

MOLECULAR DIAGNOSTICS FOR THE 21ST CENTURY

The age of the genome is with us. The technology to sequence complete genomes is being fully exploited and the range of organisms whose sequence is available is substantial. By far the most popular genomes to sequence initially were those of human bacterial pathogens, a consequence of both pharmaceutical and scientific expediency. For some species the sequences of two strains are now available, notably *Escherichia coli* K-12 and the pathogen *E. coli* O157^{1,2}. This has allowed us to compare the genomes directly and draw conclusions about the evolution of bacterial virulence. But to exploit all this information new molecular diagnostic approaches are required that will help improve the speed and accuracy of pathogen identification.

Modern molecular techniques are currently applied in a number of ways to detect and characterise microbial pathogens. Interest in the use of molecular methods for pathogen detection began with the application of DNA probes³ but was stimulated particularly with the development of DNA and RNA amplification methods in the mid 1980s⁴. The ability to detect the presence of genetic material from a micro-organism without the need for culture considerably expanded the horizon of microbial diagnostics and it is now possible to identify an infectious organism within a few hours. The early promise of amplification methods was not followed by the wholesale introduction of such tests in the routine diagnostic laboratory essentially because of the costs compared to traditional culture methods and also the need for rigorous quality assurance. However, molecular methods have found a particular niche in pathogen detection and are used extensively for their characterisation.

The basis of the amplification methods has been reviewed in the literature⁵. Essentially they are able to detect very small amounts of pathogen DNA (or RNA by first using reverse transcription to convert the RNA to so-called complementary or cDNA) by amplifying specific genes or regions of DNA that are characteristic of the pathogen. The product of the amplification reaction provides a simple yes/no or present/absent answer. Problems arise because the technique is particularly sensitive to contamination giving false positives or the presence of inhibitors in the sample generating false negatives. Amplification methods have found particular niches in the detection of non-culturable pathogens such as human papillomavirus⁶, hepatitis C⁷ and Norwalk-like virus⁸, slow growing pathogens such as mycobacteria⁹, intracellular pathogens such as chlamydia¹⁰, and pathogens in patients who have been treated with antibiotics preventing growth of the suspect organism¹¹. The problems associated with each of these situations have provided the impetus for the development of routine amplification assays with commercial kits now available for enterovirus, hepatitis C, HIV, mycobacterial and chlamydial detection. Amplification techniques have also been used to detect antibiotic resistance genes^{12,13}.

Recent developments in the field of amplification include identification of the amplification product in real-time and the ability to provide a quantitative estimate of the pathogen load. Real-time detection evolved from the need to identify the presence of an amplification product as quickly as possible and bypasses the need to run agarose gels. It requires the binding of a fluorescently labelled probe or a dye to the amplification product, which results in an increase in fluorescence directly proportional to the amount of product present. Optical systems can

detect the increase in fluorescence as the number of amplification cycles increases. The use of fluorescent probes to detect the product increases the specificity of detection since identification of the product requires the binding of three specific primers, two to amplify the product and one to detect it. Both the Taqman system (Applied Biosystems) and the LightCycler (Roche) use real-time analysis to detect amplification products. One advantage of real-time analysis is that it can be used for quantitation. The amount of pathogen in the sample can be calculated by identifying the cycle at which exponential amplification begins and comparing it with a series of standards. This is particularly relevant to infection with HIV or hepatitis B where the course of treatment may be dictated by the viral load.

A recent innovation is the development of molecular beacons to identify specific pathogens^{14,15}. The molecular beacon consists of a DNA probe that is able to bind to the amplification product. The probe has a fluorescent dye at one end and a quencher at the other. The first few bases of the probe are complementary to the last few and bind to each other bringing the quencher and the dye close together. This prevents the dye from fluorescing. At the annealing stage of the amplification reaction the beacon binds to the amplification product separating the dye and quencher allowing the dye to fluoresce. The fluorescent intensity is proportional to the amount of product in the reaction. Molecular beacons can also be used to identify single point mutations within a gene as they can discriminate between two amplification products that differ by a single base pair.

Although the area of pathogen detection has seen limited application of molecular techniques within the diagnostic laboratory, pathogen characterisation has been revolutionised by it. There are a large number of molecular techniques available to characterise pathogens. They range from the macromolecular such as pulsed field gel electrophoresis (PFGE) to DNA sequence analysis. Each technique has its advantages and limitations. Characterisation of the pathogen is performed to provide information on the diversity within the pathogen population and to distinguish amongst strains. This information is a useful and often essential component of local, national and international surveillance of a pathogen or disease.

Characterising bacterial populations is important in order to understand the spread of pathogens, to identify the emergence of particular strains and to monitor the spread of antibiotic resistance genes. Early molecular methods relied mainly on restriction patterns using either PFGE or insertion sequence typing. The introduction of multilocus sequence typing (MLST) has significantly improved our understanding of bacterial populations^{16,17}. It is based on amplification and sequencing of up to seven housekeeping genes, identifying and numbering variants and establishing sequence types based on allele profiles. It has been applied to a number of pathogens including *Staphylococcus aureus*, *Neisseria meningitidis*, *Streptococcus pyogenes*, *S. pneumoniae* and *Campylobacter jejuni* (see <http://www.mlst.net/new/index>). Because sequence data are used the results are directly comparable with little or no investigator interpretation required making the method very robust. Variants of each of the genes used can be stored and used for comparison over

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the Internet allowing labs worldwide to access the data. Results from the study of bacterial populations include identification of the major MRSA clones in circulation and their emergence from MSSA clones, the horizontal spread of capsular genes in *S. pneumoniae* and the close link between the *emm* type and MLST-defined clone in *S. pyogenes*. The ease and simplicity of the technique means that more and more bacterial pathogen populations will be subjected to this type of analysis. Viral pathogens present a much harder prospect for population characterisation. The ability to mutate within the host and the generation of so-called quasi-species means identification of discrete populations is more difficult. This is especially true for the RNA viruses such as HIV and Hepatitis C. Most viral population studies are based on characterising a single region of the genome allowing the relationships amongst isolates to be determined¹⁸.

Many molecular techniques have been used for the characterisation of pathogens for epidemiological investigations of outbreaks and surveillance. Such techniques are often applied within reference laboratories that have specialist equipment and expertise. Normally a hierarchical approach to the typing of bacterial pathogens is performed. This consists of a general method for grouping such as serotyping coupled with a technique that is able to distinguish amongst members of the same serotype. For example, *C. jejuni* are initially grouped using the Penner serotyping scheme followed by PFGE analysis. Similarly Salmonella are serotyped and subjected to PFGE. *N. meningitidis* is serogrouped and serotyped but can also be subtyped by amplification of the PorA gene and sequencing of the amplification product¹¹. Other molecular techniques used to distinguish amongst isolates include ribotyping (using 16S rRNA gene polymorphisms), RFLP, AFLP, repPCR and RAPD. Some molecular methods are able to distinguish amongst very closely related isolates. Epidemic MRSA isolates that have identical phage types and PFGE patterns can be distinguished by amplifying and digesting (or sequencing) the repeat region present in the coagulase gene or the repeat region in the Protein A gene^{19, 20}.

The wealth of information provided by the sequencing of genomes has opened up a number of possibilities for molecular diagnosis and pathogen characterisation. The identification of regions specific for individual pathogens can generate novel amplification strategies. The whole genome may also provide additional virulence factors or strain-specific regions that could also be targeted for identification. Having the complete genome of any organism could provide a starting point for an MLST approach to population characterisation, even in the case of slow-growing or non-culturable organisms.

Perhaps the most important consequence of the availability of a complete genome is the ability to examine the products from every single gene at the same time. This is achieved using microarray technology²¹. Probes for each gene, such as PCR products from the gene or oligonucleotides specific for the gene can be attached to a solid support (modified glass slides or membranes). Only a very small amount of the probe needs to attach and therefore a large number of different probes can be placed on the support. A probe for each gene in the bacterial genome can easily be accommodated on a single glass slide with its position known. In studies looking at changes in the expression of mRNA under different conditions, the mRNA is purified and reverse transcribed to cDNA. During this process a label is incorporated in the cDNA. The labelled cDNA is hybridised with the probes on the glass slide. Only those genes that are expressed will hybridise with their respective probes. Slides from samples that have been generated under different conditions can be compared and changes in the expression of particular genes under those conditions can be identified. Similarly, the slides can also be used to determine the presence or absence of genes amongst a collection of isolates. In this case the genome from one strain is digested and labelled then hybridised with the array. Comparison of the gene content of strains of *Mycobacterium* spp.²², *Helicobacter pylori*²³ and *Vibrio cholerae*²⁴ have been performed and have identified changes involved in the spread of these organisms and the diseases they cause. A drawback is that only genes that have been identified in a sequenced genome can be placed on the array and strain specific genes may be missed. However, arrays are being developed for identification and typing of bacteria and viruses^{25, 26, 27}. Comparisons of the gene content or variation in individual genes may allow closely related isolates to be distinguished.

Array technology can also be used for bacterial identification. Sequencing of ribosomal RNA (rRNA) has been used extensively for bacterial identification for

over twenty years. The ubiquitous nature of rRNA means that all bacteria are amenable to characterisation. The association of specific sequences within the rRNA gene with bacterial species has led to the development of arrays which can be used for identifying species present in samples²⁸. How might arrays be developed in the future? One could begin by developing arrays specific for individual groups of bacteria such as enteric pathogens or those associated with upper-respiratory tract infection. Each array would carry probes for different pathogens (rRNA for bacteria and gene-specific probes for viruses). Because of the large number of probes which can be attached to a slide, not only might the array tell us what is present but it could also identify specific virulence factors, antibiotic resistance genes, MLST sequence type and provide significant typing data. The development of novel primer chemistries with improved hybridisation characteristics may eventually lead to the single slide laboratory²⁹.

The whole genome sequence also provides access to all the proteins in the pathogen as well as the genes. Individual genes can be placed in expression systems and the protein purified in large quantities. Antibodies can be raised to each of the purified proteins and tested for diagnostic applicability. Arrays of different diagnostic antibodies would allow simultaneous testing for many different pathogens analogous to the microarrays described above. The expressed proteins could also be used for direct detection of antibody responses with the ability to test for many pathogens on the same array³⁰.

Although the ideas for using DNA and protein arrays have been around for a time the technology underpinning the techniques requires further improvement. There need to be improvements in the ability of the technology to deal with different samples and to reduce or eliminate sample preparation. The time required for identification needs to be minimised and the system should be portable and ideally hand-held. Costs of the testing would also need to be reduced to a level equivalent to culture methods. The information on pathogens is available, we now need the best and most appropriate technology to use it effectively³¹.

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ronmental isolates that were isolated either by ESR or referred to ESR for identification. Of these, 37 were identified to the species and serogroup level and one as an unidentified *Legionella* species (non pneumophila).

Table 2. Legionellosis cases and environmental isolates, April-June 2002

Legionella species	Clinical Cases			Environmental Isolates	
	Confirmed	Probable	Total	Number	Where isolated* – if known
<i>L. anisa</i>	-	-	-	3	
<i>L. bozemanii</i> serogroup 1	-	-	-	1	
<i>L. dumoffii</i>	-	1	1	-	
<i>L. feelei</i> serogroup 1	1	-	1	2	
<i>L. feelei</i> serogroup 2	-	-	-	1	
<i>L. hackelliae</i> serogroup 1	1	-	1	-	
<i>L. longbeachae</i> serogroup 1	2	-	2	2	2 x compost
<i>L. longbeachae</i> serogroup 2	-	-	-	2	2 x compost
<i>L. micdadei</i>	1	-	1	1	1 x compost
<i>L. pneumophila</i> serogroup 1	-	1	1	13	4 x industrial process waters
<i>L. pneumophila</i> serogroup 3	-	-	-	1	
<i>L. pneumophila</i> serogroup 4	-	-	-	1	
<i>L. pneumophila</i> serogroup 6	-	-	-	5	
<i>L. pneumophila</i> serogroup 8	-	-	-	2	
<i>L. pneumophila</i> serogroup 10	-	-	-	2	1 x compost
<i>L. pneumophila</i> serogroup 12	2	-	2	-	
<i>L. pneumophila</i> serogroup 13	1	-	1	-	
<i>L. pneumophila</i> serogroup 14	-	-	-	1	
<i>Legionella</i> sp. (non-pneumophila)	-	-	-	1	
Total	8	2	10	38	

*: All environmental *Legionella* isolates were found in cooling tower waters, unless otherwise stated.

BACTERIOLOGY

LEGIONELLOSIS AND ENVIRONMENTAL LEGIONELLA ISOLATES

During April to June 2002, laboratory testing identified 10 sporadic cases of legionellosis. Eight of the 10 cases were confirmed either by isolation of legionella organisms from respiratory tract sites (3 cases), or the demonstration of a four-fold or greater rise in antibody titres (3 cases), or consistently high antibody titres >256 (2 cases). The remaining two cases are regarded as probable cases, with one case positive by the urinary antigen test alone and the other demonstrating an increase in antibody titre from 256 to 512. All cases had presented with clinical symptoms compatible with recent or current legionellosis. A further case was notified on suspicion of legionellosis, but repeated serology testing by IFAT revealed no increase in antibody titres to indicate recent exposure.

There were two deaths resulting from infection with legionella in the April to June quarter. The first involved a previously healthy 73-year old male with a *Legionella longbeachae* serogroup 1 infection following exposure to compost, and an 81-year old female who had undergone minor surgery in hospital about a month prior to her legionellosis being diagnosed.

Table 1. Age and sex distribution of clinical legionellosis cases, April-June 2002

Age (Years)	0-25	26-50	51-75	>75	No. of Cases	Average Age	Age Range
Male	0	1	3	1	5	62.6	45 to 83
Female	0	1	2	2	5	64.6	36 to 82
Total	0	2	5	3	10	63.6	36 to 83

The average age was 63.6 years (64.6 years for females and 62.6 years for males). The age range was 36 to 83 years, with five of the ten cases being between 51 and 75 years of age. The cases involved five males and five females. The causative legionella agent was identified in all of the 15 cases. The infecting organisms are shown in Table 2.

During April to June 2002, *Legionella* species were identified in 38 envi-

SPECIAL BACTERIOLOGY

Interesting Isolates Received in the Special Bacteriology Laboratory

- *Burkholderia pseudomallei* from blood of patient M 49y who had travelled in Malaysia.
- *Corynebacterium diphtheriae* var. *gravis* from M 4y which proved to be toxigenic by PCR detection of the toxin gene. The patient had septic arthritis and the culture was obtained in pure growth from hip aspirate. The child was fully immunised and had no toxin-related symptoms.
- *Corynebacterium diphtheriae* var. *mitis* non-toxicogenic strain from M 42y with an infected tattoo.
- An atypical *Pseudomonas aeruginosa* which was colistin resistant and grew on *B. cepacia* selective agar. 16S rRNA gene sequencing was used to identify the isolate. The patient, a child with cystic fibrosis who was nebulised daily with colistin, had never been infected with *Burkholderia cepacia*, so a definitive identification was important.
- Two isolates of *Neisseria gonorrhoeae* with an unusual biochemical characteristic which were received from a Wellington laboratory. They lacked the enzyme prolyliminopeptidase which, when tested for in several widely used diagnostic kits, is listed as 99-100% positive. This caused concern that similar isolates could be mis-identified. Conventional testing confirmed the identification, and DNA typing showed the isolates to be indistinguishable. The patients, however, have not been linked by contact tracing. The auxotype determined by Auckland Hospital STD laboratory was of a kind not previously seen in New Zealand.

Erratum (incorrect species name)

There was an error in Lablink Vol. 9 No. 2, Page 3, in the section headed "Interesting Isolates Received in the Special Bacteriology Laboratory". *Brucella melitensis* was the incorrect species name, this should read *Brucella suis* biovar 3.

Listeria monocytogenes

One isolate of *L. monocytogenes* from a human case was referred in the period April-June 2002 (Table 3). The isolate was from a premature baby who died of overwhelming sepsis two days after delivery.

Table 3. *Listeria monocytogenes* from human cases, April-June 2002

Month isolated or of onset	Health district	Sex/Age	Source	O antigen serotype
April	Central Auckland	F 1d	BC and body swabs	4

ENTERIC PATHOGENS

SALMONELLA

Human Sources

There were 1,270 human isolates of *Salmonella* confirmed during January-June 2002 compared with 1,223 in 2001. The predominant isolate was again *S. Typhimurium* phage type 160 with a slight increase to 25% of total isolates (22% in 2001), spread over all Health Districts in the country.

S. Paratyphi B was isolated from a F 3 Central Auckland, and from the tank water of the family's turtle aquarium. There was one mixed infection of *S. Bovismorbificans* and *S. Derby M 2* Hawkes Bay. No travel details were given but these serotypes are uncommon in New Zealand.

S. Typhi

There were 16 isolates of *S. Typhi* during the period (Table 4).

Table 4: *S. Typhi* isolates January-June 2002

Sex/Age	Health District	Phage Type	Clinical Details
F 4	Central Auckland	E1a	Immigration
F 26	Central Auckland	E7 variant	Travel Samoa
U 17	North West Auckland	E1a	Contact, index case not received at ESR
F 14	North West Auckland	E1a	Contact, index case not received at ESR
F 8	North West Auckland	E1a	Contact, index case not received at ESR
F 11	Central Auckland	C1 variant	Travel India
F 38	North West Auckland	Untypable	Travel Indonesia
F 5	South Auckland	E7 variant	Relatives visiting from Samoa
F U	South Auckland	E7 variant	Contact F 5
M 65	South Auckland	E1a	No details
M 29	South Auckland	E7 variant	Travel Pacific Islands
M 15	South Auckland	D2	Immigration
M 11	South Auckland	D2	Immigration
M 9	Wellington	E1a	Travel India
F 20	Wellington	Untypable	Travel Indonesia
F U	Wellington	D2	Travel Indonesia

Non-Human Sources

There were 329 non-human isolates during April-June 2002 compared with 342 for the same period in 2001.

There was an increase in ovine isolates of *S. Hindmarsh*, 62 (42 in 2001) and in *S. Senftenberg* in poultry feed, 13 (1 in 2001).

S. Typhimurium phage type 160 continues to be isolated principally from birds and cats, *S. Brandenburg* from cattle, sheep and animal feeds.

ESCHERICHIA COLI

There were 21 isolates of *E. coli* O157 during April-June 2002 compared with 22 for the same period in 2001. Two cases of HUS were admitted to Starship Children's Hospital, F 3 Northland and M 2 Taranaki. There was one isolate of O130:H11 a recognised verocytotoxigenic serotype from F 47, North West Auckland. No clinical details were given. The strain was isolated from a mixed culture, cultured on EHEC agar.

Table 6. Isolates of *E. coli* O157, April-June 2002

Month	Sex / Age	District	Clinical Details	Isolates from Known Contacts
April	M 1	Canterbury	None given	F no age given
April	F 1	Tauranga	None given	
April	M 1	Tauranga	Diarrhoea	
April	F 2	Rotorua	Diarrhoea	
April	F 55	Central Auckland	None given	
April	F 1	Rotorua	Gastroenteritis	
April	M 24	Otago	None given	
May	F 3	Northland	HUS	
May	F 70	Canterbury	None given	
May	M 6	Rotorua	Diarrhoea	
May	M 15m	Manawatu	None given	
May	M 1	Waikato	None given	
May	F 2	Central Auckland	None given	
May	M 5	Canterbury	None given	
May	M 2	Waikato	None given	
May	M 2	Taranaki	HUS	
May	M 1	Waikato	None given	
May	M 1	Waikato	None given	
May	F 3	Waikato	None given	
June	F 25	Waikato	Diarrhoea	

Vibrio cholerae

Vibrio cholerae O1 Biotype El Tor subtype Ogawa was isolated from F 63 North West Auckland, who had recently travelled to India.

SHIGELLA

There were 62 *Shigella* isolates and 10 probable *Shigella* species confirmed during January-June 2002, compared with 103 for the same period in 2001. (Refer Tables 7 and 8).

A new multiplex PCR has been introduced into the Enteric Reference Laboratory to help identify enteroinvasive strains of *E. coli* (EIEC) and to confirm the identification of *Shigella* species where biochemical or serological reactions are atypical. This PCR detects the *virA* gene which is found on the virulence plasmid of *Shigella* species and EIEC; and the *ipaH* gene, also associated with virulence, which is present in multiple sites in both the plasmid and the chromosome of these organisms. The PCR is also useful for confirming the loss of the virulence plasmid, which occurs in the smooth to rough transformation of *S. sonnei*. This plasmid may also be lost in other *Shigella* species.

Table 7. *Shigella* isolates, January-June 2002

Species	Type	Number	Comment
<i>S. boydii</i>	2	1	Travel Thailand
	4	1	No details
	13	3	No details
<i>S. dysenteriae</i>	2	1	Immigration
<i>S. flexneri</i>	1b	1	Travel Thailand
	2a	15	3 travel Pacific Islands
	3	1	Immigrant
	3a	3	Immigrants
	3c	1	No details
	3d	1	No details
	4	1	Travel Tonga
	4a	1	Travel Cambodia
	6	2	1 immigration
	Y variant	1	Immigration
<i>S. sonnei</i>	Biotype a	20	2 overseas travel
	Biotype f	1	No details
	Biotype g	8	2 recent overseas travel

Table 8. Probable *Shigella* isolates January-June 2002

Species	Biochemically	PCR	Number
<i>Sonnei</i>	Biotype a	Shig V - Ipa H +	6
<i>Flexneri</i>	Biotype 4	Shig V + Ipa H +	3
<i>Flexneri</i>	Species	Shig V - Ipa H +	1

Table 5. *Salmonella* isolates, January-June 2002

Serotypes	HEALTH DISTRICTS																								Total	
	NL	MW	CA*	SA	WK	TG	BE	GS	RD	TP	RJ	HB	TK	WG	MW	WR	WN	HJ	NM	WC	CB	SC	OT	SO		
Agona			1		1	1 ^R															1 ^R				4	
Anatum				1						1 ^R															2	
Anatum 15+			1																						1	
Bareilly									1												1				2	
Birkenhead																					1				1	
Blockley															1										1	
Bovismorbificans	1	1 ^R								1		1					1							1	6	
Brandenburg		1	3	1														1			2	5	6	7 ^L	26	
Bredeney				1																					1	
Choleraesuis var Kunzendorf		1 ^B																							1	
Derby					1							1						1 ^R							3	
Ealing																							1 ^R		1	
Emek					2																				2	
Enteritidis phage type	1		1					1	1 ^R												1 ^R				4	
4		2	1	2								1		1							1				8	
6a					2 ^R																				2	
9a		2	13	3	17	4			1			2	3	2	4	2	4	2	1		7	2	3	1	73	
13a			1												1										2	
40									1	1															2	
RDNC		1 ^R	2	1				2											1						7	
Rough																								1 ^V	1	
Hadar			4																		1				5	
Heidelberg					1	1															4		2	1	9	
Hindmarsh												3									1				4	
Hvittingfoss		1				1																			2	
Infantis	8	11	14	3	11	2	3		1			1	1	7	2		3	1	1		4			1	74	
Javiana				1	1												1				1 ^R				4	
Kentucky				1 ^R		1																			2	
Mbandaka						1													1 ^R						2	
Mississippi				1															2		2		3		8	
Montevideo			3										1 ^R				2 ^R	1 ^R			1				8	
Newport	1 ^R		1	1													1				2				6	
Oranienburg		1			1 ^R																		1		4	
Orion																									1	
Oslo		1																							2	
Panama			1																						1	
Paratyphi A				1 ^R																1 ^R					2	
Paratyphi B			2	1																					3	
Paratyphi B var Java	3	1			1							1 ^R					1					1			8	
Pensacola																								1	1	
Poona			1																						1	
Reading			1	1																					3	
Rissen			1 ^R	1																					2	
Saintpaul									1		7				1		1				9	3	5	3	30	
Schwarzengrund			1 ^R																						1	
Tennessee			1																						1	
Thompson	2	2	8	3	3			1					3												22	
Typhi			4	3	6																				16	
Typhimurium phage type	1	4	6	10	8	7	2		4	1	1		4	3	2	1		9	6	84	6	28	8	5	2	201
1 variant																					1				1	
9			1 ^R		1	1	3	2		1											1	2	3		15	
12a		2	2		2								1		1			1 ^R	1		10	5	6		31	
23		4	2	2					1															1	10	
26		2																							2	
41				1																					1	
42				1	1			1							1		2				3		4	1	14	
101	2	2	2	4	2	1			2				1						3	1	4		2		26	
104							1																		1	
135	3	7	10	14	6	3	1	14	6	1	1	4	3	3	3	1	8	4			7		8	1	108	
154		1			1																1				3	
155					1																				1	
156	3	4	5	4	6					1		1	3		7	3	6	3	2		4	2		2	56	
160	10	23	32	22	22	5	1	2	10	2	1	21	13	5	17	4	25	13	14	3	41	16	9	6	317	
205																	1				1				2	
206		2																							2	
U291 ^N			2	1																					3	
RDNC			2							1											1 ^R		1		5	
RDNC WK					1																				1	
RDNC Aug 01	1																								1	
Rough												2		1							1				4	
Untypable		2	5			1			1									1	1 ^R		1			1	13	
Victoria																						1			1	
Virchow			2	2	1										1						2				9	
Waycross																							1 ^R		1	
Weltevreden		1	4	1 ^R	1 ^R																				7	
Weltevreden 15 +			6	2																					8	
Group B 4,5,12 : d : -			2	2									1								1 ^R				11	
Group B 4,12 : eh : -																								1	1	
Group B 4,12 : - : 1,2				1																	1				2	
Group C 6,7 : k : -	2	2	15	8	2	1			2			4													37	
Group E 3,10 : r : -																									1	
Group E 3,10 : - : 1,5				1																					2	
TOTAL	40	87	168	101	95	25	9	27	29	10	2	57	29	22	39	10	78	38	111	10	147	47	59	30	1270	

* Includes where precise Auckland district is not known.

S. Typhimurium 818

Others 452

R Recent Overseas Travel N This phage type has not previously been isolated in New Zealand

I Immigration B Blood culture isolate

V Incoming visitor L Includes one isolate from a leg abscess

25% of total isolates S. Typhimurium phage type 160 compared with 22% for the same period in 2001

ANTIBIOTIC RESISTANCE

MULTIRESISTANT METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS, JANUARY–JUNE 2002

During the six months January to June 2002, multiresistant methicillin-resistant *Staphylococcus aureus* (mMRSA, MRSA resistant to two or more classes of antibiotics in addition to β -lactams) from 895 people, 844 patients and 51 healthcare workers, were referred to ESR (Figure 1). The crude (not adjusted for trend) annualised incidence of mMRSA during the first half of 2002 was 47.9 per 100,000. This incidence is similar to that for 2001 (45.8 per 100,000) but there was actually a decline in isolations during the first six months of 2002 compared with the last six months of 2001 (Figure 1). The majority (73.6%) of the 844 patients with mMRSA were categorised as hospital patients (in hospital or another healthcare facility when MRSA was isolated or during the preceding three months).

Figure 1. Multiresistant MRSA isolations, January 1999–June 2002

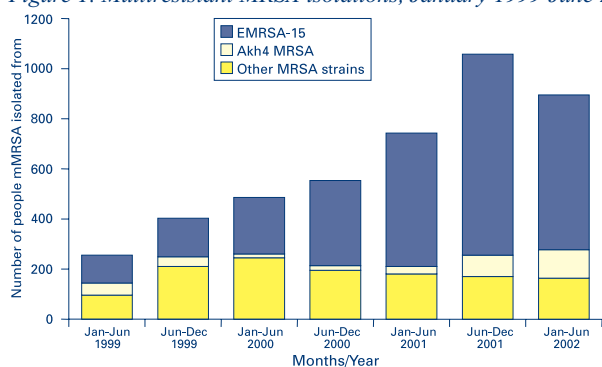


Table 9. Most commonly isolated multiresistant MRSA strains, January–June 2002¹

Strain (origin) ²	Number of people the strain isolated from (% of all mMRSA isolations)
EMRSA-15 (UK)	618 (67.5)
AKh4 (Australia)	113 (12.3)
WR/AK1	65 (7.1)
WSPP (Western Samoa)	19 (2.1)
EMRSA-16 (UK)	17 (1.9)

¹ Includes strains isolated from more than 10 people.

² Descriptions of the strains are included in previous *LabLink* issues: EMRSA-15 and WR/AK1 strains, *LabLink* 2000; 7(1): 8–9; AKh4 strain, *LabLink* 2002; 9(1): 8–9; and WSPP, *LabLink* 1997; 4(4): 25–6. EMRSA-16 is one of the UK epidemic multiresistant MRSA strains, and is currently the second most prevalent MRSA strain after EMRSA-15 in the UK. In New Zealand this strain, unlike EMRSA-15, appears to have caused only sporadic infections and has not become widespread.

The mMRSA strains most commonly isolated are shown in Table 9. The predominance of the EMRSA-15 strain decreased during the first six months of 2002, from 75.0% in 2001 to 67.5%. Among the patients with EMRSA-15, 78.3% were categorised as hospital patients. The majority (82.4%) of mMRSA isolated from healthcare workers were EMRSA-15. The hospitals and other healthcare facilities in which EMRSA-15 was isolated are shown in Table 10. EMRSA-15 was most commonly isolated from patients and staff in hospitals and other healthcare facilities in the Auckland, Hawkes Bay and Wellington areas. About one-third (32.4%) of the EMRSA-15 isolations in healthcare facilities were in facilities other than public hospitals, predominantly long-term care facilities.

The decrease in the predominance of EMRSA-15 was due to an increase in isolations of the AKh4 MRSA, which accounted for 12.3% of isolates and was isolated predominantly in Auckland healthcare facilities (Table 10). The third most commonly isolated strain was the WR/AK1 strain. This strain accounted for 7.1% of isolates and was isolated mainly from children and young adults in the community and hospitals in Auckland.

The incidence 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. Compared with 2001, rates increased in Auckland, Bay of Plenty, Wairarapa and Wellington Health Districts and decreased in Tauranga, Rotorua, Hawkes Bay and Hutt Health Districts.

Table 10. Healthcare facilities with patients and staff with EMRSA-15 and AKh4 MRSA, January–June 2002

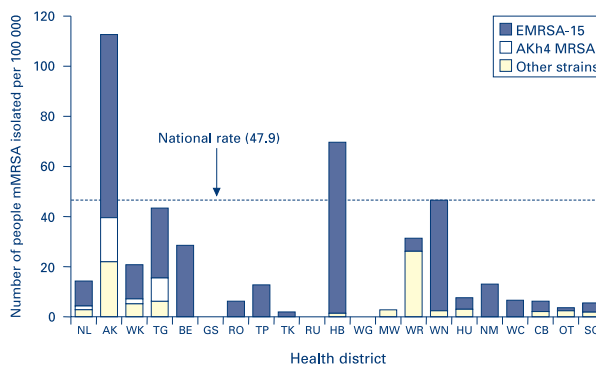
Healthcare facility ¹	Number of people EMRSA-15 isolated from (% of all EMRSA-15 isolations in healthcare facilities, n=586 ²)	Number of people AKh4 MRSA isolated from (% of all AKh4 isolations in healthcare facilities, n=116 ²)
North Shore Hospital	46 (7.8)	15 (12.9)
Waitakere Hospital	14 (2.4)	
Auckland Hospital	79 (13.5)	10 (8.6)
Green Lane Hospital	7 (1.2)	
Middlemore Hospital	77 (13.1)	57 (49.1)
Other Auckland HCFs ³	162 (27.6)	17 (14.7)
Waikato Hospital	11 (1.9)	
Tauranga Hospital	16 (2.7)	7 (6.0)
Whakatane Hospital	5 (0.9)	
Hawkes Bay Hospital	44 (7.5)	
Wellington Hospital	32 (5.5)	
Kenepuru Hospital	28 (4.8)	
Other Wellington HCFs ³	16 (2.7)	
Wairau Hospital, Blenheim	6 (1.0)	

¹ Only hospitals and other healthcare facilities (HCFs) with ≥ 5 patients or staff with EMRSA-15 or AKh4 MRSA are listed in the table. EMRSA-15 was also isolated from people in Kaitiaki Hospital (1 patient or staff), Whangarei Hospital (4), Starship Children's Hospital (3), National Women's Hospital (2), Hamilton HCFs (4), Thames Hospital (2), Tauranga HCF (1), Whakatane HCFs (2), Rotorua Hospital (2), Rotorua HCF (1), Taupo Hospital (3), Taupo HCF (1), New Plymouth Hospital (2), Hastings HCFs (3), Masterton Hospital (1), Hutt Hospital (2), Greymouth Hospital (1), Christchurch Hospital (4), Burwood Hospital (2), Timaru Hospital (1), and Southland Hospital (1). AKh4 MRSA was also isolated from people in Whangarei Hospital (1), Waitakere Hospital (3), Green Lane Hospital (1), Waikato Hospital (3), Thames Hospital (1), and Hawkes Bay Hospital (1). In these lists, 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.

Figure 2. Annualised incidence of multiresistant MRSA by health district, January–June 2002



The susceptibility of mMRSA isolates referred between January and June 2002 was not tested at ESR. However, based on previous testing, the typical resistance patterns of the most common strains are shown in Table 11. In addition to multiresistant EMRSA-15 isolates, which are typically resistant to ciprofloxacin and erythromycin, nonmultiresistant (ciprofloxacin-resistant and erythromycin-susceptible) isolates also occur. These nonmultiresistant EMRSA-15 are not included in the above analyses of mMRSA. During the January to June 2002 period, nonmultiresistant EMRSA-15 were isolated from 92 people in addition to the 618 people with multiresistant EMRSA-15 (Table 9).

Table 11. Resistance patterns of the most common multiresistant MRSA

Strain	Resistance pattern ¹
EMRSA-15	Cip Em ²
AKh4	Cip Cl Co Em Gm Tc
WR/AK1	Fa Mu ^{HL}
WSPP	- ₃
EMRSA-16	Cip Em

¹ Cip, ciprofloxacin; Cl, clindamycin; Co, co-trimoxazole; Em, erythromycin; Fa, fusidic acid; Gm, gentamicin; Mu^{HL}, high-level mupirocin; Tc, tetracycline

² EMRSA-15 also has inducible clindamycin resistance

³ Multiresistant WSPP MRSA are most commonly erythromycin resistant and either mupirocin or fusidic acid resistant. However, most WSPP MRSA remain nonmultiresistant.

VIROLOGY

RESPIRATORY VIRUSES

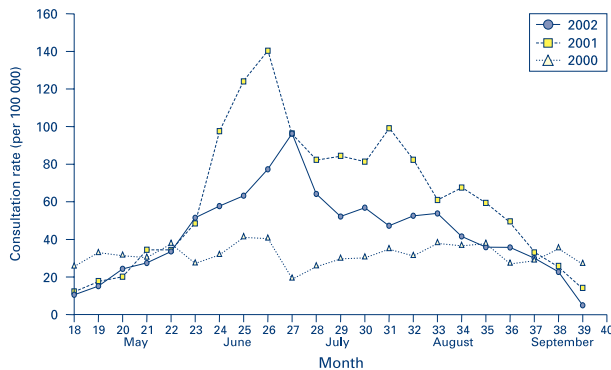
Influenza virus

National influenza surveillance in 2002 was undertaken between May and September using a sentinel network of 92 general practices. On average 88 practices, with a total patient roll of 339,954 participated each week.

Participating practices record the number of consultations for influenza-like illness each week and the age group of each suspected case. Influenza-like illness was defined as acute upper respiratory tract infection, characterised by abrupt onset, and at least two of the following: fever, chills, headache and myalgia. Each practice was asked to collect swabs from up to three patients each week. The swabs were sent to regional virus laboratories for isolation and strain identification.

During the surveillance period, 3159 consultations for influenza-like illness were reported, and the average weekly consultation rate was 43.2 per 100,000 patient population. This rate is the second lowest since rates have been available from the sentinel surveillance system which began in 1991 (refer to *NZPHR 2001, 8(1):9-12 "Influenza Surveillance and Immunisation in New Zealand, 1990-1999"*). The lowest consultation rate recorded was 32.5 in 2000. The consultation rate remained consistently low throughout the sentinel surveillance period (Figure 3) with a peak in week 27 (at the beginning of July). Influenza virus isolations in the five regional virus laboratories also peaked in Week 27 (Figure 4).

Figure 3: Weekly consultation rates for influenza-like illness in New Zealand 2000, 2001 and 2002.



Consultation rates varied between health districts, with rates above the national average in eight of the 22 health districts and a rate of more than four times the national average in Eastern Bay of Plenty (178.7 per 100,000). (Figure 5).

Pre-schoolers and children were the most likely to be seen by a general practitioner for an influenza-like illness. The age-specific average weekly consultation rates were: infants less than one year of age, 40.7 per 100,000; 1-4 year olds, 107.9; 5-19 years, 51.5; 20-34 years, 50.6; 35-49 years, 41.2; 50-64 years, 7.6, and ≥65 years, 13.5. The lower rate in those ≥65 years of age is likely to be due to higher levels of vaccination in this age group.

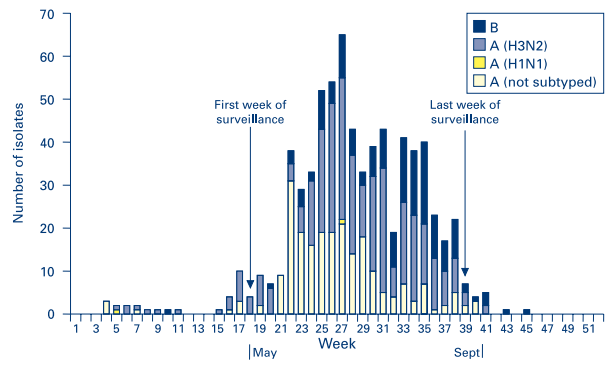
Influenza virus was isolated from 241 (25%) of the 963 swabs submitted by the sentinel practices. One hundred and ninety-two (79.7%) of these isolates were influenza A. The remaining 49 were influenza B of which 38 (77.6%) were typed as B/Hong Kong/330/01 and another 11 influenza B were untyped. Of the 192 influenza A isolates, 157 (81.8%) were sub-typed as H3N2 (A/Moscow/10/99-like strain). Based on all influenza virus isolations made by the regional virus laboratories during 2002 (Figure 4), influenza A(H3N2) predominated throughout the winter season.

Characterisation of the influenza viruses isolated during the 2002 winter indicated a need for a change in the Influenza B components of the vaccine for the 2003 winter. Accordingly, the 2003 Southern Hemisphere winter influenza vaccine has the following composition:

- A/New Caledonia/20/99 (H1N1)-like strain
- A/Moscow/10/99 (H3N2)-like strain
- B/Hong Kong/330/01-like strain

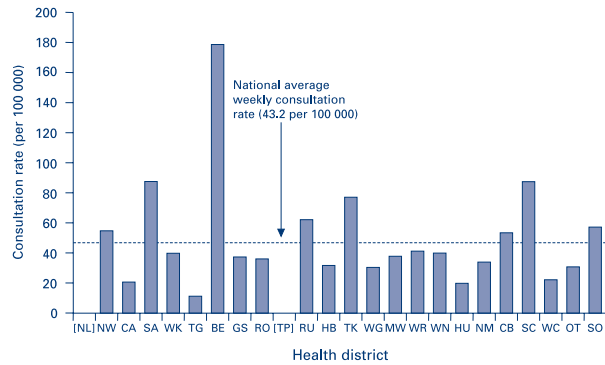
This composition differs from the vaccine used in the 2002 Southern Hemisphere winter, when the influenza B component was the B/Sichuan/379/99-like strain.

Figure 4: Total influenza isolates by type and week specimen taken, 2002.



Influenza immunisation is especially recommended for those at increased risk of complications from influenza due to their age or medical condition (see the *Immunisation Handbook* for details). Influenza vaccination has been free for people ≥65 years of age since 1997. From 1999, it has been extended to younger people with chronic illnesses who are at risk of developing complications from influenza. Optimally, influenza vaccine should be given in March or April.

Figure 5: Sentinel average weekly consultation rate for influenza-like illness by health district, 2002.



Note [] Northland and Taupo health districts did not participate in 2002.

CULTURE COLLECTION

Recent accessions to the Collection are shown in Table 12.

Table 12. NZRM new accessions

Name	NZRM No.	Source, Strain	Comments
<i>Bordetella holmesii</i>	4068	ATCC 51541	Type strain
<i>Campylobacter upsaliensis</i>	4067	NZ isolate, strain A73098	Canine faeces
<i>Candida albicans</i>	4069	ATCC 14053	QC of bioMerieux Vitek products. Control strain for identification
<i>Candida albicans</i>	4070	ATCC 64548	MIC testing, fluconazole sensitive
<i>Candida albicans</i>	4071	ATCC 64550	MIC testing, fluconazole sensitive
<i>Candida parapsilosis</i>	4072	ATCC 22019	Type strain. Susceptibility testing. Control strain for identification
<i>Escherichia coli</i>	4073	ATCC 51446	QC of Vitek test kit
<i>Haemophilus influenzae</i>	4074	ATCC 9006	QC strain for bioMerieux Vitek and IDS products
<i>Klebsiella pneumoniae</i> subsp. <i>pneumoniae</i>	4075	ATCC 33495	QC strain for BBL products
<i>Moraxella (Branhamella) catarrhalis</i>	4076	ATCC 25240	QC strain for API products
<i>Staphylococcus aureus</i>	4058	NZ isolate, 2000	MRSA, strain WGW1

New Names

No new names considered to be of relevance to Lablink readers were notified as validated in the International Journal of Systematic and Evolutionary Microbiology publications of November 2001 and January, March and May 2002.

MYCOLOGY

Table 13. Biannual summary of opportunistic mycoses in New Zealand, January – June 2002

Organism	No. of cases	Site	Clinical data
Filamentous fungi			
<i>Aspergillus niger</i>	2	Nasal cavity (1) BAL (1)	DE+ (histology), NR. Neutropenic, AML, on chemo therapy. Lung consolidation, not responding to antibiotics. DE+, tx: AmpB.
<i>Aspergillus versicolor</i>	1	CAPD	ESRF 2' to diabetes mellitus DE-
<i>Aspergillus fumigatus</i>	4	BW (3)	Post BMT, SCIDS, HVGD, on steroids, lesions in brain (1) lung transplant, DE+ x anastomotic slough (2), acute leukaemia, pneumonia with lung consolidation, tx: AmpB (1)
		Frontal brain lesion (1)	NHL, admitted to hospital with seizures. Lesion found on CT scan. DE+ (histology).
		Nasal tissue (1)	Leukaemic, DE+, also isolated with <i>Rhizopus</i> species.
Basidiomycetous fungus	1	CAPD	ESRF, isolated from multiple bags, DE+
<i>Beauveria bassiana</i>	1	Left upper arm biopsy	DE+ (histology), ALL.
<i>Fusarium solani</i>	1	Toe & AC fossa biopsies	DE+, ALL, neutropenic, black necrotic toe that was amputated, lesions in AC fossa appeared 4 days later. Tx: ambisome.
<i>Geotrichum candidum</i>	1	Shin swab	DE+, IDDM with CRF.
<i>Histoplasma capsulatum</i>	1	Post mortem lung	HIV+, Vietnamese had lived in NZ 1½ years. At PM cerebral mass also +ve for CMV & toxoplasma. Miliary nodules in lungs, also <i>Pneumocystis carinii</i> present.
<i>Mucor ramosissimus</i>	1	Shin abscess	DE+, myelodysplastic syndrome, "knocked" shin, cellulitis & nodular lesions. Tx: with AmpB.
<i>Paecilomyces species</i>	1	Sphenoid tissue	DE-, chronic sinusitis, also isolated with <i>Stenotrophomonas</i> spp.
<i>Phialophora richardsiae</i>	3	Foot nodules (1)	DE+, renal transplant patient, re-isolation 8 months after initial isolation June 2001.
		Foot ganglion – pus & biopsy (1)	DE+, diabetic admitted with gastritis. Incidental finding of lump over metatarsal head that had been present for 2 years with increased inflammation in the previous 3 months. Surgically excised & course of itraconazole for 6 weeks. Foot x-ray no bone abnormality.
		Hand dorsum cyst (1)	DE+, gardener, RA, on steroids.
<i>Scedosporium apiospermum</i>	1	Thigh & lower leg biopsies	DE+, cellulitis. Commenced on AmpB, changed to Voriconazole.
<i>Scedosporium prolificans</i>	1	BW	DE+, post-single lung transplant, CXR changes. Commenced on AmpB, changed to Itraconazole & then Voriconazole + Terbinafine.
Yeasts			
<i>Candida albicans</i>	37	Blood (31)	Line sepsis (3), lymphoma, post PBSCT (1), PUO (1), pneumonia (1), ICU patient (1), AVR, patient deceased (1), premature baby, intra-ventricular haemorrhage (1), pneumonia, stroke, gastrointestinal haemorrhage (1), post infectious encephalomyelitis (2' to EBV infection) (1), bowel surgery 2' to bowel perforations, tx: fluconazole (1), diffuse large cell lymphoma, neutropenic, on TPN, tx: fluconazole (1), Ca head and neck, line in-situ, tx: fluconazole (1), hyperosmotic uraemic acidosis, kidney stones causing obstruction, tx: fluconazole (1), pancreatic Ca, now liver metastases (1), Ca colon with liver metastases, <i>C. glabrata</i> also isolated. Portacath ruptured during CT scan, tx: fluconazole, deceased (1), Ca bowel with bowel obstruction, tx: fluconazole (1), type II diabetes, UTI, ?line sepsis (1), diabetic, Ca, abscess (1), diabetic, UTI, also isolated from supra-pubic

Organism	No. of cases	Site	Clinical data
			tissue (1), gall bladder perforation, patient deceased (1), post-op infection, patient deceased (1), diabetic (1), gall bladder calculus, patient deceased (1), cystic fibrosis (1), inguinal hernia, iliofemoral prolapse, on TPN, ?line sepsis, episodes of <i>Candida parapsilosis</i> fungaemia also (1), NR (4)
		Abdominal fluid (1)	Post-pancreas surgery.
		CAPD (2)	ESRF
		Peritoneal fluid (1)	DE-, peritonitis, also isolated with <i>P. aeruginosa</i> .
		Pleural tissue (1)	DE+, post thoracic surgery.
		AAA tissue (1)	DE+, also grew a Group D <i>Salmonella</i>
<i>Candida glabrata</i>	2	Blood (1)	Ca
		CAPD (1)	ESRF, also grew <i>E. coli</i> & <i>E. cloacae</i> .
<i>Candida guilliermondii</i>	3	Blood (1)	Line sepsis.
		CAPD (2)	Peritonitis, also isolated with a coagulase negative staphylococcus (1), perforated duodenal ulcer (1)
<i>Candida haemulonii</i>	1	Blood	Necrotic pancreatitis, line sepsis.
<i>Candida krusei</i>	1	Blood	AML
<i>Candida parapsilosis</i>	16	Blood (9)	AML (1), in neonatal intensive care (2), post BMT (1), aplastic anaemia, neutropenic, long term IV cannula, tx: fluconazole (1), premature baby, tx: AmpB (1), subacute bowel obstruction with adhesions, tx: fluconazole & CVL removed (1), spina bifida, paraplegic (1), hysterectomy complicated by bowel obstruction, line sepsis (1)
		CAPD (5)	ESRF
		Knee joint fluid (1)	Prosthetic knee infection, initial tx: AmpB, followed by fluconazole & 5FC
		Drain site swab (1)	DE+, encephalitis
<i>Candida rugosa</i>	1	CAPD	ESRF
<i>Candida tropicalis</i>	2	Blood (1)	Haematology patient.
		CAPD (1)	ESRF
<i>Cryptococcus neoformans var neoformans</i>	5	Blood (2)	Myelodysplastic syndrome, patient deceased (1), end stage AML (1)
		CSF & lymph node (1)	HIV+, CSF Crypto LA = 1:2048
		Early morning sputum (1)	Multiple myeloma, febrile.
		FNA – lung (1)	Lesion on CXR, cryptococcoma.
<i>Rhodotorula mucilaginosa</i>	1	CAPD	ESRF, repeatedly isolated.
<i>Trichosporon beigeli</i>	1	CAPD	ESRF
<i>Pneumocystis carinii</i>	6	Sputum (2)	HIV+ (1), immunocompromised (1)
		Induced sputum (1)	HIV+
		BAL/BW (3)	Neoplastic disorder, lymphoma & leukaemia (1), NHL, ARF (1), NR (1)
Aerobic Actinomycetes			
<i>Nocardia species</i>	1	Sputum	NR, 16S rDNA sequencing showed a 98% match for <i>Nocardia</i> sp. R441.
<i>Nocardia farcinica</i>	1	Sputum	DE+, cystic fibrosis.
<i>Nocardia nova</i>	9	Pleural fluid (1)	Pleural effusion, isolated from BW 2000.
		Abdominal wall (1)	DE+. Post cardiac transplant.
		Sputum (7)	Chronic cough, bronchiectasis (1), NR (6)
<i>Rhodococcus species</i>	1	Pleural tissue	Heavy growth after extended incubation, NR
<i>Gordona bronchialis</i>	1	CAPD	ESRF, repeatedly isolated

KEY:

AAA	Aortic abdominal aneurysm	5FC	5 Flucytosine
AC	Ante-cubital	IDDM	Insulin dependent diabetes mellitus
AML	Acute myeloid leukaemia	HIV	Human immunodeficiency virus
ARF	Acute renal failure	HVGD	Host vs. graft disease
AVR	Aortic valve replacement	ICU	Intensive care unit
BAL	Bronchoalveolar lavage	IV	Intravenous
BMT	Bone marrow transplant	LA	Latex agglutination
BW	Bronchial washing	NHL	Non-Hodgkin's lymphoma
Ca	Carcinoma	NR	Clinical data not received
CAPD	Continuous ambulatory peritoneal dialysis	PBSCT	Peripheral blood stem cell transplant
CMV	Cytomegalovirus	PM	Post mortem
CSF	Cerebro-spinal fluid	PUO	Pyrexia of unexplained origin
CVL	Central venous line	RA	Rheumatoid arthritis
CT	Computerised tomography	SCIDS	Severe combined immunodeficiency syndrome
CXR	Chest x-ray	TPN	Total parenteral nutrition
DE	Direct examination	Tx	Treatment
EBV	Epstein-Barr virus	UTI	Urinary tract infection
ESRF	End stage renal failure		
FNA	Fine needle aspirate		

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