Yes	1978	No
China	Mid 1970s	No	Not marketed	
Cyprus	N/O		Not marketed	
India	1962	No	1988	No
Italy	Early 1960s	No	1979	No
Malta	N/O		Not marketed	
Poland	N/O		Not marketed	
Somalia	N/O		1980	No
South Africa	1968	No	1974	No
Spain	1961	No	1974	No
Turkey	1963	No	1991	No
United Kingdom	1960	No	1982	No
West Indies	N/O		Not marketed	

N/O=data not obtained.

the Bangladeshi patients had migrated to the UK.

MINIMUM INHIBITORY CONCENTRATION SUSCEPTIBILITY TESTING

Twenty three of 24 strains produced minimum inhibitory concentration and disc sensitivity concordance using the definitions for resistance in the methods above. The one strain that showed disc and minimum inhibitory concentration discordance was deemed minimum inhibitory concentration sensitive (4 mg/l) but resistant on disc sensitivity testing (diameter of 21 mm).

Discussion

This study shows that 90% of gastric isolates of *H pylori* from patients born in Bangladesh and now living in the UK were resistant to metronidazole. This was significantly greater than the 37% resistance rate in patients born in the UK.

Our results have also shown that for patients born in the UK, previous nitroimidazole treatment, given in the UK, was associated with metronidazole resistance. An association between previous nitroimidazole use in the UK and metronidazole resistance may not have been detected in Bangladeshi patients simply because there were too few patients with sensitive strains, limiting the power of the statistical analysis. An additional problem is that as nitroimidazole use was obtained solely from GP records, hospital prescriptions, if not reported to the GP, would be excluded.

It is probable that the high prevalence of metronidazole resistance in Bangladeshi patients was acquired in their country of birth and may be attributable to the frequent use of nitroimidazoles before migration. Metronidazole is the most used antimicrobial for diarrhoeal disease in Bangladesh (Dr R Bradley Sack, personal communication) and a community study in Bangladesh has shown that 51% of the population sampled had ingested metronidazole without prescription.[10] It therefore seems probable that a high proportion of Bangladeshi patients will have been exposed to metronidazole before migrating to the UK. We do not have data on the frequency of return visits to Bangladesh and therefore we cannot estimate the importance of nitroimidazole ingestion on return visits to Bangladesh. In addition, however, Bangladeshi patients had significantly higher metronidazole usage in the UK than the indigenous population (50% *v* 26%); thus the high metronidazole resistance rate may be attributable in part to their metronidazole ingestion since migration.

Within the UK born population, we found that women were significantly more likely to harbour resistant strains; this confirms previous reports.[3 11] It has been shown that in patients with *H pylori* infection, women are more likely than men to have been treated for non-*H pylori* disease with nitroimidazoles.[11] This sex difference in metronidazole use further supports the hypothesis that previous nitroimidazole treatment for non-*H pylori* related disease is a risk factor for *H pylori* metronidazole resistance.[11]

H pylori was more frequently isolated from patients born outside the UK. There was no evidence that metronidazole resistant strains were more easily isolated than sensitive strains as there was no relation between metronidazole resistance and gastric biopsy specimen rapid urease test result.

Acquisition of antimicrobial resistance has been associated with both increased and decreased bacterial virulence.[12–16] For *H pylori* we found the distribution of significant gastroduodenal disease in metronidazole sensitive and resistant strains to be similar. These results do not support an associated between acquisition of metronidazole resistance and *H pylori* virulence.

While there is no universally accepted treatment, regimens for *H pylori* eradication including a nitroimidazole (usually metronidazole) are generally the most effective.[17] *H pylori* eradication treatment is less successful in subjects harbouring metronidazole resistant strains.[3 4 18] The high frequency of metronidazole resistant strains in our population, particularly in ethnic minority groups, constitutes a therapeutic problem to which there is as yet no clear solution. The metronidazole resistance rates for our UK born population are higher than in previous reports.[7] This may be explained by the comparatively high rate of nitroimidazole use in our UK born subgroup. An alternative but untested hypothesis is that imported resistant strains have infected subjects born in the UK.

In conclusion, this study has shown that subjects born in developing countries, infected with *H pylori*, and now living in the UK, have a high prevalence of metronidazole resistance. In addition, metronidazole resistance was more frequent when patients had a history of previous nitroimidazole treatment. The spectrum of gastroduodenal abnormality associated with *H pylori* infection seems independent of metronidazole resistance status.

We thank Professor D I Edwards and Dr G Urwin for their valuable comments during preparation of this manuscript. We thank Pfizer Pharmaceuticals, Sandwich, UK and Rhône Poulenc Rorer, Eastbourne, UK for assistance in determining nitroimidazole availability in the countries listed.

Funding for this work was provided by The Special Trustees

of The Royal London Hospital and Lederle Pharmaceuticals. Some of these data have been published in abstract form: *Gut* 1993; **34** (suppl): S37 and *Gastroenterology* 1993; **104:** A37.

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