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BACTERIOLOGY

INVASIVE INFECTIONS

The number of isolates received from cases of invasive disease caused by *Haemophilus influenzae, Neisseria meningitidis, Streptococcus pneumoniae, Streptococcus pyogenes* (Group A) and *Streptococcus agalactiae* (Group B) during January to December 2000 are shown in Table 1.

Table 1. Sterile site isolates, 2000

Organism	BC	CSF or CSF/BC	Other sterile site	Total
H. influenzae[1]	47	3	1	51
N. meningitidis	176	75	5	256
S. pneumoniae	442	18	6	466
S. pyogenes	108	1	18	127
S. agalactiae	58	4	7	69

[1] *H.influenzae*: 10 serotype b and 41 others.

The age profile of the patients from whom the isolates were obtained is given in Table 2.

Table 2. Age distribution of cases of invasive disease, 2000

Organism	<1m	1-11m	1y	2y	3y	4y	5-9y	10-24y	25-59y	≥60y
H. influenzae b	0	3	1	1	1	0	2	0	0	2
H. influenzae non b[1]	5	1	1	0	0	1	5	5	9	13
N. meningitidis	1	45	31	25	9	16	22	75	26	6
S. pneumoniae	1	56	49	18	10	8	19	28	95	182
S. pyogenes[1]	1	5	5	1	1	2	4	5	50	51
S. agalactiae	22	4	1	0	0	0	0	6	17	19

[1] Information on age was not provided with one isolate of *H. influenzae* and two isolates of *S. pyogenes*.

Haemophilus influenzae

Haemophilus influenzae serotype b (Hib) disease is notifiable. Since vaccination against this disease became available it has become particularly important to identify the serotypes of *Haemophilus influenzae* that are causing invasive disease. Laboratory data is matched with notification data to ensure that all cases from which an isolate has been received are represented in the notifiable disease dataset.

Isolates were received from 51 cases of *Haemophilus influenzae* invasive disease in 2000. Ten (19.6%) of these isolates were serotype b, including one isolate that was not serotypable with antisera but was shown to possess the genes for capsular expression. One isolate was serotype c, one was serotype e, three were serotype f, and the others were non-serotypable using serotype-specific antisera. This compares with nine serotype b (20.5%) out of a total of 44 viable organisms in 1999. There has been no increase in the number of *Haemophilus influenzae* serotype b isolates referred from cases over the last four years. Isolates that were non-serotypable were tested by PCR for the presence of the serotype b specific *cap* gene and the *bexA* gene necessary for capsular expression.

The antimicrobial susceptibilities of these isolates are reported in the Antibiotic Resistance section of this issue of LabLink.

Neisseria meningitidis

Culture-confirmed cases of meningococcal disease are those from whom a meningococcus has been isolated from a sterile site. Meningococci received at ESR from cases are serogrouped, and then serotyped and subtyped using monoclonal antibodies prepared against the following serotypes and subtypes:

serotype 1, 2a, 2b, 4, 14 and 15

subtypes P1.1, P1.2, P1.4, P1.5, P1.6, P1.7, P1.9, P1.10, P1.12, P1.13, P.14, P1.15 and P 1.16.

Subtyping by amplification of the *porA* gene is undertaken on organisms which are not serosubtypable using monoclonal antibodies. The *porA* gene encodes subtype-specific antigens. Restriction patterns obtained by digestion of the PCR product enables prediction of the subtype, which is then confirmed by DNA-DNA hybridisation for subtypes P1.2, P1.4, P1.7 and P1.16 only.

Confirmation of meningococcal disease can also be made by demonstration of meningococcal DNA in specimens of blood, CSF or tissue aspirates. The same *porA* PCR test is used to detect meningococcal DNA directly in patient specimens. It should be noted that on the surface of meningococci the P1.7b epitope is inaccessible to monoclonal antibodies and goes unrecognised, whereas the sequence-specific probes are able to detect sequences encoding this epitope. Thus, the serosubtype P1.4 detected by monoclonal antibodies equates with the sequence-specific subtype P1.7b, 4 detected by DNA-DNA hybridisation.

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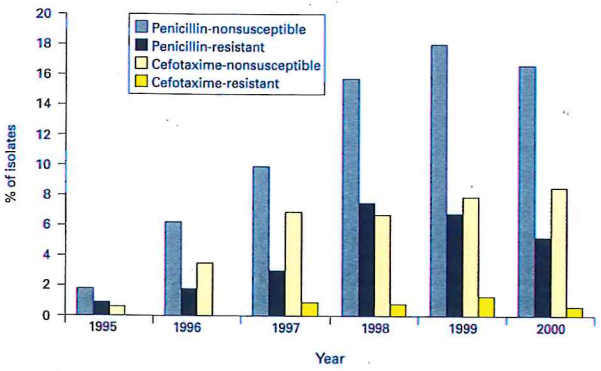
Figure 10. Penicillin and cefotaxime nonsusceptibility among pneumococci from invasive disease, 1995-2000



were tested for susceptibilities in 2000, 8 (15.7%) were ampicillin resistant and 2 (3.8%) were resistant to amoxycillin/clavulanate. All isolates were sensitive to cefotaxime, chloramphenicol and rifampicin.

Neisseria meningitidis

A total of 255 *N. meningitidis* isolates from invasive disease that were referred to ESR were tested for susceptibility to penicillin, ceftriaxone, ciprofloxacin and rifampicin. Reduced penicillin susceptibility (MIC 0.12-0.25 mg/L) occurred in 7.1% (18/255) of the isolates compared with 18.5% (24/130) of isolates that were tested in 1999. The proportion of isolates with reduced penicillin susceptibility had varied from 0.0% to 7.4% between 1991 and 1998. All isolates were sensitive to ceftriaxone, ciprofloxacin and rifampicin.

VIROLOGY

Table 22 summarises viral identification and mycoplasma infections in New Zealand in 2000. The information is based on weekly data collated from the five virology laboratories in Auckland, Waikato, Canterbury, Otago, and ESR.

RESPIRATORY VIRUSES

Influenza virus

Influenza activity during January to December 2000 was relatively low. (Figures 11 & 12). A total of 298 influenza isolates from all sources were identified in 2000 by ESR, Auckland and Canterbury virology laboratories, including swabs from sentinel surveillance. This compares with 816 isolates in 1999. As usual, most isolations (184) occurred during the sentinel surveillance period (May to September - see Lablink 2000; 7(4): 34). However, the peak of activity in week 38 was very late and a large number of isolates, 107 in total, were identified during October to December.

In 2000, there were 82 (28%) isolations of A(H1N1) and this was the predominant strain. There have been two antigenically distinct lines of influenza A(H1N1) circulating around the world in recent years , and the current

Table 22. Summary of virus identification and mycoplasma infections, 2000

	Jan	Feb	Mar	Apr	May	Jun	Jul	Aug	Sep	Oct	Nov	Dec	Total
Influenza (total)	1	6	0	0	5	8	15	68	88	66	30	11	298
Influenza A (not subtyped)	1	6	0	0	0	1	2	8	13	19	15	5	70
Influenza A H3N2	0	0	0	0	2	0	0	17	33	21	0	2	75
Influenza A H1N1	0	0	0	0	1	2	11	34	25	7	2	0	82
Influenza B	0	0	0	0	2	5	2	9	17	19	13	4	71
Parainfluenza 1	0	0	1	4	6	9	9	2	0	1	0	1	33
Parainfluenza 2	0	0	1	3	2	6	0	0	0	0	0	0	12
Parainfluenza 3	1	0	0	1	0	1	7	13	18	8	5	6	60
RSV	5	4	1	10	17	165	307	243	75	13	0	0	840
Rhino	5	5	2	9	8	10	6	4	0	2	8	3	62
Measles	0	0	0	0	0	0	0	5	0	3	0	1	9
Mumps	1	2	0	0	0	0	0	0	0	2	1	0	6
Rubella	0	0	0	0	0	0	0	0	0	0	0	0	0
Varicella Zoster	7	9	6	4	2	2	14	11	6	2	11	20	94
CMV	3	3	2	0	0	1	0	1	1	1	0	3	15
Mycoplasma	2	0	0	0	0	0	0	0	11	13	7	11	44
Adenovirus	2	2	5	3	1	16	10	29	19	13	30	23	153
Adeno type 1	2	0	2	0	1	0	3	0	1	2	5	2	18
Adeno type 2	1	1	0	0	0	1	0	1	2	3	1	1	11
Adeno type 3	3	1	0	0	0	2	1	7	1	1	0	1	17
Adeno type 4	1	0	1	0	0	0	0	0	0	0	0	0	2
Adeno type 5	0	0	2	0	1	0	1	0	1	0	2	0	7
Adeno type 7	0	0	0	0	0	1	0	0	0	0	0	0	1
Adeno type 8	1	0	0	0	0	1	0	0	0	0	0	1	3
Adeno type 9	0	0	0	0	0	0	0	0	0	1	0	0	1
Adeno type 10	0	0	0	0	0	0	0	1	0	0	0	1	2
Adeno type 11	1	0	0	1	0	0	0	0	1	1	0	0	4
Adeno type 13	0	0	0	0	0	0	0	0	3	0	0	0	3
Adeno type 15	0	1	0	0	1	0	1	0	1	0	0	0	4
Adeno type 17	0	2	0	1	0	0	0	0	0	0	0	0	3
Adeno type 18	0	0	0	0	0	0	0	0	0	0	0	1	1
Adeno type 19	0	0	3	1	0	0	1	0	0	0	0	0	5
Adeno type 21	0	0	0	0	0	1	0	0	0	2	0	2	5
Adeno type 22	0	0	0	0	0	0	0	0	0	0	0	2	2
Adeno type 29	0	0	1	0	0	0	0	0	0	0	0	0	1
Enterovirus	4	1	10	2	20	27	14	29	39	24	18	15	203
Polio 1	0	1	4	1	1	4	2	3	5	0	2	0	23
Polio 2	0	0	0	0	1	1	0	1	2	0	0	0	5
Polio 3	0	1	0	0	0	0	0	1	0	1	0	0	3
Coxsackie B2	3	0	0	3	1	2	0	0	0	1	0	0	10
Coxsackie B4	0	1	0	1	0	0	0	0	0	0	0	0	2
Coxsackie A6	0	0	0	0	0	1	0	0	0	1	0	0	2
Coxsackie A10	0	0	0	0	0	0	0	0	0	1	1	0	2
Coxsackie A16	0	0	0	0	0	0	0	0	0	0	1	0	1
Coxsackie A21	0	0	0	0	0	0	0	1	0	0	2	0	3
Echo 3	0	0	1	0	0	0	0	0	0	0	0	0	1
Echo 7	0	0	0	0	0	0	0	0	0	1	3	5	9
Echo 9	1	0	0	0	0	0	0	0	0	0	0	0	1
Echo 11	0	0	0	0	1	1	0	0	0	0	0	0	2
Echo 30	0	0	0	0	1	1	1	0	2	2	1	3	11
Echo 33	0	0	1	10	22	12	8	13	2	1	1	0	70
Enterovirus 71	0	0	0	0	1	0	0	0	0	0	0	0	1

Figure 13. Annual laboratory-confirmed RSV cases, 1990-2000

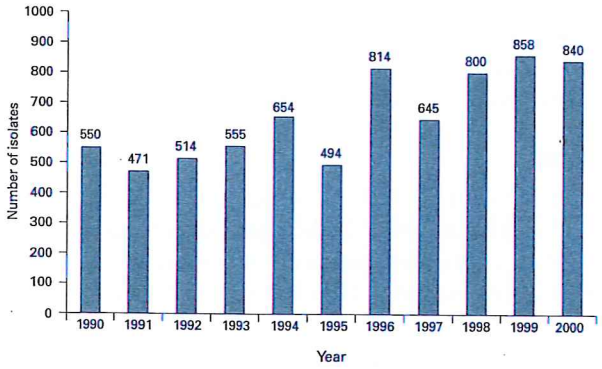
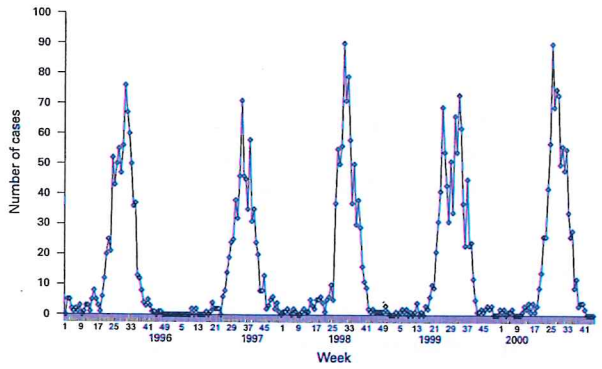


Figure 14. RSV laboratory-confirmed cases by week, 1996-2000



ENTEROVIRUSES

As previously reported in Lablink (2000 7(4):29-30), New Zealand experienced a large echovirus type 33 outbreak in 2000 with 70 confirmed cases (mainly meningitis).

Eleven isolations of E30 were reported in 2000, between May to December, from Waikato (7), Auckland (3) and Wellington (1). This is in contrast to only two isolations of E30 in 1999.

Ten isolations of CB2 were reported in 2000, between January to October, from Wellington (5), Auckland (2), Dunedin (2) and Christchurch (1). This is in contrast to no isolations at all in 1999. Clinical features ranged from respiratory illness to meningitis, and there was one death.

Nine patients with laboratory-confirmed E7 were reported in 2000, between August to December, compared with only one case in 1999. The first case in 2000, a 36 year old female, occurred in Auckland in August. Subsequently, eight more cases were reported from Waikato (3), Wellington (1), Auckland (3) and Palmerston North (1). The age of cases ranged from one month to 36 years old, and most were children. The range of clinical manifestations included sore throat, persistent mouth ulcers, sepsis and aseptic meningitis.

ADENOVIRUSES

There were a total of 153 adenovirus isolations in 2000, compared with 105 in 1999. The predominant serotypes in 2000 were adenovirus type 1 (18 isolates, 11.8%), adenovirus type 3 (17 isolates, 11.1%) and adenovirus type 2 (11 isolates, 7.2%). These serotypes also predominated in 1999, with 43 isolations of adenovirus type 3 (41.0%), 18 of adenovirus type 1(17.1%) and 16 of adenovirus type 2 (15.2%).

HEPATITIS VIRUSES

Results from hepatitis B virus (HBV), hepatitis C virus (HCV) and hepatitis D virus (HDV) testing on serum and plasma samples at ESR are now being included in Lablink.

Hepatitis B virus

Hepatitis B surface antigen (HBsAg) neutralisations were performed on 132 samples in 2000. Of 36 blood donor samples, 30 (83%) were confirmed reactive. Of 96 clinical samples, 39 (41%) were confirmed HBsAg reactive by neutralisation.

There were 374 HBV DNA PCR tests requested. Using an in-house nested PCR, which has a lower detection limit of 1000 HBV DNA copies/mL, qualitative assays were performed on 197 samples, of which 99 (50%) had detectable DNA. A quantitative assay is performed instead if the patient is receiving anti-virals and being monitored by a specialist. Using the Amplicor HBV monitor™ test, quantitative assays were performed on 177 samples, of which 144 (81%) had detectable DNA. This assay has a lower detection limit of 400 HBV DNA copies/mL.

Hepatitis C virus

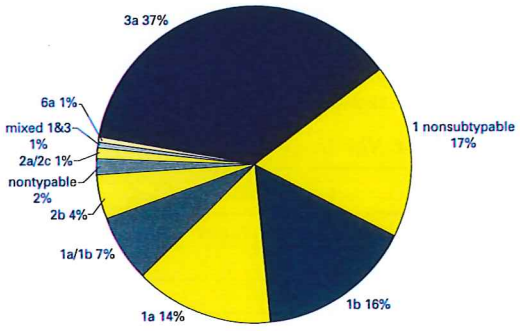
A total of 881 samples were referred to ESR for anti-HCV supplementary ('confirmatory') testing in 2000. As there is no true confirmatory test available for hepatitis C, samples are 'confirmed' using an alternative EIA and/or immunoblot antibody test. The Murex™ anti-HCV (version 4) was used as the EIA. The Chiron™ RIBA™ HCV (version 3) immunoblot assay was used as a supplementary assay when the antibody levels were low or discrepant. The consensus of all assays provides a method for determining a true anti-HCV positive result. Blood donor samples are tested by the immunoblot assay only. A total of 665 clinical samples were received, of which 63% were confirmed reactive, 18% were indeterminate, and 19% were resolved as negative in the supplementary antibody tests performed. A total of 216 blood donor samples were received, of which 21% were confirmed reactive, 27% indeterminate and 52% were resolved as negative.

HCV RNA PCR tests can also be used as a method of HCV 'confirmation', and 891 samples were tested in this way by ESR in 2000. Out of 717 clinical samples, 373 (52%) were positive, and from 174 blood donor samples 37 (21%) were positive.

HCV genotyping was performed on samples from 183 HCV infected patients by ESR in 2000. The discrimination of HCV genotype is important as it is linked to the efficacy of anti-viral treatment. Genotypes were classified using the INNO-LiPA™ line probe assay, based on the principle of reversed hybridisation. The reactivity of the amplified fragment with one or more of the genotype-specific probes on the strip allows recognition of the 6 major HCV genotypes and their most common subtypes. As depicted in Figure 15, genotype 1 was the most predominant, present in 54% of patients. Of these, 30% were subtypable, almost evenly divided between subtypes 1a and 1b. Type 3 was the next most frequent genotype (37%), all being subtype 3a. Type

2 was the third most frequent genotype (5%), predominantly subtype 2b (4%). One patient had subtype 6a and another patient showed mixed infections with types 1 and 3. Three patients had inconclusive typing results.

Figure 15. HCV genotyping 2000 (n = 183)



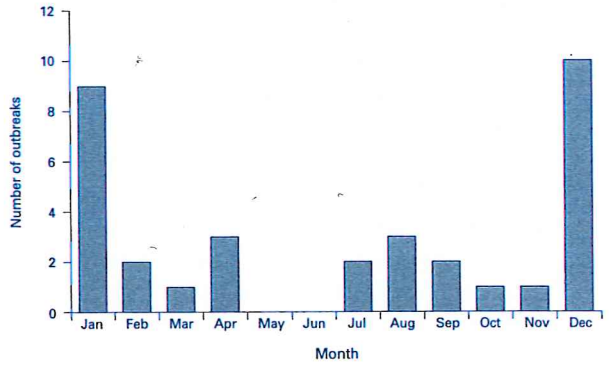
Hepatitis D virus

Anti-Delta testing was performed on 58 samples in 2000, using the Abbott™ Anti-Delta EIA. Two samples tested reactive.

NORWALK-LIKE VIRUSES

Thirty-four laboratory-confirmed Norwalk-like virus (NLV) outbreaks were recorded by health authorities during 2000. Most of these outbreaks (16, 47%) were associated with foodborne disease and occurred in restaurants or catered event settings. Person to person transmission accounted for 11 outbreaks (32%), hospitals and rest homes a further 6 (18%). One outbreak in a school camp was reported. The seasonal distribution of NLV outbreaks is shown in Figure 16. As in previous years, the majority of outbreaks (22, 63%) occurred during the summer holiday period. Several foodborne outbreaks were associated with Christmas functions.

Figure 16. Seasonal distribution of NLV outbreaks during 2000



The predominant NLV genotypes were GII/1,4 (the 'global strain' cluster), GII/3 'Mexico-like virus' and a cluster of GII/6,7,9 strains, some of which belong to the GII/6 'Napier virus' cluster, which has not been identified outside New Zealand (Table 24). Only one Genogroup I strain has been identified during 2000. However, one strain currently being sequenced also appears to belong to Genogroup I. For one large food-associated outbreak, several cases and a foodhandler were all infected with identical NLV GII/6,7,9 strains, indicating a common source of infection.

Table 24. NLV Genotypes occurring in 2000

NLV Strain	Genotype	Number (%)
Lordsdale virus 'Global strain' cluster	GII/1,4,8	12 (35)
Mexico-like virus	GII/3	9 (26)
Napier virus	GII/6	5 (15)
Florida / Gwynedd / Idaho Falls virus cluster	GII/6,7,9	6 (18)
'Cruise Ship Virus'	GI/4	1(3)
Not sequenced	GI	1 (3)
Total		34 (100)

ARBOVIRUSES

Dengue fever virus

During 2000 ESR screened 170 samples for both IgM and IgG antibodies to Dengue fever virus. Seven samples were reactive to only IgG antibodies thus indicating possible past infection with Dengue fever virus or another flavivirus.

Two samples were reactive for IgM and IgG antibodies and were confirmed as recent infections with Dengue fever virus Type 3. One case was working in Sarawak and returned to New Zealand on leave. There was no travel information available for the second case.

A total of seven cases of Dengue fever were notified during 2000. Travel details were known on six of the seven cases. Two had returned from Fiji; the other four had been to East Timor, Queensland, and Indonesia.

Ross River virus

Eighty-one samples were screened for both IgM and IgG antibodies to Ross River virus. Two samples were reactive for IgM antibodies and were confirmed as recent infections with Ross River virus. No convalescent samples were sent, therefore seroconversion could not be demonstrated. Both patients had a history of overseas travel, one to Australia and the other to Fiji.

Barmah Forest virus

Eighty-one samples were screened for both IgM and IgG antibodies to Barmah Forest virus. No samples were reactive and no cases of Barmah Forest virus infection were notified in 2000.

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