

Update of *Escherichia coli* as a faecal pathogen

Introduction

Escherichia coli (*E. coli*) is a component of the normal faecal flora of humans and other mammals, colonising the gastrointestinal tract within hours of birth. Some strains of *E. coli* have adapted to become faecal pathogens however, causing symptoms such as abdominal pain, watery diarrhoea, and bloody diarrhoea, and sometimes serious sequelae, including renal failure and death. The detection of faecal pathogenic *E. coli* and their differentiation from normal commensal strains presents a challenge to diagnostic laboratories. Some of these pathogens can be detected using methods readily available to a diagnostic laboratory, while for others diagnosis may require the use of a reference laboratory. This update briefly describes the various types of diarrhoeagenic *E. coli* and methods used to identify them, and explains why a new polymerase chain reaction (PCR) test will be introduced in the Enteric Reference Laboratory (ERL) from October 2001.

There are currently six recognised groups of *E. coli* that cause diarrhoeal disease in humans:

- enteropathogenic (EPEC)
- enterotoxigenic (ETEC)
- enteroinvasive (EIEC)
- enteroaggregative (EAEC)
- diffusely adherent (DAEC)
- enterohaemorrhagic (EHEC) - a subset of shigatoxigenic *E. coli* (STEC)

Enteropathogenic *E. coli* (EPEC)

Historically, the first group of *E. coli* recognised to cause diarrhoea were the enteropathogenic strains described in the 1940s as a cause of epidemic diarrhoea, often in nurseries.¹ Disease resulting from EPEC infection was, and still is, usually restricted to children under the age of two. Serotyping was the only way of identifying these pathogens, and the *E. coli* strains associated with these outbreaks belonged to certain serogroups: O26, O44, O55, O86, O111, O114, O119, O125-O128, O142, and O158. The importance of EPEC as faecal pathogens has decreased in the Western world since the 1970s, for reasons that are unclear.² Subsequently, few diagnostic laboratories in industrialised countries now test *E. coli* from the faeces of young children for the classical EPEC serogroups, although these strains still cause some cases of sporadic and epidemic diarrhoea.^{3,4} In the developing world they remain an important cause of infant morbidity and mortality.

It is now recognised that strains other than the classical EPEC serogroups cause human disease, and modern molecular methods have enabled EPEC to be better defined by genetic virulence markers. EPEC cause diarrhoea in part by intimate attachment to the brush border of the small intestine, which precedes production of the attaching-effacing (A/E) lesion also seen following infection with EHEC. Epithelial cell attachment requires the presence and expression of the *eaeA* gene (codes for the protein intimin) and the *bfpA* gene (codes for the pro-

duction of bundle-forming pili), both of which can be detected by PCR. The *eaeA* gene is located in a pathogenicity island on the chromosome, known as the locus of enterocyte effacement (LEE). The *bfpA* gene is present on the *E. coli* adherence factor (EAF) plasmid together with the *per* locus, a group of genes that enhance the expression of genes in the LEE.

The term "classical" (or "typical") EPEC is now used to denote those strains that carry both the *bfpA* and the *eaeA* genes and belong to the classical serogroups. These strains are associated with epidemic and sporadic diarrhoea in young children. Strains that just have the *eaeA* gene (ie. no *bfpA* gene) are known as "atypical" EPEC and only cause sporadic diarrhoea.⁵ Atypical EPEC may belong to any serogroup. There is no marker of EPEC suitable for use in the routine diagnostic laboratory. Serogrouping using pools of antisera against the classical EPEC serogroups is of limited value as strains may belong to a classical EPEC serogroup but not be pathogenic (lack the *bfpA* and the *eaeA* genes). The best way to detect these organisms in the diagnostic laboratory is to send any *E. coli* isolated in pure growth from the faeces of a child under the age of two, where no other faecal pathogen has been isolated, to a reference laboratory for further testing. The ERL will be introducing a new protocol to test for EPEC in October 2001 (see below).

Enterotoxigenic *E. coli* (ETEC)

Enterotoxigenic *E. coli* are the most common cause of traveller's diarrhoea, as well as weaning diarrhoea among children of the developing world.⁶ ETEC produce either or both heat labile (LT) and heat stable (ST) toxins. *E. coli* LT is chemically very similar to cholera toxin, but the rice water stools associated with cholera are not seen in ETEC infection. LT production can be demonstrated by the activity of the toxin against cells in tissue culture. Y1 adrenal cells or Chinese hamster ovary cells are used, with the toxin causing rounding of the former and elongation of the latter. Vero cells can also be used, and are elongated in the presence of the toxin. The LT gene can be detected by PCR. An ELISA kit is available for ST detection. Both LT and ST genes can also be detected by hybridisation to specific probes.⁷

Enteroinvasive *E. coli* (EIEC)

Enteroinvasive *E. coli*, like *Shigella* which they closely resemble, cause bacillary dysentery. The organisms invade cells in the intestinal mucosa, causing fever, severe abdominal cramps, malaise, toxemia and watery diarrhoea. Blood and mucus may be present in the stool. The carrier state exists, and some infections may be asymptomatic. The infection is also commonly polymicrobial, with other faecal pathogens such as *Salmonella* and *Shigella* present. EIEC are uncommon in developed countries, although this finding may be related to difficulty in isolating and identifying these organisms. Biochemically EIEC are very similar to *Shigella* species, being lysine negative, lactose negative and non-motile. Both EIEC and *Shigella* species possess an invasion plasmid, Inv, and PCR primers have been published that detect both

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EIEC and *Shigella* species in stool samples.⁸ ERL will be introducing a PCR test for EIEC in the near future.

Enteraggregative *E. coli* (EAEC)

Enteraggregative *E. coli* are the second most common cause of traveller's diarrhoea. The process by which EAEC cause diarrhoea is unclear. Infection enhances mucus secretion from the small bowel mucosa leading to the formation of a bacterium/mucus biofilm, and there is evidence that cytotoxic effects may also occur. EAEC are not invasive and they do not produce ST or LT. DNA probes have been described, but diagnosis is usually by examining the pattern of adherence to cells in cell culture.⁹ A characteristic layering pattern of the bacteria is seen, described as a "stacked brick configuration". Some EAEC form a pellicle on the surface of liquid media such as Mueller-Hinton or Luria broth. Diagnosis of EAEC infection can also be made by a DNA probe, which detects the large plasmids present in most EAEC strains. The clinical significance of EAEC can be difficult to determine as they are often isolated in conjunction with other faecal pathogens. Nataro and Kaper² accept that an EAEC strain is likely to be the cause of a patient's diarrhoea in three situations: (i) if the patient presents in the course of a documented outbreak, (ii) when the patient's isolate can be shown to belong to one of the common EAEC serotypes associated with disease (O3:H2, O15:H18, O44:H18, O86:HNM, O77:H18, O111:H21, O127:H2), (iii) where the isolate is the predominant organism repeatedly isolated from patients with diarrhoea in the absence of another enteric pathogen.

Diffusely Adherent *E. coli* (DAEC)

These strains of *E. coli* do not form microcolonies on Hep2 cells, but adhere in a diffuse manner, and diagnosis is by demonstrating this pattern of adherence. There are no molecular tests currently available that detect all strains of DAEC. The pathogenesis of DAEC is not yet elucidated, and their clinical significance is uncertain. Some studies have found a definite link between the presence of DAEC and diarrhoea, whereas in other studies DAEC are found with the same frequency in both diarrhoeal stools and asymptomatic controls.

Enterohaemorrhagic *E. coli* (EHEC)

Enterohaemorrhagic *E. coli* (EHEC) are a subset of STEC - probably the most familiar type of diarrhoeagenic *E. coli* to New Zealand diagnostic laboratories - and are capable of causing serious human disease, including haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura. These organisms produce shiga toxin (stx) and possess the EHEC enterohaemolysin (EHEC *hlyA*) gene and the *eaeA* gene which codes for intimin, a protein partly responsible for the attaching-effacing lesion of enterocytes caused by EHEC and EPEC. The term EHEC was coined to differentiate strains that cause disease in humans, from STEC strains that lack the *eaeA* gene and are usually not pathogenic. STEC are also known as verotoxigenic *E. coli* (VTEC), since the toxin produced by these organisms is toxic to African green monkey kidney (Vero) cells. This sometimes-confusing nomenclature has been clarified by Calderwood et al.¹⁰

The most common serotype isolated in New Zealand is O157. The H7 flagella antigen may be present, but many strains are non-motile. The frequency of isolation of this serotype may be related to the sorbitol negative phenotype, making it easier to isolate than non-O157 STEC. *E. coli* O157 can be isolated from faecal samples using sorbitol MacConkey agar (SMAC). The addition of cefixime and tellurite to this medium (CT-SMAC) enhances its selectivity. This serotype is also glucuronidase (GUR) negative, unlike most other strains of *E. coli*. A fluorescent substrate, 4-methyl-umbelliferyl- β -D-glucuronide (MUG), is now available to detect glucuronidase activity and is incorporated into some commercial media. It should be noted that sorbitol-positive, GUR-positive isolates of *E. coli* O157 have been found in Europe.

Shiga toxin production can be demonstrated using commercial EIA kits, and isolates can be screened for the O157 antigen using latex kits or by slide agglutination. Positives should be confirmed by titration. The H7 antigen is rarely detectable on primary isolation as most isolates are only sluggishly motile. Serial transfer through semisolid medium enhances the motility of the bacteria but lengthens the time required to detect H7. The two shiga toxin genes, the *eaeA* gene, and the EHEC *hlyA* gene can be detected by multiplex PCR. Washed sheep blood agar (EHEC agar, Fort Richard) is very useful for the isolation of non-O157 serotypes from stool samples.¹¹ The enterohaemolytic phenotype is the only selectable marker for these strains, and is present in 70-80% of isolates.

Current testing for diarrhoeagenic *E. coli* in the Enteric Reference Laboratory at ESR

Isolates sent to ERL for toxin testing are either pure cultures, or where no sorbitol negative colony has been isolated and patient symptoms indicate EHEC, mixed sweeps of organisms taken from a non-selective plate. Faecal samples are occasionally received.

The current protocol for testing isolates of diarrhoeagenic *E. coli* encompasses STEC and ETEC LT-producing strains. Testing includes:

For pure cultures:

- confirmation as *E. coli* biochemically
- multiplex PCR - primers for *stx1*, *stx2*, *eaeA*, EHEC *hlyA*
- latex agglutination test for serotype O157:H7
- confirmation of positives by titration
- serial subculture in semisolid medium for H7 antigen where necessary
- tissue culture (vero cells) for heat-labile toxin and shiga toxin
- serology where appropriate for shiga toxin-producing non-O157 isolates
- urgent samples may have an EIA for shiga toxin performed within 4 hours of receipt if indicated

For mixed cultures/faecal samples:

- multiplex PCR and vero cell culture
- subculture of positives to CT-SMAC and EHEC agar. Once a pure culture of STEC is isolated, testing as above.

All positives are telephoned to the referring laboratory immediately. Interim reports are normally mailed within two working days.

On occasion it may be necessary to type clusters by macrorestriction DNA analysis of *XbaI* digests using pulsed-field gel electrophoresis.

New Protocol

The introduction of a multiplex PCR in 1998 led to the identification of a number of strains that were *eaeA* positive but *stx* negative. The significance of this finding was investigated, and the results will be published in the New Zealand Journal of Medical Laboratory Science as a Fellowship dissertation.

Further to this work, as from October 2001, isolates that are *eaeA* positive but *stx* negative will be tested by *bfpA* PCR to determine whether they are classical or atypical EPEC. These isolates will be checked with classical EPEC antisera, and isolates that are *bfpA* positive will be fully serotyped.

Results will be presented as:

- *eaeA* positive, *bfpA* positive (*serotype*) - isolate is a classical enteropathogenic *E. coli*. Classical EPEC are associated with epidemic and sporadic diarrhoea in young children.
- *eaeA* positive, *bfpA* negative - isolate is an atypical enteropathogenic *E. coli*. Pathogenicity is unproven. Atypical EPEC have been associated with sporadic cases of diarrhoea.

It is hoped that the introduction of this new PCR will help to ascribe an infectious cause to some cases of childhood diarrhoea.

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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 2001, are shown in Table 1.

Table 1. Sterile site isolates, April-June 2001

Organism	BC	CSF or CSF/BC	Other Sterile Site	Total	YTD
<i>H. influenzae</i> ¹	10	0	0	10	19
<i>N. meningitidis</i>	54	21	2	77	124
<i>S. pneumoniae</i>	134	8	1	143	204
<i>S. pyogenes</i>	35	0	5	40	69
<i>S. agalactiae</i>	25	4	2	31	54

¹ *H. influenzae*: 5 serotype b and 5 non-b.

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, April-June 2001

Organism	<1m	1-11m	1y	2y	3y	4y	5-9y	10-24y	25-59y	≥60y
<i>H. influenzae</i> b	0	1	1	1	0	1	0	0	1	0
<i>H. influenzae</i> non b	1	0	0	0	0	0	0	0	0	4
<i>N. meningitidis</i>	0	13	10	3	5	1	5	24	14	2
<i>S. pneumoniae</i>	1	26	24	8	5	2	2	8	25	42
<i>S. pyogenes</i> ¹	0	4	2	1	1	0	0	4	19	8
<i>S. agalactiae</i>	8	1	0	0	0	0	0	1	11	10

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

Haemophilus influenzae

We continue to monitor *H. influenzae* isolates to determine that serotype b disease remains controlled by the vaccine. During April to June 2001, ten isolates were received from cases of *H. influenzae* invasive disease. Five of these isolates were serotype b, one was serotype f and the others were not serotypable using serotype-specific antisera. This compares with two serotype b from a total of nine isolates for the same period last year. Only one of the five cases was recorded as having had the full immunisation series.

No non-serotypable organism was shown by PCR to possess either the *bexA* gene necessary for capsular expression or the serotype b specific *cap* gene.

Neisseria meningitidis

In association with the collection of notifiable disease data ESR monitors the serogroup, serotype, and serosubtype of meningococci causing invasive disease. Currently New Zealand is experiencing an epidemic caused by serogroup B meningococci with the P1.4 serosubtype. When determined by serologic methods the P1.7 epitope associated with the P1.4 is almost never able to be detected. The sequence for this epitope is defined as P1.7b and can be detected in DNA analyses.

During April to June 2001, a total of 77 sterile site isolates were received from cases, compared with 73 for the same period last year. Of these, 67

were serogroup B, four were serogroup C, two were serogroup Y, one was serogroup A, and three were unable to be determined. Serotyping and serosubtyping results of the serogroup B and C organisms are given in Table 3. The serosubtypes of five isolates were unable to be determined by routine testing. The serogroup A isolate was typed as A:4:P1.4.

Table 3. Serotypes and subtypes of *N. meningitidis*, April-June 2001

Subtype	Serotype							Total
	1	2a	2b	4	14	15	NT	
<i>Serogroup B</i>								
P1.4	1			48	3		10	62
P1.6				1				1
P1.7						1		1
NST	1			1			1	3
Total	2			50	3	1	11	67
<i>Serogroup C</i>								
P1.5		3						3
NST							1	1
Total		3					1	4

NT - non typable

NST - non subtypable

All meningococci were tested against the following serotypes and serosubtypes using whole-cell ELISA:

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

serosubtypes 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.

Thirty-nine blood or CSF samples from culture-negative cases of meningococcal disease were tested by PCR for the presence of meningococcal DNA. Thirty-three samples were positive by *porA*-PCR. The *porA* gene encodes the subtype-specific antigens. Dot blot hybridisation showed that 28 of these samples were subtype P1.7b,4, one was P1.7, and four were negative with probes for subtypes P1.2, P1.4, P1.7 and P1.16.

Bordetella pertussis

The serotyping of all submitted isolates of *Bordetella pertussis* will be stopped as the results give no useful information relating to the use of pertussis vaccine. The predominance of serotype 1,3 is consistent with previous periods. The ages of the cases are given in Table 4. The recommended ages for vaccination against *B. pertussis* in New Zealand are at six weeks, three months, five months and 15 months.

Table 4. Age distribution of cases of *Bordetella pertussis*, April-June 2001

Age	<5m	5-<15m	15m-4y	5-9y	10-14y	15-19y	≥20y
Number	4	3	12	14	2	0	2

LEGIONELLOSIS AND ENVIRONMENTAL LEGIONELLA ISOLATES

During April to June 2001, 17 cases of legionellosis were identified and a further three cases were notified on clinical grounds only. Seven of the 17 cases were confirmed either by isolation of legionella organisms or the demonstration of a four-fold or greater increase in antibody titre. The remaining 13 were regarded as probable cases. All but one case occurred in people 50 years of age or older, the exception being a 32 year old. The age range was 32 to 84 with the average age being 63.9 years. The cases showed no gender bias.

During April and June 2001, 59 presumptive environmental isolates were received for confirmation as legionella species from other laboratories or were isolated by ESR. Of these, 38 were identified to the species and serogroup level, five legionella isolates could not be identified, and 16 did not belong to the genus.

Table 5. Legionellosis cases and environmental isolates, April-June 2001

Legionella species / serogroup	Clinical Cases			Number of environmental isolates
	Confirmed	Probable	Total	
<i>L. anisa</i>	-	-	-	4
<i>L. bozemanii</i> serogroup 1	-	-	-	4
<i>L. dumoffii</i>	0	3	3	-
<i>L. feellii</i> serogroup 2	-	-	-	1
<i>L. gormanii</i>	0	1	1	-
<i>L. longbeachae</i> serogroup 1	5	4	9	4
<i>L. longbeachae</i> serogroup 2	1	0	1	2
<i>L. micdadei</i>	0	1	1	-
<i>L. pneumophila</i> serogroup 1	-	-	-	8
<i>L. pneumophila</i> serogroup 3	-	-	-	1
<i>L. pneumophila</i> serogroup 5	-	-	-	1
<i>L. pneumophila</i> serogroup 6	-	-	-	4
<i>L. pneumophila</i> serogroup 10	-	-	-	2
<i>L. pneumophila</i> serogroup 13	0	1	1	-
<i>L. pneumophila</i> serogroup unidentified	1	0	1	7
Legionella sp.	-	-	-	5
Total	7	10	17	43

LEPTOSPIROSIS

During April to June 2001, 36 cases of leptospirosis were reported, which had either been laboratory-confirmed or notified on clinical grounds only. Twenty-eight cases were laboratory-confirmed and eight cases were notified on clinical grounds only. The known occupations for the notified cases included farmers (13), freezing workers (7), truck drivers (2), and a DOC worker (1).

The infecting *Leptospira* species and serovars were identified in 28 of the cases.

Table 6. *Leptospira* cases, April-June 2001

Leptospira species / serovar	Number of cases
<i>L. interrogans</i> sv pomona	6
<i>L. borgpetersenii</i> sv ballum	7
<i>L. borgpetersenii</i> sv hardjo	14
<i>L. borgpetersenii</i> sv tarassovi	1
Total	28

SPECIAL BACTERIOLOGY

Listeria monocytogenes

Two isolates of *L. monocytogenes* from human cases were referred in the period April to June 2001 (Table 7). One of the isolates was from an elderly adult with underlying illness, the other was a seven year old child being investigated for a cell-mediated immunity problem.

Table 7. *Listeria monocytogenes* from human cases, April-June 2001

Month isolated or of onset	Health district	Sex/Age	Source	O antigen serotype
May	Central Auckland	F 70y	BC	1/2
May	South Auckland	F 7y	CSF	1/2

ENTERIC PATHOGENS

SALMONELLA

There were 1,223 human isolates of Salmonella confirmed during January to June 2001 (Table 8) compared with 1,010 for the same period in 2000. The predominant isolate was *S. Typhimurium* phage type 160, averaging 20% of all isolates. One outbreak of 22 cases has occurred during this period. This phage type was uncommon during the same period in 2000.

There were 648 non-human isolates of Salmonella confirmed during January to June 2001 (Table 9), the same number as for the equivalent period in 2000.

The predominant isolates were *S. Brandenburg* (animal feed), *S. Hindmarsh* (sheep), *S. Typhimurium* phage type 135 (poultry environment), and *S. Typhimurium* phage type 160 (poultry environment and birds).

ESCHERICHIA COLI

There were 21 isolates of *E. coli* O157 confirmed during April to June 2001 (Table 10), compared with 14 for the same period in 2000.

Twenty-five isolates were characterised by pulsed-field gel electrophoresis (PFGE) using the enzyme XbaI. Indistinguishable patterns were seen in family groups but were distinct from each other. Two isolates from Auckland in May were indistinguishable from each other but no common link was found, similarly three isolates from Tauranga in February. Isolates from the Waikato district in May and June were distinct from each other. PFGE is used when a cluster is noted, but is not used routinely.

Table 10. Isolates of *E. coli* O157, April-June 2001

Month	Sex / Age	District	Clinical Details	Isolates from Known Contacts
April	U 2y	Southland	None given	
	M 20y	Wellington	Bloody diarrhoea	
	M 2y	Hawkes Bay	Bloody diarrhoea	
	M 1y	Otago	No details	
	F 1y	Waikato	No details	
May	M 5y	Auckland	Bloody diarrhoea	
	M 2y	Nelson	Diarrhoea	
	M 1y	Canterbury	No details	
	F 24y	Rotorua	Diarrhoea	
	M 2y	Waikato	No details	F 20, F -
	F 44y	Auckland	Bloody diarrhoea	
	M 12y	Waikato	No details	
	M 8m	Waikato	No details	
	F 1y	Canterbury	No details	
	F 68y	Auckland	Bloody diarrhoea	
June	F 1y	Waikato	Bloody diarrhoea	F 4
	M 1y	Tauranga	No details	
	M 4y	Waikato	Diarrhoea	

SHIGELLA

There were 103 isolations of Shigella confirmed during January to June 2001 (Table 11) compared with 66 for the same period in 2000. Details of the Auckland outbreak of *S. sonnei* Biotype a were published in Lablink 2001; 8:2.

Table 11. *Shigella* isolates, January-June 2001

Species	Type	Number	Comment
<i>S. sonnei</i>	Biotype a	38	3 known recent overseas travel 15 Auckland outbreak
	Biotype f	1	
	Biotype g	17	6 known recent overseas travel
<i>S. flexneri</i>	1	1	
	1a	1	Recent overseas travel
	2a	25	
	2b	1	
	3a	6	1 known recent overseas travel
	6	6	
	6 (Manchester strain)	1	
<i>S. boydii</i>	1	1	
	4	1	
	13	3	1 known recent overseas travel
	14	1	Recent overseas travel

Table 8. *Salmonella* isolates, January-June 2001

Serotypes	HEALTH DISTRICTS																				Total				
	NL	NW	CA*	SA	WK	TG	BE	GS	RO	TP	RJ	HB	TK	WG	MW	WR	WN	HJ	NM	WC		CB	SC	OT	SO
Adelaide	1																								1
Agona		2	3	2		2			1								2	1							13
Albany									2																2
Anatum		1	1		1												1				2				6
Bareilly					1				1																2
Bovismorbificans		1 ^R																		1					2
Brandenburg	1	1	2	1	2						9	1			1					4	2	12	9		45
Charlottenburg																	1								1
Derby					1																				1
Emek																	1 ^R								1
Enteritidis phage type	1		1		1										1		1			1		2	1		8
4		1	3		1												2	1				1			9
6			1	1	1 ^R	1														1					5
6a	1 ^R																								1
9a	1	3	3		15	4		1			1	6	6	1	3			3	1		8	1	5		62
9c																					1 ^R				1
13a																					1 ^R				1
14 variant		1	1	2																					4
21 variant																					1 ^R				1
40			1																		1 ^R				2
RDNC		1	2	3											1										7
Give					1																				1
Haardt		2	4		3	1						1			1		1	1		4		2			20
Hadar		4	1														1		1						8
Halfa						1																			1
Havana															1										1
Heidelberg		1	2	2				1	1										1						8
Hindmarsh			2										2		1					2		1			8
Hvitlingfloss					1													1							2
Ibadan	1			2																					3
Indiana																		1							1
Infantis	1	9	10	4	3	4	1		7			2				1	1	1				1	1	1	47
Javiana					1 ^R																				1
Kentucky			1																						1
Kiambu																		1							1
Lansing															1 ^R										1
Livingstone			1																						1
London			1																						1
Mbandaka		5	6	2																	1				14
Mississippi		1																	2		2		1		6
Montevideo				1													1	1			2				3
Muenchen																	1								1
Newport			1																1		3				5
Oranienburg			2														1								3
Orion 15 +																			1 ^R						1
Othmarschen		1 ^R																							1
Panama				1 ^R	1																				2
Papuaia	1																								1
Paratyphi A	1	1 ^R		2 ^R																	1 ^R				4
Paratyphi B																					1 ^R				1
Paratyphi B var Java			4	1	1						2			1											9
Poona																					1				1
Richmond																								1	1
Rissen			2	1																			1 ^R		4
Saintpaul	1										2						1				4	1	3		12
Sandiego				1							1										1				3
Schwarzengrund	1			1																					3
Senftenberg			2																						2
Singapore			1																	1					1
Stanley		2	3	2				1										1							9
Tennessee																								1	1
Thompson		1		1								7					1								10
Typhi		3	2	5 ^R	1 ^R				1				2								1 ^R		1 ^R		16
Typhimurium phage type	1	7	11	24	11	14	2		3			7	7	7	4		11	6		2	10		7	133	
4												1									2				2
8			1																						2
9	2	1	1	2	1									5	1			1		1	1		3	1	17
12a		1	1	1	1	1											1		1	1	7	2		1	16
23	1		3		1	1											1								6
26		1										1													2
42			3									1		1	1		9		3		6				24
42 variant		1	1	2																					4
42a		1																			3	1			5
60					1							1													2
101	2	6	10	2	4		1	1				3	1				3		8		11	1	3	1	57
135	3	9	11	13	11	3			2			5	24	1	10		5	9	18	1	1	3	7		136
154			1	4	1			1										1							9
155			3	2					1												3				10
156	6	9	12	3	9	1	1					3	5	1	7		11	8			3				79
156 variant					1																				2
160	6	18	38	46	21	8	1		1		1	1	3	3	17	1	15	19	7		43	4	13	3	269
160 variant	1																								1
177		1 ^R																							1
192																								1	1
205			1		1																1				4
RDNC					1														1		3				7
Untypable		1			1	1													1		2				6
Uganda				1																					1
Virchow	1 ^R	1	1		1												2								6
Wandswoth			1																						1
Welleveden	1		1	1													3							1	7
Welleveden 15 +			1 ^R																						1
Worthington																					1				1
Group A 2,12 : - : -			1 ^R																						1
Group B 4,12 : - : 1,2																					1				1
Group C 6,7 : k : -											4						1								5

Table 9. *Salmonella* serotypes, non-human isolates, January-June 2001

SEROTYPE	ANIMALS												Meat/bone meal	Environmental	Food	Not specified	POULTRY					TOTAL
	Alpaca	Avian	Bovine	Canine	Caprine	Cervine	Equine	Feline	Other	Ovine	Porcine	Rabbit					Neckflap	Caecae	Feed	Environmental	Miscellaneous including product	
Agona															1	1		14	6		22	
Anatum													5								5	
Anatum 15 +																		1	2		3	
Bovismorbificans																			1	1	1	
Brandenburg			9		1			2		8	1		15	7	11	16	1	9	1		81	
Derby														2		1					3	
Emek														1							1	
Enteritidis phage type 1																1					1	
9a			3					1			1			3							8	
Havana													2					1			3	
Hindmarsh			2	1		1	2	1		75					2	1					85	
Idikan																		1		1	2	
Infantis											1		15	3		3	2	9	1	2	36	
Krefeld																1					1	
Lille																		1			1	
London																			1		4	
Mbandaka														1				6		1	8	
Muenster														1				8			9	
Oranienburg					1													3			4	
Orion				1												1					2	
Orion 15+													1								1	
Oslo															4	1					5	
Ruiru																			1		1	
Saintpaul							1			3					1						5	
Schwarzengrund																1					1	
Senftenberg													2					2	5		9	
Tennessee																		14	2		17	
Thompson				1											3			2	1		7	
Typhimurium			1																		1	
Typhimurium phage type 1			8				1							1		1	4		6	3	24	
8			1							1											2	
9			2	1			1	1		1			1		1						8	
12a			2															5	1	2	11	
23		2						1											1		4	
41																					5	
42			2											5							5	
42 variant														6	1	1			2	2	14	
42a			3											4				3	1	1	6	
60			2																		2	
101			6							1				1	1		1		1	1	12	
130																			1		1	
135			9	2			1	1						1		1	17	2	8	27	87	
154							7														7	
155			2																1		3	
156			12	3		1	4														20	
160	1	12	2			2	2	8	1		1	1	5	6	3	4		5	18	11	82	
206										1											1	
RDNC		1	1		1		1									1	1			2	8	
Rough										1											1	
Untypable																			1		1	
Group B 4,5,12 :- :- (non motile)										1				1		1					3	
Group C 6,7 : k :-															2					1	3	
Group C 6,7 :- :- 1,5																1					2	
Group C 6,7 :- :- (non motile)																			2		2	
Group E 3,19 :- :- (non motile)																				4	4	
Welleveden																					1	
TOTAL	1	15	67	9	3	4	20	15	1	93	6	1	41	42	33	37	33	2	99	83	43	648

S. Typhimurium 307
 Other serotypes 341
 Poultry isolates 260
 Animal Isolates 235

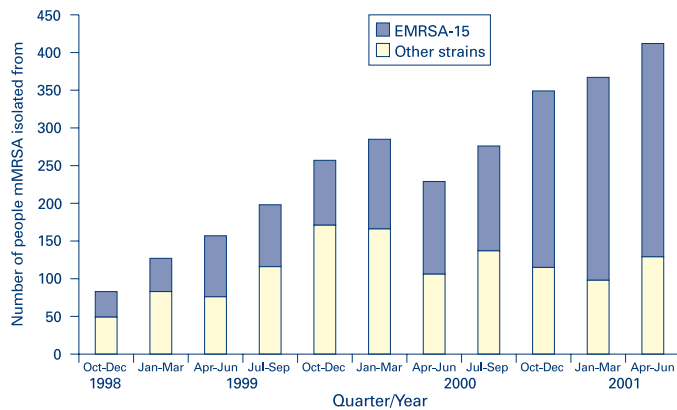
ANTIBIOTIC RESISTANCE

MULTIRESISTANT METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS, JANUARY-JUNE 2001

During the six months January to June 2001, multiresistant methicillin-resistant *Staphylococcus aureus* (mMRSA, MRSA resistant to two or more classes of antibiotics in addition to β -lactams) from 752 people (706 patients and 46 healthcare workers) were referred to ESR (Figure 1). The crude (not adjusted for trend) annualised incidence of mMRSA during the first half of 2001 was 41.6 per 100,000, which is a 50.2% increase on the 2000 rate of 27.7 per 100,000. The majority (74.5%) of the 706 patients with mMRSA were categorised as hospital patients (in a healthcare facility when MRSA was isolated or during the preceding three months).

The mMRSA strains most commonly isolated are shown in Table 12. EMRSA-15 became even more predominant during the first six months of 2001, and represented 71% of mMRSA isolations compared with 55% in 2000 (Figure 1). The hospitals and other healthcare facilities in which EMRSA-15 was isolated between January and June 2001 are shown in Table 13. EMRSA-15 was most commonly isolated from hospitals and other healthcare facilities in the Auckland area. There were also notable outbreaks in Hawkes Bay Hospital and Waikato Hospital. The Hawkes Bay Hospital outbreak commenced in late 2000 and continued into the first few months of 2001.

Figure 1. Multiresistant MRSA isolations, October 1998 - June 2001



Among the 497 patients with EMRSA-15, 83.1% were categorised as hospital patients. Over a third (165, 37.2%) of these patients were in healthcare facilities other than public hospitals, predominantly long-term care facilities. Almost all (43, 93.5%) mMRSA isolated from healthcare workers were EMRSA-15.

Table 12. Most commonly isolated multiresistant MRSA strains, January-June 2001

Strain (origin) ¹	Number of people the strain isolated from (% of all mMRSA isolations)
EMRSA-15 (Britain)	540 (71.0)
WR/AK1	47 (6.2)
TANS2 (Australia)	19 (2.5)
WSPP1 (Western Samoa)	16 (2.1)
WGWH1 (Australia)	11 (1.4)

¹ Includes strains isolated from more than 10 people. For a description of the EMRSA-15, WR/AK1, WGWH1 strains, see *LabLink* 2000; 7 (1): 8-9. The TANS2 strain is a typical Australian mMRSA, with phage pattern 83A. It was isolated from several Auckland hospitals during the second quarter of 2001. The index case was a patient transferred from a Queensland hospital to North Shore Hospital.

The second most commonly isolated strain between January and June 2001 was the WR/AK1 strain. This strain was isolated from 47 people, mostly community patients in the Auckland area or patients in Auckland hospitals.

Table 13. Healthcare facilities with patients and staff with EMRSA-15, January-June 2001

Healthcare facility ¹	Number of people EMRSA-15 isolated from (% of all EMRSA-15 isolations in healthcare facilities, n=496 ²)
North Shore Hospital	20 (4.0)
Auckland Hospital	60 (12.1)
Green Lane Hospital	5 (1.0)
Middlemore Hospital	74 (14.9)
Other Auckland HCFs ³	139 (28.0)
Waikato Hospital	34 (6.9)
Hamilton HCFs ³	7 (1.4)
Thames Hospital	7 (1.4)
Tauranga Hospital	11 (2.2)
Whakatane Hospital	7 (1.4)
Hawkes Bay Hospital	64 (12.9)
Other Hawkes Bay HCFs ³	7 (1.4)
Wellington Hospital	9 (1.8)
Kenepuru Hospital	16 (3.2)
Other Wellington HCFs ³	10 (2.0)
Timaru Hospital	5 (1.0)

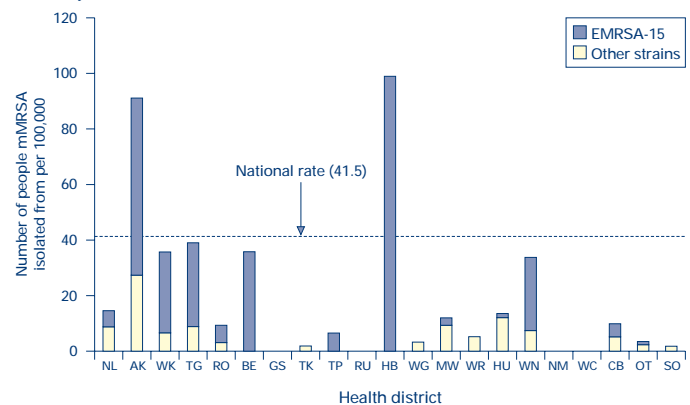
¹ Hospitals and other healthcare facilities (HCFs) with ≥ 5 patients or staff with EMRSA-15 are listed in the table. EMRSA-15 was also isolated from people in Whangarei Hospital (2 patients or staff), Whangarei HCF (1), Dargaville Hospital (1), Waitakere Hospital (3), Starship Hospital (1), National Women's Hospital (1), Tauranga HCF (1), Rotorua Hospital (3), Rotorua HCF (1), Te Kuiti Hospital (4), Wairoa Hospital (1), Hutt Hospital (1), and Burwood Hospital (1). In this list, private HCFs are not named, as many have withheld publication of their identity.

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

³ An aggregated total for private healthcare facilities in the area, many of whom have withheld publication of their name.

The incidence rates of mMRSA in the various health districts is shown in (Figure 2). Two health districts had rates above the national average: Auckland (the three combined Auckland health districts) and Hawkes Bay.

Figure 2. Annualised incidence of multiresistant MRSA by health district, January-June 2001



The susceptibility of mMRSA to an extended range of antibiotics is shown in Table 14. All EMRSA-15 isolates tested were resistant to oxacillin, ciprofloxacin and erythromycin. However, as previously noted (*LabLink* 2001; 8 (1): 11), there is some variation in macrolide sensitivity among EMRSA-15, with some isolates being sensitive to erythromycin. These isolates are not categorised as multiresistant and are therefore not included in this report on mMRSA.

The WR/AK1 strain is characteristically resistant to high-level mupirocin (MIC ≥ 512 mg/L) and fusidic acid. The TANS2 strain is usually resistant to ciprofloxacin, clindamycin, co-trimoxazole, erythromycin, gentamicin and tetracycline (Table 14). Refer to *LabLink* 2001; 8 (1): 11 for the antibiograms of other mMRSA strains, including AKAH2, AMRSA-1 and WGWH1, which are all typical Australian mMRSA. AKAH2 and WGWH1 have a similar antibiogram to TANS2, while AMRSA-1 is usually resistant to clindamycin, erythromycin and tetracycline, and variably resistant to co-trimoxazole and gentamicin.

Table 14. Resistance of multiresistant MRSA, January-June 2001¹

Antimicrobial agent (resistance breakpoint, mg/L)	Percent resistance			
	All isolates ²	EMRSA-15 (n=109)	WR/AK1 (n=47)	TANS2 (n=19)
Chloramphenicol (MIC ≥32)	1.2	0.9	0	0
Ciprofloxacin (MIC ≥4)	85.7	100	0	100
Clindamycin (MIC ≥4)	22.2	11.0 ³	0	100
Co-trimoxazole (MIC ≥4/76)	11.3	0	0	100
Erythromycin (MIC ≥8)	91.4	100	6.4	100
Fusidic acid (MIC ≥2)	10.5	0.9	100	0
Gentamicin (MIC ≥16)	11.4	0	0	89.5
Mupirocin (MIC ≥8)	13.2 ⁴	2.8 ⁴	100 ⁴	0
Rifampicin (MIC ≥4)	3.1	0.9	0	10.5
Tetracycline (MIC ≥16)	14.5	0.9	2.1	100
Vancomycin (MIC ≥32)	0	0	0	0

- ¹ A sample of 109 isolates of EMRSA-15 strain was tested. All isolates of other strains were tested.
² These resistance rates have been calculated to give an estimate of resistance among all mMRSA. The estimate adjusts for the sampling of EMRSA-15.
³ EMRSA-15 demonstrates inducible clindamycin resistance by the disc diffusion induction test.
⁴ 1.3% of isolates had low-level resistance (MIC 8-256 mg/L) and 11.8% had high-level resistance (MIC ≥512 mg/L). All mupirocin-resistant EMRSA-15 and WR/AK1 isolates had high-level resistances.

VIROLOGY

Influenza virus

Influenza sentinel surveillance currently runs from May to September. In May, influenza activity remained at the baseline level with only fourteen isolations (seven of A H1N1 and seven of A H3N2). The first two influenza isolations were reported in Waikato in Week 18 and spread quickly throughout the country. In June, influenza activity increased rapidly and peaked at Week 26 (the end of June). A total of 201 influenza isolates were reported in June, 166 of A H1N1, 5 of A H3N2, 5 of influenza A not subtyped, and 25 of influenza B. Obviously, A H1N1 was the predominant strain during April to June. A H1N1 was antigenically related to the A/New Caledonia/20/99 strain. Influenza A H3N2 was antigenically similar to A/Moscow/10/99 strain (as represented by A/Panama/2007/99 in the WHO diagnostic kit). Influenza B was antigenically related to B/Sichuan/379/99 strain (as represented by B/Johannesburg/5/99 in the WHO diagnostic kit). These strains were all covered in the influenza vaccine composition for year 2001.

Adenoviruses

During April to June 2001, a total of 45 adenoviruses were serotyped. This is significantly higher than 10 adenovirus isolations during the same period of 2000. Adenovirus type 22 was isolated from 17 patients (with conjunctivitis), all from Auckland. The specimens from these patients were taken between 2 April and 24 May 2001. The patients ranged in age from 25 to 85 years (average 45.8 years). The male (14) to female (3) ratio was 4.7. Eleven isolations of Adenovirus type 3 were reported: Auckland (5), Waikato (5), and Christchurch (1). Adenovirus type 3 was mainly associated with conjunctivitis/respiratory illness. The remaining 17 adenoviruses were typed as adenovirus type 1 (4), type 2 (3), type 5 (2), type 8 (3), type 17 (1), type 4 (1), and untypable (3).

Enteroviruses

Coxsackie B5 virus outbreak

Lablink 2001, 8(2):24 reported the Coxsackie B type 5 outbreak during the period of January to March. This outbreak continued during April to June with 7 additional cases reported: Waikato (5), Auckland (1), and Wellington (1). The specimens from these patients were taken between 15 March and 2 May 2001. The main clinical features were vomiting, headache and meningitis.

Enterovirus 71 outbreak

During March to June, Enterovirus 71 was identified from eleven patients: Waikato (6), Wellington (4), and Auckland (1). The index case was an eleven-month old boy from Waikato presenting with hand-foot-mouth symptoms. Patients ranged in age from three weeks to three years (median one year). Male (10) to female (1) ratio was 10:1. Clinical features were mainly viral meningitis and hand-foot-mouth disease.

Enterovirus 71 is relatively uncommon in New Zealand, with only one

isolate identified each year during the 1998-2000 period. Between 1991 and 1997 no isolates were identified. The virus can cause mild respiratory illness, hand-foot-mouth disease, as well as potentially life threatening illnesses such as aseptic meningitis, encephalitis, and acute flaccid paralysis. Enterovirus 71 is transmitted by faecal-oral contamination and less commonly via respiratory droplets, and has the potential to cause outbreaks or epidemics. The most recent outbreak in the Southern Hemisphere occurred in Perth, Western Australia, from February to September 1999 (McMinn P, et al. *Clin Infect Dis* 2001. Jan 15; 32(2):236-42). This outbreak included 14 cases of EV71-induced neurological disease.

Measles

During April to June, three laboratory-confirmed measles cases were reported from Christchurch. Serum was taken from a 33-year old female on 3 April, from a one-year old boy on 9 May, and from a 33-year old female on 31 May. They were all IgM positive for measles.

Mumps

During April to June, six laboratory-confirmed mumps cases were reported: Christchurch (3), Otago (2), and Rotorua (1). The patients ranged in age from 4 years to 84 years (average 25.2 years). Sera from these patients were taken between 2 April and 25 June 2001.

Norwalk-Like Virus (NLV)

There were 20 outbreaks of laboratory-confirmed NLV-associated gastroenteritis to 30 June 2001. The seasonal drop in outbreaks over the winter months observed over the last 5 years did not occur in 2001. Seventeen of the 20 outbreaks (85%) occurred in April, May and June. Only one outbreak was classed as foodborne, but a further three outbreaks were reported as both foodborne and person-to-person transmission. Six institutional outbreaks in rest homes and hospitals were reported; these were generally person-to-person transmission. The remaining 10 outbreaks were also reported as person-to-person transmission. Outbreak settings included a large barbecue attended by over 200 people, two children's party centres, a factory workplace event, and several family groups.

The range of genotypes circulating was lower than in previous years. The predominant types were the common 'Global strain', GII/1,4,8 and strains belonging to genotype GII/6,7,9 (Napier, Florida and Gwynedd viruses). However, some Genogroup I strains are again circulating in New Zealand. These strains were last seen in New Zealand in 1999. One outbreak was caused by GI/3b, Desert Shield virus, and three by GI/4, 'Cruise Ship virus'.

Table 16. NLV Genotypes occurring between January and June 2001

NLV Strain	Genotype	Number (%)
Lordsdale virus 'Global strain' cluster	GII/1,4,8	7 (35)
Napier / Florida / Gwynedd / Idaho Falls virus cluster	GII/6,7,9	6 (30)
'Cruise ship virus'	GI/4	3 (15)
Desert Shield virus	GI/3b	1 (5)
Not sequenced	Unknown	3 (15)
Total		20 (100)

Laboratory Safety

• Health Management

The Australian/New Zealand Standard AS/NZS 2243.3:1995 Safety in Laboratories Part 3: Microbiology sets out requirements, responsibilities and general guidelines relating to safety in laboratories where microorganisms are handled.

One section deals with personal health management and includes immunisation recommendations for persons working with infectious agents. Vaccination against *Neisseria meningitidis* for laboratory staff working with material (eg, blood, cerebrospinal fluid and cultures) which may contain the organism is of particular importance, as several rapidly-fatal laboratory acquired infections have been recorded in the literature.

At present most of the invasive meningococcal disease in New Zealand is caused by *N. meningitidis* Group B strains for which there is no vaccine available, but there is vaccine available for Groups A, C, W135 and Y.

• Packing and Shipping of Infectious Substances

The International Air Transport Association (IATA) has published a manual entitled "Infectious Substances Shipping Guidelines". The 2nd edition issued January 2001 may be ordered on line at www.iataonline.com or from good booksellers. IATA's internet site is www.iata.org

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