Discriminatory Power of RAPD, PCR-RFLP and Southern Blot Analyses of *ureCD* or *ureA* Gene Probes on *Helicobacter pylori* Isolates

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The genetic diversity of 33 Nigerian *Helicobacter pylori* isolates were studied using RAPD, PCR-RFLP and Southern blot analysis of *ureA* or *ureCD* gene probes. RAPD was able to distinguish the following number of isolates using the primers 3880 : 5′-AAGAGCCCGT-3′ (28), 3881 :5′-AACGCGCAAC-3′ (33) and OPH8 :5′-GAAACACCCC-3′ (25). Southern blot analysis using the *ure*CD probe was also able to distinguish the 12 isolates tested into ten different patterns. The PCR-RFLP technique distinguished all 33 isolates into six types. In conclusion, considering typeability, discriminatory power, and convenience, RAPD with the 3881 primer was considered the most useful technique.

Introduction

Helicobacter pylori is the cause of chronic gastritis and peptic ulcer disease and is a major risk factor in the development of gastric carcinoma and gastric mucosa associated lymphoid tissue (MALT) lymphoma (Marshall *et al*., 1985; Forman *et al*., 1991).

The natural history of *H. pylori* infection is not fully understood especially in regions like Africa, where several strains can colonize the same subjects. It has been emphasized in the past that it is important to be able to differentiate strains for clinical and epidemiological purposes.

Various molecular methods have been developed for this purpose i.e. restriction enzyme analysis (REA) of the whole genomic DNA (Owen *et al*., 1990; Taylor *et al*., 1995), ribotyping (Romero Lopez *et al*., 1993), pulsed-field gel electrophoresis (PFGE) (Takahami *et al*., 1994), polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis (Taylor *et al*., 1995), repetitive extragenic palindromic – polymerase chain reaction (REP-PCR) (van Doorn *et al.*, 1998) and randomly amplified polymorphic DNA (RAPD) (Akopyantz *et al*., 1995). In Africa, Kidd *et al*., (2001) reported on the use of RAPD and REP-PCR to assess the association between clonal grouping, disease and virulence fingerprints of 76 South African *H. pylori cag*A positive strains. Other reports dealt with the presence or absence of *cag*A, *vac*A genes as virulence factors (Letley *et al*., 1999; Kidd *et al*., 1999). No other study from Africa on the use of molecular typing method has been reported.

The aim of this study was to compare the properties of three molecular typing methods on Nigerian *H. pylori* isolates. These methods were chosen because of their feasibility in the Nigerian environment.

Materials and Methods

Bacteria and culture conditions

Thirty-three clinical isolates of *H. pylori* obtained from Lagos and Ife in Nigeria were analysed in this study: 13 from 12 ulcer patients and 20 from patients with gastritis only. The reference strain of *H. pylori* 26695 was also included. All isolates were grown in Columbia agar base containing 7% laked horse blood (Oxoid SR48) and supplemented with *H. pylori* supplement SR 147 E (Oxoid). Cultures were incubated at 37 °C under

microaerobic conditions in a candle extinction jar for 10 days (Coker *et al*., 1984).

Preparation of genomic DNA

The genomic DNA of the isolates were obtained by the phenol extraction method according to Covacci and Rappuoli (1996).

RAPD analysis. The method proposed by Akopyantz *et al*. (1992) was used with two of the primers described (3881 :5′-AACGCGCAAC-3′, 3880: 5′-AAGAGCCCGT-3′) as well as another primer commercially available (OPH8 :5′-GAAACACCCC-3′; Bioprobe Systems, Montreuil, France). The parameters of the cycles were 94 °C for 3 min, 94 °C for 30 sec, 36 °C for 30 sec, 72 °C for 90 min, 40 times, then a further extension for 7 min at 72 °C. After PCR, 8 μl of the PCR products were separated by 1.5% agarose gel electrophoresis. The DNA of 1kb ladder (Promega, Madison, Wis.) was used as size marker in all gels.

Southern blot analysis with ureA or ureCD probes

The protocol of Desai *et al.* (1994) was followed. Briefly, *Hind*III was used for restriction of the whole genomic DNA (10 μg) and separated by electrophoresis in 0.8% agarose gel at 30V for 16h.

Probes were prepared by amplification of a 411 bp fragment of *ureA* (Clayton *et al*., 1990) and a 1.7 kb fragment of the *ureCD* genes (Labigne *et al*., 1991). The PCR conditions were the following: for *ureA*, 30 sec at 94 °C, 1 min at 48 °C and 1.5 min at 72 °C, (× 30 cycles) for the *ureCD* fragment, 1 min at 94 °C, 1 min at 60 °C, and 2 min at 72 °C (x 20 cycles). For both fragments, there was an initial step of denaturation for 5 min at 94 °C and a final step of extension for 5 min at 72 °C. The products were analyzed by electrophoresis in 1.5% gel agarose.

Southern blotting was carried out using the method of Desai *et al*. (1994) , with the exception of the detection system, which was digoxigenin. Dig-labelled DNA was used as the molecular weight marker. The reference strain *H. pylori* 26695 was included as control.

PCR-RFLP analysis. A 1.7 kb fragment of the *ure*CD gene was amplified according to the method of Akopyantz *et al*. (1992). The restriction enzyme *Sau*3A was used in place of *Mbo*I as these were found to cut at the same restriction site. The parameters of the cycle used were as described by Desai *et al*. (1994), i.e., 1 min for 94 °C, 1 min for 60 °C, and 2 min at 72 °C (x 20 cycles).

Results

The profiles generated by RAPD with the three primers were composed of two to ten major bands for all primers. (Fig 1a, b, c). All 33 DNA from *Helicobacter pylori* isolates showed amplification with the primers used. Different patterns for all 33 isolates were obtained with primer 3881, while primers 3880 and OPH8 could discriminate 28 and 25 isolates respectively.

For the southern blot analysis, out of 16 isolates tested, the DNA from four was not digested by *Hind* III and the DNA from 12 other isolates, including the reference strain, could be separated into six groups with the *ureA* probe (Fig. 2a), while the *ureCD* probe showed a lower typeability despite the fact that the profiles were more discrminatory (Fig. 2b).

With the *ureA* probe, five isolates had the common band (4.97 kb), while for three isolates the band was slightly higher (approx. 5kb) and for three isolates the band had a size of 1.4 kb (Fig. 2a).

With *ureCD* probe, five isolates showed a similar band of about 1.5 kb, while for seven isolates a band of size less than 947 bp was present. Five isolates also shared the same band of size greater than 3.5 kb. Only one band, apart from the reference strain was greater than 4.97 kb (Fig. 2b).

PCR-RFLP led to profiles in all 33 isolates. Six different patterns could be differentiated, with one more fragment than the others (Fig 3).

Discussion

From this study, RAPD and PCR-RFLP analyses had an excellent typeability, which is further corroborated by Burucoa *et al.* (1999) who reported that PCR-based techniques had a 100% typeability. In contrast, the typeability of southern blot analysis was lower (75%) as four out of the 16 isolates could not be digested with the restriction enzyme *Hind*III.

In terms of discriminatory power, RAPD technique using the primer 3881 was the most discriminatory as all 33 strains had different patterns, vs 28 and 25 for primers 3880 and OPH8 respectively.

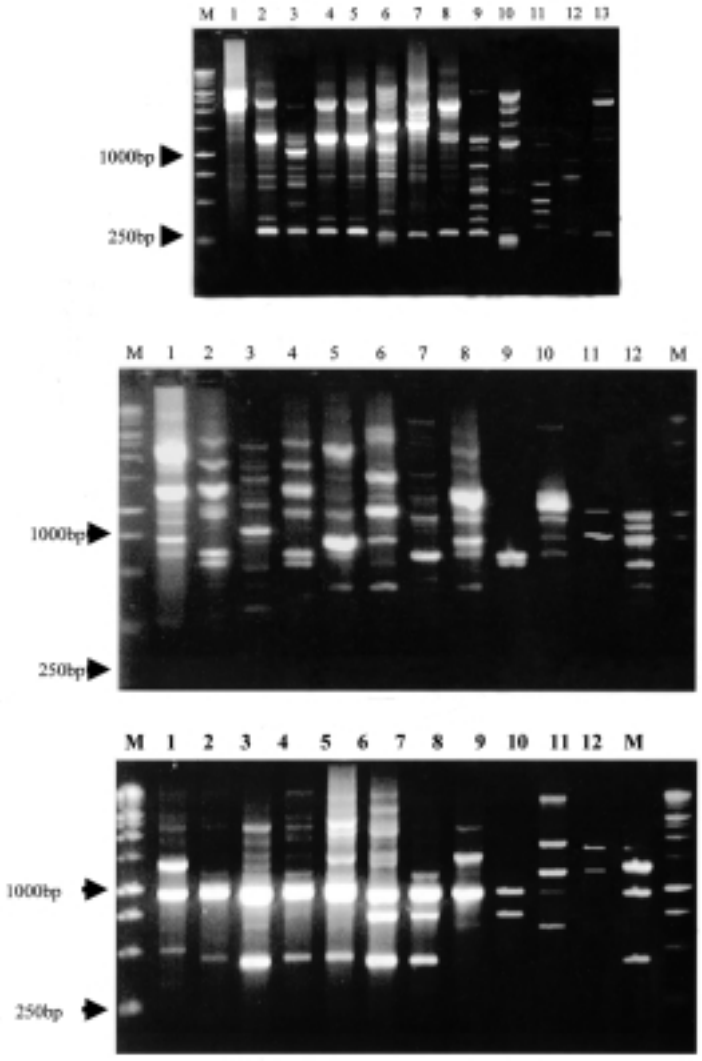

Fig. 1a, b, c. RAPD patterns (primers 3880, 3881 and OPH8 respectively) of 13 *H. pylori* isolates Lanes M, marker lane, 13, *H. pylori* 26695 standard strain, 1–12, Nigerian *H. pylori* isolates. Lanes 4 & 5 are isolates from the same patient but from different sites.

Southern blot analysis with the *ureCD* probe was also the second most discriminatory technique as ten patterns were observed from 12 isolates. A previous report by Desai *et al.* (1994) also showed that Southern blot analysis with *ureA* probe was not as discriminatory as the *ureCD* probe. However, it was able to show the heterogeneity among *H. pylori*. Burucoa *et al.* (1994), who used ribotyping also reported a pattern similar to this study.

The least discriminatory technique, was PCR-RFLP with the restriction enzyme *Sau*3A as all 33 isolates were separated into only six groups. In contrast, Akopyanz *et al.* (1992), using the same target and *Mbo*I for restriction could differentiate most of the 25 strains tested.

Gzyl *et al.* (1999), stated that the RAPD technique from their result was the most discriminatory, however, they felt that PCR-RFLP allows for

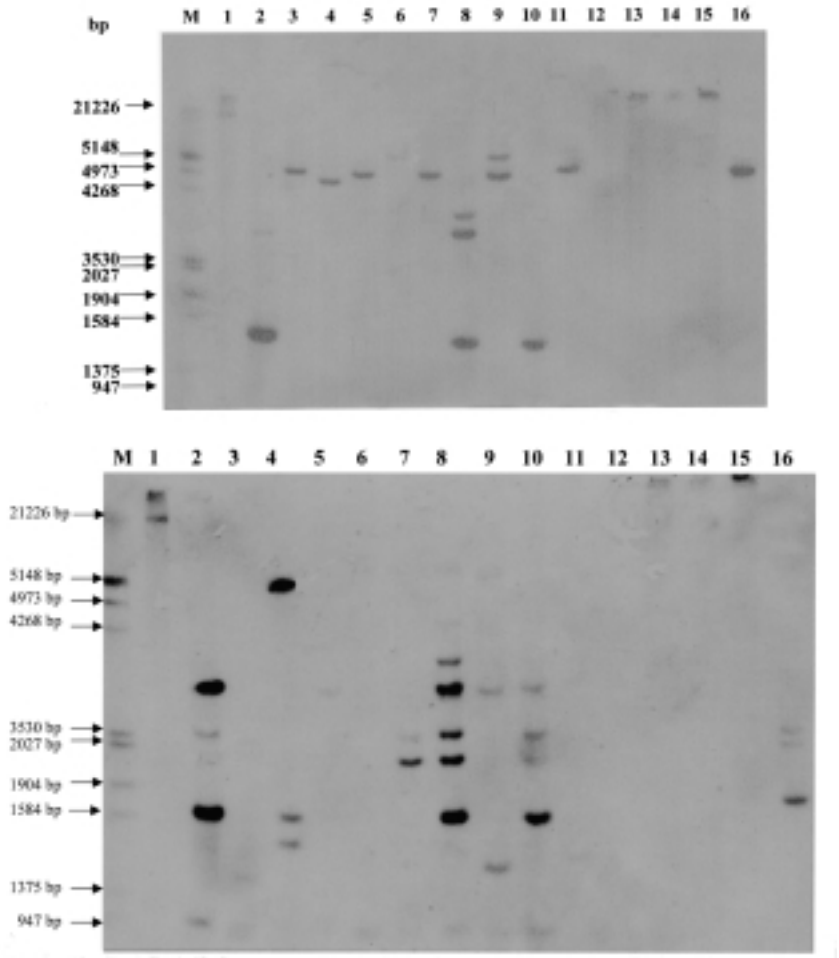

Fig. 2a, b. RFLP patterns of Nigerian *H. pylori* using the *ureA* and *ureCD* probes respectively. Lanes: 1–11, show the different digests, while 12–15 are strains not digested with *Hind*III restriction enzyme. Lane 1, 26695 standard strain, M is the diglabeled DNA molecular weight marker.

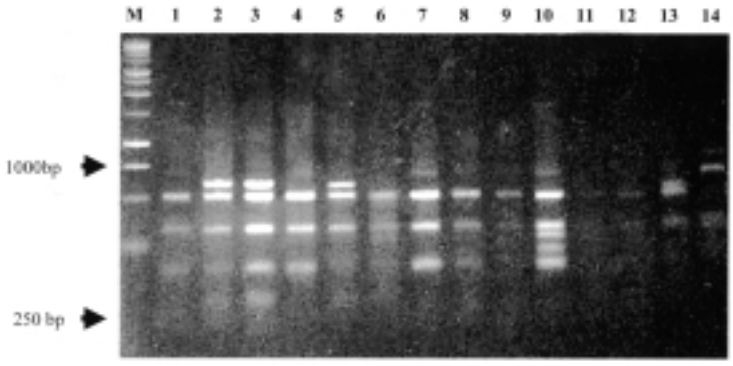

Fig. 3. PCR-RFLP patterns of Nigerian *H. pylori* strains using *ureCD* primer and *Sau*3A restriction enzyme.

easy recognition of mixed infection with two or more different strains, a view also supported by Burucoa *et al*. (1999), because of the excellent typeability of PCR-RFLP.

RAPD technique is much cheaper than the other two techniques and less time consuming.

In conclusion, RAPD analysis and southern blot analysis using *ureCD* as probe and *Hind*III enzyme, were found most useful for the typing of our strains. However, RAPD analysis was found to be the most suitable as it required only few genomic DNA and was less time- consuming. The only problem concerns the very strict and constant parameters, such as same thermal cycler, concentration of reagents, Taq polymerase etc., as suggested by previous authors (Meunier *et al*., 1993; Tyler *et al*., 1997). In an African environment, where little money can be devoted to health related issues, the typing technique most suited for our environment is RAPD with the primer 3881 as the only probe, as in addition to its typeability and discriminatory power, is the least expensive technique and requires the shortest time.

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