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Molecular typing of Nigerian *Helicobacter pylori* isolates by *glmM* restriction fragment length polymorphism

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Helicobacter pylori is recognised as the causative agent for chronic gastritis and an essential agent in the development of ulcers, and is implicated as a major risk factor for the development of gastric cancer.[1] Several typing techniques are used for epidemiological and clinical purposes and some of these have confirmed the genomic variability of *H. pylori*.[2-4] The techniques most frequently reported for typing are polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis and randomly amplified polymorphic DNA (RAPD) analysis.

A number of authors have reported on the molecular typing of *H. pylori* using the urease A and B genes but few have used *glmM*, formerly *ureC*, for epidemiological typing and for monitoring recrudescence.[5-8] *glmM* RFLP is reliable, highly reproducible and most useful in monitoring reinfection versus recrudescence.[9-11] Although the main role of *glmM* is now recognised to be related to cell-wall synthesis and not to urease activity, it is still used for genotyping.[12]

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Table 1. Restriction enzyme digest profiles of 41 *H. pylori* isolates using PCR-RFLP of the *glmM* gene

			Restriction enzyme pattern	
Isolate No.	Diagnosis	*cagA* status	*Hha*I	*Sau*3A
1	NUD	–	1	1
2	Ulcer	+	1	2
3	Ulcer	+	2	3
4	NUD	+	3	4
5	Ulcer	+	2	5
6	Ulcer	+	1	6
7	Ulcer	+	1	7
8	Ulcer	+	1	8
9	NUD	+	4	1
10	NUD	+	3	1
11	NUD	–	2	1
12	NUD	+	1	1
13	Ulcer	+	2	9
14	NUD	+	1	10
15	NUD	+	3	1
16	NUD	+	4	1
17	Ulcer	+	5	2
18	NUD	+	5	2
19	NUD	+	2	1
20	Ulcer	+	3	4
21	Ulcer	+	1	10
22	NUD	+	1	1
23	NUD	+	1	1
24	Ulcer	+	5	2
25	NUD	+	4	1
26	NUD	+	2	3
27	Ulcer	+	5	3
28	Ulcer	+	3	5
29	NUD	+	1	6
30	Ulcer	–	3	7
31	Ulcer	+	3	10
32	Ulcer	+	1	8
33	NUD	+	1	1
34	NUD	+	4	1
35	NUD	+	5	1
36	Ulcer	+	3	10
37	NUD	+	5	1
38	NUD	+	2	1
39	NUD	+	2	1
40	Ulcer	+	5	10
41	Ulcer	+	5	3

NUD: non-ulcer dyspepsia

Isolates 22 and 23, 10 and 15 and 25 and 34 were from the same patients

This study aims to type Nigerian *H. pylori* strains using a previously described *glmM* PCR-RFLP technique,[9,10] and to examine multiple isolates from the same patients with non-

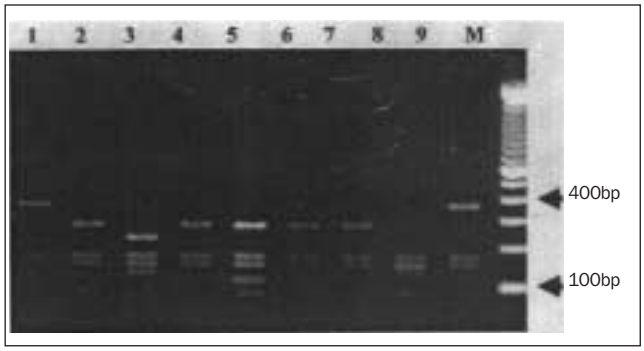

Fig. 1. *Hha*I digest patterns of Nigerian *H. pylori* strains. RFLP type 1 (lanes 1 and 9), RFLP type 2 (lanes 2, 4, 6 and 7), RFLP type 3 (lane 3) RFLP type 4 (lane 5), RFLP type 5 (lane 8); M: 100 bp molecular weight marker.

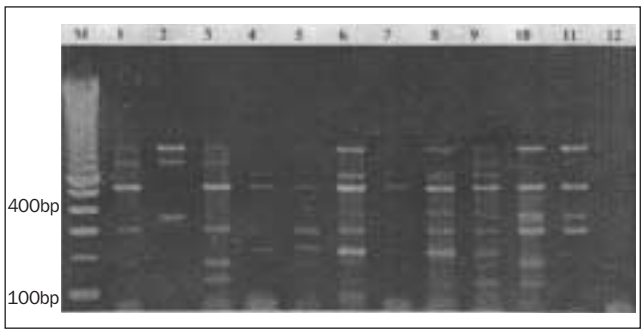

Fig. 2. *Sau*3A digest patterns of Nigerian *H. pylori* starins. RFLP type 1 (lanes 1 and 3), RFLP type 2 (lane 2), RFLP type 3 (lane 4), RFLP type 4 (lane 5), RFLP type 5 (lanes 6 and 8), RFLP type 6 (lane 7), RFLP type 7 (lane 9), RFLP type 8 (lane 10), RFLP type 9 (lane 11), RFLP type 10 (lane 12); M: 100 bp molecular weight marker.

ulcer dyspepsia (NUD) to determine whether or not there is DNA heterogeneity at different anatomical sites. The presence of the cytotoxin-associated gene (*cagA*) is also determined.

A total of 41 clinical isolates (22 from NUD and 19 from ulcer) were used in this study, obtained from 38 patients. Two biopsies/isolates each from the stomach and antrum of three patients presenting with NUD were included, while all the others comprised unrelated isolates. All patients lived in the western region of Nigeria.

Clinical *H. pylori* strains were isolated on Columbia agar base containing Dent's supplement and 7% laked horse blood. The isolates were incubated at 37 °C for 3–7 days.

Typical colonies were screened for urease, catalase and oxidase positivity. DNA was extracted using the phenol DNA method as described by Covacci and Rappuoli.[13]

The primers and conditions used for *glmM* amplification were those used by Fujimoto *et al*.[9]

The primers for *cagA* were: *cagAF* (+) TTGACCAACAACCACAAACCGAAG, and *cagAR* (–) CTTCCCTTAATTGCGAGATTCC and they amplify a 183 bp region in *H. pylori*. The PCR conditions were as described by van Doorn *et al*.[14] Statistical analysis was performed using the χ^2 test for any association of *cagA*-positivity with ulceration.

The PCR protocol successfully amplified an 820 bp product from all 41 isolates. Among these, three pairs of isolates were obtained from three patients from the stomach and antrum. All the pairs had identical restriction digestion profiles, indicating that the *H. pylori* were the same strain

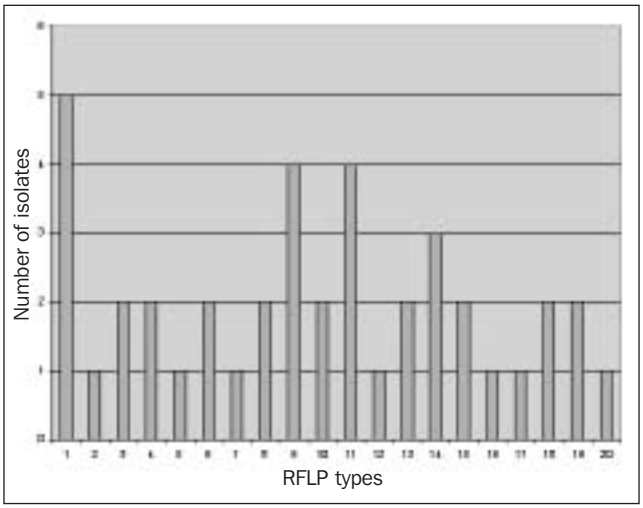

Fig. 3. Distribution of *glmM* RFLP types.

(Table 1). This supported the observations of Moore *et al*.,[15] who found that isolates obtained from patients before and after antibiotic therapy showed that two out of the three pairs had identical restriction profiles, indicating that the patient was infected with the same *H. pylori* strain, before and after antibiotic treatment.

The method used in the present study showed heterogeneity among *H. pylori*, as five and 10 different patterns were observed with *Hha*I and *Sau*3A digests, respectively (Table 1). Figures 1 and 2 show the common *glmM* PCR- RFLP patterns observed among the isolates, while Figure 3 shows the distribution of *glmM* RFLP types.

When the isolates were grouped, 20 different PCR types were observed, demonstrating the genetic diversity of the *glmM* gene in *H. pylori* when typed by PCR-RFLP. Several other studies have demonstrated this heterogeneity among *H. pylori*.[9-12, 15-16] Interestingly, this technique (with *Sau*3A) was able to partly distinguish between the NUD strains and the ulcer strains, as strains from group 1 were all NUD strains, while group 2 strains were shared between NUD and ulcer strains (Table 1).

Groups seven and eight comprised exclusively ulcer strains. However, the *Hha*I digests could not distinguish between the ulcer and NUD isolates because the strains were found to share the same restriction profiles, irrespective of disease, and this finding was supported by Stone *et al*.[11]

PCR-RFLP of the *glmM* gene has also been reported to be useful in the identification of strains in cases of reinfection with a new strain and recrudescence of the original strain that failed to respond to treatment.[9-11, 16] In the present study we were unable to look into post-treatment cases because most, if not all, patients fail to return because of the cost of endoscopy.

Analysis of the *cagA* gene by PCR showed that of the 22 isolates from patients with NUD, 20 (91%) were *cagA*-positive, and 18 (95%) out of the 19 isolates from patients with ulcer were *cagA*-positive. Thus, no significant difference was observed ($P>0.05$) for the presence of *cagA* between NUD and ulcer patients, and no significant correlation was observed between the presence of *cagA* and ulceration.

PCR-RFLP typing of *glmM* is suitable for epidemiological studies to differentiate between Nigerian *H. pylori* isolates. It also supports other studies of urease genes that show a high degree of heterogeneity among *H. pylori* isolates. □

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