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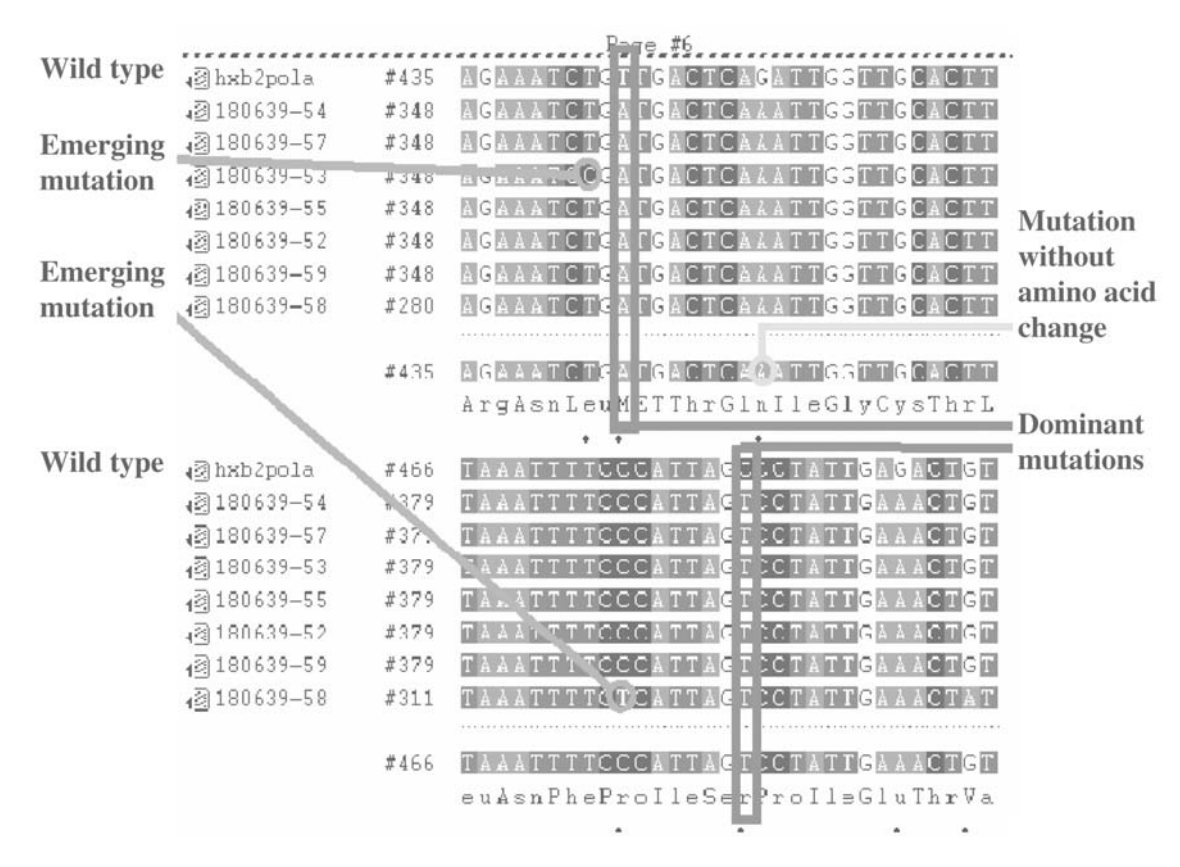
HIV-1 Strain and Primary Drug Resistance in Canada

Surveillance Report to March 31, 2004



May 2005

Canada

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Surveillance and Risk Assessment Division

National HIV and Retrovirology Laboratories

Centre for Infectious Disease Prevention and Control

Public Health Agency of Canada

To promote and protect the health of Canadians through leadership, partnership, innovation and action in public health.

Public Health Agency of Canada

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We also thank Scientific Publication and Multimedia Services for its contribution in editing and producing the report.

N.B. This document must be cited as the source for any information extracted and used from it.

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Information to the readers of *HIV-1 Strain and Primary Drug Resistance in Canada*

On behalf of the Surveillance and Risk Assessment Division and the National HIV and Retrovirology Laboratories, we are pleased to provide you with the *HIV-1 Strain and Primary Drug Resistance in Canada: Surveillance Report to March 31, 2004.* This report is part of an annual series, providing a review of the genetic diversity of HIV in Canada.

We present data that are shared by provinces participating in the Canadian HIV Strain and Drug Resistance Surveillance Program. The Field Surveillance Officers are responsible for coordinating data collection and submission to the HIV/AIDS Surveillance Section and the Strain and Drug Resistance Program Section. The HIV Strain and Drug Resistance Surveillance Section is responsible for managing and analyzing data, as well as, writing and coordinating the publication of this report. The National Laboratory for HIV Genetics conducts the strain and primary drug resistance genotyping. The National Laboratory for HIV Reference Services determines the estimated time of infection, using a combination of two commercially available kits: the Organon Technika Vironostika HIV-1-LS™ and the Abbott 3A11-LS™ assays. This laboratory also serves as a sentinel arm in monitoring the presence of unusual strains of HIV in Canada.

The major findings of the surveillance data are outlined in the section entitled *Results at a Glance.* This is followed by a series of tables summarizing the HIV-1 strain and primary drug resistance data. Each table provides specific explanatory details, as appropriate. Technical notes, references, and data sources are available in the Appendices.

A further description of HIV-1 strain and primary drug resistance in Canada is available in the *HIV/AIDS Epi Updates* reports available on our web site at **www.phac-aspc.gc.ca/hast-vsmt/public_e.html**.

The publication of this report would not be possible without the collaboration of the provinces participating in our national HIV strain and drug resistance surveillance program. Their ongoing contribution to this surveillance program is gratefully acknowledged in Appendix 7.

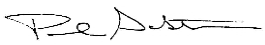
This is the third report on HIV strain and primary drug resistance surveillance in Canada. We will be working toward improving this report to reflect changes in the surveillance of HIV strain and primary drug resistance. We welcome and appreciate your comments and suggestions.

Yours sincerely,

Dr. Gayatri Jayaraman

Dr. James Brooks

Dr. Chris Archibald



Dr. Paul Sandstrom

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Results at a Glance

Introduction

This section summarizes the main findings of the surveillance data and is followed by two additional sections. The first section describes HIV-1 subtypes in Canada as determined by the Canadian HIV Strain and Drug Resistance Surveillance Program (SDR program), and outlines the results from other key studies conducted in Canada, the United States, and Western Europe. The second section describes HIV-1 primary drug resistance in Canada, as determined by the SDR program, and outlines results from other key studies in countries where highly active antiretroviral therapy is widely available.

Summary of the Main Findings

- While HIV-1 subtype B continues to predominate, 10.1% of the sampled population (n = 2,152) were infected with non-B subtypes. These non-B subtypes also include various circulating recombinant forms of HIV-1.
- Significantly higher proportions of non-B subtype infections were detected among females (compared with males), among those who were older at initial diagnosis, among African/Caribbean or mixed ethnicities (compared with Caucasians), and among those reporting heterosexual sex as their primary risk factor (compared with male-to-male sex).
- There is geographic variation across Canada in the prevalence of non-B HIV-1 subtypes. This variation likely relates to travel and migration from countries where other subtypes predominate.
- The overall prevalence of primary drug resistance to at least one antiretroviral drug has been identified in 8.6% of our sample population of 1,738 newly diagnosed individuals who had never received treatment.
- Multi-drug resistance to $\geq$ 2 classes of antiretroviral drugs has been identified in 1.3% of the sample population.
- Primary drug resistance has been observed in females and males; across different age groups, ethnicities, and exposure categories; in HIV-1 subtypes A, B, and C infections; and among recent and established HIV infections.
- The prevalence of primary drug resistance is similar to the rates observed in other countries, where highly active antiretroviral treatment is widely used.

Public Health Implications

Information on the specific HIV strains that occur in Canada is important to assess the usefulness of a potential vaccine since any vaccine will likely be strain specific. Since subtype B predominates in Canada, vaccines that are effective against this subtype would be of most use here. Vaccines against non-B subtypes would be of most use in parts of the world where these other subtypes predominate (such as Africa and Asia), but would also be of interest in Canada where 10% of newly diagnosed infections are non-B.

HIV tests need to reliably detect all the different HIV strains circulating in the country. This issue was highlighted with the discovery of HIV-2 and highly divergent group O strains of HIV-1 which required modification of existing serologic tests by the addition of new antigens to ensure detection. To help extend the search for unusual HIV variants, the SDR Program links with reference services at the National HIV and Retrovirology Laboratories where samples with unusual virologic results are tested and quality assurance and monitoring of diagnostic kits are conducted.

The assessment of primary HIV drug resistance is a new and evolving field, and has the potential to help develop more effective prevention and care programs. For example:

- Information on the prevalence of primary drug resistance can contribute to the development of population-based recommendations for initial anti-retroviral therapies (especially for pregnant women and for use in post-exposure prophylaxis);
- The prevalence of drug resistance in newly diagnosed HIV infections reflects the extent to which drug-resistant strains of HIV are being transmitted from individuals who are already diagnosed and on treatment, and so can help assess the effectiveness of prevention and counselling programs.

Further expansion of the Canadian HIV Strain and Drug Resistance Surveillance Program is ongoing and will permit the collection of more representative data, which will in turn result in an improved ability to realize the potential public health benefits of such data.

SECTION I: HIV-1 Subtypes (1984 - March 31, 2004)

Background

Since the first reported cases of HIV/AIDS in the mid-1980s, HIV has emerged as one of the most significant infectious agents, with 40 million people infected worldwide. What is key to the pathogenicity of HIV is its genetic heterogeneity, resulting from the error-prone reverse transcriptase, the rapid turnover of HIV-1 *in vivo*, recombination and the selective pressures by host.

The initial classification of HIV into two main types, HIV-1 and HIV-2, was based on the geographic distribution and the animal source of the human infection— chimpanzee *(Pan troglodytes)* for HIV-1 and sooty mangabey *(Cercocebus atys)* for HIV-2. Expanding access to diverse samples of HIV-1 and the advent of new molecular tools have led to the classification of HIV-1 into three distantly related "groups": M (for main), N (for new, non-M, non-O), and O (for outlier). The vast majority of isolates (> 90%) cluster in the "M" group. Based on partial HIV *gag* and *env* gene sequencing, the circulating genetic forms of this "M" group include nine major "clades" or subtypes (A-D, F-H, J), at least 4 different "sub-clades", and 13 circulating recombinant forms. This classification is not exhaustive; new recombinant HIV strains are arising continually, and this, coupled with the migration of populations, are powerful forces in the spread of HIV worldwide.

Although there has been no systematic surveillance for genetic diversity of HIV subtype in Canada, studies to date on high-risk populations suggest that HIV-1 subtype B is the most common subtype found in the country. Despite the predominance of HIV-1 subtype B, non-B subtypes have also been reported in Canada. With increased international travel and migration, it is inevitable that diverse HIV strains will continue to be introduced into this country. However, little is known about the distribution of HIV subtypes in Canada, how it changes over time, or its effects on particular risk groups in different regions of the country. This information is important to evaluate the utility of potential vaccines in the Canadian setting and to assess any differences in subtype-specific susceptibilities to antiretroviral drugs and in the pathogenicity of the various subtypes. Likewise, conducting the systematic surveillance of HIV genetic diversity would determine whether currently approved assays for HIV in Canada can detect all circulating strains. This includes the ability to control and manage HIV infection through approved viral load test assays and by using other tests that determine the stage and progression of the disease.

Data Tables:

This section highlights the main findings from the Canadian HIV Strain and Drug Resistance Surveillance Program (SDR program) based on specimens from individuals newly diagnosed with HIV infection between 1984 and March 31, 2004. Of note, these results represent individuals who sought testing, who were properly diagnosed, and who reported HIV positive. In addition, results include only those individuals for whom sufficient sera, taken for the purposes of diagnostic testing, was available to send to the Public Health Agency of Canada and of these samples, the subset for whom RT-PCR amplification and sequencing to identify subtypes were successful (Appendix 4 identifies additional data limitations). A total of 2,937 sera samples, from individuals who were newly diagnosed between 1984 to March 31, 2004 and corresponding non-nominal epidemiological data have been received by the Public Health Agency of Canada from British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Newfoundland, and Nova Scotia for HIV-1 subtype analysis. Discussions are underway to expand the SDR program to the remaining provinces and territories. Viral RNA was successfully amplified from 2,152 (73.2%) of the sera samples. This level of success in amplifying virus from sera specimens will likely improve further by enhancing sample quality and by identifying and using various primer combinations for RT-PCR amplification. (Appendix 2 details the laboratory methods used to identify subtypes.)

Table 1: Number and distribution of HIV-1 subtypes among newly diagnosed, treatment-naive individuals (1984 - March 31, 2004)

HIV-1 Subtype	Frequency (n)	Percentage
B	1934	89.9
C	124	5.6
A	24	1.1
AE [1]	19	0.9
AG	19	0.9
AD	12	0.6
D	6	0.3
BD	4	0.2
AB	2	0.09
AC	1	0.05
B/AG	1	0.05
BC	1	0.05
F	1	0.05
G	1	0.05
K	1	0.05
K/AE	1	0.05
K/AG	1	0.05
Total	**2152**	**100**

[1] The circulating recombinant form (CRF) AE has also been referred to as subtype E.

Table 1 illustrates the distribution of HIV-1 subtypes in our sample population. Of note, between July 1998 and December 2000, the C2-V5 region (233 amino acids) of the envelope protein was used to assess HIV subtype. Since this time, the sequence analysis of the *pol* gene (entire protease and the first 253 amino acids of reverse transcriptase) was used for subtype analysis. While most samples (89.9%) are of HIV-1 subtype B, other subtypes and circulating recombinant strains of HIV-1 have been identified. In decreasing order of prevalence, these include subtype C (5.6%), A (1.1%), AE and AG (0.9% each), D (0.3%), BD (0.2%), AB (0.09%), and AC, B/AG, BC, F, G, K, K/AE, K/AG (0.05% each).

While existing studies on high-risk populations also suggest the predominance of HIV-1 subtype B in Canada, subtype A was reported in Canada in 1995 (Montpetit M, AIDS Res Hum Retroviruses 1995, 11(11):1421-22). The vast majority of seroconverters from the POLARIS cohort (comprising men who have sex with men [MSM]) in Ontario are of subtype B (Paul Sandstrom co-investigator, POLARIS cohort, National HIV and Retrovirology Laboratories). The British Columbia Centre for Excellence in HIV/AIDS has identified subtypes A, C, and D in at least 4% of individuals linked to cohort studies and to the B.C. HIV drug treatment program (Alexander C et al. 7th Conference on Retroviruses and Opportunistic Infections. San Francisco, CA, Feb 2000, # 174). All HIV-1 sequences analysed among injecting drug users (n = 17) and MSM (n = 5) residing in Montreal were of subtype B (Bernier L et al. 8th Conference on Canadian HIV/AIDS Research, Vancouver, B.C., May 1999, #104).

Table 2: Number and distribution of HIV-1 subtypes by year of diagnosis

	HIV-1 Subtype								
	B [1]	C [2]	A [3]	AE [4]	AG	AD [5]	D	Others [6]	**Total**
Year of diagnosis	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
1995	60 (95.2)	1 (1.6)	2 (3.2)	0	0	0	0	0	**63 (100)**
1996	83 (83)	13 (13)	3 (3)	0	0	0	0	1 (1)	**100 (100)**
1997	101 (91)	2 (1.8)	7 (6.3)	1 (0.9)	0	0	0	0	**111 (100)**
1998	156 (91.8)	10 (5.9)	2 (1.2)	0	0	0	1 (0.6)	1 (0.6)	**170 (100)**
1999	348 (91.6)	21 (5.5)	4 (1.1)	3 (0.8)	0	2 (0.5)	2 (0.5)	0	**380 (100)**
2000	417 (94.6)	13 (2.9)	2 (0.5)	1 (0.2)	3 (0.7)	4 (0.9)	0	1 (0.2)	**441 (100)**
2001	325 (95.6)	10 (2.9)	0	0	1 (0.3)	0	0	4 (1.2)	**340 (100)**
2002	135 (84.4)	11 (6.9)	0	6 (3.8)	3 (1.9)	2 (1.3)	2 (1.3)	1 (0.6)	**160 (100)**
2003	143 (74.5)	25 (13)	3 (1.6)	6 (3.1)	8 (4.2)	3 (1.6)	1 (0.5)	3 (1.6)	**192 (100)**
Jan-Mar 2004	67 (75.3)	13 (14.6)	0	2 (2.2)	4 (4.5)	0	0	3 (3.4)	**89 (100)**
Total, n (%)	**1835 (89.7)**	**119 (5.8)**	**23 (1.1)**	**19 (0.9)**	**19 (0.9)**	**11 (0.5)**	**6 (0.3)**	**14 (0.7)**	**2046 (100)**

[1] Year of diagnosis was unknown for 99 individuals HIV-1 subtype B infection

[2] Year of diagnosis was unknown for five individuals with HIV-1 subtype C infection

[3] Year of diagnosis was unknown for one individual with HIV-1 subtype A infection

[4] The circulating recombinant form (CRF) AE has also been referred to as HIV-1 subtype E.

[5] Year of diagnosis was unknown for one individual with the CRF AD infection

[6] Others refers to the following HIV-1 subtypes and CRFs: BD, AB, AC, B/AG, BC, F, G, K, K/AE, and K/AG

Table 2 shows the number and distribution of HIV-1 subtypes by year of diagnosis with HIV infection. The results suggest an increase in the prevalence of non-B HIV-1 subtypes from 4.8% prior to 1996 to 25.5% during 2003. However, the samples are not representative of all newly diagnosed HIV cases in Canada in each noted year. Thus, significant associations between year of first diagnosis and HIV-1 subtypes could not be determined. This matter is being addressed in our effort to ensure that the SDR program represents all newly diagnosed cases across Canada.

Table 3: Number and distribution of HIV-1 subtypes by province

	HIV-1 Subtype								
	B	C	A	AE[1]	AG	AD	D	Others[2]	**Total**
Province	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
British Columbia	924 (94.8)	32 (3.3)	11 (1.1)	3 (0.3)	1 (0.1)	0	0	4 (0.4)	**975 (100)**
Alberta	396 (92.5)	18 (4.2)	0	5 (1.2)	2 (0.5)	0	3 (0.7)	4 (0.9)	**428 (100)**
Saskatchewan	214 (79.3)	42 (15.6)	5 (1.9)	5 (1.9)	2 (0.7)	1 (0.4)	0	1 (0.4)	**270 (100)**
Manitoba	257 (85.7)	17 (5.7)	5 (1.7)	3 (1.0)	4 (1.3)	11 (3.7)	3 (1.0)	0	**300 (100)**
Ontario	99 (74.4)	13 (9.8)	3 (2.3)	3 (2.3)	10 (7.5)	0	0	5 (3.8)	**133 (100)**
Nova Scotia	2 (50)	2 (50)	0	0	0	0	0	0	**4 (100)**
Newfoundland	42 (100)	0	0	0	0	0	0	0	**42 (100)**
Total, n (%)	**1934 (89.9)**	**124 (5.8)**	**24 (1.1)**	**19 (0.9)**	**19 (0.9)**	**12 (0.6)**	**6 (0.3)**	**14 (0.7)**	**2152 (100)**

[1] The circulating recombinant form (CRF) AE has also been referred to as HIV-1 subtype E.

[2] Others refers to the following HIV-1 subtypes and CRFs: BD, AB, AC, B/AG, BC, F, G, K, K/AE, and K/AG

Table 3 presents the number and percentage distribution of HIV-1 subtypes by province of diagnosis. The data indicate geographic variation in the distribution of non-B HIV-1 subtypes. All 42 samples from Newfoundland were identified as subtype B, and 5.2%, 7.5%, 20.7%, 14.3%, and 25.6% of the analysed samples from British Columbia, Alberta, Saskatchewan, Manitoba, and Ontario, respectively, belonged to non-B HIV-1 subtypes. However, sample sizes may not represent the total diagnosed population in each of the indicated provinces. Further, the Province of Quebec, which reports a high prevalence of HIV infection, is not presented; thus, caution is recommended in interpreting these results .

Table 4: Number and distribution of HIV-1 subtypes by age at diagnosis with HIV-1 infection

	HIV-1 Subtype								
	B [1]	C [2]	A [3]	AE [4]	AG	AD [5]	D	Others [6]	**Total**
Age (years)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
< 15	7 (53.8)	3 (23.1)	2 (15.4)	0	0	0	1 (7.7)	0	**13 (100)**
15-19	134 (91.8)	7 (4.8)	3 (2.1)	1 (0.7)	0	0	0	1 (0.7)	**146 (100)**
20-29	269 (85.4)	29 (9.2)	7 (2.2)	2 (0.6)	3 (1.0)	1 (0.3)	1 (0.3)	3 (1.0)	**315 (100)**
30-39	684 (88.5)	56 (7.2)	4 (0.5)	12 (1.6)	10 (1.3)	0	2 (0.3)	5 (0.6)	**773 (100)**
40-49	468 (93)	15 (3.0)	6 (1.2)	3 (0.6)	2 (0.4)	5 (1.0)	2 (0.4)	2 (0.4)	**503 (100)**
50-59	152 (89.9)	7 (4.1)	1 (0.6)	1 (0.6)	3 (1.8)	3 (1.8)	0	2 (1.2)	**169 (100)**
60	59 (92.2)	1 (1.6)	0	0	1 (1.6)	2 (3.1)	0	1 (1.6)	**64 (100)**
Total	**1773 (89.4)**	**118 (6.0)**	**23 (1.2)**	**19 (1.0)**	**19 (1.0)**	**11 (0.6)**	**6 (0.3)**	**14 (0.7)**	**1983 (100)**

[1] Age at diagnosis was unknown for 161 individuals HIV-1 subtype B infection

[2] Age at diagnosis was unknown for six individuals with HIV-1 subtype C infection

[3] Age at diagnosis was unknown for one individual with HIV-1 subtype A infection

[4] The circulating recombinant form (CRF) AE has also been referred to as HIV-1 subtype E.

[5] Age at diagnosis was unknown for one individual with a CRF AD infection

[6] Others refers to the following HIV-1 subtypes and CRFs: BD, AB, AC, B/AG, BC, F, G, K, K/AE, and K/AG

Table 4 shows the number and distribution of HIV-1 subtypes by age at diagnosis. Although the sample sizes in certain age categories are small and the data are not representative of all newly diagnosed cases of HIV infection in Canada, the results identified non-B subtypes of HIV-1 in all age groups.

Table 5: Number and distribution of HIV-1 subtypes by gender

	HIV-1 Subtype								
	B [1]	C [2]	A [3]	AE [4]	AG	AD [5]	D	Others [6]	**Total**
Gender	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
Male	1428 (92.4)	68 (4.4)	12 (0.8)	11 (0.7)	8 (0.5)	6 (0.4)	2 (0.1)	10 (0.6)	**1545 (100)**
Female	406 (81)	52 (10.4)	11 (2.2)	8 (1.6)	11 (2.2)	5 (1.0)	4 (0.8)	4 (0.4)	**501 (100)**
Total	**1834 (89.6)**	**120 (5.9)**	**23 (1.1)**	**19 (0.9)**	**19 (0.9)**	**11 (0.5)**	**6 (0.3)**	**14 (0.7)**	**2046 (100)**

[1] Gender was unknown for 100 individuals HIV-1 subtype B infection

[2] Gender was unknown for four individuals with HIV-1 subtype C infection

[3] Gender was unknown for one individual with HIV-1 subtype A infection

[4] The circulating recombinant form (CRF) AE has also been referred to as HIV-1 subtype E.

[5] Gender was unknown for one individual with a CRF AD infection

[6] Others refers to the following HIV-1 subtypes and CRFs: BD, AB, AC, B/AG, BC, F, G, K, K/AE, and K/AG

Table 5 identifies the number and percentage distribution of HIV-1 subtypes by gender. Although the data are not representative of all newly diagnosed cases of HIV infection, the results indicate that the prevalence of non-B subtypes may be higher among females than among males (19% vs. 7.6%, respectively). A greater percentage of females have heterosexual exposure as their primary exposure category, and this exposure category is associated with a higher proportion of non-B HIV subtypes (Table 6).

Table 6: Number and distribution of HIV-1 subtypes by exposure category

	HIV-1 Subtype								
	B [1]	**C [2]**	**A**	**AE [3]**	**AG**	**AD [4]**	**D**	**Others [5]**	**Total**
Exposure Category	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
MSM [6]	578 (97.6)	6 (1.0)	3 (0.5)	0	0	0	0	5 (0.8)	**592 (100)**
MSM/IDU [7]	67 (93.1)	2 (2.8)	1 (1.4)	1 (1.4)	0	0	0	0	**72 (100)**
IDU	572 (97.3)	9 (1.5)	2 (0.3)	2 (0.3)	0	1 (0.2)	1 (0.2)	1 (0.2)	**588 (100)**
Blood/blood products									
a) recipient of blood	6 (75)	1 (12.5)	0	0	0	0	0	1 (12.5)	**8 (100)**
b) recipient of clotting factor	4 (66.7)	2 (33.3)	0	0	0	0	0	0	**6 (100)**
Heterosexual contact/endemic									
a) origin in pattern II country	15 (17.8)	46 (54.8)	1 (1.2)	6 (7.1)	9 (10.7)	0	3 (3.6)	4 (4.8)	**84 (100)**
b) sexual contact with person at risk	278 (86.6)	22 (6.9)	5 (1.6)	7 (2.2)	3 (0.9)	4 (1.2)	1 (0.3)	1 (0.3)	**321 (100)**
Occupational exposure	1 (100)	0	0	0	0	0	0	0	**1 (100)**
NIR - HET [8]	132 (78.1)	20 (11.8)	6 (3.6)	1 (0.6)	4 (2.4)	5 (3.0)	0	1 (0.6)	**169 (100)**
Other	1 (50)	0	1 (50)	0	0	0	0	0	**2 (100)**
NIR [9]	134 (89.3)	8 (5.3)	4 (2.7)	0	1 (0.7)	2 (1.3)	0	1 (0.7)	**150 (100)**
Perinatal	1 (50)	0	1 (50)	0	0	0	0	0	**2 (100)**
Total	**1789 (89.7)**	**116 (5.8)**	**24 (1.2)**	**17 (0.9)**	**17 (0.9)**	**12 (0.6)**	**5 (0.3)**	**14 (0.7)**	**1994 (100)**

[1] Risk exposure was unknown for 145 individuals HIV-1 subtype B infection

[2] Risk exposure was unknown for eight individuals with HIV-1 subtype C infection

[3] The circulating recombinant form (CRF) AE has also been referred to as HIV-1 subtype E. Risk exposure was unknown for two individuals with a CRF AE infection

[4] Risk exposure was unknown for one individual with HIV-1 subtype D infection

[5] Others refers to the following HIV-1 subtypes and CRFs: BD, AB, AC, B/AG, BC, F, G, K, K/AE, and K/AG

[6] MSM refers to men who have sex with men

[7] IDU refers to injecting drug use

[8] NIR - HET refers non-identified risk related to heterosexual exposure

[9] NIR - refers to non-identified risk exposures, i.e., when no risk exposures were identified

Table 6 illustrates the number and percentage distribution of HIV-1 subtypes by exposure category. Although the sample sizes in certain risk exposure categories are small and the data are not representative of all newly diagnosed cases of HIV infection in Canada, the results suggest that a higher proportion of individuals infected through heterosexual contact (particularly with individuals from other countries where non-B strains of HIV-1 prevail) have non-B HIV-1 subtypes, compared with individuals who are infected through male-to-male sex or through injecting drug use.

Table 7: Number and distribution of HIV-1 subtypes by ethnicity

	HIV-1 Subtype								
	B [1]	C [2]	A [3]	AE [4]	AG [5]	AD [6]	D	Others [7]	**Total**
Ethnicity	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
White	1075 (95.9)	22 (2.0)	7 (0.6)	7 (0.6)	4 (0.4)	2 (0.2)	0	4 (0.4)	**1121 (100)**
Black	44 (28.4)	72 (46.5)	9 (5.8)	6 (3.9)	12 (7.7)	1 (0.6)	5 (3.2)	6 (3.9)	**155 (100)**
Aboriginal									
First Nations	264 (96)	7 (2.5)	1 (0.4)	1 (0.4)	0	1 (0.4)	0	1 (0.4)	**275 (100)**
Métis	48 (94.1)	1 (2.0)	0	2 (3.9)	0	0	0	0	**51 (100)**
Inuit	3 (100)	0	0	0	0	0	0	0	**3 (100)**
Unspecified	101 (90.2)	3 (2.7)	2 (1.8)	0	0	5 (4.5)	1 (0.9)	0	**112 (100)**
Asian	45 (88.2)	2 (3.9)	2 (3.9)	1 (2.0)	0	0	0	1 (2.0)	**51 (100)**
South Asian	24 (80)	5 (16.7)	0	1 (3.3)	0	0	0	0	**30 (100)**
Latin American	34 (94.4)	0	0	0	0	0	0	2 (5.6)	**36 (100)**
Other (mixed)	10 (90.9)	1 (9.1)	0	0	0	0	0	0	**11 (100)**
Total	**1648 (89.3)**	**113 (6.1)**	**21 (1.1)**	**18 (1.0)**	**16 (0.9)**	**9 (0.5)**	**6 (0.3)**	**14 (0.8)**	**1845 (100)**

[1] Ethnicity was unknown for 286 individuals HIV-1 subtype B infection

[2] Ethnicity was unknown for 11 individuals with HIV-1 subtype C infection

[3] Ethnicity was unknown for three individuals with HIV-1 subtype A infection

[4] The circulating recombinant form (CRF) AE has also been referred to as HIV-1 subtype E. Ethnicity was unknown for one individual with a CRF AE infection

[5] Ethnicity was unknown for three individuals with a CRF AG infection

[6] Ethnicity was unknown for three individuals with a CRF AD infection

[7] Others refers to the following HIV-1 subtypes and CRFs: BD, AB, AC, B/AG, BC, F, G, K, K/AE, and K/AG

Table 7 highlights the number and percentage distribution of HIV-1 subtypes by ethnicity. Although the sample sizes among certain ethnic groups are small and the data are not representative of all newly diagnosed cases of HIV infection, the results suggest that a higher proportion of African/Caribbean persons (71.6%), Asians (11.8%), and South Asians (20%) may be infected with non-B HIV-1 subtypes, compared with the Caucasian (4.1%) population. These results may be due to travel and migration from countries where non-B strains of HIV-1 prevail.

Table 8: Number and distribution of HIV-1 subtypes by recently acquired vs. established HIV-1 infections

	HIV-1 Subtype								
	B [1]	C [2]	A [3]	AE [4]	AG [5]	AD [6]	D [7]	Others [8]	**Total**
HIV-1 Infection	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
Recent infection [9]	345 (93.5)	14 (3.8)	0	3 (0.8)	3 (0.8)	1 (0.3)	0	3 (0.8)	**369 (100)**
Established infection	923 (88.4)	69 (6.6)	6 (0.6)	11 (1.1)	13 (1.2)	8 (0.8)	5 (0.5)	9 (0.9)	**1044 (100)**
Total	**1268 (89.7)**	**83 (5.9)**	**6 (0.4)**	**14 (1.0)**	**16 (1.1)**	**9 (0.6)**	**5 (0.4)**	**12 (0.8)**	**1413 (100)**

[1] Time of HIV-1 infection could not be determined for 666 individuals HIV-1 subtype B infection

[2] Time of HIV-1 infection could not be determined for 41 individuals with HIV-1 subtype C infection

[3] Time of HIV-1 infection could not be determined for 18 individuals with HIV-1 subtype A infection

[4] The circulating recombinant form (CRF) AE has also been referred to as HIV-1 subtype E. Time of HIV-1 infection could not be determined for five individuals with a CRF AE infection

[5] Ethnicity was unknown for three individuals with a CRF AG infection

[6] Ethnicity was unknown for three individuals with a CRF AD infection

[7] Time of HIV-1 infection could not be determined for one individual with HIV-1 subtype D infection.

[8] Others refers to the following HIV-1 subtypes and CRFs: BD, AB, AC, B/AG, BC, F, G, K, K/AE, and K/AG. Time of HIV-1 infection could not be determined for two individuals in this category.

[9] Due to kit availability, a combination of two assays (Organon Technika Vironostika™ and Abbott™) were used to determine recent infections. These assays were only used on specimens diagnosed since 2000.

Table 8 identifies the number and distribution of HIV-1 subtypes among recently acquired (within about 170 days of diagnostic specimen collection) versus established infections. Two commercially available kits were used to assess the time of infection: the Organon Technika Vironostika HIV-1-LS™ and the Abbott 3A11-LS™ assays. The availability of test kits aimed at determining incident infections affected the extent to which these data could be generated. Hence, the sample size does not reflect all newly diagnosed cases of HIV-1 infection of samples for which HIV-1 subtyping had been completed. For this reason, significant associations between time of infection and HIV-1 subtype could not be determined. However, 6.5% of recent infections and 11.7% of established infections were non-B HIV-1 subtype infections. Of note, serological assays, developed to detect recently acquired infections, have been based on subtype B-derived antigens and have been shown to occasionally misdiagnose incident non-B infections as established infections. To accurately detect recently acquired infections among other non-B subtypes of HIV-1, further investigation is required to determine the sensitivity of the commercially available assays.

Table 9: Number and distribution of HIV-1 subtypes by primary drug resistance

	HIV-1 Subtype								
	B [1]	C [2]	A [3]	AE [4]	AG	AD	D [5]	Others [6]	**Total**
Drug resistance mutations [7]	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
Wild type/minor mutations [8]	1414 (89.2)	101 (6.4)	11 (0.7)	17 (1.1)	18 (1.1)	9 (0.6)	5 (0.3)	11 (0.7)	**1586 (100)**
NRTI [9]	67 (95.7)	1 (1.4)	0	0	1 (1.4)	0	0	1 (1.4)	**70 (100)**
NNRTI [10]	21 (91.3)	0	0	0	0	2 (8.7)	0	0	**23 (100)**
Protease inhibitors	32 (94.1)	1 (2.9)	0	0	0	1 (2.9)	0	0	**34 (100)**
MDR [11]	22 (95.7)	1 (4.3)	0	0	0	0	0	0	**23 (100)**
Total	**1556 (89.6)**	**104 (6.0)**	**11 (0.6)**	**17 (1.0)**	**19 (1.1)**	**12 (0.7)**	**5 (0.3)**	**12 (0.7)**	**1736 (100)**

[1] Primary drug resistance testing results were not available for 378 individuals infected with HIV-1 subtype B

[2] Primary drug resistance results were not available for 20 individuals infected with HIV-1 subtype C

[3] Primary drug resistance results were not available for 13 individuals infected with HIV-1 subtype A

[4] The circulating recombinant form (CRF) AE has also been referred to as HIV-1 subtype E. Primary drug resistance results were not available for two individuals infected with CRF AE.

[5] Primary drug resistance results were not available for 1individuals infected with HIV-1 subtype D

[6] "Others" refers to the following HIV-1 subtypes and CRFs: BD, AB, AC, B/AG, BC, F, G, K, K/AE, and K/AG. Primary drug resistance results were not available for two individuals in this category.

[7] Drug resistance testing was initiated in 2001 and has been conducted on specimens largely derived from individuals diagnosed since 1999.

[8] Wild type indicates no major mutations associated with drug resistance were identified. Minor mutations refers to genetic variables not associated with drug resistance

[9] NRTI refers to nucleoside reverse transcriptase inhibitor

[10] NNRTI refers to non-nucleoside reverse transcriptase inhibitor

[11] MDR refers to multi-drug resistance and includes mutations in HIV-1 that are associated with resistance to any two of the three classes of antiretroviral drugs (NRTIs, NNRTIs, or protease inhibitors)

Table 9 provides the number and distribution of primary drug resistance among HIV-1 subtypes. Since drug resistance genotyping began in 1999, almost one year after subtype testing was initiated, not all subtyped samples have been tested for drug resistance. This implies that samples received prior to the initiation of drug resistance testing have not yet been tested for drug resistance. The data are also not representative of all newly diagnosed cases of HIV-1 infection. The results, however, indicate that multidrug resistance was identified in an individual infected with HIV-1 subtype C. Single class resistance against NRTIs, NNRTIs, or PIs has also been identified among HIV-1 subtype C and the recombinant HIV-1 subtypes AG, BC, and AG.

SECTION II: HIV-1 Primary Drug Resistance (1996 - March 31, 2004)

Background:

Drug resistance is often cited as a contributing factor to treatment failure. Drug resistance that is associated with individuals who are already on treatment and that is described in the context of treatment failure is commonly referred to as "secondary" drug resistance. A phenomenon that has received considerable attention recently is the transmission of drug-resistant HIV-1. This type of drug resistance, also called "primary" drug resistance, has been reported among individuals who have had no previous treatment for HIV infection and so presumably, these individuals have been infected with drug-resistant HIV. A glossary of terms used in this report is presented in Appendix 5.

Primary drug resistance is becoming more widespread in most countries where antiretroviral therapy is used. Persons infected with drug-resistant variants of HIV may be at an increased risk of drug failure, despite never having been treated. However, the prevalence of primary drug resistance and the variation of this prevalence over time, the geographic area, and the population risk group are not well understood.

Data Tables:

This section highlights the main findings related to the number and distribution of primary drug resistance from specimens submitted through the Canadian HIV Strain and Drug Resistance Surveillance Program (SDR program), based on cases newly diagnosed between 1996 and March 31, 2004. Note that these results represent individuals who sought testing, who were properly diagnosed, and who reported HIV positive. Further, the results include only those individuals for whom sufficient sera, taken for the purposes of diagnostic testing, was available to send to the Public Health Agency of Canada for genotyping, and of these samples, the subset for whom reverse transcriptase PCR amplification and sequencing to identify mutations associated with drug resistance were successful. (See Appendix 4 for additional data limitations.)

As of March 31, 2004, 2,272 sera samples from individuals who were newly diagnosed since 1996 and March 31, 2004 and their corresponding non-nominal epidemiologic data have been received from British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, and Nova Scotia for drug resistance. Discussions are also underway to expand the program to the remaining provinces and territories. Although the goal of the SDR program is to collect sera samples from all newly diagnosed cases, the data presented in this report are a result of convenience sampling methods and may not be representative. Viral RNA had been successfully amplified from 1,738 (76.5%) of the sera samples. This level of success in amplifying virus from sera specimens will likely improve by enhancing sample quality and by identifying and using various primer combinations for RT-PCR amplification. Appendix 2 outlines the laboratory methods used for drug resistance genotyping. For this report, major mutations identified in the protease gene and mutations identified in the reverse transcriptase genes of HIV were defined by a consensus of listings reported by the International AIDS Society - USA Drug Resistance Mutations Group (Topics in Medicine, May/June 2003; 11(3): 92-96). Appendix 6 provides a complete list of mutations associated with clinical resistance.

Table 10: Number and distribution of primary drug resistance among newly diagnosed, treatment naive individuals (1996—March 31, 2004)

Primary Drug Resistance	Frequency (n)	Percentage
Wild type/minor mutations[1]	1587	91.4
NRTI[2]	71	4.1
NNRTI[3]	23	1.3
PI[4]	34	1.9
NRTI/NNRTI	13	0.6
PI/NRTI	4	0.3
PI/NNRTI	3	0.2
PI/NRTI/NNRTI	3	0.2
Total	**1738**	**100**

[1] Wild type indicates no major mutations associated with drug resistance were identified. Minor mutations refers to genetic variables not associated with drug resistance

[2]NRTI refers to nucleoside reverse transcriptase inhibitors

[3]NNRTI refers to non-nucleoside reverse transcriptase inhibitors

[4]PI refers to protease inhibitors

Table 10 displays the number and percentage distribution of primary drug resistance among the sample of individuals who were newly diagnosed between 1996 and March 31, 2004, from jurisdictions participating in the SDR program. Mutations associated with drug resistance were present in 8.6% of the sample population of 1,738 newly diagnosed, treatment-naive individuals. Note that since these individuals have not previously been on treatment, they likely have been infected with a drug-resistant strain of HIV-1. Mutations associated with NRTIs and NNRTIs, and major mutations associated with PIs were identified among 71 (4.1%), 23 (1.2%), and 34 (1.9%) of individuals in the sample population, respectively. Of the sample, 23 (1.3%) were infected with multidrug resistant HIV-1, harbouring major mutations to 2 classes of antiretroviral drugs.

Table 11: Mutations in reverse transcriptase and in major mutations in protease

Anti-retroviral drug	Number of individuals (%)*	Major mutation(s)[1]
NRTI[2], Total	**71 (100)**	
	46	M41L[3]
	1	K65R
	2	D67N
	4	T69D
	1	K70R
	3	M184V
	19	L210W
	1	T215Y
	36	T215C/D/E/S[4]
	1	K219Q
NNRTI[5], Total	**23 (100)**	
	1	L100I
	11	K103N
	1	V106A
	5	V108I
	2	Y181C
	1	Y181I
	3	G190A
	1	M230L
PI[6], Total	**34 (100)**	
	3	D30N
	10	M46I
	7	M46L
	1	G48V
	2	I50V
	2	V82F
	11	L90M

* Does not include individuals with resistance-conferring mutations to >1 drug class (n=23). However, certain individuals have >1 resistance-conferring mutation in any given drug class

[1] Major mutations were identified by sequencing the entire protease enzyme and the first 253 amino acids of reverse transcriptase.

[2] NRTI refers to nucleoside reverse transcriptase inhibitor.

[3] M41L refers to the substitution of amino acid methionine (M) by leucine (L) at position 41 of the reverse transcriptase enzyme. Other mutation nomenclature refers to substitutions as indicated; amino acid abbreviations transcriptase enzyme. Other mutation nomenclature refer to substitutions as indicated; amino acid abbreviations are as follows: K, lysine; R, arginine; T, threonine, D, aspartic acid; N, asparagine; E, glutamic acid; H, histidine; Y, tyrosine; V, valine; I, isoleucine; A, alanine

[4] These 'transition' mutations have been shown to confer increased risk of virologic failure to certain NRTIs

[5] NNRTI refers to non-nucleoside reverse transcriptase inhibitor.

[6] PI refers to protease inhibitor.

Resistance to PIs usually requires an accumulation of major mutations for resistance to all members of this class of antiretroviral drug. The same, however, is not true for NNRTIs, where a single mutation can result in resistance to the whole class of drugs.

Table 11 shows the mutations in the reverse transcriptase gene and major mutations in the protease genes of HIV-1 that are associated with resistance to NRTIs, NNRTIs, and PIs. Of the 71 individuals harbouring HIV-1 with mutations associated with resistance to NRTIs, the majority (46, 64.7%) carried virus with an M41L mutation in reverse transcriptase associated with reduced susceptibility to zidovudine and stavudine. M41L refers to the replacement of the amino acid methionine (M) with leucine (L) at position 41 of the reverse transcriptase enzyme. A total of 23 individuals harboured virus resistant to NNRTIs. Of these individuals, the majority (11, 47.8%) carried virus with a K103N mutation associated with reduced susceptibility to delavirdine, efavirenz, and nelfinavir. K103N refers to the replacement of the amino acid lysine (K) with asparagines (N) in position 103 of the reverse transcriptase enzyme. Of 15 individuals harbouring major mutations associated with resistance to PIs, the majority (11, 73.3%) carried virus with an L90M mutation associated with resistance to nelfinavir and saquinivir. L90M refers to the replacement of leucine (L) with methionine (M) at position 90 of the protease enzyme.

Appendix 6 contains a list of mutations associated with drug resistance that were used in the generation of this report.

Table 12: Number and distribution of primary drug resistance in sample population by year of diagnosis

	Primary drug resistance					
	Wild type/minor mutations [1]	**NRTI [2]**	**NNRTI [3]**	**PI [4]**	**MDR [5]**	**Total**
Year of diagnosis	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
1996	25 (92.5)	2 (7.4)	0	0	0	**27 (100)**
1997	38 (100)	0	0	0	0	**38 (100)**
1998	82 (95.3)	3 (3.5)	0	1 (1.2)	0	**86 (100)**
1999	298 (91.7)	18 (5.5)	1 (0.3)	5 (1.5)	3 (0.9)	**325 (100)**
2000	388 (93.5)	17 (4.1)	2 (0.5)	5 (1.2)	3 (0.7)	**415 (100)**
2001	306 (90)	16 (4.7)	8 (2.4)	6 (1.8)	4 (1.2)	**340 (100)**
2002	145 (90.6)	2 (1.2)	3 (1.9)	7 (4.4)	3 (1.9)	**160 (100)**
2003	170 (88.5)	8 (4.2)	5 (2.6)	8 (4.2)	1 (0.5)	**192 (100)**
Jan-March, 2004	81 (91)	4 (4.5)	3 (3.4)	0	1 (1.1)	**89 (100)**
Total	**1533 (91.7)**	**70 (4.2)**	**22 (1.3)**	**32 (1.9)**	**15 (0.9)**	**1672 (100)**

[1] Wild type indicates no major mutations associated with drug resistance were identified. Minor mutations refers to genetic variables not associated with drug resistance. Year of diagnosis was unknown for 54 individuals infected with wild type virus or HIV-1 with minor mutation

[2] NRTI refers to nucleoside reverse transcriptase inhibitor. Year of diagnosis was unknown for one individual infected with HIV-1 harbouring a major mutation to an NRTI

[3] NNRTI refers to non-nucleoside reverse transcriptase inhibitor. Year of diagnosis was unknown for one individual infected with HIV-1 harbouring a major mutation to an NNRTI

[4] PI refers to protease inhibitor.

[5] MDR refers to multi-drug resistance and includes mutations in HIV-1 that are associated with resistance to any two of the three classes of antiretroviral drugs (NRTIs, NNRTIs, and PIs). Year of diagnosis was unknown for eight individuals harbouring multi-drug resistant HIV-1

Table 12 illustrates the number and percentage distribution of primary drug resistance in the sample population by year of diagnosis with HIV infection. However, the following observations should be made cautiously: among newly diagnosed, treatment-naive persons, resistance to NRTIs was observed as early as 1996, to PIs as early as 1998, and to NNRTIs as early as 1999. Further, multidrug resistance in this population has been observed as early as 1999. Larger and more representative sample sizes are required to conduct trend analyses and to determine significant associations between time of diagnosis and primary drug resistance. Accordingly, more representative data from additional years are required before any clear temporal trend in drug resistance can occur.

Table 13: Number and distribution of primary drug resistance in sample population by province

	Primary drug resistance					
	Wild type/minor mutations[1]	NRTI [2]	NNRTI [3]	PI [4]	MDR [5]	**Total**
Province	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
British Columbia	730 (92.2)	30 (3.8)	10 (1.3)	9 (1.1)	13 (1.6)	**792 (100)**
Alberta	380 (94.8)	4 (1.0)	3 (0.7)	9 (2.2)	5 (1.2)	**401 (100)**
Saskatchewan	123 (92.5)	4 (3.0)	2 (1.5)	4 (3.0)	0	**133 (100)**
Manitoba	229 (82.7)	28 (10.1)	4 (1.4)	12 (4.3)	4 (1.4)	**277 (100)**
Ontario	121 (92.4)	5 (3.8)	4 (3.1)	0	1 (0.8)	**131 (100)**
Nova Scotia	4 (100)	0	0	0	0	**4 (100)**
Total	**1587 (91.3)**	**71 (4.1)**	**23 (1.3)**	**34 (2.0)**	**23 (1.3)**	**1738 (100)**

[1] Wild type indicates no major mutations associated with drug resistance were identified. Minor mutations refers to genetic variables not associated with drug resistance

[2] NRTI refers to nucleoside reverse transcriptase inhibitor

[3] NNRTI refers to non-nucleoside reverse transcriptase inhibitor

[4] PI refers to protease inhibitor.

[5] MDR refers to multi-drug resistance and includes mutations in HIV-1 that are associated with resistance to any two of the three classes of antiretroviral drugs (NRTIs, NNRTIs, and PIs).

Table 13 shows the number and percentage distribution of primary drug resistance cases in the sample population by province of residence at the time of diagnosis with HIV infection. Larger sample sizes, which are more representative of all newly diagnosed cases in Canada, will help determine significant associations between province of residence and primary drug resistance. The observations are as follows: primary drug resistance was identified in British Columbia, Alberta, Saskatchewan, Manitoba, and in Ontario; and multi-drug resistance was identified among treatment-naive individuals who were newly diagnosed in British Columbia, Alberta, Manitoba, and Ontario. Of note, resistance to all three classes of antiretroviral drugs was also identified through sentinel surveillance of newly diagnosed, treatment-naive individuals in Quebec (Table 20).

Table 14: Number and distribution of primary drug resistance in sample population by age at diagnosis with HIV infection

	Primary drug resistance					
	Wild type/minor mutations [1]	**NRTI [2]**	**NNRTI [3]**	**PI [4]**	**MDR [5]**	**Total**
Age at diagnosis (years)	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
<15	9 (90)	1 (10)	0	0	0	**10 (100)**
15-19	20 (90.1)	0	1 (4.5)	1 (4.5)	0	**22 (100)**
20-29	303 (91.8)	15 (4.5)	1 (0.3)	7(2.1)	4 (1.2)	**330 (100)**
30-39	566 (90.3)	29 (4.6)	8 (1.3)	11 (1.8)	13 (2.1)	**627 (100)**
40-49	397 (91.9)	14 (3.2)	8 (1.9)	10 (2.3)	3 (0.7)	**432 (100)**
50-59	134 (92.4)	4 (2.8)	4 (2.8)	3 (2.1)	0	**145 (100)**
60	54 (91.5)	3 (5.1)	0	2 (3.4)	0	**59 (100)**
Total	**1483 (91.3)**	**66 (4.1)**	**22 (1.4)**	**34 (2.1)**	**20 (1.2)**	**1625 (100)**

[1] Wild type indicates no major mutations associated with drug resistance were identified. Minor mutations refers to genetic variables not associated with drug resistance. Age at diagnosis was unknown for 104 individuals infected with wild type virus or HIV-1 harbouring minor mutations

[2] NRTI refers to nucleoside reverse transcriptase inhibitor. Age at diagnosis was unknown for five individuals infected with HIV-1 harbouring a major mutation to an NRTI

[3] NNRTI refers to non-nucleoside reverse transcriptase inhibitor. Age at diagnosis was unknown for one individuals infected with HIV-1 harbouring a major mutation to an NNRTI

[4] PI refers to protease inhibitor

[5] MDR refers to multi-drug resistance and includes mutations in HIV-1 that are associated with resistance to any two of the three classes of antiretroviral drugs (NRTIs, NNRTIs, and protease inhibitors). Age at diagnosis was unknown for three individuals harbouring multi-drug resistant HIV-1

Table 14 presents the number and percentage distribution of primary drug resistance cases in the sample population by age at diagnosis with HIV infection. Although the data do not represent all cases newly diagnosed between 1996 and March 31, 2004, they demonstrate that primary drug resistance has been identified among individuals within a wide age range. Of note, in Ontario, only specimens from individuals who were over age 18 years at the time of first diagnosis with HIV infection are eligible for inclusion under the SDR program.

Table 15: Number and distribution of primary drug resistance in sample population by gender

	Primary drug resistance					
	Wild type/minor mutations [1]	NRTI [2]	NNRTI [3]	PI [4]	MDR [5]	**Total**
Gender	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
Male	1142 (91.4)	59 (4.7)	16 (1.3)	21 (1.7)	12 (1.0)	**1250 (100)**
Female	357 (90.8)	9 (2.3)	6 (1.5)	13 (3.3)	8 (2.0)	**393 (100)**
Total	**1499 (91.2)**	**68 (4.1)**	**22 (1.3)**	**34 (2.1)**	**20 (1.2)**	**1643 (100)**

[1] Wild type indicates no major mutations associated with drug resistance were identified. Minor mutations refers to genetic variables not associated with drug resistance. Gender at diagnosis was unknown for 88 individuals infected with wild type virus or HIV-1 harbouring minor mutations

[2] NRTI refers to nucleoside reverse transcriptase inhibitor. Gender was unknown for three individuals infected with HIV-1 harbouring a major mutation to an NRTI

[3] NNRTI refers to non-nucleoside reverse transcriptase inhibitor. Gender was unknown for one individuals infected with HIV-1 harbouring a major mutation to an NNRTI

[4] PI refers to protease inhibitor

[5] MDR refers to multi-drug resistance and includes mutations in HIV-1 that are associated with resistance to any two of the three classes of antiretroviral drugs (NRTIs, NNRTIs, and protease inhibitors). Gender was unknown for three individuals harbouring multi-drug resistant HIV-1

Table 15 illustrates the number and percentage distribution of primary drug resistance in the sample population by gender. The data demonstrate that primary drug resistance has been found among individuals of both genders. While the proportion of cases diagnosed with primary drug resistance is similar among both genders (8.6% among males and 9.2% among females), the absolute number of cases with primary drug resistance is greater among males (108 cases) compared with females (36 cases).

Table 16: Number and distribution of primary drug resistance in sample population by exposure category

	Primary drug resistance					
	Wild type/minor mutations [1]	NRTI [2]	NNRTI [3]	PI [4]	MDR [5]	**Total**
Exposure category	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
MSM [6]	435 (89.7)	30 (6.2)	7 (1.4)	4 (0.8)	9 (1.9)	**485 (100)**
MSM/IDU [7]	47 (90.4)	3 (5.8)	2 (3.8)	0	0	**52 (100)**
IDU	441 (92.6)	10 (2.1)	7 (1.5)	16 (3.4)	2 (0.4)	**476 (100)**
Blood/blood products						
recipient of blood	7 (87.5)	1 (12.5)	0	0	0	**8 (100)**
recipient of clotting factor	3 (100)	0	0	0	0	**3 (100)**
Heterosexual contact/ Endemic						
origin in pattern II country	80 (96.4)	1 (1.2)	0	1 (1.2)	1 (1.2)	**83 (100)**
sexual contact with person at risk	233 (93.2)	3 (1.2)	3 (1.2)	7 (2.8)	4 (1.6)	**250 (100)**
Occupational exposure	0	0	0	0	0	**0**
NIR - HET [8]	116 (84.7)	10 (7.3)	3 (2.2)	6 (4.4)	2 (1.5)	**137 (100)**
Other	1 (100)	0	0	0	0	**1 (100)**
NIR [9]	85 (91.4)	4 (4.3)	1 (1.1)	0	3 (3.2)	**93 (100)**
Perinatal	2 (100)	0	0	0	0	**2 (100)**
Total	**1533 (96.4)**	**68 (4.3)**	**23 (1.4)**	**34 (2.1)**	**21 (1.3)**	**1590 (100)**

[1] Wild type indicates no major mutations associated with drug resistance were identified. Minor mutations refers to genetic variables not associated with drug resistance. Risk exposure was unknown for 54 individuals infected with wild type virus or HIV-1 harbouring minor mutations

[2] NRTI refers to nucleoside reverse transcriptase inhibitor. Risk exposure was unknown for three individuals infected with HIV-1 harbouring a major mutation to an NRTI

[3] NNRTI refers to non-nucleoside reverse transcriptase inhibitor.

[4] PI refers to protease inhibitor

[5] MDR refers to multi-drug resistance and includes mutations in HIV-1 that are associated with resistance to any two of the three classes of antiretroviral drugs (NRTIs, NNRTIs, and protease inhibitors). Risk exposure was unknown for two individuals harbouring multi-drug resistant HIV-1

[6] MSM refers to men who have sex with men

[7] IDU refers to injecting drug use

[8] NIR - HET refers non-identified risk related to heterosexual exposure

[9] NIR - refers to non-identified risk exposures, i.e., when no risk exposures were identified

Table 16 displays the number and distribution of primary drug resistance by exposure category. The data are not representative of all cases that were newly diagnosed between 1996 and March 31, 2004. Further, because of the large proportion of individuals with unknown risk factors and the small sample sizes in certain cells, significant associations between risk exposure and primary drug resistance could not be determined. However, the data indicate primary drug resistance was found among the following main exposure categories: male-to-male sex, injecting drug use, and heterosexual contact, specifically heterosexual contact with a person at risk for HIV infection.

Table 17: Number and distribution of primary drug resistance in sample population by ethnicity

	Primary drug resistance					
	Wild type/minor mutations [1]	NRTI [2]	NNRTI [3]	PI [4]	MDR [5]	**Total**
Ethnicity	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
White	831 (91.1)	37 (4.1)	14 (1.5)	16 (1.8)	14 (1.5)	**912 (100)**
Black	140 (97.2)	1 (0.7)	0	1 (0.7)	2 (1.4)	**144 (100)**
Aboriginal						
First Nations	198 (91.7)	4 (1.9)	3 (1.4)	8 (3.7)	3 (1.4)	**216 (100)**
Métis	41 (95.3)	0	0	2 (4.7)	0	**43 (100)**
Inuit	3 (100)	0	0	0	0	**3 (100)**
Unspecified	94 (87)	6 (5.6)	2 (1.9)	6 (5.6)	0	**108 (100)**
Asian	35 (89.7)	3 (7.7)	0	1 (2.6)	0	**39 (100)**
South Asian	18 (94.7)	1 (5.3)	0	0	0	**19 (100)**
Latin-American	24 (85.7)	3 (10.7)	1 (3.6)	0	0	**28 (100)**
Other (mixed)	11 (100)	0	0	0	0	**11 (100)**
Total	**1395 (91.5)**	**55 (3.6)**	**20 (1.3)**	**34 (2.2)**	**19 (1.2)**	**1523 (100)**

[1] Wild type indicates no major mutations associated with drug resistance were identified. Minor mutations refers to genetic variables not associated with drug resistance. Ethnicity was unknown for 192 individuals infected with wild type virus or HIV-1 with minor mutations

[2] NRTI refers to nucleoside reverse transcriptase inhibitor. Ethnicity was unknown for 16 individuals infected with HIV-1 harbouring a major mutation to an NRTI

[3] NNRTI refers to non-nucleoside reverse transcriptase inhibitor. Ethnicity was unknown for one individual harbouring a major mutations to an NNRTI.

[4] PI refers to protease inhibitor

[5] MDR refers to multi-drug resistance and includes mutations in HIV-1 that are associated with resistance to any two of the three classes of antiretroviral drugs (NRTIs, NNRTIs, and protease inhibitors). Ethnicity was unknown for four individuals harbouring multi-drug resistant HIV-1

Table 17 provides the number and percentage distribution of primary drug resistance by ethnicity. The data do not represent all newly diagnosed cases of HIV between 1996 and March 31, 2004, and due to the small sample sizes in certain cells, significant associations between ethnicity and primary drug resistance could not be determined. The data suggest that while most primary drug resistance cases with known ethnicity were identified among the Caucasian population (63.3%), primary drug resistance has also been identified among affected Aboriginal, Asian, African/Caribbean (Black), and Latin American groups.

Table 18: Number and distribution of primary drug resistance in sample population by HIV-1 subtype

	Primary drug resistance					
	Wild type/minor mutations [1]	NRTI [2]	NNRTI [3]	PI [4]	MDR [5]	**Total**
HIV-1 subtype	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
B	1414 (90.9)	67 (4.3)	21 (1.3)	32 (2.1)	22 (1.4)	**1556 (100)**
A	11 (100)	0	0	0	0	**11 (100)**
C	101 (97.1)	1 (1.0)	0	1 (1.0)	1 (1.0)	**104 (100)**
D	5 (100)	0	0	0	0	**5 (100)**
F	1 (100)	0	0	0	0	**1 (100)**
G	1 (100)	0	0	0	0	**1 (100)**
K	1 (100)	0	0	0	0	**1 (100)**
AC	1 (100)	0	0	0	0	**1 (100)**
AD	9 (75)	0	2 (16.7)	1 (8.3)	0	**12 (100)**
AE [6]	17 (100)	0	0	0	0	**17 (100)**
AG	18 (94.7)	1 (5.3)	0	0	0	**19 (100)**
BC	0	1 (100)	0	0	0	**1 (100)**
BD	4 (100)	0	0	0	0	**4 (100)**
B/AG	1 (100)	0	0	0	0	**1 (100)**
K/AE	1 (100)	0	0	0	0	**1 (100)**
K/AG	1 (100)	0	0	0	0	**1 (100)**
Total	**1586 (91.3)**	**70 (4.0)**	**23 (1.3)**	**34 (2.0)**	**23 (1.3)**	**1736 (100)**

[1] Wild type indicates no major mutations associated with drug resistance were identified. Minor mutations refers to genetic variables not associated with drug resistance. HIV-1 subtype was unknown for one individual infected with wild type virus or HIV-1 with minor mutations

[2] NRTI refers to nucleoside reverse transcriptase inhibitor. HIV-1 subtype was unknown for one individual harbouring a major mutation to an NRTI3 NNRTI refers to non-nucleoside reverse transcriptase inhibitor.

[4] PI refers to protease inhibitor

[5] MDR refers to multi-drug resistance and includes mutations in HIV-1 that are associated with resistance to any two of the three classes of antiretroviral drugs (NRTIs, NNRTIs, and protease inhibitors).

[6] HIV-1 subtype AE has also been referred to as subtype E

Table 18 presents the prevalence of primary drug resistance by HIV-1 subtype. The data are not representative of all cases newly diagnosed with HIV infection between 1996 and March 31, 2004. Owing to small sample sizes in certain cells, significant associations between HIV-1 subtype and primary drug resistance could not be determined. The data suggest that while most primary drug resistance cases with known subtypes have been identified among HIV-1 subtype B (142 of 150, 94.7%), primary drug resistance has also been identified among individuals infected with HIV-1 subtype C and the recombinant subtypes AD, AG, and BC.

Table 19: Number and distribution of primary drug resistance in sample population by recent vs. established HIV-1 infection

	Primary Drug Resistance					
	Wild type/minor mutations [1]	NRTI [2]	NNRTI [3]	PI [4]	MDR [5]	**Total**
HIV-1 infection	n (%)	n (%)	n (%)	n (%)	n (%)	**n (%)**
Recent infection[6]	318 (88.8)	18 (5.0)	6 (1.7)	8 (2.2)	8 (2.2)	**358 (100)**
Established infection	934 (92.3)	36 (3.6)	15 (1.5)	19 (1.9)	8 (0.8)	**1012 (100)**
Total	**1252 (91.4)**	**54 (3.9)**	**21 (1.5)**	**27 (2.0)**	**16 (1.2)**	**1370 (100)**

[1] Wild type indicates no major mutations associated with drug resistance were identified. Minor mutations refers to genetic variables not associated with drug resistance. Time of HIV-1 infection was unknown for 335 individuals infected with wild type virus or HIV-1 harbouring minor mutations.

[2] NRTI refers to nucleoside reverse transcriptase inhibitor. Time of HIV-1 infection was unknown for 17 individuals infected with HIV-1 harbouring a major mutation to an NRTI

[3] NNRTI refers to non-nucleoside reverse transcriptase inhibitor. Time of HIV-1 infection was unknown for two individuals infected with HIV-1 harbouring a major mutation to an NNRTI

[4] PI refers to protease inhibitor. Time of HIV-1 infection was unknown for seven individuals infected with HIV-1 harbouring a major mutation to an NNRTI

[5] MDR refers to multi-drug resistance and includes mutations in HIV-1 that are associated with resistance to any two of the three classes of antiretroviral drugs (NRTIs, NNRTIs, and protease inhibitors). Time of HIV-1 infection was unknown for seven individuals harbouring a major mutation to an NNRTI

[6] Due to kit availability, a combination of two assays (Organon Technika Vironostika and Abbott) was used to determine recent infections. These assays were only used on specimens diagnosed since 2000.

Table 19 shows the prevalence of primary drug resistance among recently acquired (within approximately 170 days since collection of the diagnostic specimen) versus established infections. A combination of two commercially available kits was used to assess the time of infection: the Organon Technika Vironostika HIV-1-LS™ and the Abbott 3A11-LS™ assays. The availability of test kits aimed at determining incident infections has affected the extent to which these data could be generated. The sample size therefore does not reflect all cases newly diagnosed and genotyped for drug resistance between 1996 and March 31, 2004. However, the data suggest that primary drug resistance was identified in both recent and established infections. The percentage of drug resistance among recent infections was higher than that among established infections. These data also support results from other studies that suggest certain mutations may persist over time and may contribute to drug resistance.

Table 20: Summary of key studies on drug resistance among newly diagnosed, treatment naive individuals in Canada

Province*	Year of diagnosis	Risk exposures**	Sample size	RTIs†	PIs‡ %	MDR %	Total %
BC[1]	1996-1998	Mixed	423	1.9	1.9	0.2	3.5
QC[2]	1997-1999	IDU (26%) Sexual (69%)	81	20	6	9.9	-
QC[3]	1997	Mixed	50	12 (NRTI) 0 (NNRTI)	5	-5	-
	1998		42	6 (NNRTI) 0 (NRTI)	0	0	-
	1999		17	~18 (NRTI) ~14 (NRTI)	~18	~12	-
	2000		18	~12 (NRTI) ~6 (NNRTI)	~6	~5	-
	2001		18	0 (NRTI) 0 (NRTI)	~6	0	-
	2002		18	0 (NRTI) ~6 (NNRTI)	0	0	-
	2003		17	0	0	0	-
ON[4]	1997-1999	MSM	23	13	-	-	-
BC, AB, SK,MB, ON, NS[5]	1997	Mixed	38	0	0	0	0
	1998		86	3.5 (NRTI)	1.2	0	4.7
	1999		325	5.5 (NRTI) 0.3 (NNRTI)	1.5	0.9	8.2
	2000		415	4.1 (NRTI) 0.5 (NRTI)	1.2	0.7	6.5
	2001		340	4.7 (NRTI) 2.4 (NNRTI)	1.8	1.2	10.1
	2002		160	1.2 (NRTI) 1.9 (NNRTI)	4.4	1.9	9.4
	2003		192	4.2 (NRTI)	4.2	0.5	11.5

*BC = British Columbia, QC = Quebec, ON = Ontario, AB = Alberta, SK = Saskatchewan, MB = Manitoba, NS = Nova Scotia.

**Reported proportions may not add to 100% since risk exposure categories may not be mutually exclusive. IDU = injecting drug use, MSM = men who have sex with men

† RTI = reverse transcriptase inhibitors, NRTI = nucleoside reverse transcriptase inhibitor, NNRTI = non-nucleoside reverse transcriptase inhibitor. Information on NRTI and NNRTI provided where available.

‡ PI = protease inhibitors

MDR = multi-drug resistance

[1] Brumme ZL, Chan KJ, Dong WW et al. Prevalence and clinical implications of insertions in the HIV-1 p6Gag N-terminal region in drug-naive individuals initiating antiretroviral therapy. Antivir Ther 2003; 8:91-6.

[2] Salomon H, Wainberg MA, Brenner B et al. Prevalence of HIV-1 viruses resistant to antiretroviral drugs in 81 individuals newly infected by sexual contact or intravenous drug use. AIDS 2000; 14(2):F17-23.

[3] Routy JP, Machouf N, Edwardes MD et al. *Factors associated with a decrease in the prevalence of drug resistance in newly HIV-1 infected individuals in Montreal*. AIDS 2004; 2305-12.

[4] Cassol S, Calzavara L, Major C et al. *HIV-1 drug resistance in Ontario seroconverters*. Ninth Annual Canadian Conference on HIV/AIDS Research, Montreal, QC, April 27-30, 2000; #135P.

[5] Canadian HIV Strain and Drug Resistance Surveillance Program. Surveillance and Risk Assessment Division, Centre for Infectious Disease Prevention and Control, Public Health Agency of Canada, 2005

Table 20 summarizes the primary drug resistance results from the SDR program and other cohort and cross-sectional studies in Canada. This table, however, is not meant for inter-study comparisons. It is difficult to make such comparisons and arrive at firm conclusions because of differences in study design. For example, prevalence rates depend on the population being studied (high risk vs. general population); the types of laboratory tests used (genotypic and/or phenotypic testing); and differences in mutations studied and reported. The results suggest that the prevalence of mutations associated with drug resistance is similar to those described in the United States and in Western Europe (Table 21).

Table 21: Summary of key studies on drug resistance among newly diagnosed, treatment naive individuals in the United States and in Western Europe

Country	Year of diagnosis	Risk exposures*	Sample size	RTIs** %	PIs† %	MDR‡ %	Total¶ %
United States [1]	1989-1998	MSM (80%)	141	0.7 (NNRTI)	1.4	1.4	2.1
United States [2]	1995-1999	MSM (94%)	80	12.5 (NRTI) 7.5 (NNRTI)	3	3.8	16.3
United States [3]	1997-2001	Mixed	1,082	6.4 (NRTI) 1.7 (NNRTI)	1.9	1.3	8.3
United States [4]	1998	Mixed	238	3.4(NRTI) 0.4 (NNRTI)	0	0	3.8
	1999		240	8.3 (NRTI) 2.1 (NNRTI)	1.7	1.7	10
	2000		245	6.9 (NRTI) 1.2 (NNRTI)	2	1.2	9
United States (and some samples from Canada) [5]	1995-1998	MSM	377	8.5 (NRTI, n = 213) 1.7 (NNRTI, n = 176)	0.9 (n = 213)	3.8 (n = 213)	8
	1999-2000			15.9 (NRTI, n = 82) 7.3 (NNRTI, n = 82)	9.1 (n = 88)	10.2 (n = 88)	22.7
Germany [6]	1996-1998	Mixed	64	6.3 (NRTI) 3.1 (NNRTI)	1.6	1.6	12.5
France [7]	1995-1998	Mixed	48	16.6	2	-	-
France [8]	1999-2000	Mixed	251	7.6 (NRTI) 4.0 (NNRTI)	5.2	4.8	-
Spain [9]	1996-1998	Mixed	68	16.2	6	4.4	-
Spain [10]	1997-1999	Mixed	31	16.1	9.7	0	25.8
	2000-2001	Mixed	21	0	4.8	0	4.8
Switzerland [11]	1996	Mixed	193	5.6	3	-	8.6
	1997			6.9	7.7	-	14.6
	1998			6.8	2	-	8.8
	1999			3.1	1.9	-	5
Switzerland [12]	1999-2001	Mixed	200	6.5 (NRTI) 0.5 (NNRTI)	1	1.5	10
United Kingdom [13]	1996-1997	Mixed	310	9 (NRTI) 1 (NNRTI)	1	1	10
	1998	Mixed	306	8 (NRTI) 1 (NNRTI)	2	1	9
	1999	Mixed	342	9 (NRTI) 3 (NNRTI)	2	2	11
	2000	Mixed	430	12 (NRTI) 4 (NNRTI)	3	1	16
	2001	Mixed	476	12 (NRTI) 4 (NNRTI)	3	2	14
	2002-2003	Mixed	161	16 (NRTI) 8 (NNRTI)	3	3	21
Europe [14]	1996-2002	Mixed	1,369	9	2	-	11

*MSM = men who have sex with men

**RTI = reverse transcriptase inhibitors, NRTI = nucleoside reverse transcriptase inhibitor, NNRTI = non-nucleoside reverse transcriptase inhibitor. Information on NRTI and NNRTI provided where available.

†PI = protease inhibitors

‡MDR = multi-drug resistance

¶Total may include major and minor mutations associated with primary drug resistance.

[1] Little SJ, Daar ES, D'Aquila RT et al. Reduced antiretroviral drug susceptibility among patients with primary HIV infection. JAMA 1999; 282:1142-1149.

[2] Richman D, Morton SC, Terri W et al. The Prevalence of antiretroviral drug resistance in the United States. AIDS 2004:1393-1401.

[3] Bennett D, Zaidi I, Heneine W et al. Prevalence of mutations associated with antiretroviral drug resistance among men and women newly diagnosed with HIV in 10 US cities, 1997-2001 [Abstract]. Antivir Ther 2003; 8:S133.

[4] Bennett D, Zaidi I, Heneine W et al. Prevalence of mutations associated with antiretroviral drug resistance among recently diagnosed persons with HIV 1998-2000. Ninth Conference on Retroviruses and Opportunistic Infections, Seattle, WA, Feb 24-28 2002; #95.

[5] Little SJ, Holte S, Routy JP et al. Antiretroviral drug resistance among patients recently infected with HIV. NEJM 2002; 347:385-394.

[6] Duwe S, Brunn M, Altmann D et al. Frequency of genotypic and phenotypic drug-resistant HIV-1 among therapy-naive patients of the German Seroconverter Study. J Acquir Immune Defic Syndr 2001;26:266-73

[7] Tamalet C, Pasquier C, Yahi N et al. Prevalence of drug resistant mutants and virological response to combination therapy in patients with HIV-1 infection. J Med Virol 2000; 61:181-6.

[8] Caix ML, Descamps D, Deveau C et al. Antiretroviral resistance, molecular epidemiology and response to initial therapy among patients with HIV-1 primary infection in 1999-2000 in France. XI International HIV Drug Resistance Workshop, Seville, Spain, July2-5 2002. Antiviral Ther 2002; 7(Suppl 1):#166.

[9] Puig T, Perez-Olmeda M, Rubio A et al. Prevalence of genotypic resistance to nucleoside analogues and protease inhibitors in Spain. The ERASE-2 Study Group. AIDS 2000; 14:727-32.

10 De Mendoza C, del Romero J, Rodriguez C et al. Decline in the rate of genotypic resistance to antiretroviral drugs in recent HIV seroconverters in Spain. Ninth Conference on Retroviruses and Opportunistic Infections, Seattle, WA Reb 24-28, 2002: 371M.

[11] Yerly S, Vora S, Rizzardi P et al. Acute HIV infection: impact on the spread of HIV and transmission of Drug Resistance. AIDS 2001;12:2287-92

[12] Yerly S, Jost S, Telenti A et al. Transmission of Drug Resistance: impact of primary and chronic HIV infection. XI International HIV Drug Resistance Workshop, Seville, Spain. July 2-5 2002. Antiviral Ther 2002;7(Suppl 1):#183

[13] UK HIV Drug Resistance Database. HIV drug resistance in the United Kingdom. Commun. Dis Rep CDR Wkly. Oct 23, 2003; 13(43).

[14] Wensing AMJ, van der Vijver CAMC, Asjo B et al. Prevalence of transmitted drug resistance in Europe is largely influenced by the presence of non-B sequences: analysis of 1400 patients from 16 countries: the CATCH-Study. Antivir Ther 2003; 8:S131.

Table 21 shows the results from studies on primary drug resistance that were conducted in the United States and other countries in Western Europe. This table is not meant for inter-study comparisons since such interpretations are difficult due to differences in study design.

Appendices

Appendix 1. Overview of the Canadian HIV Strain and Drug Resistance Surveillance Program

As a result of the Krever Commission's recommendations for strengthening Health Canada's Blood Safety Program, the Division of Surveillance and Risk Assessment has been mandated to enhance blood surveillance activities and is developing an integrated surveillance program that will provide support to provinces and territories to develop and maintain their surveillance systems for HIV and AIDS. This development is integrated with, and supported by, an improved federal infrastructure and national monitoring of HIV subtypes and drug resistance. The Canadian HIV Strain and Drug Resistance Surveillance Program (SDR program) is a key component in a national system for the enhanced surveillance of HIV/AIDS, emerging retroviruses, and other sexually transmitted blood-borne pathogens. Initiated in 1998, the SDR program is designed to characterize and monitor the genetic diversity of the HIV epidemic in Canada. It is a collaborative effort between the provinces and territories in Canada and the Centre for Infectious Disease Prevention and Control, Public Health Agency of Canada. In addition, it was designed to serve as an integrated mechanism for the analysis of HIV genetic characteristics as they relate to the epidemiology of HIV, addressing the concerns of affected communities, public health authorities, primary care physicians, and researchers.

The program's primary goals, established during a 1998 consensus workshop in Vancouver, are as follows:

1) To enhance the safety of the blood supply

To ensure the safety of the blood supply, all HIV tests need to reliably detect the different HIV strains that are circulating in the country. The precedent for this goal was the discovery of HIV-2 and highly divergent group O strains of HIV-1, which required modifying some serologic tests by adding new antigens that would ensure detection. The reference services of the National HIV and Retrovirology Laboratories addressed this goal by testing samples with unusual virologic test results, quality assurance, and the monitoring of diagnostic kits.

2) To inform vaccine development

It is important to know the distribution of the HIV strains and clade variations to target vaccine development and testing; the efficacy and effectiveness of vaccines may be strain- and subtype specific.

3) To assess genetic markers of HIV drug resistance

Although antiretroviral therapies have led to a reduction in HIV-related morbidity and mortality in Canada, there is concern that their widespread use, the increased number of treatment failures, and high HIV infection rates may result in increased transmission of HIV drug-resistant virus. The information provided by the SDR program can be used to develop treatment guidelines at the population level for initial therapeutic regimens and for more effective HIV prevention strategies.

4) To determine rates of HIV transmission, pathogenesis, and progression to HIV-related diseases

Although genetic analyses have been used to assess the spread of HIV globally, there is limited information on whether differences in HIV clades and mutations conferring drug resistance affect the rates of transmission, pathogenesis, or HIV-related disease progression. The public health implications of such findings, including prevention and treatment strategies, are of special interest.

As of December 31, 2004, British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Newfoundland, and Nova Scotia are

participating in the SDR program. The results presented in this report represent samples for which HIV subtype analysis and primary drug resistance genotyping were completed successfully as of December 31, 2004. Samples and epidemiologic data continue to flow from participating provinces to the Public Health Agency of Canada and results from these analyses will be presented in future reprorts. Discussions are underway to expand the collection of samples and epidemiologic data to the remaining provinces and territories.

Appendix 2. Methodology

Epidemiologic data and laboratory specimen collection and transfer

The provincial partners in the Canadian HIV Strain and Drug Resistance Surveillance Program (SDR program) send sera samples taken for diagnostic testing from treatment-naive individuals with newly diagnosed HIV infection to the Centre for Infectious Disease Prevention and Control (CIDPC), Public Health Agency of Canada. Subtype analysis and primary drug resistance genotyping is conducted at the National Laboratory for HIV Genetics in the National HIV and Retrovirology Laboratories. The National Laboratory for HIV Reference Services in the National HIV and Retrovirology Laboratories conducts testing for the time of infection.

For each submitted laboratory sample, non-nominal epidemiologic information is also sent to the Public Health Agency of Canada. The data include information routinely collected on the national or provincial HIV case reporting forms and, where available, additional information that helps interpret the laboratory results, including treatment history, CD4 count and viral load at diagnosis, and previous HIV testing history. Epidemiologic analyses are conducted at the Surveillance and Risk Assessment Division.

As of December 31, 2004, British Columbia, Alberta, Saskatchewan, Manitoba, Ontario, Newfoundland, and Nova Scotia have participated in the SDR program. The results presented in this report represent samples that the Public Health Agency of Canada has received as of December 31, 2004, on which HIV subtype and drug resistance genotyping have been completed successfully.

Meanwhile, samples and epidemiologic data continue to be sent to the Public Health Agency of Canada from participating provinces, and results from these analyses will be presented in future reports. Discussions are underway to expand the collection of samples and epidemiologic data to the remaining provinces and territories.

Genetic algorithm for HIV subtyping and drug resistance testing

After extracting the RNA and an initial one-step reverse transcriptase PCR, nested PCR amplification of the *pol* gene (which encodes for the HIV protease and reverse transcriptase enzymes) is performed by using a combination of published and in-house group M consensus primers. The PCR product is directly sequenced with internal PCR primers on a Li-Cor 4200L automated sequencer. In this way, a complete double stranded sequence for the entire protease gene and the first 253 amino acids of reverse transcriptase is used to assess subtype and primary mutations associated with drug resistance. Between July 1998 and December 2000, the C2-V5 region (233 amino acids) of the envelope protein was used to assess HIV subtype. When PCR products sequence poorly, at least two additional amplification and sequencing attempts are made with increasing enhancement algorithms to sequence the PCR product. If this is unsuccessful, a final effort is made to resolve the problem by cloning the PCR product and screening 10 to 12 clones per person.

Consensus of mutations associated with drug resistance

Interpretation of results from genetic algorithms requires knowledge of the association between specific mutations and virologic response to antiretroviral drugs. The associations are often complex and not necessarily additive. Consensus drug resistance mutation lists have been published through database banks (e.g. Stanford University, http://hivdb.stanford.edu/hiv and the Los Alamos HIV Sequence Database, http://resdb.lanl.gov/Resist_DB/) and by expert committees on HIV drug resistance (e.g. International AIDS Society – USA Drug Resistance Mutations Group). However, even the experts do not always agree on these so-called "rules-based" algorithms.

For this report, major mutations associated with drug resistance that were identified in the protease gene and mutations that were identified in the reverse transcriptase genes of HIV were defined by a consensus of listings reported by the International AIDS Society – USA Drug Resistance Mutations Group[1]. Appendix 6 provides the list of mutations associated with drug resistance that are used for this report.

Determining time of infection

Recent infections were defined as those that occurred within the past 170 days of serum collection (95% CI=162-183 days) and were identified using one of two enzyme immunoassays: the Abbott 3A11-LS™ or the Organon Teknika Vironostika HIV-1-LS™.

Epidemiologic analyses

Laboratory and epidemiologic data are linked using unique identifiers. Significant associations between primary drug resistance or HIV-1 non-B subtypes and epidemiologic characteristics of individuals in the sample population were assessed using the chi-squared test and, where appropriate, Fisher's exact test. Logistic regression analyses to further define independent factors associated with primary drug resistance and with non-B infections were conducted using SPSS 8.0™ (SPSS Inc. Chicago, IL). Independent variables that were examined included age at diagnosis of HIV infection, gender, exposure category, ethnicity, and year of diagnosis of HIV infection.

Appendix 3: Technical Notes

Data Collection and Reporting

The results in this report represent individuals who sought testing, who were properly diagnosed, and who were reported as HIV positive. Further, they represent those individuals for whom sufficient serum specimen, taken for the purposes of diagnostic testing, was available to send to the National HIV and Retrovirology Laboratories and, of these, the subset for whom subtype analysis and/or primary drug resistance genotyping was completed as of June 30, 2000. The quality of samples received by the National HIV and Retrovirology Laboratories also determines whether subtype and drug resistance results can be generated. Typically, the laboratories make at least two attempts on samples that are difficult to amplify with the in-house and consensus Group M primers. The National Laboratory for HIV Genetics is examining the use of other primer sets for RT-PCR amplification, as well as other methods for sequencing the genetic material.

The epidemiologic data collected through the SDR program contain information included in the National HIV/AIDS reporting form, along with additional data that allow interpretation of the laboratory results. These additional data include the type of laboratory specimen sent, the date of the last negative HIV test, the history of seroconversion (if any), the antiretroviral treatment history (if any), and the viral load count at diagnosis.

There are several limitations to the epidemiologic data (Appendix 4) and one of the key roles of the federal Field Surveillance Officers is to work with the provincial and territorial health partners to facilitate the collection and timely reporting of these data to the Surveillance and Risk Assessment Division.

1 International AIDS society-USA Drug Resistance Mutations Group *Drug Resistance Mutations in HIV-1*, Topics in HIV Medicine 2003; 11(3): 92-96.

Exposure Category Hierarchy

HIV cases were assigned to a single exposure category according to an agreed-upon hierarchy of risk factors. The *HIV and AIDS in Canada Surveillance Report* details this hierarchy and is available by contacting the Surveillance and Risk Assessment Division or by visiting its Web site at www.phac-aspc.gc.ca/publicat/aids-sida/haic-vsac0604/index.html.

Analysis of Drug Resistance

Although both genotypic and phenotypic testing methods are well established, each has its limitations. Both kinds of test provide information only on the virus that predominated at the time of sampling and cannot identify virus that may be present as a result of past drug exposures. This is particularly important as "minority" species of virus may become predominant under selective drug pressures that do not completely inhibit viral replication. Both assays are technically difficult to perform when the concentration of virus is < 1,000 copies/mL and may require highly specialized laboratory facilities and personnel. The ability of both assays to quantify resistance to certain drugs has not yet been determined. Phenotypic testing is expensive. In genotypic testing, repeat analyses may be required because mutations that strongly associate with drug resistance continue to be "discovered," and their complex interactions are only beginning to be understood.

Interpretation of Drug Resistance

The interpretation of genotypic and phenotypic test results for patient care is still uncertain and under active research. The complexity of this task is compounded by the following factors: genotypic and phenotypic test results may not correlate with one another, clinical relevance varies from drug to drug, the concentrations at which a drug is ineffective has not been determined *in vivo*, and the extent to which pharmaceutical interactions influence resistance is not well known. Appendix 6 lists the mutations that were included as drug resistance mutations in the results presented in this report. We anticipate that this list will change as new information on drug resistance mutations becomes available over time. International expert review panels have been formed. These panels meet periodically to review the latest laboratory and clinical findings for use in developing guidelines to interpret genotypic and phenotypic drug resistance mutations for clinical management. A similar panel of experts is being assembled to identify and standardize mutations, useful for primary drug resistance surveillance.

Appendix 4: Data Limitations

The data presented in this report must be interpreted with caution for the following reasons:

- The data represent cases of newly diagnosed individuals for whom serum specimen and corresponding epidemiologic information are provided to the Public Health Agency of Canada from provincial partners participating in the Canadian HIV Strain and Drug Resistance Surveillance Program (SDR program). The data are based on convenience sampling and therefore do not include all newly diagnosed cases in a given population for any given year. Although we do not anticipate any biases introduced as a result of the convenience sampling, we need to keep in mind that the data may not be representative of all newly diagnosed cases in the population.
- The data do not include Quebec and may not be representative of cases newly diagnosed in Ontario. Together, however, these two provinces represent about two-thirds of reported HIV infections in Canada. Work is already underway on mechanisms to include representative data from these provinces.

- This report deals solely with primary drug resistance (i.e. resistance seen among individuals who have never received treatment); for this reason, analysis was conducted on the laboratory specimens collected from treatment-naive individuals at the time of initial testing for HIV. However, treatment history cannot always be verified. At least 5% of laboratory specimens from B.C., for example, are likely to have been collected from individuals who have received treatment. Missing or unknown epidemiologic data remain problematic, particularly with respect to information on previous HIV testing, date of first positive HIV test, ethnicity, risk behaviour, CD4 and viral load at diagnosis, and previous antiretroviral treatment.
- Subtype analyses are conducted on 1,053 base-pairs in the *pol* gene and reflect what is observed within this small region of the viral genome.
- The serological assays that have been developed to detect recently acquired infections have been based on subtype B-derived antigens and have been shown to occasionally misdiagnose incident non-B infections as established infections. Further investigation is required to determine the sensitivity of the commercially available assays to accurately detect recently acquired infections among other non-B HIV-1 subtypes.

Appendix 5: Glossary of Terms[1]

Cross-resistance: resistance selected by one drug that, in turn, confers resistance to one or more drugs not included in the current treatment

DNA: deoxyribonucleic acid, the genetic material of a cell

Drug resistance: decreased susceptibility to a drug

Drug resistance mutation: a change in amino acid associated with increased resistance of HIV to an antiretroviral drug

Gene: a segment of DNA coding for a particular protein or protein sub-unit

Genotype: specific sequence of nucleotides that determines the genes of HIV-1

Genotypic resistance: presence of mutations to nucleotides that increase resistance of HIV to one or more antiretroviral drugs

Genotypic tests: conducted to determine the presence of mutations in the nucleotide sequence of the viral genome

HIV: Human immunodeficiency virus

Incidence: the number of new occurrences of a disease in a given population during a specified period of time

Major mutation: mutation in the viral protease sequence that, in and of itself, is strongly associated with conferring increased resistance of HIV to protease inhibitors

Minor mutation: mutation in the viral protease sequence that, in combination with other mutations, confers increased resistance of HIV to protease inhibitors

Multi-drug resistance: increased resistance of HIV to more than one class of drugs

Mutation: genetic change in the viral nucleotide sequence

Nucleotide: a monomeric unit consisting of a sugar, phosphate, and nitrogenous base

PCR: polymerase chain reaction, a molecular technique used to amplify nucleotide sequences

Phenotype: characteristics and growth properties of HIV-1

Phenotypic resistance: when four or more times the amount of drug is required to inhibit viral growth by 50% (inhibitory concentration 50)

Phenotypic tests: used to determine the susceptibility of a virus to drug in a virus culture assay

Prevalence: the number of people with the disease in a given population who are alive during a specified period of time

Primary resistance: increased resistance of HIV to antiretroviral drugs seen in individuals who have never before received treatment and so, presumably, have been infected with drug-resistant virus

Protease: an enzyme that breaks down proteins to their subunits or component peptides

Recombinant: HIV-1 containing a sequence corresponding to a mixture of more than one subtype in the envelope gene

Reverse transcriptase: an enzyme that is unique to all retroviruses. It reads the genetic information of the retrovirus, which is RNA, and makes a DNA copy.

RNA: ribonucleic acid, a polymer of nucleotides involved in protein synthesis

RT-PCR: PCR using the enzyme reverse transcriptase (RT), a molecular technique used to amplify RNA sequence into DNA

Secondary resistance: increased resistance of HIV to drugs seen in individuals who are already receiving treatment (presumably a result of treatment failure)

Subtype: also referred to as clade, a group of related HIV variants classified according to degree of genetic similarity

Wild type virus: the most commonly occurring form of HIV-1

[1] Some definitions are adapted from the *HIV and AIDS in Canada Surveillance Report* to December 31, 2000 and from the International Consultation on Monitoring the Emergence of Antiretroviral Resistance sponsored by WHO, UNAIDS and ISS (October, 2000).

Appendix 6: List of mutations used for this report*

Reverse transcriptase	
Mutation	**Antiretroviral drug**
M41L[1]	Zidovudine, Stavudine
K65R	Dinanosie, Zalcitabine, Abacavir, Tenofovir
D67N	Zidovudine, Stavudine
T69D	Zalcitabine
K70R	Zidovudine, Stavudine
L74V	Didanosine, Zalcitabine, Abacavir
L100I	Nevirapine, Efavirenz
K103N	Delavirdine, Nevirapine, Efavirenz
V106A	Nevirapine
V108I	Nevirapine, Efavirenz
Y115F	Abacavir
Y181C	Delavirdine, Nevirapine, Efavirenz
Y181I	Nevirapine
M184I	Lamivudine
M184V	Zalcitabine, Abacavir, Lamivudine
Y188C/H	Nevirapine
Y188L	Delavirdine, Nevirapine, Efavirenz
G190A	Nevirapine, Efavirenz
G190S	Efavirenz
L210W	Zidovudine, Stavudine
T215F/Y	Zidovudine, Stavudine
K219E/Q	Zidovudine, Stavudine
P225H	Efavirenz
M230L	Delavirdine, Nevirapine, Efavirenz
P236L	Delavirdine

* **Note:** The correlation of drug resistance to genotype in this report is based on scientific consensus of mutations associated with HIV resistance to antiretroviral drugs as of June 2003. These correlations do not necessarily imply phenotypic resistance to a particular antiretroviral drug in a clinical setting.

[1]M41L refers to the substitution of amino acid methionine (M) by leucine (L) at position 41 of the reverse transcriptase enzyme. Other mutation nomenclature refers to substitutions as indicated; amino acid abbreviations transcriptase enzyme. Other mutation nomenclature refers to substitutions as indicated; amino acid abbreviations are as follows: K, lysine;R, arginine; T, threonine, D, aspartic acid; N, asparagine; E, glutamic acid; H, histidine; Y, tyrosine; V, valine; I, isoleucine; A, alanine

Other commonly used names for the indicated anti-retroviral drugs:

Zidovudine (AZT, retrovir); stavudine (d4T, zerit); zalcitabine (ddC, hivid); lamivudine (3Tc, epivir); and abacavir (ABC, 1592, ziagen); delavirdine (rescriptor); efavirenz (sustiva); nevirapine (viramune); saquinavir (invirase, fortovase); ritonavir (norvir); amprenavir (agenarase); nelfinavir (viracept); indinavir (crixivan).

Protease	
Major mutation	**Antiretroviral drug**
D30N	Nelfinavir
M46I/L	Indinivir
G48V	Saquinavir
I50L	Atazanavir
I50V	Amprenavir
V82A/F/T	Indinavir
V82A/F/S/T	Ritonavir
I84V	Amprenavir, Indinavir, Ritonavir
L90M	Saquinavir, Nelfinavir

Appendix 7: Data Sources

B.C. Centre for Disease Control
655 West 12th Avenue
Vancouver, British Columbia
V5Z 4R4

Alberta Health and Wellness
TELUS Plaza North Tower
PO Box 1360, STN Main
Edmonton, Alberta
T5J 2N3

Provincial Laboratory of Public Health (Microbiology)
8440-112 Street
Edmonton, Alberta
T6G 2J2
and
3030 Hospital Drive NW
Calgary, Alberta
T2N 4W4

Saskatchewan Health
3475 Albert St.
Regina, Saskatchewan
S4S 6X6

Public Health Services
Department of Health Canada
1690 Hollis Street
PO Box 488

Joseph Howe Building
Halifax, Nova Scotia
B3J 2R8

Communicable Disease Control Unit
Public Health Branch
Manitoba Health
4th Floor - 300 Carlton Street
Winnipeg, Manitoba
R3B 3M9

Cadham Laboratories
P.O. Box 8450
750 William Avenue
Winnipeg, Manitoba
R3C 3Y1

HIV Laboratory
Laboratory Services Branch
Ontario Ministry of Health
81 Resources Rd.
Etobicoke, Ontario
M9P 3T1

Newfoundland Department of Health
Disease Control and Epidemiology
West Block, Confederation Bldg
P.O. Box 8700
St. John's, Newfoundland and Labrador
A1B 4J6

Newfoundland Public Health Laboratory
Leonard A. Miller Centre for Health Services
100 Forest Road, P.O. Box 8800
St. John's, Newfoundland and Labrador
A1B 3T2