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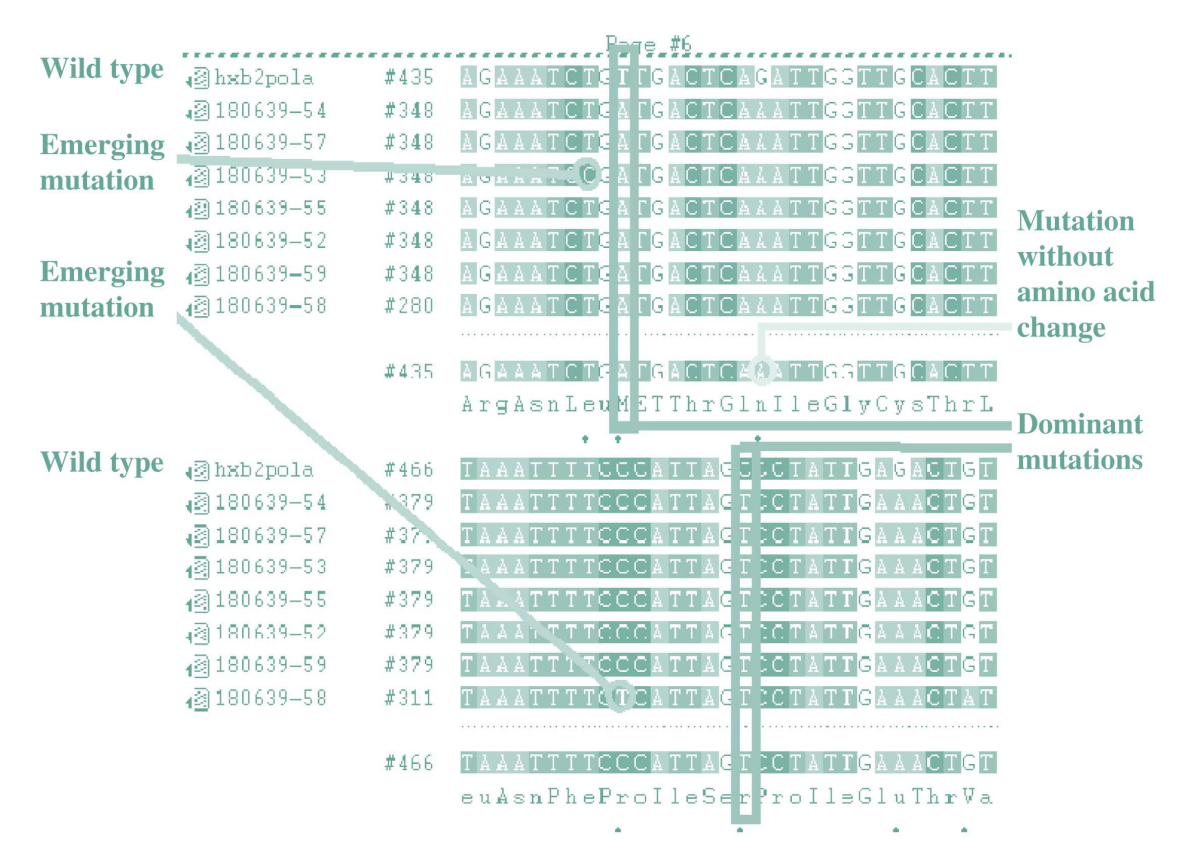
HIV-1 Strain and Primary Drug Resistance in Canada

Surveillance Report to June 30, 2001



December 2001

Canada

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Division of HIV/AIDS Epidemiology and Surveillance
National HIV and Retrovirology Laboratories
Centre for Infectious Disease Prevention and Control
Population and Public Health Branch
Health Canada

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

The Division of HIV/AIDS Epidemiology and Surveillance and the National HIV Laboratories at the Centre for Infectious Disease Prevention and Control, Health Canada, are pleased to provide you with *HIV-1 Strain and Primary Drug Resistance in Canada: Surveillance Report to June 30, 2001.* Primary drug resistance is a term used to identify resistance observed in HIV-infected individuals who have never before received treatment and so presumably have been infected with a drug-resistant strain of HIV.

This report presents data that are shared by provinces participating in the Canadian HIV Strain and Drug Resistance Surveillance Program. The Division of HIV/AIDS Epidemiology and Surveillance is responsible for data management, data analysis, writing and coordination of the publication of this report. The Division of Retrovirus Surveillance is responsible for coordinating the collection of the HIV data. The National Laboratory for HIV Genetics is responsible for conducting the strain and primary drug resistance genotyping.

The major finding in this surveillance report is that primary drug resistance to anti-retroviral drugs (nucleoside reverse transcriptase inhibitors, non-nucleoside reverse transcriptase inhibitors, protease inhibitors) has been identified in 5.9% of our sample population of 481 treatment naïve individuals. Resistance to more than one class of anti-retroviral drug has been identified in 0.2% of the sample population. With respect to HIV-1 subtypes, subtype B continues to predominate in Canada with 91.6% of the samples subtyped (n=919) belonging to this group, but HIV-1 subtypes A, C, D, E (also called recombinant A/E), recombinant A/C, and recombinant A/G have been identified across Canada. There is geographic variation in the prevalence of non-B HIV-1 subtypes, and this variation is likely related to travel and migration from countries where other subtypes predominate.

From a public health perspective, primary drug resistance may not yet be a significant problem in Canada, but it will no doubt play a significant role in shaping the course of the HIV epidemic in Canada and worldwide. For this reason, the data from the Canadian HIV Strain and Drug Resistance Surveillance Program will be valuable in guiding Canada's response to the public health problems posed by drug-resistant HIV, including our efforts to develop treatment strategies and effective prevention programs. The introduction of variant HIV-1 subtypes into Canada also requires vigilant monitoring in order to ensure that diagnostic and screening algorithms can adequately detect all circulating strains and to inform vaccine research and development.

This is the first report of results from the Canadian HIV Strain and Drug Resistance Surveillance Program. We will be working towards improving this report to reflect changes in the surveillance of HIV strain and drug resistance. We welcome and appreciate your comments and suggestions.

Yours sincerely,

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Results at a Glance

This report is organized into a summary page followed by four further sections. The first section provides an overview of Health Canada's Canadian HIV Strain and Drug Resistance Surveillance Program (CHSDRSP). The second section describes primary drug resistance in Canada as determined by the CHSDRSP and summarizes results from other key studies conducted in Canada, the United States, and Western Europe. The third section describes HIV-1 subtypes in Canada as determined by the CHSDRSP. The fourth section describes HIV subtype results from the sentinel surveillance arm of the CHSDRSP, which helps the provincial health laboratories with the testing and identification of samples from individuals with unusual clinical manifestations and/or with unusual laboratory results.

Summary of the Main Findings

- The overall prevalence of primary drug resistance to at least one anti-retroviral drug is 5.9% in the sample population of 481 newly diagnosed individuals who had never received treatment.
- Multi-drug resistance to the nucleoside reverse transcriptase inhibitors 3TC, abacavir, and adefovir and to the protease inhibitor nelfinavir has been identified in one newly diagnosed, treatment naïve individual (0.2%).
- In Canada, HIV-1 subtype B continues to predominate with 91.6% of the samples subtyped (n=919) belonging to this group; subtypes A, C, D, E (also known as the recombinant A/E), recombinant A/B, and recombinant A/G have also been identified across Canada.
- There is geographic variation with respect to the prevalence of non-B HIV-1 subtypes. This variation is likely related to travel and migration from countries where other subtypes predominate.

Public Health Implications

- The prevalence of primary drug resistance can be used to develop population recommendations for initial therapies (especially for pregnant women and for use in post-exposure prophylaxis).
- The extent to which drug-resistant strains of HIV are being transmitted can serve as an indicator to evaluate the effectiveness of prevention programs.
- HIV isolates from different populations and changes over time can be monitored to evaluate diagnostic and screening algorithms to ensure that all circulating strains are adequately detected.
- HIV strain data can be used for research into and development of vaccines.

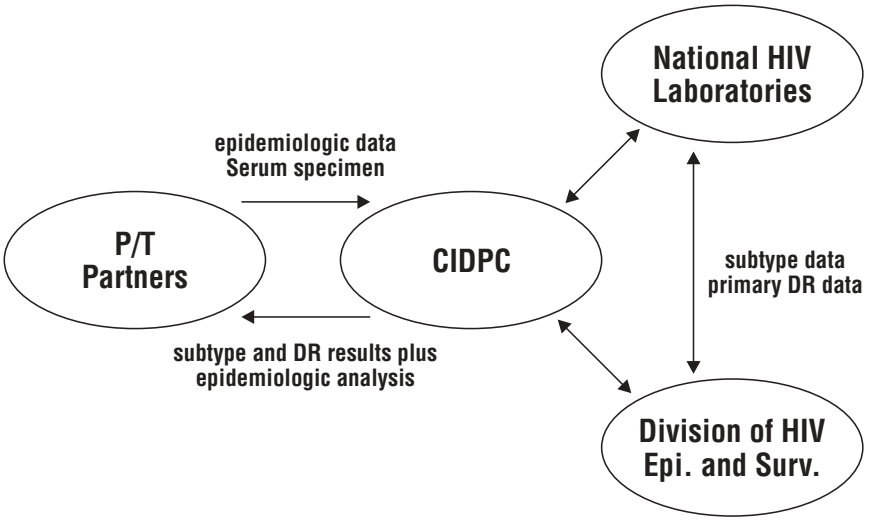
An Overview of the Canadian HIV Strain and Drug Resistance Surveillance Program

The CHSDRSP is a collaborative effort between the provinces and territories and the Centre for Infectious Disease Prevention and Control, Population and Public Health Branch, Health Canada. It forms a key component in a national system for the enhanced and integrated surveillance of HIV/AIDS, STD, emerging retroviruses, and other sexually transmitted bloodborne pathogens. The program was initiated to characterize and monitor the genetic diversity of the HIV epidemic in Canada, addressing the concerns of public health authorities, primary care physicians, and researchers.

The program consists of two key components: (1) serum specimen from individuals newly diagnosed with HIV for subtype and primary drug resistance testing, and, (2) non-nominal epidemiologic data, which include information collected through the national or provincial HIV reporting form and additional data to interpret the laboratory results. Data collection, analysis and transfer are shown in Figure 1.

The provincial and territorial (P/T) partners in the CHSDRSP send serum specimen taken for diagnostic testing from treatment naïve individuals and corresponding epidemiologic data to the Centre for Infectious Disease Prevention and Control (CIDPC). Note that only samples from individuals newly diagnosed with HIV in Canada are included under the CHSDRSP. Subtype analysis and primary drug resistance genotyping are conducted in the National Laboratory for HIV Genetics at the National HIV and Retrovirology Laboratories. Samples with unusual laboratory results are sent through the sentinel arm of the CHSDRSP and are analyzed for subtype by the National Laboratory for HIV Genetics. This information is linked using unique specimen identifiers to the epidemiologic data, and the Division of HIV Epidemiology and Surveillance conducts further analyses at the national level. Laboratory results are sent to the P/T partners for local analysis.

Figure 1. Data collection, transfer and analysis



As of June 30, 2001, British Columbia, Alberta, Manitoba, Saskatchewan, Ontario, and Newfoundland are participating in CHSDRSP. The results presented in this report represent samples on which HIV subtype analysis and primary drug resistance genotyping were completed successfully as of June 30, 2001. Samples and epidemiologic data continue to flow to Health Canada from participating provinces, and results from these analyses will be presented in future reports. Discussions are currently under way to expand the collection of samples and epidemiologic data to the remaining provinces and territories.

Goals of CHSDRSP

A 1998 consensus workshop in Vancouver established the following goals for CHSDRSP:

1) To enhance the safety of the blood supply

In order to ensure the safety of the blood supply, all HIV tests need to reliably detect the strains 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 modification of some serologic screening tests by the addition of new antigens to ensure detection. The sentinel arm of CHSDRSP, through the reference services of the National HIV Laboratories, addresses this goal by testing samples with unusual serologic, PCR (polymerase chain reaction), or

other virologic test results that are provided by the provincial health laboratories. This relationship between provincial and national laboratories also serves other external programs, including quality assurance and the monitoring of diagnostic kits.

2) To inform vaccine development

It is important to know the distribution of the viral subtypes and intrasubtype variations to target vaccine development and testing, since the efficacy and effectiveness of vaccines may be subtype-specific.

3) To assess genetic markers of HIV drug resistance

Although anti-retroviral 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 drug-resistant virus. In fact, studies have shown that transmission of drug-resistant virus is increasing. The CHSDRSP aims to assess the prevalence of primary drug resistance and the variation of this prevalence over time, geographic area, and population risk group. The resulting information can be used to develop treatment guidelines at the population level for initial therapeutic regimens and to develop more effective HIV prevention strategies, including the prevention of mother-to-child transmission.

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

Although genetic analyses have been used to assess the spread of HIV globally, there is little consensus on whether differences in HIV subtype and mutations conferring drug resistance affect the rate of transmission, pathogenesis or HIV-related disease progression. Knowing the distribution of HIV variants in Canada, along with corresponding epidemiologic information, will help to address these questions. The public health implications of such findings, including prevention and treatment strategies, are of special interest.

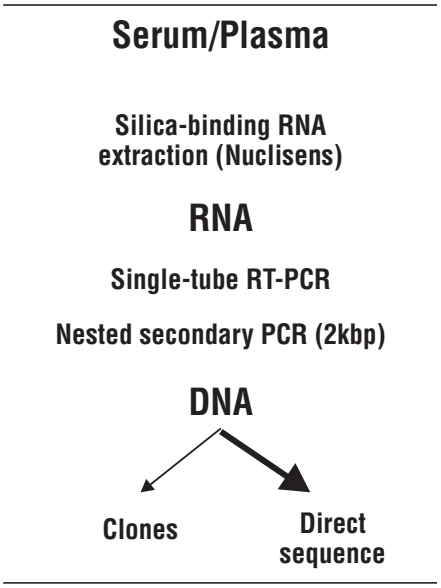
Methodology

The provincial laboratories send archived serum specimen collected from newly diagnosed, treatment naïve people to the Centre for Infectious Disease Prevention and Control at Health Canada for HIV subtype analysis and primary drug resistance genotyping. The genetic testing algorithm used during PCR amplification is shown in Figure 2. Primary mutations identified in the protease and reverse transcriptase genes of HIV are defined by the consensus of listings reported by Stanford University (http://hivdb.stanford.edu/hiv/) and the Los Alamos HIV Sequence Database (http://resdb.lanl.gov/Resist_DB/), and the results are sent back to the provinces. Although the majority of these mutations are consistent with the list of mutations identified by the International AIDS Society-USA[1], some differences exist. Appendix 2 provides the list of primary mutations used for this report.

Non-nominal epidemiologic information is also collected and sent to the Centre for Infectious Disease Prevention and Control at Health Canada. The data include information routinely collected on the national or provincial HIV case reporting forms and, where available, additional information that will help interpret the laboratory results, including treatment history, viral load at diagnosis, and previous HIV negative testing history. Laboratory and epidemiologic data are linked using unique specimen identifiers, and further analysis is conducted using the statistical package SPSS[c] (SPSS Inc. Chicago).

The algorithm used by the National Laboratory for HIV Genetics to identify subtypes and primary mutations associated with drug resistance is shown in Figure 2. After extraction of the RNA and an initial one-step RT-PCR reaction, nested PCR amplification of the *pol* gene (protease/reverse transcriptase) is performed 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 are used to assess subtype and primary mutations associated with drug resistance. Previously, the C2-V5 region (233 amino acids) of the envelope protein was used to assess HIV subtype. When PCR products sequence poorly, they are resolved by cloning the PCR product, sequencing about 10-12 clones/person.

Figure 2. Genetic testing algorithm used by the National Laboratory



[1] Resistance Mutations Project Panel. Update on drug resistance mutations in HIV-1. Topics in HIV Medicine 2001; 9(6):21-23.

HIV-1 Primary Drug Resistance in Canada

Background

HIV can develop resistance to various antiretroviral drugs as a result of mutations in the genes coding for protease and reverse transcriptase (RT), two viral proteins that are required for HIV to replicate. Current antiretroviral drugs impede viral replication by binding to these proteins and inhibiting their ability to function. However, largely because of its rapid and relatively inaccurate replication, HIV is able to mutate and diminish the ability of the drugs to interact with these viral proteins.

For some drugs (e.g. non-nucleoside reverse transcriptase inhibitors), a single mutation is sufficient to confer drug resistance. Such a mutation is called a "primary" mutation. For other drugs (e.g. protease inhibitors), a combination of mutations is required to confer resistance. Such mutations are known as "secondary" mutations. Most mutations are lethal or neutral, and the wild type strain usually dominates because it replicates more efficiently.

Laboratory Tests to Detect Drug Resistance

Two types of tests are used to detect drug resistance, genotypic and phenotypic. Genotypic tests provide information about the genetic makeup of the virus by identifying the mutations that are strongly associated with resistance. Phenotypic testing measures the ability of a virus to replicate in the presence of varying drug concentrations. Although the methodologies for both tests are well established, each has its limitations, as described in the section Data Limitations.

Primary and Secondary Drug Resistance

In general, two types of drug resistance have been described. "Primary drug resistance" is used to describe reduced susceptibility to drugs seen among patients who have never received treatment for HIV. So presumably these patients have been infected with drug-resistant HIV. "Secondary drug resistance" is the term used to describe drug resistance among patients already receiving treatment, and this phenomenon has been widely described in the context of treatment failures. (Note, these terms should not be confused with "primary" and "secondary" mutations.)

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

Data Sources

This section highlights the main findings from the CHSDRSP up to June 30, 2001. It is important to note that the results presented here represent individuals who sought testing, were properly diagnosed, and were reported as HIV positive. Furthermore, the findings include only those on individuals for whom sufficient serum specimen, taken for the purposes of diagnostic testing, was available to send to the National HIV Laboratories and, of these, the subset for whom RT-PCR amplification and sequencing to identify primary mutations were carried out successfully.

As of June 30, 2001, B.C., Alberta, Manitoba, and Saskatchewan have submitted 1,354 serum specimen from individuals newly diagnosed between 1997 and 2000 and corresponding non-nominal epidemiologic data for primary mutational analysis. These data are collected through convenience sampling methods and may not be representative. However, it is anticipated that serum specimen and corresponding enhanced epidemiologic data on ALL people newly diagnosed with HIV will be collected prospectively from the provinces currently participating in CHSDRSP and analyzed for both subtype and primary mutations. Discussions are also under way to expand CHSDRSP to the remaining provinces and territories.

At the time of writing this report (December 31, 2001) a total of 563 samples have been analyzed for primary mutations by the National Laboratory for HIV Genetics. Viral RNA had been successfully amplified from 85.4% of the serum specimen. This high level of success in amplifying virus from serum specimen specimens will likely improve further as sample quality is enhanced and various primer combinations for RT-PCR amplification are identified and used.

Primary mutations identified in the protease and RT enzymes of HIV are defined by the

consensus of listings reported by Stanford University (http://hivdb.stanford.edu/hiv/) and the Los Alamos HIV Sequence Database (http://resdb.lanl.gov/Resist_DB/). Please refer to Appendix 2 for a list of primary mutations used in this report.

The distribution of primary mutations based on sequence analysis of protease and the first 253 amino acids of RT are shown in Table 1. The results indicate that primary mutations were present in at least 5.9% of the sample population of 481 newly diagnosed, treatment naïve individuals. Note that none of these individuals had previously received treatment, and so had presumably been infected with a drug-resistant strain of HIV-1. The prevalence of primary mutations to reverse transcriptase inhibitors (RTIs) and protease inhibitors (PIs) was 4.2% and 1.5% respectively. One individual in the sample population (0.2%) was infected with virus harbouring mutations to both RTIs and PIs. Of note is that multi-drug resistant HIV was also identified in a sample from Ontario that was sent to the National Laboratory for HIV Reference Services, but since this individual had been receiving anti-retroviral treatment previously primary drug resistance could not be ascertained.

Table 1. Prevalence of primary drug resistance among newly diagnosed, treatment naïve individuals

Primary mutations	Frequency	Percentage
WT/minor mutations[1]	453	94.1
RT[2]	20	4.2
Protease	7	1.5
Protease/RT	1	0.2
Total	**481**	**100.0**

[1] WT refers to wild type virus. This variable indicates that no primary mutations were identified through sequencing the protease and RT genes. Minor mutations refers to genetic variabilities not associated with drug resistance.

[2] RT refers to reverse transcriptase enzyme

Of the individuals harbouring HIV-1 resistant to RTIs, the majority (19, or 4%) were infected with virus containing mutations associated with resistance to the nucleoside RTIs (NRTIs) AZT, ddC, 3TC, abacavir and adefovir. The remaining individual (0.2%) was infected with HIV containing mutations associated with resistance to the non-nucleoside RTI (NNRTI) foscarnet. The most common mutation that was identified in RT was the replacement of the amino acid methionine (M) with leucine (L) at position 41. This mutation, associated with a high level of resistance to AZT, was identified in 11 individuals (2.3%). Primary mutations associated with resistance to the PIs ritonavir, amprenavir, and indinavir were identified in seven individuals (1.5%). The most common primary mutation identified in protease was the replacement of methionine with isoleucine (I) at position 46, associated with a high level of resistance to ritonavir. Four individuals (0.8%) in the sample population of 481 were infected with HIV containing this mutation. Of note is that one individual (0.2%) harbouring mutations associated with multi-drug resistance was identified. The replacement of methionine (M) with valine (V) at position 184 of the RT enzyme (associated with resistance to the NRTIs 3TC, abacavir, and adefovir) and of leucine (L).with methionine (M) at position 90 of the protease enzyme (associated with resistance to nelfinavir) were identified. Other primary mutations and the anti-retroviral drug to which they confer resistance are shown in Table 2.

Table 3 shows the epidemiologic characteristics of individuals harbouring primary mutations associated with a high level of resistance to RTIs and to PIs. Univariate analysis to test for significant associations with primary drug resistance were not conducted because of

Table 2. Primary mutations in reverse transcriptase and in protease

Anti-retroviral drug	Number of individuals (%) n = 481	Primary mutation(s)[1]
RTI[2], Total	**20 (4.2)**	
AZT	11 (2.3)	M41L[3]
AZT	1 (0.2)	K70R
ddC	2 (0.4)	T69D/N
Adefovir	1 (0.2)	K70E
AZT/ddC	1 (0.2)	K70R/T69N
3TC/abacavir/adefovir	2 (0.4)	M184V
AZT/3TC/abacavir/adefovir	1 (0.2)	T215Y/M184V
Foscarnet[4]	1 (0.2)	H208Y
PI[5], Total	**7 (1.5)**	
Ritonavir	4 (0.8)	M46I
Amprenavir	1 (0.2)	I50V
Nelfinavir	1 (0.2)	N88D
Indinavir/ritonavir	1 (0.2)	V82A
RTI/PI, Total	**1 (0.2)**	
3TC/abacavir/adefovir/nelfinavir	1 (0.2)	M184V/L90M

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

[2] RTI refers to reverse transcriptase inhibitor. Other commonly used names for the nucleoside RTIs mentioned include AZT (zidovudine, retrovir); ddC (zalcitabine, hivid); 3TC (lamivudine, epivir); and abacavir (1592, ziagen).

[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 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] Foscarnet (PFA, foscavir) is a non-nucleoside analogue that is used in Canada to treat cytomegalovirus retinitis.

[5] PI refers to protease inhibitor. Other commonly used names for the PIs mentioned include ritonavir (norvir); amprenavir (agenerase); nelfinavir (viracept).

Table 3. Epidemiologic characteristics of individuals infected with HIV-1 harbouring primary mutations (n = 481)

	Sample size	Primary mutation in specified drug class (%)		
		RT	Protease	RT/Protease
Sex[1]				
Male	355	17 (4.8)	4 (1.1)	—
Female	112	3 (2.7)	2 (1.8)	1 (0.9)
Age[2]				
< 15	2	—	—	—
15-19	7	—	1 (14.2)	—
20-29	98	4 (4.1)	2 (2.0)	1 (1.0)
30-39	187	12 (6.4)	1 (0.5)	—
40-49	111	—	1 (0.9)	—
50-59	37	1 (2.7)	—	—
60-69	19	2 (10.5)	1 (5.3)	—
Ethnicity[3]				
White	283	11 (3.9)	3 (1.1)	1 (0.4)
Aboriginal[4]	68	1 (1.5)	2 (2.9)	—
Black	25	1 (4.0)	—	—
Asian[5]	19	1 (5.3)	—	—
Latin American[6]	8	—	—	—
Year of first diagnosis[7]				
1997	20	—	—	—
1998	50	4 (8.0)	—	—
1999	270	14 (5.2)	6 (2.2)	1 (0.4)
2000	131	2 (1.5)	—	—
Risk factors[8]				
MSM	132	8 (6.0)	—	—
MSM/IDU	10	—	—	—
IDU	145	1 (0.7)	3 (2.1)	—
Recipient of blood[9]	3	—	—	—
Heterosexual contact[10]	119	8 (6.7)	3 (2.5)	—
Province				
Manitoba	55	7 (12.7)	5 (9.1)	—
Saskatchewan	14	—	—	—
Alberta	94	4 (4.2)	1 (1.1)	—
BC	318	9 (2.8)	1 (0.3)	1 (0.3)
HIV-1 subtype				
A	7	—	1 (14.2)	—
B	416	19 (4.6)	5 (1.2)	1 (0.2)
C	22	1 (4.5)	—	—
D	3	—	—	—
E	3	—	—	—
PCR amplification[11]	5	—	1 (20.0)	—

[1] There were 14 individuals for whom sex was unknown, of whom 1 was infected with HIV-1 harbouring a primary mutation in protease.

[2] Age reflects age at first diagnosis and is calculated by subtracting year of birth from year at first diagnosis with HIV. There were 15 individuals for whom age was not known, of whom 1 was infected with HIV-1 harbouring a primary mutation in RT and 1 a primary mutation in protease.

[3] There were 78 individuals for whom ethnicity was not known, of whom 6 and 2 were infected with HIV-1 harbouring a primary mutation to RT and protease respectively.

[4] Includes people belonging to First Nations and Metis groups. These groups could not be differentiated because of insufficient data,

[5] Includes people of Middle-Eastern origin

[6] Includes people from Central and South America and from the Caribbean

[7] There are 10 individuals for whom year of first diagnosis was not known, of whom 1 was infected with HIV-1 harbouring a primary mutation to protease.

[8] Includes identified, mutually exclusive risk factors among adults (> 15 years). There were 72 individuals with unknown risk exposure, of whom 3 and 1 were infected with HIV-1 harbouring a primary mutation to RT and protease respectively. The risk exposure of one case infected with virus harbouring primary mutations to both RT and protease was not known.

[9] Receipt of blood may not be the primary risk factor for these individuals. This requires further investigation.

[10] Heterosexual contact could not be further subdivided into endemic exposures because of insufficient data.

[11] Envelope gene for subtype analysis could not be amplified using PCR despite ≥ two attempts.

small sample sizes in the cells. However the prevalence of primary drug resistance may be increasing over time.

The following additional observations can be cautiously made. Primary drug resistance has been identified

- among adults of both sexes aged 16-69 years at first diagnosis with HIV;
- among individuals identifying male-to-male sex (MSM), injection drug use (IDU) and/or heterosexual contact as the primary risk factors;
- across most ethnic backgrounds, including people of Caucasian, Aboriginal, African and Asian origin;
- in people with HIV-1 subtypes A, B, or C.

Results from the CHSDRSP and other cohort and cross-sectional studies suggest that, in Canada, the overall prevalence of primary drug resistance to at least RTIs is between 4.2% and 20% (Table 4). Primary drug resistance to PIs is between 1.5% and 6.5%. Primary drug resistance to more than one class of anti-retroviral drug (multi-drug resistance) has been observed in Canada, and preliminary studies suggest an overall prevalence of between 0.2% and 6%.

The studies also suggest that, in total, drug resistance (both primary and secondary drug resistance) is between 5.9% and 26% in Canada.

Table 4. Summary of key studies on HIV-1 primary drug resistance in Canada

Province	Year of first diagnosis	Risk factors	Sample size	RTIs[1,2] %	PIs[2,3] %	MDR[2,4] %	Total[5]
BC[6]	1997-1998	Mixed	423	4.6 (n = 416)	4.6	4.6	—
QC[7,8]	1997-1999	IDU (26%) Sexual (69%)	81	20	6.0	6.0	—
	1999-2000	—	61	6.7 (n = 59)	6.5	4.9	26.0
ON[9]	1997-1999	MSM	23	13	—	—	—
BC, AB, SK, MB	1997-2000	Mixed	481	4 (NRTI) 0.2 (NNRTI)	1.5	0.2	5.9

[1] RTIs refers to reverse transcriptase inhibitors. Differentiation into non-nucleoside RTI (NNRTI) and nucleoside RTI (NRTI) is only given where this information is available.

[2] Only primary mutations are identified.

[3] PIs refers to protease inhibitors.

[4] MDR refers to multi-drug resistance.

[5] Includes both primary and secondary mutations.

[6] Alexander CS et al. 8th Annual Canadian Conference on HIV/AIDS Research, Vancouver, May 1999: #B224.

[7] Saloman H et al. AIDS 2000; 142 (2):F17-23.

[8] Simon V et al. 8th Conference on Retroviruses and Opportunistic Infections. Chicago, Feb. 2001: #423.

[9] Cassol S et al. 9th Annual Canadian Conference on HIV/AIDS Research, Montreal, April 2000: #135P.

Note: Table 4 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 versus general population), the types of laboratory tests used (genotypic and/or phenotypic testing) and differences in the mutations studied and reported.

Studies from the United States and other countries in Western Europe also give similar results (Table 5). A column summarizing the total number of primary mutations could not be constructed because this information was not available for most of the studies cited.

Note: Interpretation and inter-study comparisons are difficult because of differences in study design as noted previously.

Table 5. Summary of key studies on HIV-1 primary drug resistance in the United States and in Western Europe

Country	Year of first diagnosis	Risk factors	Sample size	RTIs[1,2] %	PIs[2,3] %	MDR[2,4] %
United States[5-9]	'89-'98	MSM (80%)	141	0.7 (NNRTI)	1.4	1.4
	'95-'99	MSM (94%)	80	12.5 (NRTI) 7.5 (NNRTI)	3.0	3.8
	'97-'98		114	4.0 (NRTI) 15 (NNRTI n = 95)	10.0	5.0
	'97-'00	Mixed	603		1.2	1.2
(includes Montreal and Vancouver)	'95-'98	Mixed	389	2.5 (NRTI) 2.0 (NNRTI)	0.4	1.0
	'99-'00			7.0 (NRTI) 7.0 (NNRTI)	8.0	6.0
France[10,11]	'95-'98	Mixed	48	16.6	2.0	
Italy[12]	'99	MSM/Bisexual				
Spain[13,14]	'96-'98	Mixed	68	16.2	6.0	4.4
	'98	Mixed	126	17 (NRTI n = 52)	6.0	
Switzerland[15]	'97-'99	Mixed	52	13.5 (NRTI)		
	'96	Mixed	36	5.6	3.0	
	'97	Mixed	40	10.0	9.0	
	'98	Mixed	62	7.1	2.0	
UK[16]	'99	Mixed	59	3.4	2.0	
	'94-'96	Mixed	21	0	0	
	'97-'99	Mixed	22	13.6	0	0
	'00	Mixed	26	19.2	3.8	0

[1] RTIs refers to reverse transcriptase inhibitors. Differentiation into non-nucleoside RTI (NNRTI) and nucleoside RTI (NRTI) is only given where this information is available.

[2] Only primary mutations are identified.

[3] PIs refers to protease inhibitors.

[4] MDR refers to multi-drug resistance.

[5] Little SJ et al. JAMA 1999; 282:1142-49.

[6] Boden D et al. JAMA 1999; 282:1135-41.

[7] Wegner S et al. AIDS 2000; 14: 1009-15.

[8] Zaidi I et al. 5th workshop on HIV drug resistance and treatment strategies. Scottsdale, AZ. June 2001; Antiviral Ther. 6(suppl 1):118., #155.

[9] Little S et al. 5th workshop on HIV drug resistance and treatment strategies. Scottsdale, AZ. June 2001; Antiviral Ther. 6(suppl 1):118., #25.

[10] Tamalet C et al. J Med Virol 2000; 61: 181-6.

[11] Chaix ML et al. 8th Conference on Retroviruses and Opportunistic Infections Chicago, Feb 2001: #755.

[12] Balotta C et al. 4th Workshop on HIV drug resistance and treatment strategies. Sitges, Spain June 2000: 5-S3:144.

[13] Puig T et al. AIDS 2000; 14: 727-32.

[14] Perez-Olmeda M et al. J Med Virol 2001; 63:(2):85-7.

[15] Yerly S et al. 8th Conference on Retroviruses and Opportunistic Infections. Chicago, Feb 2001: #754..

[16] UK Collaborative Group on Monitoring the Transmission of HIV Drug Resistance. BMJ 2001; 322:1087-8.

HIV-1 Subtypes in Canada

Data Sources

This section highlights the main findings related to HIV-1 subtype from the CHSDRSP up to June 30, 2001. It is important to note that the results presented here represent individuals who sought testing, were properly diagnosed, and were reported as being HIV positive. Furthermore, they represent only those individuals for whom sufficient serum specimen, taken for the purposes of diagnostic testing was available to send to the National HIV Laboratories and, of these, the subset for whom RT-PCR amplification and sequencing to obtain subtype results were successful.

As of June 30, 2001, British Columbia, Alberta, Manitoba, Saskatchewan and Newfoundland have submitted 1,604 serum specimen from individuals newly diagnosed between 1996 and 2000 and corresponding non-nominal epidemiologic data for subtype analysis. These data are collected through convenience sampling methods and may not be representative. However, it is anticipated that serum specimen and corresponding enhanced epidemiologic data of ALL persons newly diagnosed with HIV will be collected prospectively from provinces currently participating in CHSDRSP and analyzed for both subtype and primary mutations associated with drug resistance. Discussions are also under way to expand the CHSDRSP to the remaining provinces and territories. Additional samples have been received through reference services (the sentinel arm of the CHSDRSP), and the results from the analysis of these samples are described in the next section.

At the time of writing this report (December 31, 2001), a total of 1,048 samples have been analyzed for subtype by the National Laboratory for HIV Genetics. Viral RNA has been successfully amplified from 87.7% of the serum specimen. This high level of success in amplifying virus from serum specimen will likely improve further as sample quality is enhanced and various primer combinations for RT-PCR amplification are identified and used.

The distribution of HIV-1 subtypes based on the sequence analysis of the HIV envelope gene is shown in Table 6. Although the majority (91.6%) of samples are of subtype B, other subtypes have been identified. In decreasing order of prevalence they include subtype C (4.8%), A (2.5%), E (0.5%), D (0.4%), and the recombinant A/B (0.1%). Of note is that the recombinant subtype A/G, from three individuals, has been identified in Ontario from samples sent through reference services and subtyped by the National Laboratory for HIV Genetics (Table 9).

Table 6. Distribution of HIV-1 subtypes in Canada

Subtype	Frequency	Percentage
A	23	2.5
A/B	1	0.1
B	842	91.6
C	44	4.8
D	4	0.4
E[1]	5	0.5
Total	**919**	**100.0**

[1] HIV-1 subtype E has also been referred to as the recombinant subtype A/E

Preliminary evidence shown in Table 7 indicates that there is geographic variation in the distribution of non-B HIV-1 subtypes. Whereas all 40 samples from Newfoundland were identified as subtype B, 13.2%, 7.9%, 8.6% and 8% of the analyzed samples from Manitoba, Saskatchewan, Alberta and BC respectively belonged to non-B HIV-1 subtypes. B.C. had the greatest genetic variation among the non-B HIV-1 subtypes. It should be noted however, that sample sizes are not representative of the total diagnosed population in each of the indicated provinces. Furthermore, the provinces of Quebec and Ontario, which report the highest prevalence of HIV infections, are not represented.

Results from other Canadian epidemiologic studies have also identified non-B subtypes across Canada:

- As of November 2000, all samples of 31 recent seroconverters from the POLARIS cohort in Ontario are of subtype B (Major C for POLARIS Study Group. BHST Annual Meeting, Halifax, NS, Nov 16-18, 2000)
- The B.C. 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, Jan 31-Feb 2, 2000, Abst# 174)
- All HIV-1 sequences analyzed among injection drug users (n = 17) and men who have sex with men (n = 5) residing in Montreal were of subtype B (Bernier L et al. CJID 1999; 10 (suppl B): 104).

Table 7. Prevalence of HIV-1 subtypes by provinces participating in the Canadian HIV Strain and Drug Resistance Program

	Province (%)				
HIV-1 Subtype	**Newfoundland**	**Manitoba**	**Saskatchewan**	**Alberta**	**BC**
A	0	8 (6.6)	5 (3.9)	0	10 (1.9)
A/B	0	0	0	0	1 (0.2)
B	40 (100)	105 (86.8)	117 (92.1)	106 (91.3)	474 (92)
C	0	8 (6.6)	5 (3.9)	8 (6.9)	23 (4.5)
D	0	0	0	0	4 (0.8)
E[1]	0	0	0	2 (1.7)	3 (0.6)
Total	**40 (100)**	**121 (100)**	**127 (100)**	**116 (100)**	**515 (100)**

[1] HIV-1 subtype E has also been referred to as the recombinant subtype A/E

Gender, ethnicity, year of first diagnosis with HIV, and risk factors were significantly associated with HIV-1 non-B subtype infection (Table 8). Specifically, significantly higher proportions of non-B infections were observed among (1) females (13.6% versus 6.3% of males), (2) individuals of African or Asian origin (62.5% and 19% respectively versus 0%-6.8% for other reported ethnic groups), (3) individuals newly diagnosed in 1996 (23.1% versus 4.9%-9.1% for other years), and (4) individuals who reported heterosexual contact as the primary exposure factor (18.4% versus 3.4%-6.1% for the other risk groups). The probability of infection with a non-B HIV-1 subtype was 2.3-fold higher among females than among males; 17.2-fold and 2.6-fold higher among people of African and Asian origin respectively as compared with all other reported ethnic groups; 5.7-fold higher if the individual was newly diagnosed in 1996 as compared with all other years of diagnoses; and 5.7-fold higher if heterosexual contact was the primary reason for exposure as compared with all other risk factors. Of note is that out of the five pediatric cases, three were infected with non-B subtypes. All three individuals were of African origin.

Table 8. Epidemiologic characteristics of individuals infected with HIV-1 subtype B versus non-B subtypes

	Sample size	HIV-1 non-B subtype	Univariate analysis	
			OR (95% CI)[1]	p-value
Sex[2]				**0.001**
Male	674	42 (6.3)	—	
Female	227	31 (13.6)	2.3 (1.4-3.7)	
Age[3]				**0.005**
< 15	5	3 (60.0)	16.5 (2.7-100.2)	
15-19	16	0	—	
20-29	204	21 (10.2)	—	
30-39	347	28 (8.1)	—	
40-49	189	16 (8.4)	—	
50-59	65	4 (6.1)	—	
60-69	22	1 (4.5)	—	
=0 70	8	1 (12.5)	—	
Ethnic background[4]				**0.0001**
White	504	22 (4.4)	0.47 (0.24-0.9)	
Aboriginal[5]	146	10 (6.8)	—	
Black	40	25 (62.5)	17.2 (7.2-38.4)	
Asian[6]	42	8 (19.0)	2.6 (1.2-6.1)	
Latin American[7]	16	0	—	
Year of first diagnosis[8]				**0.001**
= 1995	61	3 (4.9)	—	
1996[9]	65	15 (23.1)	5.7 (2.2-14.9)	
1997	103	8 (7.8)	—	
1998	149	10 (6.7)	—	
1999	342	31 (9.1)	—	
2000[10]	140	7 (5.0)	—	
Risk factors[11]				**0.0001**
MSM	258	10 (3.9)	0.18 (0.08-0.37)	
MSM/IDU	33	2 (6.1)	—	
IDU	262	9 (3.4)	0.16 (0.07-0.33)	
Heterosexual contact[12]	228	42 (18.4)	5.7 (3.3-9.9)	

[1] Odds ratio calculations are based on comparing variable of interest with all other variables in the particular group. Only significant OR are indicated.

[2] There were 18 individuals for whom sex was not known, of whom 3 (16.7%) were infected with an HIV-1 non-B subtype.

[3] Age reflects age at first diagnosis and is calculated by subtracting year of birth from year at first diagnosis with HIV. There were 63 individuals for whom age was not known, of whom 3 (4.8%) were infected with an HIV-1 non-B subtype.

[4] There were 171 individuals for whom ethnicity was not known, of whom 12 (7%) were infected with a non-B subtype.

[5] Includes people belonging to First Nations and Metis groups. These groups could not be differentiated because of insufficient data.

[6] Includes people of Middle-Eastern origin.

[7] Includes people from Central and South America and the Caribbean.

[8] There were 58 individuals for whom year of first diagnosis was not known, of whom 3 (5.2%) were infected with an HIV-1 non-B subtype.

[9] The data from 1996 are largely from B.C., and additional data are needed to interpret the findings from this year.

[10] Data for the year 2000 represent samples received from people diagnosed between January and March 2000 in Manitoba and Saskatchewan and between July and December 2000 in B.C.

[11] Includes identified, mutually exclusive risk factors in adults (> 15 years). Of the 5 pediatric cases, 3 were infected with a non-B subtype. There were 7 cases reporting receipt of blood (n = 5) or clotting factor (n = 2) as the only exposure categories; one was infected with a non-B subtype. There were two cases of non-medical exposure – one attributed to female-to-female sex (infected with non-B subtype) and one due to occupational exposure. There were 124 individuals with unknown risk exposure, of whom 9 (7.3%) were infected with an HIV-1 non-B subtype.

[12] Heterosexual contact could not further subdivided into endemic exposures because of insufficient data.

National HIV Reference Services

The provincial public health laboratories, the Canadian Blood Services, and HEMA-QUEBEC test thousands of samples each year and thus serve as crucial partners in the CHSDRSP. A number of factors can cause serologic tests to yield fallible results. These include samples from seroconverters, samples that are cross-reactive, for example to HIV-2 and divergent strains of HIV-1. Furthermore, genetic variants of HIV can be problematic for HIV PCR and viral load tests, often leading to discordant findings with serologic testing.

As a part of its reference services, the National HIV Laboratories test samples showing unusual serologic, PCR or other virologic test results. This function of the National HIV Laboratories is crucial in addressing one of the goals of the CHSDRSP – protection of the blood supply – since screening tests should be able to detect all circulating strains of HIV within Canada. This relationship between the provincial and national laboratories may also serve in quality assurance and diagnostic kit monitoring.

As of June 30, 2001, the National Laboratory for HIV Genetics has analyzed serologic samples from five provinces, submitted through the reference services, for subtype. The results of these analyses are shown in Table 9.

HIV-2

There is currently no active surveillance for HIV-2 in Canada. At the time of writing this report (December 31, 2001), a total of six samples submitted to the National Laboratory for HIV Reference Services had been identified as belonging to HIV type 2. This number is likely to be an underestimate: it is possible that HIV-2 cases have been reported as HIV-1 because the only approved HIV western blot kit is specific to HIV-1. Discussions are currently under way to enhance surveillance for HIV-2.

Table 9. HIV-1 subtype distribution of 26 samples submitted to the National Laboratory for HIV Reference Services

Province	HIV Subtype (# samples)	Year sample submitted
Newfoundland	HIV-1 subtype A (1)	1999
Nova Scotia	HIV-1 subtype C (1)	1998
Ontario[1]	HIV-1 subtype A (1)	1998
	HIV-1 subtype B (1)	1999
	HIV-1 subtype A(1)	1999
	HIV-1 subtype A (1)	2000
	HIV-1 subtype B (10)	2000
	HIV-1 subtype C (3)	2000
	HIV-1 recombinant subtype A/G (3)	2000
Manitoba	HIV-1 subtype C (1)	1998
	HIV-1 subtype C (1)	1999
	HIV-1 subtype B (1)	1999
Alberta	HIV-1 subtype B (1)	1998

[1] The samples received from Ontario in the year 2000 were received through the CHSDRSP.

Technical Notes

Data Collection and Reporting

It is important to note that the results presented here represent individuals who sought testing, were properly diagnosed, and were reported as HIV positive. Furthermore, they represent those individuals for whom sufficient serum specimen, taken for the purposes of diagnostic testing, was available to send to the National HIV 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 Laboratories also determines whether subtype and primary 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 currently in the process of examining the use of other primer sets for RT-PCR amplification.

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

The quality and completeness of epidemiologic data remain problematic (see Data Limitations section) and one of the key roles of 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 Health Canada.

Exposure Category Hierarchy

HIV cases were assigned to a single exposure category according to an agreed-upon hierarchy of risk factors. This hierarchy is described in more detail in the *HIV and AIDS in Canada Surveillance Report* available by contacting the Division of HIV/AIDS Epidemiology and Surveillance or electronically at www.hc-sc.gc.ca/hpb/lcdc/publicat.html

(select LCDC Periodicals & Serials, and then select HIV and AIDS in Canada).

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 are unable to 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, at a cost of about US$800 per test. In genotypic testing, repeat analyses may be required since mutations strongly associated with drug resistance continue to be "discovered" and their complex interactions are only now 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 2 lists the mutations that were included as primary drug resistance mutations in the results presented in this report. It is anticipated that this list will change as new information on drug resistance mutations becomes available over time. International expert review panels have been formed. These meet periodically to review the latest laboratory and clinical findings in the development of 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.

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 Health Canada from provincial partners participating in the CHSDRSP. 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 bear in mind that the data are not representative of all newly diagnosed cases in the population.
- The data presented are only from those individuals who are infected with HIV who then seek testing. They do not represent individuals who do not know their HIV status or choose not to seek testing. Furthermore, they do not represent individuals for whom insufficient sample is available for strain and drug resistance genotyping.
- The data do not represent information from the two provinces that bear the burden of HIV infections – Ontario and Quebec. Work is already under way on mechanisms to include data from these provinces, and it is anticipated that one or both of them will be included in the next report on strain and primary drug resistance surveillance in Canada.
- Since this report is dealing solely with primary drug resistance (i.e. resistance seen among individuals who have never before received treatment), analysis was conducted on the laboratory specimens collected from treatment naïve 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.
- At the time of writing this report, all data had been received retrospectively, so that the report actually describes what has happened historically. Although these data are still useful in informing program planning and policy formulation, their utility could be enhanced with "real-time" data. We anticipate that as the CHSDRSP moves towards the collection of prospective data, real time information will be presented in the report.
- 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 anti-retroviral 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.

Appendix 1[1]

Glossary of terms

Cross-resistance: resistance selected by one drug which, 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 anti-retroviral 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 anti-retroviral 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

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

Primary mutation: mutation in the viral nucleotide sequence that, in and of itself, is strongly associated with conferring increased resistance of HIV to an anti-retroviral drug

Primary resistance: increased resistance of HIV to anti-retroviral 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 mutation: mutation in the viral nucleotide sequence, which, in combination with other mutations, confers increased resistance of HIV to a drug

Secondary resistance: increased resistance of HIV to drugs seen in individuals 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 2

List of primary mutations used for this report

Protease

Primary mutation	Anti-retroviral drug
D30N	Nelfinavir
M46I	Ritonavir
M46L	Indinavir
I47V	Amprenavir
G48V	Saquinavir
I50V	Amprenavir
V82A/F/S/T	Indinavir, ritonavir
I84V	Amprenavir
N88D	Nelfinavir
L90M	Nelfinavir

Reverse transcriptase

Primary mutation	Anti-retroviral drug
M41L	AZT
I50V	d4T
K65R	Adefovir
T69D/N	ddC
K70E	Adefovir
K70R	AZT
L74V	ddI, ddC, abacavir
V75T	d4T
K103T	Delavirdine
K103N	Delavirdine, efavirenz, nevirapine
V106A	Nevirapine
Q151M	AZT, ddI, ddC, d4T, 3TC, FTC, abacavir
Q161L	Foscarnet
Y181C/I/L	Delavirdine, efavirenz, nevirapine
M184I/V	3TC, FTC, abacavir, adefovir
G190A/E/S	Nevirapine
H208Y	Foscarnet
T215F/Y	AZT

1. **Note:** The correlation of drug resistance to genotype in this report is based on scientific consensus of primary mutations associated with HIV resistance to anti-retroviral drugs as of June 2001. These correlations do not necessarily imply phenotypic resistance to a particular anti-retroviral drug in a clinical setting.