

FOODBORNE VIRAL DISEASE - EMERGING PATHOGENS OR EMERGING TECHNIQUES?

Foodborne viral disease has emerged as a major public health problem in recent years. What is not clear is whether the observed increase in foodborne viral illness is genuine or simply reflects improved techniques available for identification of viruses in clinical and food samples associated with outbreaks. The main viruses associated with foodborne illness are the human enteric viruses, which are excreted in human faeces and transmitted by the faecal-oral route. They include the Norwalk-like viruses (NLVs) and hepatitis A virus (HAV). Other viruses occasionally associated with foodborne disease include rotaviruses, astroviruses, picornaviruses (enteroviruses including poliovirus) and, rarely, the parvoviruses⁴. NLVs, previously known as small round structured viruses, have been identified as the predominant causal agent.

Norwalk-like viruses (NLVs)

NLVs, including Norwalk virus itself, are now the most widely recognised viral agents associated with food and waterborne outbreaks of non-bacterial gastroenteritis world-wide. These viruses have recently been classified as members of a distinct group of human calciviruses in the Calciviridae family and are positive-sense, single-stranded, non-enveloped RNA viruses with a genome of approximately 7.6 kb. The NLV group does not show the characteristic cup-shaped morphology of calciviruses but shows a 'fuzzy' morphology by electron microscopy (EM). The identification of NLVs was difficult prior to development of molecular methods because they are nonculturable, there is no animal model, and they show great genetic diversity, which limits the use of traditional immunology and serotyping assays.

NLVs are transmitted mainly by the faecal-oral route, although secondary spread may occur by airborne transmission. Gastroenteritis due to Norwalk-like virus is a significant cause of morbidity in New Zealand⁷. An estimated 53,345 cases of NLV foodborne infection occur in New Zealand each year, and an estimated 35,208 annual productivity days are lost due to potential foodborne NLV disease¹². Because the illness caused by NLVs is self-limiting, hospitalisation and notification to health authorities is rare worldwide. A recent English study estimated the ratio of cases occurring in the community to those reaching national surveillance as 1562:1¹⁵.

NLVs were the most commonly identified cause of gastrointestinal disease outbreaks in New Zealand in 1999¹¹. A number of the 1999 outbreaks were epidemiologically associated with consumption of commercially farmed New Zealand oysters. Analysis of stools from cases and oyster flesh from the same batches as consumed showed the presence of a genogroup II 'Mexico-like virus' NLV in both stools and oysters⁹. Subsequently, the same strain of NLV was detected in both farmed and feral bivalve shellfish from the same growing area in northern New Zealand¹³.

Hepatitis A virus

Hepatitis A virus is transmitted by the faecal-oral route and is responsible for outbreaks of hepatitis A but is not a major cause of disease in

New Zealand. Hepatitis A is one of the more severe foodborne infections and is a notifiable disease in most developed countries. It is listed as a Severe Hazard in Appendix V of the United States FDA Food Code⁴ and so more accurate data on its occurrence is available than for other enteric viral foodborne diseases. It is difficult to culture in the laboratory and, because of the four-week incubation period, foods are unlikely to be available for analysis.

Viral contamination of foods

Contamination of foods by human faecal material can occur at two stages (Table 1):

- **Primary contamination** of food products prior to harvest. The main products at risk are shellfish growing in faecally contaminated waters^{1,4,6,10}, fruits and vegetables contaminated in the growing stages by either polluted water or the unhygienic practices of horticultural workers⁶, and faecally contaminated water or ice^{2,3}. The use of sewage sludge as fertilizer on agricultural land has not been proven to contribute to foodborne viral infection¹.
- **Secondary contamination** by infected foodhandlers during food processing, preparation and distribution. This can result from staff not ceasing work when they are ill, poor staff hygiene practices, provision of inadequate handwashing facilities, and also indirect transmission via fomites or asymptomatic foodhandlers to food^{4,5,6}.

Table 1. Major food groups at risk of viral contamination

Food type	Contamination source
Molluscan shellfish (oysters, mussels, cockles)	Primary : preharvest at growing stages
Fresh fruit and salad vegetables	Primary : preharvest at growing stages
Water and ice	Primary : direct use
Hand-prepared cold foods and buffet dishes, salads	Secondary : foodhandling
Bakery products	Secondary : foodhandling

Detection of foodborne viruses

EM methods have been used for detection of NLVs in faeces for many years, but until recently there were no suitable methods for NLV detection in foods. Examination of foods for viral agents is a laborious procedure, and specific pathogens have rarely been recovered. Failure to confirm a viral aetiology in a foodborne outbreak has generally been due to lack of appropriate methodology^{4,6}. The first report of NLVs being recovered from a non-shellfish food item implicated in an outbreak was published only recently¹⁴.

Processing and pre-treatment of food samples

Inhibitors such as lipids, fats, polysaccharides, resistant plant materials and toxic organic compounds present in shellfish and food samples necessitate extensive pretreatment before specimens can be analysed

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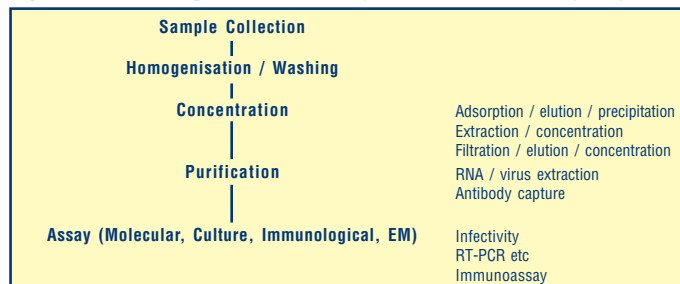
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for viruses. Shellfish contain many inhibitors, including lipids, polysaccharides and enzymes. Other foods such as salads and fruits contain plant cellulose, tannin and lignin materials that can be difficult to remove. The general approach for virus recovery (Figure 1) is to either wash virus off the food (ie, fruits and vegetables) or to homogenise the food (shellfish) matrix with buffers, elute the virus, then concentrate the resulting fluid to a small volume for analysis by either cell culture (where applicable) or molecular assay^{5,8,10}. Several methods have been developed to purify the viruses for molecular assay. One of these is antibody capture: the virus is captured onto a solid tube or bead matrix, washed to remove inhibitors, then disrupted to release nucleic acid for amplification by RT-PCR^{4,5,10}. Another approach concentrates and purifies the intact virus then releases the RNA by heating prior to reverse transcription¹⁰.

Figure 1. General steps in the isolation of human enteric viruses from foods¹⁰



Molecular methods

Generally the enteric viruses contain RNA rather than DNA. RNA is less stable than DNA and once the viral capsid is fractured, the RNA is easily degraded by ubiquitous RNases in the environment. Initial processing and extraction methods must protect the RNA by either maintaining the integrity of the virus until the RNA is transcribed to DNA, or disrupt the viral particles while protecting exposed viral RNA from degradation with chemicals. There are four main stages in the identification of enteric RNA viruses by molecular techniques:

- recovery and extraction of viral RNA from specimens
- conversion of RNA to complementary DNA (cDNA) by reverse transcription
- amplification of cDNA by PCR using specific primers for selected virus
- detection and confirmation of amplified product

Cell culture methods

Most enteric viruses which cause foodborne disease are either difficult to grow in tissue culture or cannot be grown at all and so this method is rarely used for analysis of food samples.

The **ESR Environmental and Food Virology Laboratory** has the capability to investigate viral foodborne illness by identifying viral sources of infection both in clinical specimens from gastroenteritis outbreaks and in the implicated foods (including shellfish). Many different enteric viruses can be identified in faecal samples, environmental samples (eg, sewage, water, shellfish, sediments, sludge) and foods (green salad, potato salad, fresh fruits, cooked meats). These viruses include NLVs, HAV, enteroviruses (including polioviruses), human adenoviruses, rotaviruses, astroviruses, and F-RNA phage. The viruses can be further discriminated for epidemiological investigations using molecular methods, including DNA sequencing and DNA hybridisation. Infectivity and quantification data can be provided using culture methods for enteroviruses and F-RNA phage.

The Environmental and Food Virology Laboratory is only the fifth laboratory worldwide to detect natural NLV contamination in commercially farmed oysters and directly relate it to disease outbreaks. These virological tools are available to assist with assessment of shellfish quality when there are doubts about the condition of growing waters, and to obtain useful data for development of risk assessments. In collaboration with leading overseas researchers, new methods have been implemented for virus detection that are more sensitive, provide internal controls for each assay to reduce the possibility of false negative results, and significantly reduce turnaround time for results.

The use of virological analysis has proved to be a valuable tool in establishing the source of infection for shellfish-associated gastroenteritis outbreaks and for identifying health hazards presented by viral contamination of commercial growing sites and recreational shellfish-gathering areas.

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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 April to June 2000, are shown in Table 2.

Table 2. Sterile site isolates, April-June 2000

Organism	BC	CSF or CSF/BC	Other Sterile Site	Total	Cumulative Total to June 2000
<i>H. influenzae</i> ¹	9	0	0	9	18
<i>N. meningitidis</i>	41	30	2	73	106
<i>S. pneumoniae</i>	103	4	2	109	166
<i>S. pyogenes</i>	32	1	4	37	61
<i>S. agalactiae</i>	12	2	1	15	25

¹ *H. influenzae*: two serotype b and seven 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, April – June 2000

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	1	0	0	1
<i>H. influenzae</i> non b	1	0	0	0	0	0	2	1	3	0
<i>N. meningitidis</i>	0	14	4	11	2	3	4	25	9	1
<i>S. pneumoniae</i>	1	14	14	4	3	2	4	8	22	37
<i>S. pyogenes</i> ¹	0	2	2	0	0	1	1	1	17	12
<i>S. agalactiae</i>	7	2	0	0	0	0	0	2	2	2

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

Haemophilus influenzae

During April to June 2000, nine isolates were received from cases of *H. influenzae* invasive disease. Two of these isolates were serotype b and the others were non-serotypable using serotype-specific antisera. This compares with four serotype b

from a total of 14 isolates for the same period last year.

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

Neisseria meningitidis

During April to June 2000, a total of 73 sterile site isolates were received from cases of meningococcal disease, compared with 54 for the same period last year. Of these, 69 were serogroup B, three were serogroup C and one was serogroup Y. Serotyping and serosubtyping results of the serogroup B and C organisms are given in Table 4. The serogroup Y isolate was Y:14:P1.5,2. In addition one isolate was received from a non-sterile site from a notified case. This typed as B:4:P1.4.

The three non-subtypable organisms were shown by *porA* PCR and DNA-DNA hybridisation not to be P1.2, P1.4, P1.7 or P1.16.

Table 4. Serotypes and subtypes of *N. meningitidis*, April – June 2000

Subtype	Serotype							Total
	1	2a	2b	4	14	15	NT	
Serogroup B								
P1.4	1			49	4		7	61
P1.6				1				1
P1.7,16						1		1
P1.9	1							1
P1.15	1			1				2
NST					1	1	1	3
Total	3	0	0	51	5	2	8	69
Serogroup C								
P1.2		1						1
P1.5,2							1	1
P1.15	1							1
NST								0
Total	1	1	0	0	0	0	1	3

NT - non typable

NST - non subtypable

All meningococci were tested against the following serotypes and subtypes: serotypes 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.

Twelve blood and CSF samples from culture-negative cases of meningococcal disease were tested by PCR for the presence of meningococcal DNA. Nine samples were shown to contain the meningococcal *porA* gene which encodes the subtype-specific antigens. Restriction digestion and dot blot hybridisation showed that seven of these samples were subtype P1.7,4, one was P1.7, and one was not P1.2, P1.4, P1.7 or P1.16.

PCR and culture results show that the epidemic strain (B:4:P1.4) continues to cause most disease.

LEGIONELLOSIS AND ENVIRONMENTAL LEGIONELLA ISOLATES

During April to June 2000, eight cases of legionellosis were identified and a further four cases were notified on clinical grounds only. Four of the cases were regarded as confirmed and eight as probable.

The majority of cases (75%, 9/12) were males and four (33%) were aged over 60 years. The age range was 29 to 75 years.

The infecting *Legionella* species and serogroup was identified in six of the cases (Table 5).

During April to June 2000, twenty-one presumptive environmental legionella isolates from various environmental sources were received from other laboratories or were isolated by ESR. Of these, nine could be identified to species and serogroup level, four could not be identified, and eight did not belong to the genus. These four were identified as belonging to the family Legionellaceae and are being identified using 16S rRNA gene sequencing.

Two species, *L. cherrii* and *L. maceachernii*, which had not previously been isolated in New Zealand, were identified in this quarter. Both species were

Table 5. Legionellosis cases and environmental isolates, April-June 2000

<i>Legionella</i> spp.	Clinical Cases			Number of environmental isolates
	Confirmed	Probable	Total	
<i>L. pneumophila</i> serogroup 1	1	0	1	1
<i>L. pneumophila</i> serogroup 3	0	0	0	1
<i>L. pneumophila</i> serogroup 5	0	1	1	0
<i>Legionella pneumophila</i> serogroup unidentified	1	1	2	0
<i>L. bozemanii</i> serogroup 2	1	0	1	0
<i>L. gormanii</i>	0	1	1	0
<i>L. jordanis</i>	0	0	0	3
<i>L. cherrii</i>	0	0	0	1
<i>L. maceachernii</i>	0	0	0	3
<i>Legionella</i> sp.	1	1	2	4
Total	4	4	8	13

isolated from cooling tower water. *L. cherrii* type strains were originally isolated from thermally altered water in Minnesota, USA, 1982¹, and from a potable water cistern St Croix, Virgin Islands, in 1982¹. It is not known whether this species is pathogenic or not.

L. maceachernii type strain was isolated from a home evaporator cooler in Phoenix, Arizona, in 1979. This species was also isolated in 1985 from a lung of an immunocompromised patient with fatal pneumonia^{1,2}. Therefore, this species is considered to be potentially pathogenic.

¹ Int J Syst Bacteriol 1985 ; 35 : 50-59

² Bartlett, CLR, et al. Legionella Infections. Edward Arnold Ltd; 1986.

LEPTOSPIROSIS

During April to June 2000, 31 cases of leptospirosis were identified. The infecting *Leptospira* species and serovar was identified in 28 of the cases (Table 6).

The majority of cases (94%, 29/31) were males and 19 (61%) were aged between 30-49 years. The age range was 17 to 64 years.

The occupation was known for 15 cases: agricultural/farm workers (6), meat workers (7), butcher (1), and an events organiser who had travelled overseas (1).

Table 6. Leptospirosis cases, April-June 2000

Leptospira species / serovar	Number of cases
<i>L. borgpetersenii</i> serovar ballum	6
<i>L. interrogans</i> serovar copenhageni	1
<i>L. borgpetersenii</i> serovar hardjo	15
<i>L. interrogans</i> serovar pomona	6
Total	28

SPECIAL BACTERIOLOGY

Interesting isolates received in the Special Bacteriology Laboratory

- *Acidomonas methanolica* from lymph node tissue of M 19y with chronic granulomatous disease. This isolate was identified using 16S rRNA sequencing, as it grew very poorly in ESR's usual biochemical test media. The genus *Acidomonas* contains organisms which are acidophilic, methanol-utilising gram-negative bacilli. *A. methanolica* is an unusual isolate from a human, as its usual source is industrial processes or the environment.
- *Burkholderia pseudomallei* from sputum of F 7y, a cystic fibrosis patient whose sibling was colonised (sputum) with this organism. The family had lived in the Northern Territories, Australia, where *B. pseudomallei*, the causative organism of melioidosis, is endemic.

Listeria monocytogenes

Five isolates of *L. monocytogenes* from human cases were referred during April to June 2000 (Table 7). Three of the isolates were from perinatal cases in which there were two foetal deaths. The remaining two cases were an adult with underlying illness, and a previously well infant (M 7m). The *L. monocytogenes* isolate from the seven month old baby and from cooked ham related to the case were indistinguishable by serotype and DNA type.

Table 7. *Listeria monocytogenes* from human cases, April - June 2000

Month isolated or of onset	Health District	Sex/Age	Source	O antigen serotype
April	North West Auckland	Foetus ¹	body swabs	4
April	Southland	M 7m	BC	4
May	Hutt	F neonate	BC	4
June	Canterbury	F 39y ¹	placenta	1/2
June	Hutt	M 63y	BC	1/2

¹ foetal death

Bordetella pertussis

During April to June 2000, 255 isolates of *Bordetella pertussis* were received for serotyping and surveillance, compared with 20 for the same period last year. Two isolates were serotype 1,2; two were 1,2,3, and 251 were 1,3. The ages of the 254 cases for whom age was provided are given in Table 8. The recommended ages for vaccination against *B. pertussis* in New Zealand are six weeks, three months, five months and 15 months.

Table 8. Age distribution of cases of *Bordetella pertussis* infection, April - June 2000

Age	<5m	5-<15m	15m-4y	5-9y	10-14y	15-19y	≥20y
Number	20	28	58	88	22	6	32

ENTERIC PATHOGENS

SALMONELLA

There were 1,010 human isolates of *Salmonella* confirmed during January to June 2000 compared with 1,431 for the same period in 1999 (Table 9). The predominant strain was *S. Typhimurium* phage type 135 which has been linked to food premises outbreaks in Wairarapa, Manawatu and Wellington Health Districts. *S. Typhimurium* phage type 135 isolates from these outbreaks accounted for 144 (54%) of the total 266 isolates of this phage type. Food handlers tested positive and there were several hospitalised cases in each outbreak. Overseas travel was indicated in 80 cases (7.9%).

NON-HUMAN SOURCES

There were 648 non-human isolates of *Salmonella* confirmed during January to June 2000 compared with 684 for the same period in 1999 (Table 10). *S. Typhimurium* phage type 135 was the predominant type in poultry, *S. Hindmarsh* in sheep, and *S. Typhimurium* phage type 101 in cattle.

E. COLI O157

There were 14 isolates of *E. coli* O157 confirmed during April to June 2000 (Table 11), compared with 23 isolates during April to June 1999.

Table 11. Isolates of *E. coli* O157, April-June 2000

Month	Sex / Age	Health District	Comments
April	M/10m	Otago	No details
April	M/22y	Waikato	No details
April	M/5y	Waikato	No details
April	M/9y	Auckland	Bloody diarrhoea
April	F/47y	Wellington	No details
April	F/84y	Auckland	Nausea and diarrhoea
April	M/61	Auckland	No details
May	M/2y	Otago	No details
May	F/48y	Waikato	No details
May	M/2y	Waikato	Diarrhoea
May	M/1y	Waikato	No details
May	F/2y	Taranaki	Bloody diarrhoea
May	F/1y	Waikato	No details
June	F/1y	Tauranga	Bloody diarrhoea

SHIGELLA

There were 65 *Shigella* isolates confirmed during January to June 2000 (Table 12), compared with 142 for the same period in 1999.

Table 12. *Shigella* isolates, January-June 2000

Species	Type	Number	Comment
<i>S. sonnei</i>	Biotype a	22	1 overseas traveller
	Biotype g	25	3 overseas travellers 1 refugee
<i>S. flexneri</i>	1b	6	4 refugees
	2a	6	2 household cases
	2b	1	
	3a	1	
	4a	1	
<i>S. boydii</i>	1	1	immigrant
		1	did not react with type specific antisera
<i>S. dysenteriae</i>	12	1	immigrant

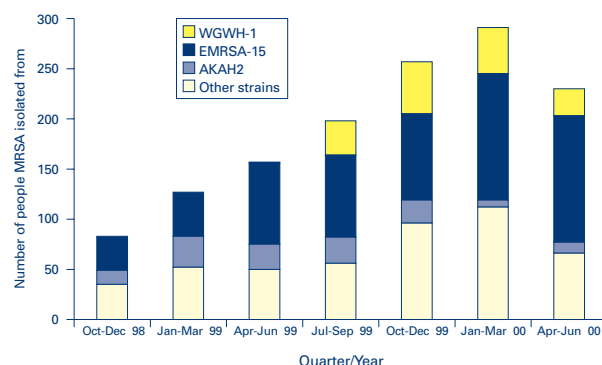
ANTIBIOTIC RESISTANCE

EPIDEMIOLOGY OF MULTIRESISTANT METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS, JANUARY-JUNE 2000

During the first six months of 2000, multiresistant methicillin-resistant *Staphylococcus aureus* (MMRSA, MRSA resistant to two or more classes of antibiotics in addition to β -lactams) from 477 people (444 patients and 33 healthcare workers) were referred to ESR. The majority (73.9%) of the 444 patients were classified as hospital patients, as they had been in a hospital or another healthcare facility (HCF) either when their MRSA was isolated or in the preceding three months.

Until the most recent quarter (April-June 2000), there was a trend of an increasing number of MMRSA isolations (Figure 2). The decrease in the April-June 2000 quarter was mainly due to the control of outbreaks of two MRSA strains (EMRSA-15 and WGH1) in Wellington Hospital. These outbreaks, and outbreaks of the WGH1 strain in Wanganui and Palmerston North, contributed a large proportion of the increase in MMRSA between October 1999 and March 2000 (Figure 2).

Figure 2. Multiresistant MRSA isolations, October 1998-June 2000



The MMRSA strains that were most commonly isolated between January and June 2000 are shown in Table 13. In 1999, 40.0% of all MMRSA isolated were the epidemic strain, EMRSA-15. During the first six months of 2000, this proportion rose to 48.0%. Among the 214 patients from whom EMRSA-15 was isolated, 81.8% were hospital patients. The hospitals and other HCFs in which EMRSA-15 was isolated during the first half of 2000 are shown in Table 14. As was observed in 1999, EMRSA-15 was most frequently isolated from HCFs in the Auckland and Wellington areas.

Table 9. *Salmonella* isolates, January-June 2000

Serotypes	HEALTH DISTRICTS																				Total					
	NL	MW	CA*	SA	WK	TG	BE	GS	RD	TP	RJ	HB	TK	WG	MW	WR	WN	HJ	NM	WC		CB	SC	OT	SO	
Agona			3		1			2									1								7	
Albany	1																								1	
Anatum			1	1																					2	
Bareilly									1										1						2	
Berta																		2							2	
Bovismorbificans		1			1												1								3	
Brandenburg			3	2	2																5	1	13	1	29	
Bredeney																							1		1	
Emek			2																						2	
Enteritidis phage type 1		2																							3	
4			1	2				1					1	1			2	1			5			1	15	
6a		1																							1	
8																					1				1	
9a	1	6	5	3	14	5	1		1				2		7	2	3		1		9	2	1	1	64	
22																	1								1	
RDNC		1				1																			2	
Hadar			1	1	2													3	2						10	
Hartford			1																						1	
Havana																			1			1			2	
Heidelberg			1																						1	
Hindmarsh			1	1																	1				3	
Hull																					1				1	
Ibadan			1																						1	
Infantis		1	1	2	1	10	1	1	2						1		1	1			2	1	1		26	
Liverpool	1																								1	
London		1	1										1												3	
Mbandaka			1																						1	
Meleagridis									1																1	
Mississippi				1													1				2		1		5	
Montevideo			1																						1	
Muenchen			1		1																				2	
Newport		1	1										1												3	
Ohlstedt			1																						1	
Oranienburg					1																				1	
Oslo		1																1							2	
Panama			1			1																		1	3	
Paratyphi B		1	1																1						3	
Paratyphi B var. Java				1	1												1								3	
Pensacola			1																						1	
Reading																					1				1	
Saintpaul								1				2			2			1	1		3	1	7		18	
Sandiego					1																				1	
Sangalkam			1																						1	
Schwarzengrund				1													1				1		1		4	
Senftenberg					1												1								2	
Singapore			1																						1	
Stanley		2																			2				4	
Thompson					1	1							1					1							4	
Typhi			5	5													1								11	
Typhimurium phage type 1	4	9	13	4	14	4		4	2			2	6		11		6		2		11	1	3		96	
8		2	1	2	2							1	2												10	
9					3								1				2	1	5		6	1	38	3	60	
12a		2	1			1									1			1	5		1	8	2	2	21	
13			1																						1	
23	1	1	3		4	3											3								15	
26			1																						1	
41		1																							1	
42			2	4	2									1	4	2	6	1			11	4	3	1	41	
42a	1		2														1	1			1				6	
43			1																						1	
101	2	4	5	2	2	1			1						3			3	6		19	6	6		60	
120															1										1	
129																								1	1	
135	1	22	17	10	16	2	2	1	3	1		12	4	3	46	29	46	13	7		21	4	5	1	266	
150																13		2				1			16	
155		1	3	1	3									1			1	1			2				13	
156	3	4	10	4	17	3						6	1	2	4	2	7	6	1		6		3		79	
160																					7				7	
196																									1	
205																					1	1			3	
206																									1	
RDNC			1			1	1						1					1						1	6	
Rough																					1				1	
Untypable																						1	2	1		4
Victoria																					1				1	
Virchow		1	3	2					3					1							1				12	
Weltvedren		2	1	1									1												5	
Worthington															1										1	
Group B 4,12 : d : -			1	1																					2	
Group B 4,12 : - : 1,2																					1				1	
Group B 4,5,12 : - : -			2																						2	
Group C 6,7 : k : -	1				1							1												3	3	
Group C 6,7 : - : - (non motile)															1										1	
Group D 9,12 : - : - (non motile)															1										1	
Group D 9,12 : l,v : -						1																			1	
Group E 3,10 : r : -			1			2							1					1							6	
Group E 3,15 : r : -			2																						2	
Subspecies IIIb 61 : l,v : 1,5																						1			1	
TOTAL	15	68	108	51	90	37	5	10	14	1	1	31	14	9	85	48	94	38	28	1	133	28	87	14	1010	

Table 10. *Salmonella* serotypes, non-human isolates, January-June 2000

SEROTYPE	ANIMAL									Meat/bone meal	Environmental	Food	Spice	Not Specified	POULTRY					TOTAL
	Avian	Bovine	Canine	Caprine	Cervine	Equine	Feline	Ovine	Porcine						Neckflap	Caecae	Feed	Environmental	Miscellaneous including product	
Agona												1	1		15		8	4	4	33
Anatum										2	3									5
Anatum 15+		1								1										2
Bergen												1	1							2
Brandenburg			1	1		1			20	4	10	16		1	4	3	6	1	7	75
Cerro																	3			3
Derby										2										2
Eastbourne											1									1
Enteritidis phage type 9a		2					1							1						4
Give 15+																	1			1
Havana										2			4				3		1	10
Heidelberg									1											1
Hindmarsh									52		1	5							1	59
Idikan																			1	1
Infantis									1	1	4			1	6	1	6		3	23
Johannesburg						1														1
Lille									6	5										11
London												1					2			3
Mbandaka																	4			4
Mississippi											1									1
Muenster																		1		1
Newport								1	1											2
Oranienburg										1			12				3			16
Orion 15+		1						1		2	3									7
Ruiru																		1		1
Saintpaul		1						1			2	2						1		7
Senftenberg											1	1		4			5	2	15	28
Singapore			1																	1
Tennessee														1			8	1	7	17
Thompson														1			2			3
Typhimurium phage type 1	1	3	1			1	1	2			23	6		2	2		2	5		48
9								4			1	5		1			1	8	1	21
12a			1								1	1					1		3	7
41											7		1	1						9
42		4			1			1			77	1						1	1	86
42a																	1			1
101	1	7	1	1				5			7	5			7		1	4	15	54
135		1						1			2	2		2	16		3	3	8	38
155												1								1
156	2	4				1		1			9			1						18
160							1										2	1		4
199											7									7
RDNC											3									3
Rough											3									3
Untypable																		2		2
Weltevreden											1	1								2
Group B 4,12 : - : - (non motile)																		2		2
Group B 4,5,12 : - : - (non motile)											1									1
Group B 4,12 : l,v : -			1					1												2
Group B 4,12 : r : -								1												1
Group C 6,7 : k : -											1									1
Group C 6,7 : - : e,n,z15			1																	1
Group C 8 : - : 1,5								1												1
Group E 3,19 : - : - (non motile)																			1	1
Group K 6,18 : - : - (non motile)															1					1
Group X 47 : z4,z23 : -													2							2
Subspecies IIIb 61 : l,v : z35											2									2
Rough : l,v : e,n,z15											1						1			2
Rough : r : -																		1		1
Rough : z4,z23 : -																	1			1
TOTAL	3	24	7	2	1	3	4	98	2	21	169	52	21	16	51	4	64	38	68	648

S. Typhimurium 302
 Other 346
 Poultry isolates 225

Table 13. Most commonly isolated multiresistant MRSA strains, January-June 2000

Strain ¹ (origin)	Number of people the strain isolated from (% of all MMRSA isolations)
EMRSA-15 (Britain) ²	229 (48.0)
WGWH1 (Australia) ²	70 (14.7)
AMRSA-1 (Australia) ²	23 (4.8)
WSPP1 (Western Samoa)	18 (3.8)
AKAH2 (Australia) ²	17 (3.6)
AKNW1 ³	13 (2.7)
phage pattern HS 42E ⁴	13 (2.7)
WR/AK1 ²	12 (2.5)

¹ Includes strains isolated from more than five people.

² For a description of strains EMRSA-15, WGWH1, AMRSA-1, AKAH2 and WR/AK1 see LabLink 2000; 7: 8-9.

³ The AKNW1 strain was first identified in 1999, and has been mainly isolated from babies in the Neonatal Intensive Care Unit at National Women's Hospital.

⁴ The phage pattern HS 42E strain was first identified in 1999, and has been mainly isolated in Auckland Hospital.

Table 14. Healthcare facilities with patients and staff with EMRSA-15, January-June 2000

Hospitals ¹	Number of people EMRSA-15 isolated from (% of all EMRSA-15 isolations in healthcare facilities) ²
*Bay of Islands	1 (0.4)
Whangarei	2 (0.9)
North Shore	8 (3.6)
Waitakere	7 (3.1)
Auckland	29 (13.0)
Middlemore	22 (9.9)
Otara Spinal Unit	8 (3.6)
Other Auckland HCFs ³	55 (24.7)
Auckland subtotal	129 (57.8)
Hamilton	1 (0.4)
Rotorua	5 (2.2)
Other Rotorua HCFs ³	3 (1.3)
Hawkes Bay	3 (1.3)
*Other Hawkes Bay HCFs ³	3 (1.3)
*Palmerston North	2 (0.9)
Horowhenua	1 (0.4)
*Other Palmerston North HCFs ³	1 (0.4)
Wellington	29 (13.0)
*Hutt	1 (0.4)
Kenepuru	14 (6.3)
*Porirua	2 (0.9)
Other Wellington HCFs ³	22 (9.9)
Wellington subtotal	68 (30.5)
*Blenheim	1 (0.4)
Christchurch	2 (0.9)
*Burwood	2 (0.9)
Total	223

¹ Asterisked hospitals are those in which EMRSA-15 has not been isolated previously.

² The same person may be recorded in more than one HCF.

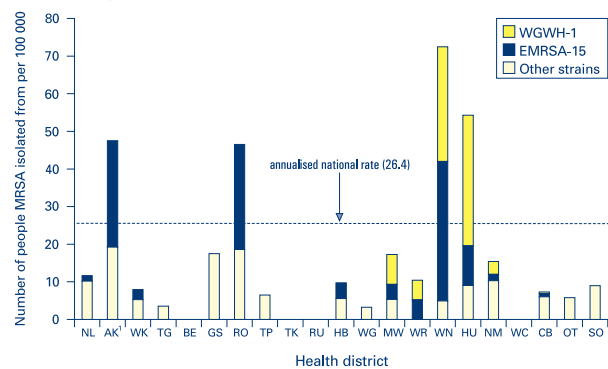
³ HCFs in the area which have withheld publication of their name.

EMRSA-15 was newly isolated in several HCFs during the first half of 2000 (Table 14), although there did not appear to be extensive spread within any of these facilities. Precise information about MRSA in private HCFs is not available, as the identity of these facilities is often not disclosed. Over a third (84, 37.7%) of the isolations of EMRSA-15 from hospital patients and healthcare workers were made in private HCFs, predominantly long-term care facilities.

The second most commonly isolated strain between January and June 2000 was the WGWH1 strain (Table 13). This strain was isolated from 70 people, and was mostly confined to hospitals in the Wellington area.

The incidence rates of MMRSA in the various health districts are shown in Figure 3. Rates were above the national average in four health districts: Auckland (the three combined Auckland health districts), Rotorua, Wellington and Hutt.

Figure 3. Annualised incidence of multiresistant MRSA by health district, January-June 2000



¹ AK includes all Auckland health districts

Compiled by Helen Heffernan and Heather Davies
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ANTIBIOTIC-RESISTANT BACTERIA MONITORING SCHEME

Multiresistant methicillin-resistant *Staphylococcus aureus*

During January to June 2000, a total of 475 multiresistant MRSA were tested for susceptibilities. Among the MRSA tested were 226 EMRSA-15, 68 WGWH1, 23 AMRSA-1, and 17 isolates each of the AKAH2 and WSPP1 strains.

The percent resistance of the isolates tested is shown in Table 15. Mupirocin resistance occurred in 11.2% (53) of the isolates and 9.7% (46) exhibited high-level resistance (MIC \geq 512 mg/L). High-level mupirocin resistance occurred in many strains including 12 AKNW1, 11 WR/AK1, six WSPP1, and six EMRSA-15 strains. Fusidic acid resistance occurred in 6.3% (30) of the isolates.

The majority (89.4%) of the EMRSA-15 isolates were resistant to oxacillin, erythromycin and ciprofloxacin. As reported previously, the majority of the EMRSA-15 isolates exhibited a low clindamycin MIC but showed inducible clindamycin resistance when tested by a disc diffusion test¹. Some variation in the macrolide resistance phenotype was shown in this period; eight EMRSA-15 isolates were erythromycin-sensitive and another seven exhibited clindamycin MIC of 16-32 mg/L.

Table 15. Resistances of 475 multiresistant MRSA referred in January-June 2000

Antimicrobial agent (Resistance breakpoint, mg/L)	% Resistance		
	All isolates (n = 475)	EMRSA-15 (n=226)	WGWH1 (n = 68)
chloramphenicol (MIC \geq 32)	1.5	0	0
ciprofloxacin (MIC \geq 4)	77.5	100	100
clindamycin (MIC \geq 4)	35.8	3.1 ¹	97.6
co-trimoxazole (MIC \geq 4/76)	25.9	0.9	61.0
erythromycin (MIC \geq 8)	90.1	96.4	100
fusidic acid (MIC \geq 2)	6.3	1.8	0
gentamicin (MIC \geq 16)	31.6	0.4	100
mupirocin (MIC \geq 8)	11.2	3.6	4.4
rifampicin (MIC \geq 4)	2.9	0.4	5.9
vancomycin (MIC \geq 32)	0	0	0

¹ strain demonstrates inducible clindamycin resistance by disc diffusion induction test

The WGWH1 isolates were resistant to six to eight antimicrobials. The most common antibiogram was resistance to oxacillin, erythromycin, clindamycin, ciprofloxacin, gentamicin, tetracycline, and trimethoprim/sulphamethoxazole. The AKAH2 isolates exhibited resistance to six to nine different antimicrobials. All the AKAH2 isolates were resistant to oxacillin, ciprofloxacin, erythromycin and clindamycin. The AMRSA-1 isolates were less multiresistant than the WGWH1 and AKAH2 strains and were resistant to three to seven antimicrobials. There were 17 multiresistant WSPP1 isolates; resistance occurred to oxacillin, erythromycin, mupirocin, tetracycline and gentamicin. There were also eleven WR/AK1 isolates, which were resistant to oxacillin, fusidic acid and mupirocin.

Penicillin-non-susceptible Streptococcus pneumoniae (PNSP)

A total of 251 penicillin-non-susceptible *Streptococcus pneumoniae* (PNSP), defined as pneumococci with penicillin MIC ≥ 0.12 mg/L, were referred in January to June 2000. Among the 251 PNSP were 143 penicillin-resistant (MIC ≥ 2 mg/L) and 108 penicillin-intermediate (MIC 0.1-1 mg/L) pneumococci. The trend of increased prevalence in cefotaxime resistance that was first noted in 1997 continued in 2000. Cefotaxime resistance (MIC ≥ 2 mg/L) occurred in 22.7% (57) and cefotaxime intermediate resistance (MIC 1 mg/L) in 44.2% (111) of the isolates. Of the 57 cefotaxime-resistant isolates, 44 (77.2%) exhibited cefotaxime MIC ≥ 4 mg/L.

Of the 143 penicillin-resistant pneumococci, 95.8% (137) were resistant to at least three antibiotics and 72.7% (104) were resistant to more than five antibiotics. Nearly 94% (134) of the penicillin-resistant pneumococci were nonsusceptible to cefotaxime (MIC ≥ 1 mg/L) and 38% (55) had cefotaxime MIC ≥ 2 mg/L. Among the 108 penicillin-intermediate pneumococci, 55.6% (60) were resistant to at least three antibiotics and 16.7% (18) were resistant to more than five antibiotics. Two isolates had cefotaxime MIC ≥ 2 mg/L and 32 had cefotaxime MIC of 1 mg/L.

¹ LabLink 2000; 6: 4-5

Compiled by Maggie Brett
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VIROLOGY

RESPIRATORY VIRUSES

Influenza

Influenza sentinel surveillance started in May and continued until the end of September. During May and June, influenza activity was the lowest, compared with the last 10 years. The first influenza isolate was identified as influenza A H3N2 from Canterbury in the week ending May 7. A total of five influenza isolates were identified from Canterbury (1) and Central Auckland (4) in May: two were influenza AH3N2, one was influenza AH1N1, and two were influenza B. In June, there were only 10 isolates reported: influenza A not subtyped (1), influenza AH1N1 (3), and influenza B (6). Influenza A H3N2 was antigenically related to the A/Sydney/5/97 strain, influenza A H1N1 was similar to the A/New Caledonia/20/99 strain, and influenza B was antigenically related to the B/Beijing/184/93. These strains were all covered in the year 2000 influenza vaccine composition.

ENTEROVIRUSES

Echovirus type 33

Echovirus type 33 (family *Picornaviridae*: genus *Enterovirus*) was isolated from 34 people during March to June 2000.

ADENOVIRUSES

During April to June 2000, 10 adenoviruses were identified, compared with 26 adenoviruses isolations during the same period in 1999. There was one isolate each of Adenovirus types: 1, 2, 3, 5, 7, 8, 11, 15, 17, and 19.

ARBOVIRUS SEROLOGY

Dengue fever virus

One case of primary dengue fever virus infection was detected in April 2000. A helicopter pilot working in Sarawak experienced a flu-like illness with fatigue. Both IgM and IgG antibodies were detected in the screening assays. On

confirmation, the IgM antibody titre was very high and the infection was due to dengue virus Type 2.

The assays used for routine screening at ESR are the Panbio Dengue IgM capture ELISA and the Panbio Dengue IgG ELISA. These kits are not specific for dengue virus and may cross-react with other flavivirus such as Japanese encephalitis virus, Murray Valley Encephalitis virus, kunjin, and yellow fever virus. Samples which are reactive in the IgM assay are sent to the Arbovirus and Emerging Diseases Unit at Westmead Hospital, Sydney, for confirmation.

It is important that all dengue virus infections are serotyped, as infection with one serotype does not protect an individual from subsequent infection from a different serotype. There are four dengue serotypes, 1 – 4, and it is not uncommon for different serotypes to be present in different areas of a country. Reinfection also greatly increases the risk of developing dengue haemorrhagic fever.

Ross River virus

One case of Ross River virus infection was detected between April and June 2000. The screening assays used at ESR are the Panbio IgM and IgG ELISAs. Reactive sample are all referred to the Westmead Laboratory for confirmation. The patient was an Australian holidaying in New Zealand. He was staying in Napier at the time the screening assays were positive. On further investigation it was found that IgM antibodies had been detected two weeks earlier in Australia. Although IgM antibodies may be present for several weeks with infection caused by Ross River virus, viraemia is often short, usually 3-7 days.

HIV INFECTION

Several screening assays are available in New Zealand for HIV 1/2 antibody detection. They range from the Abbott AXSYM to rapid assays which are sometimes used by small laboratories for a quick screen. At ESR all referred samples are tested with the Serodia Particle Agglutination Assay, Abbott/Murex HIV1/2 EIA and Genelab Diagnostics HIV Western blot. For a Western blot to be defined as positive for HIV-1, a combination of two of the three following bands need to be present: p24, gp41 and gp120/160.

From April to June, seven new cases of HIV infection were detected at ESR. One case showed an interesting seroconversion pattern. The initial sample had reactive screening assays but the Western blot was indeterminate with bands only in the gp 120/160 position. However, a second sample taken seven days later showed a full set of bands (p17, p24, p31, gp41, p51, p66 and gp120/160) for a positive Western blot.

CULTURE COLLECTION

Recent accessions to the Collection are shown in Table 16.

Table 16. NZRM new accessions

Name	NZRM No.	Source, Strain	Comments
<i>Acinetobacter baumannii</i>	3697	NZ isolate, 1998	Multiresistant outbreak strain
<i>Acinetobacter haemolyticus</i>	3971	ATCC 17906	Type strain
<i>Acinetobacter</i> species	3972	ATCC 33305	
<i>Aeromonas trota</i>	3968	NZ isolate, 2000	
<i>Aeromonas veronii</i>	3699	NZ isolate, 2000	Biogroup sobria
<i>Staphylococcus aureus</i> subsp <i>aureus</i>	3969	NZ isolate, 1999	MRSA, mannitol negative strain

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