

# MENINGOCOCCAL DISEASE DIAGNOSIS

## Introduction

Invasive meningococcal disease follows exposure and colonisation of the naso-pharyngeal mucosa by a virulent meningococcus, passage of that meningococcus through the mucosa followed by multiplication in the bloodstream.<sup>1</sup> The process of infection is influenced by a number of organism and host factors including organism characteristics, a preceding or concomitant viral infection, the immune status of the individual and other contributing factors, such as mucosal damage by active or passive smoking.<sup>2-4</sup> Once viable meningococci have reached the bloodstream different disease manifestations develop.<sup>1</sup> In some patients a transient meningococcaemia occurs, characterised by a short febrile flu-like illness.<sup>1</sup> If the bacteremia is not cleared, clinically overt disease occurs resulting in either meningitis or meningococcal septicaemia (meningococcaemia) without signs of meningitis. Frequently, meningococcal disease occurs as a combination of meningitis and meningococcaemia. Meningitis involves the multiplication of meningococci in the outer lining of the brain and the spinal cord. Meningococcaemia involves the multiplication of meningococci in the bloodstream with rapid progression to fulminant disease involving skin haemorrhages and other signs of disseminated intravascular coagulation.<sup>1</sup> As household contacts of cases have an increased risk of disease the prompt delivery of chemoprophylaxis to eradicate the disease-causing strain in contacts, is necessary. Thus public health intervention requires both the timely notification of cases occurring and prompt and informative laboratory confirmation. This article provides information on diagnostic tests currently available in New Zealand for the confirmation of clinical disease and their respective values in the overall control and surveillance of disease.

## Diagnostic testing to confirm meningococcal disease

Clinical laboratories play a vital role in supporting the diagnosis of meningococcal disease in patients presenting with relevant symptoms. Critical to any diagnostic test result is the collection of an appropriate specimen, its storage and transportation in a fashion that will not compromise the results.<sup>5</sup> Personnel in diagnostic laboratories should ensure that appropriate specimens are taken and are immediately transferred to the laboratory. **It is recommended that all patients with suspected meningococcal infection have blood and CSF (if appropriate) taken for microscopy, culture, nucleic acid testing using PCR, and for serum antibody determinations (Table 1).** In most cases only the gram-stain result will be received in time to impact upon patient management but other laboratory results allow confirmation of disease diagnosis and provide information for public health action.

Diagnosis is confirmed by the isolation of *Neisseria meningitidis* from

CSF, blood or other normally sterile sites. For a clinically compatible illness and in the absence of a positive culture, the presence of meningococcal-specific DNA in CSF, blood or an aspirated specimen, or the demonstration of gram-negative intracellular diplococci in CSF or an aspirated specimen, is consistent with confirmation of disease. Serum antibody tests, based on demonstration of a single high IgM titre or a raised IgG titre in convalescent serum, provide retrospective confirmation of meningococcal disease (Table 2). Meningococcal serum antibody testing is being set up at ESR in 2002. Throat swabs may reveal the presence of a meningococcus but cannot confirm the disease agent as there is no certainty that the meningococcus recovered is the causative organism although there is usually a high correlation.<sup>6</sup> As administration of antibiotics, prior to specimen taking, will reduce the likelihood of culturing a meningococcus, DNA detection is an important means by which aetiological confirmation can occur. PCR testing requires the availability of uncentrifuged CSF or anticoagulated blood.

## CSF and aspirated joint fluids or skin lesions

In cases of meningococcal meningitis the CSF usually has a high neutrophil count, low glucose, and high protein. Low or absent white blood cell counts and normal CSF parameters, particularly in the early stages of disease, do not exclude meningococcal disease.<sup>8</sup> Gram-negative diplococci may be seen in a smear of CSF or an aspirate. The sensitivity of the gram stain for demonstration of intracellular diplococci is around 65% whereas the sensitivity of culture in untreated cases of disease is around 95%.<sup>7</sup> In cases of meningococcal meningitis a CSF culture is more likely to be positive than is blood. Timing is critical for obtaining a positive culture particularly after antibiotics. Sensitivity of the PCR test is around 90% and may be positive up to 72h after antibiotics.<sup>9</sup> A PCR-positive test on CSF, aspirated fluids or lesions confirms the diagnosis of meningococcal disease. Punch biopsy specimens or aspirates of skin lesions may be culture-positive up to 13 hours after administration of antibiotics.<sup>10</sup> Taylor et al, 1997 reported skin scrapings of petechial rashes were gram-stain positive in 80% (24/30) of instances.<sup>11</sup>

## Blood

Blood cultures should be obtained for all cases of suspected disease. The sensitivity of blood culture is reported to be only about 50% in untreated cases of meningococcal disease and falls rapidly to zero once antibiotics have been administered.<sup>7</sup> Most PCR tests on blood specimens have a sensitivity of 70-80% and specificities of >95% depending on the test used. Desirably a separate blood specimen should be taken specifically for PCR to prevent use of contaminated specimens.

## Serum antibody tests

A variety of specialised antibody tests can be used to measure functional antibody. However, the measurement of non-functional antibody based on enzyme immunoassay, using outer membrane proteins as the antigen, was developed in the United Kingdom.<sup>12</sup> The test specificity has been calculated at 95% and sensitivity at >97% in adults and children ≥4 years of age.<sup>7</sup> Reactions compatible with a recent meningococcal infection are a positive IgM test on a single serum or seroconversion if paired sera are available. IgM reaches diagnostic levels about 5-7 days after onset of disease. The test lacks sensitivity in very young children. **Serum**

Table 1. Specimens used for the diagnosis of meningococcal disease



<b>Leading Article</b>									
Meningococcal disease diagnosis	33	Special Bacteriology	37	WHO Gonococcal Antimicrobial Surveillance Programme, 2000	38				
<b>Bacteriology</b>	35	<i>Listeria monocytogenes</i>	37	<b>Virology</b>	39				
Invasive Infections	35	<b>Enteric Pathogens</b>	37	Respiratory Viruses	39				
<i>Bordetella pertussis</i>	36	Salmonella	37	Norwalk-like Virus	39				
Nosocomial Infections	36	<i>Escherichia coli</i>	37	<b>Culture Collection</b>	40				
Legionellosis and Environmental		Shigella	38	New Names	40				
Legionella isolates	36	<b>Antibiotic Resistance</b>	38	<b>Mycology</b>	40				
Leptospirosis	37	Resistance among Group A Streptococci	38						

collected within 24 hours of admission and a convalescent serum sample taken 14 to 21 days after admission to hospital are required.

#### PCR testing methods available

PCR testing is highly specific and sensitive, providing appropriate methodologies are used. PCR tests not only confirm diagnosis in clinically compatible culture-negative cases, but can provide information that guides public health action and policies and assist in the epidemiologic description of disease. Internationally, the tests most often used diagnostically are the *ctrA*<sup>13</sup> with or without the *siaD* test<sup>14</sup> and the *porA*<sup>15</sup> test. The *ctrA* test, is often used as a screening test. It identifies the capsular transfer gene *ctrA*. The *siaD* assay is based on restriction fragment length polymorphism analysis of the sialyltransferase (*siaD*) gene which defines the capsular serogroup of the invading meningococcal DNA. It is particularly informative when vaccination using the quadrivalent vaccine for serogroups A,C,Y,W135 is being considered. The *porA* PCR amplifies the gene encoding the PorA protein and enables definition of the PorA subtype of the organism informing on the type of meningococcus causing the disease. This has particular importance in the New Zealand context where meningococci with the PorA subtype P1.7b,4 are currently causing most disease.<sup>16</sup> When the Insertion Sequence IS1106 PCR test is used the results should be confirmed by an alternative test. The reason is that the insertion sequence is not specific for meningococci. It is a mobile genetic element and is found in other *Neisseria* spp and other pathogens including the pneumococcus.<sup>17</sup> Alternative and less informative PCR tests have also been devised but are not reported here.

Currently there is no commercially available PCR test. Thus standardization at the amplification and detection stages of 'in-house' methods requires careful optimisation. Furthermore the rapid evolution of reagents and testing procedures makes it very difficult to standardize across methodologies. Amplification primers must be selected to give the highest specificity for the target nucleic acid. Methods used should provide useful information and results that can be compared between laboratories. The recent introduction of 'real-time PCR' with both TaqMan and Light Cycler technologies is revolutionising results obtainable at the diagnostic testing level. The TaqMan and Light Cycler processes combine amplification and detection steps, thus shortening the turn-around time for diagnostic use and add the option of quantitation not available using gel visualisation.

In a recent inter-laboratory blind comparison with Waikato Hospital a 100% comparability of results occurred for the 20 CSF specimens sent from the Waikato laboratory and for 10 other CSF specimens sent from ESR. Waikato tested the specimens by the *ctrA* test using a Light Cycler and ESR by the *porA* nested PCR test. PCR testing of blood produced a few discrepancies which are being investigated. The nested *porA* method used at ESR is more sensitive than the *ctrA* PCR and this could account for the discrepant results as PCR testing of blood is less sensitive than testing of CSF. ESR receives extracted DNA from Auckland and Christchurch Hospital laboratories both of whom use the IS1106 test. Over the

Table 2. Value of tests available for the diagnosis of meningococcal disease

Diagnostic test	Specimen	Availability	Value
Gram stain	CSF, skin lesion, joint fluid	All diagnostic laboratories	Rapid test Confirms diagnosis in a clinically compatible case Sensitivity in CSF around 65% Sensitivity in skin lesions 80%
Culture	CSF, blood, skin lesion, joint fluid, or other normally sterile site	All diagnostic laboratories	Results in 24-48 hours Confirms diagnosis if positive Against clinically compatible disease with true meningism the: • sensitivity in CSF, if no prior antibiotics, near to 100% • sensitivity in blood, if no prior antibiotics, around 50% <sup>7</sup>
PCR test	CSF, blood (EDTA or citrated) or aspirate	Diagnostic testing by arrangement: Auckland, Waikato or Christchurch Hospitals. Delayed-diagnostic and characterisation testing at ESR	Positive result confirms diagnosis in a clinically compatible case. Specificity should be 100% but sensitivity varies with test used and specimen (see section Interpreting Diagnostic Results). Information can be gained on serogroup, serotype and subtype from DNA in patient specimens or untypable isolates.
Antigen test	CSF	Diagnostic laboratories	Unreliable result Corroborates diagnosis if other laboratory parameters positive
Serum antibodies	Blood	ESR only	Single positive IgM or rising convalescent titre confirms diagnosis in a clinically compatible case. Specificity 95%; sensitivity >97% in children ≥ 4 years and adults.

last three years most results have been confirmed using the *porA* PCR. For those not confirmed, possible reasons include degradation of DNA prior to receipt at ESR and a previously false positive result.

#### Interpreting meningococcal diagnostic results

The definitive result confirming meningococcal disease is the culture of a meningococcus from an invasive site. However, CSFs are frequently not taken, or may not be an appropriate specimen, and culture of blood is relatively insensitive.<sup>7</sup> The use of antibiotics prior to specimen taking also reduces the likelihood of obtaining a viable culture. In New Zealand, for the years 1996-2000, 24.9% of 586 cases treated with antibiotics prior to admission were culture-positive compared with 60.0% of 1502 cases not receiving antibiotics.<sup>18</sup> Thus, the positive PCR test becomes the most important result after culture.

**Sensitivity and specificity of PCR testing:** PCR tests are more sensitive than are culture methods and the PCR result is less affected by antibiotics. Thus PCR tests are often genuinely positive when conventional tests are negative due to the higher sensitivity of the PCR test. Rangunathan and co-workers showed that PCR-based techniques increased the overall number of microbiologically confirmed cases of meningococcal disease by 44%.<sup>19</sup> Comparison of the sensitivity of a PCR test against the gold standard culture results, under-estimates the true sensitivity of PCR testing. Corless and co-workers<sup>20</sup> showed that when compared against culture-confirmed cases the *ctrA* PCR on CSF was 88.9% sensitive and on whole-blood EDTA was 82.4% sensitive. ESR showed a similar result with the *porA* PCR when results from specimens received were compared against diagnostic laboratory culture-positive results. The *porA* PCR was 100% sensitive for CSF and 82.4% sensitive for whole-blood (Martin and Walker, unpublished). Difficulties when comparing results from whole-blood assays occur due to differences in timing of the specimens taken as whole-blood for PCR assay is not often taken at the same time as the blood culture draw. Furthermore, differences in sensitivity occur between different PCR assays undertaken on the same specimens. Guiver et al<sup>17</sup> showed that the sensitivity of the Taqman *ctrA*, IS1106, and *siaD* assays from culture-confirmed cases were 64, 69 and 50% compared with 26, 67, and 43% for the corresponding PCR-ELISA assays.

The higher sensitivity of PCR does allow detection of DNA in a specimen even at a clinically insignificant level. In such cases clinical parameters and other tests may clarify the situation. However, it should not be overlooked that samples positive by PCR, but negative by conventional methods, may be falsely positive due to contamination of the specimen prior to testing, laboratory error, or use of a test with poor specificity. False negative results also occur mostly due to inhibitors present in patient specimens.

Of the remaining diagnostic tests the gram-stain may be falsely interpreted due to overstaining of gram-positive bacteria although careful examination of organism shape should alert the viewer to the error. The serum antibody test relies on a differential between IgG antibody levels occurring in the acute phase of disease and those occurring in the convalescent phase. Timing of serum samples is the major factor influencing the value of this testing.<sup>7</sup>

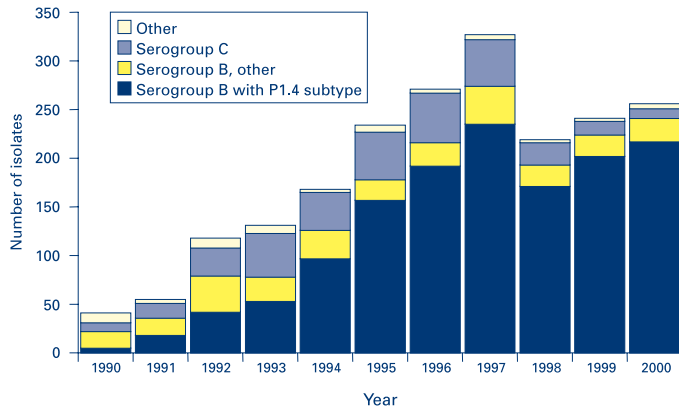
#### Simultaneous infection with meningococci and viruses

Simultaneous infections of meningococci and various viruses, particularly enteroviruses, are documented in the literature.<sup>3, 21-23</sup> Such reports are based on either culture of the organisms involved or serologic confirmation and pre-date the use of more sensitive PCR technology for viral and bacterial DNA. With increasing use of PCR it is likely that there will be more reports of simultaneous infections. The existence of dual infection in a few patients is currently being investigated in New Zealand. An outbreak of enterovirus infections is superimposing itself on an existing high background rate of meningococcal infection in many parts of the country. Definition of a single aetiological agent for a small number of cases is in conflict with the laboratory results which suggest the possibility of dual infection with meningococcus and enterovirus. The validity of some laboratory results, particularly those obtained by PCR from blood, are being called into question. A predominance of polymorphonuclear cells in both bacterial and early stage enteroviral meningitis may be an additional confounder<sup>24</sup> An investigation using additional laboratory testing, assessment of clinical presentations and treatment responses is in progress.

#### Meningococci causing New Zealand's epidemic of disease

Early in the epidemic the only tools available to the Meningococcus Reference Laboratory in ESR for defining the epidemic strain were monoclonal antibodies. Thus, New Zealand's epidemic was defined as being caused by a strain with

Figure 1. Meningococcal disease isolate serogroup and dominant subtype, 1990-2000



phenotype B:4:P1.4 where B is the serogroup, 4 is the serotype, and P1.4 the subtype.<sup>25</sup> Antigenic variation of the PorB (Class 2 or 3) outer membrane protein (OMP) and the PorA by the Class 1 OMP determine the serotype and subtype respectively. The subtype is recognised by the use of P1 in front of the antigen type. All meningococci have a PorA protein which has two epitopes on its protein structure defining the P1 type. Meningococci causing New Zealand's epidemic have the two epitopes, 7b, and 4, giving it the subtype P1.7b,4.<sup>25</sup> Sequence variations can occur in the gene encoding the PorA protein and these may directly affect the amino acid sequence of the epitope making it unrecognisable or may alter the position of the epitopes on the mature Por A protein. This latter situation occurs with the P1.7b. The shift in the positioning of the 7b epitope means it cannot be detected by epitope-specific monoclonal antibody used for serotyping. Thus, when serologic typing is being used only the second epitope, the P1.4, is detected. When DNA sequencing methods are used both epitopes on the P1.7b,4 PorA protein can be defined. Results sent out following DNA-DNA hybridisation gives the subtype of the *porA* PCR as P1.7,4. The reason is that the probe used does not differentiate the sequence as 7b or an alternative 7. Analysis by sequencing shows that virtually all isolates with the P1.4 subtype defined by sero-subtyping, or as P1.7,4 by DNA-DNA hybridisation, are P1.7b,4. Note, the P1.7b,4 PorA subtype may occur in combination with other serotypes and occasionally with another serogroup. Examples of combinations among New Zealand isolates are: B:14:P1.7b,4; B:1:P1.7b,4; C:2a:P1.7b,4. The impact of meningococci causing disease in New Zealand with the P1.7b,4 PorA protein subtype is illustrated in Figure 1.

**Prospects for controlling the meningococcal epidemic**

The Ministry of Health has entered into an agreement with Chiron Corporation in association with the National Institute of Public Health, Norway, to produce a vaccine for New Zealand that is intended to control the current epidemic. The most important antigen contained in the vaccine is the PorA protein P1.7b,4. Antibodies elicited by the PorA protein are the most important for protecting against serogroup B disease. The vaccine should protect against all meningococci expressing the P1.7b,4 PorA subtype regardless of their serogroup or serotype. In 2000 such organisms caused 84.6% of all cases of disease for whom this was able to be ascertained and preliminary results for 2001 indicate a similar proportion occurred. ESR is dependent on the goodwill of diagnostic laboratories for the provision of isolates and samples for DNA analysis. We are very grateful for the co-operation and assistance we get from laboratory staff. Without this, New Zealand would not have such a comprehensive surveillance system.

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**BACTERIOLOGY**  
**INVASIVE INFECTIONS**

Numbers of isolates received from cases of invasive disease caused by *Haemophilus influenzae*, *Neisseria meningitidis*, *Streptococcus pneumoniae* during July to September 2001, are shown in Table 3.

Table 3. Sterile site isolates, July-September 2001

Organism	BC	CSF or CSF/BC	Other Sterile Site	Total	YTD
<i>H. influenzae</i> <sup>1</sup>	17	1	1	19	38
<i>N. meningitidis</i>	84	28	3	115	239
<i>S. pneumoniae</i>	193	11	2	206	410

<sup>1</sup> *H. influenzae*: 1 serotype b and 18 non-b

The age profile of the patients from whom the isolates were obtained is given in Table 4.

Table 4. Age distribution of cases of invasive disease, July–September 2001

Organism	<1m	1-11m	1y	2y	3y	4y	5-9y	10-24y	25-59y	≥60y
<i>H. influenzae</i> b	1									
<i>H. influenzae</i> non b	7	1	10							
<i>N. meningitidis</i>	1	14	13	12	5	3	8	39	17	3
<i>S. pneumoniae</i>	1	25	18	7	4	1	11	12	48	79

### Haemophilus influenzae

We continue to monitor *H. influenzae* isolates to determine whether serotype b disease remains controlled by the vaccine. During July to September 2001, 19 isolates were received from cases of *H. influenzae* invasive disease. One of these isolates was serotype b, and the others were not serotypable using serotype-specific antisera. This compares with three serotype b from a total of 25 isolates for the same period last year.

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

During July to September 2001, a total of 115 sterile site isolates were received from cases, compared with 76 for the same period last year. Of these, 107 were serogroup B, seven were serogroup C, and one was serogroup Y. Serotyping and serosubtyping results of the serogroup B and C organisms are given in Table 5.

Table 5. Serotypes and subtypes of *N. meningitidis*, July–September 2001

Subtype	Serotype							Total
	1	2a	2b	4	14	15	NT	
<i>Serogroup B</i>								
P1.4	1			70	3	4	21	99
P1.5				1				1
P1.6				1				1
P1.7						1		1
P1.14				2	1			3
P1.15				1				1
NST								0
Total	1			75	4	5	21	106
<i>Serogroup C</i>								
P1.5		5						5
P1.7						1		1
NST		1						1
Total		6				1		7

NB: Serogroup B totals do not include one isolate which was found to have two serotypes (4 and 15) associated with the P1.4 serosubtype.

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.

Fifty-four culture-negative cases provided blood and/or CSF samples for testing by PCR for the presence of meningococcal DNA. Forty-five of these cases were positive by *porA*-PCR. The *porA* gene encodes the subtype-specific antigens. Dot blot hybridisation showed that 36 of the samples were subtype P1.7b,4, and nine 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* has been stopped as the results give no useful information relating to the use of pertussis vaccine. The recommended ages for vaccination against *B. pertussis* in New Zealand are at six weeks, three months, five months and 15 months.

## NOSOCOMIAL INFECTIONS

Commencing in this issue of *LabLink*, we are including short reports on outbreaks of hospital-acquired infections from which isolates have been referred to ESR for typing.

### ESBL outbreak in Hawkes Bay Hospital

There was a prolonged outbreak of a strain of *Escherichia coli* with extended-spectrum  $\beta$ -lactamase (ESBL) in Hawkes Bay Hospital during 2001. During the year isolates of this strain were referred from 40 patients. The majority (23/40, 58%) of isolates were from urine. The others were from a variety of sites. Twenty-six of the isolates have been typed by DNA macrorestriction with *XbaI* enzyme using pulsed-field gel electrophoresis (PFGE). All 26 isolates were indistinguishable.

The strain has a distinctive  $\beta$ -lactam resistance phenotype. Compared to some other ESBL producers, it is highly resistant to both cefotaxime and ceftazidime, with MICs typically  $\geq 512$  and 64–128 mg/L, respectively. In the presence of clavulanic acid, sensitivity to these cephalosporins is restored, with MICs of  $\leq 0.25$  for cefotaxime/clavulanic acid and 0.5–1.0 for ceftazidime/clavulanic acid. In the NCCLS disc test recommended for ESBLs, the difference in zone sizes for cefotaxime with and without clavulanic acid is in the range of 15–20 mm; for ceftazidime with and without clavulanic acid the difference is in the range of 10–15 mm. The strain is also resistant to gentamicin, tobramycin, quinolones and trimethoprim.

No common source of the organism has been found. Sixty-two patients who had been hospitalised >7 days were screened by rectal swabs and 271 environmental swabs were taken. The strain was not isolated from any of the environmental swabs. It was isolated from three of the 62 patients screened, two of whom were already known to have the organism.

Outbreak control measures have included emphasising strict adherence to contact precautions and to catheter management techniques, routine screening of ICU patients and ward patients who are in hospital for >7 days, and isolation of colonised patients. (Reported by Dr Richard Meech, Hawkes Bay Hospital.)

## LEGIONELLOSIS AND ENVIRONMENTAL LEGIONELLA ISOLATES

During the period 1 July to 30 September 2001, laboratory testing identified six sporadic cases of legionellosis, including the death of a 43 year old immunosuppressed female. Five of the six cases were confirmed either by isolation of *Legionella* organisms (1 case) or the demonstration of consistently high antibody titres >512 in convalescent phase sera (4 cases). The remaining case was regarded as a probable case, with a single antibody titre >2048 and presenting with atypical pneumonia. Only three cases were notified although all were associated with a clinically compatible illness fitting legionellosis.

All but one case occurred in people 50 years of age or older, the exception being a 43 year old. The age range was 43 to 84 with the average age being 66.4 years. The cases involved four males and two females. The causative *Legionella* agent was identified in five of the six cases, with the unknown case not being serotyped to identify the causative agent. For the known cases, the infecting organism was *Legionella longbeachae* serogroup 1 in all but one case, the exception being *L. bozemanii*. This is a species like *L. longbeachae*, that is more commonly associated with composted material and soils.

During the period 1 July to 30 September 2001, *Legionella* species were identified in 35 environmental isolates that were either isolated by ESR or referred to ESR for identification. Of these, 27 were identified to the spe-

Table 6. Legionellosis cases and environmental isolates, July–September 2001

<i>Legionella</i> species	Clinical Cases			Environmental Isolates	
	Confirmed	Probable	Total	Number	Where isolated – if known
<i>L. anisa</i>	-	-	-	1	
<i>L. bozemanii</i>	1	0	1	1	Compost (1)
<i>L. longbeachae</i> serogroup 1	4	0	4	6	Compost (6)
<i>L. micdadei</i>	-	-	-	3	Compost (3)
<i>L. pneumophila</i> serogroup 1	-	-	-	8	Hot water system (1), Extruder cooling water system (1)
<i>L. pneumophila</i> serogroup 5	-	-	-	1	Chiller water (1)
<i>L. pneumophila</i> serogroup 6 (5), Cooling tower water (1)	-	-	-	7	Hot water system
<i>L. pneumophila</i> serogroup unidentified	-	-	-	4	Cooling tower water (1),
<i>Legionella</i> sp. (non- <i>pneumophila</i> )	-	-	-	1	
Unknown <i>Legionella</i> species	0	1	1	3	Cooling tower water (2)
Total	5	1	6	35	

cies and serogroup level, four were identified as *L. pneumophila* of an unknown serogroup, one as an unknown non-*pneumophila legionella* isolate, and three *legionella* isolates could not be identified further.

## LEPTOSPIROSIS

During July to September 2001, 31 cases of leptospirosis were reported, which had either been laboratory-confirmed or notified on clinical grounds only. Twenty-four cases were laboratory-confirmed and seven cases were notified on clinical grounds only.

The known occupations of the notified cases included farmers (9), freezing workers (8), life style block owners (2), stock agent (1), and a fencer (1).  
 Table 7. *Leptospira* cases, July-September 2001

<i>Leptospira</i> species / serovar	Number of cases
<i>L. interrogans</i> sv pomona	12
<i>L. borgpetersenii</i> sv ballum	3
<i>L. borgpetersenii</i> sv hardjo	9
<b>Total</b>	<b>24</b>

## SPECIAL BACTERIOLOGY

### Interesting isolates received in the Special Bacteriology Laboratory

- Corynebacterium diphtheriae* var *mitis* non-toxicogenic strain from an infected tattoo in F35y, and a var *gravis* non-toxicogenic strain from infected leg in M13y.
- Corynebacterium kroppenstedtii* from a breast infection in F36y was confirmed by 16S rRNA sequencing. This lipophilic skin diphtheroid was first described in 1998 from sputum and a pathogenic role was not determined at that time. A review of cases seen at the submitting laboratory has revealed that the species has been isolated in several other breast infections dating back as early as 1995. The species may be differentiated from other small-colony skin diphtheroids such as *Corynebacterium jeikeium* and *Corynebacterium* group G by a positive aesculin hydrolysis reaction.
- Dietzia* species most similar to *D. maris* identified by 16S rRNA sequencing from the blood of F87y. This aerobic actinomycete was formerly known as *Rhodococcus maris* and is a rare human pathogen. Only two reports of human infection have been made. These were septicaemia associated with the presence of a catheter, and bone infection in a patient hospitalised for a total hip prosthesis.
- Burkholderia pseudomallei* from sputum of cystic fibrosis patient M38y who had recently been in Northern Territory, Australia, where this organism, the causative agent of melioidosis, is endemic.
- Campylobacter concisus* identified by 16S rRNA sequencing from osteovertebral joint of F69y. This anaerobic species has been associated primarily with periodontal disease, but has also been isolated from patients with septicaemia and gastrointestinal tract infections.
- Bacillus megaterium* isolates from cutaneous sites (2). This species appears morphologically similar to the *Bacillus cereus* group, which includes *Bacillus anthracis*. It may be non-motile and non-haemolytic like *B. anthracis*, but is clearly distinguished from *B. anthracis* by producing acid from mannitol. Growth of *B. megaterium* is inhibited on selective and differential agars containing polymyxin B used for the isolation of the *B. cereus* group, which assists in differentiation from this group.

## LISTERIA MONOCYTOGENES

Five isolates of *L. monocytogenes* from human cases were referred in the period July to September 2001 (Table 8). Four of the isolates were from  
 Table 8. *Listeria monocytogenes* from human cases, July-September 2001

Month isolated or of onset	Health district	Sex/Age	Source	O antigen serotype
July	Taranaki	F 67y	BC	4
August	Northland	F neonate	Body swab	4
August	Gisborne	M 78y	BC	4
September	Central Auckland	M 67y	BC	4
September	Otago	F 90y	BC	4

elderly adults, three of whom had underlying illness identified. The remaining case was in a full-term infant with respiratory distress, who made a good recovery after antibiotic therapy.

## ENTERIC PATHOGENS

### SALMONELLA

#### Human

The usual winter drop in cases of salmonellosis has not occurred in 2001. There were 598 isolates of *Salmonella* confirmed during July to September, compared with 409 for the same period in 2000. This increase can be attributed to an increase in prevalence of:

- S. Heidelberg* throughout the country, with 98 cases being confirmed compared with two cases in 2000.
- S. Typhimurium* phage type 160 isolates which have increased from 46 in 2000 to 192 in 2001. Isolations have been received from all Health Districts in the country except Gisborne, compared with pockets of infection in Wellington, Manawatu, Canterbury and Otago during the same period in 2000. Results of a case-control study will be published at a later date.

#### Non-Human

Isolates of *S. Brandenburg* from sheep and cattle abortions in the Southland/Otago region continued for the fifth season. Sheep isolates dropped from 501 in 2000 to 326 in 2001. Conversely, cattle isolates increased from 73 isolates in 2000 to 99 in 2001.

Animal and bird isolations of *S. Typhimurium* phage type 160 have increased from 20 in 2000 to 106 in 2001. The most significant increase has been in domestic cats presenting with gastroenteritis (two cases in 2000, 37 in 2001).

### ESCHERICHIA COLI

There were 20 isolates of *E. coli* O157 (Table 9) confirmed during July to September 2001, compared with 14 for the same period in 2000.

Pulsed-field gel electrophoresis (PFGE) using the enzyme *XbaI* on two unrelated human isolates from Canterbury in August, showed they were indistinguishable from a strain isolated from raw milk.

Table 9. Isolates of *E. coli* O157, July-September 2001

Month	Sex / Age	Health District	Comments
July	F 10m	Waikato	No details
August	M 1y	Canterbury	No details
	M 4m	Canterbury	Contact of the above
	M 3y	Otago	Rural water supply
	M 4y	Waikato	HVS
	? 3y	Waikato	No details
	M ?	Waikato	No details
	F 3y	Taranaki	No details
	M 2y	Waikato	No details
	M 1y	Wanganui	Diarrhoea
	M 62y	Otago	Overseas tourist
	M 16y	Canterbury	Consumed raw milk
	M 1y	Canterbury	Raw milk fed to household cats
	September	F 39y	Waikato
F 91y		Northland	Bloody diarrhoea
F 1y		Gisborne	Diarrhoea
M 1y		Bay of Plenty	Diarrhoea
M 1y		Bay of Plenty	Bloody diarrhoea
F 5y		Bay of Plenty	Bloody diarrhoea
F U		Auckland	No details

## SHIGELLA

During July to September 2001 there were 52 isolations of Shigella species compared with 35 in the same period in 2000 (Table 10).

Table 10. Shigella isolates, July-September 2001

Species	Type	Number	Comment
S. boydii	2	1	Immigration
S. boydii	4	2	Immigration
S. boydii	13	1	
S. dysenteriae	7	1	Overseas travel
S. flexneri	1b	3	Immigration
S. flexneri	2a	15	2 cases overseas travel
S. flexneri	2b	1	Overseas travel
S. flexneri	3a	1	
S. flexneri	6	2	Immigration
S. flexneri	Species	1	Reacted with polyvalent antisera only
S. sonnei	Biotype a	8	6 cases overseas travel
S. sonnei	Biotype g	16	8 cases overseas travel

## ANTIBIOTIC RESISTANCE

### RESISTANCE AMONG GROUP A STREPTOCOCCI

Group A streptococci (*Streptococcus pyogenes*) are universally susceptible to penicillin - the antibiotic of choice for the treatment of infections with this organism. Macrolide antibiotics are indicated for patients allergic to penicillin, when penicillin therapy fails, or in cases of multiple recurrences. While the prevalence of erythromycin resistance remains low in most parts of the world, high rates have been reported in several countries.

In March-April 2001, isolates were collected for a national survey of antimicrobial resistance among group A streptococci in New Zealand. A total of 474 isolates, from 30 hospital and community laboratories, were tested by a standard agar dilution method. The majority (94%) of isolates were reported to be community acquired, 48% were from skin/wound/abscess sites, and 45% were from respiratory sites. Just over 40% were from children less than 10 years of age.

None of the 474 isolates tested were resistant to penicillin, cefotaxime, cephalothin, chloramphenicol, clindamycin, mupirocin, or trimethoprim-sulphamethoxazole (Table 11). Based on the results of tests for inducible macrolide-lincosamide (ML) resistance, three (0.6%) of the 474 isolates were considered to be erythromycin resistant: one with the inducible ML resistance phenotype and two with the erythromycin-resistant, clindamycin-sensitive, or so called M, phenotype. Fifty-nine (12.5%) isolates were tetracycline resistant. Isolates from children less than 8 years of age were significantly less resistant to tetracycline than isolates from older people ( $p=0.04$ ).

Compared with a previous survey in 1990, erythromycin resistance was lower Table 11. MIC range, MIC<sub>50</sub>, MIC<sub>90</sub> and resistance among group A streptococci, 2001

Antimicrobial agent (resistance breakpoint, mg/L)	MIC (mg/L)			Percent (number) resistance
	range	MIC <sub>50</sub>	MIC <sub>90</sub>	
Cefotaxime (MIC ≥1)	0.008-0.03	0.016	0.016	0 (0)
Cephalothin (MIC ≥1)	0.06-0.25	0.12	0.25	0 (0)
Chloramphenicol (MIC ≥16)	1-4	2	4	0 (0)
Clindamycin (MIC ≥1)	0.03-0.12	0.06	0.06	0 (0)
Erythromycin (MIC ≥1)	0.06-8.0	0.06	0.06	0.6 (3) <sup>1</sup>
Mupirocin (MIC ≥8)	0.03-4	0.12	0.25	0 (0)
Penicillin (MIC ≥0.25)	0.004-0.03	0.016	0.016	0 (0)
Tetracycline (MIC ≥8)	0.06-64	0.25	16	12.5 (59)
Trimethoprim-sulphamethoxazole (MIC ≥4) <sup>2</sup>	0.03-0.5	0.06	0.12	0 (0)

1 Based on the results of tests for inducible macrolide-lincosamide resistance.

2 The MICs for trimethoprim-sulphamethoxazole refer to the trimethoprim content in a ratio of 1 part trimethoprim to 19 parts sulphamethoxazole.

(0.6% vs 4.1%) and tetracycline resistance was higher (12.5% vs 6.2%) in 2001. Among the antimicrobials to which no resistance was detected in 2001, a comparison of the MIC values obtained in the two surveys indicates there has been no change, or even an increase, in susceptibility to these antimicrobials during the last 10 years. The one exception to this trend was a decrease in mupirocin susceptibility, as indicated by an increase in the upper end of the MIC range from 0.25 mg/L in 1990 to 4 mg/L in 2001.

The results of this survey of group A streptococci indicate that this organism remains extremely sensitive to the antibiotics most used for therapy, that is, penicillin and macrolides. The decrease in susceptibility to mupirocin is a concern and is likely to be the result of the high, and until recently unrestricted, use of this antibiotic in New Zealand.

Thank you to all the laboratories who contributed isolates for this survey. A full report is available on request to the Antibiotic Reference Laboratory, ESR.

## WHO GONOCOCCAL ANTIMICROBIAL SURVEILLANCE PROGRAMME, 2000

The World Health Organization (WHO) Gonococcal Antimicrobial Surveillance Programme (GASP) is a multicentre long-term programme that has been monitoring the antimicrobial susceptibility of *Neisseria gonorrhoeae* since 1992. New Zealand is one of 15 countries in the Western Pacific Region (WPR) which regularly tests and submits data to the programme. The New Zealand data are provided by Microbiology, LabPlus, Auckland Healthcare, and include isolates from the Auckland Sexual Health Clinics, hospital and community laboratories in Auckland, and Waikato Hospital.

In 2000, around 11,000 isolates were tested. An increasing number of isolates in many countries were resistant to quinolones and penicillins, continuing the trend observed since 1992. Resistance to penicillin (MIC ≥1 mg/L), both plasmid-mediated production of penicillinase (PPNG) and chromosomally mediated resistance due to other mechanisms (CMRNG), remains widespread. Very high rates of penicillin resistance were recorded in Korea (91%), Philippines (89%), China (80%), Brunei (63%), Singapore (58%), Hong Kong SAR (54%), and Vietnam (48%). In New Zealand resistance was 7.9%, made up of 2.9% PPNG and 5.0% CMRNG. Penicillin resistance in Australia was 19.5% and predominantly CMRNG.

The proportion of PPNG has been declining in some centres, but CMRNG have become more prominent. As a point of reference, the WHO recommends that an antibiotic should no longer be used for treatment when 5% of isolates are resistant to its action. In 2000, the majority of the 15 countries participating in the programme reported rates of penicillin resistance over this 5% threshold. In contrast, several Pacific Island states reported low rates of penicillin resistance.

Resistance to the quinolone antibiotics, represented by ciprofloxacin (MIC ≥1 mg/L), has become a major problem in parts of the WPR in recent years and worsened in 2000. Resistance was detected in 11 of the 12 countries which tested quinolone susceptibility. The countries with high rates of resistance included China (85%), Hong Kong SAR (80%), Vietnam (43%), Japan (40%), Philippines (38%), and Korea (26%). The resistance rates for Australia and New Zealand were 8.2% and 2.3%, respectively. In China, Hong Kong, Philippines, Japan, Vietnam, Korea and Australia, the proportion of strains resistant to quinolones is increasing. For example, quinolone resistance in Hong Kong increased from about 50% in 1998 to 80% in 2000. Similarly, quinolone resistance is increasing among gonococci in New Zealand, with resistance having risen to 8.7% in the Auckland area during the first nine months of 2001. A representative sample of the ciprofloxacin-resistant isolates was phenotypically and genotypically typed. This typing showed that a single clone was predominant in Auckland.

No resistance to ceftriaxone was reported in the WPR in 2000, although a small number of isolates were found to have decreased susceptibility, with MIC values up to 0.25 mg/L in Singapore, Brunei, China, Australia and New Zealand. This is a concern, given that the third-generation cephalosporins are important agents in the treatment of infection with gonococci resistant to other antibiotics, such as penicillin and ciprofloxacin.

Tetracyclines are not recommended treatment for gonorrhoea, yet their ready availability and low cost means that they are often used in the informal health sector in a number of countries. The programme only monitors high-level tetracycline resistance (MIC ≥16 mg/L, TRNG), which has been reported in Malaysia, Singa-

pore, Brunei, China, Papua New Guinea and Vietnam, with prevalences ranging from 25 to 70% in 2000. Rates in Australia and New Zealand were 9.2% and 3.3%, respectively. It should be noted that low-level chromosomally mediated resistance to tetracycline (MIC 1-8 mg/L) is high in New Zealand and as many as 30% of isolates are resistant.

There has been an increase in the incidence of gonorrhoea over last three years, both in New Zealand and overseas. This, combined with increasing resistance to quinolones and the emergence of reduced susceptibility to third-generation cephalosporins, is of concern.

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## VIROLOGY

### RESPIRATORY VIRUSES

#### Influenza virus

During the 2001 winter season, the overall influenza activity was low to moderate. Figure 2 shows weekly consultation rates for influenza-like illness in New Zealand from 1992 to 2001. Clearly, the level of influenza-like illness in 2001 was higher than in 2000, but lower than in 1999. The consultation rate remained at the baseline level from week 18 to week 23. Then it increased rapidly and peaked in Week 26 (at the end of June) at 140 per 100,000 patients. The influenza activity remained at a low to moderate level till Week 35 (the end of August) and then dropped down to the baseline level in September. In 2001, the national average consultation rate was 62.1 per 100,000 patient population, compared with 32.5 per 100,000 patient population in 2000.

Figure 2. Weekly consultation rates for influenza-like illness in New Zealand, 1992-2001

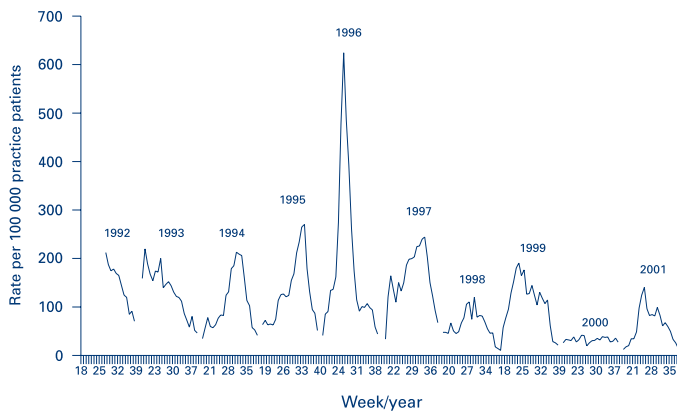
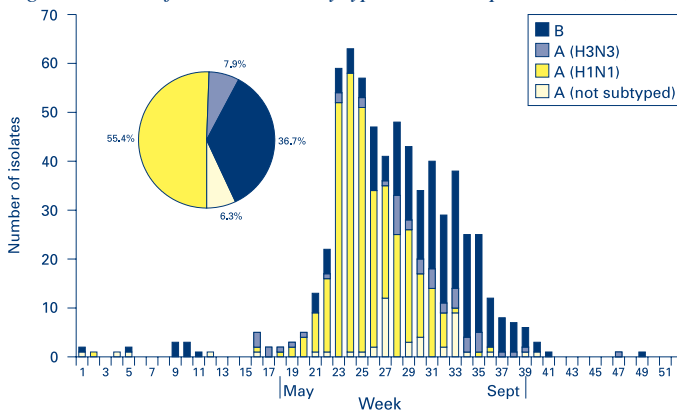


Figure 3 shows weekly influenza isolates by types during May to September 2001. There are three interesting features: 1) In June and July (Week 21 to 31), influenza A(H1N1) was the predominant strain. 2) In August and

Figure 3. Total influenza isolates by type and week specimen taken, 2001

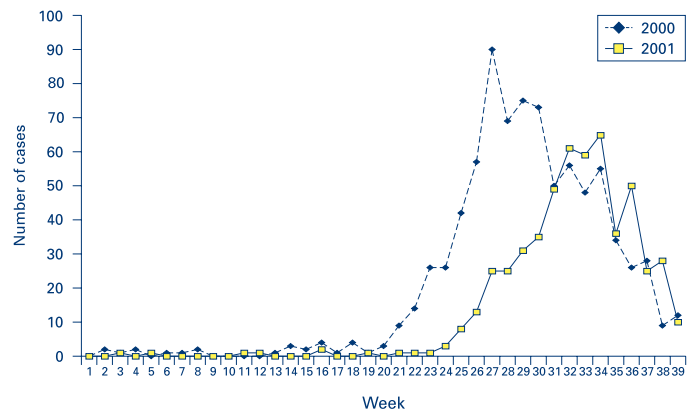


September (Week 32 to 41), influenza B was the predominant strain. 3) Influenza A(H3N2) remained at a low level throughout the winter season. During the period of May to September 2001, a total of 636 influenza isolates were analysed by five virology laboratories around the country. Figure 3 showed the percentage of types/subtypes of influenza isolates. The majority of them were influenza A (417 isolates). Among them, most Influenza A (377 isolates) were subtyped and 40 isolates were not subtyped. Influenza A(H1N1) was the predominant subtype (330, 55.4% of total typed/subtyped isolates). Influenza A(H3N2) consisted of a small proportion (47, 7.9% of total typed/subtyped isolates). Influenza B co-circulated with influenza A with 219 isolations (36.7% of total typed/subtyped isolates).

#### Respiratory Syncytial Virus (RSV)

Respiratory syncytial virus infections in 2001 were lower than that of 2000. During January to September, a total of 533 RSV infections were reported compared with 830 during the same period in 2000 (Figure 4). The RSV activity peaked in Weeks 32-34 (August), five weeks later than the peak in 2000. The RSV activity remained at the high level till Week 36 (early September). Since then, the number of RSV cases has rapidly declined.

Figure 4. RSV laboratory-confirmed cases by week, 2000 and 2001



### NORWALK-LIKE VIRUS

#### Characterisation of Norwalk-like strains from gastroenteritis outbreaks occurring between 1 January and 30 June 2001

There were 20 outbreaks of laboratory-confirmed NLV-associated gastroenteritis to 20 June 2001. The seasonal drop in outbreaks over the winter months observed over the last five 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. Settings for these 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', GI/1,4,8, and strains belonging to genotype GI/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 12. NLV Genotypes occurring, January-June 2001

NLV Strain	Genotype	Number (%)
Lordsdale virus 'Global strain' cluster	GI/1,4,8	7 (35)
Napier / Florida / Gwynedd / Idaho Falls virus cluster	GI/6,7,9	6 (30)
'Cruise ship virus'	GI/4	3 (15)
Desert Shield virus	GI/3b	1 (5)
Awaiting sequence identity from CDC		3 (15)
<b>Total</b>		<b>20 (100)</b>

# CULTURE COLLECTION

Recent accessions to the Collection are shown in Table 13.

Table 13. NZRM new accessions

Name	NZRM No.	Source, Strain	Comments
<i>Aeromonas jandaei</i>	4019	NZ isolate, 2000	Wound, human
<i>Arcobacter butzleri</i>	4017	CCUG 30485	Type strain
<i>Arcobacter cryaerophilus</i>	4018	CCUG 17801	Type strain
<i>Bifidobacterium bifidum</i>	3930	DSM 20082	Type strain
<i>Clostridium sordelli</i>	4032	NZ isolate, 2000	Blood, human
<i>Enterococcus faecium</i>	4037	NZ isolate, 2001	Multi-resistant, vancomycin resistant enterococcus
<i>Neisseria gonorrhoeae</i>	4033	AGSP QC00/3	Ciprofloxacin resistant
<i>Salmonella</i> species	4030	NZ isolate, 2000	Non-motile
<i>Salmonella</i> Typhimurium	3970	NZ isolate, 2000	Phage type 104, human source. Antibiotic resistant strain.
<i>Salmonella</i> Typhimurium	4022	NZ isolate, 2000	Phage type 160, sparrow
<i>Staphylococcus aureus</i>	4039	NZ isolate, 2000	Methicillin resistant <i>S. aureus</i> . Mec probe negative.
<i>Vibrio cholerae</i>	4031	NZ isolate, 2001	Seafood-associated gastroenteritis

## NEW NAMES

The names in Table 14 have either been published in the International Journal of Systematic and Evolutionary Microbiology (IJSEM), formerly the International Journal of Systematic Bacteriology (IJSB), or validated by announcement in the IJSEM having been previously effectively published elsewhere.

Many names are validated in each bi-monthly publication of the IJSEM. The ones listed below, notified as validated in the IJSEMs of March, May, July and September 2001, are those considered to be of relevance to LabLink readers.

Table 14. New names notified as validated, March-September 2001

Name	Previous name	Reference
<i>Geobacillus stearothermophilus</i>	<i>Bacillus stearothermophilus</i>	Nazina et al. IJSEM 2001; 51 : 433-46
<i>Peptoniphilus asaccharolyticus</i>	<i>Peptostreptococcus asaccharolyticus</i>	Ezaki et al. IJSEM 2001; 51 : 1521-8
<i>Raoultella terrigena</i>	<i>Klebsiella terrigena</i>	Drancourt et al. IJSEM 2001; 51 : 925-32
<i>Rhizobium radiobacter</i>	<i>Agrobacterium radiobacter</i>	Young et al. IJSEM 2001; 51 : 89-103

## MYCOLOGY

Table 15. Biannual summary of opportunistic mycoses in New Zealand, January-June 2001

Organism	No. of cases	Site	Clinical data
<b>Filamentous fungi</b>			
<i>Acremonium kiliense</i>	1	Liver biopsy	Post liver-transplant, lymphoma. DE+. Liver abscess.
<i>Alternaria</i> species (probable <i>A. alternata</i> )	2	Aspirate finger PIP joint (1) Leg aspirate (1)	Thorn embedded in PIP joint of finger. Renal transplant, nodules on leg.
<i>Aspergillus fumigatus</i>	3	FNA from lung (1) Brain (1) CAPD (1)	Renal transplant increased steroids. AML post bone marrow transplant. Tx: ambisome. CRF. Tx: fluconazole, amphotericin B. Patient deceased.
<i>Fonsecaea pedrosoli</i>	1	Arm biopsy	Warty lesion on arm.
<i>Fusarium solani</i> and <i>Paecilomyces lilacinus</i>	1	Right thigh	Burns patient ( <i>Candida albicans</i> also isolated from blood cultures).
<i>Microsporum gypseum</i>	1	Forearm biopsy	Gardener.
<i>Nattrassia mangiferae</i>	1	Left thigh	Burns patient - deceased.
<b>Yeasts</b>			
<i>Candida albicans</i>	35	Sub hepatic fluid (1) Blood culture (20)	NR Pancreatitis (1), pancreatitis. Tx: fluconazole now deceased (1), liver transplant (1), premature baby (2), bowel fistulas (1), NR (2), gallbladder calculus - patient deceased (1), burns patient - deceased (2), burns patient - survived, also had <i>F. solani</i>

Organism	No. of cases	Site	Clinical data
<i>Candida albicans</i> (cont.)		CAPD (5) Ascitic fluid (2) Hip aspirate (1) Intra-peritoneal collection (1) Pleural fluid (1) Peritoneal aspirate (1) Sternal aspirate (1)  Bile (1)  Tissue and aspirate from chest (1)  CAPD	and <i>P. lilacinus</i> isolated from thigh (1), cancer - deceased (2), AML - deceased (1), bullous pemphigoid (1) multiple myeloma (1), diverticulitis - Tx fluconazole (2) Ca bladder - renal failure (1) ESRF(4), NR (1) NR (2) MRSA in hip. Metalware removed. Multiple abdominal surgery. Recurrent (R) pleural effusion. Perforated small bowel. Cardiac surgery complicated by mediastinal infection. Ca pancreas blocked biliary duct. Mixed with <i>K. oxytoca</i> and <i>Citrobacter</i> sp. Empyema ruptured oesophagus. Mixed with <i>E. faecalis</i> . Perforated colon.
<i>Candida albicans</i> and <i>Candida glabrata</i>	1	CAPD	
<i>Candida albicans</i> and <i>Rhodotorula</i> species	1	Blood culture	AML in remission. Patient well, Hickman line removed. Discharged.
<i>Candida glabrata</i>	2	Blood culture (1) Mucus from gallbladder (1)	Short bowel syndrome. Empyema gallbladder.
<i>Candida glabrata</i> and <i>Candida tropicalis</i>	1	Aspirate pancreatic pseudocyst	Acute pancreatitis - deceased.
<i>Candida guilliermondii</i>	2	CAPD (2)	ESRF (2)
<i>Candida parapsilosis</i>	24	CAPD (11) Catheter tip (1) Pleural fluid (1) Blood culture (10)  Bone fragments (1)	ESRF (8), CRF (2), NR (1) Premature baby. Lung transplant. On TPN, (2), NR (2), Necrotising pancreatitis (1), Renal failure (1), Premature baby (1), Bronchiectasis(1), Ca oesophagus - Tx: fluconazole, deceased (1), CRF on CAPD - deceased (1) Compound fractures.
<i>Candida</i> species	1	Blood culture	Pneumonia.
<i>Candida tropicalis</i>	3	Blood culture	AML post bone marrow transplant. Tx: ambisome and itraconazole. (1), aplastic anaemia (1), redo MVR (1)
<i>Cryptococcus laurentii</i>	1	CAPD	ESRF also isolated with <i>C. indologenes</i> and <i>S. maltophilia</i> .
<i>Cryptococcus neoformans</i> (untyped)	1	CSF	Sarcoidosis.
<i>Cryptococcus neoformans</i> var <i>gattii</i>	1	Lung of Brown Kiwi	Deceased. Second isolation from a kiwi bird in NZ.
<i>Cryptococcus neoformans</i> var <i>neoformans</i>	2	CSF (1) Blood culture (1)	AIDS, LA 1:2048. HIV +
<i>Exophiala</i> ( <i>Wangiella</i> ) <i>dermatitidis</i>	1	Bronchial washings	Cystic fibrosis. 3+ yeast cells seen in DE.
<i>Saccharomyces cerevisiae</i>	1	Blood	Ca
<i>Trichosporon inkin</i>	1	CAPD	CRF
<i>Pneumocystis carinii</i>	4	Bronchial washing (2) Sputum (1) Induced sputum (1)	Liver transplant patient (1), HIV+ (1) NR Liver transplant patient.
<b>Actinomycetes</b>			
<i>Actinomyces</i> sp. similar to <i>Actinomyces israelii</i>	1	Pleural aspirate	Multiple pleural abscess with pleural effusion.
<i>Nocardia asteroides</i> complex	6	Bronchial washing (1)  Sputum (4)  Leg wound (1)	Post liver transplant, cavity pneumonia, immunosuppressed. Chest infection (1), NR but <i>N. farcinica</i> isolated from lung biopsy in 1998 (1), NR also isolated from sputum in 1996 (1), NR (1) Cellulitis.
<i>Nocardia farcinica</i>	3	Blood culture and lung brushings (1) Bronchial wash (1) Blood culture (1)	Immunocompromised post brain tumour surgery. NR x TB MGIT broth ?significance. Febrile, pneumonia.
<i>Nocardia nova</i>	5	Aspirate - finger (1) Sputum (4)	Abscess - patient not admitted. Short of breath (1), NR - isolates were from TB MGIT broths ?significance (3)

### KEY:

AML	Acute myeloid leukaemia	HIV	Human immunodeficiency virus
Ca	Carcinoma	LA	Latex agglutination
CAPD	Continuous ambulatory peritoneal dialysis	NR	Clinical data not received
CRF	Chronic renal failure	TPN	Total parenteral nutrition
DE	Direct examination	Tx	Treatment
ESRF	End stage renal failure	FNA	Fine needle aspirate

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