NATIONAL CENTRE FOR STREPTOCOCCUS

ANNUAL REPORT FOR APRIL 1, 2001 TO MARCH 31, 2002

Introduction

The National Centre for Streptococcus (NCS) continues to meet on-going interest in characterization of *Streptococcus pneumoniae*, group A and group B *Streptococcus* isolated from invasive disease. Our specimen load has been similar for the past three years, and reflects participation in both provincial and national notifiable disease programs that have been implemented to monitor these important infections.

Goals and Objectives for the Past Year

Over the past year, we have continued to focus our limited resources on enhanced surveillance of antibiotic resistance in *S. pneumoniae*, *S. pyogenes* (GAS) and *S. agalactiae* (GBS), important areas of interest to the health care community. Unfortunately this increased workload has compromised our ability to meet some of the project goals established last year. Those that continue to have importance and/or relevance to surveillance of invasive *Streptococcus* disease will be included in our future planning for the coming year.

Service

1. Current surveillance data is available on-line through our website at www.provlab.ab.ca. View the National Centre for Streptococcus via the Virtual Lab option. Quarterly and annual reports may be accessed as well as our Guide to Services and expected turn around times. We have also recently added a summary of publications from the National Centre for Streptococcus.

2. Effective April 1, 2001, analyses of antibiotic resistance data for non-beta lactam antibiotics were added to our quarterly report for *S. pneumoniae*, *S. pyogenes* (GAS) and *S. agalactiae.*

Investigation

1. We completed the investigation of two new yellow-pigmented *Enterococcus* species isolated from Canadian patients. This report was recently published.

2. Investigation of new *Aerococcus* species in collaboration with Dr. Richard Facklam, CDC Atlanta, is almost complete. A preliminary report of our data will be presented at the 2002 American Society for Microbiology meeting in Salt Lake City. We expect to publish this manuscript later this year.

3. Our manuscript describing M type distribution in Canada over the past eight years is in its final stages of revision. This should be submitted for publication within the next three months.

4. Surveillance for the new GAS *emm* type *st2967* continues as part of our routine GAS serotyping program. Publication of our experience with this new serotype was planned, but conflicting priorities over the past year prevented us from reaching this target. This will remain on our goal list for 2002-03.

5. Investigation of macrolide resistance in pneumococci is incomplete. Further molecular work is required and we expect to pursue this project over the coming months.

6. Review of requests for Pneumococcal EIA testing over the past year revealed some physician-related ordering practices. Concern about steadily increasing test load combined with the high cost of this test in relation to its limited clinical utility have resulted in a plan to survey users to determine the clinical circumstances in which optimum cost benefit from this testing can be obtained. We hope to initiate this survey within the next 2-3 months.

7. Significant workload increases in our Provincial Laboratory Molecular Department prevented the implementation molecular investigation of nontypable invasive group B streptococcus isolates last year. This remains an area of interest, and we hope to find the additional resources that will be necessary to carry out this testing in the future.

Activities

a. Reference Services

After steadily increasing numbers of specimens received at the NCS between 1991 and 1998, our test load has stablized over the last three years, averaging about 3900 isolates per year. Ninety-five percent of the isolates examined over the past year were submitted by Canadian agencies. On-going surveillance of invasive *Streptococcus* disease in Canada combined with newly implemented vaccine programs targeted at prevention of invasive pneumococcal disease in young children is expected to result in continued utilization of the testing offered by the NCS. The community interest in *S. pneumoniae* is evident in table 1; all externally funded research projects processed by the NCS last year involved pneumococcus serotyping. Comparison of specimen numbers for the past four years is presented in Table 1. There was a notable increase (37%) over last year in requests for pneumococcal serology. Services provided for research projects for 2001/2002 and the proportion of the total testing dedicated to this function are also identified. Only externally funded research projects are listed.

Table 1. Specimen Volume and Research Testing

					2001/02 Research	
Total Test Requests	**1998/99**	**1999/00**	**2000/01**	**2001/02**	**# Specimens**	**% Testing**
Group A Serotyping	926	1140	1111	1103	0	0
Group B Serotyping	319	473	425	209	0	0
Pneumococcal Serotyping	1434	1734	1916	1980	1029	52%
Identification	249	243	238	226	0	0
Pneumococcal Serology	159	299	256	351	0	0
Other	0	28	3	11	0	0
Total Isolates Received	**3087**	**3917**	**3949**	**3880**	**1029**	**26.5%**

b. Laboratory Surveillance

All of the data presented in this section reflect passive surveillance only. The majority of all isolates tested at the NCS are recovered from, or associated with, invasive disease. Occasionally noninvasive isolates are submitted due to atypical characteristics. Wherever possible, only one isolate was counted per patient, however specimen coding may have prevented interpretation of this information for some isolates.

Group A *Streptococcus*

Historically the majority of the GAS that were submitted to the NCS for serotyping have come from Alberta, Ontario and Quebec (Figure 1). Between April 1, 2001 and March 31, 2002, we observed a substantial increase in GAS submitted from both British Columbia and Saskatchewan. We believe that this increase represents enhanced surveillance, and may not necessarily reflect an increase in the incidence of invasive GAS disease in those provinces. GAS isolates submitted from these five provinces account for 98% of the 2001/02 collection.

Figure 1.

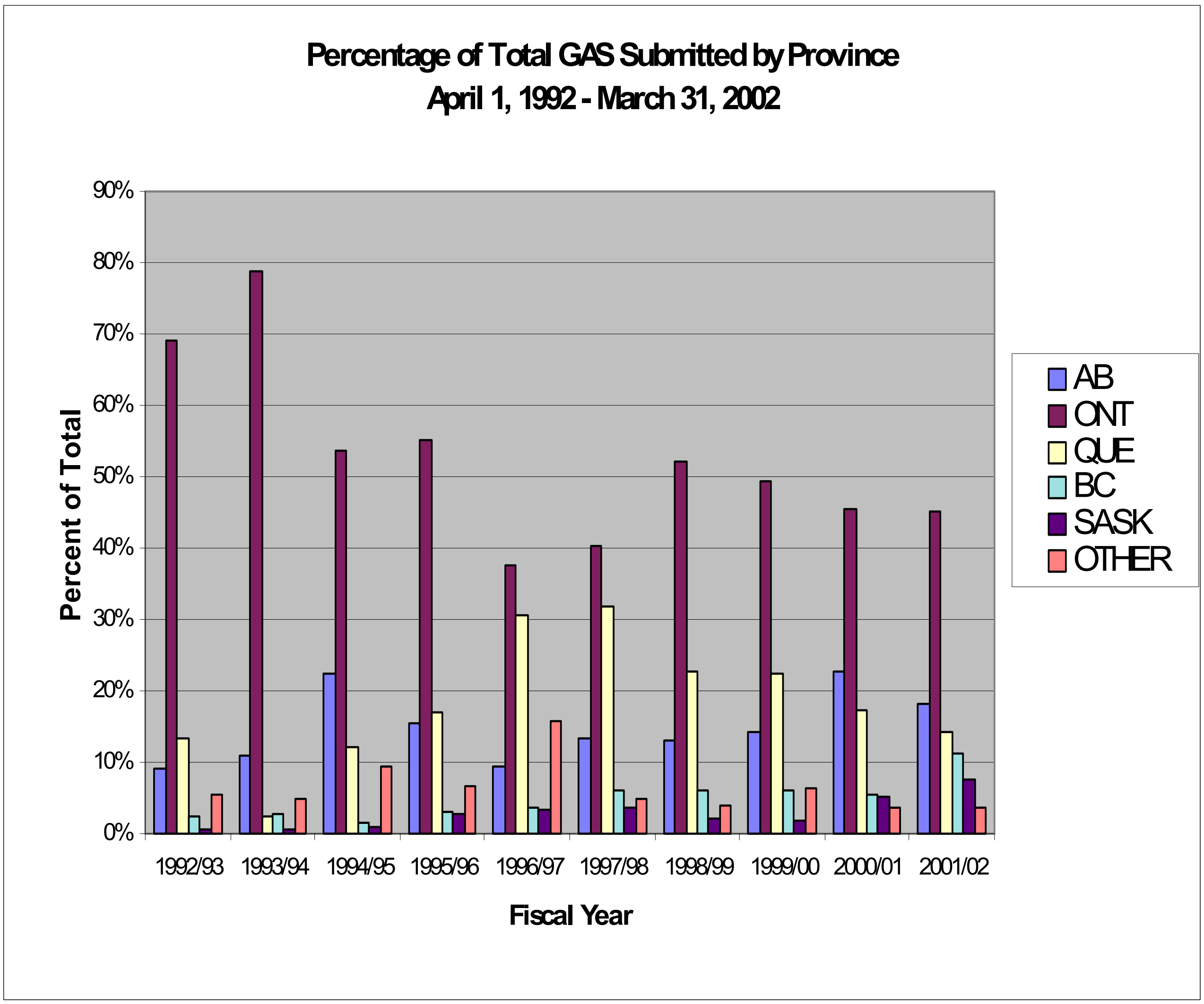


Table 2 presents M type distribution for the past year and comparative data for 2000/01 and 1999/2000. Specific M types are known to be poorly antigenic, making it difficult to prepare the M antisera necessary for serological M type classification. Serotypes M28 and M77 fall into this category. These serotypes are more easily classified according to the AOF type, using antisera specific for the serum opacity factor produced by OF positive strains. The AOF type is, with few exceptions, consistent with the M type, and strains typed as 28 or 77 by either method have been listed together in this report.

Table 2. Group A Streptococcus M type Distribution

	2001/02			2000/01			1999-2000		
M type	# Cases	Rank	% of total	# Cases	Rank	% of total	# Cases	Rank	% of total
M1	186	1	19.0	173	2	18.9	216	1	27.3
M3	104	2	10.6	174	1	19.0	79	3	10.0
M12	79	3	8.1	62	4	6.8	69	4	8.7
M4	64	4	6.5	53	6	5.8	36	7	4.6
M5	62	5	6.3	40	7	4.4	9	12	1.1
AOF†28	56	6	5.7	76	3	8.3	81	2	10.3
M/AOF†77	40	7	4.1	16	14	1.7	25	8	3.2
PT2967	35	8/9	3.6	56	5	6.1	NA		
M89 (PT4245)	35	8/9	3.6	21	12	2.3	40	6	5.1
M6	30	10	3.1	27	8	3.0	18	9	2.3
M49	24	11	2.5	10	17	1.1	7	15	0.9
M nt*	80		8.2	48		5.2	79		10.0
Other	183		18.7	159		17.4	131		16.5
Total	**978**		**100**	**915**		**100**	**790**		**100**

NA = not available

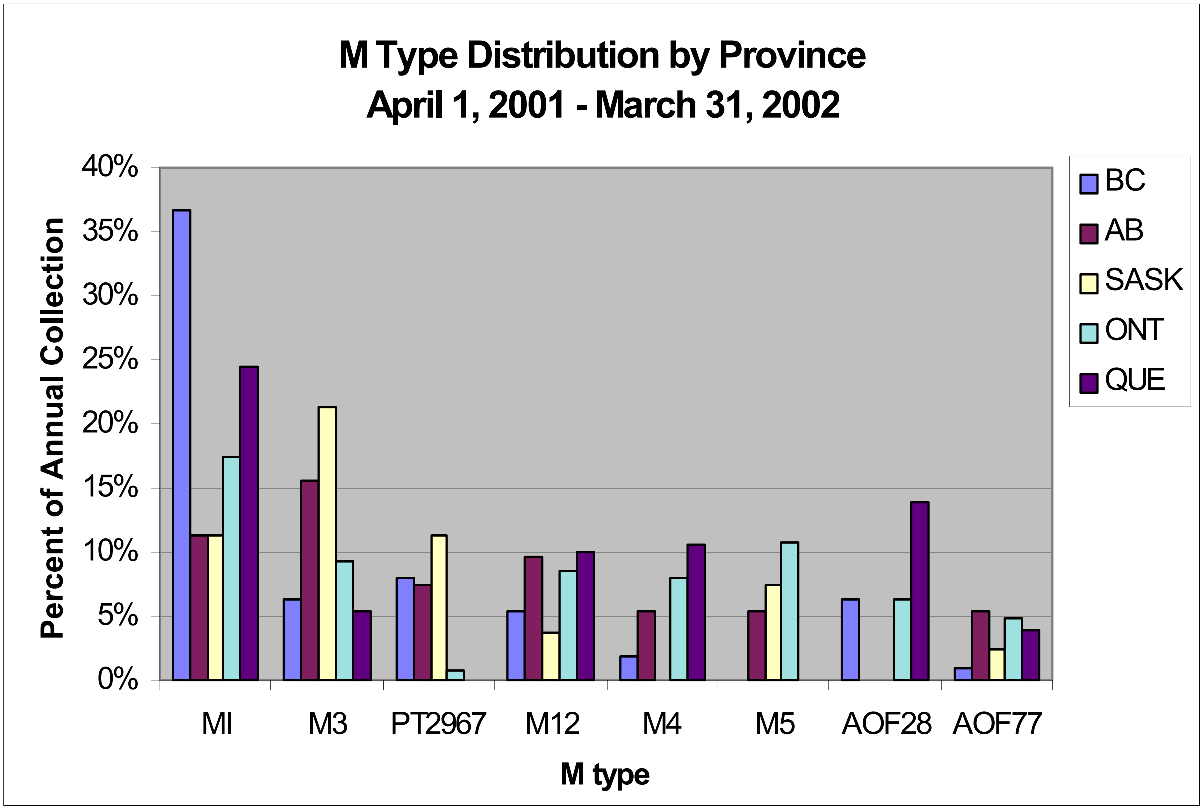
†AOF = Anti Opacity Factor type

*nt = not typable

After falling to second place in 2000/01, M1 has regained its first place ranking. Except for 2000/01, this M type has consistently been the most frequently encountered serotype since the NCS began reporting national GAS seroprevalence in 1992.

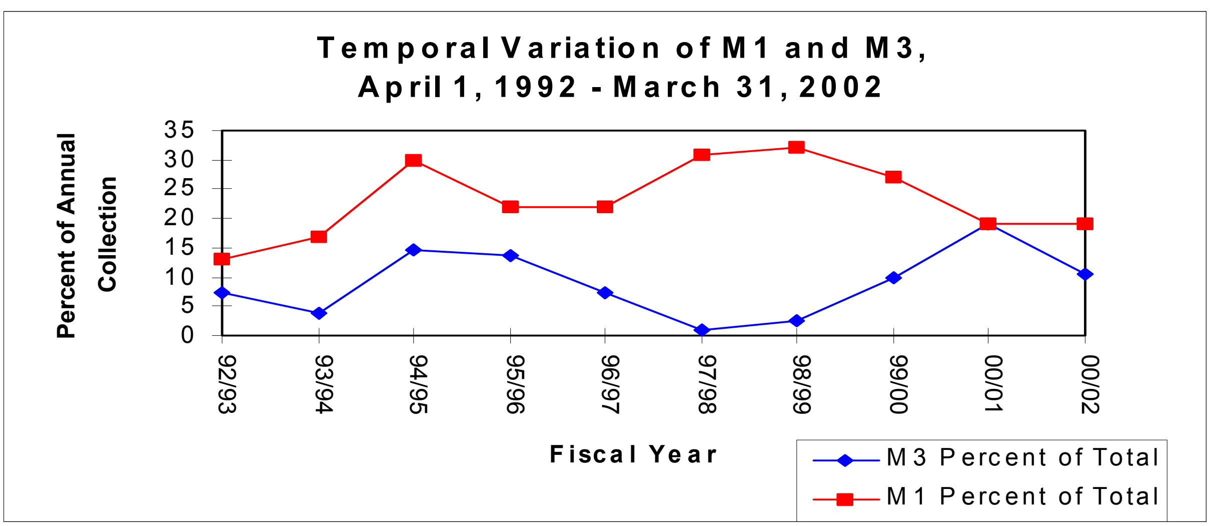
The Provincial distribution of the top 8 ranking M types in the 2001/2002 collection, are presented in Figure 2. There is obvious geographic variation across Canada. The predominance of M1 in British Columbia last year is striking; this type accounted for 37% of isolates submitted from that province. M3 was the top ranking M type in both Alberta and Saskatchewan accounting for 16% and 21% of their isolates respectively. PT2967 continues to be frequently encountered in Western Canada, but not in Ontario or Quebec. The distribution of AOF28 is interesting; it was a common serotype in Eastern Canada, ranking 2nd in Quebec, and on the west coast in British Columbia where it ranked 3rd, but there were no AOF28 isolates submitted from the other Western Provinces.

Figure 2.



The temporal variation of M1 over the past 10 years is compared with that of M3 in Figure 3. For the first four years of our surveillance, the ratio of these two types was relatively proportionate but in 1996/97, the prevalence of M3 began to decline followed by an increase in the prevalence of M1. During 2000/01 M3 was equally as important as M1 as a cause of invasive disease in Canada. The decline in M3 observed last year suggests a 4-5 year pattern in seroprevalence variation for this M type.

Figure 3.



GAS and Antibiotic Resistance

Antibiotic susceptibility of all GAS submitted for serotyping was determined by the disk diffusion method. Penicillin, erythromycin, clindamycin, chloramphenicol and vancomycin were routinely tested. Resistance to erythromycin was associated with MPT2967 and M58. Because MPT2967 has been prevalent in Western Canada over the past two years, those regions experienced higher erythromycin resistance rates than the rest of Canada. Data are compared in Table 3.

Table 3. Proportion (%) of Antibiotic Resistance by Region for Group A Streptococci; April 1/01 – March 31/02 (comparative data for April 1/00 - March 31/01)

Antibiotic	BC	AB	SK	ON	QB	Other	Total
Erythromycin	16.1(10.2)	11.8(23.5)	20.0(38.7)	8.8(8.5)	9.3(9.6)	0(21.7)	11.0(14.1)
Clindamycin	1.8(3.4)	0.5(0.5)	0(0)	0.7(0)	2.0(0)	0(0)	0.9(0.3)
Chloramphenicol	0(0)	0(0)	0(0)	0(0)	0.7(0)	0(0)	0.1(0)
Penicillin	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Vancomycin	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)	0(0)
Total # isolates tested	112(59)	187(200)	80(52)	430(414)	151(167)	18(23)	978(915)

All erythromycin-resistant isolates were also screened for inducible resistance to clindamycin using the double disk test. Inducible resistance was detected in 67 of 108 (62%) of the erythromycin-resistant isolates. This resistance mechanism was demonstrated for all MPT2967 isolates examined over the past two years.

Group B *Streptococcus*

The data presented in table 4 represents the number of cases of invasive disease for which isolates were submitted to the NCS for serotyping. These were primarily from the province of Alberta. Isolates from 133 of 179 cases (74%) received from April 1, 2001 to March 31, 2002 and isolates from 114 of 148 cases (77%) examined between April 1, 2000 and March 31, 2001 were from that province. The data therefore may not be representative of national trends. Only one isolate per case was included in the analysis.

Table 4. Group B Streptococcus Serogroup Distribution by Age for April 1/01 – March 31/02 (Comparative data for April 1, 2000 - March 31, 2001)

Serotype	<3 mon	3 mon-20 yr	21-50 yr	>50 yrs	Age Not Specified	Total
V & V / R	6(4)	1(0)	14(7)	36(23)	0(0)	57(34)
Ia & Ia / c	5(5)	0(2)	13(10)	24(13)	0(1)	42(31)
III & III / R	12(14)	3(1)	5(11)	10(6)	0(0)	30(32)
Ib / c	3(1)	1(0)	8(3)	9(11)	0(0)	21(15)
II	1(4)	0(0)	5(5)	4(12)	0(0)	10(21)
IV	0(0)	0(0)	0(2)	2(2)	0(0)	2(4)
VI	0(0)	0(0)	0(0)	2(0)	0(0)	2(0)
Not typable*	1(1)	0(0)	2(2)	12(8)	0(0)	15(11)
TOTAL	**28(29)**	**5(3)**	**47(40)**	**99(75)**	**0(1)**	**179(148)**

*Not typable = carbohydrate antigen not detected

Types V, Ia and III (with and without the c or R protein antigens) account for 72% of the disease represented by this sample. Isolates belonging to serotypes V, Ia, Ib, and II were associated with adult disease; 114 of 130 isolates (88%) belonging to these serotypes were recovered from patients ≥21 years of age. Nontypable isolates are most frequently encountered in older adults. Twelve of 30 isolates (40%) belonging to serotype III and III/R were isolated from the youngest age group (<3 months).

Five isolates from cerebrospinal fluid were submitted. Three of these were from children <3 months old; two belong to serotype III/R and one to serotype V/R. One isolate was from a 6 month old child (seroytpe III). The fifth CSF isolate was nontypable and was cultured from a 71 year old adult.

GBS and Antibiotic Resistance

Antibiotic susceptibility of all GBS submitted for serotyping was determined by the disk diffusion method. Penicillin, erythromycin, clindamycin, chloramphenicol and vancomycin were routinely tested. Because the majority of the isolates were submitted from Alberta, data are presented for that province separately from the rest of Canada (TROC) in Table 5. Resistance rates for TROC should be interpreted with caution due to the small sample size.

Erythromycin resistance has increased substantially over 2000/01, and was encountered in all of the most common serotypes and in nontypable isolates. Clindamycin resistance was encountered in four of the five most common serotypes. There is no obvious association between serotype and resistance to either of these antibiotics.

Table 5. Proportion (%) of Antibiotic Resistance by Region for Group B Streptococci; April 1/01 – March 31/02 (comparative data for April 1/00 - March 31/01)

Antibiotic	Alberta	TROC	Total
Erythromycin	21.8(14.8)	17.4(6.1)	20.7(12.8)
Clindamycin	7.5(9.6)	8.7(3.0)	7.8(8.1)
Chloramphenicol	0(0)	0(0)	0(0)
Penicillin	0(0)	0(0)	0(0)
Vancomycin	0(0)	0(0)	0(0)
Total # isolates tested	**133(115)**	**46(33)**	**179(148)**

Streptococcus pneumoniae

The following analyses for April 1, 2000 to March 31, 2002 exclude data from isolates received from Laboratoire de Santé Publique du Québec (LSPQ), where serotyping for their provincial pneumococcal surveillance program is performed. Only isolates of less common serotypes are submitted to the National Centre for Streptococcus for factoring; data from these uncommon serotypes have been excluded in an effort to eliminate the resulting bias. Data specific for Quebec may be obtained by contacting the LSPQ directly. Please note that comparative data from previous years do not exclude isolates from Québec, and this must be considered when interpreting the data.

Seroprevalence for pneumococcal isolates recovered from blood and CSF for the past five years is presented in Table 6. With the exception of serotypes 1, 3 and 8, these same serotypes have consistently been among the top 11 for the past 5 years with only slight changes in ranking. The increase in the occurrence of type 1 over the past two years is due to a larger number of isolates received from the Canadian Arctic regions, where this serotype is very common. The reason for an apparent increase in the prevalence of types 3 and 8 is unclear. All serotypes listed in table 6 are included in the currently available 23-valent vaccine. Overall, vaccine coverage can be expected for 93% of the total cases represented in the 2001/02 collection including expected cross-protection for serotype 6A, and 90% if serotype 6A is excluded.

Table 6. Comparative Ranking Of Seroprevalence April 1, 1997 - March 31, 2002

Serotype	**2001-02**	**2000-01**	**1999-2000**	**1998-1999**	**1997-1998**
Type 14	1	1	1	1	1
Type 4	2	2	5	4	7
Type 6B	5	3	2	2	2
Type 3	6	11/12	11	10	11
Type 19F	7	4	3	3	3
Type 9V	3	5	4	5	4
Type 18C	4	6	7	8	6
Type 1	9	8	13	21	12
Type 22F	8	9	8	11	8
Type 8	10	11/12	16	24	18
Type 23F	11	10	6	6	5
Total cases	**703**	**670**	**772**	**876**	**856**

As in previous years, Alberta was disproportionately represented in this collection, presumably because of proximity and community awareness of national and provincial surveillance programs. Fifty-nine percent (416 of 703 isolates) of the 2001/02 sample was from Alberta. Because of this obvious bias, Table 7 presents Alberta data separately from the rest of Canada (TROC). Types 14 and 4 are the most common serotypes across Canada. The top 8 serotypes are the same for both Alberta and TROC with the exception of type 22F in Alberta, and type 1 from the other provinces. The prevalence of type 22F in Alberta has doubled from the previous year (2000-01); the reason for this is not clear. Type 1 ranks higher in other Provinces than it does in Alberta primarily because this serotype is very common in the Canadian Arctic regions.

Table 7. Serotype Distribution in Alberta compared with the rest of Canada (TROC) for 2001/02
Rank and Percent of the total for 2001/02 (comparable data for 2000/01)

	Alberta		**TROC**	
Serotype	**Rank**	**Percent of Total**	**Rank**	**Percent of Total**
Type 14	1 (1)	17.6 (20.1)	1 (1)	12.9 (15.9)
Type 4	2 (2)	13.0 (10.2)	2 (2)	11.9 (11.6)
Type 18C	3 (8/9)	7.2 (4.4)	7 (7)	5.6 (7.0)
Type 22F	4 (12)	6.3 (3.4)	12 (8)	3.5 (5.0)
Type 3	5 (7)	6.0 (4.6)	8 (14)	5.2 (1.6)
Type 9V	6 (5)	5.5 (6.6)	3 (5/6)	9.8 (7.4)
Type 6B	7 (3/4)	5.3 (7.5)	4 (3)	8.0 (8.9)
Type 19F	8 (3/4)	4.3 (7.5)	5 (4)	7.3 (7.8)
Type 6A	9 (10/11)	4.1 (3.6)	13 (12/13)	2.1 (1.9)
Type 8	10 (8/9)	3.6 (4.4)	9/10 (12/13)	4.2 (1.9)
Type 23F	11 (10/11)	3.4 (3.6)	9/10 (9)	4.2 (3.9)
Type 1	12 (13)	2.6 (2.7)	6 (5/6)	5.9 (7.4)
Other Types		21.1 (21.4)		19.4 (19.7)
Total # cases		**416 (412)**		**287 (258)**

The serotypes that cause invasive disease in young children are known to be different from those causing disease in older patients. The data for April 1, 2001 – March 31, 2002 have been presented in tables 8 and 9 sorted according to patient age. Comparative data for the previous year are also provided.

Table 8. Serotype Distribution for Children (≤16 years) for April 1, 2001 - March 31, 2002 (Comparative data for April 1, 2000 - March 31, 2001)

	≤ 5 years	6 - 16 years	Total ≤ 16 years	
Serotype	# Cases	# Cases	# Cases	Rank
Type 14*	55(60)	1(5)	56(65)	1(1)
Type 18C*	25(16)	6(2)	31(18)	2(5)
Type 6B*	27(33)	2(1)	29(34)	3(2/3)
Type 19F*	23(31)	1(3)	24(34)	4(2/3)
Type 4*	16(14)	2(5)	18(19)	6(4)
Type 9V*	16(14)	3(0)	19(14)	5(6)
Type 19A	7(2)	2(1)	9(3)	8/9(11-13)
Type 1	3(1)	6(10)	9(11)	8/9(8)
Type 6A	9(2)	1(1)	10(3)	7(11-13)
Type 23F*	8(11)	0(2)	8(13)	10(7)
Other Types	21(18)	11(6)	32(24)	
Total No. Cases	210(202)	35(36)	245(238)	

* Serotypes included in the heptavalent conjugate vaccines

Serotypes 6B, 18C, 19A and 19F were associated with invasive disease in children and were encountered less frequently in adults.

Eighty-one percent (170 of 210) of the isolates recovered from children ≤5 years of age belong to the seven serotypes which are included in the heptavalent conjugate vaccines.

Table 9. Serotype Distribution for Adults (≥17 years) for April 1, 2001 - March 31, 2002
(Comparative data for April 1, 2000 - March 31, 2001)

Serotype	17-64 years # Cases	≥65 years # Cases	Total ≥ 17 years # Cases	Rank
Type 4*	58(35)	12(18)	70(53)	1(2)
Type 14*	27(30)	27(29)	54(59)	2(1)
Type 3*	17(13)	18(9)	35(22)	3(6/7)
Type 22F*	21(12)	12(10)	33(22)	4(6/7)
Type 9V*	18(19)	14(13)	32(32)	5(3)
Type 8*	20(18)	5(5)	25(23)	6(5)
Type 1*	15(17)	4(2)	19(19)	7(9)
Type 23F*	8(7)	10(5)	18(12)	8(14)
Type 7F*	13(25)	3(4)	16(29)	9/10(4)
Type 6B*	9(9)	7(11)	16(20)	9/10(8)
Type 18C*	10(13)	5(5)	15(18)	11/12(10)
Type 19F*	10(10)	5(7)	15(17)	11/12(11)
Other Types	60(63)	50(43)	109(106)	
Total No. Cases	286(271)	172(161)	458(432)	

* Serotypes included in the 23-valent vaccine

Serotypes 3, 7F, 8 and 22F are commonly recovered from the adult population, but not from young (≤5 yrs) children. The 23-valent vaccine would provide coverage for 89% (407 of 458) of these invasive adult isolates, assuming cross-protection for types 6A and 6B.

Thirty-one isolates from cerebrospinal fluid were submitted. These belonged to 15 different serotypes. Fifteen of the 31 cases had an accompanying blood isolate; the serotype of these isolates always matched the serotype of the CSF isolate. Cases were distributed over all age ranges; 16 (52%) were from patients ≤16 years of age including 13 from children ≤5 years. Of 15 isolates from the ≥17 year old age group, only 3 patients were ≥65. Table 10 compares the serotype with the age range of the patients from which the pneumococci were isolated.

Four of 31 CSF isolates (12.9%) had reduced susceptibility to penicillin; two of these were fully resistant (MIC 2.0µg/ml) and were from children ≤2 years of age.

Table 10. Comparison of serotype and age range for pneumococci isolated from CSF for April 1,2001 - March 31, 2002.

Serotype	**≤2 years**	**3-5 years**	**6-16 years**	**17-64 years**	**≥65 years**	**Total**
Type 6B	2	1	1	0	1	5
Type 18C	4	0	0	0	0	4
Type 22F	0	2	0	2	0	4
Type 23F	0	0	0	2	2	4
Type 19F	1	0	1	1	0	3
Type 14	0	1	0	1	0	2
Other*	2	0	1	6	0	9
Total	**9**	**4**	**3**	**12**	**3**	**31**

*includes 9 serotypes represented by a single isolate

Streptococcus pneumoniae and Antibiotic Resistance

As of April 1, 2000 susceptibility testing of chloramphenicol, clindamycin, erythromycin, ofloxacin, trimethoprim-sulfamethoxazole and vancomycin was implemented for all invasive pneumococci submitted to the NCS for serotyping (excluding Quebec). The minimum inhibitory concentration was determined by the National Committee for Clinical Laboratory Standards (NCCLS) recommended broth microdilution method.

Because isolates from Alberta account for the majority of this collection, antibiotic resistance data have been analyzed separately in Tables 11 and 14-17. The proportion of intermediate and full resistance to seven antibiotics for Alberta compared with the rest of Canada (TROC) is presented in Table 11. These data are analyzed separately for children (≤16 yrs) and adults (≥17 yrs) in tables 14-17. As expected, all isolates were susceptible to vancomycin.

Table 11. Proportion (%) of Antibiotic Resistance by Region for Pneumococci;
Analysis for All Ages: from April 1, 2001 – March 31, 2002
(Comparative data for April 1/00 - March 31/01)

Antibiotic	**Interpretive Category**	**Alberta** # of isolates = 416(412)	**TROC** # of isolates = 287(258)	**Total for Canada** # isolates = 703(670)
Penicillin	Intermediate Resistant **Total**	4.1(6.3) 6.0(4.6) **10.1(10.9)**	5.6(5.4) 5.2(6.2) **10.8(11.6)**	4.7(5.9) 5.7(5.2) **10.4(11.2)**
Ceftriaxone* **	Intermediate Resistant **Total**	6.5(4.2) 0.7(0.2) **7.2(4.4)**	5.2(6.2) 1.1(0) **6.3(6.2)**	6.0(4.9) 0.8(0.2) **6.8(5.1)**
Chloramphenicol	Intermediate Resistant **Total**	0(0) 1.7(0.7) **1.7(0.7)**	0(0) 1.1(1.2) **1.1(1.2)**	0(0) 1.4(0.9) **1.4 (0.9)**
Clindamycin	Intermediate Resistant **Total**	0 (0) 1.2(1.2) **1.2(1.2)**	0(0) 1.7(0.8) **1.7(0.8)**	0(0) 1.4(1.0) **1.4(1.0)**
Erythromycin	Intermediate Resistant **Total**	0(0) 7.9(8.3) **7.9(8.3)**	0(0.4) 6.6(8.9) **6.6(9.3)**	0(0.2) 7.4(8.5) **7.4(8.7)**
Ofloxacin	Intermediate Resistant **Total**	3.1(2.7) 0.2(0.5) **3.3(3.2)**	7.0(2.3) 0.3(0.4) **7.3(2.7)**	4.7(2.5) 0.3(0.4) **5.0(3.0)**
Trimethoprim-Sulfamethoxazole	Intermediate Resistant **Total**	5.0(4.4) 10.6(9.7) **15.6(14.1)**	7.3(5.4) 11.2(9.3) **18.5(14.7)**	6.0(4.8) 10.8(9.6) **16.8(14.3)**

*** Ceftriaxone category interpretation based on NCCLS 2001 guidelines (M100-S11).

In January, 2002, the NCCLS modified the interpretive standard for pneumococci when testing ceftriaxone, cefotaxime and cefepime (Document M100-S12). The MIC breakpoints for these drugs for pneumococci isolated from patients with meningitis are now interpreted differently from pneumococci isolated from nonmeningitis cases. The new interpretation for all three antibiotics is provided in Table 12.

Table 12.Jan,2002 NCCLS ceftriaxone, cefotaxime & cefepime interpretive standards for *S. pneumoniae*

	Susceptible MIC breakpoint	**Intermediate MIC breakpoint**	**Resistant MIC breakpoint**
Meningitis	≤0.5 µg/ml	1.0 µg/ml	≥2.0 µg/ml
Nonmeningitis	≤1.0 µg/ml	2.0 µg/ml	≥4.0 µg/ml

It is not possible for a laboratory to make a definitive antibiotic interpretation based on clinical disease presentation since that information is inconsistently provided on laboratory requisitions. However if we assume that cases of meningitis are accurately represented by isolates recovered from CSF, the overall rate of reduced susceptibility to ceftriaxone in our 2001- 2002 collection presented in table 13 is markedly reduced from that reported in table 11 (1.1% versus 6.8%).

Table 13. Ceftriaxone interpretation for according to NCCLS Document M100-S12, January, 2002

Source/Assumed Diagnosis	# Isolates (Apr 1/01-Mar 31/02)	Intermediate (# isolates/%)	Resistant (# isolates/%)
Blood/nonmeningitis	672	6 (0.9%)	0 (0%)
CSF/meningitis	31	2 (6.5%)	0 (0%)
Total	703	8 (1.1%)	0 (0%)

Table 14. Proportion (%) of Antibiotic Resistance by Region for Pneumococci;
For children (≤16 yrs); from April 1/01 – March 31/02;
(comparative data for April 1/00 - March 31/01)

Antibiotic	**Interpretive Category**	**Alberta** # of isolates = 141(130)	**TROC** # of isolates = 104(108)	**Total for Canada** # isolates = 245(238)
Penicillin	Intermediate Resistant **Total**	5.7(3.9) 8.5(3.9) **14.2(7.7)**	9.6 (2.8) 6.7 (13.0) **16.3(15.7)**	7.3(3.3) 7.8(8.0) **15.1(11.3)**
Ceftriaxone* **	Intermediate Resistant **Total**	8.5(3.1) 2.1(0) **10.6(3.1)**	5.8(10.2) 1.9(0) **7.7(10.2)**	7.4(6.3) 2.0(0) **9.4(6.3)**
Chloramphenicol	Intermediate Resistant **Total**	0(0) 1.4(0) **1.4(0)**	0(0) 1.0(1.9) **1.0(1.9)**	0(0) 1.2(0.8) **1.2(0.8)**
Clindamycin	Intermediate Resistant **Total**	0(0) 2.8(0.8) **2.8(0.8)**	0(0) 2.9(1.9) **2.9(1.9)**	0(0) 2.9(1.3) **2.9(1.3)**
Erythromycin	Intermediate Resistant **Total**	0(0) 9.9(10.8) **9.9(10.8)**	0(0) 12.5(11.1) **12.5(11.1)**	0(0) 11.0(10.9) **11.0(10.9)**
Ofloxacin	Intermediate Resistant **Total**	1.4(2.3) 0(0) **1.4(2.3)**	6.7(1.9) 0(0) **6.7(1.9)**	3.7(2.1) 0(0) **3.7(2.1)**
Trimethoprim-Sulfamethoxazole	Intermediate Resistant **Total**	5.7(5.4) 13.5(10.8) **19.2(16.2)**	13.5(6.5) 11.5(11.1) **25.0(17.6)**	9.0(5.9) 12.6(10.9) **21.6(16.8)**

*** Ceftriaxone category interpretation based on NCCLS 2001 guidelines (M100-S11).

In Alberta, there was an alarming trend toward increasing beta-lactam resistance in the younger age group (Table 11). The rate of reduced susceptibility to penicillin in children in Alberta has doubled over last year (14.2% versus 7.7%) and reduced susceptibility to ceftriaxone has tripled (10.6% versus 3.1%). Conversely, the proportion of reduced susceptibility to penicillin in adults from Alberta (Table 16) was reduced (8.0% versus 12.4%), and ceftriaxone rates were unchanged.

Comparison of beta-lactam resistance for children's isolates submitted from regions outside of Alberta shows a relatively stable rate of reduced susceptibility to penicillin (≈16%) over the previous fiscal year, and a slightly lower rate of reduced susceptibility to ceftriaxone (7.7% versus 10.2%).

The rate of reduced susceptibility to ceftriaxone in the pediatric population is substantially decreased if the new 2002 NCCLS interpretation is applied based on specimen source (Table 15). Reduced susceptibility to ceftriaxone was encountered more frequently in the younger age group compared to the adult population (9.4% versus 5.5%). This observation is consistent with higher rates of penicillin resistance in children (15.1%) compared with adults (7.9%) in the 2001-2002 collection.

Table 15. Ceftriaxone interpretation for pneumococci from **children (≤16 yrs)** (April 1/01-March 31/02) by specimen source according to NCCLS Document M100-S12, January, 2002
Number of isolates (%)

	ALBERTA			**TROC**			**TOTAL**		
Specimen Source	# Isolates	Inter-mediate	Resis-tant	# Isolates	Inter-mediate	Resis-tant	# Isolates	Inter-mediate	Resis-tant
Blood/nonmeningitis	131	3	0	98	2	0	229	5	0
CSF/meningitis	10	1	0	6	1	0	16	2	0
Total	**141**	**4(2.8%)**	**0**	**104**	**3(2.9%)**	**0**	**245**	**7(2.9%)**	**0**

Table 16. Proportion (%) of Antibiotic Resistance by Region for Pneumococci;
For adults (≥17 yrs); from April 1/01 – March 31/02
(comparative data for April 1/00 - March 31/01)

Antibiotic	**Interpretive Category**	**Alberta** # of isolates = 275(282)	**TROC** # of isolates = 182(150)	**Total for Canada** # isolates = 457(432)
Penicillin	Intermediate	3.3(7.4)	3.3(7.3)	3.3(7.4)
	Resistant	4.7(5.0)	4.4(1.3)	4.6(3.7)
	Total	**8.0(12.4)**	**7.7(8.7)**	**7.9(11.1)**
Ceftriaxone* **	Intermediate	5.5(4.6)	5.0(3.3)	5.3 (4.2)
	Resistant	0(0.4)	0.6(0)	0.2(0.2)
	Total	**5.5(5.0)**	**5.6(3.3)**	**5.5(4.4)**
Chloramphenicol	Intermediate	0(0)	0(0)	0(0)
	Resistant	1.8(0.8)	1.1(0.7)	1.5(0.7)
	Total	**1.8(0.8)**	**1.1(0.7)**	**1.5(0.7)**
Clindamycin	Intermediate	0(0)	0(0)	0(0)
	Resistant	0.4(1.4)	1.1(0)	0.7(0.9)
	Total	**0.4(1.4)**	**1.1(0)**	**0.7(0.9)**
Erythromycin	Intermediate	0(0)	0(0.7)	0(0.2)
	Resistant	6.9(7.1)	3.3(7.3)	5.5(7.2)
	Total	**6.9(7.1)**	**3.3(8.0)**	**5.5(7.4)**
Ofloxacin	Intermediate	4.0(2.8)	7.1(2.7)	5.3(2.8)
	Resistant	0.4(0.7)	0.6(0.7)	0.4(0.7)
	Total	**4.4(3.5)**	**7.7(3.3)**	**5.7(3.5)**
Trimethoprim-Sulfamethoxazole	Intermediate	4.7(3.9)	3.8(4.7)	4.4(4.2)
	Resistant	9.1(9.2)	11.0(8.0)	9.8(8.8)
	Total	**13.8(13.1)**	**14.8(12.7)**	**14.2(13.0)**

*** Ceftriaxone category interpretation based on NCCLS 2001 guidelines (M100-S11).

Table 17. Ceftriaxone interpretation for pneumococci from **adults (≥17 yrs)** (April 1/01-March 31/02) by specimen source according to NCCLS Document M100-S12, January, 2002
Number of isolates (%)

	ALBERTA			**TROC**			**TOTAL**		
Specimen Source	# Isolates	Inter-mediate	Resis-tant	# Isolates	Inter-mediate	Resis-tant	# Isolates	Inter-mediate	Resis-tant
Blood/nonmeningitis	268	0	0	174	1	0	442	1	0
CSF/meningitis	7	0	0	8	0	0	15	0	0
Total	**275**	**0(0%)**	**0**	**182**	**1(0.6%)**	**0**	**457**	**1(0.2%)**	**0**

Reduced susceptibility to penicillin was detected in 73 isolates belonging to 8 different serotypes. Ninety-nine percent of these (72 of 73) are covered by the 23-valent vaccine if one assumes cross-protection for serotype 6A. Fifty-five percent (40 of 73) of these strains are fully resistant to penicillin (MIC ≥2.0 µg/ml); this was observed most frequently for types 9V, 14 and 6B.

Trimethoprim-sulfamethoxazole continues to be the antibiotic to which pneumococci are most frequently resistant in all age groups. In adults the proportion of intermediate and full resistance was relatively unchanged over the past year, however there was a 5% increase in overall reduced trimethoprim-sulfamethoxazole susceptibility for isolates recovered from children between 2001-02 and 2000-01 (21.6% versus 16.8%). This may be associated with common pediatric use of this drug.

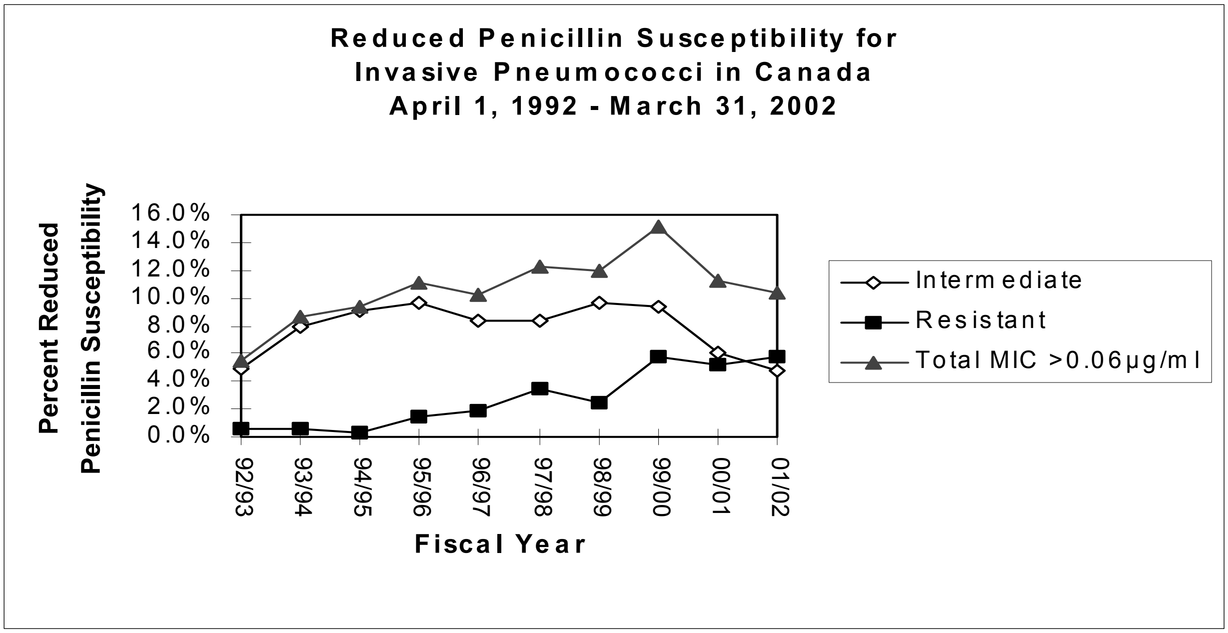
As reported last year, resistance to erythromycin and to trimethoprim-sulfamethoxazole frequently occurred in the absence of reduced susceptibility to penicillin. Twenty-seven (52%) of 52 erythromycin-resistant pneumococci and sixty of 118 (51%) trimethoprim-sulfamethoxazole-resistant pneumococci were susceptible to penicillin. This may be clinically relevant if antibiotic resistance observed for our invasive isolates can be extrapolated to pneumococci causing non-invasive disease. Both drugs are frequently used to treat respiratory infections.

An increase in intermediate ofloxacin resistance was observed from provinces other than Alberta for all age groups. This change was not apparent for isolates from Alberta. Reduced susceptibility to ofloxacin was found in 35 isolates but only 2 of these were fully resistant to this drug (MIC ≥8.0 µg/ml). Both resistant isolates were recovered from adults. Reduced susceptibility to ofloxacin is not associated with reduced susceptibility to penicillin. Only 1 of 35 isolates in our 2001-02 collection was not susceptible to penicillin.

We have defined multiple resistance as intermediate or full resistance to three different classes of antibiotics. Twenty-nine of 703 isolates (4.1%) were multiply resistant; 24 of these had reduced susceptibility to penicillin. Multiple resistance was demonstrated in serotypes 6B (8 isolates), 14 (8 isolates), 9V (6 isolates), 23F (3 isolates), 19F (2 isolates), 6A (1 isolate) and type 3 (1 isolate). Antibiotic resistance in serotype 3 is very unusual; this strain was susceptible to penicillin, but resistant to chloramphenicol, eythromycin and clindamycin.

Regardless of the dramatic increase in penicillin resistance observed in children from Alberta, the overall national rate continues to fall (Figure 4). It is, however, of concern that the proportion of pneumococci that are fully resistant to penicillin (MIC ≥2.0μg/ml) now exceeds the intermediate resistant category (MIC 0.12 - 1.0μg/ml).

Figure 4.



c. Outbreak Investigation

Tracking outbreak and/or clusters of streptococcal infection is sometimes difficult since a description of the event is not always submitted. When non-invasive isolates are received in this laboratory, follow up is initiated to determine the reason for the test request. It is often only after this type of contact with the submitting agency that we are able to identify the group of specimens as part of an outbreak investigation. We encourage laboratory directors to reinforce the importance of submitting outbreak documentation to the NCS, and recruiting the assistance of their local outbreak investigation teams in this regard.

During the past year the NCS was asked to provide laboratory testing for 15 epidemiological investigations conducted in 5 provinces. This was slightly higher than for 2000-2001 when requests for investigation of 11 events were received from 4 provinces and territories. Listed in Table 18 are the investigations conducted during 2001/02.

Table 18. Summary of Epidemiologic Investigations April 1, 2001 - March 31, 2002

Investigation Number	Province Requesting Investigation	Test Request	No. of Samples	Causative Agent
01-507	Ontario	GAS serotyping investigation of family cluster of infections	4	M12 T12 OF -
01-508	Ontario	GAS serotyping investigation of nursing home outbreak	17	M77 T9/13/14/28 AOF 77 OF+ (16 of 17 isolates)
01-509	Alberta	Pneumococcal serotyping and PFGE Contacts of fatal case	3	Serotype 6B (2 of 3 isolates)
01-510	Ontario	GAS serotyping investigation of family cluster of infections	5	M3 T3/B3264 OF- (4 of 5 isolates)
01-511	New Brunswick	GAS serotyping Investigation of nosocomial infection	3	All isolates different serotypes
01-512	British Columbia	Pneumococcal serotyping investigation of regional increased disease incidence	12	Random serotype distribution – no pattern
01-513	British Columbia	Pneumococcal serotyping investigation of regional increased disease incidence	19	Random serotype distribution – no pattern
01-514	Ontario	GAS serotyping outbreak investigation in nursing home	10	M1 T1 OF – (7 of 10 isolates)
01-515	Ontario	GAS serotyping outbreak investigation in day care centre	7	M12 T12 OF –
02-500	Ontario	GAS serotyping outbreak investigation of primary school	6	Random distribution of 4 different M types - no pattern
02-501	British Columbia	GAS serotyping investigation cluster of infections	3	M1 T1 OF – (2 of 3 isolates)
02-502	Ontario	GAS serotyping outbreak investigation in nursing home	4	Random distribution of 3 different M types - no pattern
02-503	Ontario	GAS serotyping outbreak investigation in nursing home	25	M1 T1 OF –
02-504	Prince Edward Island	Pneumococcal serotyping investgation of cluster of penicillin-resistant infections	3	Serotype 19F

d. Research

Table 19 lists completed and/or on-going research projects in which the National Centre for Streptococcus participated during 2001 - 2002.

<u>Streptococcus pneumoniae</u>

Table 19. Summary of Research Projects April 1, 2001 - March 31, 2002

Researcher/Agency	Study Description	Services Required
Pan American Health Organization with funding from the Canadian International Development Agency	SIREVA Project - detemination of pneumococcal seroprevalence and antibiotic resistance rates in Latin American children <5 yrs of age	SIREVA Network now includes 23 countries. NCS continues to provide leadership and technical resources for the Quality Assurance program
Immunization Monitoring Program, Active (IMPACT) Dr. David Scheifele	Surveillance of invasive pneumococci recovered from Canadian children ≤16 yrs of age	Pneumococcal serotyping and antibiotic susceptibility testing
Dr. Jim Kellner, Alberta Children's Hospital, Calgary, Alberta	Surveillance of invasive pneumococcal disease within the Calgary Regional Health Authority	Pneumococcal serotyping
Dr. Allison McGeer, Mount Sinai Hospital, Toronto, Ontario	Retrospective analysis of invasive pneumococci associated with Evaluation of the Ontario Pneumococcal Vaccination program	Pneumococcal serotyping
Dr Fred Aoki, Health Sciences Centre, Winnipeg, Manitoba	Morbidity, Mortality and Health Care Costs of Invasive Pneumococcal Disease in Manitobans	Pneumococcal serotyping
Dr. Alan Parkinson, Arctic Investigations Program, Anchorage, Alaska	International Circumpolar Surveillance of Invasive Pneumococcal Disease	Pneumococcal serotyping and antibiotic susceptibility testing
Dr. Daryl Hoban, Health Sciences Centre, Winnipeg, Manitoba	CROSS national study – selected isolates for characterization	Pneumococcal serotyping
Dr. Don Low, Mount Sinai Hospital, Toronto, Ontario	Quinolone Resistance in Pneumococci	Pneumococcal serotyping
Sandra March, Newfoundland Public Health Lab, St. John's Newfoundland	Prevalence of antibiotic resistant pneumococci in day car centres	Pneumococcal serotyping
NCS in-house study	Investigation of pneumococci with an unusual erythomycin/clindamycin resistance phenotype	Antibiotic susceptibility testing and molecular testing of selected strains

Group A Streptococci (*Streptococcus pyogenes*)

Researcher/Agency	**Study Description**	**Services Required**
Dr. Richard Facklam, Centres for Disease Control, Atlanta, Georgia	Validation of new *emm* sequences to official *emm* type	GAS serotyping Collaborative study
Dr. Alan Parkinson, Arctic Investigations Program, Anchorage, Alaska	International Circumpolar Surveillance of Invasive GAS Disease	GAS serotyping and antibiotic susceptibility testing
Mark Reddish ID Biomedical/ID Vaccine Corp. Bothell, Washington	StreptAvax™ GAS Vaccine trials	Phase 1 Safety trials - GAS serotyping Contribution to design and participation in Phase 2 Canadian trials planned
NCS in-house study	Investigation of emm *st2967*	GAS serotyping, antibiotic susceptibility testing and molecular analysis

Group B Streptococcus (*Streptococcus agalactiae*)

Researcher/Agency	**Study Description**	**Services Required**
Dr. Dele Davies Alberta Children's Hospital, Calgary, Alberta	Group B Streptcoccus Invasive Disease in Alberta	Group B serotyping
Dr. Alan Parkinson, Arctic Investigations Program, Anchorage, Alaska	International Circumpolar Surveillance of Invasive GBS Disease	GBS serotyping and antibiotic susceptibility testing
Dr. Michelle Alfa St. Boniface Research Centre Winnipeg, Manitoba	Group B Streptococcus carriage in pregnancy	Group B serotyping

Other Investigations

Researcher/Agency	**Study Description**	**Services Required**
Dr. Richard Facklam Centres for Disease Control, Atlanta, Georgia	Collaborative investigation of new *Aerococcus* species	Identification

e. Training

There were no formal training events held during 2000/01.

f. Other Highlights

Nothing to report.

Quality Indicators Monitored

1. Turn Around Time

   The average turn around times (TAT) for the past year are compared with those for 2000/01 in Table 20. Isolates that are associated with designated outbreak investigations are given priority status and the TAT will be significantly reduced from the averages reported here. With the exception of pneumococcal serology, the NCS maintained similar TATs to 2000/01. There was a 37% increase in requests for pneumococcal serology during 2001-02. This test is very labor intensive and the increased TAT observed over the past year is a direct result of the difficulties we encountered in managing the additional workload within our existing staffing component.

Table 20. Average Turn Around Time (TAT) for April 1, 2001 - March 31, 2002 compared to 2000/01

Test Request	**Apr 1/01 - Mar 31/02 Avg. TAT in days**	**Apr 1/00 - Mar 31/01 Avg. TAT in days**
Group A Serotyping	13	13
Group B Serotyping	10	11
Streptococcus pneumoniae Serotyping	11	14
Identification	13	14
Pneumococcal Serology	30	19

2. Proficiency Testing Programs

   Unfortunately the international Quality Control program for group A *Streptococcus* serotyping that was implemented in 1997 for Streptococcus Reference laboratories located in England, New Zealand, the Czech Republic, the United States and Canada was not active last year. The promised support from the World Health Organization (WHO) has not yet been approved. International shipment of bacterial isolates is very expensive and this has been a significant barrier to the continued coordination and participation in this valuable Quality Assurance initiative. As members of the WHO Laboratory Working group, we will continue to push for WHO funding.

   Streptococcus pneumoniae Serotyping and Susceptibility testing - The NCS continues to participate in a collaborative Quality Control program involving the Laboratoire de Santé Publique du Québec and the Arctic Investigation Program laboratory in Anchorage, Alaska. Over the past year three panels have been distributed. Correlation for serotyping data for all three labs was 100%. This is the second year that all centres have achieved this excellent agreement. The three laboratories achieved 91-100% correlation with MIC values within +/- one log_2 dilution for nine antibiotics. This is not as good as the 2000/01 correlation of 96-100%, and poorer performance was related to macrolide discrepancies for one of the three annual distributions. We look forward to continued particpation in this important quality assurance initiative.

The NCS continues to serve as a resource for both education and Quality Assurance for the Sireva Project in Latin America. Consistent with the new model established in 1999, we work primarily with the three Quality Control Centres (Mexico, Colombia and Brazil), and continue to coordinate an external quality control program with semiannual distributions of pneumococci for serotyping and MIC testing.

Future Plans

The following projects have been identified for the next 12 months:

1. Design a questionnaire for clinicians who order pneumococcal EIA testing to determine the reasons for testing and how to meet this need within exisitng limited resources.

2. Implement levofloxacin MIC testing for all invasive pneumococcal isolates. This will replace ofloxacin that is currently being tested.

3. Complete and submit the manuscript reporting our experience with M type distribution in Canada over the past 8 years.

4. Work with Dr. Richard Facklam, CDC, Atlanta, Georgia to compile a manuscript describing our collaborative *Aerococcus* investigation.

5. Compile a manuscript describing our investigation of GAS *emm st2967* and our proposal that this type should gain official international status as a provisional M type.

6. Continue the investigation of pneumococci with an unusual erythomycin/clindamycin resistance phenotype.

Publications

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3. Scheifele, D. S. Halperin, L. Pelletier, J. Talbot, M. Lovgren, W. Vaudry, T. Jadavji, B. Law, N. MacDonald, R. Gold, E. Wang, E. Mills, M. Lebel, P. Dery, and R. Morris. 2001. Reduced susceptibility to penicillin among pneumococci causing invasive infection in children - Canada, 1991 to 1998. Canadian Journal of Infectious Disease;12:241-246.

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5. Hortal, M., M. Lovgren, F. de la Hoz, C. I. Agudelo, M. Brandileone, T. Camou, S. Casagrande, E. Castañeda, A. Corso, G. Echaniz-Aviles, J. Hormazabal, J. Pace, R. Palacio, G. Perez-Giffoni, R. Ruvinsky, J.L. di Fabio and the PAHO SIREVA-Vigia Study groups. 2001. Antibiotic resistance in *Streptococcus pneumoniae* in six Latin American countries: biological and therapeutic implications. Microbial Drug Resistance 7:391-401.

6. Purcell, L., J.P. Finley, R. Chen, M. Lovgren, and S.A. Halperin. *Gemella* species endocarditis in a child. 2001. Canadian Journal of Infectious Disease;12:317-320.

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