**ABSTRACT**

The subtypes of 141 isolates of human immunodeficiency virus type-1 (HIV-1) from Jamaica were determined by a combination of env and gag heteroduplex mobility analysis (HMA) genotyping. The majority of HIV-1 isolates were subtype B (131/141, 93.0%); one (0.8%) isolate each of subtypes C, D and E was found and 7 (4.9%) were indeterminate. These results and the failure of the sets of primers used to amplify some of the HIV-1 isolates provide strong evidence of genetic diversity of the HIV/AIDS epidemic in Jamaica. Surveillance of the circulating HIV-1 genetic subtypes is a prerequisite for developing regional vaccine strategies and understanding the transmission patterns of the virus. This is the first study of its kind in Jamaica and the findings complement data from other Caribbean countries. This work supports the view of colleagues from the French and Spanish-speaking Caribbean that an epidemiological network supported by regional laboratories will help track this epidemic accurately with positive outcomes for the public.

**INTRODUCTION**

The extensive genetic variation which characterizes the Human Immunodeficiency Virus Type-1 (HIV-1) is an important feature of the HIV/AIDS epidemic (1, 2). The genetic variability of HIV-1 is generated by the lack of proof reading ability of the reverse transcriptase, the rapid turnover of HIV-1 in vivo, host selective immune pressures and...
the recombination events during viral replication (1–5). Based on differences in genetic sequences, HIV-1 isolates have been classified into three different groups including the major (M) group of HIV-1, responsible for the majority of infections in the epidemic, outlying groups O (outlier) and N (non-M/non-0). Currently, it is known that the M group contains 9 sub-types, several non-recombinant sub-subtypes, and at least 16 circulating recombinant forms (CRFs) (1–7). The genetic variation of HIV-1 has several public health implications although the biological significance is not fully understood (1, 8). It is known that HIV-1 subtypes vary in their geographical distribution (1, 2). The differences in HIV-1 sub-types and geographical location impact on the design of efficacious vaccines, diagnostic testing and the generation of antiviral resistance and aid the surveillance of patterns of transmission of the virus (1).

Several techniques have been used in the subtyping of HIV-1 (1, 2, 9). The definitive method involves genetic sequencing of the envelope (env), group antigen (gag) or polymerase (pol) genes but this is expensive and time consuming and requires highly qualified personnel (1, 8, 9). The heteroduplex mobility assay (HMA) has been shown to be a reliable, effective, rapid, inexpensive and standardizable method of HIV-1 subtyping. This method correlates well with genetic sequencing and characterization by phylogenetic analysis. The HMA has been introduced by UNAIDS in several developing countries as a tool for monitoring subtype distribution. The combination of results from gag HMA and env HMA allows the recognition of inter-subtype recombinant strains. This is not possible when only one genomic region is typed (1, 2, 8, 9). We used the HMA in the present study which is the first of its kind to be done in Jamaica in what is still a growing HIV/AIDS epidemic.

METHODS
Between August 2001 to January 2005, 3 ml EDTA blood samples were received by the Microbiology Department, University Hospital of the West Indies (UHWI), for leucocyte immunophenotyping for immune monitoring of a cohort of HIV-1 infected patients who were attending the HIV adult and paediatric outpatient clinics at the UHWI and other healthcare facilities throughout Jamaica. In each case, the peripheral mononuclear blood cells (PBMC) were separated from the remnant EDTA blood samples using density gradient centrifugation with Histopaque® 1077 (Sigma- Aldrich Inc., St Louis, Mo, USA) and the cells were kept frozen at – 20°C. A total of 276 consecutive PBMC samples were retrieved for HIV-1 subtyping.

Two microlitres of the PBMC were used as the DNA template for two-step nested polymerase chain reactions (PCR) carried out in a Perkin Elmer 9600 Thermal Cycler (Perkin-Elmer Corp. Norwalk, Conn.) using the PCR mixtures and amplification programmes described in the HIV-1 env/gag HMA subtyping kits manuals (10, 11). The PCR amplicons were genotyped using the HMA as previously described with modifications (10, 11). The HIV-1 HMA subtyping kits which include plasmid clones of the complete genome of HIV-1 subtypes A-D of the major M group of HIV-1 sequences and primer pairs for PCR amplification of the HIV-1 env and gag genes were obtained from the National Institutes of Health (NIH) AIDS Research and Reference Program (10, 11). The PCR core reagents used were commercially prepared (Invitrogen, Life Technologies, Grand Island, NY). For PCR amplification of the env gene, the primer pair used in the first round PCR was ED3/ED14, and ED5/ED12 in the second round. When ED5/ED12 failed, the ES7/ES8 primer pair was used (10). For amplification of the gag gene, the primers from the subtyping kit including the HIG777/HIP202 primer pair in the first round and HIGag 1584/g17 in the second round were used. Failure of the gag primers from the subtyping kit led to their replacement with DT1/DT7 and DT3/DT6 as first and second round primers, respectively (12).

The plasmids from the env and gag subtyping kits were amplified using second round primers. The second round PCR amplification of the env gene yielded a 1.2 Kb fragment spanning the V1-V5 coding region of HIV-1 gp120. Second round amplification of the gag gene resulted in a 748 bp fragment of the HIV-1 p17/ p24 gene. The PCR products were resolved by agarose gel electrophoresis.

The heteroduplexes/DNA hybrids which were generated by combining aliquots of the second round PCR products of samples of unknown subtype and the reference subtypes were separated by Criterion™ precast polyacrylamide gel electrophoresis (5% polyacrylamide, 6 M urea; Bio-Rad Laboratories, Hercules, Ca). The heteroduplexes were visualized under UV light after ethidium bromide staining. The HIV-1 subtype assigned was that of the heteroduplex with the highest electrophoretic mobility.

RESULTS
The HIV-1 env gene was amplified successfully in 139/259 (53.7%) PBMC samples, the gag gene in 154/276 (55.8%), both env and gag genes in 111/276 (40.2%) and neither env or gag were amplified in 47/276 (17.0%). As shown in Fig 1, all gag (35/35,100%) and env (21/21, 100%) reference subtype plasmids were amplified successfully by all second round primers.

The env and gag HMA were performed on the PCR amplicons of 141 samples and of these 134 (95.0%) were unambiguously genotyped. The HMA was indeterminate in 7 samples (4.9%). The majority of HIV-1 isolates were subtype B (131/141, 93.0%) while 1 (0.8%) isolate each of subtypes C, D and E was found (Fig. 2.).

DISCUSSION
A panel of primer sets from North American and British sources was used in the PCR amplifications. Nonetheless, just over 50% of the isolates were successfully amplified.
The low sensitivity of the PCR amplification of the HIV-1 isolates and the relative high level of non-concordance between the PCR results for the gag and env regions might reflect the marked genetic heterogeneity of the virus (9, 12). This is supported by the fact that all NIH reference plasmids were successfully amplified using the same primer sets which failed to amplify the HIV-1 isolates.

In another study done by McCutchan et al., a PCR fingerprinting approach for genetic characterization of HIV-1 was carried out to compare HIV-1 isolates from North America and Zambia using primer pairs in gag gene. The authors observed that several primer pairs which consistently amplified North American isolates were unable to amplify most of the isolates from Zambia due to primer mispairing. The DNA sequencing of these isolates revealed that consistent differences from the primer sequences in the Zambian isolates were responsible for their distinctive PCR fingerprint. Based on their observations, they suggested that Zambian HIV-1 isolates and other genetically diverse isolates should be included in the matrix of experimental approaches needed to define vaccine requirements (13). Other workers have reported the problem of unamplifiable HIV-1 strains with env primers of the current HMA kits due to the broad heterogeneity within the gp120 region of the HIV-1 env gene (13). In some geographical locations prior exposure to antiretroviral drugs contributes to heterogeneity of HIV-1 strains. However this would not have applied in the present study since at the time of sampling most patients had not begun to receive antiretroviral drugs. These drugs only became widely accessible in Jamaica under the National HIV/AIDS Prevention and Control Project in the middle of 2004 (14).

The majority of HIV-1 isolates from patients in Jamaica were found to be genetic subtype B. This is the predominant subtype in other parts of the Caribbean, North America, Western Europe, Australia and South America (15–20). Jamaica, therefore, is positioned to benefit when the HIV-1 subtype B prophylactic and therapeutic vaccines, including those currently in preclinical and clinical trials, become available (21–23).

Although the number of HIV-1 sub-types C, D and E strains was low. The presence of HIV-1 non-B subtypes in the HIV/AIDS population in Jamaica is important. The present study indicates that HIV-1 non-B subtypes are beginning to contribute to the genetic and antigenic diversity of the HIV/AIDS epidemic in Jamaica. The HIV-1 subtype C, the dominant subtype in China and India, is the most prevalent subtype worldwide accounting for approximately 50% of infections (24, 25). Subtype D is generally limited to East and Central Africa with sporadic cases observed in Southern and Western Africa (26). Subtype E is a recombinant strain (CRF01_AE), and not a distinct subtype as initially thought. Subtype E co-circulates with subtype B within the intravenous drug user (IDU) population and among fishermen in Thailand and it also occurs in Vietnam and South East Asia (1, 27, 28). At the time of writing, the transmission patterns
of the HIV-1 subtypes in Jamaica are not known and were beyond the scope of the present study.

We intend to report the results of further studies involving nucleic acid sequencing and phylogenetic analysis which will provide more detailed information on the genetic diversity and origins and transmission patterns of these viruses in Jamaica. In addition, the authors concur with colleagues from the Francophone Caribbean that an epidemiological network supported by regional laboratories will help to continue tracking the HIV epidemic accurately (29). Information derived from combined efforts will be of strategic value to policy makers and will bring positive benefits to the Caribbean public as the knowledge is applied to issues such as the use of antiretroviral drugs and vaccines.

REFERENCES