NZIMLS
THE NEW ZEALAND INSTITUTE OF MEDICAL LABORATORY SCIENCE (INC)

ISSN 1171-0195 Volume 72 Number 1 April 2018

Established in 1946

New Zealand Journal of Medical Laboratory Science

Official Publication of the New Zealand Institute of Medical Laboratory Science Incorporated

1

STA Compact Max³

Max Efficiency

Routine and specialised Haemostasis tests
All common tubes size accepted

Max Performance

Higher loading capacity
Ready to operate 24/7

Max Innovation

Automated validation of results using expert rules, with integrated STA Coag Expert

Max Reliability

"Gold Standard" viscosity based detection system

Innovation born from Expertise

EPC module: Expert Preanalytical Check module
(Sample integrity check: Haemolysis, Icterus, Lipaemia)

Stago
At the Heart of Haemostasis

Diagnostica Stago S.A.S.
RCS Nanterre B305 151 409
3, allée Thérésa
92600 Asnières sur Seine
France
Ph.: +33 (0)1 46 88 20 20
Fax: +33 (0)1 47 91 08 91
webmaster@stago.com
www.stago.com

About the Journal
The *New Zealand Journal of Medical Laboratory Science* (the Journal) is the official publication of the New Zealand Institute of Medical Laboratory Science (NZIMLS). The Journal is peer reviewed and publishes original and review articles, case studies, technical communications, and letters to the Editor on all subjects pertaining to the practice of medical laboratory science. The Journal is open access (www.nzimls.org.nz/nzimls-journal.html) and is published three times per year in April, August, and November. Hard copies are circulated to all NZIMLS members and universities and research units in New Zealand and overseas. Current circulation is about 2,200 copies per issue. Printing is by Griffin Press, Christchurch on environmentally responsible paper using elemental chlorine free third party certified pulp sourced from well managed and legally harvested forests and manufactured under the strict ISO14001 Environmental Management System. The Journal is indexed by CINAHL, EMBASE, SCOPUS, Informit, Thomson Gale, EBSCO and Biosis Citation Index, and the Journal Editors are members of the World Association of Medical Editors (www.wame.org).

Brief instructions to authors
The Journal accepts original submissions from anyone and anywhere. Comprehensive instructions can be found on the NZIMLS website (www.nzimls.org.nz/instructions-to-authors.html). All submissions will undergo single-blind peer review and possibly plagiarism checking with iThenticate™ software. If accepted for publication, copyright is vested in the author(s) under terms of the Creative Commons Attribution License (www.creativecommons.org/licenses/by/2.5/legalcode). The authors are responsible for the scientific content and views. Opinions expressed in the Journal are not necessarily those of the Editors, Editorial Board, or Council of the NZIMLS.

Advertising and subscription
Advertisement bookings and enquiries should be addressed to the NZIMLS Executive Officer at fran@nzimls.org.nz. Enquiries regarding subscription and address changes should be addressed to sharon@nzimls.org.nz. Address for the NZIMLS is PO Box 505, Rangiora 7440, New Zealand. Phone +64 3 313 4761.

New Zealand Journal of

Medical Laboratory Science

Volume 72 Number 1
November 2018
ISSN 1171-0195

Review article

Original articles

Case studies

Obituary

Regular features

In this issue

Rob Siebers, Editor

Shiga-toxigenic Escherichia coli (STEC), also known as Verotoxigenic E. coli (VTEC), are a toxin-producing subset of a normally commensal enteric bacterium, recognised to be a significant contributor to diarrhoeal disease worldwide. In this issue in their review article, Rowan Thomas and colleagues from Otago University trace the history of STEC, describe the relationship between major virulence factors and pathology, comment on disease course and current therapy, and discuss the epidemiology of these pathogens in New Zealand and elsewhere, highlighting distinctive differences.

The steady rise in the prevalence of Gram-negative bacteria producing extended spectrum beta-lactamase (ESBL), AmpC beta-lactamase and carbapenemases means newer antibiotics like extended spectrum cephalosporins, cephamycins, monobactams and carbapenems may not achieve their therapeutic purpose. In this issue Ephraim Ibudin and colleagues determined the prevalence rate of ESBL, AmpC β-lactamase and MBL enzymes among bacterial isolates recovered from the urine samples of patients with signs and symptoms of urinary tract infection (UTI) in Nigeria. They found an overall prevalence of ESBL, AmpC β-lactamase and MBL among Gram-negative bacteria causing UTI of 51.6%, 15.1% and 35.7% respectively. The authors recommend prudence in the use of antibiotics in hospital and community settings.

The emphasis on using haemoglobin (Hb) as the only indicator for anaemia is reported in The World Health Organisation's anaemia reports. The three-fold conversion of the haematocrit (Hct) to yield the "derived Hb" has been suggested in settings where measured Hb results cannot be rapidly provided. Nosaiba Al-Ryalat and colleagues from Jordan studied the usefulness of the derived Hb in detecting anaemia compared to the measured Hb in 1695 patients and sought factors that could affect the measured and the derived Hb values. Using the derived Hb,the authors were able to detect anemic patients with 85.94% sensitivity, 94.50% specificity, and a positive predictive value of 88.8%. Accuracy of the derived Hb was higher at lower Hct levels, and was not affected by patient's age, gender, hydration status or kidney function. The three-fold conversion was also reliable for assessment of anaemia in elderly patients and in patients with impaired kidney function.

Mammary analogue secretory carcinoma (MASC) is a recently classified entity with unique genetic characteristics of a ETV6-NTRK3 fusion gene, identical to that found in secretory breast carcinoma. MASC, also shares immunohistochemical and histologic features with secretory breast carcinoma. In a case study, Sharda Lallu and colleagues from Wellington Hospital present the typical cytologic features of a relatively recently recognised salivary gland tumour, which displayed cytological, histologic, immunophenoptic and genetic features similar to secretory breast carcinoma. MASC and secretory breast carcinoma share a t (12;15) (p13; q25) translocation and both are positive for S100 protein and GATA3 and but with a "triple negative" (ER/PR/Her2) phenotype.

Natasha Dutt and Samarina Musaad from Labtests, Auckland present a case of apparent acute kidney injury that was due to interference with the Jaffe creatinine method. In spite of improvements in the Jaffe assay it is prone to interferences that may lead to erroneous results. Inaccurate creatinine results lead to incorrect estimation of the glomerular filtration rate (eGFR) which is the hallmark of assessing kidney function and detecting acute kidney injurie. Therefore, scientists need to be aware of the circumstances that can jeopardise analytical analysis, particularly when it comes to specific filtration markers, such as creatinine.

Michael Legge, one of the Editorial Board Members, has agreed to take on the role of Deputy Editor. Mike is learning the roles of editorship to cover for when required, and assist, the Editor. I welcome Mike in this role given his extensive authorship and reviewing knowledge and look forward to working productively with him (and the rest of the Editorial Board and Sharon Tozer at the NZIMLS Office).

Advertisers in this issue

Advertising rates are available at:
www.nzimls.org.nz/advertising-rates-pre-press-specifications

REVIEW ARTICLE

Shiga (Vero) -toxigenic Escherichia coli: epidemiology, virulence and disease

Rowan R Thomas, Mabel LH Gaastra and Heather JL Brooks

Department of Pathology, University of Otago, Dunedin

ABSTRACT

Shiga (Vero) –toxigenic *Escherichia coli* (STEC) most likely evolved as diarrhoea causing pathogens before the 1950s through the transfer of virulence genes. However, their significance to human health was not recognised until the 1980s. Bacteriophages (viruses which infect bacteria) have played an important role in gene transfer. The origin of STEC and evolution of sorbitol fermenting versus sorbitol non-fermenting O157 strains is briefly discussed. Production of cell adhesion factors and the damaging effects of the Shiga toxins on vascular endothelium and intestinal cells are the main STEC virulence characteristics. Many other putative virulence factors and accessory genes have been identified. The modes of action and routes of entry of Shiga toxins are summarised. STEC clinical spectra range from diarrhoea to haemorrhagic and neurological complications, with haemolytic uraemic syndrome an important sequel in some patients. Treatment options are limited and mainly supportive. Clinical presentations of STEC infections, including the enterohaemorrhagic subset, are explored. The incidence of STEC infection in New Zealand appears to be increasing. In contrast to many other countries where food is the main source, STEC infection occurs mainly through contact with animals or contaminated agricultural environments in New Zealand. The diversity of STEC serogroups and their occurrence in New Zealand and elsewhere is discussed. Both O157 and non-O157 STEC are significant pathogen groups in New Zealand. The high mobility of virulence genes in diarrhoeagenic *E. coli* indicates new STEC pathotypes will likely emerge in the future.

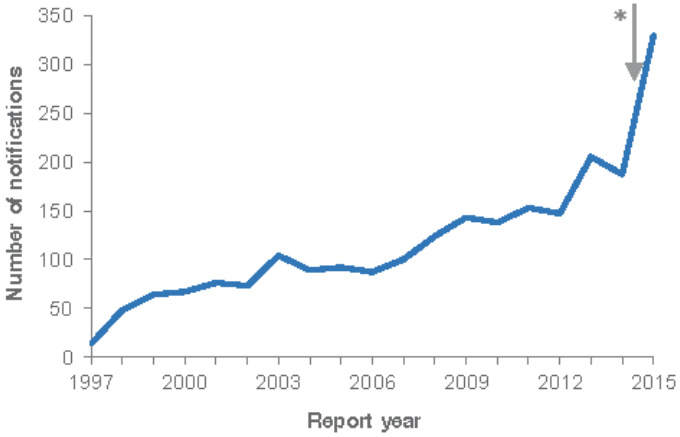
Keywords: *Escherichia coli,* Shiga toxin, infection, New Zealand.

N Z J Med Lab Sci 2018; 72: 03-10

INTRODUCTION

Shiga-toxigenic *Escherichia coli* (STEC), also known as Vero-toxigenic *E. coli* (VTEC), are a toxin-producing subset of a normally commensal enteric bacterium. This subset is recognised to be a significant contributor to diarrhoeal disease worldwide. Much of the epidemiological and diagnostic effort regarding STEC is directed towards serogroup O157, because of its common association with severe forms of gastrointestinal disease, including haemorrhagic colitis (HC), and extra-intestinal disease, such as haemolytic uraemic syndrome (1,2).

In recent years, international researchers and surveillance networks have noted increases in the number of cases of diarrhoea and more severe symptoms caused by STEC belonging to serogroups other than O157 (collectively referred to as 'non-O157') (3). Non-O157 STEC contribute to a significant proportion of STEC-related illness in developed countries (4), with some strains considered to have a pathogenic potential comparable to that of the most virulent O157 serotype, O157:H7 (4,5). Much of the disease burden presented by non-O157 *E. coli* remains under-reported in many countries (2,3), mainly due to limitations in popular diagnostic techniques to detect these serotypes (4,5). However, this is changing with the introduction of molecular assays targeting Stx genes (4). *E. coli* O157:H7 related disease is notifiable in New Zealand, and national data accumulated since the first case in 1993 indicates that the incidence of all reported STEC has been steadily increasing, as shown in Figure 1 (6).



*Introduction of screening of all faecal specimens using PCR in an Auckland laboratory.

Figure 1. Number of all STEC infections reported to ESR by community and hospital based diagnostic laboratories 1997-2015. The number of notifications of STEC infections has been increasing since 1997 (6).

The purpose of this review is to trace the history of STEC, describe the relationship between major virulence factors and pathology, comment on disease course and current therapy, and discuss the epidemiology of these pathogens in New Zealand and elsewhere, highlighting distinctive differences.

Table 1. Diarrhoeagenic *Escherichi coli* pathotypes

***E. coli* pathotype**	**Acronym**	**Disease**	***eaeA*, *stx* genes***
Shiga-toxigenic/ Enterohaemorrhagic	STEC/EHEC	Watery diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome	*eaeA*$^{+/-}$, *stx*$^{+}$
Enteropathogenic (typical/atypical) †	EPEC	Watery diarrhoea	*eaeA*$^{+}$, *stx*$^{-}$
Enteroinvasive‡	EIEC	Dysentery	*eaeA*$^{-}$, *stx*$^{-}$
Enteroaggregative	EAEC *syn.* EAggEC	Traveller's diarrhoea, (haemolytic uraemic syndrome)	*eaeA*$^{-}$, *stx*$^{-}$ (serotype O104:H4 *stx*$^{+}$)
Enterotoxigenic	ETEC	Persistent watery diarrhoea	*eaeA*$^{-}$, *stx*$^{-}$
Diffusely adherent	DAEC	Persistent watery diarrhoea in children (Crohn's in adults?)	*eaeA*$^{-}$, *stx*$^{-}$

*Important genetic markers of virulence for STEC and EPEC
†Typical EPEC possess *E. coli* adherence factor plasmid pEAF, atypical EPEC do not
‡Closely related to *Shigella* spp.; *S. dysenteriae stx*$^{+}$

HISTORY

Many major *E. coli* pathotypes have emerged over time through the transfer of virulence factors from other bacterial species by way of mobile genetic elements such as pathogenicity islands, transposons, and plasmids (7). The different pathotypes associated with human diarrhoea have been extensively reviewed by Croxen et al (8) and are summarised in Table 1. There is uneven geographical distribution of some of these pathotypes; for example, enterotoxigenic *E. coli* (ETEC) are particularly problematic in developing countries (8). On the other hand, STEC have been reported to occur world-wide (9).

The role of STEC in human health emerged in 1983, when Karmali et al (10), discovered a substance acutely lethal to African green monkey kidney (Vero) cells in the filtered stools of children suffering from haemolytic uraemic syndrome. Vero cytotoxic *E. coli* recovered from patient stool samples belonged to serogroups O26, O111, O113 and O157 (10). This study was published in the same year as another investigation by Riley et al, where an unusual serotype of *E. coli*, expressing the somatic (O) antigen 157 and the flagellar (H) antigen 7, was reckoned to be the cause of bloody diarrhoea (11). Subsequent investigations confirmed the link between STEC infection and cases of diarrhoea, haemorrhagic colitis, haemolytic uraemic syndrome and thrombotic thrombocytopenic purpura (12), and also revealed there are two main types of Shiga toxin, Stx1 and Stx2. Stx1 is antigenically similar to Shiga toxin produced by *Shigella dysenteriae* while Stx 2 is less closely related and antigenically distinct (12). The genes encoding these toxins (*stx*1 and *stx*2) appear to be highly mobile.

According to Law (13), STEC O157:H7 arose from the enteropathogenic serotype *E. coli* (EPEC) O55:H7. The chromosomes of enteropathogenic *E. coli* encode the cell adhesin intimin, a virulence factor also important in STEC. Originally, an *E. coli* O55:H7 is believed to have been infected with a *stx*2 carrying bacteriophage (a virus which can infect bacteria) then later acquired the large virulence plasmid pO157 and a gene encoding the O157 antigen. This progenitor is thought to have divided into two lineages: 1. non-motile, sorbitol fermenting Stx 2 producing strains seen in Germany and parts of Eastern Europe; 2. sorbitol non-fermenting strains producing both Stx1 and Stx2, the former toxin having crossed over from *Shigella dysenteriae*. The next step along the evolutionary pathway was the loss of the enzyme beta-glucuronidase to give the common beta-glucuronidase/MUG negative, sorbitol non-fermenting O157:H7 clone. Non-O157 STEC have not been so extensively studied, but from the wide diversity of *E. coli* serogroups found to harbour Stx, it can be surmised that transfer of *stx* by bacteriophages is a common event and occurred quite recently in an O104:H4 strain (13,14). Although the timescale for these events is not known, a large, post-war outbreak of diarrhoea with symptoms of haemolytic uraemic syndrome in the USA suggests they occurred prior to the 1950s (15).

VIRULENCE AND PATHOLOGY

In STEC, Stx1 and Stx2 are encoded on two mobile genetic elements (H19B and 933W, respectively) derived from bacteriophages (16). These prophages have the ability to insert themselves into the chromosomal DNA of *E. coli* through the action of recombinase enzymes (17). The prophages permit the host *E. coli* to express Stx and can also multiply and excise themselves from *E. coli* genomes (18-20). The toxins expressed form a protein complex composed of two subunits; a single A subunit attached to five identical B subunits (21). The B subunit pentamer component of the toxin binds to glycosphingolipid globotriosylceramide (Gb_3) receptors of absorptive villi and Paneth cells in the mammalian intestine, and globotetraosylceramide (Gb_4) surface receptors in renal glomerular cells and brain endothelia (18,21-23). Binding by B subunits promotes the internalisation of the toxin complex in an early endosome. A proposed alternative route for Stx entry is through bacterial invasion of the host cell (24). From the endosome, the toxin is transported to the trans-Golgi network, then through the Golgi apparatus to the endoplasmic reticulum, where the released A subunit induces a number of host cell responses including inhibition of protein synthesis, apoptosis (programmed cell death), autophagy (degradation of cellular components) and increases in chemokine and cytokine production (21,23,24) (Figure 2). The toxins cause bloody and non-bloody diarrhoea through the killing of intestinal endothelial cells either directly, or indirectly through inducing mesenteric ischaemia in the regional vasculature (2). The toxins are also able to translocate across the gastrointestinal endothelia and enter the systemic circulation, causing a host of pathological thrombotic and immunomodulatory effects on both the renal glomerulus and systemic microvasculature (21, 23). The mechanism by which the toxins traverse to the circulation is unknown (25), and the systemic effects are not usually accompanied by bacteraemia (26).

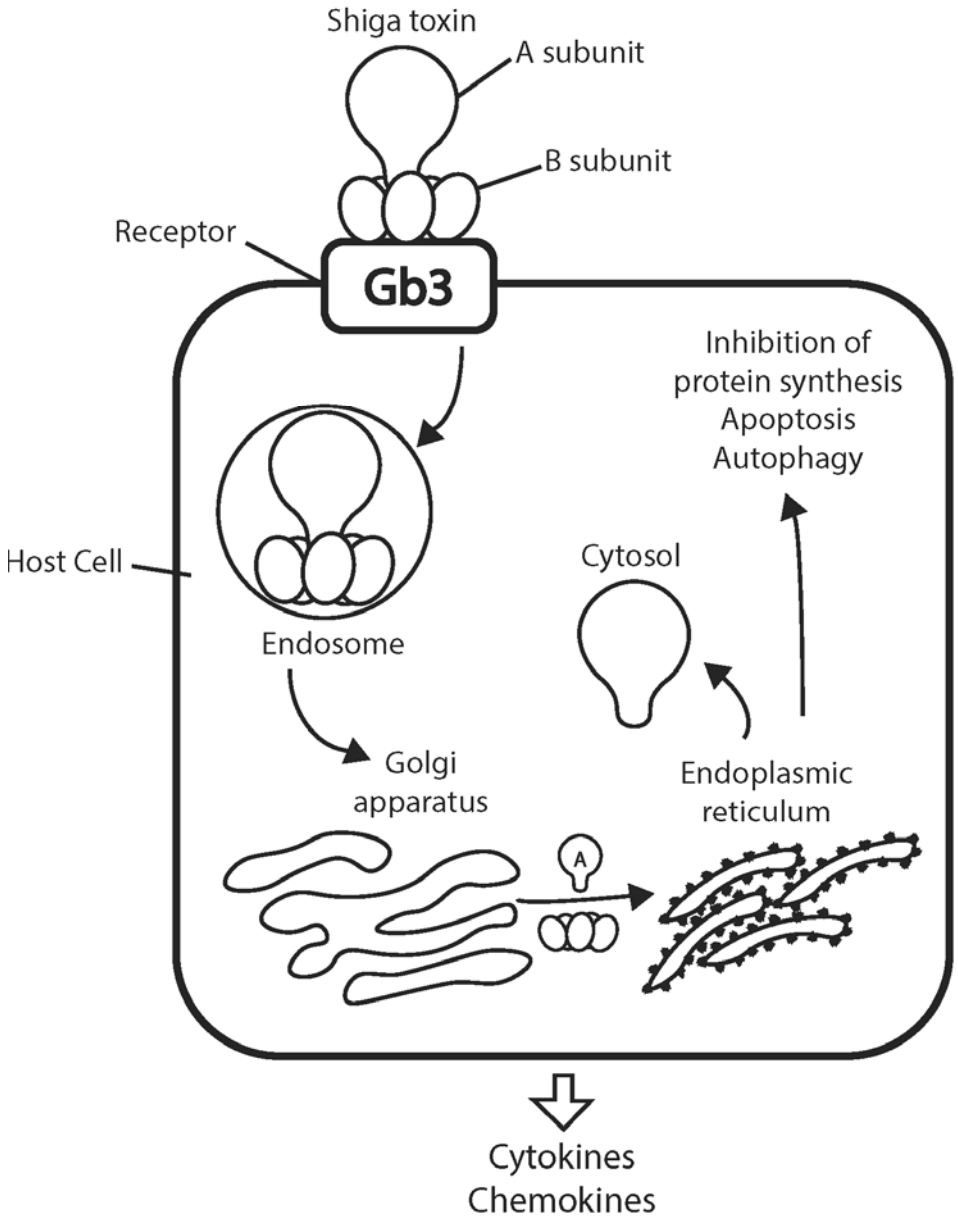
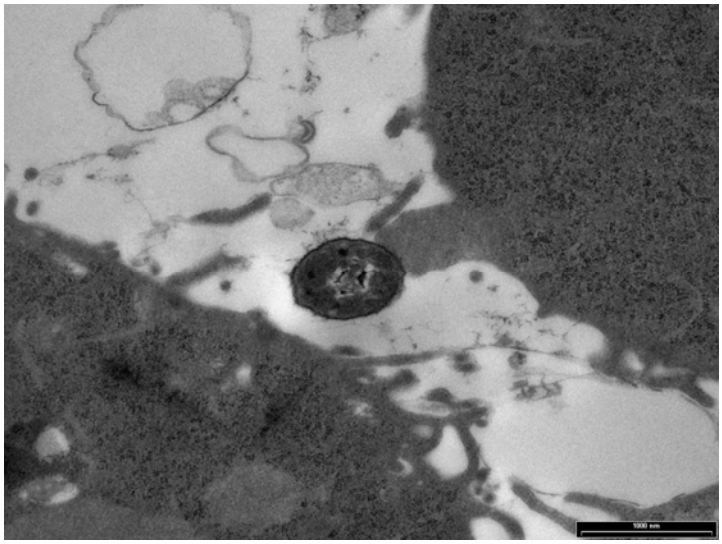


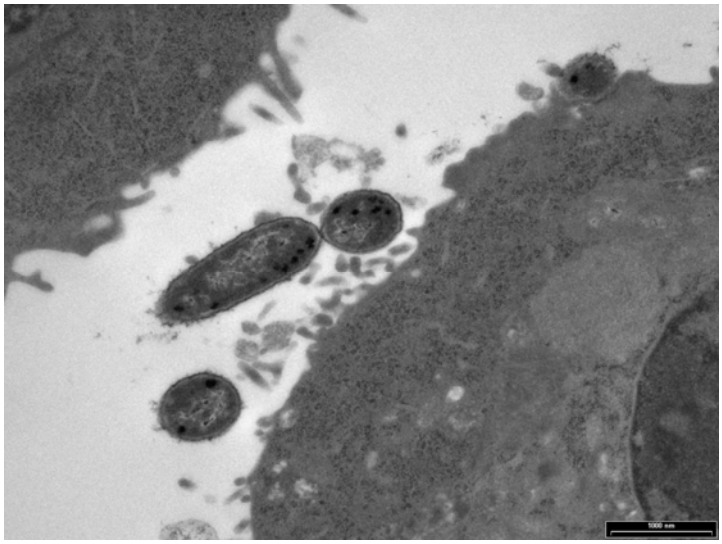
Figure 2. Proposed mode of action of Shiga toxin. Shiga toxin binds to the host cell receptor Gb3 (globotriaosylceramide, P^k blood group antigen). Following internalisation in an early endosome, the toxin is transported to the Golgi apparatus then to the endoplasmic reticulum where the dissociated A subunit inhibits protein synthesis by destroying the function of ribosomal RNA (28S subunit). The toxin A subunit is subsequently released into the cytosol. Host cell responses include autophagy (degradation of cell constituents), apoptosis (programmed cell death), ribotoxic and endoplasmic reticulum stress, and release of pro-inflammatory cytokines and chemokines (reviewed by Lee et al (24)).

Stx2 has at least a 100-fold higher toxicity against renal endothelial cells than Stx1 (1,21) due to differences in the DNA sequences encoding the toxin components (27). As a result, Stx2 is the variant most often associated with clinical cases of STEC and the progression of such cases to haemorrhagic colitis and haemolytic uraemic syndrome (21,27). Stx2 is also noted to have a number of subvariants (Stx2, stx2c2, stx2d, stx2e, and stx2f) each with varying toxicity (28, 29). Perhaps unsurprisingly, O157 serotypes associated with clinical symptoms are generally found to produce Stx2, usually in preference to Stx1. Stx2 may occur in other serogroups but the frequency is lower than Stx1 (27,30,31).

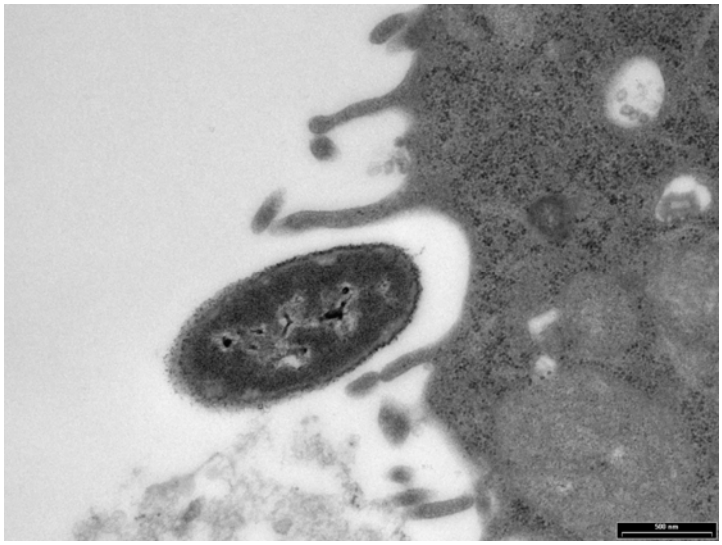
A virulence factor that STEC and EPEC can have in common is the adherence factor intimin, which has the encoding sequence *eaeA*. Intimin is a bacterial outer membrane protein encoded within a chromosomal pathogenicity island referred to as the Locus of Enterocyte Effacement (LEE) (1). The LEE encodes a syringe-like type III secretion system, capable of injecting over 50 bacterial effector proteins directly into the cytosol of the human host cell (21,32). In strains possessing LEE, intimin anchors bacteria to host cells (33,34) facilitating injection of the effector proteins. These reshape the actin cytoskeleton of intestinal epithelial cells to form characteristic 10 µm pedestal formations on the cell surface, as shown in Figure 3 (33,35). The close bacterium/host cell association formed by the components of the LEE mechanism are recognised to greatly aid in the delivery of Stx to enterocytes and the underlying mesenteric vasculature (33). Most of the strains isolated from outbreaks of haemorrhagic colitis and haemolytic uraemic syndrome, and those infections with more severe symptoms, typically express both Stx and intimin along with carriage of pO157 (30,31,36), and are usually designated as the pathotype enterohaemorrhagic *E. coli* (EHEC) (37). The tendency for haemolytic uraemic patients to form strong antibody responses to intimin and other LEE components underscores the importance of the adhesion determinant in advanced STEC infections (32).



(a)



(b)



(c)

Figure 3. Formation of actin pedestals. Formation of actin pedestals (a) due to the interaction of intimin with translocated intimin receptor (22). This is preceded by an initial loose attachment (b), then firm attachment with underlying effacement of the microvilli (c). Figure shows serogroup O111 *Escherichia coli* attached to the surface of Hep-2 tissue culture cells (prepared for transmission electron microscopy using standard fixation and osmium tetroxide staining by M. Gaastra).

The high virulence of serotype O157:H7 is attributed to Stx2 production, LEE and accessory virulence factors carried on a plasmid, pO157 (21,38). pO157 encodes putative virulence factors such as proteases, enterohaemolysin (*ehx*) and cell adhesins on a heterogeneous mix of prophages, transposons and genetic elements thought to have evolved to aid intestinal colonisation in cattle (21,32,39). However, haemolytic uraemic syndrome-causing strains do not consistently have the full complement of known virulence factors (37). A second large plasmid, pO113, is believed to encode other virulence factors which may allow LEE negative STEC to cause haemolytic uraemic syndrome (40).

DISEASE COURSE AND THERAPY

Due to the confluence of host and virulence factors, the clinical spectrum of STEC is appreciably broad and patient outcomes may be difficult to predict. Symptoms can range from asymptomatic or subclinical (and hence unreported) diarrhoea to severe haemorrhagic and neurological complications and death. Clinical cases of STEC infection typically present as self-limiting, with painful abdominal cramps and non-bloody diarrhoea that occur 1-8 days post ingestion (35,41), but may progress to bloody diarrhoea without fever or raised leucocyte count after a further 2-7 days (21,35). The progression to haemorrhagic colitis and haemolytic uraemic syndrome is difficult to predict (19), but is especially frequent in children <10 years of age (35,42), the immunocompromised and the elderly, with the highest rates associated with EHEC (*stx*$^+$, *eaeA*$^+$) and particularly O157 serotypes (21,35). The rate of progression to haemorrhagic colitis and haemolytic uraemic syndrome is generally 10-15%, although this is subject to patient factors, the accessory virulence factors present, and the Stx variant expressed (18,42,43). Haemolytic uraemic syndrome is characterised by the onset of microangiopathic anaemia (damaged erythrocytes), thrombocytopenia and may be accompanied in adults by thrombotic thrombocytopenic purpura (a diffuse formation of microangiopathic thrombi, often with concurrent neurological abnormalities) (12,44). Haemolytic uraemic syndrome is the clinical manifestation of Stx-induced damage of the kidney glomerular vasculature, and may even result in long term sequelae such as renal insufficiency and neurological aberrations (35). Overall, 3-5% of cases are fatal (21).

Generally, non-O157 infections have a milder course than that of O157:H7. In the latter, diarrhoea has a greater likelihood of being bloody (>90% chance with O157 vs 60%), abdominal cramping is usually more severe, and the need for hospitalisation (43% vs 18%) and progression to haemolytic uraemic syndrome (10-15% vs <10%) is more pronounced (3). Combined *eae* and *stx2* carriage is regarded as a stronger predictor for bloody diarrhoea and haemolytic uraemic syndrome than the presence of serogroup O157 (45). The haemolytic uraemic syndrome that accompanies severe STEC infection can mimic other intestinal diseases such as Crohn's disease and induce elevated faecal calprotectin levels, while chronic STEC infections may result in irritable bowel syndrome (IBS) (21). Many physicians have an incomplete understanding of STEC infection and do not consider non-O157:H7 infection as part of a differential diagnosis (46). The clinical presentation of STEC infection may also potentially lead to confusion with other gastrointestinal disorders such as intussusception, appendicitis, inflammatory bowel disease (IBD), or infection with *Clostridium*, *Shigella*, *Yersinia*, *Salmonella*, or *Campylobacter* species (12,19,47,48).

Treatments options for STEC infections are largely supportive, as antibiotics that induce DNA damage in *E. coli* have a tendency to activate a lysogenic SOS response in the Shiga

prophage and cause upregulation of the expression of Stx (29). As a result, treatments involving quinolones, ciprofloxacin, in addition to anti-motility agents, are contraindicated in STEC infection, having been associated with increased incidences of progression to haemolytic uraemic syndrome (21,26,49). Parenteral volume expansion with liberal amounts of intravenous fluids (21,50), particularly in conjunction with peritoneal dialysis is the recommended course for advanced cases of STEC infection (19, 25) . Early administration of the monoclonal anti-Shiga toxin antibody Eculizumab is also recognised to increase platelet counts and limit toxin-mediated neurological effects (25,50). The development of vaccines capable of stimulating a strong antibody response against pathogenic *E. coli* antigens such as intimin (29,51), is currently ongoing, albeit hampered by the difficulty in finding an appropriate animal model (35).

EPIDEMIOLOGY

The global impact of STEC infection is difficult to estimate because of inconsistencies in reporting methods between countries and the varying prevalence of detected and undetected serotypes within such countries. A recent estimate, a meta-analysis of papers and databases of 21 countries, places the global number of acute STEC infections at approximately 2.8 million per year, with an estimated 3890 cases of haemolytic uraemic syndrome, 270 cases of end-stage renal disease, and 230 mortalities (52). STEC infections are usually sporadic and generally affect children and the elderly, with the typical source of infection being the ingestion of contaminated foodstuffs (35). The natural reservoir of STEC is the intestine and rectum of cattle, and the contamination of processed beef carcasses combined with modern mass distribution is recognised as the classic cause of larger outbreaks (1,53,54). STEC may also be carried by a variety of farmyard animals including sheep, goats, chickens, pigs and deer, with outbreaks traced to contact with these animals or consumption of improperly prepared foodstuffs (42,55,56).

World-wide, STEC outbreaks have also been associated with person-to-person spread, as well as contaminated or unpasteurised milk, juice, sprouts and other vegetables (43). The durability of STEC in bovine faecal matter and groundwater is thought to permit the spread of STEC to mass-produced crops via effluent run-off and exposures through environmental water sources (29,51,57,58). Perhaps the most notable case of an outbreak of STEC infection was the 2011 O104:H4 outbreak in Germany, involving an especially virulent strain of non-O157 *E. coli* that caused over 4000 STEC illnesses, 908 cases of haemolytic uraemic syndrome and 90 deaths (49). The source of infection was ultimately traced to a batch of contaminated fenugreek sprouts (49), and although the strain did not employ intimin as its primary attachment factor (59), the outbreak served to highlight the contribution of non-O157 STEC to human disease in developed countries. Non-O157 STEC infection is usually dominated by the serogroups O26, O45, O103, O111, O121 and O145, which together typically comprise ~70% of the non-O157 STEC infections in many countries and are well represented amongst isolates retrieved from sufferers of haemolytic uraemic syndrome, thrombotic thrombocytopenic purpura and bloody diarrhoea (27,31). These serogroups, known as the 'Big 6' possess many of the virulence factors and associations to complicated enteric disease seen in O157:H7 strains (5), although the dominant strain may vary from country to country (Table 2).The contribution of the 'Big 6' to STEC disease is noted to be underreported (3), and international data suggests that the proportion of STEC disease caused by non-O157 serogroups is increasing (3,4,60,61). It's important to note that serogroup alone is not a determinant of virulence as carriage of virulence genes varies between strains of the same serogroup.

Table 2. Prevalent and predominant Shiga-toxigenic *Escherichia coli* serogroups by country

Country/Continent	Prevalent sero-groups	Predominant serogroup
United States of America	O157, O26, O111, O103, O121, O45, O145	O157
Canada	O157, O55, O125, O26, O126, O128, O18	O157
South America	O1, O2, O15, O25, O26, O49, O92, O11	Non-O157
United Kingdom	O157	O157
Continental Europe	O157, O26, O111, O104, O103, O128, O91, O113, O2, O9, O145	Both O157 and non-O157
Australia	O157, O111, O26	Both O157 and non-O157
Japan	O157, O26, O111	O157

Table adapted from Vanaja et al (41)

In New Zealand, the annual notification rate for all STEC infection has been increasing since 1997, with the highest number notified in the most recent Annual Surveillance Report produced by Environmental and Science Research (330 cases; 7.2/100,000 population) (6). However, this significant increase is most probably due to the introduction of PCR screening of all faecal samples in an Auckland laboratory (Figure 1) (6). Most STEC cases are unrelated or occur in small outbreaks confined to a family or geographic region with exposure to contaminated farmland or private water supplies as primary risk factors (62). Infection peaks in the summer months when outdoor activities increase (63). ESR identified common risk factors in 2015 as contact with pets, farm animals and manure (6).

The Enteric Reference Laboratory at ESR identified 53% of received STEC isolates as serotype O157:H7 in 2015, with the remainder confirmed as non-O157 *E. coli* (29.3%) or of undetermined serotype (17.7%) (6). In a recent qPCR study of over 500 diarrhoeic stool samples received by Southern Community Laboratories, Dunedin, the only STEC serogroups detected using molecular techniques were O103 and O157 (both in two patients). A further six stool samples from six patients were positive for *stx* but non-typable, indicating they did not belong to serogroup O157 or any of the 'Big 6' (63). This lack of dominance of the 'Big 6' serogroups among non-O157 STEC is compatible with previous studies of human cases, STEC types found in in retail meat and animal carriage and is likely related to agricultural practices in New Zealand (64-67).

CONCLUSIONS

E. coli O157:H7 was the first STEC to be discovered but it is now clear that non-O157 STEC are capable of causing disease, which can be severe if they produce Stx2 and encode intimin or other cell adherence factors. The roles of Stx, intimin and accessory gene products have been well described and a clear link to the pathology and clinical manifestations established. However, the virulence genotype(s) of the enterohaemorrhagic subset, EHEC, has not been fully defined and STEC strains are designated as EHEC mainly based on clinical associations. Some of the prophages encoding *stx* are able to infect other *E. coli*. This, and the potential mobility of other virulence factors, points to future evolutionary changes in the STEC pathotype (68). An example of this type of horizontal gene transfer is provided by the O104:H4 enteroaggregative *E. coli* strain, which acquired *stx* and caused a massive outbreak of illness in Europe, including haemolytic uraemic syndrome (69).

Compared to most other countries, there are distinctive differences in NZ with regard to the dominant risk factors and serogroups of non-O157 STEC. Elsewhere in the world food is the most significant transmission vehicle for O157 and the 'Big 6' serogroups, but in New Zealand STEC infection occurs with a greater diversity of serogroups mainly through contact with contaminated agricultural environments or animals. So far, New Zealand has not experienced the large STEC outbreaks seen in other countries, probably because of differences in food production and/or regulation of the industry. With few treatment options available, STEC represent an important and previously underestimated pathogen group. Due to the varying clinical picture, diagnosis can be difficult but it is anticipated that the adoption of molecular diagnostic techniques for STEC detection will lead to correct diagnosis and better reflect the true incidence of STEC infection.

ACKNOWLEDGEMENTS

Rowan Thomas thanks the University of Otago and Dunedin Basic Medical Sciences for Masters and Sandy Smith scholarships respectively. The authors thank Douglas McComish for construction of Figure 2 and the Otago Centre for Electron Microscopy for electron micrograph images (Figure 3).

AUTHOR INFORMATION

Rowan R Thomas, BMLSc MMLSc[1]
Mabel LH Gaastra, BSc PGDipSci, Medical Student[2]
Heather JL Brooks, BSc(Hons) PhD, Senior Lecturer and Course Director for Medical Laboratory Science[3]

[1]Department of Microbiology and Immunology, School of Biomedical Sciences, University of Otago
[2]Dunedin School of Medicine, University of Otago
[3]Department of Pathology, Dunedin School of Medicine, University of Otago

Correspondence: Dr Heather Brooks, Department of Pathology, University of Otago, PO Box 913, Dunedin 9054, New Zealand.

REFERENCES

1. Mainil JG, Daube G. Verotoxigenic Escherichia coli from animals, humans and foods: who's who? *J Appl Microbiol* 2005; 98: 1332-1344.
2. Tarr PI, Gordon CA, Chandler WL. Shiga-toxin-producing Escherichia coli and haemolytic uraemic syndrome. *Lancet* 2005; 365: 1073-1086.
3. Gould LH, Mody RK, Ong KL, Clogher P, Cronquist AB, Garman KN, et al. Increased recognition of non-O157 Shiga toxin-producing Escherichia coli infections in the United States during 2000-2010: epidemiologic features and comparison with *E. coli* O157 infections. *Foodborne Pathog Dis* 2013; 10: 453-460.
4. Johnson KE, Thorpe CM, Sears CL. The emerging clinical importance of non-O157 Shiga toxin-producing Escherichia coli. C*lin Infect Dis* 2006; 43: 1587-1595.
5. Kobayashi N, Lee K, Yamazaki A, Saito S, Furukawa I, Kono T, et al. Virulence gene profiles and population genetic analysis for exploration of pathogenic serogroups of Shiga toxin-producing Escherichia coli. *J Clin Microbiol* 2013; 51: 4022-4028.
6. Annual Surveillance Report. The Institute of Environmental Science and Research Ltd. Notifiable Diseases in New Zealand: Annual Report 2015, Porirua, New Zealand.
7. Buvens G, Pierard D. Virulence profiling and disease association of verocytotoxin-producing Escherichia coli O157 and non-O157 isolates in Belgium. *Foodborne Pathog Dis* 2012; 9: 530-535.
8. Croxen MA, Law RJ, Scholz R, Keeney KM, Wlodarska M, Finlay BB. Recent Advances in understanding enteric pathogenic Escherichia coli*. Clin Microbiol Rev* 2013; 26: 822-880.
9. Majowicz SE, Scallan E, Jones-Bitton A, Sargeant JM, Stapleton J, Angulo FJ etal. Global incidence of human shiga toxin–producing Escherichia coli Infections and Deaths: A systematic review and knowledge synthesis *Foodborne Pathog Dis* 2014; 11: 447-455.
10. Karmali MA, Petric M, Lim C, Fleming PC, Steele BT. Escherichia coli cytotoxin, haemolytic-uraemic syndrome, and haemorrhagic colitis. *Lancet* 1983; 2: 1299-1300.
11. Riley LW, Remis RS, Helgerson SD, McGee HB, Wells JG, Davis BR, et al. Hemorrhagic colitis associated with rare Escherichia coli serotype. *N Engl J Med* 1983; 308: 681-685.
12. Tarr PI. Escherichia coli O157:H7: Clinical, diagnostic and epidemiological aspects of human infection. *Clin Infect Dis* 1995; 20: 1-8.
13. Law D. The history and evolution of *Escherichia coli* O157 and other Shiga toxin-producing E. coli*. World J Microbiol Biotechnol* 2000; 16: 701–709.
14. Kaper JB, O'Brien AD. Overview and historical perspectives. *Microbiol Spectr* 2014; 2: doi:10.1128/microbiolspec.EHEC-0028-2014.
15. Johnson RP, Clarke RC, Wilson JB, Read SC, Rahn K, Renwick SA, et al. Growing concerns and recent outbreaks involving non-O157:H7 serotypes of vero- toxigenic Escherichia coli. *J Food Prot* 1996; 59: 1112-1122.
16. Strockbine N, Marques LRM, Newland JW, Smith HW, Holmes RK, O'Brien A. Two toxin-converting phages from Escherichia coli O157:H7 strain 933 encode antigenically distinct toxins with similar biologic activites. *Infect Immun* 1986; 53: 135-140.
17. Herold S, Karch H, Schmidt H. Shiga toxin-encoding bacteriophages--genomes in motion. *Int J Med Microbiol* 2004; 294: 115-121.
18. Melton-Celsa A, Mohawk K, Teel L, O'Brien A. Pathogenesis of Shiga-toxin producing Escherichia coli. *Curr Top Microbiol Immunol* 2012; 357: 67-103.
19. Thorpe CM. Shiga toxin-producing Escherichia coli infection. *Clin Infect Dis* 2004; 38: 1298-1303.
20. Wagner PL, Livny J, Neely MN, Acheson DWK, Friedman DI, Waldor MK. Bacteriophage control of Shiga toxin 1 production and release by Escherichia coli. *Mol Microbiol* 2002; 44: 957-970.
21. Ho NK, Henry AC, Johnson-Henry K, Sherman PM. Pathogenicity host responses and implications for management of enterohemorrhagic Escherichia coli O157:H7 infection. *J Gastroenterol* 2013; 27: 281-285.
22. Croxen MA, Finlay BB. Molecular mechanisms of Escherichia coli pathogenicity. *Nat Rev Microbiol* 2010; 8: 26-38.
23. Sears CL, Kaper JB. Enteric bacterial toxins: Mechanisms of action and linkage to intestinal secretion. *Microbiol Rev* 1996; 60: 167-215.
24. Lee M-S, Koo S, Jeong DG, Tesh VL. Shiga toxins as multi-functional proteins: induction of host cellular stress responses, role in pathogenesis and therapeutic applications *Toxins (Basel)* 2016; 8: 77; doi:10.3390/ toxins8030077.
25. Trachtman H, Austin C, Lewinski M, Stahl RA. Renal and neurological involvement in typical Shiga toxin-associated HUS. *Nat Rev Nephrol* 2012; 8: 658-669.
26. Ibarra C, Amaral MM, Palermo MS. Advances in pathogenesis and therapy of hemolytic uremic syndrome caused by Shiga toxin-2. *IUBMB Life* 2013; 65: 827-835.
27. Eklund M, Leino K, Siitonen A. Clinical Escherichia coli strains carrying stx genes: Stx variants and stx-positive virulence profiles. *J Clin Microbiol* 2002; 40: 4585-4593.

28. de Sablet T, Bertin Y, Vareille M, Girardeau JP, Garrivier A, Gobert AP, et al. Differential expression of stx2 variants in Shiga toxin-producing Escherichia coli belonging to seropathotypes A and C. *Microbiology* 2008; 154: 176-186.
29. Serna IV A, Boedeker EC. Pathogenesis and treatment of Shiga toxin-producing Escherichia coli infections. *Curr Opin Gastroenterol* 2008; 24: 38-47.
30. Anjum MF, Jones E, Morrison V, Tozzoli R, Morabito S, Toth I, et al. Use of virulence determinants and seropathotypes to distinguish high- and low-risk Escherichia coli O157 and non-O157 isolates from Europe. *Epidemiol Infect* 2014; 142: 1019-1029.
31. Brooks JT, Sowers EG, Wells JG, Greene KD, Griffin PM, Hoekstra RM, et al. Non-O157 Shiga toxin-producing Escherichia coli infections in the United States, 1983-2002. *J Infect Dis* 2005; 192: 1422-1429.
32. Kaper JB, Nataro JP, Mobley HL. Pathogenic Escherichia coli. *Nat Rev Microbiol* 2004; 2: 123-140.
33. Farfan MJ, Torres AG. Molecular mechanisms that mediate colonization of Shiga toxin-producing Escherichia coli strains. *Infect Immun* 2012; 80: 903-913.
34. Finlay BB, Ruschkowski S, Kenny B, Stein M, Reinscheid DJ, Stein MA, et al. Enteropathogenic E. coli exploitation of host epithelial cells. *Ann N Y Acad Sci* 1996: 797: 26-31.
35. Nataro JP, Kaper JB. Diarrheagenic *Escherichia Coli. Clin Microbiol Rev* 1998; 11: 142-200.
36. 36. Boerlin P, McEwen SA, Boerlin-Petzold F, Wilson JB, Johnson RP, Gyles CL. Associations between virulence factors of Shiga toxin-producing *Escherichia coli* and disease in humans. *J Clin Microbiol* 1999; 37: 497-503.
37. Karmali MA, Mascarenhas M, Shen S, Ziebell K, Johnson S, Reid-Smith R, et al. Association of genomic O island 122 of Escherichia col*i* EDL 933 with verocytotoxin-producing Escherichia coli seropathotypes that are linked to epidemic and/or serious disease. *J Clin Microbiol* 2003; 41: 4930-4940.
38. Cookson AL, Bennett J, Thomson-Carter F, Attwood GT. Molecular subtyping and genetic analysis of the enterohemolysin gene (ehxA) from Shiga toxin-producing *Escherichia coli* and atypical enteropathogenic E. coli. *Appl Environ Microbiol* 2007; 73: 6360-6369.
39. Lim JY, Yoon JW, Hovde CJ. A brief overview of Escherichia coli O157:H7 and its plasmid O157. *J Microbiol Biotechnol* 2010; 20: 5-14.
40. Newton HJ, Sloan J, Bulach DM, Seemann T, Allison CC, Tauschek M, Robins-Browne RM, et al. Shiga toxin–producing Escherichia coli strains negative for locus of enterocyte effacement. *Emerg Infect Dis* 2009; 15: 372-380.
41. Vanaja SK, Jandhyala DM, Mallick EM, Leong JM, Balasubramanian S. Enterohemorrhagic and other Shiga toxin-producing Escherichia col*i*. In: Donnenberg M, ed. Escherichia coli: Pathotypes and principles of pathogenesis. Academic Press, Cambridge, MA, USA, 2013: 121-182.
42. Mathusa EC, Chen Y, Enache E, Hontz L. Non-O157 shiga toxin–producing Escherichia coli in foods. *J Food Prot* 2010; 73: 1721-1736.
43. Gould LH, Bopp C, Strockbine N, Atkinson R, Baselski V, Body B, et al. Recommendations for diagnosis of Shiga toxin-producing Escherichia coli infections by clinical laboratories. *MMWR Recomm Rep* 2009; 58: 1-14.
44. Tarr PI, Neill MA. Perspective: The problem of non-0157:H7 Shiga toxin (Verocytotoxin)-producing Escherichia coli. *J Infect Dis* 1996; 174: 1136-1139.
45. Ethelberg S, Olsen KEP, Scheutz F, Jensen C, Schiellerup P, Engberg J, et al. Virulence factors for hemolytic uremic syndrome, Denmark. *Emerg Infect Dis* 2004; 10: 842-847.
46. Clogher P, Hurd S, Hoefer D, Hadler JL, Pasutti L, Cosgrove S, et al. Assessment of physician knowledge and practices concerning Shiga toxin-producing Escherichia coli infection and enteric illness, 2009, Foodborne Diseases Active Surveillance Network (FoodNet). *Clin Infect Dis* 2012; 54 Suppl 5: S446-452.
47. Kallinowski F, Wassmer A, Hofmann MA, Harmsen D, Heesemann J, Karch H, et al. Prevalence of enteropathogenic bacteria in surgically treated chronic inflammatory bowel disease. *Hepatogastroenterology* 1998; 45: 1552-1558.
48. Paton JC, Paton AW. Pathogenesis and diagnosis of shiga toxin-producing Escherichia coli infections. *Clin Microbiol Rev* 1998; 11: 450-479.
49. Jandhyala DM, Vanguri V, Boll EJ, Lai Y, McCormick BA, Leong JM. Shiga toxin-producing Escherichia coli O104:H4: an emerging pathogen with enhanced virulence. *Infect Dis Clin North Am* 2013; 27: 631-649.
50. Gerritzen A, Wittke JW, Wolff D. Rapid and sensitive detection of Shiga toxin producing Escherichia coli directly from stool samples by real-time PCR in comparison to culture, enzyme immunoassay and Vero cell cytotoxicity assay. *Clin Lab* 2011; 57: 993-998.
51. Beutin L. Emerging enterohaemorrhagic Escherichia coli, causes and effects of the rise of a human pathogen. *J Med Vet* 2006; 53: 299-305.
52. Majowicz SE, Scallan E, Jones-Bitton A, Sargeant JM, Stapleton J, Angulo FJ, et al. Global incidence of human Shiga toxin-producing Escherichia coli infections and deaths: A systematic review and knowledge synthesis. *Foodborne Pathog Dis* 2014; 11: 447-455.
53. Acheson DW, Keusch GT. Which Shiga toxin-producing types of E. col*i* are important? *ASM News* 1996; 62: 302-306.
54. Ethelberg S, Smith B, Torpdahl M, Lisby M, Boel J, Jensen T, et al. Outbreak of non-O157 Shiga toxin-producing Escherichia coli infection from consumption of beef sausage. *Clin Infect Dis* 2009; 48: e78-81.
55. Cookson AL, Taylor SC, Bennett J, Thomson-Carter F, Attwood GT. Serotypes and analysis of distribution of Shiga toxin producing Escherichia coli from cattle and sheep in the lower North Island, New Zealand. *N Z Vet J* 2006; 54: 78-84.
56. DebRoy C, Roberts E. Screening petting zoo animals for the presence of potentially pathogenic Escherichia coli. *J Vet Diagn Invest* 2006; 18: 597-600.
57. Fremaux B, Prigent-Combaret C, Beutin L, Gleizal A, Trevisan D, Quetin P, et al. Survival and spread of Shiga toxin-producing Escherichia coli in alpine pasture grasslands. *J Appl Microbiol* 2010; 108: 1332-1343.
58. Fukushima H, Hoshina K, Gomyoda M. Long-term survival of Shiga toxin-producing Escherichia coli O26, O111, and O157 in bovine feces. *Appl Environ Microbiol* 1999; 65: 5177-5181.
59. Guy L, Jernberg C, Arven Norling J, Ivarsson S, Hedenstrom I, Melefors O, et al. Adaptive mutations and replacements of virulence traits in the Escherichia coli O104:H4 outbreak population. *PloS One* 2013; 8: e63027.
60. Johnson RP, Clarke RC, Wilson JB, Read SC, Rahn K, Renwick SA, et al. Growing concerns and recent outbreaks involoving non-O157:H7 serotypes of verotoxigenic Escherichia coli. *J Food Prot* 1996; 59: 1112-1122.
61. Stigi KA, MacDonald K, Tellez-Marfin AA, H. LK. Laboratory practices and incidence of non-O157 Shiga toxin-producing Escherichia coli infections. *Emerg Infect Dis* 2012; 18: 477-479.
62. Baker M, Eyles R, Bennett J, Nicol C, Wong W, Garrett N. Emergence of verotoxigenic *Escherichia coli* (VTEC) in New Zealand. *New Zealand Public Health Report* 1999; 6: 9-12.
63. Thomas R, Brooks HJL, O'Brien R. Prevalence of Shiga toxin-producing and enteropathogenic Escherichia coli marker genes in diarrhoeic stools in a New Zealand catchment area. *J Clin Path* 2017; 70: 81–84.
64. Cookson AL, Cao M, Bennett J, Nicol C, Thomson-Carter F, Attwood GT. Relationship between virulence gene profiles of atypical enteropathogenic *Escherichia coli* and Shiga toxin-producing E. coli isolates from cattle and sheep in New Zealand. *Appl Environ Microbiol* 2010; 76: 3744-3747.

65. Cookson AL, Croucher D, Pope C, Bennett J, Thomson-Carter F, Attwood GT. Isolation, characterization, and epidemiological assessment of Shiga toxin- producing Escherichia coli O84 isolates from New Zealand. *J Clin Microbiol* 2006; 44: 1863-1866.
66. Jaros P, Cookson AL, Campbell DM, Besser TE, Shringi S, Mackereth GF, et al. A prospective case-control and molecular epidemiological study of human cases of Shiga toxin-producing Escherichia coli in New Zealand. *BMC Inf Dis* 2013; 13: 450-465.
67. Brooks HJL, Mollison BD, Bettelheim KA, Matejka K, Paterson KA, Ward VK. Occurrence and virulence factors of non-O157 Shiga toxin-producing Escherichia coli in retail meat in Dunedin, New Zealand. *Lett Appl Microbiol* 2001; 32: 118–123.
68. Asadulghani M, Ogura Y, Ooka T, Itoh T, Sawaguchi A, Iguchi A, et al. The defective prophage pool of Escherichia coli O157: prophage-prophage interactions potentiate horizontal transfer of virulence determinants. *PLoS Pathog* 2009; 5: e1000408.
69. Grad YH, Lipsitch M, Feldgarden M, Arachchi HM, Cerqueira GC, Fitzgerald M, et al. Genomic epidemiology of the Escherichia coli O104:H4 outbreaks in Europe, 2011. *Proc Natl Acad Sci USA* 2012; 109: 3065–3070.

NZIMLS ASM

Icons, Geniuses and Mavericks

A Christchurch Scientific Experience

21 - 24 August 2018

Join us at the Air Force Museum, Wigram, Christchurch for the NZIMLS Annual Scientific Meeting 21-24 August 2018

Be enthralled by the speakers!

Be amazed by the displays!

Catch up with colleagues!

Enjoy a memorable evening at the Conference dinner!

Registration available at:

www.nzimls.org.nz

Register early to avoid late rates!

ORIGINAL ARTICLE

Prevalence of extended spectrum β-lactamase, AmpC β-lactamase and metallo-β-lactamase enzymes among clinical isolates recovered from patients with urinary tract infections in Benin City, Nigeria

Ephraim Ehidiamen Ibadin[1], Richard Omoregie[1,2] and Idahosa Onaiwu Enabulele[2,3]

[1]Medical Microbiology Unit, Medical Laboratory Services, University of Benin Teaching Hospital; [2]School of Medical Laboratory Sciences, University of Benin Teaching Hospital and [3]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

ABSTRACT

Objective: Urinary tract infection (UTI) is one of the most common type of infection in humans, and a major reason that patients residing in the community seek medical care. The worldwide rise in resistance to routinely administered antibiotics would suggest a need to ascertain local resistance prevalence. This study was therefore conducted to determine the prevalence rate of extended spectrum β-lactamase (ESBL), AmpC β-lactamase, and metallo-β-lactamase (MBL) enzymes, among bacterial isolates recovered in samples of urine from patients with UTI, in Benin City, Nigeria.

Methods: Clean-catch urine samples were collected from 554 patients (249 males and 305 females) with signs and symptoms of UTI and transported to the medical microbiology laboratory, University of Benin Teaching Hospital, Nigeria. Samples were processed using standard techniques. Emergent bacteria were identified, antimicrobial susceptibility tests were carried out, and ESBL, AmpC β-lactamase and metallo-beta-lactamase (MBL) enzymes were screened for using phenotypic methods.

Results: Of the 554 urine samples, 205 (37.0%) were culture positive, including 126 samples with Gram-negative bacilli (GNB). *Klebsiella* spp was the most prevalent ESBL and MBL producer with 83.3% and 63.9% respectively, and 30% of *P. aeruginosa* hyper expressed AmpC β-lactamase. Co-production of two of these three enzymes was observed in 27.5% of GNB, while one isolate each of *E. coli* (1.8%), *Klebsiella* spp (2.8%) and *P. aeruginosa* (10.0%) showed simultaneous production of all three enzymes. The most effective antibacterial agents for these extended β-lactamase-producing GNB were the carbapenems (imipenem-52.8%, meropenem-53.9%).

Conclusion: The overall prevalence of ESBL, AmpC β-lactamase and MBL among GNB causing UTI was 51.6%, 15.1% and 35.7% respectively. Prudence in the use of antibiotics in hospital and community settings is strongly recommended.

Key words: urinary tract infection; extended spectrum β-lactamase; AmpC β-lactamase; metallo-β-lactamase; Nigeria.

N Z J Med Lab Sci 2018; 72: 11-16

INTRODUCTION

Urinary tract infection (UTI) is one of the commonest type of infection in humans, and a major reason that patients residing in the community seek medical care (1). Infection of the urinary tract is almost exclusively due to bacteria and the rise in antibacterial resistance worldwide means that therapeutic options are getting fewer (1,2). Beta-lactam antibiotics are drugs of choice for the treatment of many bacterial infections (3). Production of beta-lactamase enzyme(s) is a major mechanism of bacterial resistance to this class of antibiotics (1,2. The steady rise in the prevalence of Gram-negative bacteria producing extended spectrum beta-lactamase (ESBL), AmpC beta-lactamase and carbapenemases means newer antibiotics like extended spectrum cephalosporins (ESCs) (e.g. ceftriaxone, cefotaxime, ceftazidime), cephamycins (e.g. cefoxitin and cefotetan), monobactams (e.g. aztreonam) and carbapenems (e.g. ertapenem, imipenem and meropenem) may not achieve their therapeutic purpose when they are administered to patients (1,3,5).

ESBL enzymes, which are largely plasmid mediated, are capable of hydrolyzing ESCs and aztreonam, but are inhibited by clavulanic acid, tazobactam or sulbactam (5). AmpC β-lactamases are either plasmid- or chromosomally mediated and, when hyper-produced, confer resistance to a wide variety of β-lactam antibiotics including penicillins, ESCs, cephamycins, and monobactams (3). Metallo-beta-lactamase (MBL) enzymes are a type of carbapenemase enzyme which are also either plasmid or chromosomally mediated (6,7). They are characterised by their ability to hydrolyse carbapenem antibiotics, inhibition by metal ion chelators such as EDTA, and resistance to all currently available β-lactamase inhibitor drugs (6,7). Mobile genetic elements harboring genes that code for any of these enzymes have a high propensity for transfer within and between bacterial species.

Any bacterium that harbors any of the aforementioned beta-lactamase enzymes would compulsorily have resistance to a

number of antibiotics. This could invariably lead to prolonged hospital stay, worsened health conditions or even death (3,5,6). Diagnostic laboratories in Nigeria, however, do not routinely screen for these enzymes (3,4,8).

A previous study in Libya showed ESBL prevalence among the predominant uropathogens *E. coli* and *Klebsiella* spp as 6.7% and 21.7% respectively (1). A similar study in Nepal showed a prevalence of 13.51% and 16.55% for *E. coli* and *K. pneumoniae* respectively (9). A North-West Nigerian study showed a prevalence of 17.6% and 50.0% for ESBL and AmpC respectively among *Enterobacteriaceae* recovered from urine specimens (10). Another study in the same region showed 38.3% prevalence of MBL among bacterial isolates recovered from urine specimens (4). There is however paucity of information on the distribution of these three enzymes among Gram-negative bacilli (GNB) causing UTI in South-South region, Nigeria. This study was therefore conducted to determine the prevalence rate of ESBL, AmpC β-lactamase and MBL enzymes among bacterial isolates recovered from the urine samples of patients with signs and symptoms of UTI in Benin City, Nigeria.

MATERIALS AND METHODS

Study population

A total of 554 patients with signs and symptoms of UTI either admitted in wards or attending out-patient clinics in University of Benin Teaching Hospital (UBTH) were recruited for this study. This comprised 249 males and 305 females. The study was conducted between 15th February and 14th May, 2016. Informed consent was obtained from all patients and research protocols were carried out in accordance with the Helsinki declaration.

Specimen collection and processing

Clean-catch urine samples were collected into sterile screw-cap universal containers containing boric acid crystals and transported to the medical microbiology laboratory, University of Benin Teaching Hospital for processing. A loopful (0.001 ml) of well-mixed un-centrifuged urine was streaked on blood agar and cysteine lactose electrolyte deficient medium (Lab M, United Kingdom). The plates were incubated aerobically for 24 hours at 37°C and counts were expressed in a colony forming unit (CFU) per milliliter (mL). A count of ≥ 10^5 CFU/mL was considered significant to indicate UTI. For the cell count, each urine sample (10 mL) was centrifuged at 1000 g for 5 minutes. The supernatant was thereafter discarded and the deposit was examined microscopically at high magnification for red blood cells, pus cells, casts, crystals, and epithelial cells (11). A count of pus cells ≥ 5 per high power field (X 40 objective) was considered to indicate infection. UTI was diagnosed if the bacteria count, pus cells, or both were significantly high in an individual.

Identification of bacterial isolates

Identification of bacterial colonies was performed using standard techniques, including Gram staining, and biochemical tests (12). The isolates were thereafter preserved on nutrient agar slants for further analysis.

ESBL detection

Bacterial isolates were screened for ESBL enzymes using the double disc diffusion method as previously described with 30 µg ceftazidime and cefotaxime discs (Abtek Biologicals Ltd, Liverpool, UK) used as the indicator cephalosporins (13). The positive control strain *K. pneumoniae* ATCC 700603 was included.

Test for AmpC β-lactamase

The cefoxitin-cloxacillin inhibition test was carried out as previously described (14). Briefly, the test isolate was seeded on Mueller Hinton agar (MHA) as for ESBL detection and two 30 µg cefoxitin disks (Abtek Biologicals Ltd, Liverpool, UK) were placed on the surface of the seeded MHA plate. One of the cefoxitin discs was supplemented with 200 µg cloxacillin. The plate was thereafter incubated at 37^{0}C overnight. Production of AmpC β-lactamase was inferred if the zone of the cefoxitin disc supplemented with cloxacillin was ≥4mm greater than that of cefoxitin disc alone.

Metallo-β-lactamase detection

A modification of a previously described method was used for the detection of metallo-β-lactamase (15). Briefly, each bacterial isolate was seeded on the surface of MHA plate as in ESBL and AmpC detection. Imipenem 10µg and meropenem 10µg discs were placed on either side of a 1,900mg EDTA disc, 10mm apart from the EDTA disc (edge–to–edge) on the seeded plate. The plate was incubated overnight at 37^{0}C. A synergistic zone of inhibition between the EDTA disc and one or both discs was taken as indicative of metallo-b-lactamase production when compared with the control strain *Escherichia coli* ATCC 25922 which did not show synergism.

Disc susceptibility test

Antimicrobial susceptibility tests were performed using antibacterial discs namely imipenem (IPM) (10µg), meropenem (MEM) (10µg), Amoxicillin-clavulanate (AMC) (30µg), ceftazidime (CAZ) (30µg), cefuroxime (CXM) (5µg), gentamicin (GEN) (10µg), ofloxacin (OFX) (5µg), ceftriaxone (CRO) (30µg), and ciprofloxacin (CIP) (5µg), using the British Society for Antimicrobial Chemotherapy (BSAC) method (16). Bacterial isolates which showed resistance to ≥ 3 classes of antibacterial agents were deemed multi-drug resistant (MDR).

RESULTS

A total of 554 consecutive non-repetitive urine samples (249 males and 305 females) were received during the study period. Of this number, 205 (37.0%) were culture positive and 126 were infected with GNB.

The distribution of GNB and beta-lactamase enzyme type is shown in Table 1.*Escherichia coli* showed the highest prevalence among GNB causing UTI (43.7%). *Klebsiella* spp was the most prevalent ESBL producer (83.3%). *P. aeruginosa* and *Klebsiella* spp showed the highest rate of AmpC and MBL production with 30.0% and 63.9 respectively.

In relation to the combined effect of beta-lactamase enzymes, among all bacterial isolates screened, 35 (27.8%) of isolates showed production of two out of the three β-lactamase enzymes screened for in this study. *Klebsiella* spp had the highest prevalence for ESBL + MBL (38.9%), but only one isolate showed simultaneous production of AmpC + MBL enzymes (2.8%). One isolate each of *E. coli* (1.8%), *Klebsiella* spp (2.8%) and *P. aeruginosa* (10.0%) showed simultaneous production of all three enzymes (Table 2).

The distribution of MDR clinical isolates is shown in Table 3. *Klebsiella* spp showed the highest prevalence among MDR beta-lactamase positive bacteria with 44.9%. Compared to other bacteria, the majority of *P. aeruginosa* were MDR.

Table 1. Distribution of Gram-negative bacilli according to beta-lactamase type produced.

Organism	Number tested (% of total)	ESBL	AmpC	MBL
Citrobacter spp	1 (0.8)	1 (100)	0	0
Escherichia coli	55 (43.7)	22 (40.0)	7 (12.7)	15 (27.3)
Klebsiella species	36 (28.6)	30 (83.3)	8 (22.2)	23 (63.9)
Proteus mirabilis	6 (4.8)	2 (33.3)	0	1 (16.7)
Proteus vulgaris	5 (4.0)	2 (40.0)	0	1 (20.0)
Providencia spp	6 (4.8)	4 (66.7)	1 (16.7)	1 (16.7)
Alcaligenes spp	5 (4.0)	3 (60.0)	0	0
Pseudomonas aeruginosa	10 (7.9)	1 (10.0)	3 (30.0)	4 (40.0)
Pseudomonas fluorescens	1 (0.8)	0	0	0
Acinetobacter species	1 (0.8)	0	0	0
Total	**126**	**65 (51.6)**	**19 (15.1)**	**45 (35.7)**

Number in bracket: percentage. ESBL: extended spectrum beta lactamase. MBL: metallo-β-lactamase. AmpC: AmpC β-lactamase.

Table 2. Proportion of GNB in relation to combined effect of beta-lactamase enzymes.

Organism	Number tested	ESBL + AmpC	ESBL+ MBL	AmpC + MBL	ESBL+AmpC+ MBL
Citrobacter spp	1	0	0	0	0
E. coli	55	3 (5.5)	10 (18.2)	0	1 (1.8)
Klebsiella spp	36	2 (5.6)	14 (38.9)	1 (2.8)	1 (2.8)
Proteus mirabilis	6	0	1 (16.7)	0	0
Proteus vulgaris	5	0	1(20.0)	0	0
Providencia spp	6	1 (16.7)	0	0	0
Alcaligenes spp	5	0	0	0	0
Pseudomonas aeruginosa	10	0	2 (20.0)	0	1 (10.0)
Pseudomonas fluorescens	1	0	0	0	0
Acinetobacter spp	1	0	0	0	0
Total	**126**	**6 (4.8)**	**28 (22.2)**	**1 (0.8)**	**3 (2.4)**

Number in bracket: percentage. ESBL: extended spectrum beta lactamase. MBL: metallo-β-lactamase. AmpC: AmpC β-lactamase.

Table 3. Proportion of multidrug resistant (MDR) bacterial isolates recovered from uropathogens according to beta-lactamase production.

Organism	MDR with ESBL/ampC/ or MBL (n = 78)	MDR ESBL/ampC/MBL negative (n = 18)
Citrobacter spp	1 (1.3)	0
E. coli	26 (33.3)	5 (27.8)
Klebsiella spp	35 (44.9)	1 (5.5)
Proteus mirabilis	2 (2.6)	2 (11.1)
Proteus vulgaris	2 (2.6)	2 (11.1)
Providencia spp	4 (5.1)	0
Alcaligenes spp	4 (5.1)	1 (5.6)
Pseudomonas aeruginosa	4 (5.1)	6 (33.6)
Pseudomonas fluorescens	0	0
Acinetobacter spp	0	1 (5.5)

Number in bracket: value in percentage. MDR: multi-drug resistant.

The most effective antibacterial drug for the GNB which produced ESBL, ampC or MBL, were the carbapenems (imipenem-52.8%, meropenem-53.9%), while the least effective was amoxicillin-clavulanate (13.5%). Also, the most effective antibacterial drug for β-lactamase negative GNB were the carbapenems (imipenem-78.4%, meropenem- 63.9%) while the least effective was amoxicillin-clavulanate (35.1%) (Figure 1).

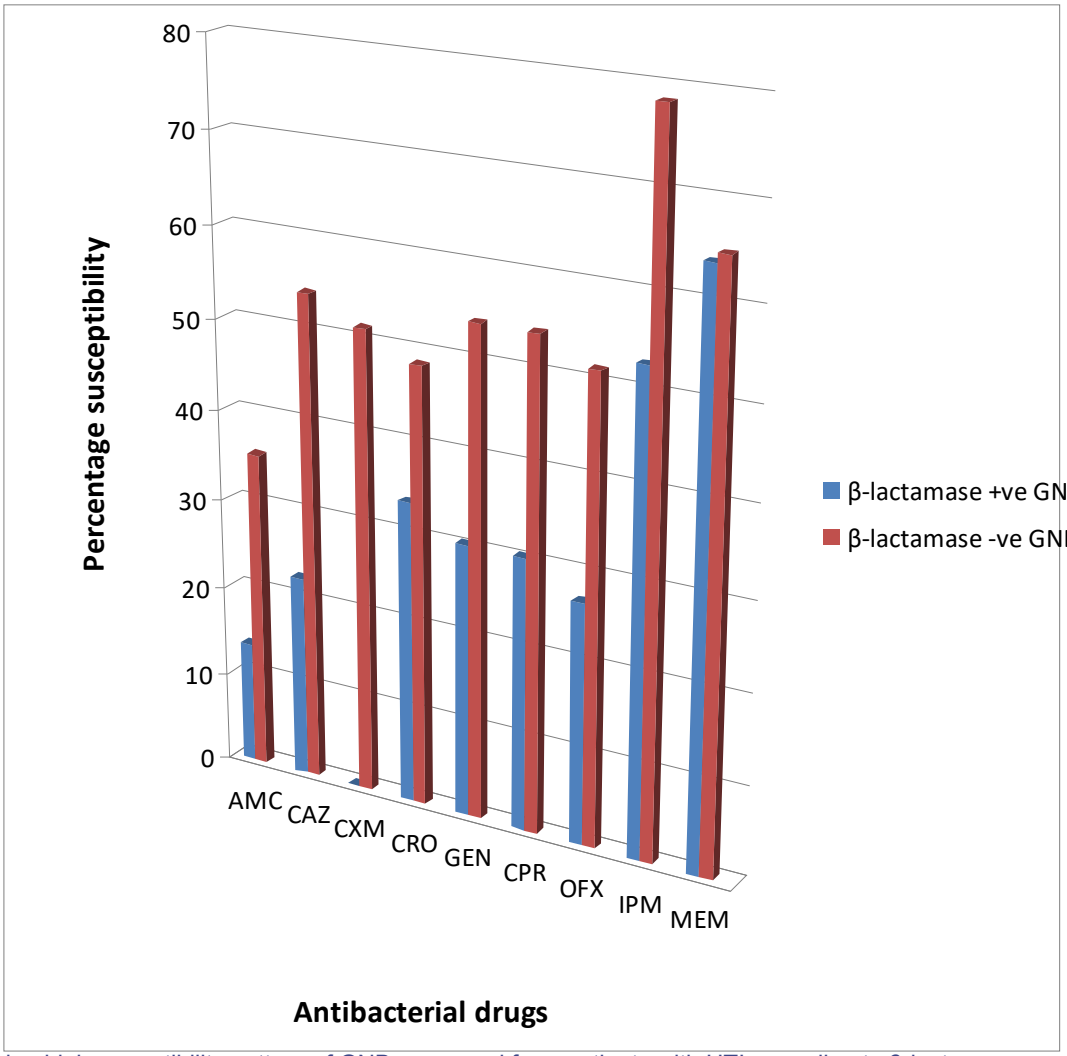


Figure 1. Antimicrobial susceptibility pattern of GNB recovered from patients with UTI according to β-lactamase production.

DISCUSSION

In this study, the prevalence of UTI was 37.0%. This observation agrees with a previous study in Benin which observed 39.0% prevalence of UTI among symptomatic patients and another in Okada, a rural community in Edo State, which reported 39.7% prevalence (17,18). *E. coli* was the most frequently isolated uropathogen among GNB causing UTI during this study. This finding aligns with previous studies in Nigeria and around the world (9,19). The occurrence of ESBL production among uropathogens was 51.6%, with *Klebsiella* spp showing a significantly high rate of ESBL producers, with 83.3% prevalence. This observation differs slightly from a previous study in Benin which showed an overall prevalence of GNB producing ESBL as 41.6% among uropathogens, with *Enterobacter* spp showing the highest prevalence (20). Though *Enterobacter* spp was not isolated during the study period, this study shows a rise in ESBL-producing *Klebsiella* spp in comparison with the same study in which 30.4% was observed for *Klebsiella* spp. The prevalence rate is also higher than that found in an Indian study which reported 52.8% for *Klebsiella pneumoniae* recovered from UTI (21).

The overall prevalence of GNB producing AmpC β-lactamase and MBL was 18.3% and 35.7% respectively. Among the Enterobacteriaceae *Klebsiella* spp showed the highest rate of AmpC β-lactamase (22.2%) and MBL (63.9%) production respectively. Two previous Northern Nigerian studies showed the prevalence of GNB-producing AmpC β-lactamase and MBL as 50.0% and 38.1% among uropathogens respectively (4,10). Our study therefore shows a comparatively lower prevalence rate of AmpC β-lactamase.

The co-existence of different β-lactamase enzyme phenotypes on a single bacterium was noteworthy in this study and implies markedly limited therapeutic options for patients with UTI. *Providencia* spp were more likely to show simultaneous production of ESBL and AmpC β-lactamase in comparison with other organisms. Uncommon genetic determinants of ESBL in our region (VEB-1 and OXA-10) have been recently demonstrated among *Providencia* spp recovered from catheter tips and wound specimens (22). This bacterium has also been previously implicated in UTI in previous studies in Benin (19,20). The finding of more than one β-lactamase enzyme phenotype in a bacterium therefore underscores the need for screening bacterial isolates for these three enzymes routinely when GNB are isolated from UTI cases in Nigeria as the drugs of choice for treatment, the β-lactam antibiotics, are failing. Over-the-counter sale of antibiotics without prescription is rife in Nigeria (8,19). This may have exerted selective pressure over time on bacterial pathogens, leading to the proliferation of these multidrug resistant GNB.

The majority of bacterial isolates causing UTI in this study were MDR. Our observation that the majority of *Klebsiella* spp were MDR and ESBL positive is in tandem with a lot of studies worldwide and shows that more attention should be accorded this organism in our region (7, 9). Noteworthy was the co-resistance to various other classes of antimicrobials, including fluoroquinolones and aminoglycosides. The existence of plasmids which bear genes conferring resistance to multiple classes of antibiotics alongside genes coding for ESBL, AmpC or MBL has been demonstrated in previous studies (5, 6). In comparison with other bacteria, *P. aeruginosa* was more likely to be MDR. This bacterium has been demonstrated to have intrinsic resistance to different classes of antibacterial drugs through mechanisms such as multidrug resistance efflux pumps, decreased permeability and the loss of the OprD2 (outer-membrane porin) protein (23).

In our region, the drugs of last resort are carbapenem antibiotics (imipenem, ertapenem and meropenem). The poor susceptibility of the carbapenems, the high prevalence of MBL as well as the co-existence with other resistance markers and other β-lactamase enzymes in uropathogens is a wake-up call for the need to review our antibiotic policy at both institutional and national levels.

The limitation of this study is its reliance on phenotypic methods for the detection of ESBL, AmpC and MBL. Molecular techniques remain the gold standard as genes implicated can be detected thereby aiding epidemiological surveys, more so phenotypic methods do not detect all enzymes.

CONCLUSIONS

E. coli was the most frequently encountered GNB causing UTI in this study. The overall prevalence of ESBL, AmpC β-lactamase and MBL among GNB was 51.6%, 15.1% and 35.7% respectively. The co-existence of more than one β-lactamase enzyme type in a bacterium was demonstrated. We strongly recommend laboratory guidance in administration of antibacterial drugs and advocate prudence in the use of antibiotics both in the hospital and community setting. Continued surveillance of resistance prevalence is essential in order to monitor the spread of resistance in our region.

AUTHOR INFORMATION

Ephraim Ehidiamen Ibadin, BMLS MSc AIMLS, Medical Laboratory Scientist[1]
Richard Omoregie, MPhil FIMLS, Lecturer[1,2]
Idahosa Onaiwu Enabulele, MSc PhD, Professor[2] and Lecturer[3]

[1]Medical Microbiology Unit, Medical Laboratory Services, University of Benin Teaching Hospital, Benin City, Nigeria
[2]School of Medical Laboratory Sciences, University of Benin Teaching Hospital, Benin City, Edo State, Nigeria
[3]Department of Microbiology, Faculty of Life Sciences, University of Benin, Benin City, Nigeria

Corresponding author: Ephraim Ibadin.
E-mail: ibadinsmailbox@yahoo.com.

REFERENCES

1. Abujnah AA, Zorgani A, Sabri MAM, El-Mohammady H, Khalek RA, Ghenghesh KS. Multidrug resistance and extended-spectrum β-lactamases genes among Escherichia coli from patients with urinary tract infections in Northwestern Libya. *Libyan J Med* 2015; 10: 26412.
2. Hawkey PM, Jones AM. The changing epidemiology of resistance. *J Antimicrob Chemother* 2009; 64 Suppl 1: i3–i10.
3. Ogefere HO, Osikobia JG, Omoregie R. Prevalence of AmpC β-lactamase among Gram-negative bacteria recovered from clinical specimens in Benin City, Nigeria. *Trop J Pharm Res* 2016; 15: 1947-1953.
4. Yusuf I, Yusha'u M, Sharif AA, Getso MI, Yahaya H, Bala JA, Aliyu IA, Haruna M. Detection of metallo betalactamases among Gram-negative bacterial isolates from Murtala Muhammad Specialist Hospital, Kano and Almadina Hospital Kaduna, Nigeria. *Bayero J Pure Appl Sci* 2012; 5: 84-88.
5. Paterson DL, Bonomo RA. Extended-spectrum β-lactamases: a clinical update. *Clin Microbiol Rev* 2005; 18: 657-686.
6. Queenan AM, Bush K. Carbapenemases: The versatile β-lactamases. *Clin Microbiol Rev* 2007; 20: 440-58.

7. Fukigai S, Alba J, Kimura S, Iida T, Nishikura N, Ishii Y, et al. Nosocomial outbreak of genetically related IMP-1 beta-lactamase-producing Klebsiella pneumoniae in a general hospital in Japan. *Int J Antimicrob Agents* 2007; 29: 306–310.
8. Ibadin EE, Omoregie R, Anogie NA, Igbarumah IO, Ogefere HO. Prevalence of extended spectrum β-lactamase, AmpC β-lactamase and metallo-β-lactamase among Gram-negative bacilli from clinical specimens in a tertiary hospital in Benin City, Nigeria. *Int J Enteric Pathog* 2017; 5: 85-91.
9. Chander A, Shrestha CD. Prevalence of extended spectrum beta lactamase producing Escherichia coli and Klebsiella pneumoniae urinary isolates in a tertiary care hospital in Kathmandu, Nepal. *BMC Res Notes* 2013; 6: 487.
10. Yusuf I, Haruna M, Yahaya H. Prevalence and antibiotic susceptibility of ampC and ESBL producing clinical isolates at a tertiary health care center in Kano, North-West Nigeria. *Afr J Clin Exp Microbiol* 2013; 14: 109-119.
11. Cheesbrough M. District Laboratory Practice in Tropical Countries, Part 2, 2nd Edition. Cambridge University Press, Cambridge, UK.
12. GI Barrow, RKS Feltham (Editors). Cowan and Steel's Manual for Identification of Medical Bacteria (3rd Edition), 2003. Cambridge University Press, Cambridge, UK.
13. Livermore DM, Brown DF. Detection of β–lactamase–mediated resistance. *J Antimicrob Chemother* 2001; 48 Suppl 1: 59–64.
14. Tan TY, Ng LS, He J, Koh TH, Hsu LY. Evaluation of screening methods to detect plasmid-mediated AmpC in Escherichia coli, Klebsiella pneumoniae, and Proteus mirabilis. *Antimicrob Agents Chemother* 2009; 53: 146–149.
15. Lee K, Lim YS, Yong D, Yum JH, Chong Y. Evaluation of the Hodge test and the imipenem-EDTA double-disk synergy test for differentiating metallo-ß-lactamase-producing isolates of Pseudomonas spp. and Acinetobacter spp. *J Clin Microbiol* 2003; 41: 4623-4629.
16. Andrews JM. BSAC standardized disc susceptibility testing method (version3). *J Antimicrob Chem* 2004; 53: 713-728.
17. Otajevwo FD. Urinary tract infection among symptomatic out-patients visiting a tertiary hospital based in mid western Nigeria. *Glob J Health Sci* 2013; 5: 187-199.
18. Oladeinde BH, Omoregie R, Olley M, Anunibe JA. Urinary tract infection in a rural community of Nigeria. *N Am J Med Sci* 2011; 3: 75-77.
19. Omoregie R, Igbarumah IO, Egbe CA, Ogefere H. Urinary tract infections among the elderly in Benin City, Nigeria. *Fooyin J Health Sci* 2010; 2: 90−93.
20. Ogefere HO, Aigbiremwen PA, Omoregie R. Extended-spectrum beta-lactamase (ESBL)–producing Gram-negative isolates from urine and wound specimens in a tertiary health facility in southern Nigeria. *Trop J Pharm Res* 2015; 14: 1089-1094.
21. Sasirekha B. Prevalence of ESBL, AmpC β-lactamases and MRSA among uropathogens and its antibiogram. *EXCLI J* 2013; 12: 81-88.
22. Aibinu IE, Pfeifer Y, Ogunsola F, Odugbemi T, Koenig W, Ghebremedhin B. Emergence of β-lactamases OXA-10, VEB-1 and CMY in Providencia spp. from Nigeria. *J Antimicrob Chemother* 2011; 66: 1931–1932.
23. Qu TT, Zhang JL, Wang J, Tao J, Yu YS, Chen YG, et al. Evaluation of phenotypic tests for detection of metallo-β-Lactamase-producing Pseudomonas aeruginosa strains in China. *J Clin Microbiol* 2009; 47: 1136–1142.

NZIMLS Journal Prize

Council of the NZIMLS has approved an annual Journal prize to the value of NZ$300 for the best peer-reviewed article published in the Journal during the calendar year. The article can be a review article, original article, case study, research letter, or technical communication. Excluded are Fellowship dissertations.

Many studies are presented at the Annual Scientific Meeting, SIG meetings, and the North and South Island Seminars, yet are rarely submitted to the Journal for wider dissemination to the profession.

Consider submitting your presentation to the Journal. If accepted, you are in consideration for the NZIMLS Journal Prize and will also earn you valuable CPD points.

Please contact the Editor or any Editorial Board Member for advice and help. Contact details are on the NZIMLS web site (www.nzimls.org.nz), as are instructions to authors.

No formal application is necessary but you must be the 1st author and a current financial member of the NZIMLS to be eligible. All articles accepted and published during the calendar year (April, August and November issues) will be considered. The Editor, Deputy Editor and the President of the NZIMLS, who themselves are ineligible, will judge all eligible articles in December. Their decision will be final and no correspondence will be entered into.

The 2017 winner of the NZIMLS Journal Prize was Melanie Adriaansen from Waitemata Laboratories for her article "Binary male-female laboratory reference ranges do not reflect reality for transgender individuals on sex-hormone therapy. *N Z J Med Lab Sci* 2017; 71(3): 101-105."

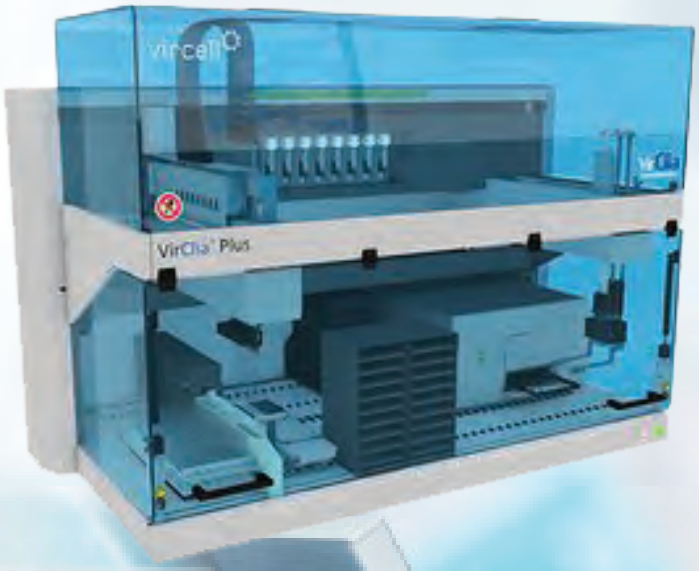
VirClia
Chemiluminescence

Chemiluminescence in Monotest

The perfect combination for infectious serology

VirClia® Monotest

More than 80 references available for your serology testing panel



VirClia® Plus

High throughput automation of immunoassay-based infectious disease testing

Distributed by Abacus dx
0800 222 170 | info@abacusdx.com | www.abacusdx.com

abacus dx

ORIGINAL ARTICLE

The haematocrit to haemoglobin conversion factor: a cross-sectional study of its accuracy and application

Nosaiba Al-Ryalat, Saif Aldeen AlRyalat, Lna Waleed Malkawi, Hana Abu-Hassan, Osama Samara and Azmy Hadidy

University of Jordan, Amman, Jordan

ABSTRACT

Background: The emphasis on using haemoglobin (Hb) as the only indicator for anaemia is reported in The World Health Organisation's (WHO) anaemia reports. The three-fold conversion of the haematocrit (Hct) (Hct / 3) to yield the "derived Hb" was suggested in settings where measured Hb results cannot be rapidly provided, such as: patients living in the rural areas and low-income countries where Hb-measuring machines are not available in all healthcare centers.

Methods: We studied the usefulness of the derived Hb in detecting anaemia compared to the measured Hb in 1695 patients and studied the factors that might affect the measured and the derived Hb values. For each patient, we retrospectively obtained demographic data, Hb, Hct and kidney function test results. We used the three-fold conversion to calculate the Hb from the Hct by dividing Hct level by 3 (Hb = Hct / 3), and from this the absolute difference between the measured and the derived Hb values (Delta Hb) was calculated. We calculated the sensitivity, specificity, and positive predictive value of the derived Hb for diagnosis of patient's anaemia.

Results: In this study, the three-fold conversion of the Hct to provide the derived Hb was not affected by the patient's age, gender, and kidney function. Hct level was the only factor found to influence the accuracy of the three-fold conversion. Our study showed that at lower Hct levels, the derived Hb was almost equal to the measured Hb (mean difference 0.2 g/L) in comparison to higher Hct levels (mean difference 4.5g/L). Using the derived Hb, we were able to detect anemic patients with 85.94% sensitivity, 94.50% specificity, and a positive predictive value of 88.8%.

Conclusions: The derived Hb can be conveniently used for assessment and follow up of a large group of patients, as its accuracy escalates at lower Hct levels, and for being not affected by patient's age, gender, hydration status and kidney function. The three-fold conversion is also reliable for assessment of anaemia in elderly patients and in patients with impaired kidney function.

Keywords: Anaemia; Haemoglobin; Haematocrit; Three-fold conversion; Sensitivity; Specificity; Positive Predictive Value.

N Z J Med Lab Sci 2018; 72: 18-21

INTRODUCTION

The definition of anaemia, a decrease of circulating red blood cell mass (1), does not change throughout life. However, the two main parameters commonly used interchangeably by medical practitioners to indicate anaemia, namely haemoglobin concentration (Hb) and haematocrit (Hct), are variable among different age groups and both genders. The World Health Organisation's (WHO) definition of anaemia uses Hb but not Hct in all its reports on the subject matter since 1968 (2). Four reference ranges are used to differentiate normal Hb from anaemia for each age group. In contrast, if both Hb and Hct are used for anaemia interpretation, eight reference ranges results for each age group and gender which further complicates the interpretation of anaemia, especially for those not in healthcare professions.

Furthermore, studies comparing Hb and Hct in detecting anaemia report that the use of Hb is more sensitive than Hct (3,4). However, Hct holds one advantage over Hb due to being less expensive and more widely used than Hb (5), especially for screening purposes; although this advantage is diminishing as new and cheaper devices are being introduced in the markets for Hb measurement, e.g. HemoCue (5). In order to emphasize the use of Hb as the only parameter for anaemia assessment without losing the advantage of Hct measurements, the use of the three-fold conversion to equate the two measures has come into practice (6). To the best of our knowledge, most of the studies that have used the three-fold conversion method to derive the Hb have been exclusively done in malaria-endemic countries (6). Accurate determination of Hb level is an essential element in assessing the extent of anaemia and making a decision whether treatment is necessary or not (5). We believe that such an imminent decision needs a reliable, rapidly testable, and a widely available laboratory test. Accordingly, investigating the accuracy and applicability of the three-fold conversion use in other areas of the world is worthwhile, particularly in the middle East where a large proportion of the population lives in rural areas where laboratory measuring devices might not be always available.

The three-fold conversion, derived Hb = Hct/3, a ratio that combines two main laboratory parameters, can be used in the assessment and diagnosis of anaemia. This ratio can be used

to provide a derived Hb value when the Hct is the only measured value available. . Its use could provide results to diagnoses anaemia according to the WHO recommendations leaving just four reference ranges for healthcare practitioners to use in daily practice. The aim of our retrospective study was to evaluate the accuracy and to determine factors that might affect the applicability of conversion of the Hct to provide a derived Hb for the diagnosis of anaemia in middle-eastern populations, particularly in situations where a measured Hb value might not be readily available.

MATERIALS AND METHODS

We retrospectively obtained the haematology data for 1695 patients from the University of Jordan Hospital computer system, including general demographics (i.e. age, gender), and kidney function tests results. Each patient had to have had a complete blood count (CBC) and kidney function tests within a previous 24-hour period and not to have been diagnosed with or suspected to have malaria. In addition, pregnant women were excluded. The three-fold conversion of the Hct to obtain the Hb was conducted and the absolute difference between the measured Hb and the derived Hb was calculated to provide the "Delta Hb" according to the following formula: Delta Hb = (measured Hb – (Hct/3).

We compared the derived Hb for each patient to the corresponding measured Hb, then calculated the sensitivity, specificity, and positive predictive value of the derived Hb for the purposes of anaemia detection using the following formulae:

Sensitivity = true positive / (true positive + false negative)
Specificity = true negative / (false positive + true negative)
Positive predictive value = true positive / (true positive + false positive)
Where:

- True negative; measured and derived Hb indicates normal.
- False positive; derived Hb indicates anaemia while measured does not.
- True positive; measured and derived Hb indicates anaemia.
- False negative; measured Hb indicates anaemia while derived value does not.

Normal and anemic Hb levels were determined based on the haemoglobin reference ranges presented in WHO report (7). The following reference ranges were considered for aneamia diagnosis:

- Children 6-59 months: below 110 g/L.
- Children 5-11 years: below 115 g/L.
- Children 12-14 years and women ≥15 years: below 120 g/L.
- Men ≥15 years: below 130 g/L.

The Siemens' high-volume haematology analyzer (ADVIA® 2120i System with Autoslide) was used to provide the patients' Hb and Hct.

Statistical analysis of the data was done using SPSS for Windows release 21.0 (SPSS Inc., Chicago, IL, USA). Descriptive statistics were used to describe our sample populations, including mean and standard deviation for continues variables, and proportions for other types of variables. We used the independent sample t-test to study gender differences in both the Hb and Hct values. Due to a non-linearity relationship we used Spearman's test to study the relationship between Hb and Hct with age, creatinine, and the blood urea nitrogen to creatinine ratio (BUN/Cr). We also used Spearman's to study the relationship between the difference between the measured and derived Hb (Delta Hb) with Hct, age, creatinine, and the BUN/Cr) ratio. A p-value of <0.05 was considered statistically significant. This study was approved by our institutional ethical committee and conducted in accordance with latest update (2013) of the declaration of Helsinki.

RESULTS

A total of 1695 patients were included, these were 849 (50.1%) male and 846 (49.9%) female with a combined mean age of 56.7 years (±20.48). The mean Hb and Hct values were 128g/L (± 21) and 39.1% (± 6.3) , respectively. The gender difference for Hb was significant (*p* value <0.001), as mean Hb for males was 134 g/L (± 22.4) and for females was 122 g/L (± 17.6). The gender difference for Hct was also significant (*p*<0.001), as mean Hct for males was 40.69% (± 6.66) and for females was 37.37% (± 5.49). On Spearman's test, both the Hb and Hct were significantly correlated with age, creatinine, and BUN/Cr ratio, as shown in Table 1.

Table 1. Correlation between both H and Hct and age, creatinine, and BUN/Cr ratio.

Factor	Hb/Hct	rho	*p*
Age	Hb	-0.213	<0.001
	Hct	-0.188	<0.001
Creatinine	Hb	-0.098	<0.001
	Hct	-0.076	0.002
BUN/Cr	Hb	-0.187	<0.001
	Hct	-0.181	<0.001

Rho = Spearman's correlation coefficient. BUN/Cr = blood urea nitrogen to creatinine ratio.

63% of the sample had a derived Hb higher than the measured Hb and 37% had a measured Hb higher than the derived Hb. The Delta Hb value was significantly correlated to Hct levels (*p*= 0.001) with a Spearman's coefficient of 0.08 (Figure 1). There was no significant correlation between Delta Hb and patient's age, creatinine, or BUN/Cr ratio (*p* values of 0.08, 0.32, and 0.41, respectively).

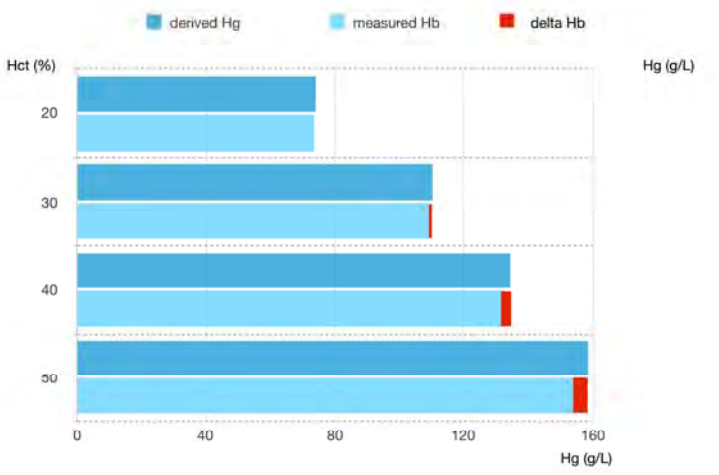


Figure 1a. Illustrates the mean measured and derived hemoglobin (Hb) values at different hematocrit (Hct) levels. The difference between derived Hb and measured Hb (mHb) is labelled in red as (Delta Hb).

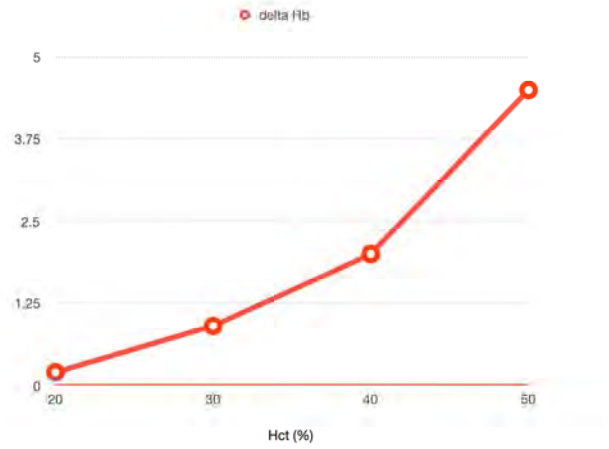


Figure 1b. Shows Delta Hb (Red labels in figure 1a). Delta Hb increases at higher Hct levels, thus widening the gap between measured and derived Hb. The greater the gap between measured and derived Hb (Delta Hb), the less accurate the three-fold conversion becomes.

Based on the WHO definition of anaemia , 33.6% (a total of 563 patients) had a measured Hb below the reference range for anaemia, whereas 32.5% (a total of 544 patients) were anaemic using the their derived Hb value. Using the derived Hb values the method provided 85.94% sensitivity, 94.50% specificity and a positive predictive value of 88.8%.

DISCUSSION

Worldwide, the laboratory gold standard method used to screen for anaemia is the Hb and/or Hct levels (8). In 2001 a WHO report recommended the use of the Hb value in clinical surveys claiming that the Hct value only added complexity, thus making results more challenging for decision-makers to interpret in the classification of anaemia (9). The use of Hb as a parameter to assess potential anaemia is sometimes not possible in the clinical setting where the Hct value is the only means available for assessment of anaemia . This might be also of particular importance for assessment and follow up of patients in regions where malaria is common. Under such circumstances a simple conversion formula is needed to provide Hb values, such as the three-fold conversion formula (2). This study has shown that the well-known three-fold conversion of the Hct to derive a Hb value provided results that are not affected by the patient's age, gender, hydration status as measured by the BUN/Cr ratio and kidney function. We also showed that the Hb value derived from the Hct is able to detect anaemic patients with 85.94% sensitivity, 94.50% specificity and a positive predictive value of 88.8%.

More importantly, we found that the accuracy of the derived Hb value increases at lower Hct levels. Delta Hb, the difference between measured and derived Hb values, is positively correlated to Hct levels, therefore, Delta Hb declines at lower Hct levels. This implies that the accuracy of the three-fold conversion increases as Hct levels decrease, making it a reliable measure to calculate the Hb for patients within the anaemia range; such as upon assessment of patients from geographic regions and age groups were anaemia is common, e.g. malaria-endemic regions and elderly age group, respectively. In another study a similar association was found in malaria-infected children where the difference between the measured and the calculated (derived) Hb was found to decrease as the anaemia worsened in severity (10).

Unexplained anaemia, where no specific aetiology can be identified, is common and accounts for one third of anaemia cases in elderly populations (11). Thus, it is worthwhile to investigate the accuracy and applicability of the three-fold conversion in this group. Recent studies suggest age-related progressive erythropoietin resistance leads to a decline in RBC production and RBC number (11). Additionally, microcytic anaemia (low mean cell volume, MCV) is common in the elderly. As many automated haematology analysers calculate Hct by multiplying the red cell count by the MCV (5, 1), a reduction in either or both of these indices may affect the Hct and consequently, the derived Hb and Delta Hb. Our results showed that Delta Hb is not correlated to age, thus the derived Hb can be reliably used in assessment of elderly populations.

A study by Hsu *et al.* showed that the loss of kidney function leads to a decrease in Hct levels and the development of anaemia (12). In our study, we found that an increase in creatinine (impaired kidney function) was associated with a decrease in both Hb and Hct. Furthermore, loss of kidney function did not affect Delta Hb, thus, it might not affect the accuracy of using derived Hb in those patients. This makes the derived Hb a reliable parameter to assess Hb levels in patients with impaired kidney function. Our findings also showed that Delta Hb is independent of a patient's hydration status (as measured by the BUN/Cr ratio), gender, or age.

The widespread use of the Hb value to reflect the blood oxygen carrying capacity and indirectly anaemia has multiple advantages. Firstly, it is easier for multidisciplinary health practitioners to communicate with each other using a standard parameter that can be derived irrespective of the devices being used in the clinical setting. Second, Hb is identified by the WHO as the parameter used principally for defining anaemia among different age groups, and so it is necessary to calculate the Hb when only the results of the Hct levels are available. Third, having a single universal parameter in research will make published research more comprehensible to a wider group of readers, so that studies that utilize only Hct in its results can be compared with studies only utilizing Hb by virtue of the three-fold conversion.

Interestingly, most of the studies that have used the three-fold conversion method to derive the Hb have been conducted in malaria-endemic countries. Recent studies on malaria-infected children show that the gap between the derived and measured Hb values decreases as the severity of anaemia increases (6,10). On the other hand, Carneiro *et al*. demonstrated that this gap increases in malaria-infected children below the age of 5 years, where the drop in the derived Hb levels does not go hand in hand with the actual drop in their measured Hb levels (6). Thus, the use of derived Hb for evaluation of children below age of 5 might lead to underestimation of the prevalence of anaemia in malaria-infected children (6).

Worth mentioning is that a recent study suggests another conversion formula that is more reliable for use in malaria-endemic regions; Hct = 5.62 + 2.6 * Hb. This formula has since been found to be comparable to the three-fold conversion but with the latter being more practical and simplified by comparison (13).As a contribution for to the epidemiologic data available in the literature regarding mean Hb levels of different populations worldwide, we compared the mean Hb of our middle-Eastern sample to some of the mean values available in literature for other populations. Noticeably, by comparing the mean Hb of our sample and that of the malaria-endemic region (sub-Saharan Africa) in the Carneiro *et al*. study (6), a difference of 23 g/L between the mean Hb values of the two regions was found (128 g/L in the middle East compared to 105 g/L in a malaria endemic region). Conversely, generally higher Hb levels were noted among American population for both whites (149 g/L in males, 135 g/L in females) and African Americans (145 g/L in males, 127 g/L in females) (14).

There are some limitations for our study that need to be taken into account. These include the inability to assess the hydration status of our patients clinically, only the BUN/Cr ratio was used to reflect the hydration status of the patients. Moreover, as this study was retrospective, the confounding effect of some other factors, such as polycythemia, haemoglobinopathies, or blood sample haemolysis, could not be assessed. We recommend that future studies be held in other populations to assess the validity of the three-fold conversion in different regions of the world, and to take the afore mentioned potential interferences into account.

CONCLUSIONS

WHO reports recommend the use of the Hb alone for the interpretation of anaemia. However, not all facilities have access to Hb measurement, instead relying only on Hct. We recommend the use of the three-fold conversion to derive Hb, so that the derived Hb can be used as a reliable parameter between healthcare practitioners. The derived Hb can be conveniently used for the assessment and follow up of a large group of patients, as its accuracy increases at lower Hct levels, and for being not affected by patient's age, gender, hydration status, or kidney function. The three-fold conversion is also reliable for assessment of anaemia in elderly patients and in patients with impaired kidney function.

ACKNOWLEDGMENTS

The authors thank the Deanship for Research at the University of Jordan for their guidance regarding the statistical analysis.

AUTHOR INFORMATION

Nosaiba Al-Ryalat, MD, Associate Professor
Saif Aldeen AlRyalat, MD, Clinician
Lna Waleed Malkawi, MD, Clinician
Hana Abu-Hassan, MD MBBS MRCGP JBFM, Assistant Professor
Osama Samara, MD, Associate Professor
Azmy Hadidy, MD FRCR, Professor

Department of Radiology and Nuclear Medicine and Department of Family Medicine, Faculty of Medicine, University of Jordan, Amman, Jordan

Correspondence: Saif Aldeen Saleh AlRyalat.
P.O. Box no.1669 Tela Al Ali 11953 Amman, Jordan.
Email: Saifryalat@yahoo.com

REFERENCES

1. Billett HH. Hemoglobin and hematocrit. In: Walker HK, Hall WD, Hurst JW, editors. Clinical Methods: The History, Physical, and Laboratory Examinations. 3rd ed. Butterworth and Heinemann, Boston, 1990.
2. WHO: Nutritional anaemias: Report of a WHO Scientific Group. Geneva, World Health Organization; 1968.
3. Graitcer PL, Goldsby JB, Nichaman MZ. Hemoglobins and hematocrits: are they equally sensitive in detecting anemias? *Am J Clin Nutr* 1981; 34: 61-64.
4. Quinto L, Aponte JJ, Menendez C, Sacarlal J, Aide P, Espasa M, et al. Relationship between haemoglobin and haematocrit in the definition of anaemia. *Trop Med Int Health* 2006; 11: 1295-1302.
5. Nkrumah B, Nguah SB, Sarpong N, Dekker D, Idriss A, May J, et al. Hemoglobin estimation by the HemoCue® portable hemoglobin photometer in a resource poor setting. *BMC Clin Pathol* 2011; 11: 5.
6. Carneiro IA, Drakeley CJ, Owusu-Agyei S, Mmbando B, Chandramohan D. Haemoglobin and haematocrit: is the threefold conversion valid for assessing anaemia in malaria-endemic settings? *Malar J* 2007; 6: 67.
7. World Health Organization. Haemoglobin concentrations for the diagnosis of anaemia and assessment of severity. 2011. Download from: http://www. who. int/vmnis/indicators/ haemoglobin. pdf. 2015.
8. Herzog B, Felton B. Hemoglobin screening for normal newborns. *J Perinatol* 1994; 14: 285-289.
9. World Health Organization. Iron deficiency anaemia: assessment, prevention and control: a guide for programme managers. 2001.
10. Rodriguez-Morales AJ, Sánchez E, Arria M, Vargas M, Piccolo C, Colina R, et al. Haemoglobin and haematocrit: the threefold conversion is also non valid for assessing anaemia in Plasmodium vivax malaria-endemic settings. *Malar J* 2007; 6: 166.
11. Cappellini MD Motta I. Anemia in clinical practice—definition and classification: does hemoglobin change with aging?. *Semin Hematol* 2015; 52: 261–269.
12. Hsu CY, Bates DW, Kuperman GJ, Curhan GC. Relationship between hematocrit and renal function in men and women. *Kidney Int* 2001; 59: 725-731.
13. Lee SJ, Stepniewska K, Anstey N, Ashley E, Barnes K, Binh TQ, et al. The relationship between the haemoglobin concentration and the haematocrit in Plasmodium falciparum malaria. *Malar J* 2008; 7: 149.
14. Beutler E, West C. Hematologic differences between African-Americans and whites: the roles of iron deficiency and alpha-thalassemia on hemoglobin levels and mean corpuscular volume. *Blood* 2005; 106: 740-745.

The Editors have identified three author errors in Figures 1a and 1b at the time of going to print. Figure 1a, cHg for the key should read cHb and on the x-axis cHg should also read cHb. In Figure 1b on the y-axis cHg should read cHb.

CASE STUDY

Fine needle aspiration cytology of mammary analogue secretory carcinoma of the parotid.

Sharda Lallu, Sarla Naran and Peter Bethwaite

Department of Anatomic Pathology, Wellington Southern Community Laboratories

ABSTRACT

We described the cytomorphological features of mammary analogue secretory carcinoma (MASC) on fine needle aspiration (FNA) cytology in a 38 year old male who presented with a slow growing solitary mass within the superficial aspect of the left parotid gland. There were no associated symptoms or previous history of tumour. FNA of the parotid mass revealed a cellular sample composed of a double cell population with clusters of columnar to polygonal cells with low-grade features and groups of large polygonal cells with high-grade features. There were intermixed prominent blood vessels, occasionally traversing the tumour cell groups.

Lesional cells were positive for vimentin, several cytokeratins [AE1/AE3, CAM 5.2, CK7, CK5/6], EMA, S100 and negative for melanoma markers on immunohistochemical staining. An initial working diagnosis of squamous cell carcinoma was favoured. On subsequent biopsy, the tumour was diagnosed as MASC with the aid of molecular and immunohistochemical studies.

Key words: Mammary analogue secretory carcinoma; parotid; fine needle aspiration; ETV6-NTRK3.

N Z J Med Lab Sci 2018; 72: 22-25

INTRODUCTION

Mammary analogue secretory carcinoma (MASC) is a recently classified entity with unique genetic characteristics of a ETV6-NTRK3 fusion gene, identical to that found in secretory breast carcinoma (1-9). MASC also shares immunohistochemical and histologic features with secretory breast carcinoma (10). Both tumours are strongly positive for S100 protein, vimentin, mammaglobin and cytokeratin markers (10). Histologically both are composed of neoplastic cells arranged in solid, microcystic and papillary patterns associated with intraluminal secretions. These features overlap with those of other salivary gland neoplasms such as pleomorphic adenoma, low-grade mucoepidermoid carcinoma, myoepithelial carcinoma, low-grade cribriform cystadenocarcinoma, oncocytic carcinoma, salivary duct carcinoma, and acinic cell carcinoma.

In this report, we review the cytohistologic features, immunohistochemical and molecular profile of MASC, emphasising those useful in making the diagnosis and distinguishing MASC from the other salivary gland tumours.

CASE REPORT

A 38 year old male presented with a slow growing solitary (11 mm diameter) mass within the superficial aspect of the left parotid gland. There were no other surrounding lesions. The remaining salivary glands were normal. There was no cervical lymphadenopathy and CT imaging showed no abnormality in the pharynx, skull base, larynx, thyroid gland, axilla, mediastinum, lung, liver, or abdomen. Fine needle aspiration (FNA) of the left parotid mass was performed to support the clinical impression of a pleomorphic adenoma. Based on an FNA diagnosis of malignancy a superficial left parotidectomy was performed and subsequently a left preauricular and left neck lymph node excision for diagnostic clarity of tumour type.

MATERIALS AND METHODS

The FNA sample was collected in Surepath preservative (BD Diagnostics TriPath, Burlington, NC) for liquid-based thin-layer preparation. The aspirate sample was spun at 600 rcf for 5 minutes. From the sediment, a Surepath slide was prepared and stained (Papanicolaou) using the SurePath Prepstain machine. The remainder of the sediment was used to make a cell block, fixed in 10% formalin, embedded in paraffin, routinely processed and stained with Hematoxylin-Eosin (H & E).

Immunohistochemical studies were carried out on the cell block sections using antibodies to CK AE1/AE3, CK5/6, Cam 5.2, CK7, S100, Melan A, HMB45, EMA, vimentin, CEA and Alpha 1AT. PAS and PASD cytochemistry were also performed on cell block sections.

Immunohistochemical studies were carried out on the biopsy sections using antibodies to CK8/18, CK7, S100, SOX10, CK5/6, GATA3, GCDFP-15, CD117, p40, SMA, AR, ER, PR, DOG1, Her2. Mucicarmine cytochemistry staining was also performed on histology sections.

RESULTS

Cytological findings

The Surepath and cell block preparations were hypercellular including macrophages, groups of normal salivary gland acinar cells, many dissociated and clustered uniform columnar to polygonal cells with basophilic cytoplasm, round to oval bland nuclei with fine chromatin, and inconspicuous nucleoli. Some cells with plasmacytoid appearances were present. In addition, there were admixed groups of polygonal tumour cells with abundant cytoplasm showing high-grade nuclear feature including enlarged pleomorphic nuclei, abnormal chromatin, and centrally located prominent nucleoli. There were prominent blood vessels in the background, occasional vessels traversing the tumour cell groups (Figures 1a and 1c). Occasional cells demonstrated intracytoplasmic vacuole formation, or intranuclear inclusions (Figure 1b), and focal cytoplasmic haemosiderin pigment mimicking melanin. No matrix material or stromal tissue was present. Based on morphologic features, an initial diagnosis of metastatic malignant melanoma was favoured and immunohistochemical stains were requested on the cell block material.

Immunohistochemical findings

Immunohistochemical staining on the cell block material showed the tumour cells to be positive for vimentin, CKAE1/AE3, EMA, CAM 5.2 and CK7. They were focally positive for CK5/6 and weakly positive for S100 protein. The tumour cells were negative for Melan A, HMB45, SMA, A1AT, GFAP, CD117 and CEA. The basophilic mucin stained positive with Periodic Acid Schiff and Periodic Acid Schiff Diastase (Figure 1d). Based on the initial immunophenotype, a diagnosis of squamous cell carcinoma was favoured.

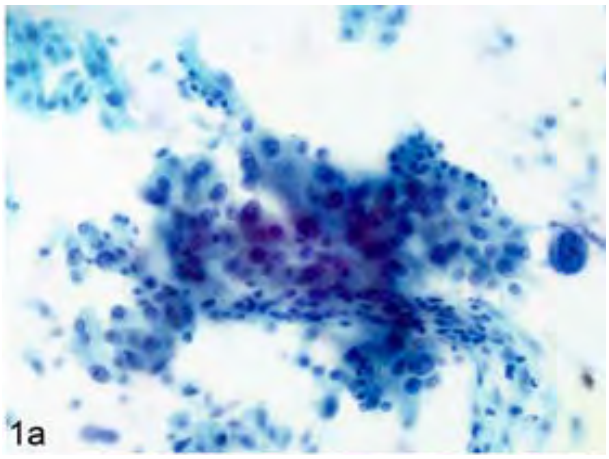


Figure 1 a. Surepath preparations showing groups of cells with low-grade and high-grade nuclear features and blood vessels traversing the tumour cell groups (Papanicolaou stain x 400).

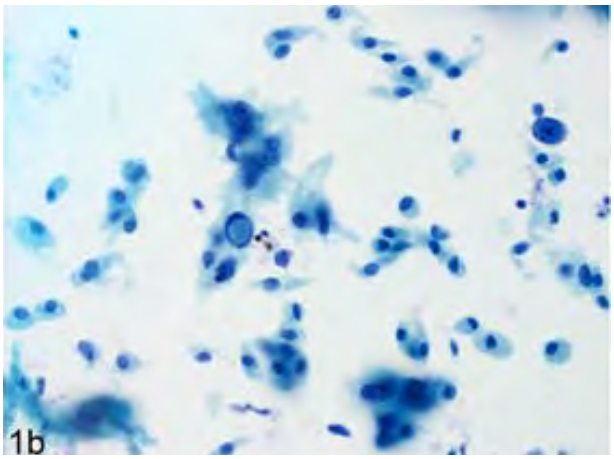


Figure 1 b. Surepath preparations showing rare cells with intranuclear inclusions (Papanicolaou stain x 400).

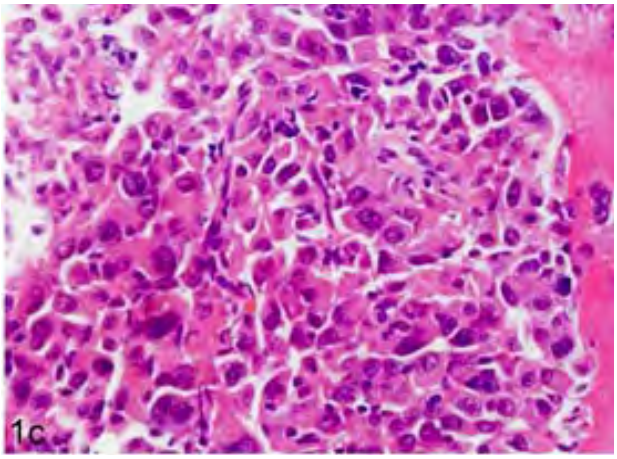


Figure 1 c. Cell block preparations showing groups of cells with low-grade and high-grade nuclear features (Haematoxylin-eosin stain x 400).

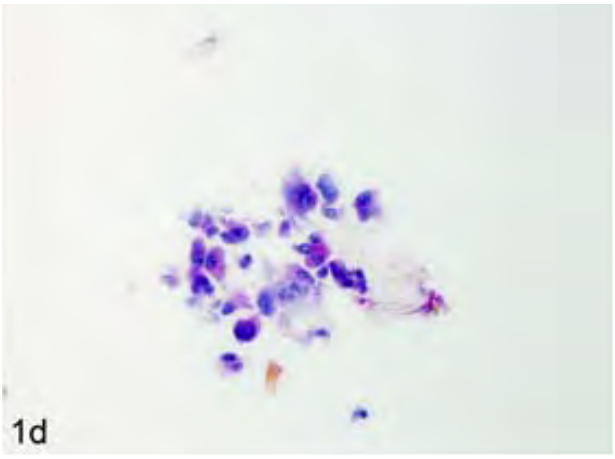


Figure 1 d. Histochemical stain on cell block section, cells showing positive staining for PASD (PASD stain x 400).

Histologic findings

On gross examination the tumour was ovoid firm, tan-yellow in colour, 11 x 11 x 8 mm in size. H & E stained sections showed parotid gland parenchyma with parotid-associated lymphoid tissue surrounded by fibroadipose tissue. The parotid was subtly infiltrated by a well circumscribed but non-encapsulated neoplasm. The tumour had a solid, largely microcystic, pattern (Figure 2a) associated with evident basophilic mucin. The tumour cells showed ovoid nuclei, discernible nucleoli, occasional intranuclear inclusions, scant mitotic activity, and amphophilic cytoplasm. Similar to the cytology sample there were admixed large neoplastic cells with pleomorphic nuclei and evident nucleoli. The neoplastic nodules were variably surrounded by a conspicuous rim of basal cells and set within a fibroconnective matrix. Fibrosis, chronic inflammation and hemosiderin deposition was identified. No tumour was identified in the lymph node sampling.

Immunohistochemical staining showed the lesional cells were positive for CK 8/18, CK7, S100, GATA3 (diffusely weak), SOX10 (Fig 2c, 2d), CK 5/6, p40 (patchy) and were negative for GCDFP-15, CD117, SMA, AR, ER, PR, Her2 and DOG1. The proliferative index by KI-67 staining was 20%. The rim of basal cells expressed P40 and CK5/6. The basophilic mucin weakly stained with mucicarmine stain (Figure 2b).

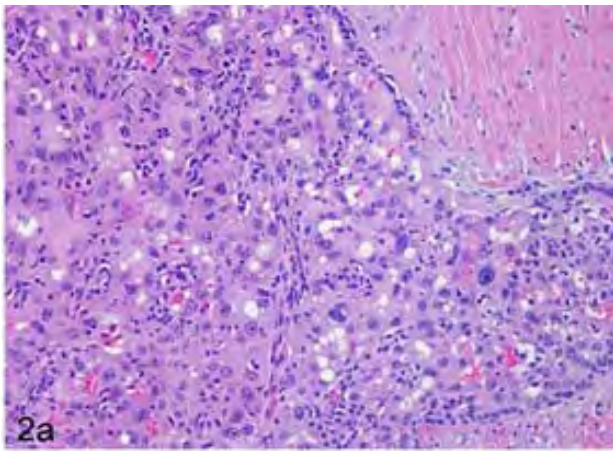


Figure 2 a. Section of parotidectomy specimen showing the tumour with microcystic pattern, admixed low-grade, high-grade nuclear features (Haematoxylin-eosin stain x 200).

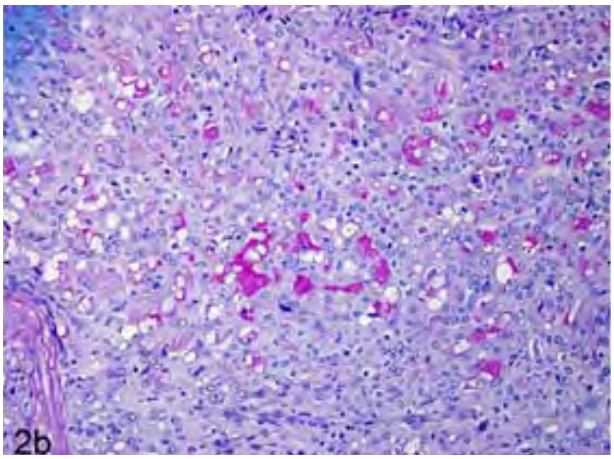


Figure 2 b. Histochemical stains on sections of parotidectomy specimen, secretions within cystic space showing positive staining for mucicarmine (Mucicarmine stain x 200).

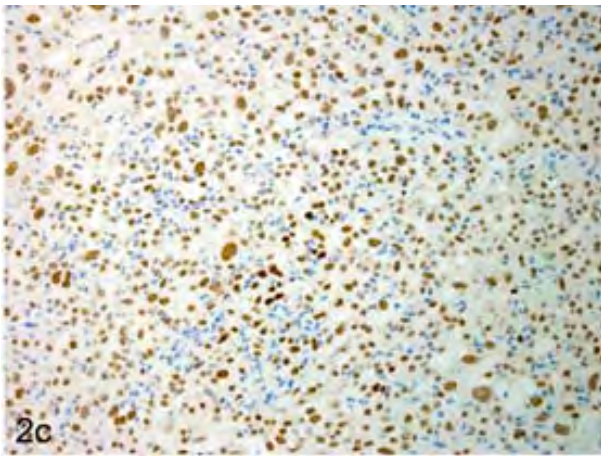


Figure 2 c. Immunohistochemical stain on section of parotidectomy specimen, tumour cells, showing nuclear positive staining for GATA3 (GATA3 x 200).

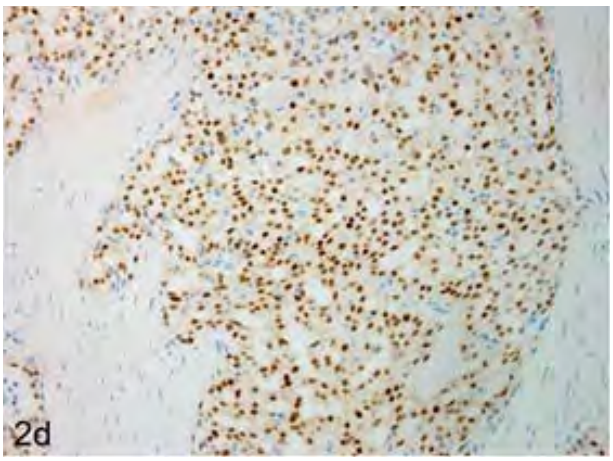
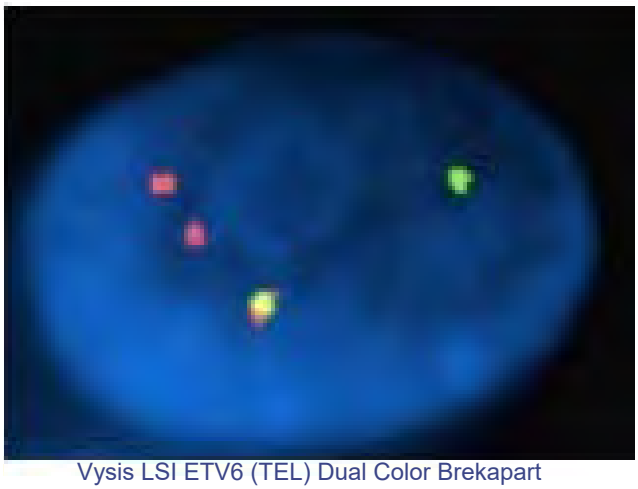


Figure 2 d. Immunohistochemical stain on section of parotidectomy specimen, tumour cells showing nuclear positive stains for SOX10 (SOX10 x 200).

Molecular analysis

ETV6 (NTRK 3) analysis was performed at IGENZ, Auckland on formalin-fixed paraffin-embedded tissue sections. Fluorescence in-situ hybridization (FISH) testing revealed gene rearrangement of ETV6 (12p13) in all 100 cells examined (Figure 3).



Vysis LSI ETV6 (TEL) Dual Color Brekapart

Figure 3. ETV6 FISH analysis for the 12q13 cytogenetic location showing positive translocation.

DISCUSSION

Mammary analogue secretory carcinoma (MASC) is a recently classified tumour first described in 2010 by Skalova *et al.* which harbours a t(12;15) (p13;q25) translocation, resulting in the ETV6-NTRK3 gene fusion (2,4,9). The same cytogenetic abnormality is described in secretory carcinoma of the breast (SCB) (10). The resultant fusion protein ETV6-NTRK3 has transforming activity, not only in epithelial but also in mesenchymal and blood cell lineages (7). Previously, the ETV6-NTRK3 translocation has been described in infantile fibrosarcoma (11), congenital mesoblastic nephroma, and acute myelogenous leukaemia (2,7,8). ETV6 is genetically unstable and fuses not only with NTRK3 but also with other genes, including ABL1, EGFR3, PAX5, SYK and JAK2 in leukaemia, myelodysplastic syndromes, and sarcomas (7). Therefore MASC and SCB also share immunohistochemical features including positivity for S100 protein, EMA, and vimentin, while being (ER, PR, Her2) “triple negative”(13).

MASC is more common in males, with a male: female ratio of 1.5: 1 occurring predominantly in the parotid gland followed by the oral cavity (lip, soft palate and buccal mucosa), and submandibular gland (3-8,12,13). The lesion typically presents as a painless, slow growing mass. Grossly, all the tumours are circumscribed mimicking salivary gland adenoma (5).

On cytological preparation, MASC have been reported as variably cellular with two different architectural patterns, including sheets of tumour cells arranged in papillary, cystic, tubular, solid growth arrangements (4,5,12,13), and dispersed dissociated cells. These cells contained abundant cytoplasm and round nucleus pushed to periphery giving a plasmacytoid appearances with nuclear atypia and pleomorphism. Tumour cells with intracytoplasmic or intraluminal mucin have been reported to be positive with PAS (diastase resistant) and Mucicarmine (3,6,13). Our case contained many abnormal dissociated cells with plasmacytoid appearances, multinucleation, predominant nucleoli, intranuclear inclusions, and pigment, which raises the possibility of metastatic melanoma. In our case melanoma markers were negative, but cells expressed cytokeratins AE1/AE3 and CK5/6 suggesting squamous differentiation. We did not perform more immunohistochemistry and suggested histological assessment for further classification.

The cytopathologic differential diagnosis of MASC includes many low-grade epithelial neoplasms including pleomorphic adenoma, low-grade mucoepidermoid carcinoma, acinic cell carcinoma, myoepithelial carcinoma, low-grade cribriform cystadenocarcinoma, oncocytic carcinoma, and salivary duct carcinoma (2-9).

Pleomorphic adenoma and MASC contain bland cells and vacuolated cells but we did not observe typical chondromyxoid stroma and fibrillary extracellular matrix admixed with spindle myoepithelial cells, as seen in pleomorphic adenoma. Additionally, the tumour cells were negative for GFAP making pleomorphic adenoma unlikely. Low-grade mucoepidermoid carcinoma and MASC share the features of hypocellular samples, vacuolated cytoplasm, macrophages, and proteinaceous material in the background. MASC does not have the intimate mixture of epidermoid cells, intermediate cells and mucinous cells that characterise mucoepidermoid carcinoma. Also, a lack of squamoid areas with intercellular bridges and/or basal like intermediate cells support a diagnosis of MASC. Mucoepidermoid carcinoma often harbours at (11;19) translocation resulting in CRTC1-MAML2 fusion transcript (3).

Acinic cell carcinoma may be distinguished by DOG1 positivity. Myoepithelial carcinoma will show positivity for smooth muscle actin. Oncocytic carcinoma, salivary duct carcinoma, and mucoepidermoid carcinoma all are SOX10 negative. Metastatic clear renal cell carcinoma could be considered, given the low-grade appearance with clear cells and histiocyte-like cells observed in MASC. Additionally, CD10 and PAX8 immunostains on cell block material would help to resolve the differential diagnosis.

GCDFP-15 or BRST-2 has variable staining strength. In the study by Skalow *et al.* eight out of eleven samples were positive, while others have found positive GCDFP-15 staining restricted to a minority of cases (14). Our case was negative for GCDFP-15. The proliferative index of our case is in keeping with the majority of studies which demonstrate a low proliferation index by Ki67 staining (5-28%) (14). Most studies have detected negative staining for calponin, CK15, smooth muscle actin, ER, PR, HER2, and CK5/6 (14). Our case was positive for CK5/6 and a weak expression of GATA3. p63 is generally negative in tumour cells; however, Chiosa *et al.* found that 28% of MASC cases had positive staining for peripherally located entrapped non-tumour basal cells, which could represent intraductal extension or possibly a ductal epithelial origin for this tumour (14,15).

CONCLUSIONS

We have presented the typical cytologic features of a relatively recently recognised salivary gland tumour which displays cytological, histologic, immunophenotypic, and genetic features similar to secretory breast carcinoma. MASC and secretory breast carcinoma share a t (12;15) (p13; q25) translocation and both are positive for S100 protein and GATA3, but with a “triple negative” (ER/PR/Her2) phenotype. FNA is a common investigation in the workup of salivary gland tumours and it is important to recognise the cytoarchitectural features of this tumour to avoid confusion from other primary or secondary tumours at these sites. Failure to appreciate these features initially led us to an erroneous diagnosis of metastatic squamous cell carcinoma and the true diagnosis was revealed only on subsequent definitive surgery.

ACKNOWLEDGEMENT

The authors acknowledge Louise Goossens for her excellent photographic assistance.

AUTHOR INFORMATION

Sharda Lallu, BSc CT(FIAC), Cytotechnologist
Sarla Naran, BSc CT(FIAC), Cytotechnologist
Peter Bethwaite, MBChB PhD FFSc(RCPA) FRCPA, Cytopathologist

Department of Anatomic Pathology, Wellington Southern Community Laboratories

Author for correspondence: Dr Peter Bethwaite
Email: Peter.Bethwaite@wellingtonscl.co.nz

REFERENCES

1. Skálová A, Vanecek T, Sima R, Lako J, Weinreb I, Perez-Ordonez B, et al. Mammary analogue secretory carcinoma of salivary glands containing the ETV6-NTRK3 fusion gene: a hitherto undescribed salivary gland tumour entity. *Am J Surg Pathol* 2010; 34: 599-608.
2. Bishop JA, Yonescu R, Batista DA, Westra WH, Ali SZ. Cytopathologic features of mammary analogue secretory carcinoma. *Cancer Cytopathol* 2013; 121: 228-233.
3. Levine P, Fried K, Krevitt LD, Wang B, Wenig BM. Aspiration biopsy of mammary analogue secretory carcinoma of accessory parotid gland: another diagnostic dilemma in matrix-containing tumors of the salivary glands. *Diagn Cytopathol* 2014; 42: 49-53.
4. Takeda M, Kasai T, Morita K, Takeuchi M, Nishikawa T, Yamashita A, et al. Cytological features of mammary analogue secretory carcinoma - review of literature. *Diagn Cytopathol* 2015; 43: 131-137.
5. Oza N, Sanghvi K, Shet T, Patil A, Menon S, Ramadwar M, et al. Mammary analogue secretory carcinoma of parotid. Is preoperative cytological diagnosis possible? *Diagn Cytopathol* 2016; 44: 519-525.
6. Bajaj J, Gimenez C, Slim F, Aziz M, Das K. Fine -needle aspiration cytology of mammary analogue secretory carcinoma masquerading as low-grade mucoepidermoid carcinoma: case report with a review of the literature. *Acta Cytol* 2014; 58: 501-510.
7. Majewska H, Skálová A, Stodulski D, Klimková A, Steiner P, Stankiewicz C, et al. Mammary analogue secretory carcinoma of salivary glands: a new entity associated with ETV6 gene rearrangement. *Virchows Arch* 2015; 466 : 245-254.
8. Pinto A, Nosé V, Rojas C, Fan YS, Gomez-Fernandez C. Searching for mammary analogue secretory carcinoma of salivary gland among its mimics: *Mod Pathol* 2014: 27: 30-37.
9. Salat H, Mumtaz R, Ikram M, Din NU. Mammary analogue secretory carcinoma of the parotid gland: A third world country perspective-a case series. *Case Rep Otolaryngol* 2015; 2015: 697254.
10. Skálová A. Mammary analogue secretory carcinoma of salivary gland origin: an update and expanded morphologic and immunhistochemical spectrum of recently described entity. *Head Neck Pathol* 2013; 7 Suppl 1: S30-S36.
11. Bourgeois JM, Knezevich SR, Mathers A, Sorensen PH. Molecular detection of the ETV6-NTRK3 gene fusion differentiates congenital fibrosarcoma from other childhood spindle cell tumors. *Am J Surg Pathol* 2000; 24: 937-946.
12. Stevens TM, Kovalovsky AO, Velosa C, Shi Q, Dai Q, Owen RP, et al. Mammary analogue secretory carcinoma, low-grade salivary duct carcinoma, and mimickers: a comparative study. *Mod Pathol* 2015; 28: 1084-1100.
13. Jackson BS, Pratt TL, Van Rooyen A. Mammary analogue secretory carcinoma: a rare salivary gland tumour. *S Afr Med J* 2017; 107: 304-306.
14. Sethi R, Kozin E, Remenschneider A, Meier J, VanderLaan P, Faquin W, et al. Mammary analogue secretory carcinoma: update on a new diagnosis of salivary gland malignancy. *Laryngoscope* 2014; 124: 188-195.
15. Chiosea SI, Griffith C, Assaad A, Seethala RR. Clinicopathological characterization of mammary analogue secretory carcinoma of salivary glands. *Histopathology* 2012; 61: 387-394.

CASE STUDY

Cephalosporin interference in the Jaffe creatinine method: a case study

Natasha Dutt and Samarina Musaad
Labtests, Auckland

ABSTRACT

This is a case of apparent acute kidney injury (AKI) due to interference with the Jaffe creatinine method. In spite of improvements in the Jaffe assay it is prone to interferences that may lead to erroneous results. Inaccurate creatinine results lead to incorrect estimation of the glomerular filtration rate (eGFR) which is the hallmark of assessing kidney function and detecting AKI. Therefore, scientists need to be aware of the circumstances that can jeopardise analytical analysis particularly when it comes to specific filtration markers, such as creatinine.
Key words: eGFR, kidney failure, interference, creatinine, analytical variables, Jaffe assay.

N Z J Med Lab Sci 2018; 72: 26-28

INTRODUCTION

Creatinine is a waste product of muscle that is normally filtered from the blood by the kidneys. Urea is a waste product of protein metabolism that is also removed by the kidneys. Both increase when kidney function is compromised although urea is less specific to kidney dysfunction because it can also increase under other conditions e.g. gastrointestinal bleeding (1). Nevertheless blood creatinine concentration along with blood urea concentration have historically been used as first-line tests to diagnose and monitor kidney disease (2).

When kidney function is compromised, creatinine is not filtered leading to high blood creatinine levels. However, blood creatinine alone does not accurately reflect kidney function in some clinical situations as it is influenced by several pre-analytical factors associated with muscle mass such as age e.g. it can be low in the frail elderly in spite of impaired kidney function, sex e.g. it is lower in females compared to males, race e.g. it is higher in Africans because of a physiologically higher muscle mass compared to Caucasians, and body size e.g. it is very low in children and neonates because of their small muscle bulk, and haemodynamics e.g. it is lower in pregnancy than the non-pregnant state because of increased filtration (3). Thus, creatinine has high specificity but is not a sensitive marker for kidney function.

Elevated creatinine in muscular individuals can incorrectly suggest kidney injury while the low or normal creatinine concentration in thin individuals can mistakenly suggest healthy kidney function (4). For instance, patients with a relatively lower muscle mass can have a serum creatinine concentration within normal reference range, although their renal function is severely compromised (5). Furthermore, an increased serum creatinine is suggestive of kidney damage but only occurs after a significant proportion of renal function has already been lost i.e. there is a lag phase in which creatinine levels are “normal” but the kidneys are deteriorating (5).

The rate of glomerular filtration is a marker for how effectively the filtering units, nephrons, filter (remove) unwanted material over a period of time (1). The relationship between serum creatinine is inversely proportional to eGFR, which starts to drop before creatinine significantly rises in the early stages of injury, proving eGFR calculations to be more sensitive than creatinine alone (4).

This is because eGFR includes other factors apart from creatinine that influence kidney function namely age, sex. One of the most important tools for measuring renal function is the eGFR. eGFR helps screen for and diagnose kidney injury and monitor the progression of chronic kidney disease (CKD) (5). CKD is defined as an eGFR less than 60 mL/min/1.73m^2 for over three months (6).

Several equations are now widely used to indirectly measure eGFR using serum creatinine concentration. The historical timeline starts from the Cockcroft-Gault (CG) equation followed by the Modification of Diet in Renal Disease (MDRD), then the *modified* MDRD (with a factor for creatinine measurement) equation when creatinine measurement became standardised, and finally the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equations (7). CG equation is not often used in routine clinical practice because it requires a patient’s weight, which is not usually provided in request forms and can be a labile (5). The main reason for the continued need for the CG equation is that most recommendation for drug dosing use it hence pharmacists still rely on it heavily (6).

The MDRD equation provides an eGFR based on serum creatinine concentration, age, sex and is normalised adult body size of 1.73m² (3). Ideally it includes the patient’s ethnicity but this is another parameter that is not usually provided on request forms and hence the equation in use does not factor it in (6). Because MDRD does not require a measurement of body weight or height, laboratories can report eGFR using information that is routinely provided. A limitation of the MDRD (and its modified version) was that it under estimated kidney function in healthy individuals (5). The reason for that was that the population used to generate the equation was mostly unwell. The CKD-EPI equation was found to be more accurate than the MDRD equation for patients with an eGFR of more than 60 mL/min/1.73m^2 i.e. for healthy people, and for females and younger people (6). CKD-EPI also factors in creatinine, age and sex. Nonetheless, improvements and further modification of the formulas need to be researched for better refining eGFR (8). Consequently, according to Australasian eGFR Consensus reporting eGFR using CKD-EPI equation is recommended and

became standard in most laboratories in Australasia, all laboratories in New Zealand use the CKD-EPI equation (7).

eGFR is done through computer-based calculations. When the analysers connect to laboratory information system (LIS), the test results of serum creatinine can automatically generate eGFR (5). The automation of eGFR enables a high workload and effective reporting of the results for better patient management. This also reduces potential errors of manually calculating the eGFR (8).

Even though eGFR has proven to be one of the best single measures for kidney function it has its limitations that are common to all equations. These limitations include all pre-analytical, analytical and post-analytical challenges that creatinine measurement introduces; their lack of suitability for use in pregnant women, children and in the unstable patient; and their limited application to some ethnicities. It is prudent to interpret eGFR against the backdrop of these limitations (6).

CASE STUDY

The purpose of this case study was to investigate a sudden drop in eGFR with a high creatinine concentration of 873 umol/L.

Mr X was a 70-year-old male patient with hypertension, CKD, longstanding emphysema, chronic sinusitis, gastroesophageal reflux disease, and diverticulitis. He had no history of recent surgery. He did not have diabetes mellitus. He was a lifelong smoker who quit smoking in 2004. A computed tomography scan in 2014 showed severe emphysema with mild sub-plural fibrosis. The emphysema was treated with salbutamol sulphate, fluticasone and tiotropium bromide; drug inhalation medication for chronic obstructive pulmonary diseases (9). His longstanding hypertension and emphysema probably contributed to his CKD (5,10). He was prescribed the antihypertensive medication, doxazosin mesylate (11). Mr X's eGFR had been in the 60-78 ml/min/1.73m^2 range for at least the previous 8 years.

Laboratory findings

On 4th December 2011, upon routine monitoring, Mr X was found to have an eGFR of 5 mL/min/1.73m^2, an approximate 93% reduction from his usual eGFR. This drop was immediately communicated to his General Practitioner who admitted him to the hospital the same day for assessment. Table 1 summarises Mr X's results. A repeat renal profile demonstrated a return of the eGFR to the patient's baseline. The patient was discharged within hours.

Table 1. Timeline of renal function tests

	RR	Lab A	Lab A	Lab B	Lab A	Lab B	Lab A	Lab A
Date		**25/5/10**	**4/2/11**	**21/9/11**	**4/12/11**	**4/12/11**	**26/9/12**	**21/3/13**
Sodium mmol/L	135-145	138		143	**164**	144	140	140
Potassium mmol/L	3.5-5.2	4.8		4.3	3.9	4.2	4.8	4.5
Creatinine umol/L	60-105	102	86	89	**873**	87	99	87
eGFR mL/min/ 1.73m^2	> 90	**64**	**78**		**5**		**66**	**76**

RR= reference range.

The Jaffe assay, a colorimetric assay used for measuring creatinine, is prone to interferences due to glucose, ketoacids, albumin, and by antibiotics like cefoxitin (cephalosporin) and streptomycin, and ascorbic acid, that provide an inaccurate figure for creatinine (1). All these possible interferences can potentially interfere with alkaline picrate to give false increase in serum creatinine (4). Different companies have attempted to modify the reaction to minimise interferences but they have never been totally eliminated. However, creatinine remains the most convenient marker at the present time and evidence has demonstrated that it can be used to calculate eGFR (5). Enzymatic assays may be used instead of the Jaffe method to avoid the effect of most interferences such as bilirubin but they are less available and usually more expensive than Jaffe technology (3).

DISCUSSION

As demonstrated in Table 1, on the same day there were different test results obtained from two different laboratories. Inter-laboratory differences, in addition to pre-analytical, analytical and post-analytical errors, should be considered for Mr X's discrepant results. Possibilities of pre-analytical errors include sample mix up or contamination from a urine sample leaking if placed in the same bag as the blood sample. Consuming a high protein meal before a blood test is a patient related factor which may have played an important role in the increase in serum creatinine and drop in eGFR in Mr X's case.

The analytical phase may also be prone to inaccuracy due to interference in the assay as stated above. Furthermore, the technician or scientist needs to monitor and check the analyser for the results for calibration and quality control abnormalities as inaccuracies in creatinine measurement reflect on the eGFR calculation.

Post-analytical phase may also be considered to see if there were any information technology (IT) errors as transcription errors are unlikely to occur in fully automated laboratories.

Hypertensive kidney damage happens over time and so cannot explain the sudden drop in eGFR. Hypertension contributes significantly to kidney failure by scarring and narrowing of the blood vessels in the kidneys (1). If the kidneys blood pressure is impaired, the normal homeostasis of the kidney is at risk causing glomerular diseases (12).

AKI seemed the most likely explanation assuming the sudden drop in eGFR was genuine and not due to an analytical error. AKI is defined as a drop in eGFR within a short time frame and is potentially reversible condition and timely intervention is warranted (13).

After the discrepancy in creatinine measurement between the laboratories was discovered and the investigation for apparent interferences or errors did not reveal any, the test requestor was contacted who subsequently discussed the circumstances with the patient. The patient reported recently seeing another doctor.

The flawed diagnosis was due to interference of cephalosporin with the Jaffe creatinine method at Lab A with abnormally high creatinine level and low eGFR due to cephalosporin falsely increasing creatinine measurements (4). The test requestor was unaware of the antibiotic prescription and unaware of the effect cephalosporins can have on the Jaffe method.

It was noted that cephalosporin was given intravenously a couple of hours before his blood test. The antibiotic was prescribed by a locum doctor and was not communicated to the patients' General Practitioner who had requested the renal function test months in advance as part of routine monitoring. A few more hours had passed by the time the patient was re-tested in hospital, reducing the potential effect of the antibiotic on the laboratory Jaffe method. Furthermore, different platforms using the same basic method have different assay modifications and it is possible that in this case the hospital laboratory platform demonstrated less interference with cephalosporins.

Limitation

While clinical notes did not suggest the presence of interfering substances, such as high glucose, ketones, or ascorbic acid, it would have been ideal to test for them. However, by the time the investigation was underway the sample was aging and its volume was insufficient for such testing.

This case report presents findings for one patient but there are probably other similar scenarios in our everyday practice that go undetected. Different platforms do not have identical clinical results for the same analyte. This is because laboratories are different in terms of the methods used on different machines, differences in modifications (often patented technology) for the same method on different machines, in addition to differences in processes and operators; all these factors result in slightly different results (14). In case of known interfering agents, like antibiotics with the Jaffe method, it may be worth considering to what degree the blood antibiotic level, in this case a cephalosporin, interferes in either platform and quantitate the change in concentration with time elapsed after a specified dose. This may help clinicians choose a suitable platform if their patients are on cephalosporins (or streptomycin).

Cystatin C has been proposed as a potential alternative for serum creatinine as a filtration marker. Most studies show that serum levels of cystatin C are not affected by muscle mass and more closely correlated with GFR than serum creatinine. However, comparison studies between two markers are still ongoing to ensure its accuracy (15).

CONCLUSION

This case study highlights the significance of assay interferences that affect creatinine measurement using the Jaffe method that reflect on the eGFR calculation; an important limitation of eGFR. It is important to be aware of pre-analytical and analytical errors, and interferences that can jeopardise analysis when it comes to specific filtration markers, such as creatinine. Further studies need to be carried out to investigate what blood level with varying amounts of cephalosporin can affect the Jaffe assay.

ACKNOWLEDGMENTS

The authors acknowledge laboratories A and B test results that contributed to this case study by helping justify the reasons for writing this case study.

AUTHOR INFORMATION

Natasha Dutt, BMLS, Medical Laboratory Scientist
Samarina Musaad, PGDipPH FAACB FRCPA, Chemical Pathologist
Department of Biochemistry and Immunology, Labtests, Auckland

Corresponding author: Natasha Dutt.
Email: duttnatasha@yahoo.co.nz

REFERENCES

1. Burtis CA, Ashwood ER, Bruns DE. Tietz Textbook of Clinical Chemistry and Molecular Diagnostics. 5th Edition. Elsevier Health Sciences; 2012.
2. Kaplan LA, Pesce, A, Kazmierczak, S. Clinical Chemistry: Theory, Analysis, Correlation. Mosby; 2003.
3. Jones GR. Estimating renal function for drug dosing decisions. *Clin Biochem Rev* 2011; 32: 81-88.
4. Peake M, Whiting M. Measurement of serum creatinine–current status and future goals. *Clin Biochem Rev* 2006; 27: 173184.
5. Johnson DW, Jones GR, Mathew TH, Ludlow MJ, Doogue MP, Jose MD, et al. Chronic kidney disease and automatic reporting of estimated glomerular filtration rate: new developments and revised recommendations. *Med J Aust* 2012; 197: 222-223.
6. Traynor J, Mactier R, Geddes CC, Fox JG. How to measure renal function in clinical practice. *BMJ* 2006; 333: 733-737.
7. Anavekar N, Bais R, Carney S, Davidson J, Eris J, Gallagher M, et al. Chronic kidney disease and automatic reporting of estimated glomerular filtration rate: a position statement. *Clin Biochem Rev* 2005; 26: 81-86.
8. Wu I, Parikh CR. Screening for kidney diseases: older measures versus novel biomarkers. *Clin J Am Soc Nephrol* 2008; 3: 1895-901.
9. Kamin W, Schwabe A, Krämer I. Physicochemical compatibility of fluticasone-17-propionate nebulizer suspension with ipratropium and albuterol nebulizer solutions. *Int J Chron Obstruct Pulmon Dis* 2007; 2: 599-607.
10. Chandra D, Stamm JA, Palevsky PM, Leader JK, Fuhrman CR, Zhang Y, et al. The relationship between pulmonary emphysema and kidney function in smokers. *Chest* 2012; 142: 655-662.
11. Chung M, Vashi V, Puente J, Sweeney M, Meredith P. Clinical pharmacokinetics of doxazosin in a controlled-release gastrointestinal therapeutic system (GITS) formulation. *Br J Clin Pharmacol* 1999; 48: 678-687.
12. Dorgalaleh A, Mahmudi M, Tabibian S, Khatib ZK, Tamaddon GH, Moghaddam ES, et al. Anaemia and thrombocytopenia in acute and chronic renal failure. *Int J Hematol Oncol Stem Cell Res* 2013; 7: 34-39.
13. Basile DP, Anderson MD, Sutton TA. Pathophysiology of acute kidney injury. *Compr Physiol* 2012; 2: 1303-1353.
14. Miller WG, Myers GL, Ashwood ER, Killeen AA, Wang E, Thienpont LM, et al. Creatinine measurement: state of the art in accuracy and interlaboratory harmonization. *Arch Pathol Lab Med* 2005; 129: 297-304.
15. Stevens LA, Coresh J, Schmid CH, Feldman HI, Froissart M, Kusek J, et al. Estimating GFR using serum cystatin C alone and in combination with serum creatinine: A pooled analysis of 3,418 individuals with CKD. *Am J Kidney Dis* 2008; 51: 395-406.

Science Digest

Contributed by Michael Legge

A new approach to tissue analysis

Traditional histology approach to tissue preparation and analysis is both labour and time consuming. A research group in Texas has developed a non-destructive technique for cancer diagnosis using a modified mass spectroscopy system (1). The researchers have developed a mass spectrometer probe in the form of a pen, which is connected to the analyzer. The pen is used to touch the tumour and identifies potential cancer biomarkers and could be used for identifying tumour margins. The biomarkers relate to metabolites such as lipids and proteins with individual tumours having their own metabolic signature. An in-vivo trial using mice demonstrated that the technique was reliable for both ex-vivo and in-vivo diagnosis. The tumours analyzed in this research were breast, lung, ovary and thyroid with the technique demonstrating high sensitivity (96.4%) and high specificity (96.2%) from the metabolic profiles.

Epstein-Barr virus (EBV) and nasopharangeal cancer

Historically EBV infections have been identified as a critical factor in the pathogenesis of nasopharengeal cancer. Although this cancer is rare in North American and European Caucasian populations it is one of the most common cancers in Chinese and South East Asian populations. Previous research has identified that regardless of the geographical population all tumour cells contain EBV DNA and that initiation of nasopharangeal cancer comes from a single clonal population of EBV-induced cellular proliferation. Early detection of nasopharengeal cancer (Stage 1) has a 94% survival rate whereas Stage IV survival is approximately 60%. This cancer is asymptomatic in the early stage, however 80% of patients present with advanced or metastatic stage at diagnosis. Research from Hong Kong has investigated the use of 'liquid biopsies' whereby EBV DNA was analyzed from plasma samples (2). Based on a sensitivity 0f 97.1% and a specificity of 98.6 % the researchers concluded that screening plasma for EBV DNA was useful for an at-risk population and that the majority of those identified with nasopharengeal cancer were identified at significantly earlier stages of the disease.

Eye metabolism – waste for sight

The retina is made up of two important cell types, rods and cones that are part of the retinal epithelium. The energy requirement of the rods and cones is very high and until recently not fully understood. Previous research has demonstrated that the retinal pigment epithelium (RPE) was very efficient at transferring glucose to the rods and cones to meet their energy demands, which was via aerobic glycolysis, which produces high lactate concentrations. This raised a question as to why retinal pigment epithelium did not use the glucose to meet its own energy demands? Recent research from the USA has demonstrated that the RPE utilizes lactate (normally regarded as a metabolic waste product) and not glucose for a preferred energy source (3). The metabolic feedback from the rods and cones with lactate suppresses the requirement of the RPE for glucose thereby supplying a high energy substrate (glucose) for vision and utilizing the metabolic product (lactate) for RPE energy requirements.

New cure for sleeping sickness

Sleeping sickness is caused by *Trypanosoma brucei gambiense* which is spread by tsetse flies. The disease is widespread through Africa especially in the poorer regions. Typically, the disease affects the central nervous system causing sleepiness during the day and insomnia at night with some people developing psychiatric symptoms. Untreated, people eventually become comatose and die. For decades, the treatment was arsenic-based drugs which killed about 5% of people being treated. More recently a new therapy NECT (niturtimox-eflornithine) made significant cure rates but was a course of pills and 14 intravenous infusions. An alternative therapy has recently been presented to the European congress on Tropical Medicine and Health (4). Oral use of a drug, fexinidazole, cured 91% of people with severe sleeping sickness (compared to 98% using NECT) and 99% who were in the early stages of the disease. Trials are underway for final approval for routine use.

Disarming host defense proteins

For pathogenic bacteria to be effective they must first overcome the host immune defense system, in particular host defense proteins. Many of these proteins signal that a pathogen is in action. New research has identified that pathogens are capable of immobilizing host defense proteins. Using *Shigella flexneri* (the causative agent of shigellosis, bacterial dysentery) the researchers investigated the mechanism for the infection. Typically, *Shigella flexneri* invades the intestinal epithelium cells causing severe inflammation, thereby disrupting cellular function. This is achieved by being encapsulated in a membrane, which disrupts once in the cell cytoplasm. The defense response is to attempt to destroy the bacteria, however the bacteria can produce proteins to immobilize the host defense proteins. The new research identified that following infection guanylate-binding proteins (GBP) were completely inactivated but other infections (*Listeria monocytogenes* and *Salmonella enteria*) were removed by the GBP (5). After testing 1300 strains of *Shigella flexneri* containing mutations of different genes, the researchers identified that an enzyme E3 ubiquitin ligase (IpaH9.8) inactivates the GBP causing it to degrade, indicating a mechanism for bacteria to avoid the host immune response.

Gene therapy for spinal muscular atrophy (SMA)

SMA is an inherited progressive motor neuron disease with variable onset, the earliest form occurring in infancy and death usually by the age of 2 when mechanical ventilation is required. It is due to a mutation in a gene on chromosome 5 coding for a protein SMN (survival of motor neuron) which is necessary for normal motor neuron function. Although there are four identified SMAs' (1-4), SMA1 is the earliest onset disease. The incidence is approximately 1 in 10000 live births. Using gene therapy information from a mouse model of SMA, researchers in the USA have used an adeno-associated viral vector with DNA coding for SMN (6). Following a single intra-venous infusion, all 15 children in the trial passed the median age for survival without ventilation, and with the exception of one, all achieved meaningful clinical motor function which was maintained at the age of 2 years. While the gene therapy indicates some success, the researchers caution that long-term durability and safety are still unknown factors at this stage.

The dangers of mail order DNA

Small pox is now considered a disease that has been eradicated since 1980. Researchers in Canada have reported that it would be possible to bring back an extinct virus (7). In their research, they synthesized the horse pox virus, which is believed to no longer exist. Using this technology, it is proposed

that the small pox virus could be synthesized using 'mail order DNA'. They purchase a series of overlapping DNA fragments from an overseas company then synthesized the 212000 base-pair horse pox virus in culture. The researchers claim that this technology was not difficult and has raised concerns about biosecurity. However, a pharmaceutical company (TONIX Pharmaceuticals, USA) has announced that the synthetic biology approach to creating viruses such as small pox will lead to developing new and safer vaccines.

Hope for a novel stem cell therapy

Junctional epidermolysis bullosa (JSB) is a life threatening disease caused by a mutation in the basement membrane protein laminin-332 (*LAMB*3 gene), which is characterized by blisters and erosion of the skin and mucosa within the lamina lucinda of the basement membrane. This leads to massive chronic skin wounds and infections as well as a predisposition to skin cancer. As there is no cure for JSB it is usually lethal in early life with approximately 40% of children dying before adolescence. In a recent publication, a seven-year old boy with JSB was admitted to hospital in Germany having lost almost all his skin (8). Researchers removed an area of unaffected skin from the boy and established keratinocyte cultures from which they derived epidermal stem cells that were transduced with *LAMB*3 cDNA using a retroviral vector. From the epidermal skin stem cell culture, the researchers harvested transgenic epidermal skin sheets and progressively grafted them to the boy's affected areas. In total approximately 80% of the boy's total body skin area was replaced. Currently the boy is well with no evidence of a return of the disease. The researchers were cautious that the full impact long-term of the transgenic epidermal replacements and the relative risks of the use of retroviral vectors are still to be determined.

Glyoxal – an alternative fixative to paraformaldehyde

Paraformaldehyde is a common fixative for immunostaining and fluorescence microscopy, however there are concerns relating to the loss of epitopes, changes in tissue morphology and, slow and incomplete fixation. The use of a paraformaldehyde/ glutaraldehyde mixture largely overcomes some of these issues associated with paraformaldehyde, however the efficiency of immunostaining may be reduced. Research from Germany in a multicenter trial, have tested the use of the aldehyde, glyoxal for use in fluorescence immunostaining (9). The researchers found that a glyoxal solution at pH 4 to 5 with ethanol provided superior fixation for fluorescence immunostaining when compared with paraformaldehyde; with 51 of the target tissues better stained, 19 equally stained and 12 of the target tissues stained worse. The overall conclusion was that glyoxal was a superior fixative with an added benefit as being safer to use.

Lymphocyte mitochondrial DNA as an inflammatory response signal

The cellular receptors of the innate immune system are programmed to recognize and respond to pathogen – associated molecular patterns (PAMPS) on foreign microbes. In addition, the innate immune system recognizes danger associated molecular patterns (DAMPS) released by damaged cells. In 2015 it was discovered that mitochondria (mt) participate in the inflammatory response by releasing mtDNA via the GAS/STING pathway, participating in the cell – intrinsic pathway triggering of the innate immune responses, antiviral signaling and triggering a type I IFN release. mtDNA as DAMP is significant in trauma, autoimmune disease, HIV and some cancer patients. Recent research from Sweden has demonstrated that human lymphocytes (B, T and natural killer cells as well as monocytes and neutrophils) will rapidly eject a mtDA web filament structure in response to oligodeoxynucleosides of class C, which provoked antiviral type I IFN production in peripheral mononuclear cells (10). The researchers conclude that lymphocytes may have an alternative role in antiviral signaling using their mtDNA to communicate danger.

Macrophage cytoplasm as a cause of metastases

In many cancers the cause of death is via metastases whereby primary cancer cells migrate to other cellular sites. Although many factors influence metastatic progression, the microenvironment of the cancer has an important role. A collaborative research project between researchers in Austria and the USA has identified a novel factor in the establishment of metastases (11). Using animal models, the researchers identified that macrophages can donate cytoplasm to primary melanoma cells both in-vivo and in vitro. Although macrophage interaction with cancer progression has been previously identified the recent research has identified that 70% of invasive melanoma cells had received macrophage cytoplasm. The researchers caution that although the research has identified the phenomena the precise mechanism is still to be determined.

Reducing apoptosis in haematopoietic stem cells

The use of allogenic haematopoietic stem cell (HSC) transplantation (HSCT) is a widely used treatment. However, the significant issues of HSCT are the survival of the HSC and their ability to repopulate the haematopoietic stem cell niche. A major issue is that HSC out of their normal environment are subject to significant apoptosis. Research to prolong the survival of HSC has investigated the use of direct protein transduction of the anti-apoptosis protein BCL-XL in to HSC (12). This protein blocks the apoptotic protein cascade mediated by the pro-apoptotic proteins BIM and BMF, which resulted in both prolonged survival of HSC and improved ability of HSC to differentiate in-vitro. This research has provided the opportunity to consider the control of apoptosis in HSC and the potential for use in other cell-therapy treatments.

References

1. Zhang J, Rector J, Lin JQ, Young JH, Sans M, Katta N, et al. Non-destructive tissue analysis for ex vivo and in vivo cancer diagnosis using a handheld mass spectrometry system. *Sci Transl Med* 2017; 9(406): eaan 3968.
2. Chan KC, Woo JK, King A, Zee BC, Lam WK, Chan SL, et al. Analysis of plasma Epstein-Barr virusDNA to screen for nasopharyngeal cancer. *N Engl J Med* 2017; 377: 513-522.
3. Kanow MA, Giarmarco MM, Jankowski CS, Tsantilas K, Engel AL, Du J, et al. Biochemical adaptations of the retina and retinal pigment epithelium support a metabolic ecosystem in the vertebrate eye. *eLife* 2017; 6: e28899.
4. Maxmen A. Sleeping sickness can now be cured with pills. *Nature* 2017; 550: 441.
5. Li P, Jiang W, Yu Q, Liu W, Zhou P, Li J, et al. Ubiquitination and degredation of GBPs by a Shigella effector to suppress host defence. *Nature* 2017; 551: 378-383.
6. Mendell JR, Al-Zaidy S, Shell R, Arnold WD, Rodino-Klapac LR, Prior TW, et al. Single-dose gene-replacement therapy for spinal muscular atrophy. *N Engl J Med* 2017; 377: 1713-1722.
7. Tonix Pharmaceuticals Holding Corp. Press release, 2017.
8. Hirsch T, Rothoeft T, Teig N, Bauer JW, Pellegrini G, De Rosa L, et al. Regeneration of the entire human epidermis using transgenic stem cells. *Nature* 2017; 551: 327-332.
9. Richter K, Revelo NH, Sietz KJ, Helm MS, Sarkar D, Saleeb RS, et al. Glyoxal as an alternative fixative to formaldehyde in immunostaining and super-resolution microscopy. *EMBO J* 2018; 37: 139-159.
10. Ingelsson B, Södenberg D, Strid T, Södenberg A, Bergh AC, Loitto V, et al. Lymphocytes eject interferogenic mitochondrial DNA webs in response to CpG and non-CpG oligodeoxynucleotides of class C. *Proc Natl Acad Sci U S A* 2018; 115: E478-E487.
11. Roh-Johnson M, Shah AN, Stonick JA, Poudel KR, Karql J, Jang JH, et al. Macrophage-dependant cytoplasmic transfer during melanoma invasion in vivo. *Dev Cell* 2017; 43: 549-562.
12. Murphy WJ. Making a better hematopoietic stem cell – timing is everything. *N Engl J Med* 2018; 378: 89-91.

Fellowship of the NZIMLS

The NZIMLS encourages members to consider Fellowship as an option for advancing their knowledge and career prospects. Fellowship provides an attractive option to academic postgraduate qualifications at a fraction of the cost.

Fellowship of the NZIMLS may be gained by thesis, by peer reviewed publications; or by treatise in case of a member holding an appropriate postgraduate or professional qualification.

Fellows may use the nominals FNZIMLS if a current financial member of the Institute.

Thesis

The thesis must be based on the style of Master of Science by thesis requirement of New Zealand universities.

Publications

A minimum of ten peer reviewed publications in international or discipline acknowledged biomedical journals. The candidate must be the 1st or senior author of at least six of these publications. A comprehensive review of the submitted publications is also required.

Treatise

By submission of a treatise in the form of a dissertation of 3000 - 5000 words on a medical laboratory science subject. The dissertation may take the form of a review, a scientific study, development of a hypothesis, or any other presentation that meets with the approval of the Fellowship Committee.

Candidates applying for Fellowship by this route must be holders of at least a Master's degree in medical laboratory science or a closely related subject, or have a professional qualification such as Fellowship of the following professional bodies: Australian Institute of Medical Science; Institute of Biomedical Science; Faculty of Science of the Royal College of Pathologists of Australasia, Australiasian Association of Clinical Biochemists; Royal Institute of Biology, London.

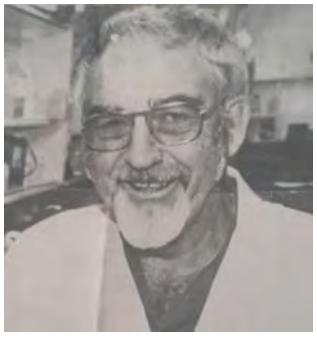
For full Fellowship regulations and the application process visit the NZIMLS web site at www.nzimls.org.nz

Current Fellows of the NZIMLS

Jenny Bennett
Jillian Broadbent
Brett Delahunt (Honorary)
Jan Deroles-Main
Susan Evans
Sheryl Khull
Christine Leaver
Dennis Mok
Maxine Reed
Robert Siebers
Andrew Stewart
Vasanthan Thuraisamy
Jacqueline Wright
Sheryl Young
Mark Bevan
Ailsa Bunker
Jennifer Castle
Marilyn Eales
Christine Hickton
Michael Legge
Ron Mackenzie
Howard Potter
Mohammad Shahid
Mary Stevens
Vanessa Thomson
Emil Wasef
Rubee Yee

Obituary

John Edwin Horner
Honorary Member NZIMLS



John died peacefully in his home on 4 February 2018 at the age of 90.

John spent his formative years in Waipukurau and moved to Wellington when he started training as a Medical Laboratory Technologist in the Wellington hospital laboratory. Following his qualification as a Technologist John moved to Wairau hospital in Blenheim and then to Ashburton hospital. This latter move proved to be an excellent choice as it was here he met his wife, Helen.

In 1966 they moved to Tauranga following John's appointment as Charge Technologist in the hospital laboratory. During this period of his career John was involved in the planning of three laboratories, none of which came to fruition. This was a source of disappointment for John, who retired in 1988 without the excitement of moving into a new laboratory. Last year I had the pleasure of taking him to the new Pathlab for a personal tour which was a great thrill for him.

John got on well with his pathologists and was respected by the hospital staff. He always included himself on the afterhours roster, so he could keep in touch with the 'coal face'. He had a great turn of phrase that made listening to his anecdotes an entertaining experience.

John was an active member of the Institute and helped organise the annual conference when it was held in Tauranga. He was also very community-minded, joining the Round Table while still in Ashburton and he was the inaugural president of the Tauranga branch when he moved to the Bay of Plenty. John joined the Greerton Lions Club and held most offices over the years. He was a keen music lover, the NZSO being one of the organisations he supported. John enjoyed the outdoors, especially walking in the bush with friends when opportunities presented.

John will be remembered with respect and affection for his commitment to laboratory technology, his sense of humour and his interest in people.

Jeremy Whimster

NZIMLS
Barrie Edwards & Rod Kennedy Scholarship

The Barrie Edwards & Rod Kennedy scholarship is one of the most significant awards offered by the NZIMLS. The scholarship provides the winner with support to attend an international or national scientific meeting up to a maximum value of $7,500.


Barrie Edwards


Rod Kennedy

Application for this prestigious scholarship is invited from Fellows, Members and Associate Members of the NZIMLS. Applicants must be a current financial member of the NZIMLS and have been a financial member for at least two concurrent years prior to application. To be eligible applicants must make an oral presentation or present a poster as 1st author at their nominated scientific meeting.

All applications will be considered by a panel consisting of the President and Vice-President of the NZIMLS and the Editor of the New Zealand Journal of Medical Laboratory Science (who are ineligible to apply for the scholarships). The applications will be judged on your professional and academic abilities together with your participation in the profession. The panel's decision is final and no correspondence will be entered into.

Application is by letter. Please address all correspondence to:

NZIMLS Executive Officer
PO Box 505
Rangiora 7440

There is one scholarship awarded in each calendar year. Closing date is December 20th in any given year.

In your application letter please provide the following details:

- Full name, position, work address, email address and contact phone number
- The length of time you have been a financial member of the NZIMLS
- The conference you wish to attend – please provide dates
- A budget comprising airfares, conference registration and accommodation costs
- The abstract of your intended oral or poster presentation and whether it has been accepted for presentation (proof required)
- Your intentions to publish your results
- State briefly your history of participation in the profession over the last 5 years
- State the reasons why you wish to attend your nominated scientific meeting

Successful applicants will be required to provide a full written report on return which will be published in the Journal. If not intended to publish elsewhere, successful applicants will be required to submit their study results for consideration by the *New Zealand Journal of Medical Laboratory Science* .

Previous recipients
2014. Maxine Reid, Aotea Pathology
2013. Julie Creighton, Canterbury Health Laboratories
2012. Holly Perry, Auckland University of Technology
2011. Bernard Chambers, Middlemore Hospital
2010. Sandy Woods, Canterbury Health Laboratories

Two medical laboratory scientists and NZIMLS members were recently awarded the Scholarship. They were:

Jackie Wright from ESR, Porirua who will be attending the upcoming VTEC meeting in Florence, Italy.

Rebecca O'Toole from Wellington SCL who will be attending the 27th International Society in Thrombosis and Haemostasis Scientific Meeting in Melbourne, Australia.

Both recipients are presenting at those meetings and will subsequently publish a report in the Journal.

A Weekend in The Bay!

You are invited to spend a weekend in Napier,
the beautiful Art Deco capital of the World

Whilst here, you would not want to miss the awesome

North Island Seminar

at the recently renovated
Napier Conference Centre

Saturday 5 May 2018

There is a wide selection of accommodation choices available, ranging from Te Pania hotel right across the road, to a number of hotels and motels and back-packers within easy walking distance of the venue. You will be just a short stroll from the centre of town, with shopping, bars and restaurants on the doorstep.

On Sunday morning after a leisurely start, you could pop over to Hastings and check out the Farmers Market, one of the largest and oldest in the country, or put on your walking shoes or hire a bike and explore the Coastal pathway, or maybe just find a friendly winery for a relaxed lunch before heading home.

We would love to see you here!

So get a team together and diary it now,

We would hate it if you missed out.

There is still a space in the programme for you to present that new method or interesting case study – contact shelli.turner@hbdhb.govt.nz ASAP to reserve a spot!

Registration is available at www.nzimls.org.nz

NICE 2018

You are invited to a 'T' Party!

The 29th NZIMLS Annual

NICE (National Immunohaematology Conference Education) Weekend

25 May 2018 – 27 May 2018

Bayview Wairakei Resort, Taupo

What's included?

- Two night's accommodation – twin share basis
- Breakfast, morning and afternoon teas, and lunches on Saturday and Sunday
- Great presentations on topics on Transfusion Science
- Meet other Transfusion Science enthusiasts from around the country
- CPD points!!
- Friday night NICE games – Prizes!
- Dinner and disco on Saturday night!

This year's dress theme is A 'T' party!

THE CATCH: Present a poster or 2-5 minute oral presentation on any topic on Immunohaematology or Transfusion Science (can also be a question or problem etc…)

REGISTRATIONS CLOSE 9th APRIL 2018

NICE Convenor:

Raewyn Cameron (Rotorua) - *raewyn.cameron@pathlab.co.nz Tel: 07 349 7908

*NB – please note e-mail address change

Submit your applications online at www.nzimls.org.nz

NZIMLS Annual Scientific Meeting, 2018

Mark it in your diary!

Air Force Museum, Christchurch, 21-24 August 2018

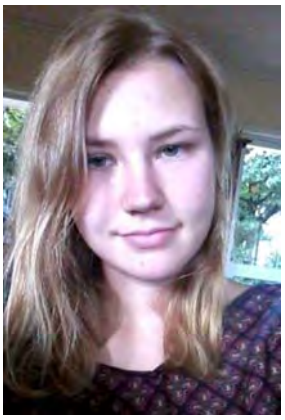
Email: jacquie.leaman@sclabs.co.nz
fran@nzimls.org.nz

News from the Universities

Massey University

Amy Aperloo has won the NZIMLS prize for fourth year BMLSc students at Massey University this year. She plans to do Haematology and then Microbiology at Canterbury Health Laboratories in 2018.

Short bio. Amy Aperloo



I attended Ponatahi Christian School from 5 yrs to 18 yrs of age starting from Year 1 and staying until the end of Year 13. I am interested in anything Biology related as well as subjects involving Chemistry, Agriculture, and Human Nutrition. During high school I became fascinated with microorganisms and wanted a career that involved this, I also felt that I had to give back to the community so my career had to entail this in some way . Because of this the most logical choice for me was doing a BMLSc so I could work in a diagnostic lab and become part of the process of helping people who are ill in some way.

I chose Microbiology for the 4th year of BMLSc because my passion for microorganisms was the reason I started BMLSc in the first place. Haematology was my other choice because I really enjoyed this subject in my 3rd year so wanted to continue this in my 4th year. I have many career aspirations including furthering my study and doing a masters and/or PhD, working in countries in need, moving to research in diseases/treatments, etc. I want to keep my options open and take opportunities as they present themselves.

University of Otago

News from Otago University's Medical Laboratory Science Programme

Cat Ronayne, Senior Teaching Fellow Department of Pathology Dunedin School of Medicine

The Otago BMLSc programme started 2017 with pizza and laser tag. We had various other social events throughout the year, including a movie night and a cocktail party at the Savoy. Our students designed new hoodies for our programme and these have received lots of attention and compliments around campus. Six students were selected to attend the NZIMLS South Island Seminar in Invercargill in March and found it to be an enjoyable and rewarding experience. The end of the teaching year was marked with the our bi-annual Amazing Race, which pits teams of students in a treasure hunt-style race around the city.

Since teaching ended, we have conducted a review and standardization of the 4th year clinical placement logbooks. Lisa Gallagher and Cat Ronayne presented excellent talks regarding innovations in teaching at the Dunedin School of Medicine Heath Profession Education Research Symposium.

In 2018, a new a paper will be introduced to the second year programme, to ensure that students are better prepared for changes within the profession. We are continuing to explore options for internationalization of the curriculum and are finding new ways to implement this. We are collaborating with the university's Pacific student support centre, fostering relationships to enhance links with labs in the pacific region.

The BMLSc class of 2017 graduated on Saturday 9th December, with the majority already finding full-time employment. They were:

BMLSc with Distinction

Vanessa Francia
Alice Gee
Katie Lennon
Bethany Mills
Philip Shaw
Rachel Spain
Farah Sethi Wang
Sharon Ward

BMLSc with Credit

David Chen
Lindsay Daysh
Theresa Diekermann
Kate MacLean
Ying Ting Tang

Prizes

Roche Diagnostics Haematology Prize (top haematology student of 3rd and 4th year): Philip Shaw

Professor Sandy Smith Microbiology Prize (top medical microbiology student of 3rd and 4th year): Farah Wang

Colin Watts Prize (top student over 3 years): Bethany Mills

Prince of Wales Nomination: Bethany Mills

Hugh Montgomery nomination (3rd year leadership and academic excellence): Nikki Montalba

NZIMLS Prize for the top 4th year student: Philip Shaw
NZIMLS Prize for the top 3rd year student: Iva Anjani
NZIMLS Prize for the top 2nd year student: Rafaella Liew

Top graduates' interviews

Bethany Mills

What made you decide to become a medical laboratory scientist?

I went into university not knowing much about medical laboratory science but learnt more about it from a friend who had spent time working in a hospital lab and realised that med lab was the perfect career choice for me as I loved working in a lab but still wanted to be playing a role within the public health sector.

You are originally from Hamilton. What attracted you to Otago University?

I liked that in first year I could get a taster of each of the different scientific disciplines before I had to commit to one of the health science professional programmes.

Which aspects of the course did you like best and which did you find most challenging?

I found all of the content really fascinating and especially enjoyed the opportunities given to apply the concepts in practical labs. For me, the biggest challenge was having to memorise all the bones and muscles for 2nd year anatomy.

Your clinical placements were in immunology and haematology at Canterbury Health Labs. What was that like?

In immunology, I enjoyed learning to recognise the different patterns in the indirect immunofluorescence assays and in haematology I loved working with the analysers and improving my morphology skills.

What are you up to now?
I am now working in Haematology at Canterbury Health Laboratories, where I did my placement.

What are your plans for the future?
I am enjoying my time at CHL and hope to stay working in Christchurch in the future.

Farah Sethi Wang
What made you decide to become a medical laboratory scientist?
I'm a patterns person and I love how in diagnostic laboratory science, everything fits together. I was really intrigued by medical laboratory science as diagnostic medicine is so dependent on it. I always wanted to work in the health field and in a career which enabled me to help improve the wellbeing of people.

You are originally from Wellington, then Brisbane. What attracted you to Otago University?
I was drawn to the Health Science First Year programme which enabled me to delve further into human health and discover what I was truly passionate about; especially as I had not done Biology in high school but had a deep interest in it. The opportunity to study away from home allowed me to grow and mature in a truly unique student environment.

Which aspects of the course did you like best and which did you find most challenging?
The course load was intense, but I really appreciated how much we were pushed to ensure we were fully equipped with the background knowledge needed for our placements. I really enjoyed diagnostic chemistry and haematology as they involved a lot of case-based tutorials and we developed a deeper understanding of the meaning of laboratory results in a clinical context. Similarly, microbiology laboratory sessions gave us a taste for diagnostic microbiology. We had to problem-solve our way in identifying particular pathogens based on limited patient history.

Your clinical placements were in microbiology and haematology in the NSW Health pathology laboratories. What was that like?
The most challenging aspect was the lack of lectures and structure I was used to during my years on campus, but it was a really worthwhile insight of a hospital diagnostic laboratory setting. I obtained a broader view of the knowledge I had obtained from University and was able to gain insight into the interdisciplinary involvement of patient care in addition to the more technical aspects of laboratory diagnostics. The hospital staff were very accommodating and I was asked to present my research project; the role of Public Health Units, Microbiology and Haematology laboratories in the management of an STEC outbreak; to the Haematology and Microbiology staff at the Prince of Wales Hospital, and the Microbiology department at St George Hospital.

What are you up to now?
I'm currently working in the Microbiology department at St George Hospital in Sydney.

What are your plans for the future?
I am planning on furthering my experience at St George Hospital Microbiology and am thinking of pursuing further study in the area of Public Health.

Philip Shaw

What made you decide to become a medical laboratory scientist?
Before enrolling at the University of Otago, I had had some previous experience as a patrol medic while serving as a soldier in the NZ Army. My training placed me alongside health professionals and introduced to the field of health sciences. I subsequently decided to study in this field and during the first year of my study I found that I really enjoyed working in the laboratory environment. I was initially unaware of medical laboratory science as a profession until I came across a brochure from the university careers office. I investigated this option a little further and I was kindly given a tour of the Dunedin hospital laboratory. After the tour, I decided that this was a great fit for me.

You are originally from North Canterbury. What attracted you to Otago University?
In 2005, in my late teens, I joined the NZ Army. After I had completed all of my initial training I was posted to Burnham, just outside of Christchurch, for four years before I took a posting to Auckland for another four years. After a total of eight years in the NZ Army, I decided to go to University for a change in career and chose Otago as it has a good reputation, it offered a broad range of courses and the cost of living in Dunedin was much cheaper than Auckland!

Which aspects of the course did you like best? Which aspects did you find most challenging?
Thought out the medical laboratory science course I found I really enjoyed learning about both the theoretical concepts and practical elements of physiology, how physiology relates to pathology, and how laboratory medicine is used to diagnose and monitor abnormalities or variations in health. During the course of my study, I faced numerous challenges such as exam preparation, assignments, presentations, and laboratory work. However, these challenges were easily overcome by extra support services that were provided by the university. I sort to make good use of these resources and I am thankful for those that gave me the advice I was seeking throughout my study.

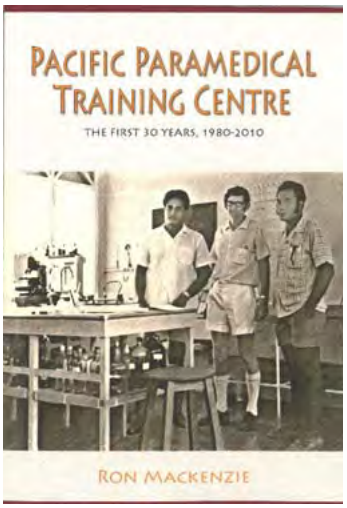
Your clinical placements were in ? and ?. What were they like?
The fourth-year papers I chose were Microbiology and Haematology. These were great papers and I really enjoyed the structure of the way the fourth year was taught. I had two very enjoyable research projects and I was supported throughout my projects by both the university teaching staff and the laboratory staff. Each of my papers also had four internally assessed written assignments which I also really enjoyed. These assignments provided an opportunity for some very in-depth learning. They were very fun to write!

What are you up to now?
Since graduating from the University of Otago, I have been working full time in a clinical biochemistry laboratory as a Medical Laboratory Scientist. I am currently practicing as an intern scientist and hope to achieve full certification this this year. I am also looking into post graduate study in fields such as business administration and, of course, medical laboratory science.

What are your plans for the future?
In the future, I plan to become proficient in my current role as I gain more experience, however, I also plan to continue my education and build my knowledge in several other areas that will complement my role as a medical laboratory scientist. So far, I am enjoying this profession and I am fortunate enough to have found employment working with a fantastic team of scientists and technicians that have been very supportive in my transition from student to scientist!

THE Pacific WAY

The PPTC has recently published a book which covers the past 30 years of its history and training activities at the Wellington Centre, the South Pacific and Asian regions. This account will be of interest to the many members of the Institute who have contributed so much to the success of the PPTC over this period.



The intention of the book is twofold: to provide a record of this period and to foster a continuing interest in the development of the health laboratory services of the Pacific Island and Asian regions. Despite it's small size and limited resources, the Centre has become a major player in the development of medical laboratories and blood transfusion services in the Pacific Island region. It is also a small but significant part of the New Zealand overseas development aid programme.

The PPTC began with the aim of providing short-term technical training programmes in basic medical laboratory disciplines which would be appropriate, affordable and sustainable and could bring immediate benefits to the work settings in which they would be used. While the PPTC has broadened its laboratory training programmes to meet the changing needs and new demands of the region's health services, this philosophy still remains the Centre's focus in 2018. The Centre has international respect for the excellence of its programmes and has provided a continuing source of training and technical expertise for health laboratory workers throughout the Pacific Island and Asian regions.

This contribution was recognised when the Centre was designated a Collaborating Centre of the World Health Organization in 1990 and it continues in this role to the present time. In recording these events it is fitting to say that none of this would have been possible without the initial and continuing support of the following organisations and people:

- New Zealand Ministry of Foreign Affairs and Trade
- New Zealand Red Cross
- World Health Organization
- New Zealand Institute of Medical Laboratory Science
- Wellington Hospital
- Department of laboratory Services , Wellington Hospital
- New Zealand Blood Transfusion Service
- Norman Kirk Trust

About the Author

Dr Ron Mackenzie (QSO, PhD, FNZIMLS) the co-founder of the PPTC and author of the book is a retired medical laboratory scientist who led the PPTC from its earliest days on the Wellington Hospital campus.

He is a life member of the NZIMLS and worked in hospital medical laboratories from the early 1950's in Kaitaia, Auckland, Masterton, Invercargill and Wellington. He worked as a WHO and Red Cross medical laboratory consultant and was a member of the first New Zealand Civilian Surgical Team to Qui Nhon, Vietnam in 1963. Ron was awarded the QSO in 1993 for the major role he had played in the development of the medical laboratory and blood transfusion services of the South Pacific and Asian regions.

Newly appointed staff to the PPTC

It is with pleasure that the PPTC Board of Governance welcomes Vichet Khieng to join the laboratory specialist team. Vichet graduated in 2008 with a Bachelor of Medical Laboratory Science from the University of Otago and majored in both advanced clinical biochemistry & diagnostic molecular pathology. In 2010, soon after gaining registration as a medical laboratory scientist while working at Dunedin hospital, he acted as technical manager at the Hawkes Bay community laboratories. He was responsible for the biochemistry department including reviews of the internal and external QC, manuals, and preparation for IANZ auditing. Vichet relocated to Wellington in 2012 and joined the department of biochemistry at Wellington hospital. He has had wide experience in NZ hospital and community laboratories and will be a valuable addition to the PPTC staff.

External Quality Assessment in Haematology

Elizabeth Tough is a New Zealand registered medical laboratory scientist who has over 50 years experience in the leadership of blood film examination and evaluation. In 1967 Elizabeth underwent five years of professional training at Wellington hospital and on completion became scientist in charge of the general haematology laboratory and chief morphologist in the years to follow. She also became the hospital laboratory expert in blood parasitology and bone marrow preparation for microscopic evaluation. Elizabeth has worked with the PPTC as a lecturer in blood parasitology and more recently has accepted the shared role of co-ordinator and haematology consultant for the PPTC's haematology EQA programme.

New Zealand based training courses 2018:

Laboratory Health & Safety; and Quality
Management Systems .....9 April - 4 May 2018 (4 weeks)
Biochemistry.....21 May – 15 June 2018 (4 weeks)
Effective Laboratory Management..... 2 – 27 July 2018 (4 weeks)
Haematology and Blood
Cell Morphology.....6 August – 14 September 2018 (6 weeks)
Microbiology ..... 24 September – 19 October 2018 (4 weeks)
Blood Transfusion Science ..... 5 – 30 November 2018 (4 weeks)

Journal Questionnaire

Below are ten questions based on articles from the April 2018 issue. Read the articles carefully as most questions require more than one answer.

Answers are to be submitted through the NZIMLS website. 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 re-submitting 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 22nd June 2018. You must get a minimum of eight questions right to obtain five CPD points.

The Editor sets the questions but the CPD Co-ordinator, Jillian Broadbent, marks the answers. Direct any queries to her at cpd@nzimls.org.nz

APRIL 2018 JOURNAL QUESTIONS

1. The three-fold conversion of the haematocrit to derive a haemoglobin value is not affected by which factors?
2. What were the limitations of the haematocrit to haemoglobin conversion factor study?
3. What pre-analytical factors associated with muscle mass can influence blood creatinine levels?
4. The Jaffe assay used for measuring creatinine is prone to which interfering substances?
5. How do clinical cases of Shiga-toxigenic *Escherichia coli* infection typically present clinically?
6. Which therapeutic medications are contra-indicated in Shiga-toxigenic *Escherichia coli* infections, and why?
7. Shiga-toxigenic *Escherichia coli* toxins cause bloody and non-bloody diahrroea through which mechanisms?
8. Expended spectrum ß-lactamase enzymes are capable of hydrolysing, but are inhibited by which antibiotics?
9. *P. aeruginosa* has been demonstrated to have intrinsic resistance to different classes of anti-bacterial drugs through which mechanisms?
10. The cytopathologic differential diagnosis of mammary analogue secretory adenoma includes which low-grade epithelial neoplasms?

NOVEMBER 2017 JOURNAL QUESTIONNAIRE AND ANSWERS

1. Which therapies can adult transgender individuals undergo to help have their physical bodies more closely align with their identified gender?
   Hormone treatment, mastectomy, hysterectomy, orchiectomy, oophorectomy, gender reassignment surgery.
2. What are amongst the most common of chromosome abnormalities that continue during pregnancy?
   Trisomy 21 (Down syndrome), trisomy 18 (Edward syndrome), trisomy 13 (Patau syndrome), Turner syndrome, triploidy.
3. Typically, infants with Down syndrome may have which symptoms?
   Heart defects, thyroid gland dysfunction, digestive tract problems, facial and hearing defects, intellectual disability, repeated infections.
4. What is the main drawback of ANA testing, and why?
   Limited specificity for SARD (Systemic Autoimmmune Rheumatic Disease). The presence of ANA directed against intracellular antigens is associated with a wide range of disorders.
5. The reticulocyte haemoglobin equivalent provides a measure of what?
   Bioavailability of iron during erythropoiesis.
6. What could the future clinical utility of the reticulocyte haemoglobin equivalent be?
   As a screening test for the iron deficient states. Also as a negative predictor of the iron deficient state when the reticulocyte heamoglobin equivalent (RET-He) falls within the reference range in anaemic patients.
7. The use of anti-human globulin in the complement dependant cytotoxicity assay enhances what?
   Detection of weak or low-titred antibodies as well as non-complement fixing antibodies, including cytotoxicity negative adsorption positive (CYNAP) antibodies.
8. The finding of early reaction errors on the Sysmex CS 2100i analyser leads to what?
   Additional sample preparation steps to resolve the issue, or may lead to a sample recollect.
9. What is an area of concern with the MAST indirect carbapenemase test?
   The number of equivocal (false positives) and the weak positive results for some of the OXA-48-like group.
10. To reliably distinguish carbapenemase-producing organisms from carbapenem-resistant-non- carbapenemase-producing organisms, what do the authors recommend?
    That the modified carbapenem inactivation method (mCIM) is used in tandem with the MAST indirect carbapenemase test (MAST ICT), or with another high performing assay such as Carba NP rather than stand-alone tests.

Index to Volume 71, 2017

2018 NZIMLS CALENDAR *(Dates may be subject to change)*		
DATE	***COUNCIL***	***CONTACT***
3/4 May 2018 19/20 August 2018	Council Meeting	fran@nzimls.org.nz
DATE	***SEMINARS***	***CONTACT***
5 May 2018	North Island Seminar, Napier Conference Centre, Napier	shelli.turner@hbdhb.govt.nz
25-27 May 2018	NICE Weekend, Wairakei Resort, Taupo	raewyn.cameron@pathlab.co.nz
10 November 2018	Mortuary SIG, Wellington City Hospital	
17 November 2018	Immunology SIG, Wellington City Hospital	sarah.burge@wellingtonscl.co.nz
DATE	***CONFERENCE***	***CONTACT***
21-24 August 2018	Annual Scientific Meeting, Air Force Museum, Christchurch	jacquie.leaman@sclabs.co.nz fran@nzimls.org.nz
DATE	***MEMBERSHIP INFORMATION***	***CONTACT***
28 January	Membership and CPD enrolment due for renewal by 28 February	sharon@nzimls.org.nz
31 January	CPD points for previous year 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
15 June	Material for the August Journal must be with the Editor	rob.siebers@otago.ac.nz
23 June	Nomination forms for election of Officers and Remits to be with the Membership (60 days prior to AGM)	fran@nzimls.org.nz
13 July	Nominations close for election of officers (40 days prior to AGM)	fran@nzimls.org.nz
2 August	Ballot papers to be with the membership (21 days prior to AGM)	fran@nzimls.org.nz
10 August	Annual Reports and Balance Sheet to be with the membership (14 days prior to AGM)	sharon@nzimls.org.nz
17 August	Ballot papers and proxies to be with the Executive Officer (7 days prior to AGM)	fran@nzimls.org.nz
15 September	Material for the November Journal must be with the Editor	rob.siebers@otago.ac.nz
DATE	***NZIMLS EXAMINATIONS***	***CONTACT***
03 November 2018	QMLT Examinations	fran@nzimls.org.nz

UN-Series

Fully Automated Urinalysis Workflow

Flexible

Modular and scalable, the UN-Series allows you to configure a complete urinalysis solution that best suits your lab environment and size, with the complete freedom to up- and down-scale.

Innovative

Leading-edge fluorescence flow cytometry means the UN-Series provides consistent and specific particle differentiation allowing your lab to analyse samples with accuracy, precision and speed.

Intelligent

The UN-Series delivers full automation analysis, significantly reducing manual review and letting staff set up samples and walk away, increasing your lab's productivity.

Contact us to find out more about the UN-Series and how your lab could benefit from the flexible, innovative and intelligent urinalysis solution.

sysmex

www.sysmex.co.nz