

Migrant workers play a key role in HIV-1 strain diversity in Bangladesh

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Abstract

Introduction: Bangladesh is a low prevalence country for human immunodeficiency virus (HIV), and genotyping data is limited which is incomplete and mainly subtype C based on gag gene. In the present paper, we characterized HIV-1 strains obtained from individuals attending three HIV testing and counseling (HTC) centers from different geographic regions in Bangladesh comprehensively based on three genes.

Material and methods: A total of 40 HIV-positive blood specimens with demographic and migration history were available from individuals who attended HTC centers during 2005-2007. DNA was extracted from whole blood and sequenced using primers specific for gag, pol, and env region of HIV genome.

Results: Sub-type C was the most predominant strain (45%) followed by G-related recombinants (35%). Other genotypes were A1 (5%), B (2.5%), CRF16_A2D (7.5%), and unique recombinant form (5%). Phylogenetic analysis indicates that the origins of these strains varied and most of them might have been imported from the Middle East on a regular basis by migrant workers.

Conclusions: This is the first study to describe comprehensive characterization of Bangladeshi HIV-1 strains. Adapted intervention programs relating to appropriate counseling and provision of information relating to risk of transmission of HIV need to aim at returnee migrant workers.

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Introduction

The number of people currently living with human immunodeficiency virus (HIV)/acquired immunodeficiency syndrome (AIDS) in Bangladesh is estimated as 14,000, and that the HIV prevalence among key populations (KPs) is less than 1% [1], which is lower compared to other countries of the Asia Pacific region. However, high-risk behaviors associated with HIV, such as needle/syringe sharing, and unprotected sex are highly prevalent among KPs in Bangladesh [1]. This has already been reflected recently in a national surveillance data, which indicated a remarkable increase of HIV rate

in people who inject drugs (PWID) from 7% in 2011 to 27% in 2016 [1].

Existing data on genetic characterization of HIV isolates from KPs indicate that most of the strains are subtype C, which was observed based on a partial sequence of the gag gene only [2-4]. In order to obtain better characterization of the virus, particularly for circulating recombinant forms (CRFs), at least three genetic regions – gag, pol, and env need to be considered. Here, we report the comprehensive characterization of HIV strains by sequencing three major HIV genes and provide evidence that: (i) diversity of HIV strains in the country is huge; (ii) a number of new re-

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combinant lineages were identified; (iii) most of them are imported by migrant workers especially from the Middle East.

Material and methods

Sample collection

HIV-positive blood samples were obtained from HIV testing and counseling (HTC) unit of International Centre for Diarrhoeal Disease Research, Bangladesh (icddr,b), which were collected from May, 2005 to February, 2007, and stored at -20°C as described elsewhere [4]. Majority of the participants were migrant workers who returned home permanently or enjoyed a vacation.

Informed and signed consent was obtained from all study participants prior to drawing blood, and in the case of children ($n = 3$), a consent was obtained from parents/guardians. The summary of the consent paper was read out for those who could not read, and a left thumb fingerprint was obtained from those who could not sign. This study was approved by the ethical review committee of icddr,b (research protocol number: 2008-051).

Sub-typing

Pro-viral DNA was extracted from whole blood samples and was amplified by nested polymerase chain reaction (PCR) in the gag, pol, and env genes, using specific primers (Table 1). The amplified DNA was purified and sequenced

on an ABI 377 automated DNA sequencer. Chromatogram sequencing files were inspected using Chromas 2.23 (Technelysium, Queensland, Australia), and consensus sequences were prepared using SeqMan II (DNASTar, Madison, WI, USA). Multiple sequence alignments were calculated using ClustalX1.81 [6]. Partial sequences of gag, pol, and env genes (at least 300 nucleotide bases) were utilized for sub-typing, using HIV genotyping tools (<http://www.ncbi.nih.gov/projects/genotyping/formpage.cgi>) of the National Centre for Biotechnology Information (NCBI; National Institutes of Health, Bethesda, MD, section 2.7). All sub-types determined by genotyping tools were confirmed through a manual phylogeny, with reference strains from the Los Alamos HIV sequence database (www.hiv.lanl.gov). Sequences at the 5' and 3' ends, which could not be aligned unambiguously due to length variability of sequences were omitted from the configuration, and approximately 350 base pair sequences of 40 HIV strains were finally analyzed. Phylogenetic analyses were conducted using a neighbor-joining method, and genetic distances were calculated with a nucleotide p-distance model in a MEGA6 software package [7]. The nucleotide sequences reported in this paper were submitted to GenBank, with MK370744-MK370841 accession number.

Results

The study population consisted of 27 males and 13 females, with ages ranging from 5 years to 75 years. The majority

Table 1. Oligonucleotide primers used in the study for PCR amplification (gag, env, and pol)

Primer	Gene	Position (nt)	Ref.	Strand	Accession #	Sequences (5'-3')
529	gag	884-903	HXB2	Plus	K03455	AACATMTARTRTGGCAAGC
530	gag	764-782	HXB2	Plus	K03455	GACTAGCGGAGGCTAGAAG
531	gag	796-818	HXB2	Plus	K03455	GCGAGAGCGTCAGTATTAAGCGG
532	gag	836-857	HXB2	Plus	K03455	GGGAAAAAATTCGGTTAAGGCC
533	gag	1249-1270	HXB2	Minus	K03455	CTTCTACTACTTTTACCCATGC
CS1	gag	1585-1610	HXB2	Minus	K03455	ACTATTTTATTTAATCCCAGGATTAT
CS2	gag	1631-1658	HXB2	Minus	K03455	TTTGGTCCTGTCTTATGTCCAGAATGC
CS4	gag	1822-1844	HXB2	Minus	K03455	CCCTGACATGCTGTCAATTC
815	gag	1359-1398	HXB2	Plus	K03455	AGTGGGGGACATCAAGCAGCCATGCAAT
1114	gag	1631-1658	HXB2	Minus	K03455	TTTGGTCCTGTCTTATGTCCAGAATGC
ED5_F	env	6517-6542	O6FR-CRN	Plus	EU448296	ATGGGATCAAAGCCTAAAGCCATGTG
ED12_R	env	7742-7771	O6FR-CRN	Minus	EU448296	AGTGCTTCTGCTGCTCCCAAGAACCCAAG
ED31_F	env	6386-6414	Cu43	Plus	AY586543	CCTCAGCCATTACACAGGCCTGTCCAAAG
633_R	env	7521-7538	HXB2	Minus	K03455	GGAGGGGGCATACTTGC
826_F	env	6967-6998	HXB2	Plus	K03455	CGCTAGGAATTCGGCCAGTAGTATCAACTCAA
308_R	env	7362-7382	HXB2	Minus	K03455	ATTACAGTAGAAAAATTC
poli5	pol	4177-4200	MN	Plus	M17449	CACACAAAGGRATTGGAGGAAATG
poli8	pol	5210-5232	MN	Minus	M17449	TAGTGGGATGTGTACTTCTGAAC
poli7	pol	4201-4223	MN	Plus	M17449	AACAAGTAGATAAATTAGTCAGT
poli6	pol	5122-5145	MN	Minus	M17449	ATACATATGRTGTTTTACTAARCT

Table 2. Summary of HIV sub-types distribution

HIV sub-type	Country of work/Migration history			n	%
	Middle East ^a	India	None		
A1	1		1	2	5.0
B			1	1	2.5
C	4	3	11	18 ^b	45
CRF02AG	2		3	5	12.5
CRF06cpx_A,G,J,K	1			1	2.5
CRF09_A,G,U	3			3	7.5
CRF13cpx_A,AE,G,J,U	1			1	2.5
CRF16_A2D	1		2	3	7.5
CRF25_A,G,U	2		1	3	7.5
CRF43_AG	1			1	2.5
Unique	2			2	5.0
Total	18	3	19	40	100

^a – UAE, Saudi Arabia, Bahrain, Jordan, Kuwait; ^b – one HTC patient carrying sub-type C visited Saudi Arabia and India

(67.5%) of participants were from Dhaka, followed by Sylhet (30%) and Chattagram (2.5%). Thirty-four (85%) of them were heterosexual, three (7.5%) children of HIV-positive mothers, one (2.5%) PWID, one (2.5%) homosexual, and one (2.5%) with unknown risk behavior. Sub-type C was the most predominant strain (45%), followed by G-related recombinant strains (35%). Other genotypes included A1 (5%), B (2.5%), CRF16_A2D (7.5%), and unique recombinant form (5%).

The migration history is summarized in Table 2. Most of the HIV-positive individuals (18 out of 40; 45%) travelled to the Middle East (Bahrain, Jordan, Kuwait, Saudi Arabia, and UAE), and they harbored a wide varieties of HIV strains. Among 26 migrant workers, 18 were male. All male migrant workers admitted buying sex from female sex workers while abroad. Only three (3 out of 40; 7.5%) who visited India had sub-type C strains. Remaining (19 out of 40; 47.5%) were non-migrants and spouses or sex partners of migrant workers, and majority of them (11 out of 19; 58%) presented with sub-type C strains.

Three dendrograms were constructed based on gag, pol, and env regions of Bangladeshi strains along with the reference strains from different geographical regions available from the GenBank database. For better clarity of findings, only the gag tree is presented (Figure 1). Phylogenetic analysis revealed some interesting facts about the origin and transmission of the virus. First, HIV strains from our study samples dispersed all through the tree indicating extreme polyphyletic origins of local HIV strains. They clustered with HIV strains from every corner around the globe. Second, migrant workers were one of the carrier and importers of HIV strains to the country and they were likely to transmit the virus to their spouses/sex partners and children. All six spouses and partners of the returnee migrants (the Middle East) clustered together with Saudi Arabian or African strains (Figure 1, sub-type A1: strain 07BD2562, CRF02_AG: strains 06BD348 and 06BD2154, CRF043_02G: strain 06BD347, sub-type C: strains

06BD2423 and 05BD952). The strain 07BD2562 (sub-type A1) was isolated from a female HTC client whose HIV-positive husband worked in the UAE and later died. We also found two strains isolated from two male children (strains 05BD1179, 05BD1230) and clustered with Indian strain (sub-type C) and Bangladeshi strain 06BD1423 whose client was a 22-year-old female. Her HIV-positive husband had travel history to India and had heterosexual contact with a sex worker there. It is presumed that the husband transferred the virus to his wife. We also identified one person with strain 07BD2718 (sub-type C) who had homosexual contact in the Saudi Arabia more likely to be infected through unprotected anal intercourse. Third, besides our male migrant workers, female migrant workers were also contributing in importing foreign HIV strains. The strain 06BD1884 (CRF16_A2D) was collected from a female who worked in Bahrain as a sex worker and is now residing in Bangladesh. It clustered with strains from a Bangladeshi couple harboring nearly identical strains 06BD2241-42 (CRF16_A2D). Another Bangladeshi strain 05BD939 (sub-type C) was collected from a female sex worker from a brothel in Mumbai.

Discussion

In this study, a broad range of HIV-1 genotypes was identified suggesting multiple transmission routes for HIV epidemics in Bangladeshi population. In Bangladesh, the HIV epidemic is concentrated mainly among PWID and at some extent in female sex workers (FSW) who have been the focus for an active surveillance [8, 17]. However, when the passively-reported cases were analyzed, another population group appeared to be vulnerable, namely migrant workers with most cases being identified through HIV testing centers; this migrants' population group who have acquired HIV while working abroad or from their spouse [8, 10]. Approximately

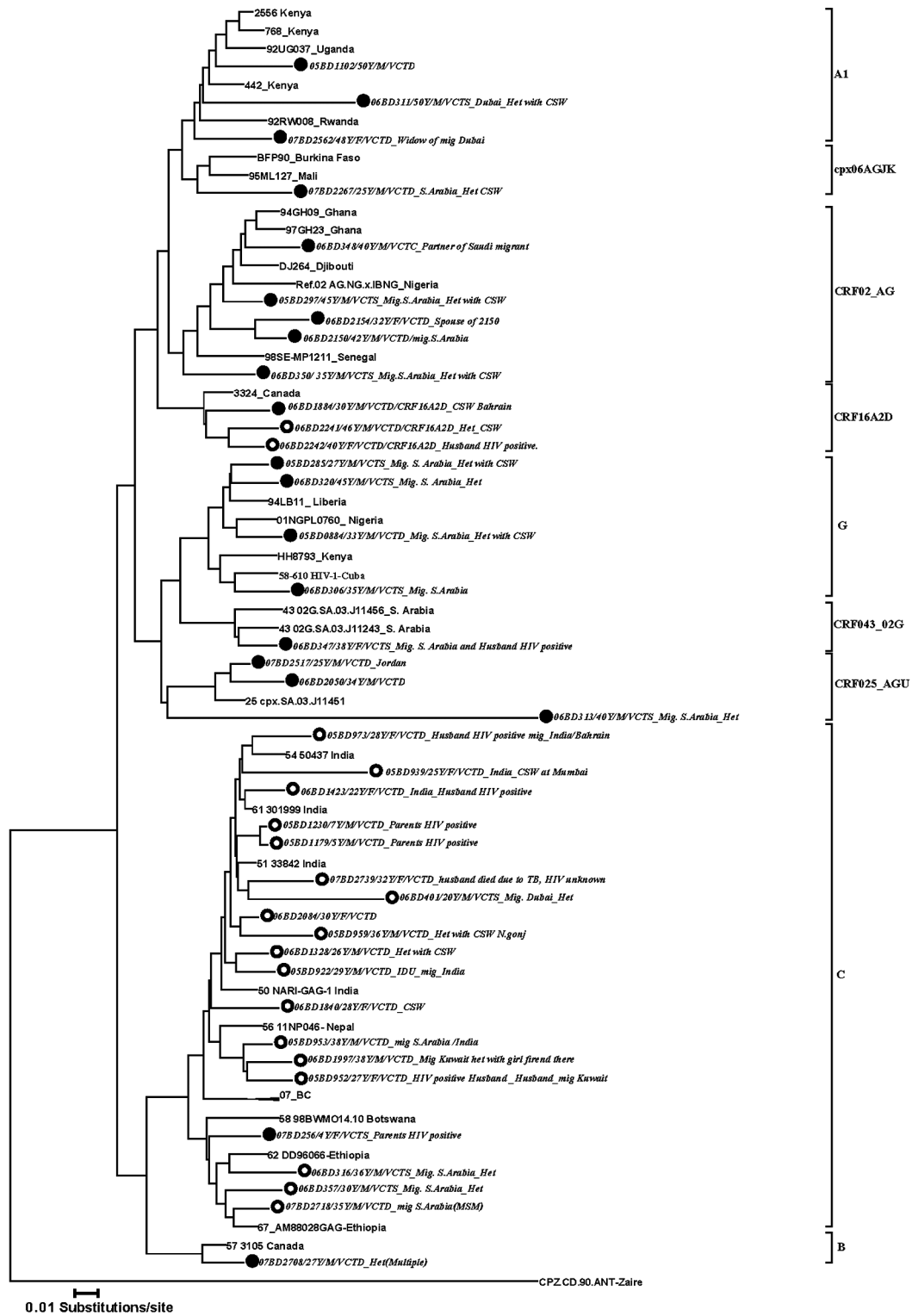


Figure 1. Neighbor-joining phylogenetic tree based on nucleotide sequences of the partial gag encoding gene (350 nt bases) for HIV-1 sub-type A1, B, C, G, CRF02_AG, cpx06AGJK, CRF16A2D, CRF25_AGU, CRF043_02G. The dendrogram is rooted using simian immunodeficiency virus CPZ.CD.90.ANT. CSW – commercial sex worker, mig – migrant, Het – heterosexual transmission, VCTS – HTC unit of Sylhet, VCTD – HTC unit of Dhaka, VCTC – HTC unit of Chattagram. MSM – male having sex with male. M – male, F – female. The Bangladeshi strains are with a filled circle. Some strains published earlier are with an open circle

79% of people attending the icddr,b's HTC unit who were diagnosed as being infected with the HIV virus were returnee-migrants [14]. A recent study from Nepal found that a high proportion of men working abroad had sex with sex worker(s) and presented with higher rates of HIV infection than those who had not left Nepal [11].

Work-related migration is very common in Bangladesh. In a previous icddr,b study, among 1,200 married women from two districts, nearly 11% had their husband living abroad. Another study undertaken among married couples [12] showed that a large proportion of married men who travelled either within Bangladesh or abroad, reported sex with female sex workers. A few men used a condom during sex with a sex worker (24, 31%) or with their spouse (28, 31%). Although not nearly as frequent, women whose husbands were away also reported more extramarital sex compared with those women who had not spent time apart from their husbands [8]. The findings of this study was that migrants from Bangladesh was, indeed, vulnerable to HIV; however, there is still no strategy for reducing risk for migrants and their spouses without inducing a stigma.

A large number of Bangladeshi citizens are employed in the Gulf States (e.g., Saudi Arabia, Kuwait, and the United Arab Emirates). It was estimated that in Saudi Arabia, one quarter of the population are foreign nationals who make up over 50% of the work force [15], and more than 99% of them were male [16]. Before getting a resident permit, foreign nationals entering the Gulf States have to undergo HIV testing in their country of origin and if positive, they are not given a resident permit. Furthermore, migrant workers are required to renew their work permit and take a HIV test every 2 years. If anyone is found to be HIV-positive, they are deported immediately to their country of origin without counseling or treatment [13]. While abroad, these men are away from their family and likely to have unprotected sex with multiple partners, usually sex workers and occasionally, other men. Sex workers in the Gulf States are typically short-term residents with a temporary tourist visa who do not require a work permit or HIV test. Sex industry import women from nearby countries (e.g., Thailand, Myanmar, Nepal, and India) for short-term work in the Gulf States [13]. Condom use or other barrier methods are rarely used, thereby increasing their chance of infection.

One limitation of the study was using blood samples archived for more than one decade, the quality of which could be deteriorated. Therefore, we were unable to amplify 50% DNA of the stored samples, which were excluded from analysis.

Conclusions

The regular transmission of HIV by both returnee migrant workers and foreigners is expected to increase the prevalence and diversity of HIV among the Bangladeshi population. Adapted intervention programs, such as counseling and provision of information relating to risk of contracting HIV, need to aim at migrant workers at different points of travelling: when leaving home-country, while working overseas, upon their return to home-country, and while staying at home-country. In conclusion, the study revealed a broad genetic diversity of HIV-1

strains in Bangladeshi population, which was mainly caused by returnee migrant workers. This finding warrants the inclusion of migrant workers as a key population for HIV in Bangladesh.

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Conflict of interest

The authors declare no conflict of interest with respect to the research, authorship, and/or publication of this article.

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