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New Zealand Journal of

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Editorial
Call for papers. Are there any clinical biochemists or transfusion medicine specialists out there?
Rob Siebers and Collette Bromhead 77

TH Pullar Memorial Address
The value of people: taking charge of our destiny
Holly Perry 78-80

Original article
Comparison of four phenotypic tests, three biochemical tests and Cepheid Xpert™ Carba-R for detection of carbapenemase enzymes in Gram-negative bacteria
Julie Creighton and Shalika Jawawardena 81-88

Case studies
Bronchial cytologic diagnosis of adenoid cystic carcinoma of the breast metastatic to the lung: a case report
Sharda Lallu, Sarla Naran and Peter Bethwaite 90-92

Panton-Valentine leukocydin-positive *Staphylococcus aureus*. A case study
Rebecca Busch 94-96

Obituary
Harry Edwin Hutchings
Contributed by Colvin Campbell 97

Report
Minutes of the NZIMLS ASM, 20 August 2015, Auckland 99-100

Regular features

Advertisers in this issue 76

In this issue 76

Pacific Way column 102-104

Journal questionnaire 101

NZIMLS Calendar 107

In this issue

Rob Siebers, Editor

The increased consumption of carbapenems has resulted in the consequent increase in the level of carbapenem-resistant organisms worldwide. For treatment and infection control and prevention purposes, it is imperative that true carbapenemase-producing Gram-negative organisms are promptly differentiated from non-carbapenemase producing Gram-negative organisms with reduced susceptibility to carbapenems. In this issue, Julie Creighton and Shalika Jayawardena from Canterbury Health Laboratories evaluated and compared a variety of tests for the confirmation and detection of carbapenemase producing Gram-negative organisms in order to implement a method best suited for a diagnostic laboratory with a low prevalence resistance. They tested 19 carbapenemase producing isolates and 31 non-carbapenemase producing isolates against a panel of phenotypic tests. They found that of the phenotypic tests, the modified Hodge test provided the highest sensitivity and specificity. Based on the results of their study the authors suggest a revised testing strategy consisting of immediate testing with the Carba NP test, with direct inoculation into lysis buffer from a five hour bacterial culture. They conclude that a positive result can then be followed with the Xpert®Carba-R test, providing a more definitive confirmation within hours; and that a modified Hodge test is also a useful test addition, in case of false negative Carba NP results.

Panton-Valentin leucocidin is a cytotoxin that can destroy white blood cells and cause extensive tissue necrosis and severe infections. Panton-Valentin leucocidin cytotoxin-positive *S.aureus* is usually associated with community acquired infections and generally affects previously healthy young children and adults. In this issue Rebecca Busch from CHL Ashburton presents a case study of two half-siblings presenting to their GP multiple times over a two year period with recurrent skin infections and abscesses. Swabs were often taken and *Staphylococcus aureus* was the only organism isolated. From a tissue biopsy from one of the siblings the *S. aureus* was found to be Panton-Valentin leukocidin cytotoxin-positive. In New Zealand Panton-Valentin leucocidin is not routinely screened for but should be considered if a patient has recurrent skin abscesses.

Adenoid cystic carcinoma arising in the breast is an uncommon primary tumour accounting for 0.1% of all breast malignancies. In this issue Sharda Lallu and colleagues from Wellington Hospital present a case study of adenoid cystic carcinoma of the breast in a 59 year old female presenting with shortness of breath, cough, increasing hemoptysis, weight loss, lethargy, night sweats and fevers in whom breast cancer was diagnosed in 1998. Repeated bronchoscopy was done for cytology and histology. The patient's history and the cytodiagnostic features, including cribriform, epithelial clusters, epithelial balls, branching epithelial cylinders and cellular hyaline mucoid globules, led to the correct diagnosis.

Each year Council of the NZIMLS invites a prominent person associated with medical laboratory science to deliver the TH Pullar Memorial Address at the Annual Scientific Meeting. This year's recipient was Holly Perry from the Auckland University of Technology who's Memorial Address was delivered at the South Pacific Congress in Auckland in August and is in this issue. In the article, Holly examined where our profession has come from and where it is headed.

There had been no clinical biochemistry or transfusion articles published in the Journal since 2013. Most articles in the last two years have been microbiology in nature. In this issue, in an Editorial, the Editors, Rob Siebers and Collette Bromhead, put out a call for biochemists and blood bankers to consider submitting articles to redress this imbalance in medical laboratory science disciplines lately in the Journal.

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EDITORIAL

Call for papers: Are there any biochemists or transfusion medicine specialists out there?

Rob Siebers[1] and Collette Bromhead[2]

[1]University of Otago, Wellington and [2]Massey University, Wellington

The New Zealand Journal of Medical Laboratory Science aims to publish articles on all subjects pertaining to the practice of medical laboratory science and since becoming open access in 2011 an increasingly number of our published articles have attracted citations in the international medical and biomedical literature (1).

However, in a recent Editorial, it was noted that there had been no clinical biochemistry or transfusion articles published in the NZIMLS Journal since 2013 (2). This issue of the Journal, the last for 2015, contains predominantly microbiology articles. Over the last two years (April 2014 - November 2015), out of 30 articles, 14 (46.7%) were on microbiology, only one on clinical biochemistry (from overseas) and none on transfusion medicine. Perhaps we should rename our Journal the *New Zealand Journal of Medical Microbiology*!

It is important to note that papers published in this Journal are representative of the submissions the Editors receive. Therefore, in search of a greater variety of submitted papers, this Editorial issues a call for articles, case studies, reviews, technical communications and letters to the Editor from the fields of biochemistry and transfusion science. We know that many excellent presentations are made at the yearly Special Interest Groups meetings, yet no clinical biochemistry or transfusion articles have appeared from the BSIG or the TSIG groups lately.

So the challenge is to biochemists and transfusion medicine specialists to redress the imbalance in medical laboratory science disciplines published in the Journal. Think about writing up your presentation as a Journal article. The Editors are 'user friendly' and more than willing to give a guiding hand, as are members of the Editorial Board. In addition to seeing your name in 'lights', you will gain valuable CPD points and the satisfaction that your message has potentially reached thousands of readers worldwide, given the open access nature of our Journal.

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N Z J Med Lab Sci 2015; 69: 77

The New Zealand Institute of Medical Laboratory Science (Inc)

2016 Annual Scientific Meeting

Energy Events Centre, Rotorua

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The value of people: taking charge of our destiny

Holly Perry
Auckland University of Technology

Tēnā koutou, Talofa lava, Malo e lelei, Bula vinaka, Kia Orana, Salām 'alaykum, Nín hǎo, Anyoung haseyo, Namaste, Good morning.

I am honoured to be standing in front of you to give the TH Pullar address today. I have attended many NZIMLS and South Pacific conferences over the years and admired our many mentors who have stood in front of us to deliver this address. I never imagined that I would be the one delivering it one day. So thank you very much to the organising committee for this invitation.

The theme of our South Pacific Congress this year is the Value of Medical Laboratory Science, a topic we all feel passionate about. I want to spend the next 20 minutes or so examining where our profession has come from, and where it's headed. I plan to be provocative, to brain storm some ideas, outline some challenges, and offer some insights.

Pathology is an ancient science. Wikipedia tells us that the history of pathology can be traced to the earliest application of the scientific method to the field of medicine, with the scientific method being defined as a method of inquiry based on empirical and measurable evidence (1). This method gradually replaced less scientific notions of bodily humors, disease as punishment for sin and cures such as blood letting. The first to practice pathology were Muslim peoples during the Islamic Golden Age of the 13th century and the Italians during the renaissance of the 14th century. Pathologists were invented in the late 19th century, but medical laboratory technologists/ technicians/call us what you will, not until the 1930s.

Medical librarian Frances Delwiche in her 2003 paper "Mapping the literature of clinical laboratory science" tells us, and I quote: *"in the late 1890s pathologists began to train assistants, primarily young women, to perform some of the simpler laboratory tasks"*! (2). In 1926 the American College of Surgeons accreditation standards stated that laboratories must be under the direction of a physician, preferably a pathologist. In 1928 the American Society of Clinical Pathologists established a college to train medical technologists and our profession was born, at least in the United States.

In New Zealand our profession was legally mandated in 1946, with the issue of a Certificate of Incorporation for the then New Zealand Association of Bacteriologists. I am indebted to Ross Hewett for publishing this piece of hard to find information in his Editorial in our latest journal (3). In the early nineties the name medical laboratory technologist changed to medical laboratory scientist in New Zealand. As you know, training programmes across the world have largely evolved from apprenticeship style to degrees.

At the same time as training has been evolving, so too has the science of pathology; and the latter at a much faster rate. When I began my training in 1981 manual techniques and mouth pipetting were the norm. Not surprisingly, colleagues caught diseases whilst at work. There was no immunology or molecular department and although I enjoyed a thorough practical training in chemistry, haematology, blood bank and microbiology, I never set foot in histology, which was strictly the domain of the male pathologist in our hospital. We relied always on the phenotype, never the genotype, and on paper request and report forms as there were no computerised laboratory information systems in those days. Quality assurance programmes were rudimentary. We made most of our own reagents, washed our glassware and threw away a good deal of expired blood, as this was well before the introduction of a national blood service. Today we work in a world of ready-made and quality-controlled kits and advanced automation. When I visit students around the country I ogle at systems such as "the track" for automation of specimen transport and storage. We know that fully automated bacterial identification systems are a reality for microbiology and planned for implementation in New Zealand within the next 5 years. Smart fridges for blood matching and dispensing are increasing in popularity around the world. At the patient bedside, handheld devices already exist for pathology ordering and even genetic testing. Genetic knowledge is on an upward spiral (not unlike the DNA helix itself) and "omics" technologies such as genomics, proteomics and pharmacogenomics are increasing the diversity of tests every day. What one does with all this genetic information is another question and one that Don Love may address in the next talk. The technology of laboratories is a far cry from 1981 and even further from 1928. And yet this has all happened in less than 100 years. I think this leaves us with many challenges as a profession.

In 1981 each region of New Zealand had its own hospital, each with its own laboratory. Each town usually also had its own private laboratory, owned and operated by the local pathologist. Gradually pathology services were rationalised and amalgamated. A count of the IANZ directory of accredited human medical testing laboratories reveals there are currently ten DHB laboratories, the New Zealand Blood Service, two non-DHB Australian owned providers with their labs in many regions and two non-DHB NZ owned providers. This comes to 15 organisations providing traditional pathology services. Driving the reduction of the number of employing organisations is the change of philosophy from a lab in each district to centralized services, with an accompanying tender process every few years. Bucking this trend are smaller organisations providing new genetic tests, for example IGENZ and Sequenom.

Is the tendering and laboratory rationalization a good or a bad thing? Almost certainly the patient is better off today than they were in 1981. For one thing, patient rights, ethics and cultural considerations are rightly conferred with much greater importance. Better systems are in place to identify patients and report their results. With the introduction of information technology or IT, patient results can be traced anywhere within the country. National services such as the New Zealand Blood Service further enhance patient safety and dramatically reduce wastage of resources. Quality assurance systems are much more advanced and the design and sophistication of automated assays has reduced assay variability and failure.

How about our profession? Were we better off as medical laboratory technicians and technologists in 1981 than we are as medical laboratory technicians and scientists in 2015? Almost certainly we are safer now in terms of better protection against infectious diseases. Employers and employees both carry responsibilities to stay safe and informed about disease transmission risks and protection, occupational overuse syndrome prevention and safe rostering.

In 1981 there was full employment from training schemes, and I believe salaries were more aligned with other professions which had five year training schemes. But the visibility of our profession was low to the public.

In the 1990s we engaged in applied research. We developed new methods and regulations of the day made it relatively easy for these to be put into routine use. I well remember one of my mentors, Dr Graeme Woodfield as director of the Auckland Regional Blood Centre, charging me with developing DNA assays for HLA typing for organ transplant patients. We also investigated distribution of HLA types amongst different ethnic groups and published this during my time there. Dr Woodfield and my current mentor Professor Steve Henry published many papers during the 1980s. My point is we were able to do this in the context of our regular job, practicing as medical laboratory staff. Perhaps we weren't as busy. Maybe we didn't have to think as much about the budgetary bottom line as much as we do today.

Today employment rates seem to be much lower. I started work at AUT as transfusion lecturer and programme leader for the Bachelor of Medical Laboratory Science in 1998. 17 years ago our BMLS graduates were all snapped up into employment as soon as they finished their clinical placement year. This is no longer the case. Whilst our degree still enjoys high employment rates compared to many degrees, some students take up to one year to find a position post-graduation and more are accepting work as technicians rather than scientists. Ironically, the degree has never been more popular, with application rates soaring. I think this means our profession now enjoys higher visibility in the public eye than it did previously. This is thanks to the efforts of the NZIMLS councils in their promotion of the profession at career fairs and also individual laboratories, who run open days and participate in work experience programme for school students. Our commercial suppliers also invest in this, with campaigns such as Roche's "The Power of Knowing" and Stago's "Medical Laboratory Professional Week". I am sure there are others and so apologies to those I have not mentioned. The enhanced visibility and subsequent higher application rates to BMLS mean we are now able to select more academically able students in the degree. Other opportunities have arisen for medical laboratory professionals as a result of the dot com revolution. Jobs like health IT advisors at companies like Sysmex did not exist in 1981.

What about threats to us as professionals in the face of rapid technology change? To me, there do not seem to be as many opportunities for research. However, there are exceptions. For example, the Waitemata DHB laboratory that initiated a novel cross-professional approach to encouraging research in their organization, inviting lab staff, doctors, research companies and universities to participate in joint projects.

A pessimistic view of the threats we face is that our profession could cease to exist at a significant level. Some skills are already significantly threatened. Experts in gynaecological cytology currently face the replacement of their microscopy skills with molecular viral testing. University graduates of medical laboratory science specializing in cytology face uncertain employment prospects, and for this reason, AUT has not offered the specialisation for the past 4 years.

With the algorithm-based approach for the automated systems I have mentioned, it is conceivable that engineers, IT specialists and medics might be able to design and run simple pathology services. A student on a recent lab visit asked the question "*Now that we have all these machines, what do the scientists do*?" It was a fair question and I believe there are threats to the autonomy of our profession if we do not evolve. We come from a patriarchal past where we worked for the pathologist. We have also lost power over our own destiny to some extent with the monopoly that large commercial companies now have on diagnostic testing contracts. Years ago, when we designed our own tests and made our own reagents, things inevitably went wrong with our recipe formulations and batch to batch variation was high. These were drivers for a change to production in the commercial world. Again, improved quality is better for the patient, but it hasn't enhanced our problem solving abilities as scientists. When we were in charge of our own reagents and tests we had to troubleshoot the problems. Troubleshooting involved utilising a different set of skills. If something goes wrong with an analyser or a batch of reagent we do troubleshoot up to a point, but then our responsibility is to report the problem to the company and scientists who work for the company will conduct the full root cause analysis. Many problems with kits are resolved at the company level before the kit reaches us. This of course is good customer service. But it means we as scientists didn't have to think about it too much.

I would like to refer again to Ross's editorial in our current journal issue and I quote Ross in saying "*Recognition of our science and our profession is our responsibility, no one else will do it for us because they don't know what we do*" (3). So how do we take charge of our own destiny? Somehow we have to stop being the back-room girls (and boys).

I believe the answer to professional visibility lies in becoming active in research. Currently, staff who want to pursue research have to leave the diagnostic laboratory to do that, as biomedical research and diagnostics are mostly separate. My vision is for a diagnostic laboratory in which we are active in research and that research environment seamlessly overlaps with the diagnostic testing. If you are research active, you do have to nut out experiments that don't work and reagents that don't behave as you expected them to. You also have to be resourceful, as the equipment or raw materials you need for a particular experiment are not always available and you have to think about how to adapt the resources you do have to meet a need. This leads to innovation, which in turn generates material that you can publish. Publications and other research outputs may help to increase our scientific kudos, our political clout and our public profile. If you are visible by an internet search of your research outputs, international colleagues are more likely to be aware of you and the press are more likely to contact you for comment. Whilst not everybody may see this as a good thing, I believe it is important. When DML lost the Auckland community contract to Labtests a few years ago, I was very distressed to hear only pathologists interviewed about it. I kept waiting to hear the voices of those who perform the testing; (our voices) in the media, but it never happened.

As Nadia-Al Anbuky from LabPlus said to me and a group of students recently (N. Al-Anbuky, personal communication, July 2015), automation will free us up from many of the repetitive tasks we perform in the diagnostic laboratory allowing us to focus more on research and innovation. I know some of you here are already forging ahead in your laboratories with this progressive and exciting work, particularly in the development of new genetic tests.

The automated future may provide the time to focus on research, but where is the money coming from? I hear you ask. I imagine it will be the same place it comes from for other health professionals who engage in research, through grant applications. Applying for grants is part of being a scientist.

Publishing is also part of being a scientist. I have heard Don Love challenge us before to write and publish and I agree that if we have the title medical laboratory scientist, this should be part of our role. Often work is published by medical teams who commissioned the lab to run the samples. This is our work too, we need to be a part of the team and take ownership with a voice in the publications.

Undergraduate MLS degrees prepare students to think critically, read the literature and be consumers of research. They are ready to start thinking about performing research themselves and to undertake postgraduate study as the next step in their career. We have a small number of students in postgraduate MLS programme at AUT. The biggest barrier for postgraduate students is getting time off work and the challenges that poses for managers in staffing their departments. I understand that challenge and hope that more research conducted in the diagnostic environment will mean that people can be enrolled in postgraduate study and investigate research topics at work, on a topic that the employer has an interest in. In other words, win-win!

Some of us have the title of medical laboratory technician, but do very similar work on a day to day basis as the medical laboratory scientist. Although the scope of practice may be different on paper, we sometimes struggle to differentiate in practice. The pay differential and lack of career prospects for technicians is another thing that distresses me about our profession. I am not sure this two tier scoping will serve us well in the future. My personal vision would be to have only one scope of practice, with the level at which people work within the scope determined by education, experience and performance. This would mean everyone has the opportunity to advance without hitting a glass ceiling so soon after qualifying.

I leave you with my visions for the future. Firstly, a team approach to pathology practice and education with laboratory people, doctors, engineers and IT people in the team. The team works together to strategically develop new technology. Universities respond to the challenge in the type of graduates they produce, looking to double degrees that span the knowledge gaps. Staff are engaged in postgraduate study, research and publishing. I think the future for our young scientists is incredibly exciting and I urge us all to take charge of our destiny. I know that Dr Pullar was a pathologist and one who encouraged medical laboratory people to upskill and I hope he would approve of my vision today. As people, we are the face and the value of pathology services.

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NZJ Med Lab Sci 2015; 69: 78-80

NZIMLS SEMINARS TO BE HELD IN NOVEMBER:

Histology Special Interest Group

Where: Commodore Hotel, Memorial Avenue Christchurch
When: Saturday 7 November 2015
Contact: Mary Sim
mary.sim@sclabs.co.nz

Mortuary Special Interest Group

Where: LabPlus Conference Centre, Auckland City Hospital
When: Saturday 7 November 2015
Contact: Shane French
shanef@adhb.govt.nz

ORIGINAL ARTICLE

Comparison of four phenotypic tests, three biochemical tests and Cepheid Xpert® Carba-R for detection of carbapenemase enzymes in Gram-negative bacteria

Julie Creighton and Shalika Jayawardena

Canterbury Health Laboratories, Christchurch

ABSTRACT

Background and aims: The increased consumption of carbapenems has resulted in the consequent increase in the level of carbapenem resistant organisms worldwide. For treatment and infection control and prevention purposes, it is imperative that true carbapenemase producing Gram-negative organisms (CPO) are promptly differentiated from non CPO with reduced susceptibility to carbapenems. Amongst the variety of methods available, no single test allows for reliable, prompt and economical identification of CPO. We evaluated and compared a variety of tests for the confirmation and detection of CPO in order to implement a method best suited for a diagnostic laboratory with a low prevalence resistance.

Methods: 50 isolates including 19 carbapenemase producing isolates (with a representation of KPC, NDM, VIM, IMP, and OXA-25,-27,-58,-181 strains) and 31 non-carbapenemase producing isolates that exhibit reduced susceptibility to one or more carbapenem due to other resistance mechanisms, were chosen. Each isolate was evaluated against a panel of phenotypic tests including the modified Hodge test (MHT), combination disk tests (CDT), the ROSCO KPC/MBL Confirm (RKMC) kit, temocillin and piperacillin/tazobactam (TPT) screen test; biochemical tests including the Carba NP test using two method variations (method A with a lysis incubation step and method B without) and the ROSCO Rapid CARB test. The Xpert®Carba-R molecular assay was also evaluated for the detection of KPC, NDM, VIM, IMP-1 and OXA-48.

Results: Overall, of the phenotypic tests, the MHT provided the highest sensitivity and specificity (100% and 93.5%, respectively). The CDT and the RKMC kit both correctly classified 11/12 CPO (sensitivity 91.7%); however, both tests falsely classified some of the non-carbapenemase *P. aeruginosa* strains as CPO, providing specificities of 87.1% and 80.6%.

The TPT test identified 21 isolates as possible carbapenemase resistant, 16 of which were CPO; resulting in a sensitivity of 84.2% and specificity of 83.9%. Of the biochemical tests the Carba NP method B provided 100% sensitivity and specificity, proving to be the superior method. The ROSCO Rapid CARB method and the CARBA-NP method A both failed to classify one NDM-1 producing *P. mirabilis* (94.7%); both methods provided 100% specificity. The Xpert®Carba-R produced 100% sensitivity, but only for those enzymes it is designed to detect and produced no false positive results.

Conclusion: Based on the results of our study our suggestion of a revised testing strategy consists of immediate testing with the Carba NP test, with direct inoculation into lysis buffer from a five hour bacterial culture. A positive result can then be followed with the Xpert®Carba-R test, providing a more definitive confirmation within hours. A MHT is also a useful test addition, in case of false negative Carba NP results. Any suspicious isolates, especially those from patients with high risk factors, could be forwarded on to a reference laboratory for confirmatory testing.

Key words: carbapenemase enzymes; Cepheid Xpert™ Carba-R, Gram-negative bacteria

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INTRODUCTION

In most institutions, the use of carbapenem antimicrobials such as ertapenem, imipenem and meropenem is restricted for the treatment of life threatening infections caused by multi-drug resistant (MDR) Gram-negative isolates; especially those isolates possessing extended-spectrum beta-lactamase (ESBL) enzymes and MDR *Pseudomonas aeruginosa*. Carbapenems have a broad spectrum of activity against both Gram-negative and Gram-positive bacteria, thus their use in the treatment of MDR organisms has been essential (1). With an alarming increase in the prevalence of Gram-negative pathogens producing ESBLs, there has been a corresponding increase in the use of carbapenems (1). Understandably, resistance to carbapenems is of international concern, especially if that resistance is due to acquisition of carbapenem hydrolysing enzymes (carbapenemases) such as KPC, VIM, IMP, NDM and, more recently, the OXA-48-like group (1-3).

The carbapenemase enzymes are a very diverse group. They can confer resistance to almost all other β-lactam agents, including β-lactamase inhibitor combinations and extended-spectrum cephalosporins (e.g. KPC group). They may be associated with plasmids that are also transporting other resistance mechanisms, culminating in co-resistance to fluoroquniolones, aminoglycosides and trimethoprim/sulfamethoxazole (e.g. NDM group or other metallo-β-lactamase (MBL) enzymes). However, some carbapenemases may only express low levels of carbapenem resistance and test susceptible to extended spectrum cephalosporins (e.g. OXA-48-like group) (3). Consequently the isolation of organisms harbouring these highly transmissible enzymes is a patient management problem; for both infection control outbreak prevention/containment issues and for the very limited range of currently available treatment options (1,4).

To date, only a small number of clinical isolates possessing carbapenemase enzymes have been found in New Zealand. Fortunately these isolates have all been associated with patients who have recently returned from countries where carbapenemase-producing organisms (CPO) are common or endemic, and there has been no localised spread (5).

Resistance to carbapenems can also be due to intrinsic chromosomal mechanisms such as reduced permeability due to mutations in outer membrane porins and/or due to up-regulated efflux pumps. Clinical isolates with these mechanisms, most commonly *P. aeruginosa*, or *Enterobacteriaceae* that also have hyper production of AmpC enzyme and/or ESBL, are a more common finding in our institution. The differentiation of non-carbapenemase causes of carbapenem resistance from the more concerning CPO can be a huge challenge for a routine laboratory. The large diversity of enzymes means that there is no single phenotypic test that can simply and accurately confirm and differentiate the group (4). Moreover, with no local spread or frequently found enzyme type, laboratories in New Zealand must be vigilant in screening for all CPO and make informed choices of suitable test methods for confirmation of carbapenem resistance.

Phenotypic based tests such as the modified Hodge test (MHT), recommended by the Clinical and Laboratory Standards Institute (CLSI), and various disk approximation tests or combination disk tests using enzyme inhibitor compounds such as EDTA, dipicolinic acid (DPA), aminophenylboronic acid (APBA), or phenylboronic acid (PBA) and cloxacillin, offer the advantage of being cheap and simple to perform, but they lack sensitivity, especially for OXA-48-like group, can be non-specific, take 24 hours or more for a result and demand a high level of experience and technical expertise (4,6,7). In addition, CPO with multiple resistance mechanisms can obscure or mask the effects of inhibitor based tests (8).

A disk diffusion test utilising both temocillin and piperacillin/ tazobactam (TPT) zone diameters has been suggested as a screen to aid detection of OXA-48 producers, but it may lack sensitivity in low prevalence areas such as New Zealand (9).

More promising methods of carbapenemase detection include two recently published biochemical tests; the Carba NP and Blue Carba (10,11). Both are based on enzymatic hydrolysis of imipenem and subsequent colour change of a pH indicator (phenol red and bromothymol blue respectively). Evaluations of both tests have shown excellent sensitivity and specificity for detection of most carbapenemases (12) but have been less successful with OXA-48 type (13). A commercial version of the Carba NP, the ROSCO Rapid CARB screen has recently become available which may suit some laboratories where a standardised kit format is more convenient than making in-house reagents.

Molecular detection methods are sensitive and specific but have the disadvantages of cost, equipment, time and technical expertise, and are limited to the detection of enzymes that are already characterised. However, the recently introduced Xpert®Carba-R assay is a simple and rapid real-time PCR that detects and differentiates the most frequently found carbapenemase enzymes, namely KPC, NDM, VIM, IMP-1 and OXA-48.

The aim of this study was to find a limited range of tests that would be sensitive enough for a low prevalence population, be simple to perform and interpret and to also provide results in a timely manner. We chose to compare and evaluate four phenotypic tests: MHT; combination disk tests (CDT) consisting of a carbapenem disk supplemented with cloxacillin or APBA, combined with DPA potentiation test; the ROSCO KPC/MBL confirm kit (RKMC); and a disk diffusion test using temocillin and piperacillin/tazobactam (TPT). We also evaluated three biochemical tests: ROSCO Rapid CARB screen; Carba NP, with a 30 minute lysis incubation step (method A); and Carba NP without lysis pre-incubation (method B). We also evaluated a molecular test: Cepheid Xpert® Carba-R. All methods were evaluated against a panel of 50 Gram-negative bacteria: 19 carbapenemase producing and 31 non-carbapenemase producing isolates.

METHODS AND MATERIALS

Bacterial isolates

A total of fifty non-duplicate isolates, consisting of 15 *Pseudomonas aeruginosa*, 30 *Enterobacteriaceae* and 5 *Acinetobacter baumannii* were included in the study (Table 1). The panel included 19 carbapenemase-producing strains: NDM (*n* = 5), KPC (*n* = 3), VIM (*n* = 2), IMP (*n* = 2), OXA-25 (*n* = 2), and one each of OXA-24, OXA-27, OXA-58, OXA-48 and OXA- 181; and 31 non-carbapenemase producing isolates that produced other resistant mechanisms such as ESBL, plasmid-mediated AmpC, de-repressed AmpC, and K1 chromosomal β-lactamase. Twenty one of the non-carbapenemase strains were non-susceptible to one or more carbapenem. All carbapenemase-producing isolates used in the study were either reference strains provided by the Institute of Environmental Science and Research Ltd. (ESR) or clinical isolates obtained from Canterbury Health Laboratories (carbapenemase enzyme confirmed and sequenced by ESR). *Klebsiella pneumoniae* BAA1705 (KPC positive) and *K. pneumoniae* BAA1706 (KPC negative/ESBL positive) were included in the panel and used as positive and negative controls respectively. Isolates were stored at -80°C and subbed twice onto Columbia base blood agar (Fort Richard Laboratories Ltd.) before tests were performed.

Modified Hodge test

The modified Hodge test was performed according to CLSI guidelines (6), with the slight modification of testing only one isolate per plate and incorporating three different carbapenem disks. Briefly, *E.coli* ATCC 25922 was applied to each Mueller Hinton agar plate (Difco, Fort Richard Laboratories Ltd.), then the test organism was streaked in two parallel lines, each with a fresh loop; then disks of ertapenem 10µg, imipenem 10µg and meropenem 10µg were applied onto the streaks (Figure 1). For testing of *P. aeruginosa* isolates, *K. pneumoniae* ATCC 700603 replaced *E. coli* as the base organism.

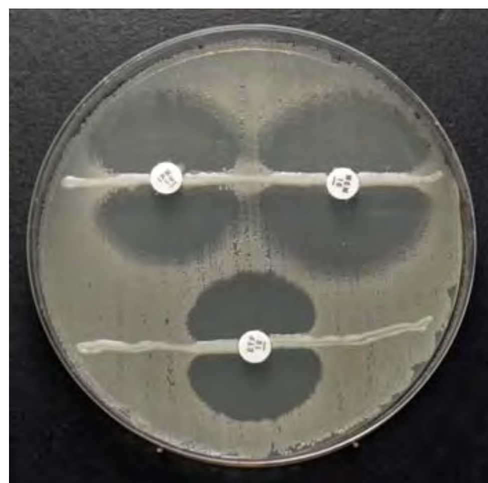

Figure 1. MHT with test isolate (*K. pneumoniae*-producing KPC -3) streaked in two parallel lines, and disks consisting of imipenem 10 g (IMP10), meropenem 10 g (MEM10) and ertapenem 10 g (ETP10) applied to the streak.

Combination disk tests (CDT)

Tests were performed on Mueller-Hinton agar (MHA). For detection of KPC and AmpC β-lactamase, carbapenem disks (ertapenem for *Enterobacteriaceae* and both imipenem and meropenem for *Pseudomonas spp.* and *Acinetobacter spp.*) were used in triplicate: one supplemented with 400μg cloxacillin (cloxacillin sodium salt monohydrate, Sigma Aldrich), one with 400μg APBA (3-aminophenylboronic acid hydrochloride, Sigma Aldrich) and one disk un-supplemented (cloxacillin is an inhibitor of AmpC production and APBA is an inhibitor of both AmpC and KPC enzymes). The test was considered positive for KPC if the zone for carbapenem plus APBA inhibitor was 5mm more than the zone without inhibitor. For AmpC the test was considered positive if the zone for carbapenem plus cloxacillin was 4mm more than the zone without inhibitor, with or without a corresponding zone enhancement for APBA. For detection of DPA enzyme inhibition, disk diffusion was performed with a DPA sensitab (ROSCO, Ngaio Diagnostics Ltd, New Zealand), with one each of meropenem 10 g disk and imipenem 10 g disk at 5mm distance apart from the DPA, and one meropenem disk at 10mm. Potentiation or distortion of the carbapenem zone was regarded as a positive test for a MBL.

The ROSCO KPC/MBL confirm (RKMC) kit contains four disks: meropenem 10 g and meropenem in combination with phenylboronic acid, cloxacillin and dipicolinic acid. The kit is designed to identify AmpC, MBL and KPC. The testing was performed, read and interpreted according to manufacturer's instructions.

Temocillin and piperacillin/tazobactam (TPT) screen test

Disk diffusion was performed according to CLSI methods, using temocillin 30 g and piperacillin/tazobactam 110 g disks (Oxoid, ThermoFisher Scientific). For the purposes of this study a temocillin zone of <12mm together with a piperacillin/tazobactam zone of <16mm was regarded as a positive test, indicating a potential carbapenemase producer.

Biochemical enzymatic tests

The Carba NP test was performed in duplicate. Method A was performed with a 30 minute lysis-incubation step (in order to compare directly against the ROSCO Rapid CARB method) and for method B the isolates were inoculated directly into lysis buffer, followed by the addition of respective reagents as recently recommended by Dortet *et al.* (14). A further variation included the direct suspension of *Acinetobacter spp* into saline, thus removing the bacterial lysis step and no difference between method A and method B for *Acinetobacter spp*, as recommended by Dortet *et al.* (15).

The ROSCO Rapid CARB test (Ngaio Diagnostics Ltd, New Zealand) was performed according to the manufacturer's instructions, with a slight modification for *Acinetobacter spp* where 7-10 l of test organism was directly inoculated into 2 x 150 l of saline in Eppendorf tubes, then vortexed before the addition of respective negative control and imipenem/indicator tablets.

All biochemical enzymatic tests were performed on isolates which were freshly plated onto MHA or blood agar and grown for five hours before testing. Use of a five hour culture has been shown to enhance detection of carbapenemases (16). Preliminary testing with the *Acinetobacter spp.* in our study showed this variation to improve time to detection, thus all study isolates were tested using this modification.

Molecular test

The Xpert™Carba-R detects the most prevalent and widespread carbapenemase enzymes: KPC, NDM, VIM, IMP-1 and OXA-48 (17). The assay is designed as a screening test to detect these enzymes from rectal swabs. Our modified sample preparation, from culture plates, consisted of using a swab to lightly touch a single colony of test strain from a blood agar plate, then emulsified into the sample reagent vial. The manufacturer's instructions were then followed, using the Cepheid GeneXpert Dx system for PCR processing and interpretation (18).

RESULTS

Phenotypic tests

Results of all phenotypic tests evaluated are shown in Table 1. Overall the most sensitive method was the MHT, with all 19 (100%) CPO isolates giving a positive reading. Only two non-carbapenemase producing isolates were considered positive, giving a specificity of 93.5%. The MHT performed particularly well with our study isolates producing KPC, OXA-48, OXA-181, VIM and IMP, giving clear zone distortion. However, interpretation was more difficult with some of the isolates producing NDM and most of the OXA-producing *Acinetobacter baumannii* due to weak zone distortion.

The APBA/cloxacillin CDT correctly classified 2/3 KPC producers. The remaining 16 CPO were all negative, showing no zone enhancement with APBA alone. Among the 31 non-carbapenemase isolates, there were 20 that were both AmpC producers and resistant to one or more carbapenem. The CDT correctly classified all of these isolates as AmpC. The DPA approximation test correctly classified all 9 MBL producers, but we observed false positive results with 4 non-carbapenemase producing *P. aeruginosa*, resulting in an overall specificity of 87.1% for this test.

The RKMC kit correctly classified all three KPC-producers, and 8/9 MBL-producers, failing to identify one *E.coli* IMP-4 (91.7% sensitivity). The kit does not include oxacillinase identification, but none of the 7 OXA-producers were misclassified as KPC or MBL. False-positive classifications occurred with 6/31 non-carbapenemase producing isolates, all of which were *P. aeruginosa,* giving 5mm zones with meropenem/DPA disks (80.6% specificity).

Using resistance to both temocillin and piperacillin/tazobactam to detect possible CPO classified 21 isolates as possible carbapenemase resistant, 16 of which were CPO, giving a sensitivity of 84.2%. An *E.coli* producing IMP-4 and a *Proteus mirabilis* producing NDM, were susceptible to both antibiotics, whereas a *P. aeruginosa* producing IMP-7 was susceptible to piperacillin/tazobactam. Five non-carbapenemase producers were resistant to both antibiotics, indicating false positives for the context of this test (83.9% specificity).

Biochemical tests

Results for the biochemical tests are shown in Table 2. Method A, Carba NP performed with a 30 minutes lysis incubation step (except for *A. baumannii*), was positive at two hours for 18/19 (94.7%) of the carbapenemase producers. *Proteus mirabilis* producing NDM-1 was consistently negative (repeated three times). The five OXA-producing *A. baumannii* strains required direct inoculation into saline instead of lysis buffer in order to produce reliable results (Table 3).

The ROSCO Rapid CARB was also positive for 18/19 (94.7%) of the CPO, with *Proteus mirabilis* producing NDM-1 failing to generate a positive reaction. The five OXA-producing *A. baumannii* strains also required direct inoculation into saline instead of lysis buffer.

In comparison, Carba NP method B without the lysis incubation step detected all 19 carbapenemase producers (100% sensitivity) with all *Enterobacteriaceae* or *P. aeruginosa* strains turning a distinct yellow in the imipenem containing solution by the 30 minute reading. The five *A. baumannii* isolates were all positive at the one hour reading (when suspended directly into saline). Using fresh five hour cultures further enhanced the positive reactions for the *A. baumannii* CPO (Table 3).

Table 1. Phenotypic test results for carbapenemase and non-carbapenemase-producing isolates tested.

Resistance mechanism(s)	Species	n	Phenotypic Test Results			
			MHT	CDT/DPA	RKMC	TPT
Carbapenemase						
NDM-1	*K.pneumoniae*	1	+	+	+	+
	P.mirabilis	1	+	+	+	-
	E.coli	3	+	+	+	+
KPC-2	*K.pneumoniae*	2	+	+	+	+
KPC-3	*K.pneumoniae*	1	+	-	+	+
IMP-4	*E.coli*	1	+	+	-	-
IMP-7	*P.aeruginosa*	1	+	+	+	-
VIM-4	*P.aeruginosa*	1	+	+	+	+
VIM-5	*P.aeruginosa*	1	+	+	+	+
OXA-48	*K.pneumoniae*	1	+	NA	NA	+
OXA-181	*K.pneumoniae*	1	+	NA	NA	+
OXA-24	*A.baumannii*	1	+	NA	NA	+
OXA-25	*A.baumannii*	2	+	NA	NA	+
OXA-27	*A.baumannii*	1	+	NA	NA	+
OXA-58	*A.baumannii*	1	+	NA	NA	+
Total (% sensitivity)		19	**19/19 (100%)**	**11/12 (91.7%)**	**11/12 (91.7%)**	**16/19 (84.2%)**
Non-carbapenemase						
AmpC (±porin/efflux)	*P.aeruginosa*	12	-	4/12 (DPA)	6/12 (DPA)	4/12
Chromosomal K1	*K.oxytoca*	2	-	-	-	-
ESBL	*E.coli*	3	-	-	-	-
	P.vulgaris	1	-	-	-	-
	K.pneumoniae	2	-	-	-	-
ESBL/pAmpC	*E.coli*	1	-	-	-	-
	K.pneumoniae	1	-	-	-	-
ESBL/AmpC	*E.cloacae*	3	1/3	-	-	1/3
pAmpC	*Citrobacter koseri*	1	-	-	-	-
	K.pneumoniae	1	-	-	-	-
	E.coli	2	-	-	-	-
AmpC derepressed	*E.cloacae*	2	1/2	-	-	-
Total (% specificity)		31	**29/31 (93.5%)**	**27/31 (87.1%)**	**25/31 (80.6%)**	**26/31 (83.9%)**

N=number of strains; AmpC, Ambler class C cephalosporinase; pAmpC, plasmid-mediated AmpC

Table 2. Xpert™ Carba-R and biochemical test results for carbapenemase and non-carbapenemase-producing isolates tested.

Resistance mechanism(s)	Species	n	Molecular: Xpert® Carba-R	Phenotypic Test Results: CarbaNP A 30min lysis step	CarbaNP B direct lysis step	ROSCO CARBA
Carbapenemase						
NDM-1	*K.pneumoniae*	1	**NDM**	+	+	+
	P.mirabilis	1	**NDM**	-	+	-
	E.coli	3	**NDM**	+	+	+
KPC-2	*K.pneumoniae*	2	**KPC**	+	+	+
KPC-3	*K.pneumoniae*	1	**KPC**	+	+	+
IMP-4	*E.coli*	1	**IMP**	+	+	+
IMP-7	*P.aeruginosa*	1	**IMP**	+	+	+
VIM-4	*P.aeruginosa*	1	**VIM**	+	+	+
VIM-5	*P.aeruginosa*	1	**VIM**	+	+	+
OXA-48	*K.pneumoniae*	1	**OXA-48**	+	+	+
OXA-181	*K.pneumoniae*	1	NEG	+	+	+
OXA-24	*A.baumannii**	1	NEG	+	+	+
OXA-25	*A.baumannii**	2	NEG	+	+	+
OXA-27	*A.baumannii**	1	NEG	+	+	+
OXA-58	*A.baumannii**	1	NEG	+	+	+
Total (% Sensitivity)		19	**13/13 (100%)#**	**18 (94.7%)**	**19 (100%)**	**18 (94.7%)**
Non-carbapenemase						
AmpC (±porin/efflux)	*P.aeruginosa*	12	NEG	-	-	-
Chromosomal K1	*K.oxytoca*	2	NEG	-	-	-
ESBL	*E.coli*	3	NEG	-	-	-
	P.vulgaris	1	NEG	-	-	-
	K.pneumoniae	2	NEG	-	-	-
ESBL/pAmpC	*E.coli*	1	NEG	-	-	-
	K.pneumoniae	1	NEG	-	-	-
ESBL/AmpC	*E.cloacae*	3	NEG	-	-	-
pAmpC	*Citrobacter koseri*	1	NEG	-	-	-
	K.pneumoniae	1	NEG	-	-	-
	E.coli	2	NEG	-	-	-
AmpC derepressed	*E.cloacae*	2	NEG	-	-	-
Total (% Specificity)		31	**100%**	**100%**	**100%**	**100%**

n= Number of strains

* All *A.baumannii* suspended in saline for biochemical tests so no difference in CarbaNP A and CarbaNP B methods

sensitivity of Xpert® Carba-R calculation limited to enzymes claimed to be detected by the manufacturer at time of testing.

Table 3. Comparison of biochemical tests performed on oxa-producing *Acinetobacter baumnanii* study isolates

Carbapenemase	Species	ROSCO CARBA, 5hr			CarbaNP, 5hr			CarbaNP, 24hr		
		30 min	1 hr	2 hr	30 min	1 hr	2 hr	30 min	1 hr	2 hr
OXA-24	*A.baumananii*	+	+	+	wk+	+	+	-	-	+
OXA-25	*A.baumananii*	+	+	+	+	+	+	-	+	+
OXA-25	*A.baumananii*	+	+	+	+	+	+	-	+	+
OXA-27	*A.baumananii*	-	-	wk+	-	wk+	+	-	-	+
OXA-58	*A.baumananii*	-	+	+	wk+	+	+	-	-	+

All tests performed with direct organism suspension into saline.
Shaded cells indicate where young 5 hour growth cultures produced quicker results compared to 24 hour old cultures.

There were no false positive results in any of the biochemical test methods. However, several isolates had to be repeated due to uninterpretable results; e.g. the negative control tube was more orange/yellow than the indicator tube. This was especially apparent with some non-carbapenemase *P. aeruginosa* strains that appeared to be weakly positive after one hour, but after two hours incubation the colour of the negative control tube matched that of the imipenem tube. Any uninterpretable results were repeat tested.

Xpert™ Carba-R

The Xpert™ Carba-R kit detected all 13 (100%) of the gene sequences it was expected to detect; i.e. KPC, VIM, IMP, NDM and OXA-48. The OXA-25, -27,-58 and -181 tested negative as they are not currently included in the mix and were not regarded as false negative results in our study. A new kit version, Xpert™ Carba-R v2, has recently been modified to detect OXA-181 and OXA-232 (17,18). There were no false positive results (100% specificity).

DISCUSSION

The global spread and increasing prevalence of CPO demands rapid detection and confirmation of resistance mechanisms in a clinical laboratory. The diversity of the carbapenemase genes in terms of both resistance profiles and lack of a single phenotypic test, in addition to possible co-resistance due to the presence of other resistance enzymes, porin loss or efflux up-regulation, makes detection a challenge for a routine laboratory.

Phenotypic tests

Our evaluation of four phenotypic tests (MHT, CDT, TPT screen, and RKMC) revealed the MHT as the most sensitive of the methods tested, detecting all 19 CPO. The MHT is recommended by the CLSI to aid confirmation of CPO, but only for *Enterobacteriaceae* and only for laboratories who have not updated to the new breakpoints. Clinical breakpoints have been set by both the CLSI and EUCAST for treatment guidelines so that antimicrobial results can be reported without delay (19). However, it is vitally important for both infection control implementation and epidemiological purposes to characterise the type of carbapenem resistance present. The MHT has been shown to be excellent for KPC confirmation (12). Bartolini *et al.* tested 101 carbapenemase-producing *Enterobacteriaceae*, finding an overall sensitivity of 94% for the MHT with all 87 KPC producers giving positive results (20). But the test was less successful with other enzymes (VIM, NDM, OXA-48 and KPC/VIM). Other studies have also called into question the ability of the MHT to detect all types of the carbapenemase enzymes (7). Pasteran *et al.* tested 72 carbapenemase-producing *P. aeruginosa* finding only 78% sensitivity with the MHT in addition to many indeterminate results, resulting in a low specificity of 57% (21). However, there was no mention of whether *E.coli* ATCC 25922 had been replaced with *K.pneumoniae* ATCC 700603 as the base organism, which could have helped to negate some of the indeterminate results, as subsequently shown by Pasteran *et al.* (22).

Nordmann and Poirel believe that due to the lack of overall sensitivity of the MHT and its time-consuming nature, the test should not be used (23). Certainly, reading of the test can be quite subjective and a positive result may depend on the reader's interpretation of the amount of zone distortion required. In our study we used three different carbapenem disks and found that some isolates produced distorted zones for only one or two of the disks (Figure 2). Hence increasing the number of carbapenem disks used may increase the sensitivity of the test. Conversely the specificity could be compromised, but we found only 2/31 false positives for the non-carbapenemase strains tested. A disadvantage of the MHT is that it cannot characterise the types of carbapenemase enzyme detected.

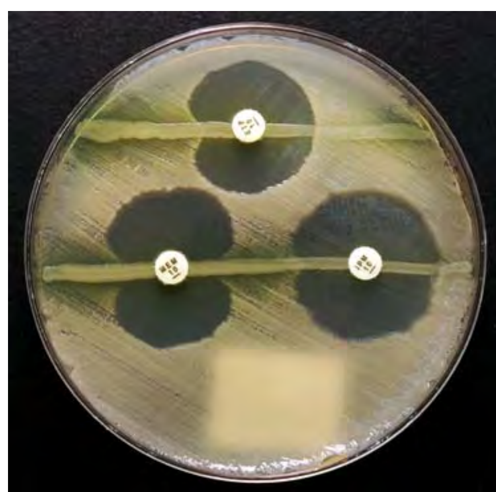

Figure 2. MHT with VIM-5-producing *P. aeruginosa* showing different levels of zone distortion with different carbapenem disks and even different sides of the zone (ertapenem).

We found less success with the TPT disk diffusion test (84.2% sensitive). This test has been suggested as a useful addition to detection algorithms, especially with OXA-48-like enzymes, with Huang *et al.* finding the test to have excellent negative predictive value of 99.2% in ruling out an isolate as a CPO if both tests were susceptible (9). Of note is that the seven OXA-type enzymes in our study were detected, indicating a possible value for the test if OXA-48-like enzymes become established in our population as has happened in many countries, including Turkey, North Africa and India (2,3).

The CDT was included in our study to differentiate KPC and AmpC producers. One of the three *K. pneumoniae* KPC-producers did not show synergy with APBA, which could be due to the masking effect of porin loss or possibly the use of APBA instead of phenylboronic acid (PBA) (8). However, other studies have shown 600 g of ABPA to be a sensitive indicator of KPC in *K. pneumoniae* and *P. aeruginosa* (21,24). Synergy with either cloxacillin alone or together with APBA was an effective way to detect AmpC producers in our study strains and to differentiate from carbapenemase producers, making this simple test a useful addition to the workflow. DPA, tested by disk approximation method, was successful in differentiating the MBL producers in this study. Previously we have observed DPA to be a useful test with *Enterobacteriaceae* (data not shown), but have found the compound to be less helpful for *Pseudomonas spp* as it generates numerous false positives or results that are difficult to interpret.

The overall usefulness of the RKMC kit was reduced as this kit format does not include identification of OXA-producers. For the KPC and MBL isolates sensitivity at 91.7% was lower than for the MHT. Bartolini *et al.* (20) and Giske *et al.* (24) found excellent performance with the RKMC kit on their respective panels of predominantly KPC-producing *Enterobacteriaceae*, but did not achieve high sensitivity with other enzymes. In comparison, Miriagou *et al.* used modifications, including the use of PBA, to aid detection of *K. pneumoniae* co-producing KPC and VIM (8). Using a broader range of CPO, Doyle *et al.* (25) concurred with previous studies in that all KPC producers were detected (25). However, only 58% of VIM and IMP were detected by RKMC and several KPC or NDM producers were misclassified as KPC/NDM co-producers. It should be noted that a newer kit version, the ROSCO KPC/MBL and OXA-48 Confirm kit includes temocillin 30 g disks for OXA-48 differentiation. If we combined the TPT disk test results with RKMC, then 18/19 (94.7%) strains would have been detected.

Biochemical tests

We observed superior results with CarbaNP method B, omitting the bacterial lysis incubation step, detecting all CPO. In addition, the test showed quicker time to results for OXA-48 and OXA-181 strains (data not shown). For *Acinetobacter spp.*, saline suspension with no bacterial lysis was essential, as recommended by Dortet *et al.* (15). The use of five hour growth cultures instead of 18-24 hour cultures provided quicker time to positive results for *Acinetobacter spp.*, but this modification was not compared for all of the study isolates and final endpoint results were the same. However, Lee *et al.* showed quicker results with their selection of *Enterobacteriaceae* strains (16), therefore it would appear to be a method variation worth considering despite the extra time taken, especially on doubtful negative results that were initially performed on 18 - 24 hour cultures.

The introduction of the Carba NP test by Nordmann *et al.* has revolutionised testing for CPO in the routine laboratory (10). The original publication reported 100% sensitivity and specificity against a panel of 162 carbapenemase-producing *Enterobacteriaceae*. However, subsequent studies have failed to reproduce this high performance, particularly with mucoid strains or isolates harbouring OXA-48-like enzymes with low carbapenemase activity (13,26). Subsequent modifications of the method (12,14,16) and adjustments for *Acinetobacter spp* (15) have improved the overall performance of the assay. Indeed, Dortet *et al.* suggested that Carba NP could be used as an initial test on any carbapenem-resistant *Enterobacteriaceae*, with negative results not investigated further as these can be considered carbapenemase-negative with resistance due to other mechanisms (27). Any positive isolates would require follow up with molecular characterisation. The Carba NP has the added benefit of being extremely cost effective to make as an in-house reagent. Solutions can be prepared, aliquoted into Eppendorf tubes and stored at -70oC until required.

Although the ROSCO Rapid CARB test achieved a high level of accuracy in our study, we found the tablets sometimes difficult to dissolve or were often stuck in the Eppendorf tube, making reactions difficult to read. In addition, the slightly yellowish colour of the tablets and cloudy deposit made for more indeterminate tests and the need for repeat testing, compared to Carba NP.

Molecular tests are required for more definitive confirmation of phenotypic and/or biochemical tests. While sensitive and specific for recognised enzymes, they do not detect novel emerging enzymes. Although the Xpert® Carba-R kit has a limited range of gene sequences, it does detect the most prevalent enzymes in New Zealand so far and is superior in terms of labour requirements and time to results compared to our in-house PCR. The method is extremely easy and the assay can also be used directly from rectal swabs as a screening test for carbapenemase isolate colonisation.

Limitations of this study include the limited number of carbapenemase-producing isolates tested and the lack of any isolates harbouring dual carbapenemases, which can interfere with the interpretation of phenotypic tests. In addition, some of the methods used are not validated and defer from previously published methods or kit instructions. A further limitation with this type of method comparison study is the lack of a gold standard for characterisation and detection of carbapenemases, which makes final result interpretation and determination of true positive and negative results a challenge.

In summary, only low numbers of CPO have been found to date in New Zealand, with no locally established carbapenemase type. This makes it imperative to utilise tests that are sensitive, accurate, rapid and simple to interpret. Furthermore, laboratories must be vigilant in the detection of all enzyme types. Current cumbersome phenotypic methods at our institution led us to evaluate and compare a variety of phenotypic, biochemical and molecular methods. The results of our study have shown that a testing strategy should include immediate testing by Carba NP, followed by a molecular assay such as the Xpert™ Carba-R. This would detect and classify the most prevalent CPO within a day. An overnight MHT is still a useful test to include in our environment. Any isolates with carbapenemase not detected and resistant to carbapenems but have other risk factors, such as isolated from a patient who has recently returned from overseas, could be sent to a reference laboratory for final confirmation.

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CASE STUDY

Cytologic diagnosis of adenoid cystic carcinoma of the breast metastatic to the lung by bronchial cytology: a case report

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ABSTRACT

The application of bronchial washings, brushings, trans-bronchial fine needle aspiration cytology in the detection of respiratory tract cancer is now routine. A pulmonary metastasis from a breast adenoid cystic carcinoma is very uncommon. We document a case of pulmonary metastasis of adenoid cystic carcinoma in the breast in a 59 year old female on bronchial brushings, washings and trans-bronchial fine needle aspiration with subsequent histologic confirmation. The patient's history and the cytodiagnostic features, including cribriform, epithelial clusters, epithelial balls, branching epithelial cylinders and cellular hyaline mucoid globules, led to the correct diagnosis. This case is of interest because of rare occurrence of lung metastasis of the breast adenoid cystic carcinoma, diagnosed on bronchial washings, brushings and trans-bronchial fine needle aspiration cytology.

Key words: Adenoid cystic carcinoma, breast, metastasis, lung, bronchial cytology, fine needle aspiration.

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INTRODUCTION

Adenoid cystic carcinoma (ACC) arising in the breast is an uncommon primary tumour accounting for 0.1% of all breast malignancies. In comparison to other sites, ACC of the breast has a good prognosis and shows low incidence of spread to regional lymph nodes. A proportion of these tumours are complicated by local recurrence or metastatic spread to distant sites (1,2). The mean time span between the initial mastectomy and distant metastasis is 6.8 years (3). Morphologic features of cytology and histologic appearances of ACC in the breast are similar to the ACC of other anatomical sites such as salivary glands, lacrimal glands, lung, nasal cavity, skin, trachea, uterine cervix, prostate glands, oesophagus and Bartholin's gland (4-5). ACC was previously termed cylindroma, initially described by Billroth in 1856 with the first description of breast ACC, credited to Geschickter in 1945 (6-9). ACC has previously been reported as negative for the receptors ER and PR (1,8) as seen in our case in the primary breast ACC. In this study we report a case of ACC of the breast metastatic to the lung after 9 years following mastectomy and clearance of lymph nodes, diagnosed by bronchial washings, brushing and trans-bronchial FNA in a 59 year old female. Metastatic ACC was confirmed on bronchial biopsy and she was treated with radiotherapy. Six years later the patient came back with metastasis of ACC to the scalp, which was excised.

CASE REPORT

A 59 year old female smoker presented with shortness of breath, cough, increasing hemoptysis, weight loss, lethargy, night sweats and fevers. Chest x-ray showed nodular opacity adjacent to the right hilum, which may represent a cluster of smaller nodules, each measuring approximately 1.4 cm in diameter, a lung lesion in the apical segment of the right lower lobe and also an abnormality in the mediastinum and lesions in the thoracic cavity. The possibilities were either metastatic disease from the previous breast cancer or a primary lung malignancy with metastasis to the mediastinum. The lungs otherwise appeared clear with no evidence of failure. She had asthma as a child and breast cancer was diagnosed in 1998. Bronchoscopy was performed and revealed some irregular mucosa in the apical segment in the right lower lobe. The bronchial washings, brushing and trans-bronchial FNA were performed via the bronchoscope for cytology examination and diagnosed as suspicious for metastatic ACC. A subsequent bronchial biopsy was negative, therefore repeated bronchoscopy was done for cytology and histology. Bronchial biopsy confirmed the diagnosis of metastatic ACC. The patient completed radiotherapy to the right hilum/mediastinum. One year later the patient came back with hip pain. Chest x-ray revealed radiation pneumonitic changes of her right lung and no metastases seen in her left hip. Six years later she presented with a painful tender mass to her left frontal scalp and excision of this mass showed metastatic ACC. On a bone scan there was definitively a L4 lesion and increased activity bilaterally in the sacrum. She also had abnormal increased activity in the proximal left femur and proximal humerus which looked most consistent with metastatic disease. On CT scan of the chest the right lower lobe was completely collapsed with a mass obstructing the bronchus and was most likely a recurrence of ACC. CT scan of the abdomen and pelvis showed multiple bilateral soft tissue lesions arising from the kidneys and were most likely deposits of cancer.

MATERIALS AND METHODS

Bronchial washings, brushings and trans-bronchial FNA samples were collected in 30% ethyl alcohol in physiologic saline and filter preparations were made on size 5 µm Sartorius AG-Cellulose Acetate filters (Sartorius, Germany) using the cytosieve method and stained by the Papanicolaou method. The remainder of the sample was spun down and from the sediment a cell block was made and fixed in 10% formalin, routinely processed and stained with hematoxylin-eosin (H & E). The cell block preparation was insufficient.

CYTOLOGIC FINDINGS

Papanicolaou stained filters from all samples showed many cohesive three dimensional clusters of uniform cells with balling effects and smooth border (Figures 1a and 1b), cystic spaces,

cribriform clusters, branching cylinders and solid clusters with ciliated bronchial cells in the background. The cribriform clusters were relatively large and contained round pale areas corresponding to the "cystic" spaces (Figures 2a and 2b). Another characteristic finding was the presence of pale blue or pale purple hyaline globules associated with small nests of tumour cells. Naked globules were also identified. Tumour cells exhibited a delicate cytoplasm. The nuclei were relatively uniform with a finely granular, evenly distributed chromatin and cystic spaces containing cyanophilic amorphous material.

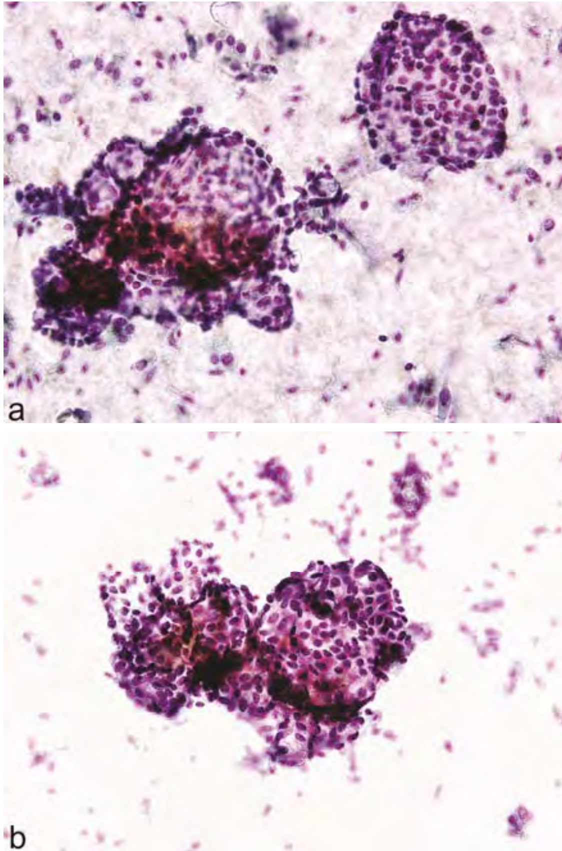
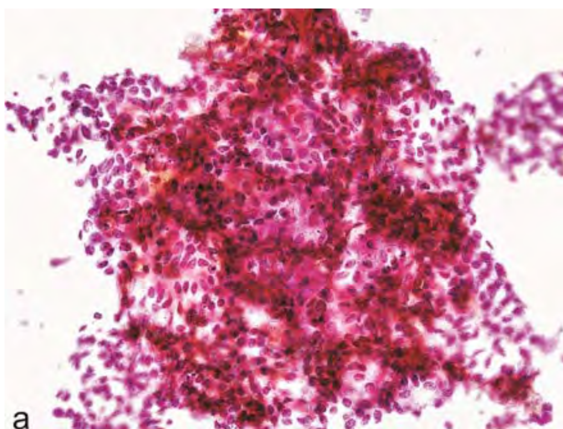


Figure 1a and 1b: Filter preparation showing three dimensional clusters of uniform cells with balling effect and smooth border (Papanicolaou stain X 400)

HISTOLOGIC FINDINGS

H & E stained sections showed fragments of respiratory epithelium in which there were islands of atypical cells comprising angulated nuclei and forming variably sized well demarcated lumen, with features consistent with metastatic ACC. Sections from scalp excision biopsy showed skin with fatty subcutis, the typical structure of scalp. The dermis was extensively infiltrated by ACC, showing perineural infiltration focally (Figure.3).



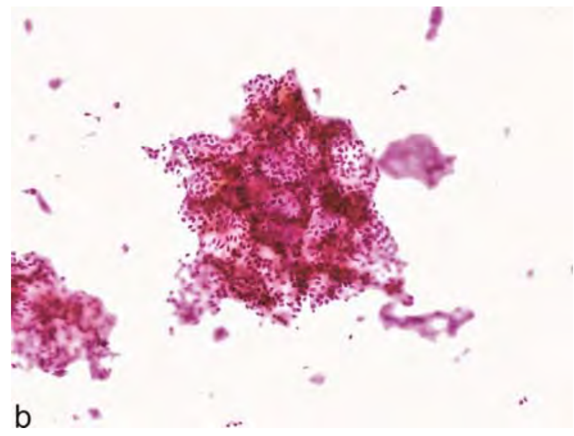


Figure 2a and 2b: Filter preparation showing three dimensional, cribriform cluster with cystic spaces (a: Papanicolaou stain X 400; b**:** Papanicolaou stain X 200)

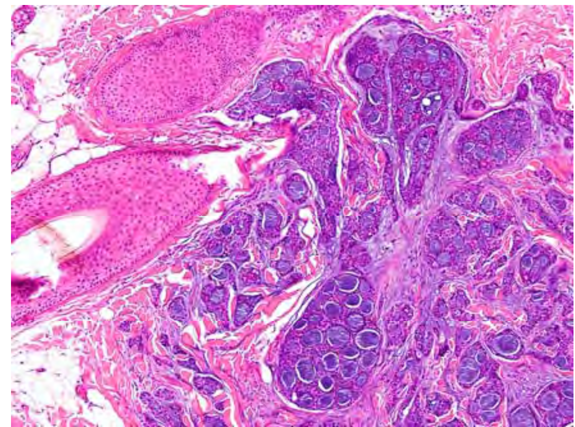


Figure 3: Scalp excision biopsy showing skin with fatty subcutis, the typical structure of scalp and the dermis was extensively infiltrated by ACC, with perineural infiltration focally (haematoxylin-eosin X 100)

DISCUSSION

Adenoid cystic carcinoma (ACC) of the breast is rare, accounting for less than 0.1% of all breast cancers. This variant of adenocarcinoma is typically seen in salivary glands but has been reported in other organs including breast, skin, lung, cervix, larynx, prostate and Bartholin gland. Unlike ACC in the salivary gland, ACC of the breast has a good prognosis, with axillary metastasis being rare. Distant metastasis, usually to the lungs, can occur without positive axillary nodes and local recurrence is more likely (1). Axillary metastasis has been reported in rare cases suggesting that distant metastases predominantly develop by a hematogenous dissemination (10).

The extra-mammary tumours are highly fatal due to frequent metastases and extensive local invasiveness, with an overall 5 year survival rate of 40%. ACC of the breast is much less malignant than histologically identified neoplasms in other sites possibly due to the factors such as the relatively small size of these tumours when first noticed in the breast and their location, which enables total excision (5). ACC has previously been reported as negative for ER and PR. One review of the literature, however, found that more than half of ACCs of the breast were positive for at least one of these receptors. Despite the relative ER-PR negativity of this special type of breast carcinoma, the prognosis is good. It is believed that the tumour is ER negative not a result of poor differentiation, but possibly due to preserved polarity and differentiation of the component epithelial and myoepithelial cells (1).

ACC of the breast, occur predominantly in woman aged 60 to 66 year and mean age is 64 and may be bilateral. It tends to develop in the peri areolar area. Cytology and histological appearances of ACC of the breast are similar to the adenoid cystic carcinoma of other anatomic sites. Recent reports describe characteristic features on cytology to enable preoperative diagnosis comprising cellular aspirate with tightly cohesive aggregates of cells with enclosed spheres and interconnecting cylinders of acellular material. The principal cell type represent the epithelial cells and minor proportion of cells are ovoid to spindle shaped with hyperchromatic nuclei, representing myoepithelial cells. Another characteristic features is the numerous bare nuclei in the background.

The histological features of ACC of the breast are the intercellular cystic spaces lined by basement membrane material and biphasic cellularity with myoepithelial cells intermixed with epithelial cells (2). In addition, the arrangement of tumour cells around cores or spheres of homogenous acellular material and the presence of cystic spaces with cyanophilic amorphous material with positivity of mucin is a very valuable feature in the diagnosis of this rare variant of breast carcinoma (4). In exfoliate specimens of the respiratory tract, the cytodiagnosis of ACC may be difficult due to the frequent intact mucosa overlying the tumour ,which prevents exfoliation of the tumour cells. The tendency of the exfoliated tumour cells lose their orientation in relationship to the cystic spaces and the often bland appearance of individual tumour cells may be difficult to discern among the normal or reactive respiratory tract epithelial cells (11, 12).

The differential diagnosis of pulmonary ACC includes reserve cell hyperplasia, where there are sheets of compactly arranged small cells with scant cytoplasm, round nuclei and a high nuclear/cytoplasmic ratio. The presence of attached columnar cells will favor a diagnosis of reserve cell hyperplasia and the presence of tubular and cribriform structures militates against a diagnosis of reserve cell hyperplasia. The tumour cells in carcinoid usually display uniform, small cells with rounded nuclei and a stippled chromatin with marked cell dissociation. Bare nuclei are uncommon. The nuclear features seen in carcinoid are different from those in ACC and the presence of mucoid globules within glandular structures favors a diagnosis of ACC. Small cell carcinoma is composed of small cells with nuclear molding, stippled chromatin, traumatic nuclear streaking and scant cytoplasm. In contradistinction, the neoplastic cells in ACC do not show nuclear molding and lack traumatic artifacts, they are composed of cells with dark nuclei. Acellular balls of basement membrane material within the cell clusters are not seen in small cell carcinoma. In well-differentiated adenocarcinoma, the glandular structures are usually seen cytologically without solid cores of pseudocyst material and the cells are usually larger and the cytoplasm more prominent than in ACC. Also, the nuclei are usually eccentric with a prominent nucleolus (11-14).

Before making a diagnosis of primary ACC or metastatic ACC, clinical and radiological correlation is essential. In our case a known history of primary ACC of the breast helped in making a definitive diagnosis. This report illustrates the biologic behavior of which this tumour is capable. Progression was extremely slow. Signs of metastases did not appear until nine years after removal of the primary tumour and the patient survived for six years after metastases in the lung.

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CASE STUDY

Panton-Valentin leukocidin cytotoxin-positive *Staphyloccus aureus*. A case study

Rebecca Busch
Canterbury Health Laboratories, Ashburton

ABSTRACT

Two Ashburton half-siblings presented to their GP multiple times over a two year period with recurrent skin infections and abscesses. The abscesses required incision/surgical drainage and despite prolonged antibiotics, still recurred. Multiple laboratory investigations were carried out to discover what was causing the abscesses and why the infections were so severe. It was proposed that the patients could be immunocompromised in some way. Swabs were often taken and *Staphylococcus aureus* was the only organism isolated. An incisional tissue biopsy of the eldest child's right forearm was carried out and bacteria were noted to be present right down to the fat layer, which was unusual. Isolates were referred to the Institute of Environmental Science and Research (ESR) for investigation and the *S. aureus* was found to be Panton-Valentin leukocidin (PVL) cytotoxin-positive. In New Zealand PVL is not routinely screened for but should be considered if a patient has recurrent skin abscesses. A national surveillance of *S.aureus* infections as well as local alerts of outbreaks may need to be investigated.

Key words: *Staphylococcus aureus*; Panton-Valentin leukocidin cytotoxin; skin abscess.

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INTRODUCTION

In 2012 child X (half Samoan/half European) presented to the GP at age 11 years with multiple large abscesses on her trunk. The abscesses were successfully treated with antibiotics (erythromycin) but recurred numerous times over the next year on different body sites (each time treated with erythromycin). Several of these abscesses required drainage and the patient was eventually put on prolonged antibiotic treatment (10 days of erythromycin). Swabs taken of the exudates always showed growth of *Staphylococcus aureus*, susceptible to flucoxacillin and erythromycin, but resistant to penicillin.

In 2013 Patient X's 18 month old half-brother (European) then developed a similar abscess on his wrist which required hospitalisation, surgical drainage and 3 days of IV flucoxacillin. As with his sister, biopsy of tissue and swabs showed *S.aureus*. For the next year both brother and sister had multiple events of abscess treated with erythromycin. In both children, when untreated, the abscesses spread rapidly and caused great pain. Due to the depth of the abscesses scarring occurred after antibiotic treatment.

The GP was unsure as to why these abscesses kept recurring and why the infections were so severe. Both children had compromised skin integrity due to having eczema thus it was considered that this may have been a contributing factor.

In early 2014 the older child's case was referred to a dermatologist who was equally puzzled by the recurring abscesses. The dermatologist considered the following:

- Hidradentitis suppurative, which is characterised by abscesses but commonly occurs in sweat gland- bearing skin
- An underlying immunodeficiency that increased the severity of these infections.
- An atypical mycobacterial infection or even a deep fungal infection.

It was noted the adults in the family showed no symptoms.

Multiple laboratory diagnostic tests were requested by the dermatologist including protein profiles, diabetic screens, thyroid function tests, hepatitis screens, HIV testing, serology/ immunology including TAA/ANA/complement levels/ANCA/ AMA/AP3Ab, B12/folate levels and lipid screens. All tests came back within normal limits for age and apart from the abscesses, the patient was systemically well.

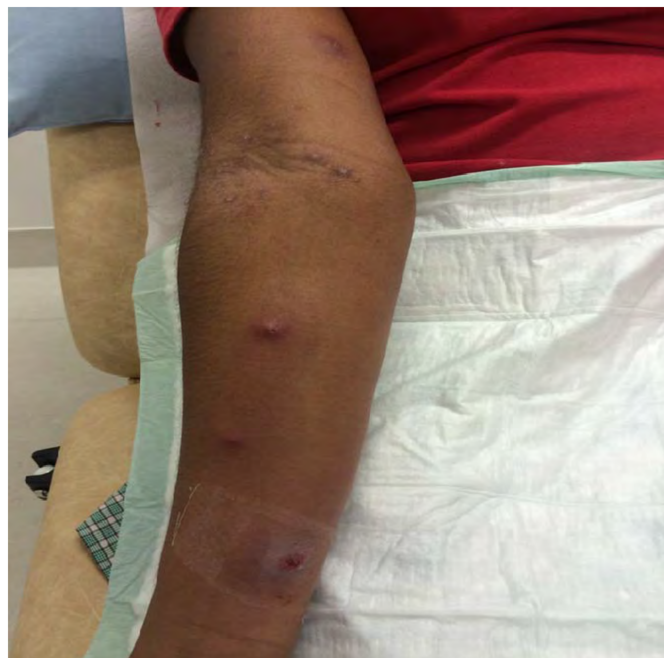

Figure 1. Abscess on right forearm of child X

In February 2014 an incisional biopsy of an abscess on the patients forearm was carried out by a tertiary hospital-based dermatologist and sent for histological investigation. Fungal, bacterial and atypical mycobacterial cultures were requested and swabs taken. The histology specimen showed marked

inflammation and necrosis was seen extending into the fat layer of the tissue. Many Gram-positive cocci were present, diagnostic of a bacterial skin infection. Stains for fungi and mycobacteria were negative.

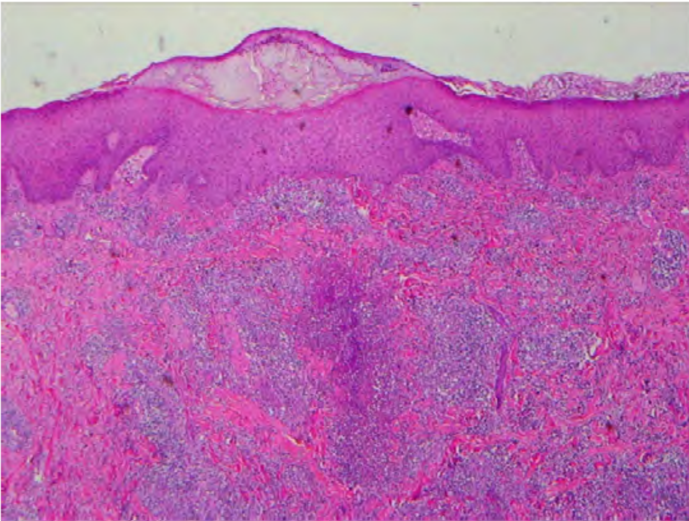

Figure 2. Histology slide of incisional biopsy of forearm showing *S.aureus* infiltrating skin. 40x magnification. Hematoxylin and eosin stain

These results raised further questions. The presence of Gram-positive cocci bacteria in the fat layer of the skin, as demonstrated in Figure 2, as well as the extensive necrosis required further investigation. The swab again grew *S.aureus* (indentified using the coagulase method and MALDI-TOF technology) but this time the dermatologist had requested the additional test for PVL toxin detection and an isolate was referred to the Institute of Environmental Science and Research (ESR) for investigation. The *S.aureus* was found to be Panton-Valentine leukocidin (PVL) cytotoxin gene positive by the PVL PCR assay. The younger sibling was subsequently swabbed and also tested positive for PVL cytotoxin positive *S.aureus.*

The family underwent a household decolonisation procedure over a week which involved washing sheets/towels/facecloths and clothes in hot water on day one then again at the end of seven days. Everybody in the family had to wash daily with chlorhexidine or diluted bleach and personal items such as razors or skin creams that may have been contaminated were disposed of. Mupirocin ointment was applied nasally twice a day by everybody in the family to eradicate any colonisation of *S.aureus* from their noses and the children were given a PVL cytotoxic positive *S.aureus* specific antibiotic (co-trimoxazole). The last swabs taken from the children in 2014 before the decontamination process and co-trimoxazole therapy showed both children had developed resistance to erythromycin. Both children were monitored by their GP for one year post-antibiotic therapy and have yet to have a recurrence of *S.aureus* infection, suggestingthat the PVL cytotoxin *S.aureus* had been eradicated.

DISCUSSION

PVL is a cytotoxin that can destroy white blood cells and cause extensive tissue necrosis and severe infections. PVL cytotoxin-positive *S.aureus* is usually associated with community acquired infections and generally affects previously healthy young children and adults. The majority of outbreaks have been associated with skin/tissue infections, but PVL can also cause septic arthritis, osteomyelitis, bacteraemia and often fatal necrotising pneumonia.

A 2010 Auckland study noted that "*Those patients with PVL-positive Methicillin susceptible S.aureus (MSSA) infection were more likely to be of Pacific ethnicity, be younger in age, have community- onset infection, have skin and soft tissue infection (SSIT) and need surgical intervention*" (4). This study also found that "*patients with PVL-positive MSSA infections were 3.9 times more likely to require surgery than those with PVL-negative MSSA SSTI.*"

A 2014 a New Zealand wide study also showed that 25% of *S.aureus* strains tested were PVL-positive and that 91% of the *S.aureus* tested were MSSA, regardless of PVL positivity (6). The presence of PVL cytotoxin has also been associated with strains of community acquired methicillin-resistant *S.aureus* (MRSA) and has been observed internationally with increasing frequency over that past two decades (3). Other Auckland studies have suggested that *S.aureus* isolates in New Zealand have a high prevalence of PVL cytotoxin-positive genes,37% in the 2009 study and 56% from children in the 2013 study (4,5).

Risk factors for developing infection with *S.aureus* PVL cytotoxin are overcrowding, skin to skin contact, such as close contact sports and compromised skin integrity, such as eczema (3). PVL typing is not routinely carried out in New Zealand on *S.aureus* isolates but perhaps should have been considered earlier in this case as it is known to cause skin abscesses.

The two general practitioners involved in the care of these children are now aware of PVL cytotoxin but this case provides evidence that it is not well understood as a cause of recurrent boils. Hospital consultants involved in the admission of the youngest child did not consider PVL cytotoxin as a cause of his large abscess that required surgical drainage. Had the diagnosis been made earlier the children would not have to have undergone so many rounds of antibiotics, incisions to drain abscesses, hospital stays, scarring and pain.

In New Zealand there is potential for improvement in local alerts, case ascertainment initiatives and monitoring PVL related disease. For example, in England the Public Health Office has developed microbiology algorithms used by GP's and hospitals. These state that if patients present with recurrent boils/abscesses or necrotising skin /soft tissue infections and the cultures come back for positive for *S.aureus*, then it is to be sent for PVL testing (3). Perhaps similar algorithms could be used in New Zealand.

CONCLUSIONS

These two cases illustrate know aspects of *S.aureus* PVL cytotoxin-positive infection, recurrent abscesses requiring surgical intervention and multiple antibiotic treatment. Earlier prompt recognition would have resulted in earlier decontamination process and resolution without the ongoing severe infections causing long term scarring and the emergence of antimicrobial resistance.

In New Zealand PVL is not routinely screened for but it should be considered if a patient has recurrent skin abscesses. A national surveillance of *S.aureus* infections as well as local alerts of outbreaks may need to be investigated.

ACKNOWLEDGMENTS

Grateful thanks to Jackie Wright, Canterbury Health Laboratories, Ashburton for her helpful review and suggestions regarding this case study.

AUTHOR INFORMATION

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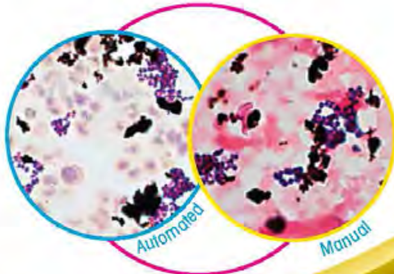

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OBITUARY

Harry Edwin Hutchings, FNZIMLS

One of our longest standing members passed away in Wellington on 16th July, 2015. Harry was born in Canada on the 7th of August, 1925. He was educated in Hawkes Bay and began his working life as a cadet in the Inland Revenue Department. Fortunately for our profession he changed direction after a year. He commenced his laboratory training at Waipukurau Hospital under the tutelage of Gordon McKinley in 1945. He came to Palmerston North in 1948 where he gained his Certificate of Proficiency in Medical Laboratory Technology in 1951. Harry was an outstanding technologist who not only introduced new techniques to the laboratory but also gave many hours of his own time in helping the profession and its members to develop.

After qualifying he went to the Middlesex Hospital, UK for 18 months in haematology and blood transfusion research. This OE was strongly encouraged by Dr Pullar, with whom Harry developed a great working relationship. He took charge of this work on his return to Palmerston North where he introduced Coombs testing, a new technique in New Zealand at that time.

When Os Jarrett left the Palmerston North Hospital laboratory to start Clinical Laboratories in 1955, Harry was appointed to the Principal Technologist position though retaining oversight of haematology and blood transfusion. The total laboratory staff at that time had grown to 21, with four qualified medical laboratory technologists.

In 1968 he was in Glasgow, UK for six months on a Churchill Scholarship learning cytogenetic techniques, which he introduced on his return. This was appropriate as Harry was a very keen and competent photographer who was able to use his hobby as an integral part of his work. Other hobbies included woodwork, playing squash, announcing on local radio and taking part in amateur theatre as actor and director. He left Palmerston North to become Head of Health Sciences at CIT in 1972 although he always maintained that our profession should be university qualified. It took a long time for this to be recognised and achieved.

Professionally he was on the NZIMLT Council for 14 years including as President from 1969 to 1971. He gave the TH Pullar Memorial Address in 1974 being the first non-medical person to do so (1). He was made a Life Member of the Institute in 1977. Harry also served on the Medical Laboratory Technologists Board from 1970 to 1984 being Chairman from 1973 to 1976. He also took an active role in salary negotiations for medical laboratory workers.

After a number of years at CIT he was appointed to the Education Department where he helped introduce the forerunner of NCEA. He and his wife Beryl retired to Tauranga in the mid 80's where they pursued many and varied interests with family and friends. He continued with his painting, photography and sculpture with the same enthusiasm and zeal which he had applied to his professional life. About four years ago he moved back to Wellington where most of his family were based.

He was certainly a major influence on our profession during the 60's and 70's. A celebration of his life took place on the 22nd of July, 2015 where his many achievements were recognised.

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Contributed by Colvin Campbell

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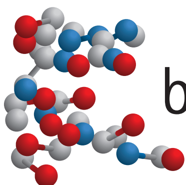

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MINUTES OF THE NZIMLS ANNUAL GENERAL MEETING HELD AT THE LANGHAM, AUCKLAND ON THURSDAY 20 AUGUST 2015 AT 7.30AM

PRESENT
The President presided over approximately 35 members.

APOLOGIES
Apologies were received from Sue Melvin and Sandy Woods

PROXIES
Ailsa Bunker - 2
Nadia Al-Anbuky - 2
Tony Barnett - 5

MINUTES OF THE AGM HELD 13 AUGUST 2014
Motion:
Moved T Barnett, seconded R Siebers
That the minutes of the AGM held on 13 August 2014 be received.
Carried

Motion:
Moved T Barnett, seconded R Siebers
That the minutes of the AGM held on 13 August 2014 be accepted.

BUSINESS ARISING FROM THE MINUTES
Nil

REMITS AS CIRCULATED

Motion:
Moved T Barnet, seconded T Taylor
That Policy Decision Number 4 be reaffirmed.
Policy Decision No 4 (1991): That the Code of Ethics as circulated to all members be adopted by the New Zealand Institute of Medical Laboratory Science (Inc.)
Carried

Motion:
Moved T Barnett, seconded K Beechey
That Policy Decision Number 6 be reaffirmed.
Policy Decision No 6 (1979): That the Council must be informed in advance of national workshops, seminars or similar gatherings which are being conducted under the aegis of the NZIMLS.
Carried

Motion:
Moved T Barnett, seconded A Bunker
That an amendment be made to the NZIMLS rule 13(b) that Council members be elected to a two yearly basis be accepted.
Rule 13(b) to read:
"All members shall retire from office after two years and shall be eligible for re-election." Council members do have the option to stand down or not seek re-election after a one year term.
Carried

Motion:
Moved T Barnett, seconded R Hewett
That further amendments be made to the rules as circulated to the membership.
Explanation: During 2014 Council reviewed the NZIMLS rules. The changes made are more points of clarification, to include the CPD programme and bringing the Finance and Membership Administrator's responsibilities into the Rules.
Carried

The following new accounting legislation was noted for member's information.

Part of the current legislation states that in the Notes to Accounts, the NZIMLS must state how the accounts have been prepared. Currently the NZIMLS states that the accounts have been prepared "in accordance with generally accepted accounting practices (GAAP) in New Zealand". This will now change to "in accordance with A Special Purpose Framework for use by Not-For-Profit Entities published by the New Zealand Institute of Chartered Accountants. The financial statements have been prepared for use by the NZIMLS members."

PRESIDENTS REPORT
Motion:
Moved R Hewett, seconded T Taylor
That the President's Report be received.
Carried

ANNUAL REPORT
Motion:
Moved T Barnett, seconded MA Janssen
That the Annual Report be received.
Carried

FINANCIAL REPORT
Motion:
Moved T Barnett, seconded R Siebers
That the Financial Report be received.
Carried

ELECTION OF OFFICERS
The following members of Council were elected unopposed:

President	Ross Hewett
Vice President	Terry Taylor
Treasurer / Secretary	Tony Barnett
Region 2 Representative	Mary-Anne Janssen
Region 4 Representative	Michael Awadalla
Region 5 Representative	Sue Melvin

It was noted that the position of Region 3 Representative is vacant.

Motion:
Moved T Barnet, seconded
That the election of officers elected unopposed be approved.
Carried

The results of the election for Region 1 election is as follows:
Ailsa Bunker – 71
Nadia Al-Anbuky - 93

Motion:
Moved T Barnett, seconded A Bunker
That the election of N Al-Anbuky to the position of Region 1 Representative be accepted.
Carried

T Barnett thanked A Bunker of her time on Council.

HONORARIA
Motion:
Moved R Siebers, seconded R Hewett
That no honoraria be paid.
Carried

AUDITOR
Motion:
Moved T Barnett, seconded M Awadalla
That Hilson Fagerlaund Keyse be reaffirmed as the NZIMLS Auditors.
Carried

GENERAL BUSINESS

R Siebers, on behalf of the PPTC, thanked the NZIMLS for the donation to the QA programme for the South Pacific. P Wakem has also sent a letter of thanks to Council for this contribution.

R Hewett noted the passing of Harry Hutchings and offered condolences to his family.

H Perry thanked the NZIMLS and LabPlus for the sponsorship for AUT students to a half day at the South Pacific Congress.

A Bunker thanked the members for their support for her year on Council as the Region 1 Representative. She will continue to support the NZIMLS and offered her help to N Al-Anbuky as the new Region 1 Representative.

2016 Annual Scientific Meeting
To be held in at the Energy Events Centre, Rotorua, 16-19 August 2016.

2017 Annual Scientific Meeting
No offers were received to organise the 2017 conference.

The meeting closed at 7.58am

Journal Questionnaire

Below are 10 questions based on articles from the November 2015 issue. Read the articles carefully as most questions require more than one answer.

Answers are to be submitted through the NZIMLS web site. Make sure you supply your correct email address and membership number. It is recommended that you write your answers in a word document and then cut and paste your answers on the web site.

The site has been developed for use with Microsoft's Internet Explorer web browser. If you are having problems submitting your questionnaire and you are using the Firefox web browser, try resubmitting from a computer or system using Microsoft's Internet Explorer.

You are reminded that to claim valid CPD points for successfully completing the journal questionnaire you must submit an individual entry. It must not be part of a consultative or group process. **In addition, members who have successfully completed the journal questionnaire cannot then claim additional CPD points for reading the articles from which the questions were derived.**

The site will remain open until Friday 18th March, 2016. You must get a minimum of eight questions right to obtain five CPD points.

The Editors set the questions but the CPD Coordinator, Jillian Broadbent, marks the answers. Please direct any queries to her at cpd@nzimls.org.nz.

NOVEMBER 2015 JOURNAL QUESTIONS

1. Which carbapenem antimicrobials are restricted for the treatment of life threatening infections caused by multi-drug resistant Gram-negative isolates?
2. Resistance to carbapenems can also be due to which intrinsic chromosomal mechanisms?
3. Which test has been suggested as a screen to aid detection of OXA-48 producers?
4. What are the test principles of the Carba NP and Blue Carba biochemical tests for carbapenemase detection?
5. What were the limitations of the Creighton and Jayawardena article?
6. What are the biological actions of Panton-Valentin leucocidin cytotoxin?
7. What are the risk factors for developing *S. aureus* Panton-Valentin leucocidin cytotoxin?
8. Possibly why is adenoid carcinoma of the breast much less malignant than histologically identified neoplasms of other sites?
9. What are the histological features of adenoid carcinoma of the breast?
10. The differential diagnosis of adenoid carcinoma of the breast includes which feature?

August 2015 Journal Questionnaire Answers

1. How do *Yersinia* spp. appear on *Yersinia* isolation agar? **Characteristic dark-red colonies with a transparent border giving a 'Bulls-eye' appearance.**
2. Name a specific limitation of the *Yersinia* isolation agar. **That many non-*Yersinia* enteric organisms are able to grow and form colonies which are difficult to differentiate from *Yersinia* spp., specifically *Enterobacter* spp., *Serratia* spp. and *Citrobacter* spp.**
3. What are the most common causative agents of Yersiniosis and how is it transmitted?
   ***Yersinia enterocolitica* and *Y. pseudotuberculosis*. Transmitted by the faecal oral route.**
4. What were the limitations of the *Yersinia* rapid urea broth study?
   The subjective interpretation of cultures or that that *Yersinia* spp. may be occasionally encountered that fail to produce a urease enzyme or that the urea broth result is misinterpreted.
5. What are the main features of *Yersinia* spp. bacteria? **Gram-negative, non-spore forming bacilli, smaller than most others in the *Enterobacteriaceae* family and relatively slower growing.**
6. Which factors affect the prevalence of lower respiratory tract infections?
   Age, gender, season, indoor air quality, crowding.
7. Name three other risk factors for lower respiratory tract infections.
   Malnutrition. Exposure to environmental pollutants such as smoke from domestic cooking with firewood. Poor parental income and education. Man-made or natural disasters with consequent living in squatter/ refugee conditions.
8. Children with lower respiratory tract infections may present with which life-threatening complications.

   Massive para-pneumonic or pleural effusion, sepsis empyema, pericarditis with cardiac tamponade, venous thromboembolism.
9. The higher prevalence of lower respiratory tract infections in males in Nigeria has been attributed to which factors. **Decreased local immunity in the respiratory tract due to smoking, use of tobacco and alcohol consumption.**
10. What is the proposed reason why children who were in-patients had a significantly higher prevalence of lower respiratory tract infections than their out-patient counter-part.
    In-patient children may be in the intensive care unit and be immuno-compromised, have other immunosuppressive conditions such as cancer, or have debilitating conditions that may increase their susceptibility to LRTIs.

THE Pacific WAY

Wellington based training courses 2015

Microbiology Course 2015

A Microbiology update course was provided by the PPTC in September of this year at its centre in Wellington, and the following students attended:
Surila Sharan and Seini Rawasoi from Vet Pathology Fiji, Luisa Vailanu from Tonga, Itibwerere Amota from the Marshall Islands, Johnson Makaen from Papua New Guinea and Carmin Pipit from Palau.

The Microbiology course provided students with an update on developments in microbiological procedures. The theoretical and practical aspects of current methods used in the isolation, identification and antimicrobial susceptibility testing of microorganisms were covered along with discussions on emerging and re-emerging bacterial organisms likely to cause infectious diseases.

Serological and other rapid methods for the identification of bacterial and viral diseases including Hepatitis A, B, and C, HIV and other STIs, were discussed as well as the role of the Microbiology laboratory in the surveillance of nosocomial infections and identification of infections of public health importance.

The PPTC Board of Management would like to thank Russell Cole the PPTC's Laboratory Quality Manager and Microbiology specialist for the excellent contribution made in developing and teaching this course.

The PPTC sincerely thanks Nicky Beamish (Aotea Pathology), Rachel Roth (Wellington Hospital), and Grant Mackie (Hutt Hospital) for taking the students on tour of their respective Microbiology Laboratories.

It was the greatest pleasure to have, Koen Van Der Werff (Wellington hospital) present to the students their certificates at the final farewell ceremony.

Centre based courses for the remainder of 2015:

Blood Transfusion: [2nd November – 27th November]
The course will include units of study covering the theoretical and practical aspects of the following topics; routine blood grouping, blood group antigens, crossmatch techniques, antibody detection, transfusion reactions, haemolytic disease of the newborn, screening blood for infectious agents, blood donor selection, organisation of a blood bank and the appropriate use of blood components in transfusion medicine. Practical sessions will also be provided, focusing on correct technique and fundamental basic procedure.

One week of the course will be set aside for an overview of current techniques in the detection of transfusion transmissible infections including, HIV, Syphilis, Hepatitis B and C.

We are sincerely grateful to Susan Evans and the NZ Blood Service staff, Wellington Hospital, for the excellent tuition and practical training that is provided throughout the duration of this course.

Overseas travel

Tonga

Haematology as adiagnostic science continues to be weak in performance throughout all Pacific laboratoriesand this is due to a devastating lack of expertise in "Blood film examination and interpretation". Apart from the general challenges that continuously face both the PPTC and Pacific Laboratories over all laboratory disciplines, Haematology continues to progress at an extremely slow rate if at all and this is specifically due to a lack of resident quality champions or drivers of Haematology excellence, and more specifically inadequate training opportunities to improve performance in blood cell recognition, and interpretative skill. The PPTC through its teaching and training programmes provides much needed education in diagnostic Haematology and this is delivered in one of three ways (ie) through a 4 week teaching course offered each year at its centre in Wellington, through its well established distance learning programme (Diploma of Medical Laboratory Science), and finally through in-country training. Blood cell identification and interpretation is a continual learning process which requires many years to acquire a level of excellence in performance, and unfortunately Pacific Island laboratories do not have permanent resident experts who are able to mentor and add to this learning experience once their students return to their working environment from the courses theyhave attended.

Phil Wakem, the PPTC's CEO and Haematology Technical Specialist, travels extensively throughout the Pacific region providing Haematology workshops to Pacific Laboratories currently on its programme. Phil accompanied by a senior Wellington Hospital Consultant Haematologist, Dr Julia Phillips visited Tonga from the 24th August – 28th August, to provide a series of Haematology lectures and practical workshops to the laboratory staff of Vaiola Hospital. This laboratory currently has 10 newly employed trainee technicians in addition to its senior staff and in order to gain the most benefit from the workshops without disruption to the daily work processes, a roster was set in place to divide the teaching and training equally between the two groups of personnel. The workshops involved continual identification of both normal and abnormal blood cell populations and associated disease states including, Anaemias, Leukaemias Myeloproliferative disorders, and Myelodysplastic disorders, to name but a few. Much discussion focused on correct interpretation and reporting of results that would offer maximum benefit to hospital clinicians in terms of diagnosis and management of their patients. Phil also identified technical process issues within the laboratory that required resolution and gave an insight to participating students on the subject of good practise that would enhance quality performance within the laboratory's diagnostic processes. Julia as a Clinical Haematologist was most valuable in providing to students a clinical perspective with reference to Haematological disease and the importance of significant findings characteristically identified in selected disease states. Julia also participated in both a paediatric and adult ward round with hospital clinicians and offered advise where appropriate to help establish diagnosis and effective treatment management of their patients. Her presence was most valued by both technical and medical staff throughout the duration of the week, and her efforts to establish a strengthened linkage between laboratory and hospital clinicians proved to be most successful. This linkage was reinforced through a lunch time presentation that was provided by her to hospital clinicians which primarily focused on the diagnostic support that the laboratory is expected to provide, the clinically correct utilisation of laboratory results in the establishment of a diagnosis or monitoring of a disease process and finally the maintenance of a reciprocal relationship that must exist between both hospital clinicians and the laboratory in order for communication lines to remain open and productive.

Students observing blood cell populations using the multi headed microscope

Dr Julia Phillips selecting blood films for the training workshop

As the CEO of the PPTC, Phil was asked by the laboratory management to formally present the PPTC's Diploma of Medical Laboratory Science to students who had successfully completed the two year study programme. This was carried out at a cultural evening provided by the laboratory as a thankyou to both Phil and Julia, and it was extremely rewarding to recognise the effort and dedication the graduating students had contributed towards their professional development over the previous two years.

Samoa

Filipo Faiga (Section Head, Biochemistry section, Wellington Hospital) travelled to Samoa on the 27th July to the 7th August to provide technical assistance both in training and teaching to the Biochemistry section of Samoa Hospital laboratories at Upolo and Savaii as well as assist in laboratory general practise in accordance with LQMS requirements. Filipo is both a PPTC Board member and Biochemistry Consultant and the PPTC is most appreciative of the valuable contribution he continues to make in the strengthening and capacity building of our Pacific laboratories.

Phil Wakem and Julia Phillips continued their journey to Samoa on the 21st Sept to provide the same training in Haematology as was given to the participating laboratory staff of Vaiola hospital. Phil and Julia were absolutely delighted that Lavea'i Ioane the PPTC's MFAT development officer was able to accompany them on this visit, to gain a clear insight into the valued work and contribution made by the PPTC towards the quest for excellence in Laboratory Quality performance.

Vanuatu

Russell Cole and Navin Karan visited Vanuatu between the 6th and 10th July to assess progress of Laboratory Quality Management implementation in the laboratories of both Port Vila and Espiritu Santo. Visits to Vanuatu have been placed on hold in past months due to the devastation of Cyclone Pam and the disruption to services within Vanuatu. The country is beginning to recover slowly and the PPTC is now continuing its work in promoting quality throughout each of the laboratories diagnostic processes.

Port Vila's brand new Laboratory, Vanuatu

Cambodia and the PPTC's regional External Quality Assessment Programme

The PPTC now that it has 30 Cambodian laboratories on its REQA programme considers that regular physical presence in Cambodia is essential to strengthen the interaction between the PPTC and its participating laboratories, address any REQA issues that require resolution, provide on going education in both Biochemistry and Haematology and overall actively support each of the laboratories in the REQA programme. On the 27th July to the 7th August 2015 Phil Wakem and Navin Karan (PPTC Programme Manager) travelled to Phnom Penh to deliver both Haematology and Biochemistry Cambodia workshops to representatives of 16 Cambodian laboratories at the National Institute of Public Health. This was very successful and a further consolidation visit is scheduled for February 2016.

Haematology workshop training in Phnom Penh

WHO Representatives , PPTC Lecturers and Cambodian Students attending Haematology / Biochemistry workshops at the National Institute of Public Health, Phnom Penh

Fiji

On the 20 – 22nd July, Russell Cole on behalf of the PPTC, attended the 2015 LABNET meeting which was held in Nadi Fiji. The aim of this meeting was to provide an update on the implementation of LQMS (Laboratory Quality Management Systems in terms of training, and implementation of national policies , national strategic plans and quality standards) and SHIP (Strengthening Health Interventions in the Pacific) training Programmes.

LABNET Meeting 2015, Nadi, Fiji

Solomons

On the 7th – 11th September, Navin Karan travelled to Honiara and Gizo to continue Laboratory Quality Management implementation in both hospital laboratories. The staff were very appreciative of the support given by the PPTC and look forward to a very productive future .

Gizo Laboratory, Solomon Islands

To Contact the PPTC:

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Contact: Rebecca Busch

Email: Rebecca.busch@cdhb.health.nz

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2016 NZIMLS CALENDAR

Dates may be subject to change

24 DECEMBER	*OFFICE CLOSED FOR CHRISTMAS / NEW YEAR BREAK*	
11 JANUARY	*OFFICE RE-OPENS FOR 2016*	
DATE	**SEMINARS**	**CONTACT**
12 March	South Island Seminar, Ashburton Hotel, Ashburton	rebecca.busch@cdhb.health.nz
May	North Island Seminar	
13-15 May	NICE Weekend, Wairakei Resort	raewyn.cameron@lsr.net.nz natalie.fletcher@sclabs.co.nz
June	Biochemistry SIG Seminar	
June	Microbiology SIG Seminar	
June	Molecular Diagnostics SIG Seminar	
October	Histology SIG Seminar	
October	Haematology SIG Seminar	
October	PreAnalytical SIG Seminar, Auckland	
November	Immunology SIG Seminar	
November	Mortuary SIG Seminar	
DATE	**NZIMLS EXAMINATIONS**	**CONTACT**
20 May	Applications close for QMLT/QSST Examinations	fran@nzimls.org.nz
07 November	QMLT and QSST Examinations	fran@nzimls.org.nz
DATE	**COUNCIL**	**CONTACT**
March	Council Meeting	fran@nzimls.org.nz
May	Council Meeting	fran@nzimls.org.nz
14 &15 August	Council Meeting, Rotorua	fran@nzimls.org.nz
18 August	Annual General Meeting, Rotorua	fran@nzimls.org.nz
November	Council Meeting	fran@nzimls.org.nz
DATE	**EVENTS**	**CONTACT**
16-19 August	Annual Scientific Meeting, Rotorua	raewyn.cameron@lsr.net.nz joanne.hartigan@lsr.net.nz fran@nzimls.org.nz
DATE	**MEMBERSHIP INFORMATION**	**CONTACT**
January	Membership and CPD enrolment due for renewal by 28 February	sharon@nzimls.org.nz
January	CPD points for 2015 to be entered before 31 January	cpd@nzimls.org.nz
15 February	Material for the April issue of the Journal must be with the Editor	rob.siebers@otago.ac.nz
17 June	Nomination forms for election of Officers and Remits to be with the Membership	fran@nzimls.org.nz
15 June	Material for the August Journal must be with the Editor	rob.siebers@otago.ac.nz
7 July	Nominations close for election of officers	fran@nzimls.org.nz
27 July	Ballot papers to be with the membership	fran@nzimls.org.nz
3 August	Annual Reports and Balance Sheet to be with members	sharon@nzimls.org.nz
10 August	Ballot papers and proxies to be with the Executive Officer	fran@nzimls.org.nz
15 September	Material for the November Journal must be with the Editor	rob.siebers@otago.ac.nz

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