

LEPTOSPIROSIS IN NEW ZEALAND: EPIDEMIOLOGY AND DIAGNOSIS

Leptospirosis

Leptospirosis is the most commonly notified zoonotic disease in New Zealand. The disease was first described in the late 1800s by Weil, a German scientist, and was called Weil's disease.¹ A relationship between bathing, water, sewage, rats, occupations, and seasons of the year to outbreaks of Weil's disease was noted, and in 1915 German and Japanese scientists independently identified the mode of infection and described the organism.¹ During the ensuing years the animal reservoirs and transmission routes were identified.¹

Description of the pathogen

Leptospira belongs to the order Spirochaetales and family Leptospiraceae and is closely related to *Leptonema*, *Borrelia* and *Treponema*.^{1,2} The genus *Leptospira* is divided into seven pathogenic and several non-pathogenic species and further subdivided into serogroups and serovars.^{1,2} Currently, approximately 230 serovars are described worldwide.^{1,2} Only seven serovars have been isolated from human cases in New Zealand and six have been isolated from animals (Table 1). The mode of transmission to humans is by direct contact with contaminated animal urine and/or water and soil contaminated by animal urine. *Leptospira* enter the body through cuts and abrasions on the hands and feet and through the mucous membranes and conjunctivae.^{1,2}

Table 1. *Leptospira* serovars isolated from animal reservoirs and/or humans in New Zealand³

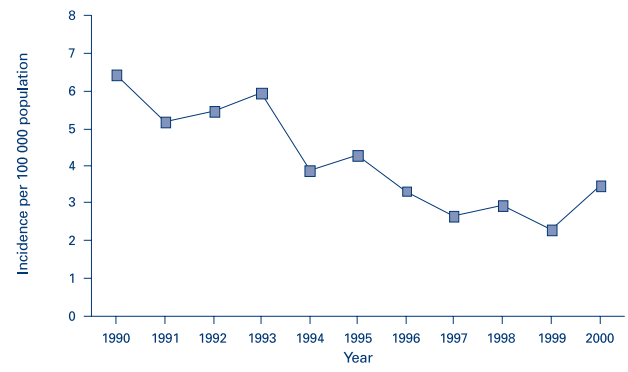
Serovar	Animal Reservoirs	Human Isolates
<i>L. borgpetersenii</i> sv hardjo	Cattle, sheep, deer	Yes
<i>L. interrogans</i> sv pomona	Pigs, cattle, sheep, deer	Yes
<i>L. borgpetersenii</i> sv tarassovi	Pigs	Yes
<i>L. interrogans</i> sv copenhagenii	Rodents, dogs	Yes
<i>L. borgpetersenii</i> sv ballum	Hedgehogs, rodents, pigs	Yes
<i>L. interrogans</i> sv canicola ¹		Yes
<i>L. interrogans</i> sv australis ¹		Yes
<i>L. borgpetersenii</i> sv balcanica	Possum	None

¹ Never isolated from animals in New Zealand; reservoirs in other countries are dogs and cattle, respectively

Epidemiology of leptospirosis

The incidence of leptospirosis has decreased since the highs of the 1970s of around 20 cases per 100,000, to 6.5 cases per 100,000 in the early 1990s, and to 3.5 cases per 100,000 in 2000 (Figure 1).^{3,4} Most cases in New Zealand represent occupational acquisition of leptospirosis. The average annual

Figure 1. Incidence of Leptospirosis, 1990-2000^{3,4}

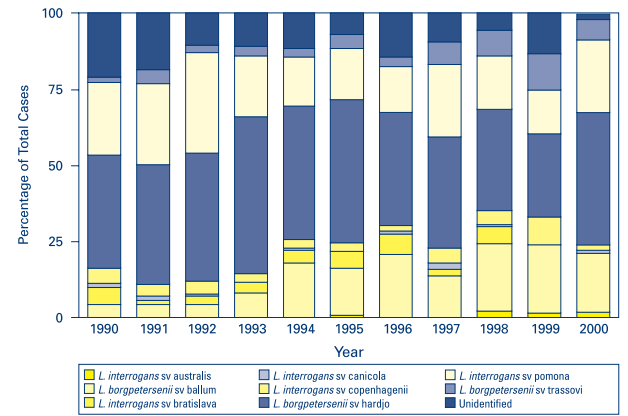


incidence rate for specific occupational groups between 1990-1998 has been calculated to be:⁴

- meat workers – 165 cases per 100,000 per year.
- livestock farmers – 92 cases per 100,000 per year.
- forestry related occupations – 24 cases per 100,000 per year.
- other occupations – 1 case per 100,000 per year.

In 2001, the Occupational Safety and Health Service, Department of Labour, released *Guidelines for the Control of Occupationally Acquired Leptospirosis*. Copies of these guidelines are available from their website (www.osh.dol.govt.nz). Serovar distribution has been changing between 1990 and 2000.^{3,4} The proportion of infections caused by *L. borgpetersenii* sv ballum and *L. borgpetersenii* sv tarassovi

Figure 2. Proportion of cases caused by each *Leptospira* species and serovar, 1990-2000^{3,4}



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have increased over the last 10 years (Figure 2). *L. interrogans* sv australis has also been identified more frequently in the last three years. There has been little change in the proportion of cases caused by *L. borgpetersenii* sv hardjo and *L. interrogans* sv pomona.^{3,4}

Clinical features of leptospirosis

The incubation period is commonly 5-14 days, but ranges from 2-30 days.¹ Disease in humans varies in severity according to the infecting serovar and the age, health, and nutrition of the patient.¹ Leptospirosis ranges from a mild sub-clinical illness to either a self-limited systemic illness (90% of cases) or a severe, potentially fatal condition accompanied by multi-organ failure. The predominant early features are: sudden onset of headache, muscle pain and tenderness, fever, rigors, nausea, conjunctival suffusion, a transient skin and mucosal rash, photophobia and other signs of meningism. Severe cases may progress to renal failure, as well as pulmonary complications and respiratory failure. The mortality rate in severe disease is reported to be approximately 5-40%.¹

Case definition of leptospirosis

The case definition currently in use in New Zealand is:⁵

Clinical description: An illness characterised by fever, headaches, chills, myalgia, conjunctival suffusion, and less frequently meningitis, jaundice, or renal insufficiency.

Laboratory test for diagnosis:

Isolation of leptospirae from a clinical specimen, or

A fourfold or greater rise in leptospiral microscopic agglutination titre (MAT) between acute and convalescent sera, or

A single high titre of ≥ 800 in the MAT

Case classification:

Probable - A clinically compatible illness with a single raised titre of ≥ 400 in MAT.

Confirmed - A clinically compatible illness that is laboratory-confirmed by isolation of leptospira or fourfold or greater rise in MAT or a single high titre of ≥ 800 in the MAT.

Laboratory diagnosis of leptospirosis

1. Isolation of the organism

Isolation of leptospira from clinical specimens provides the definitive diagnosis of leptospirosis.^{1,2} Leptospira are fastidious organisms and are difficult to isolate. Blood, CSF and urine may yield the organism during the first 10 days of the illness. Specimens must not be refrigerated before culture and culture media must be warmed.^{1,2}

(1) **Blood:** Leptospira are usually found in the blood for 7-14 days after infection.^{1,2} It is recommended that two tubes of EMJH (Ellinghausen McCollough Johnson Harris media) culture media be inoculated, one receiving 2 drops of whole blood and the other 5 drops (too much blood can be inhibitory). If the specimen has clotted, remove the serum and dissect the clot into 1 mm cubes. Place a small piece of the clot into the culture medium.^{1,2}

(2) **Urine:** After the first week of illness Leptospira may be shed in the urine for up to 30 days.^{1,2} Since Leptospira are shed intermittently, two or three samples should be taken over several days if possible. Aseptically collect a mid-stream urine sample (**not** the first sample in the morning). As soon as possible transfer 3 drops and 1 ml into separate culture media bottles (EMJH) or semi-solid media bottles (EMJH + 0.15% agar) (make sure the media contains antibiotic, as overgrowth with contaminating organisms is common).^{1,2}

(3) **CSF:** In the first 5-10 days of illness Leptospira can be cultured from CSF; 0.5 ml of CSF should be inoculated into culture EMJH or semi-solid culture EMJH media.^{1,2}

Media should be checked initially for growth or contamination after 1, 3 and 5 days. Cultures should be viewed using darkfield microscopy at weekly intervals for six weeks and sub-cultured if growth is observed. Isolates should be sent to the Leptospira Reference Laboratory, ESR (please send unrefrigerated) and are identified using a reverse MAT. If ESR cannot identify the isolate, it is sent to one of the WHO Leptospira laboratories for identification. This can take up to several months as the isolate is inoculated into rabbits for identification.

2. Antibody detection

Several kits exist for the detection of early antibodies in leptospirosis.^{1,2} One of these, the dipstick test developed by the WHO Leptospira laboratory in Amsterdam, was validated in a multi centre validation trial in which ESR took part. This test has been shown to have good specificity and sensitivity. However, the antibody screen result needs to be confirmed using MAT, so that the infecting serovar can be identified. An acute serum sample needs to be taken in the first week of the illness, and sometimes several convalescent serum samples need to be taken to show a seroconversion.^{1,2} The timing of convalescent samples is important. The first convalescent sample needs to be taken at least 2-3 weeks after the acute sample, as titres should peak at this time. A second convalescent sample is taken 1-2 weeks later, which may show a fall in titres, and can be used for the identification of the infecting serovar.^{1,2} The second sample is important as early on in the disease cross-reacting antibodies may be present, making it difficult for the infecting serovar to be identified.

3. PCR technology

PCR has successfully been used in the diagnosis of leptospirosis. Two main primer sets exist. The first is the G1/G2 set that was developed by the Amsterdam WHO Leptospira laboratory.^{6,7} A more recent set of primers uses the conserved 16S rRNA gene and amplifies a fragment of this which is then probed to confirm the amplification.² Alternatively, the amplified product can be sequenced to reveal the species identification of the infection.² ESR has evaluated this method and found the detection limit with probing to be a lot more sensitive than the conventional PCR. A drawback of these methods is that the infecting serovar cannot be identified, but they can be used to establish an early diagnosis. An outline of each method is available upon request from ESR. The samples that can be used for PCR are the same as for isolation, except that samples can be refrigerated but blood should not contain heparin.²

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References:

1. Faine S, B Adler, C Bolin, et al. *Leptospira* and Leptospirosis. 1999: MediSci.
2. Postic D, F Merien, P Perolat, et al. Biological Diagnosis Leptospirosis - Lyme Borreliosis. 2000: Institut Pasteur.
3. Maas EW, Unpublished data. 2001.
4. Thornley CN, Human Leptospirosis in New Zealand: Surveillance, Epidemiology, and Prevention, in Public Health Medicine. 2000, University of Otago: Wellington.
5. Communicable disease control manual. Wellington: Public Health Group Ministry of Health, 1998.
6. Gravekamp C, H Van de Kemp, M Franzen, et al. Detection of seven species of pathogenic leptospirae by PCR using two sets of primers. J Gen Microbiol, 1993. **139**: p. 1691-700.
7. Brown PD, C Gravekamp, DG Carrington, et al. Evaluation of the polymerase chain reaction for early diagnosis of leptospirosis. J Med Microbiol, 1995. **43**(2): p. 110-4.

BACTERIOLOGY

INVASIVE INFECTIONS

Numbers 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 March 2001, are shown in Table 2.

Table 2. Sterile site isolates, January-March 2001

Organism	BC	CSF or CSF/BC	Other Sterile Site	Total
<i>H. influenzae</i> ¹	5	3	1	9
<i>N. meningitidis</i>	31	14	1	46
<i>S. pneumoniae</i>	59	2	0	61
<i>S. pyogenes</i>	26	0	3	29
<i>S. agalactiae</i>	19	2	2	23

¹ *H. influenzae*: 1 serotype b and 8 non-b

The age profile of the patients from whom the isolates were obtained is given in Table 3.

Table 3. Age distribution of cases of invasive disease, January-March 2001

Organism	<1m	1-11m	1y	2y	3y	4y	5-9y	10-24y	25-59y	≥60y
<i>H. influenzae</i> b	0	0	0	0	0	0	0	0	1	0
<i>H. influenzae</i> non b	0	1	0	1	0	0	1	1	2	2
<i>N. meningitidis</i>	0	7	6	9	1	1	8	9	5	0
<i>S. pneumoniae</i>	0	6	9	2	4	0	1	3	15	21
<i>S. pyogenes</i>	0	1	1	1	0	0	1	6	9	10
<i>S. agalactiae</i> ¹	8	3	0	0	0	0	0	1	4	6

¹ Information on age was not provided with one isolate of *S. agalactiae*

Haemophilus influenzae

During January to March 2001, nine isolates were received from cases of *H. influenzae* invasive disease. One of these isolates was serotype b, one was serotype f, and the others were non-serotypable using serotype-specific antisera. This compares with three serotype b from a total of nine isolates for the same period last year.

None of the non-serotypable organisms was shown by PCR to possess either the *bexA* gene necessary for capsular expression or the serotype b specific *cap* gene.

Neisseria meningitidis

During January to March 2001, a total of 46 sterile site isolates were received from cases of meningococcal disease, compared with 33 for the same period last year. Of these, 41 were serogroup B, three were serogroup C, and two were serogroup Y. Serotyping and serosubtyping results of the serogroup B and C organisms are given in Table 4. The serogroup Y isolates were Y:14:P1.5 and Y:1:P1.5,2. In addition, one isolate was received from a respiratory site from a notified case. This was B:4:P1.4.

The three non-subtypable isolates were shown by *porA* PCR and DNA-DNA hybridisation not to be P1.2, P1.4 or P1.7.

All meningococci were tested 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, P1.14, P1.15, and P 1.16.

Nineteen blood, CSF, and post-mortem samples from culture-negative cases of invasive meningococcal disease were tested by PCR for the presence of meningococcal DNA. Fifteen samples were shown to contain the meningococcal *porA* gene, which encodes the subtype-specific antigens. Dot blot

Table 4. Serotypes and subtypes of *N. meningitidis*, January-March 2001

Subtype	Serotype						Total	
	1	2a	2b	4	14	15		NT
<i>Serogroup B</i>								
P1.4				31	2		6	39
NST				1			1	2
Total	0	0	0	32	2	0	7	41
<i>Serogroup C</i>								
P1.5		1						1
P1.13				1				1
NST							1	1
Total	0	1	0	1	0	0	1	3

NT - non typable

NST - non subtypable

hybridisation showed that 12 of these samples were subtype P1.7,4 and three were negative with probes for subtypes P1.2, P1.4 and P1.7.

The current testing shows that the epidemic strain (B:4:P1.4) continues to cause most disease.

LEGIONELLA

During January to March 2001, 15 cases of legionellosis were laboratory-confirmed, and a further five cases were notified based on clinical grounds only. Ten of the laboratory-confirmed cases fitted the "confirmed" case definition and five fitted the "probable" case definition. This compares with six confirmed and four probable cases in the same period last year. Ten (67%) of the laboratory-confirmed cases were notified compared to a 70% notification rate last year. The majority (65%) of cases were male, and nine (45%) of the cases were aged 60 years and over.

The infecting *Legionella* species and serogroup was identified in 14 laboratory-confirmed cases but could not be identified in one case (Table 5). In ten (67%) cases the infecting species was identified as *L. longbeachae*. This compares with 40% of cases caused by this species in the same period last year. *L. longbeachae* continues to be the most frequently identified species causing infection.

During January to March 2001, 83 presumptive environmental *Legionella* isolates from various environmental sources were received from other laboratories or were isolated by ESR. Of these 66 (80%) were identified as belonging to the genus *Legionella*. However, 15 of these could not be identified to species level. These isolates are currently being identified using 16S rRNA gene sequencing. The remainder of the isolates were identified to species and serogroup level (Table 5).

Table 5. Laboratory-confirmed legionellosis cases and environmental *Legionella* isolates, January - March 2001

<i>Legionella</i> species / serogroup	Clinical Cases			Number of environmental isolates
	Confirmed	Probable	Total	
<i>L. pneumophila</i> serogroup 1	0	0	0	6
<i>L. pneumophila</i> serogroup 2	0	0	0	1
<i>L. pneumophila</i> serogroup 5	0	0	0	1
<i>L. pneumophila</i> serogroup 6	0	0	0	6
<i>L. pneumophila</i> serogroup 8	0	0	0	2
<i>L. pneumophila</i> serogroup 13	1	0	1	0
<i>L. pneumophila</i> serogroup unidentified	2	0	2	5
<i>L. anisa</i>	0	0	0	2
<i>L. bozemanii</i> serogroup 1	0	0	0	4
<i>L. bozemanii</i> serogroup 2	0	0	0	1
<i>L. feeleii</i> serogroup 1	0	0	0	2
<i>L. feeleii</i> serogroup unidentified	0	0	0	2
<i>L. gormanii</i>	0	1	1	0
<i>L. longbeachae</i> serogroup 1	5	2	7	14
<i>L. longbeachae</i> serogroup 2	0	0	0	1
<i>L. longbeachae</i> serogroup unidentified	2	1	3	2
<i>L. micdadei</i>	0	0	0	1
<i>L. taurinensis</i>	0	0	0	1
<i>Legionella</i> sp.	0	1	1	15
Total laboratory-confirmed	10	5	15	66

LEPTOSPIROSIS

During January to March 2001, 33 cases of leptospirosis were laboratory-confirmed and a further two cases were notified based on clinical grounds only. Twenty-three of the 33 laboratory-confirmed cases (70%) were notified. This compares with 31 laboratory-confirmed cases and an 81% notification rate in the same quarter last year.

The infecting *Leptospira* species and serovar was identified in 25 of the laboratory-confirmed cases (Table 6). Three cases from Waikato were diagnosed by both serology and isolation. The isolates have been identified as two isolates of *L. interrogans* sv pomona and one isolate of *L. borgpetersenii* sv hardjo. The infecting *Leptospira* species and serovar could not be identified in eight cases.

The majority of cases (86%) were male and 22 (63%) were aged between 30-49 years. The 20 cases for whom occupation was known could all be considered to be occupationally acquired. Nine were farmers, eight freezing workers/butcher, one forestry worker, one possum trapper, and one fisherman. Four cases could be considered to be recreationally acquired leptospirosis, through rafting, swimming in contaminated water, trail bike riding, and contact with donkey urine. One death due to leptospirosis was recorded during this period, which is the first death since 1990.

Table 6. Laboratory-confirmed leptospirosis cases, January-March 2001

Leptospira species / serovar	Number of cases
<i>L. interrogans</i> sv australis	1
<i>L. borgpetersenii</i> sv ballum	2
<i>L. borgpetersenii</i> sv hardjo	5
<i>L. interrogans</i> sv pomona	9
<i>L. borgpetersenii</i> sv tarassovi	8
Unidentified	8
Total	33

SPECIAL BACTERIOLOGY

Interesting Isolates received in the Special Bacteriology Laboratory

- Corynebacterium diphtheriae* var *mitis* non-toxicogenic strain from the infected leg of a patient (F 9y). Toxigenicity was initially determined by the traditional Elek plate immunodiffusion test and the absence of the diphtheria toxin gene was confirmed by PCR assay.
- Gordonia sputi* identified by 16S rRNA sequencing from CAPD fluid of a patient (F 49y). Aerobic actinomycetes can be difficult to identify by phenotypic methods (traditional biochemical reactions) and molecular methods such as sequencing can provide valuable answers for significant isolates. *Gordonia* species have been implicated as a cause of human infections, including pulmonary disease.
- Burkholderia multivorans* (*B. cepacia* genomovar II) from sputa of several cystic fibrosis patients. The diversity of species within the *B. cepacia* complex has recently been described, and at least seven genomovars demonstrated. Certain biochemical tests can now be used to distinguish one genomovar from another, and this may have implications for patient management.

Useful reference material on the *B. cepacia* complex can be found in:

- Henry DA, Mahenthalingam E, Vandamme P, et al. Phenotypic methods for determining genomovar status of the *Burkholderia cepacia* complex. J Clin Microbiol 2001; 39:1073-8.
- Whittier S. Update on the microbiology of cystic fibrosis: traditional and emerging pathogens. Clin Microbiol Newslett 2001; 23:67-71.

Listeria monocytogenes

Five isolates of *L. monocytogenes* from human cases were referred to ESR in the period January to March 2001 (Table 7). One of the isolates

was from a pregnant woman who responded to treatment. The remaining cases were in three adults who had an underlying illness and/or were elderly, and in a ten-year-old child in whom no risk factors were identified.

Table 7. *Listeria monocytogenes* from human cases, January-March 2001

Month isolated or of onset	Health district	Sex/Age	Source	O antigen serotype
January	Central Auckland	F 34y	BC	1/2
January	Canterbury	M 86y	BC	1/2
January	Central Auckland	M 71y	BC	1/2
March	South Canterbury	M 77y	BC	1/2
March	Central Auckland	F 10y	CSF	4

Bordetella pertussis

During January to March 2001, isolates from 158 cases of *Bordetella pertussis* were received for serotyping and surveillance, compared with 334 for the same period last year. Sixty four of these isolates were serotyped; one was serotype 1,2, and 63 were serotype 1,3. The ages of the cases are given in Table 8. The recommended ages for vaccination against *B. pertussis* in New Zealand are at six weeks, three months, five months and 15 months.

Table 8. Age distribution of cases of *Bordetella pertussis*, January - March 2001

Age	<5m	5-<15m	15m-4y	5-9y	10-14y	15-19y	≥20y
Number	15	12	62	49	8	0	12

ENTERIC PATHOGENS

SALMONELLA

During January to March 2001, 737 isolates of Salmonella were received compared with 481 for the same period in 2000. Of particular interest:

- S. Typhi* – There were 11 cases of *S. Typhi*; eight indicating overseas travel, one eating food brought from the Pacific Islands, and two with no travel details.
- S. Typhimurium* phage type 160 – Isolates of this phage type have been significant during this period, representing 24.1%, 19.1%, and 22% of total Salmonella isolates in January, February, and March respectively. There was a significant outbreak (36 confirmed cases) during March in Central and South Auckland health districts, following a social function. The strain was also recovered from cooked chicken and potato salad served at the function.
- S. Typhimurium* phage type 135 – Two separate outbreaks, totalling 19 cases, were reported from the Taranaki health district. Ten cases were associated with a supermarket bakery.
- S. Infantis* – No common source was proven for a cluster of 12 isolates of this serotype in Auckland during January.

There have been a number of isolates from unusual sites including:

- S. Brandenburg* – lumbar disc aspirate and a wound swab.
- S. Typhimurium* phage type 1 from cerebrospinal fluid.
- S. Dublin* from a knee aspirate.

Non-Human Sources

There were 306 isolates of Salmonella during January to March 2001 compared with 321 for the same period in 2000. Of note are the following, which are also the phage and serotypes causing significant human infection:

- S. Typhimurium* phage type 160 – There were 31 isolates of this phage type, including isolates from birds, cattle, cats, a horse, goat, otter,

poultry feed and poultry product. The first non-human isolate of this phage type was from a cat in May 2000.

- *S. Typhimurium* phage type 135 – Isolates of this phage type increased to 31 during January to March 2001 from 15 in the same period last year. Twenty-six of these were from the poultry environment.
- *S. Infantis* – Isolates of this serotype increased to 22 during January to March 2001 from seven in the same period last year. Ten of these were from meat/bone meal and nine were from the poultry environment.

E. COLI O157

There were 18 isolates of *E. coli* O157 confirmed during January to March 2001 (Table 9) compared with 29 for the same period in 2000.

Table 9. Isolates of *E. coli* O157, January-March 2001

Month	Sex / Age	District	Clinical Details	Isolates from known contacts
January	F 5y	Otago	None given	
	M 3y	Southland	None given	M 9m
	F 4y	Otago	None given	
February	M 10m	Waikato	None given	M 31y, F 33y, F 2y
	M 86y	Tauranga	Bloody diarrhoea	
	F 6y	Tauranga	Bloody diarrhoea	
	F 11y	Auckland	Bloody diarrhoea	
	F 2y	Tauranga	No details	
March	F 2y	Rotorua	Diarrhoea	
	F	Waikato	Diarrhoea	
	M 1y	Waikato	None given	
	F 2y	Hawkes Bay	Bloody diarrhoea	
	M 1y	Waikato	HUS	
	F 2y	Canterbury	None given	

SHIGELLA

Shigella sonnei Biotype a

During January-March 2001, two geographically distinct outbreaks of *Shigella sonnei* Biotype a were detected in the Auckland region. Fifteen children and fifteen staff from a children's health camp became sick in late January, out of a total of 96 attendees. Four staff and four residents became sick in a rest home in early February. The two outbreaks were situated 40km apart, but the unusual event of two instances of shigellosis within a similar time frame prompted investigation. Epidemiological studies and DNA typing have suggested a common link. Dr Philip Hill, Public Health Medicine Registrar at Auckland District Health Board, has been the lead investigator of these outbreaks and will be publishing his findings at a later date.

ANTIBIOTIC RESISTANCE

ANTI-TUBERCULOSIS DRUG RESISTANCE, 2000

Surveillance of anti-tuberculosis drug resistance is based on the results of susceptibility testing of isolates cultured and identified in the Mycobacteriology Laboratories at Green Lane, Wellington, and Waikato Hospitals. These laboratory data are matched with tuberculosis notifications. In 2000, 353 cases of tuberculosis were notified. The causative organism was isolated and identified from 250 cases, and the antimicrobial susceptibility of 249 isolates (242 *Mycobacterium tuberculosis* and seven *M. bovis*) was tested.

The percentage resistance to each antimicrobial tested is shown in Table 10.

Table 10. Resistance among isolates from tuberculosis cases, 2000

Antimicrobial	Number tested	Number resistant ¹	% resistance ¹
Isoniazid	249	25	10.0
Rifampicin	249	1	0.4
Ethambutol	249	3	1.2
Pyrazinamide	249	10 ²	4.0
Streptomycin	249	21	8.4

¹ includes resistance alone or in combination with other antimicrobials

² includes all seven *M. bovis* isolates

There was an apparent increase in the prevalence of resistance in 2000, with 16.9% of the isolates being resistant to at least one antimicrobial compared with 9.0% in 1999. One isolate (0.4%) was multidrug resistant (MDR-TB, defined as resistance to at least isoniazid and rifampicin) (Table 11). The isolate was from a patient born in China. Since data collation began in 1995, all MDR-TB have been from patients born overseas. There have been a total of 11 MDR-TB during the six years 1995-2000, with no more than three isolates in any one year.

Table 11. Resistance patterns among isolates from tuberculosis cases, 2000

	Number (%)	Resistance pattern ¹	Number (%) with each pattern
Fully sensitive	207 (83.1)		
Resistant to 1 agent	30 (12.0)	H	13 (5.2)
		S	11 (4.4)
		Z	6 (2.4) ²
Resistant to 2 agents	9 (3.6)	HS	7 (2.8)
		HZ	2 (0.8) ³
Resistant to 3 agents	1 (0.4)	HSE	1 (0.4)
Resistant to 4 agents	1 (0.4)	HZSE	1 (0.4)
Resistant to 5 agents	1 (0.4)	HRZSE	1 (0.4) ⁴

¹ H, isoniazid; S, streptomycin; Z, pyrazinamide; E, ethambutol; R, rifampicin

² includes five *M. bovis* isolates

³ both were *M. bovis* isolates

⁴ MDR isolate

Among the 249 isolates tested, 232 (93.2%) were from new cases of tuberculosis and 17 (6.8%) from reactivations. Of the 232 isolates from new cases, 84.5% (196) were sensitive to all agents tested, 8.6% were isoniazid resistant, and 0.4% were rifampicin resistant. The one MDR isolate was from a new case. Of the 17 isolates from reactivations, 64.7% (11) were sensitive to all the agents tested, 29.4% were isoniazid resistant, and there was no resistance to rifampicin.

SURVEILLANCE OF ANTIBIOTIC RESISTANCE IN THE WHO WESTERN PACIFIC REGION, 2000

Twenty laboratories submitted their antimicrobial resistance data for 2000. These data were collated to provide estimates of national rates of resistance (Table 12). As well as providing useful national information, these data contribute to the World Health Organization's surveillance of antimicrobial resistance in the Western Pacific Region. Thank you to all the New Zealand laboratories who supplied their data.

Notably the 2000 data indicated:

- *Streptococcus pneumoniae*: a further small increase in penicillin resistance (from 22.6% in 1999 to 25.7%), 18.4% erythromycin resistance, and 15.5% tetracycline resistance.
- *Staphylococcus aureus*: small increases in resistance to methicillin (from 5.8% in 1999 to 6.9%), fluoroquinolones (from 4.3 to 5.2%) and mupirocin (from 19.3 to 21.5%); and 1.5% co-trimoxazole resistance.
- *Escherichia coli*: an increase to >1% fluoroquinolone resistance among urinary and non-urinary isolates; amoxicillin/clavulanic acid resistance of 8.9% and 17.8% among urinary and non-urinary isolates, respectively; stable levels of cefuroxime resistance (3.2%) and 0.2%

Table 12. Antimicrobial resistance data from hospital and community laboratories, 2000¹

	Percent resistance (number tested ²)																
	amikacin	ampicillin	amoxicillin/ clavulanic acid	ceftazidime	ceftriaxone/ cefotaxime	cefuroxime/ cefamandole	cephalothin	co-trimoxazole	fluoroquinolone	gentamicin	imipenem/ meropenem	nitrofurantoin	piperacillin	tetracycline	ticarcillin	tobramycin	trimethoprim
<i>Acinetobacter</i> spp. ³	2.8 (144)			14.6 (239)				14.0 (507)	8.6 (465)	12.2 (524)	4.4 (205)		33.2 (184)			10.9 (175)	
<i>Citrobacter freundii</i> ³	0 (126)	83.2 (297)	55.6 (286)		14.5 (138)	32.9 (164)	86.3 (161)	6.0 (184)	1.5 (264)	3.0 (234)	1.8 (112)						
<i>Enterobacter</i> spp. ³	0.2 (591)	93.6 (1976)	82.4 (1804)		12.6 (818)	35.1 (761)	93.5 (688)	6.4 (1144)	3.4 (1274)	3.9 (1293)	0.4 (547)		7.8 (230)			3.7 (271)	
<i>Escherichia coli</i> non-urinary	0 (1572)	56.3 (4501)	17.8 (3936)		0.2 (2129)	3.2 (2440)	24.8 (1835)	19.2 (3756)	1.4 (3175)	1.4 (3695)	0.6 (1567)		15.6 (1013)			1.1 (1066)	
<i>E. coli</i> urinary	0 (3647)	55.2 (69909)	8.9 (67758)		0.1 (5726)	1.0 (4624)	18.1 (9369)	23.1 (4534)	1.2 (65995)	1.0 (15076)		1.5 (68650)	17.9 (537)			0.7 (1757)	22.5 (69465)
<i>Klebsiella</i> spp.	0.1 (861)	89.4 (4971)	7.8 (4449)		1.8 (1257)	6.3 (1168)	16.1 (1196)	5.0 (1668)	1.9 (3852)	1.1 (2141)	0.1 (953)		9.8 (254)			1.0 (199)	
<i>Morganella morganii</i> ³	2.1 (145)	96.5 (607)	94.0 (563)		1.0 (197)	76.3 (278)	97.5 (201)	12.2 (369)	6.4 (376)	11.0 (354)	1.9 (159)						
<i>Proteus mirabilis</i>	1.0 (697)	13.7 (5180)	2.7 (4920)		0.7 (1018)	3.7 (902)	6.0 (1717)	12.6 (1711)	1.0 (4578)	0.9 (2072)	3.2 (785)		92.6 (418)			2.9 (456)	
<i>Pseudomonas aeruginosa</i>	6.3 (1692)			3.9 (4389)					10.9 (6718)	11.6 (8280)	5.2 (1737)		5.0 (4279)			13.5 (222)	3.6 (3290)
<i>Serratia</i> spp. ³	4.3 (303)	94.2 (1228)	90.7 (1088)		37.1 (574)	88.6 (581)	98.3 (410)	21.1 (796)	23.2 (789)	2.7 (915)	0.3 (388)		85.1 (194)			9.8 (255)	

	Percent resistance (number tested ²)													
	ampicillin	amoxicillin/ clavulanic acid	chloramphenicol	clindamycin	co-trimoxazole	erythromycin	fluoroquinolone	gentamicin	methicillin/ oxacillin	mupirocin	nitrofurantoin	penicillin	tetracycline	vancomycin
Coagulase-negative Staphylococci (blood isolates)				24.8 (302)	22.7 (850)	41.2 (1718)	17.7 (631)	28.5 (1212)	49.0 (1720)			83.5 (1362)	14.0 (399)	0 (1232)
<i>Campylobacter</i> spp.						2.9 (175)								
<i>Enterococci</i> spp.	2.1 (6487)							30.4 (313) ⁴			1.2 (5298)		59.3 (864)	0.3 (1807)
<i>Haemophilus influenzae</i> (non-invasive) ⁵	23.3 (10016)	0.7 (4612)	0.5 (5127)		17.4 (8463)								0.9 (4721)	
<i>Moraxella catarrhalis</i>	90.5 (1203)					1.7 (1058)							1.7 (1141)	
<i>Staphylococcus aureus</i>			1.8 (9805)	4.1 (2843)	1.5 (29944)	11.8 (67962)	5.2 (10362)	2.6 (16294)	6.9 (74415)	21.5 (32183)		89.4 (66818)	2.5 (22555)	
<i>Streptococcus pneumoniae</i> (non-invasive) ⁵			5.2 (2922)		46.5 (4273)	18.4 (5780)						25.7 (5830)	15.5 (2446)	
<i>Streptococcus pyogenes</i>						1.5 (10710)						0 (10754)		

¹ Data supplied by Auckland, Christchurch, Middlemore, North Shore, Rotorua, Southland, Taumarunui, Waikato, Wanganui, and Whakatane Hospitals; and Auckland Diagnostic Medical, Dunedin Southern Community, Medlab Bay of Plenty, Medlab Central, Medlab Northland, Medlab Wellington, Nelson Diagnostic, Rotorua Diagnostic, Valley Diagnostic and Wanganui Diagnostic laboratories

² Data presented only if available for ≥100 isolates

³ ESCAPPM organisms with potential for inducible cephalosporinases and stably derepressed mutants producing high-levels of cephalosporinases

⁴ high-level resistance

⁵ Susceptibility of isolates from invasive disease tested at ESR and reported in LabLink 2001; 8(1): 12-3

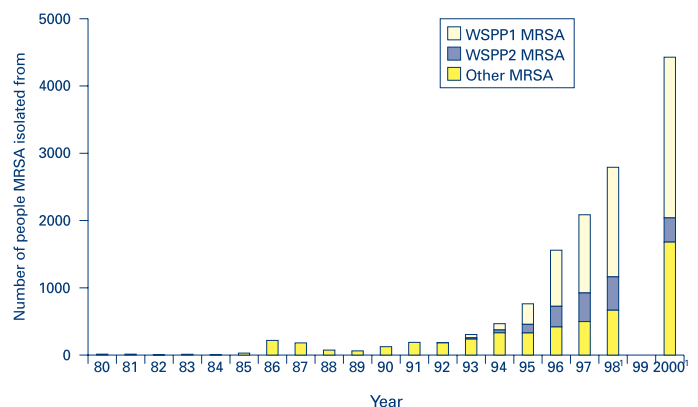
cefotaxime/ceftriaxone resistance among non-urinary isolates; and stable levels of nitrofurantoin resistance (1.5%) and trimethoprim resistance (22.4%) among urinary isolates.

- *Klebsiella*: 1.8% ceftriaxone/cefotaxime resistance.
- *Pseudomonas aeruginosa*: 11.6% gentamicin resistance, 3.9% ceftazidime resistance, 5.2% imipenem resistance, and 10.9% fluoroquinolone resistance.

SURVEY OF NON-MULTIRESISTANT AND MULTIRESISTANT MRSA, JULY 2000

In October 1998, the national surveillance of methicillin-resistant *Staphylococcus aureus* (MRSA) was rationalised to routinely cover only multiresistant MRSA. At the time, regular (approximately annual) short-term surveys of all MRSA were planned to provide up-to-date information on the overall epidemiology of MRSA in New Zealand.

Figure 3. MRSA isolations, 1980-2000



¹ annualised data

The first of these surveys was held during July 2000. Over the one-month period, MRSA were referred from 369 people (364 patients and 5 staff). This number of referrals equates to an annual rate of 122.4 per 100,000: a 59% increase on the rate in 1998 (77.1 per 100,000), the last year for which comparable data are available (Figure 3). MRSA was reported to be causing infection in 91.1% of the 190 people for whom this information was reported.

Among the 364 patients with MRSA, 40.1% were categorised as hospital patients and the other 59.9% as community patients (see LabLink 2000; 8(1): 10 for the definitions of hospital and community patients).

The majority (229, 61.7%) of the MRSA isolates were one of the two WSPP MRSA: 53.6% (199) were WSPP1 and 8.1% (30) were WSPP2. The next most common strain was EMRSA-15, which accounted for 15.4% (57) of isolates. In comparison, in 1998 WSPP MRSA accounted for 76.1% of MRSA isolations and EMRSA-15 just 2.5% of MRSA isolations. The majority (73.4%) of WSPP MRSA in July 2000 were from people in the community, whereas most (73.7%) EMRSA-15 were isolated from hospital patients or staff (Table 13).

Table 13. Distribution of WSPP MRSA and EMRSA-15 among hospital patients/staff and community patients, July 2000

	Number of people with:	
	WSPP MRSA	EMRSA-15
Hospital patient or staff	61 (26.6%) ¹	42 (73.7%)
Community patient	168 (73.4%)	15 (26.3%)
Total	229 (100%)	57 (100%)

¹ proportion of all isolations of the strain

The geographic distribution of people from whom MRSA was isolated during July 2000 is shown in Figure 4.

Figure 4. Annualised incidence of MRSA by health district, 2000

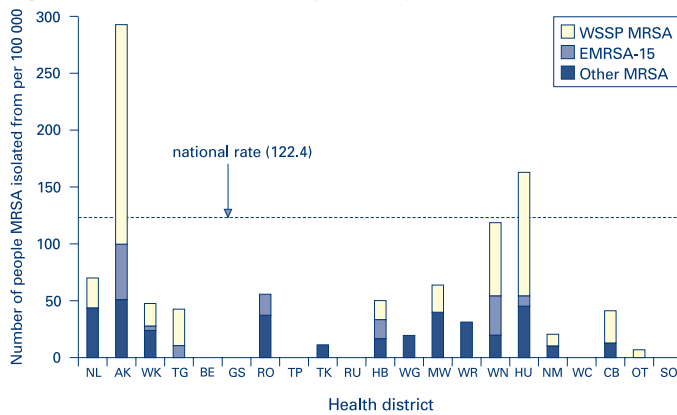


Table 14. Resistance among MRSA, referred July 2000

Antimicrobial agent (resistance breakpoint, mg/L)	Percent resistance		
	All isolates (n = 371) ¹	WSPP (n = 229)	EMRSA-15 (n = 57)
Chloramphenicol (MIC ≥ 32)	0.3	0	0
Ciprofloxacin (MIC ≥ 4)	20.5	0	100
Clindamycin (MIC ≥ 4)	4.9	0	5.3 ²
Co-trimoxazole (MIC ≥ 4/76)	3.0	0	0
Erythromycin (MIC ≥ 8)	25.9	5.7	89.5
Fusidic acid (MIC ≥ 2)	5.1	0.4	0
Gentamicin (MIC ≥ 16)	2.7	0.4	0
Mupirocin (MIC ≥ 8)	12.4	3.5	5.3
Rifampicin (MIC ≥ 4)	0.3	0	0
Tetracycline (MIC ≥ 16)	4.9	0.4	1.8
Multiresistant ³	25.3	1.3	89.5

¹ Two of the 369 people with MRSA had two strains, therefore there were 371 isolates from these 369 people

² EMRSA-15 exhibits inducible clindamycin resistance by a disc approximation test

³ Resistant ≥2 classes of antibiotics in addition to β-lactams

As previously observed in New Zealand, the majority of MRSA are not multiresistant (resistant to ≥2 classes of antibiotics in addition to β-lactams) (Table 14). However, due to the increase in the prevalence of multiresistant strains, in particular EMRSA-15, the proportion of MRSA which are multiresistant has increased from 16.5% in 1998 to 25.3% in 2000. However, the WSPP MRSA still remain predominantly non-multiresistant (Table 14).

VIROLOGY

RESPIRATORY VIRUSES

Influenza

During January to March 2001, 14 isolations of influenza virus were reported compared with seven isolations during the same period in 2000. These were from Taranaki (4), Christchurch (4), Waikato (3), Auckland (2), Northland (1). Nine isolations of influenza B and five isolations of influenza A were identified. One influenza A from Christchurch was further subtyped as A/New Caledonia/20/99 (H1N1)-like virus. There was a localised outbreak of influenza B in Stratford, Taranaki, with four influenza B isolations which were further identified as B/Yamanashi/166/98-like virus using the 2000 WHO influenza diagnostic reagents. The 2001 influenza vaccine should provide good protection against current circulating influenza strains.

ENTEROVIRUSES

Coxsackie B5 virus

During January to March 2001, a total of 14 cases of Coxsackie B type 5 virus infections were reported from Waikato (6), Auckland (6), Taranaki (1), and Wanganui (1), compared with 24 cases between 1995 and 2000, (8 cases 1995, 12 cases 1998). Coxsackie B5 can cause severe illness such as aseptic meningitis, meningoencephalitis, paralysis, myocarditis and pericarditis. The patients ranged in age from eight days to seven years (average 3.2 years) and the majority were male (9). The range of clinical features included meningitis (8), hemiplegia (1), and hand-foot-mouth disease (1).

One school in the Waikato Health District reported they had 11 children unwell with symptoms of vomiting, headache and stomach pains between 7 and 9 March. Another two schools in the same health district were known to have experienced similar outbreaks during February and March, however the total number of children involved was not known at the time of this report. Coxsackie B5 was isolated from four cases from these school outbreaks.

Echovirus type 7

During January to March 2001, the increased case numbers of Echovirus type 7 (E 7) continued. Nine cases were reported, Waikato (4), Auckland (1), New Plymouth (1), Manawatu (1), Wellington (1), and Dunedin (1). It is worth noting that cases have spread to the South Island. The patients ranged in age between 28 days to 14 years old (average 3.9 years) and all were male. The clinical features ranged from tonsillitis to meningitis. E7 was isolated from the tracheal ring, lung, and bowel of an 8 month-old baby boy with sudden infant death syndrome.

ADENOVIRUSES

During January to March 2001, a total of 53 adenovirus isolates were serotyped. This is significantly higher than 22 adenovirus isolations during the same period in 2000. Adenovirus type 19, which can cause epidemic keratoconjunctivitis, was isolated from 25 patients from Auckland (23), Christchurch (1), and Dunedin (1). The specimens from these patients were taken between 8 December 2000 and 20 February 2001. The patients ranged in age from 8 months to 86 years (average 46.8 years). Seven isolations of Adenovirus type 1 were reported from Auckland (3), Waikato (1), Wellington (2), and Christchurch (1). Adenovirus type 1 is mainly associated with respiratory illness and conjunctivitis, however two isolates of Adenovirus type 1 were isolated from a 4 year and a 14 year old girl with diarrhoea. The remaining 21 isolates were typed as adenovirus type 3 (9), type 2 (7), type 4 (2), type 22 (2) and type 8 (1).

MYCOLOGY

A summary of the opportunistic mycoses detected during July to December 2000 is shown in Table 16. Data were collated from replies received from 13 sentinel laboratories throughout New Zealand.

The significance of some of the isolates is difficult to determine due to the absence of clinical details supplied.

Table 16. Biannual summary of opportunistic mycoses in New Zealand, July - December 2000.

Organism	No. of cases	Site	Clinical data
Filamentous fungi			
<i>Absidia corymbifera</i>	1	endotracheal aspirate	DE-, NR
<i>Aspergillus flavus</i>	1	pelvic cavity swab	DE-, NR
<i>Aspergillus niger</i>	1	(R) maxillary sinus swab	maxillary sinusitis
<i>Exophiala jeanselmei</i>	1	thigh biopsy	DE+, post-renal transplant, splinter of wood in thigh. Tx: debridement & itraconazole.
<i>Fusarium species</i> (most closely resembles <i>F. solani</i>)	1	corneal scrape	DE-, NR
Unidentified phaeohyphomycete	1	(L) 4 th toe abscess	DE+, recurring abscess now isolated 3x (May & Oct 1999 & Dec 2000). Previously treated with debridement & itraconazole. Tx: amputation of toe. Isolate remains sterile after many attempts to induce sporulation.
Unidentified hyalohyphomycete	1	(R) sphenoid mycetoma tissue	NFES in direct gram but detected in histology. Unable to be speciated. (Lynne Sigler, UAMH)
<i>Scopulariopsis species</i>	1	CAPD	DE-, NR
Black mycelial fungus - previously reported has been identified by Lynne Sigler (UAMH) as most closely resembles <i>Exophiala jeanselmei</i>	1	(R) elbow subcutaneous lump	recurrent chromoblastomycosis, typical muriform bodies seen in direct exam.
Yeasts			
<i>Candida albicans</i>	29	blood culture (19) sternal wound fluid (1) pancreatic cyst fluid (1)	pre-mortem (2), pre-mortem with extensive burns (1), short bowel syndrome (1), biliary adenocarcinoma (1), cardiac patient - line sepsis (1), line sepsis, also isolated with <i>Candida parapsilosis</i> (1), long term ICU patient (1), nephrology patient (1), mediastinitis (1), NR (9) DE+, NR NR
		CAPD fluid (8)	ESRF, peritonitis (3), ESRF, peritonitis, also isolated with <i>Candida glabrata</i> (1), ARF, 2 weeks old in PICU; also isolated with <i>Staphylococcus epidermis</i> (1), renal failure, PEG tube through peritoneal cavity, also isolated with <i>Enterococcus faecalis</i> (1), NR, also isolated in sputum (1), NR (1)
<i>Candida glabrata</i>	5	blood culture (3) CAPD fluid (2)	isolated 7x over a 2 month period, subacute bowel obstruction, 2° to surgery for reproductive tract Ca (1), bowel obstruction (1), NR (1) ESRF, peritonitis
<i>Candida parapsilosis</i>	2	blood culture (8)	home TPN patient, line sepsis (1), HIV (1), premature baby in ICU (2), NR (4)

Organism	No. of cases	Site	Clinical data
		CSF (1) CAPD (9) pleural fluid (2) nephrostomy site (1)	premature baby, skin broken down ESRF, peritonitis DE-, NR DE+
<i>Candida pelliculosa</i>	1	blood	paediatric oncology neuroblastoma
<i>Candida tropicalis</i>	2	blood	decreased LOC, hypotensive ?urinary sepsis, also isolated in catheter urine & post-mortem perinephric pus (1), AML (1)
<i>Cryptococcus neoformans</i>	2	CSF	HIV, LA 1:256 (1), HIV, LA 1:4096, CD4 = 8 (1)
<i>Rhodotorula mucaliginosa</i>	1	CAPD	ESRF, peritonitis
<i>Rhodotorula species</i>	1	blood	line sepsis
<i>Yarrowia lipolytica</i>	1	blood	IV drug abuser, unwell post-injecting drugs
<i>Pneumocystis carinii</i>			
	2	sputum	HIV (1), aplastic anaemia (1)
Actinomycetes			
<i>Nocardia asteroides</i>	1	sputum	cystic fibrosis, also isolated with <i>Scedosporium prolificans</i>
<i>Nocardia asteroides</i> - most closely resembles	1	sputum	LLL pneumonia, past history of TB, isolated from TB cultures
<i>Nocardia farcinica</i>	1	sputum	NR
<i>Nocardia nova</i>	6	sputum	DE-, NR (2), isolated from direct TB cultures (1), isolated from TB MGIT bottles (3)
<i>Nocardia species</i>	1	bronchial washing	previous MAI & testicular Ca

KEY:

AML	Acute myeloid leukaemia	IV	Intravenous
Ca	Carcinoma	LA	Latex agglutination
CAPD	Continuous ambulatory peritoneal dialysis	LLL	Left lower lobe
DE	Direct examination	LOC	level of consciousness
ESRF	End stage renal failure	MAI	Mycobacterium avium-intracellulare
HIV	Human immunodeficiency virus	NR	Clinical data not received
ICU	Intensive care unit	TPN	Total parenteral nutrition
		Tx	Treatment
UAMH	University of Alberta Microfungus Collection & Herbarium, Canada		

Contributed by Dinah Parr and, Karen Rogers, Mycology Reference Laboratory, Microbiology Department, Auckland Hospital.

ESR NEWS

Fiona Thomson-Carter has joined ESR as Communicable Disease Programme Manager. Fiona was previously principal clinical scientist at the Scottish Reference Laboratory for Campylobacter and Escherichia coli.

Maggie Brett, senior scientist Antibiotic Reference Laboratory, has left ESR. Helen Heffernan has been appointed to a new combined position of senior scientist of the Antibiotic Reference Laboratory and Nosocomial Infections Laboratory. Helen will commence in this position gradually as appointments can be made to the work she will be relinquishing.

Changes have been made to the LabLink Editorial Committee. We welcome Darren Hunt, Medical Associate, and farewell Maggie Brett, Yvonne Galloway and Charlotte Kieft.

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