

NATIONAL CENTRE FOR STREPTOCOCCUS

ANNUAL REPORT FOR APRIL 1, 2000 TO MARCH 31, 2001

Introduction

The National Centre for Streptococcus continues to meet an increasing interest in characterization of *Streptococcus pneumoniae*, group A and group B *Streptococcus* isolated from invasive disease. Our specimen load was similar to that reported last year and reflects increased 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 focused 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. In order to meet this increased workload, we were forced to reevaluate 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 are now available on-line. Both quarterly and annual reports may be accessed through our website at <http://bugs.uah.ualberta.ca>. View the National Centre for Streptococcus via the Virtual Lab option. Our Guide to Services and expected turn around times are also available at this site.
2. Effective January 1, 2001, the reporting format of the serological classification of GAS was revised to include both the M type and the anti-OF type. The anti-opacity factor (AOF) represents the type specificity of the serum opacity factor (SOF) produced by SOF positive strains. Until recently the AOF type was believed to predict the M type, and this approach continues to be valid for the majority of isolates. However exceptions do exist (Beall B. *et al.* 2000. Microbiology; 146:1195-1209) and for this reason, the results of both serological classification methods are reported.

Investigation

1. Both M and AOF antisera have been prepared for the serological classification of the GAS *emm* st2967 and, to date, the NCS is the only lab worldwide to have included these reagents in their antisera inventory. Our investigation demonstrates that this new provisional M type is very common in Alberta. Isolates collected from 1993 to the end of 2000 have been examined, and we are currently working on a manuscript for publication of our results.
2. Screening of all M non-typable GAS for newly recognized M types 83, 89, 90 and 92 has been completed for our archived collections from 1996 through 2000. A manuscript reporting our experience with M type distribution of invasive GAS is currently being written for publication.
3. Erythromycin, clindamycin and chloramphenicol resistance for GAS and GBS has been analyzed for the past fiscal year (April 1, 2000 - March 31, 2001) and this information is presented in this annual report. Review of these data for previous years is incomplete at this time.

4. Effective April 1, 2000, broth microdilution MIC testing of eight antibiotics was implemented for all invasive pneumococci submitted as part of the national surveillance program. Prior to this past year, MIC testing was limited to pneumococci with reduced susceptibility to penicillin. These data are presented in this annual report.
5. Molecular analysis of non-typable invasive GBS isolates was not completed this year, however we recognize the relevance this information may have to potential vaccine development, and this investigation will remain on the planning list for the coming year
6. We had planned to design a clinical information form to be used specifically for requests for pneumococcal serology. In evaluating this project, we noted that most submitters provide appropriate clinical information when requesting this test. While the use of a consistent format to collect this information may facilitate more complete data collection, it is not a priority at this time. We will continue to monitor this over the coming year.
7. Our plan to explore the usefulness of PspA typing as a classification method for *S. pneumoniae* is considered to be of lower priority at this time and will be pursued only as available resources allow.

Activities

a. Reference Services

The total testing volume for 2000/2001 slightly exceeded that of last year. Ninety-one percent of these strains were submitted by Canadian agencies, and we believe that the significant increase we have observed over the past two years is due to implementation of new provincial and national surveillance programs. The increase in the number of group B streptococci submitted over the past two years is due primarily to research being conducted in Alberta. Comparison of specimen numbers for the past four years is presented in Table 1. Services provided for research projects for 2000/2001 and the proportion of the total testing dedicated to this function are also identified. Some, but not all research projects are externally funded.

Table 1. Specimen Volume and Research Testing

Total Test Requests	1997/98	1998/99	1999/00	2000/01	2000/01 Research	% Testing
Group A Serotyping	765	926	1140	1111	418	37.6%
Group B Serotyping	89	319	473	425	384	90.4%
Pneumococcal Serotyping	1507	1434	1734	1916	1308	68.3%
Identification	274	249	243	238	17	7.1%
Pneumococcal Serology	93	159	299	256	0	0
Other	0	0	28	3	0	0
Total Isolates Received	2728	3087	3917	3949	2127	53.9%

b. Laboratory Surveillance

All of the data presented in this section reflect passive surveillance only, and must be interpreted with caution. 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 occasionally prevented interpretation of this information for some isolates.

Group A *Streptococcus*

The majority of the GAS that we receive are submitted from Alberta, Ontario and Quebec (Figure 1). These three provinces account for 78-92% of the isolates we have examined each year since 1992. Consequently differences in annual seroprevalence reported here may be attributed primarily to variations seen in those provinces. A joint effort between the NCS and Alberta Health and Wellness to enhance surveillance of invasive GAS disease in that province has resulted in a >70% increase in the number of isolates received from Alberta over the past year. We are also pleased to note that an increased number of GAS isolates were received from Saskatchewan and Manitoba, although proportionately this contribution represents <8% of the annual collection.

Figure 1.

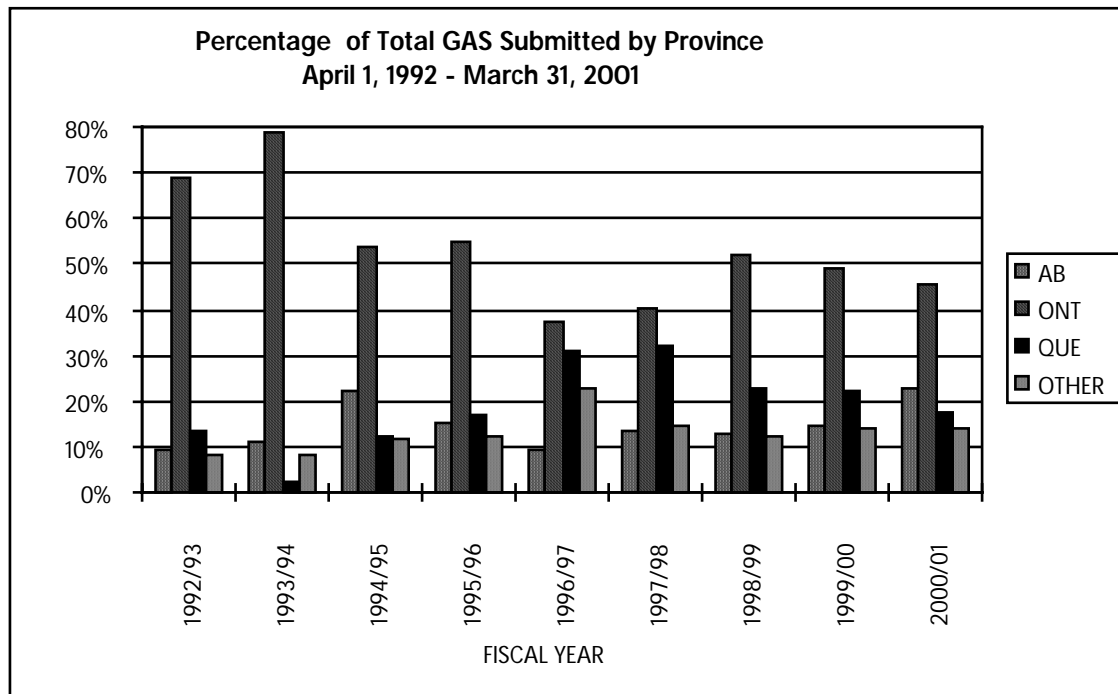


Table 2 presents M type distribution for the past year and comparative data for 1999/2000 and 1998/99. Our revised serological reporting format, as described on page one of this report, is reflected in this table. Specific M types are known to be poorly antigenic, making it difficult to prepare the M antisera necessary for serological M type classification. Serotypes M11 and M28 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 11 or 28 by either method have been listed together in this report.

Table 2. Group A Streptococcus M type Distribution

M type	2000/01			1999/2000			1998/99		
	# Cases	Rank	% of total	# Cases	Rank	% of total	# Cases	Rank	% of total
M3	174	1	19.0	79	3	10.0	20	8	2.4
M1	173	2	18.9	216	1	27.3	261	1	31.9
AOF [†] 28	76	3	8.3	81	2	10.3	93	2	11.4
M12	62	4	6.8	69	4	8.7	75	3	9.2
PT2967	56	5	6.1	NA			NA		
M4	53	6	5.8	36	7	4.6	36	5	4.4
M5	40	7	4.4	9	12	1.1	8	13	1.0
M6	27	8	3.0	18	9	2.3	9	17	1.1
M/AOF [†] 11	24	9/10	2.6	42	5	5.3	56	4	6.9
M75	24	9/10	2.6	8	13	1.0	2	-	0.2
M91 (PT5757)	22	11	2.4	NA			NA		
M89 (PT4245)	21	12	2.3	40	6	5.1	27	7	3.3
M nt*	48		5.2	79		10.0	111		13.6
Other	115		12.6	113		14.3	119		14.6
Total	915		100	790		100	817		100

NA = not available

[†]AOF = Anti Opacity Factor type

*nt = not typable

The M type distribution reported for 2000/01 is significantly different from previous years. For the first time, since surveillance began at the NCS in 1992, M1 did not rank first in overall seroprevalence. This serotype has been replaced by a striking increase in the proportion of M3 isolates submitted primarily from Ontario and Quebec; 144 of 174 M3 isolates (83%) were submitted from those two provinces. We believe that our data represent a real increase in invasive disease caused by M3 because the number of isolates submitted from Ontario and Quebec and the surveillance programs in those provinces have not changed significantly over the past three years.

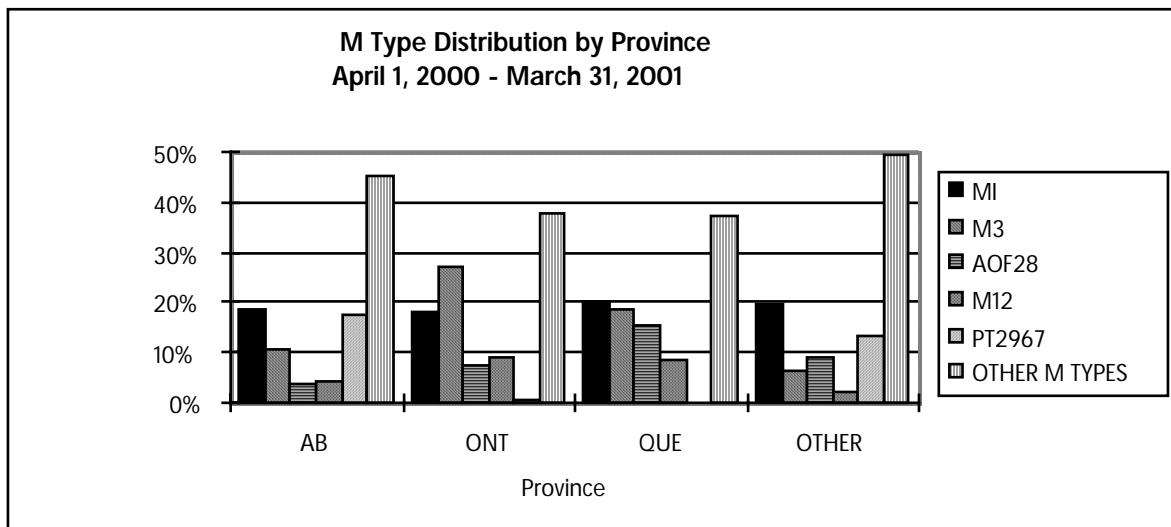
Since last year, we have added two new antisera to our testing inventory, PT2967 and M91 (PT5757). Isolates submitted from Alberta and Saskatchewan account for the majority (89%) of the PT2967 isolates

in our collection. In Alberta this serotype is as common as M1 and represents 17% of the isolates submitted from that province.

The increase in the number of M5 and M75 examined this year were due to outbreak investigations occurring in long term care facilities in Ontario

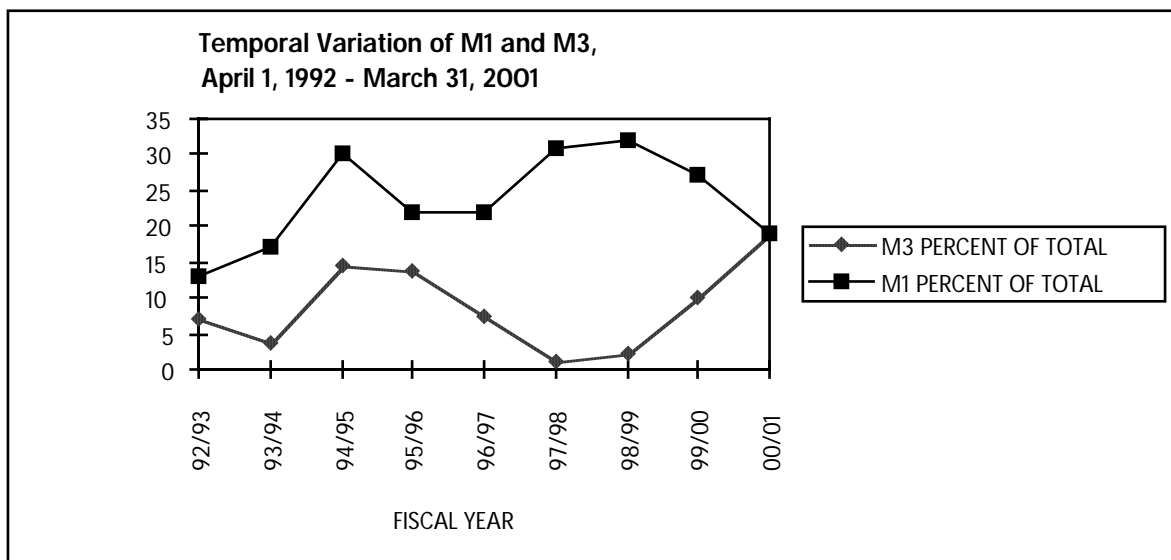
The Provincial distribution (Alberta, Ontario, Quebec and Other Provinces) of M1, M3, AOF/M28, M12 and MPT2967, the top 5 ranking M types in the 2000/2001 collection, are presented in Figure 2.

Figure 2.



The temporal variation of M3 over the past 9 years is compared with that of M1 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 with a concurrent increase in the prevalence of M1. Over the past two years the proportion of M3 has increased so that it is now equally as important as M1 as a cause of invasive disease in Canada.

Figure 3.



GAS and Antibiotic Resistance

Antibiotic susceptibility of all GAS submitted for serotyping is determined by the disk diffusion method. Penicillin, erythromycin, clindamycin, chloramphenicol and vancomycin are routinely tested. As expected, all GAS were susceptible to penicillin and vancomycin, and were also susceptible to chloramphenicol. Resistance to clindamycin was low at 0.3% (3 isolates). Resistance to erythromycin was significant at 14.3% and was associated with M4, M11, M58 and MPT2967. Because MPT2967 is prevalent in Alberta and Saskatchewan, these provinces experienced higher resistance rates than the rest of Canada (Table 3).

Table 3. Regional Variation of Erythromycin Resistance in Group A Streptococci; April 1/00-March 31/01
Number of Isolates (%)

Region	Total Number of Isolates Tested	Intermediate	Resistant	Total Resistance
Alberta	198	1 (0.5%)	47 (23.7%)	48 (24.2%)
Saskatchewan	52	1 (1.9%)	20 (38.5%)	21 (40.4%)
Other Provinces	665	0 (0%)	62 (9.3%)	62 (9.3%)
Total	915	2 (0.2%)	129 (14.1%)	131 (14.3%)

All erythromycin-resistant isolates were also screened for inducible resistance to clindamycin using the double disk test. Inducible resistance was detected in 98 of 131 (75%) of the erythromycin-resistant isolates. This resistance mechanism was demonstrated for all M58 and MPT2967 isolates in this collection.

Group B *Streptococcus*

Serotyping requests for invasive group B streptococci are received routinely from Alberta and Saskatchewan, but infrequently from the other provinces. Isolates from 148 cases of invasive disease were available for analysis after data for non-invasive isolates from specified research studies were excluded; 115 (78%) of these were from Alberta. The seroprevalence reported in Table 4 therefore, does not represent national trends.

Types V, III and Ia (with and without the c or R protein antigens) account for 66% of the disease represented by this sample. Isolates belonging to serotypes V, Ia and II were associated with adult disease; 71 of 86 isolates (83%) belonging to these serotypes were recovered from patients ≥ 21 years of age. Fourteen of 32 isolates (44%) belonging to serotype III and III/R were isolated from the youngest age group (<3 months). Three isolates from cerebrospinal fluid were submitted; 2 of these were from children <3 months old; both belonged to serotype III/R. The third CSF isolate belonged to serotype Ib/c and was cultured from a 71 year old adult.

Table 4. Group B *Streptococcus* Serotype Distribution by Age for 2000/01 (Comparative data 1999/00)

Serotype	< 3 mon	3 mon-20 yrs	21-50 yrs	> 50 yrs	Age Not Specified	Total
V & V / R	4 (3)	0 (1)	7 (12)	23 (22)	0 (0)	34 (38)
III & III / R	14 (14)	1 (2)	11 (6)	6 (8)	0 (0)	32 (30)
Ia & Ia / c	5 (2)	2 (2)	10 (7)	13 (16)	1 (0)	31 (27)
Ib / c	1 (2)	0 (3)	3 (3)	11 (9)	0 (1)	15 (18)
II	4 (2)	0 (2)	5 (3)	12 (6)	0 (0)	21 (13)
IV	0 (0)	0 (0)	2 (0)	2 (1)	0 (0)	4 (1)
Not typable*	1 (2)	0 (3)	2 (3)	8 (4)	0 (1)	11 (13)
TOTAL	29 (25)	3 (13)	40 (34)	75 (66)	1 (2)	148 (140)

*Not typable = carbohydrate antigen not detected

GBS and Antibiotic Resistance

Antibiotic susceptibility of all GBS submitted for serotyping is determined by the disk diffusion method. Penicillin, erythromycin, clindamycin, chloramphenicol and vancomycin are routinely tested. As expected, all GBS were susceptible to penicillin and vancomycin; they were also susceptible to chloramphenicol. Resistance to erythromycin and clindamycin was detected in 12.8% and 8.1% of the isolates respectively. Inducible clindamycin resistance was detected in 6 of 19 (31.2%) erythromycin-resistant isolates, the remaining 12 erythromycin-resistant isolates were fully resistant to clindamycin.

Erythromycin resistance was associated with serotype V. Ten of 34 isolates (29%) belonging to that serotype were resistant to erythromycin; 6 of these were also resistant to clindamycin.

Streptococcus pneumoniae

The following analyses for April 1, 2000 to March 31, 2001 exclude data from isolates received from Laboratoire de Santé Publique du Québec, 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 Laboratoire de Santé Publique du Québec 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 the past five years is presented in Table 5. With the exception of a slight variation for serotype 1, these same serotypes have consistently been among the top 11 for the past 5 years with only slight changes in ranking. There is an increase in the occurrence of type 1 over last year due to a larger number of isolates received from the Northwest Territories, where this serotype is very common. All serotypes listed in table 5 are included in the currently available 23-valent vaccine. Overall, vaccine coverage can be expected for 92% of the total cases represented in this sample including expected cross-protection for serotype 6A, and 89% if serotype 6A is excluded.

Table 5. Comparative Ranking Of Seroprevalence April 1, 1996 - March 31, 2001

Serotype	2000-01	1999-2000	1998-1999	1997-1998	1996-1997
Type 14	1	1	1	1	1
Type 4	2	5	4	7	3
Type 6B	3	2	2	2	4
Type 19F	4	3	3	3	7
Type 9V	5	4	5	4	2
Type 18C	6	7	8	6	6
Type 7F	7	9	7	9	8
Type 1	8	13	21	12	12
Type 22F	9	8	11	8	9
Type 23F	10	6	6	5	5
Total cases	670	772	876	856	737

As in previous years, Alberta is disproportionately represented in this collection, presumably because of proximity and community awareness of national and provincial surveillance programs. Sixty-one percent (412 of 670 isolates) of the 2000/01 sample is from Alberta. Because of this obvious bias, Table 6 presents Alberta seroprevalence compared with data for isolates submitted from the rest of Canada for the past two years. Ranking is similar for the 5 most common serotypes. Types 3, 7F and 8 rank higher and represent a larger proportion of the total sample from Alberta than from the other provinces.

Table 6. Serotype Distribution in Alberta Compared with the Rest of Canada for 2000/01 & 1999/00
Rank and Percent of the total for 2000/01 (comparable data for 1999/00)

Serotype	Alberta		Other Provinces	
	Rank	Percent of Total	Rank	Percent of Total
Type 14	1 (1)	20.1 (15.5)	1 (1)	15.9 (18.0)
Type 4	2 (3)	10.2 (9.0)	2 (6/7)	11.6 (5.2)
Type 6B	3/4 (2)	7.5 (9.7)	3 (2)	8.9 (11.3)
Type 19F	3/4 (5)	7.5 (6.8)	4 (3)	7.8 (8.7)
Type 9V	5 (4)	6.6 (8.7)	5/6 (5)	7.4 (6.3)
Type 7F	6 (9)	5.8 (4.8)	10/11 (10)	2.7 (3.5)
Type 3	7 (10)	4.6 (3.9)	14 (11)	1.6 (3.3)
Type 18C	8/9 (7)	4.4 (5.8)	7 (6/7)	7.0 (5.2)
Type 8	8/9 (13/14)	4.4 (2.3)	12/13 (NA)	1.9 (0.9)
Type 23F	10/11 (6)	3.6 (6.1)	9 (4)	3.9 (6.5)
Type 6A	10/11 (11)	3.6 (2.9)	12/13 (9)	1.9 (4.3)
Type 22F	12 (8)	3.4 (5.5)	8 (8)	5.0 (4.8)
Other Types		18.3 (19.0)		24.4 (22.0)
Total # cases		412 (310)		258 (462)

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, 2000 - March 31, 2001 are presented in tables 7 and 8 sorted according to patient age. Comparative data for the previous year are not available at this time.

Table 7. Serotype Distribution for Children (≤ 16 years) for April 1/00 - March 31/01

Serotype	≤ 5 years	6 - 16 years	Total ≤ 16 years	
	# Cases	# Cases	# Cases	Rank
Type 14*	60	5	65	1
Type 6B*	33	1	34	2/3
Type 19F*	31	3	34	2/3
Type 4*	14	5	19	4
Type 18C*	16	2	18	5
Type 9V*	14	0	14	6
Type 23F*	11	2	13	7
Type 1	1	10	11	8
Other Types	22	8	30	
Total No. Cases	202	36	238	

* Serotypes included in the heptavalent conjugate vaccines

Eighty-nine percent (179 of 202) of the isolates recovered from children ≤ 5 years of age belong to the seven serotypes which are included in the heptavalent conjugate vaccines.

Table 8. Serotype Distribution for Adults (≥ 17 years) for April 1/00 - March 31/01

Serotype	17-64 years	≥ 65 years	Total ≥ 17 years	
	# Cases	# Cases	# Cases	Rank
Type 14	30	29	59	1
Type 4	35	18	53	2
Type 9V	19	13	32	3
Type 7F	25	4	29	4
Type 8	18	5	23	5
Type 3	13	9	22	6/7
Type 22F	12	10	22	6/7
Type 6B	9	11	20	8
Type 1	17	2	19	9
Type 18C	13	5	18	10
Type 6A	8	9	17	11/12
Type 19F	10	7	17	11/12
Type 12F	10	3	13	13
Type 23F	7	5	12	14
Other Types	45	31	76	
Total No. Cases	271	161	432	

Serotypes 3, 7F, 6A, 8, 12F and 1 are commonly recovered from the adult population, but not from young (≤ 5 yrs) children. The 23-valent vaccine would provide coverage for 90.3% (390 of 432) of these invasive adult isolates, assuming cross-protection for types 6A and 6B.

Thirty-three isolates from cerebrospinal fluid were submitted. These belonged to 14 different serotypes. Ten of the 33 cases had an accompanying blood isolate; the serotype of these isolates always matched the serotype of the CSF isolate. Nineteen isolates (58%) were recovered from patients ≤ 16 years of age and 18 of these were from children ≤ 5 years. Of 14 isolates from the ≥ 17 year old age group, only 3 patients were ≥ 65 . Three of 33 CSF isolates (9.1%) had reduced susceptibility to penicillin.

Streptococcus pneumoniae and Antibiotic Resistance

Because isolates from Alberta account for the majority of this collection, antibiotic resistance data have been analyzed separately in Tables 9 and 10. The proportion of reduced susceptibility to penicillin is similar in Alberta when compared with the rest of Canada, but there are differences when these data are examined by age group. Full resistance to penicillin appears to be more common in isolates recovered from the ≤16 year old age group that were submitted from other provinces than from this age group in Alberta, with the opposite observation for the older age group. This may be a result of sampling bias for isolates received from other Provinces. The size of the sample from Alberta suggests that it is representative of real trends in that Province. The overall rate of reduced susceptibility to penicillin was lower this year compared with the previous fiscal year, however full penicillin resistance is still frequently encountered throughout the country.

Table 9. Reduced Susceptibility to **Penicillin** for Pneumococci from Blood or CSF received from Alberta compared with those from other provinces from April 1, 2000 - March 31, 2001
Comparative data (for all age groups) for April 1, 1999 - March 31, 2000 are presented below the quarterly total: Number of isolates (%)

Age	Alberta				Other Provinces			
	Isolates Rec'd	*Inter-mediate	*Resistant	Total	Isolates Rec'd	*Inter-mediate	*Resistant	Total
≤ 16 years	130	5(3.9)	5(3.9)	10(7.7)	108	3(2.8)	14(13.0)	17(15.7)
≥ 17 years	282	21(7.4)	14(5.0)	35(12.4)	150	11(7.3)	2(1.3)	13(8.7)
Total	412	26(6.3)	19(4.6)	45(10.9)	258	14(5.4)	16(6.2)	30(11.6)
Comparative data for April 1, 1999 - March 31, 2000								
Total	310	26(8.4)	19(6.1)	45(14.5)	462	46(10.0)	26(5.6)	72(15.6)

*Penicillin intermediate resistance = 0.12 - 1.0 µg/ml; resistant = ≥2.0 µg/ml

The rate of intermediate resistance to ceftriaxone is similar to that observed in the previous fiscal year (Table 10). Intermediate resistance to ceftriaxone was demonstrated only in pneumococci with a penicillin MIC ≥ 1.0 mg/L and most commonly in those strains that were fully resistant to penicillin (MIC ≥ 2.0 mg/L). Resistance to ceftriaxone (MIC ≥ 2.0 mg/L) is rarely encountered in Canadian strains. The single isolate with full resistance to ceftriaxone in this report belonged to serotype 9V and had a penicillin MIC of 4.0 mg/L.

Table 10. Reduced Susceptibility to **Ceftriaxone** for Pneumococci from Blood or CSF received from Alberta compared with those from other provinces from April 1, 2000 - March 31, 2001
Comparative data (for all age groups) for April 1, 1999 - March 31, 2000 are presented below the quarterly total: Number of isolates (%)

Age	Alberta				Other Provinces			
	Isolates Rec'd	*Inter-mediate	*Resistant	Total	Isolates Rec'd	*Inter-mediate	*Resistant	Total
≤ 16 years	130	4(3.1)	0(0)	4(3.1)	108	11(10.2)	0(0)	11(10.2)
≥ 17 years	282	13(4.6)	1(0.4)	14(5.0)	150	5(3.3)	0(0)	5(3.3)
Total	412	17(4.2)	1(0.2)	18(4.4)	258	16 (6.2)	0(0)	16 (6.2)
Comparative total data for April 1, 1999 - March 31, 2000								
Total	310	14(4.5)	0(0)	14(4.5)	462	27(5.8)	0(0)	27(5.8)

*Ceftriaxone intermediate resistance = 1.0 $\mu\text{g/ml}$; resistant = ≥ 2.0 $\mu\text{g/ml}$

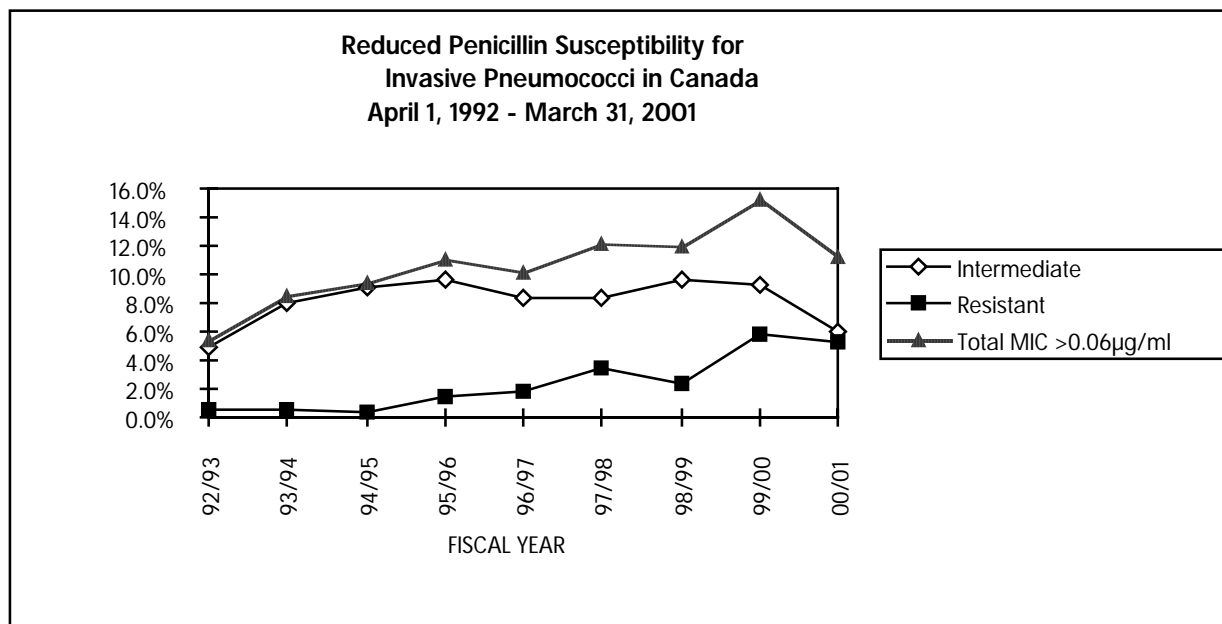
The proportion of reduced susceptibility to penicillin observed for isolates submitted to the NCS between April 1, 1992 and March 31, 2001 is provided in Table 11. Resistance rates for pneumococci submitted from Alberta are compared to those submitted from the rest of Canada for the past five years. From 1996 to 1999, the rate of reduced susceptibility to penicillin was much higher in Alberta than it was in the rest of Canada. Over the past two years the proportion of resistant isolates that we have examined has become similar across Canada.

Table 11. Reduced penicillin susceptibility (MIC >0.06 µg/ml) for isolates from blood or CSF submitted from Alberta and the rest of Canada (TROC) between April 1, 1992 and March 31, 2001
Number of isolates (%)

Fiscal Year	Alberta		TROC		All of Canada	
	Total	>0.06 mg/L (%)	Total	>0.06 mg/L (%)	Total	>0.06 mg/L (%)
Apr 1/00 - Mar 31/01	412	45 (10.9)	258	30 (11.6)	670	75 (11.2)
Apr 1/99 - Mar 31/00	310	45 (14.5)	462	72 (15.6)	772	117 (15.2)
Apr 1/98 - Mar 31/99	204	33 (16.2)	457	46 (10.1)	661	79 (12.0)
Apr 1/97 - Mar 31/98	259	46 (17.8)	493	46 (9.3)	752	92 (12.2)
Apr 1/96 - Mar 31/97	171	28 (16.4)	468	37 (7.9)	639	65 (10.2)
Apr 1/95 - Mar 31/96	Not Available		Not Available		594	66 (11.1)
Apr 1/94 - Mar 31/95	Not Available		Not Available		319	30 (9.4)
Apr 1/93 - Mar 31/94	Not Available		Not Available		162	14 (8.6)
Apr 1/92 - Mar 31/93	Not Available		Not Available		181	10 (5.5)

After steadily increasing rates of reduced susceptibility to penicillin in Canada since 1992, it is reassuring to note a decrease in both intermediate and full resistance over the past year (Figure 4).

Figure 4.



Reduced susceptibility to penicillin was detected in 75 isolates belonging to 9 different serotypes. Ninety-six percent of these (32 of 75) are covered by the 23-valent vaccine. Forty-seven percent (35 of 75) of these strains are fully resistant to penicillin (MIC ≥ 2.0 mg/L); this was observed most frequently for types 9V, 14 and 19F. The level of resistance observed for each serotype and comparative data for 1999 - 2000 are presented in Table 12. Intermediate resistance is presented as two categories to demonstrate an observed trend toward full resistance which is particularly evident for serotype 9V.

Since 1996 we have observed a significant trend toward high level resistance for type 9V in Western Canada and especially in Alberta. This serotype accounts for 63% of the isolates (22 of 35) with reduced susceptibility to penicillin that were received from that province.

Table 12. Serotype Distribution and MIC Range for Isolates with Reduced Penicillin Susceptibility.
Data for 2000-2001 (1999-2000)

Type	Total # Reduced Susceptibility	Intermediate		Resistant ≥ 2.0 mg/L
		0.12 - 0.5 mg/L	1.0 mg/L	
Type 9V*	35 (46)	2 (0)	12 (26)	21 (20)
Type 6B*	13 (15)	6 (7)	4 (4)	3 (4)
Type 19F*	10 (16)	5 (9)	0 (3)	5 (4)
Type 14*	7 (16)	1 (3)	2 (1)	4 (12)
Type 19A*	5 (13)	5 (12)	0 (1)	0 (0)
Type 35B	2 (1)	0 (0)	2 (0)	0 (1)
Type 23F*	1 (5)	0 (2)	0 (0)	1 (3)
Type 6A*	1 (1)	1 (1)	0 (0)	0 (0)
Type 35A	1 (0)	0 (0)	0 (0)	1 (0)
Type 35F	0 (1)	0 (0)	0 (1)	0 (0)
Type 15B*	0 (1)	0 (1)	0 (0)	0 (0)
Type 22F*	0 (1)	0 (1)	0 (0)	0 (0)
Nontypable	0 (1)	0 (1)	0 (0)	0 (0)
Total	75 (117)	20 (37)	20 (36)	35 (44)

*Coverage expected for 23-valent vaccine

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 NCCLS recommended broth microdilution method. Data for Alberta are compared with the rest of Canada in Table 13. As expected, all isolates were susceptible to vancomycin.

Table 13. Resistance to non-beta lactam antibiotics for 670 blood or CSF isolates submitted from Alberta and the rest of Canada (TROC) between April 1, 2000 and March 31, 2001
Number of isolates (%)

Antibiotic	Interpretive Category	Alberta # isolates=412	TROC # isolates=258	Total for Canada # isolates=670
Chloramphenicol	Intermediate Resistant	0 (0) 3 (0.7)	0 (0) 3 (1.2)	0 (0) 6 (0.9)
	Total	3 (0.7)	3 (0.1.2)	6 (0.9)
Clindamycin	Intermediate Resistant	0 (0) 5 (1.2)	0 (0) 2 (0.8)	0 (0) 7 (1.0)
	Total	5 (1.2)	2 (0.8)	7 (1.0)
Erythromycin	Intermediate Resistant	0 (0) 34 (8.3)	1 (0.4) 23 (8.9)	1 (0.2) 57 (8.5)
	Total	34 (8.3)	24 (9.3)	58 (8.7)
Ofloxacin	Intermediate Resistant	11 (2.7) 2 (0.5)	6 (2.3) 1 (0.4)	17 (2.5) 3 (0.4)
	Total	13 (3.2)	7 (2.7)	20 (3.0)
Trimethoprim-Sulfamethoxazole	Intermediate Resistant	18 (4.4) 40 (9.7)	14 (5.4) 24 (9.3)	32 (4.8) 64 (9.6)
	Total	58 (14.1)	38 (14.7)	96 (14.3)

We have defined multiple resistance as intermediate or full resistance to three different classes of antibiotics. Twenty-two of 670 isolates (3.3%) were multiply resistant; 20 of these had reduced susceptibility to penicillin. Multiple resistance was demonstrated in serotypes 6B (9 isolates), 9V (4 isolates), 14 (4 isolates), 19F (3 isolates) and 23F (2 isolates).

It may be clinically important to note that resistance to erythromycin was frequently encountered in penicillin-susceptible pneumococci. Thirty-seven of 58 erythromycin-resistant isolates (64%) were susceptible to penicillin. Resistance to erythromycin was demonstrated most frequently with serotypes 6B, 9V, 12F and 14.

As with erythromycin, susceptibility to trimethoprim-sulfamethoxazole cannot be assumed just because the isolate is susceptible to penicillin. Forty of 96 isolates (42%) with reduced susceptibility to trimethoprim-sulfamethoxazole were susceptible to penicillin.

Our experience with this collection of isolates suggests that reduced susceptibility to ofloxacin was not associated with reduced penicillin susceptibility, nor was it associated with any particular serotype. Sixteen of 17 isolates with intermediate resistance and all 3 isolates that were fully resistant to ofloxacin were susceptible to penicillin.

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 eleven epidemiological investigations conducted in 4 provinces. This was slightly higher than for 1999/2000 when requests for investigation of 9 events were received from 3 provinces and territories. Listed in Table 14 are the investigations conducted during 2000/01.

Table 14. Summary of Epidemiologic Investigations April 1, 2000 - March 31, 2001

Investigation Number	Province Requesting Investigation	Test Request	No. of Samples	Causative Agent
00-505	British Columbia	GAS serotyping investigation of possible nosocomial event	3	M1 T1 OF - (2 of 3 isolates)
00-506	Manitoba	GAS serotyping investigation of necrotizing fasciitis case and contacts	6	M1 T1 OF -
00-507	Ontario	GAS serotyping outbreak investigation in long term care facility	7	M3 T3/B3264 OF -
00-508	Quebec	Pneumococcal serology investigation of increased pneumonia-like illness	8	Variable antibody response; type 19F antibody level highest in 7 of 8 patients
01-500	Ontario	GAS serotyping outbreak investigation in long term care facility	14	M58 T58 OF +
01-501	Ontario	GAS serotyping outbreak investigation in long term care facility	7	M77 T9/13/28 OF + (6 of 7 isolates)
01-502	Ontario	GAS serotyping outbreak investigation in long term care facility	23	M5 T5/27/44 OF -
01-503	Ontario	GAS serotyping outbreak investigation in long term care facility	6	M49 T11/14 OF +
01-504	Ontario	GAS serotyping outbreak investigation in long term care facility	6	Mnt T28 AOF28 OF - (4 of 6 isolates)
01-505	Ontario	GAS serotyping outbreak investigation in long term care facility	17	M75 T25/Imp19 OF + (15 of 17 isolates)
01-506	British Columbia	GAS serotyping investigation of possible nosocomial event	4	Mnt T11/28 OF + (2 of 4 isolates)

d. Research

Table 15 lists completed and/or on-going research projects in which the National Centre for Streptococcus participated during 2000 - 2001.

Streptococcus pneumoniae

Table 15. Summary of Research Projects April 1, 2000 - March 31, 2001

Researcher/Agency	Study Description	Services Required
Pan American Health Organization with funding from the Canadian International Development Agency	SIREVA Project - determination 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. Upton Allen, Hospital for Sick Children, Toronto, Ontario	Pneumococcal Resistance and Associated Serotypes among Caribbean Children	Pneumococcal serotyping
Dr. Don Low, Mount Sinai Hospital, Toronto, Ontario	Quinolone Resistance in Pneumococci	Pneumococcal serotyping
Dr. Alison Bell, Arctic Investigations Program, Anchorage, Alaska	International Circumpolar Surveillance of Invasive Pneumococcal Disease	Pneumococcal serotyping and antibiotic susceptibility testing
NCS in-house study	Investigation of pneumococci with an unusual erythromycin/clindamycin resistance phenotype	Antibiotic susceptibility testing and molecular testing of selected strains

Group A Streptococci (*Streptococcus pyogenes*)

Researcher/Agency	Study Description	Services Required
Dr. James Musser Baylor College of Medicine, Houston, Texas	Investigation of clonal relationships of M1 Invasive GAS in Canada	Serotyping and molecular investigation Collaborative study
Dr. Bernie Beall, Centres for Disease Control, Atlanta, Georgia	<i>emm</i> and <i>sof</i> gene sequence variation in relation to serologic typing of opacity factor positive group A streptococci	GAS serotyping Collaborative study
Dr. Richard Facklam, Centres for Disease Control, Atlanta, Georgia	Validation of new <i>emm</i> sequences to official <i>emm</i> type	GAS serotyping Collaborative study
Dr. Horacio Lopardo, Buenos Aries, Argentina Dr. Bernie Beall, Centres for Disease Control, Atlanta, Georgia	Investigation of invasive and carriage isolates of <i>S. pyogenes</i>	GAS serotyping Collaborative study
Alberta Research Council Edmonton, Alberta	Investigation of laboratory derived strains	GAS serotyping
NCS in-house study	Screening M-nontypable isolates for new M types 83, 89, 90 & 92 in 1992-1999 isolate collection	GAS serotyping
NCS in-house study	Investigation of <i>emm st2967</i>	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 Streptococcus Invasive Disease in Alberta	Group B serotyping
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 <i>Aerococcus</i> species	Identification

e. Training

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

f. Other Highlights

The NCS was invited to speak at an international forum held in Bogotá, Colombia in June, 2000. The conference was entitled 'Quality in Clinical Laboratories' and involved all disciplines of laboratory medicine. We were asked to present an overview of Quality Assurance in the Bacteriology laboratory. The meeting was attended by over 200 delegates from Spain, Mexico, Brazil and Colombia. We were able to share our experience with the implementation of a QA program for the SIREVA project, as well as provide our perspective on laboratory QA in North America.

Quality Indicators Monitored

1. Turn Around Time

The average turn around times (TAT) for the past year are compared with those for 1999/00 in Table 16. Isolates that are associated with designated outbreak investigations are given priority status and the TAT will be significantly reduced from the averages reported here. A staffing increase of 0.5 FTE was partially responsible for a significant improvement in the TAT for GAS and GBS serotyping and for pneumococcal serology.

Table 16. Average Turn Around Time (TAT) for April 1, 2000 - March 31, 2001 compared to 1999/00

Test Request	Apr 1/00 - Mar 31/01 Avg. TAT in days	Apr 1/99 - Mar 31/00 Avg. TAT in days
Group A Serotyping	13	22
Group B Serotyping	11	27
<i>Streptococcus pneumoniae</i> Serotyping	14	12
Identification	14	12
Pneumococcal Serology	19	34

2. Proficiency Testing Programs

We continue to participate in 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. This program is currently being coordinated through the PHLS Central Public Health Laboratory in London, England and is being supported, at least in part, by funding provided by the World Health Organization. In addition to serological classification, this program provides a mechanism for validating *emm* gene typing. Because of this additional quality assurance tool, participation has grown to include Streptococcus Laboratories in Italy, Germany, Russia and Malaysia. There continues to be excellent agreement worldwide between the serological methods and *emm* sequence based methods for classification of group A streptococci.

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 an improvement over the serotyping correlation of 85-100% that was obtained for the three distributions in 1999/00. There were two instances for typable isolates which failed to type after multiple passages prior to distribution. This event served as a valuable reminder to submitting laboratories that subculturing should be kept to a minimum in order to optimize the serotyping procedure. The three laboratories achieved 96-100% correlation with MIC values within +/- one log₂ dilution for nine antibiotics, and compares with 1999/00 correlation of 95-100%. We look forward to continued participation 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. Complete the manuscript describing our investigation of GAS *emm st2967* and our proposal that this type should gain official international status as a provisional M type.
2. Complete the manuscript reporting our experience with M type distribution in Canada over the past 8 years.
3. Draft a manuscript describing our experience with the SIREVA project and implementation of a Quality Assurance in Latin America
4. Complete the investigation of pneumococci with an unusual erythromycin/clindamycin resistance phenotype.
5. Continue *Aerococcus* investigation in collaboration with Dr. Richard Facklam, CDC, Atlanta, Georgia
6. Initiate molecular investigation of non-typable invasive group B streptococci.

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8. Efstratiou, A., R.R. Facklam, E.L. Kaplan, P. Kriz, **M. Lovgren**, A. Totolian, B. Beall, D. Johnson, R.C. George, S. Gowan, **G. Tyrrell** and D. Martin. 2000. Report of an international quality assurance programme for group A streptococcal characterization. In Streptococci and streptococcal diseases entering the new millennium; proceedings of the XIV Lancefield International Symposium on Streptococci and Streptococcal Diseases. Auckland, New Zealand.
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