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PATHOLOGY *focus*

Medical Newsletter

ANTIMICROBIAL RESISTANCE

*By Dr Sudha Pottumarthy-Boddu
and Dr Linda Dreyer*

Special
Edition

**Accurate and timely diagnoses
of winter respiratory illnesses
strengthen antimicrobial stewardship**

Every winter respiratory virus season is unique, especially influenza trends. Well-established influenza surveillance systems all over the world aim to gauge, predict, report, and ultimately provide guidance for protection (vaccine development) for the upcoming influenza season. The ongoing COVID-19 pandemic adds another layer of complexity in making an accurate clinical diagnosis of winter respiratory illness, necessitating a robust, multiplex respiratory PCR assay to enable accurate and timely diagnosis and pathogen-directed specific therapy.

Article continues over page

How COVID-19 has influenced antimicrobial resistance

The 2022 Special Report COVID-19 U.S. Impact on Antimicrobial Resistance¹ by the Centers for Disease Control and Prevention (CDC) reports on the impact of the pandemic on alarming trends in antibiotic resistance and on antibiotic prescribing practices.

The number of hospital onset-infections due to resistant pathogens increased by at least 15% from 2019 to 2020 (13% increase for MRSA to 78% increase for carbapenem-resistant *Acinetobacter* sp.).

The pandemic also impacted antibiotic prescribing, where antibiotics were often the first treatment prescribed for any febrile pulmonary illness, which often turned out to be COVID-19, a viral illness where antibiotics had no effect.

The importance of preserving and prolonging the efficacy of the currently available antibiotics by being responsible antibiotic stewards is emphasised.

The latest Australian influenza statistics for 2023



Figure 1. Percent of notifications of laboratory-confirmed influenza, Australia, 1 January to 30 April 2023, by subtype and state or territory*²

The Australian Influenza Surveillance report for reporting weeks 16 and 17, 2023, notes 32,047 laboratory-confirmed influenza cases, with 32 influenza-associated deaths

identified in the year-to-date in the NNDSS (National Notifiable Diseases Surveillance System)². Of the laboratory-confirmed influenza cases, 77% were influenza A (94% influenza A (unsubtyped)) and 22.5% were influenza B (see Figure 1). The number of laboratory-confirmed notifications year-to-date is higher than the 5-year average, but community influenza-like-illness (ILI) activity remains within the historical ranges, with the highest notification rates among the 5-9 year age group, followed by the 0-4 years and 10-14 years age groups.

Given the rising number of influenza notifications in Australia in 2023, in the face of resumed worldwide travel and limited social restrictions, the trends of winter respiratory illnesses remain largely unpredictable at this time.

The use of the multiplex respiratory PCR assay allows for an accurate and timely diagnosis of respiratory viral illness, early administration of appropriate antivirals if indicated, and at the same time limits the unnecessary use of antibiotics.

Ordering Respiratory Virus PCR Testing

How to order: To assist the laboratory during flu outbreaks, please limit testing to suspected pathogens to ensure rapid result delivery.

Specimens required: Nose/throat or nasopharyngeal.

Additional tests: If you suspect a lower respiratory infection, the appropriate sample is sputum for MCS. If the patient presents with pharyngitis symptoms, obtain a swab from the throat for culture.

Test cost: Medicare bulk billing available, subject to Medicare guidelines and criteria.

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Dr Sudha Pottumarthy-Boddu comes to us from Houston, Texas, where she was Assistant Professor in the Department of Pathology and Laboratory Medicine at the University of Texas, School of Medicine. She was also the Technical Director of the Clinical Laboratory Services at the Houston Department of Health and Human Services. After graduating from medical school in India, Dr Pottumarthy-Boddu migrated to New Zealand and completed her Pathology/Microbiology Fellowship training with the Royal College of Pathologists of Australasia. She is a recipient of various awards and scholarships, including the Neil Prentice Memorial Prize of RCPA. She is also a Diplomate of the American Board of Medical Microbiology. Over the last 10 years she gained experience in various hospital, research, and public health laboratories in the US, publishing over 30 articles in peer-reviewed journals and presenting at various national and international conferences. Detection of the first USA isolate of *Enterobacter* spp. with NmcAcarbapenem hydrolyzing enzyme and establishing clinical significance of *Nocardia verterana* are noteworthy. Dr Pottumarthy-Boddu's main research interests are antimicrobial susceptibility trends and molecular methods in the diagnosis of infectious diseases.

Antimicrobial resistance (AMR) has emerged as one of the principal public health problems of the 21st century.

With a rapidly growing aging population, Residential Aged Care Facilities (RACFs) are becoming an increasingly important part of the healthcare system. RACFs are also recognised as an important community setting for monitoring antimicrobial resistance and antimicrobial use. The high prevalence of infections and colonisation caused by antimicrobial-resistant organisms is well-known in residents of these facilities.

Growing concern over antimicrobial resistance

The 2019 Aged Care National Antimicrobial Prescribing Survey highlighted concerning levels of inappropriate antimicrobial use and the resultant increase in the potential for antimicrobial resistance. Alarming, about 40–75% of antibiotic use in RACFs has been considered inappropriate. Common clinical indications reported were cystitis, skin, soft-tissue, or mucosal infections, and non-surgical infections. 20% of antimicrobials were prescribed for prophylactic use (rarely recommended), and one-third of all prescriptions were for topical antimicrobials (indicated for a limited number of conditions).

General Practitioners (GPs) have an important role as antimicrobial stewards as they are the key prescribers of antibiotics in outpatient settings.

Areas of concern in prescribing

Antibiotic therapy for asymptomatic bacteriuria	<ul style="list-style-type: none"> • In 2018, the Australian Commission on Safety and Quality in Health Care (ACSQHC) found that 2% of all prescriptions in aged care were for asymptomatic bacteriuria • Four out of five of these prescriptions were for prophylactic antibiotics for asymptomatic bacteriuria • Asymptomatic bacteriuria is common in the elderly and is defined as the growth of organisms at specified quantitative counts ($\geq 10^5$ colony-forming units [CFU]/mL or $\geq 10^3$ CFU/L) in an adequate urine specimen, in patients without symptoms consistent with a urinary tract infection (UTI). • It is diagnosed when urine samples are sent for microscopy and culture for patients who do not have clinical symptoms of UTI • It only requires treatment in very limited circumstances.
Widespread use of prophylactic antibiotics for UTI	<ul style="list-style-type: none"> • This is rarely indicated, and in the absence of infection risk, prolonged antibiotic use selects for resistant organisms
Empiric antibiotics without microbiological investigation	<ul style="list-style-type: none"> • Causative agents should be identified, especially in symptomatic UTIs
Widespread antibiotics prescribing for URTI and chronic bronchitis	<ul style="list-style-type: none"> • Differentiation between viral and bacterial origin of presumed RTI is essential to reduce inappropriate antibiotic use
Prolonged duration of antibiotic treatment	<ul style="list-style-type: none"> • Risk of side effects and resistance are increased
Widespread use of quinolones as empirical therapy for UTIs	<ul style="list-style-type: none"> • High levels of resistance to quinolones were detected in aged care and should not be used as first-line treatment
Broad-spectrum antibiotic treatment for elderly residents with advanced dementia or end-stage illness	<ul style="list-style-type: none"> • Antibiotic therapy is controversial for residents with advanced dementia and may be best guided by advance care directives.

Managing infection in aged care

Managing RACF residents presents a whole set of challenges for GPs, as patients tend to be prone to infection due to factors such as advanced age, poor functional status, the presence of multiple co-morbidities, compromised immune systems, and the use of urinary tract catheters and other invasive devices. Other challenging factors that affect the decision to prescribe antibiotics are difficulties in establishing symptoms due to cognitive impairment, language barriers, and frequent staff turnover.

The symptoms of infection in the elderly can be very non-specific and can present as delirium, functional decline, falls, and behavioural changes, usually in the absence of fever. In these scenarios, empirical antimicrobial therapy is often initiated without adequate pathology investigations.

Consequences of inappropriate antibiotic use in aged care

1. Increased antibiotic resistance
2. Adverse drug reactions and drug interactions
3. *Clostridioides difficile* infection
4. Increased healthcare costs
5. Diminished quality of care



Recommendations for improved prescribing practices

1. Adhere to clinical guidelines
2. Implement antimicrobial stewardship programs (AMS)
 - PCR for Respiratory viruses/SARS-CoV2
 - Sputum/throat microscopy and culture
 - Urine when symptomatic
3. Educate staff, patients, and families
4. Encourage diagnostic testing
5. Regularly reassess and de-escalate therapy

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[Fact Sheet – Asymptomatic bacteriuria – 2020 | Australian Commission on Safety and Quality in Health Care](#)

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Dr Linda Dreyer completed her undergraduate studies in 1996, receiving a Bachelor's degree in Medicine and Surgery (MBCChB) from the Faculty of Health Sciences, University of Pretoria, South Africa. Following four years of clinical practice as a Medical Officer in the Department of Family Medicine, she commenced specialisation in 2000. She was appointed as Registrar in Clinical Virology at the University of Pretoria/Gauteng Province, where she worked for two years. In 2003, she was appointed as Senior Registrar in Microbiology. Dr Dreyer received her Master's degree in Clinical Microbiology (MMed (Path)) from the University of Pretoria in 2006. She worked as a consultant for the National Health Laboratory Services (NHLS) in Pretoria until January 2008. During her time at NHLS, she was involved in teaching medical students and microbiology registrars, and gave lectures to nursing staff, medical students, and specialists. She also sat on the Infection Control Committee and the Antimicrobial Stewardship Committee of the Pretoria Academic Hospital. In 2008, she came to Melbourne and joined Australian Clinical Labs (formerly Healthscope Pathology) as a Senior Registrar, and obtained Fellowship of The Royal College of Pathologists of Australasia (FRCPA) in 2010. Dr Dreyer has special interests in the appropriate use of antimicrobials, infection control, and molecular diagnostic assays in contemporary clinical microbiology.

Iron infusions and hypophosphataemia

By Associate Professor Chris Barnes

Iron deficiency anaemia is a common condition that can have a significant impact on a patient's quality of life. The incidence of iron deficiency anaemia in Australia is estimated to be 12% in non-pregnant and up to 15% in pregnant females.¹ Iron deficiency without anaemia may be up to three times more common but is often undiagnosed.²

While oral iron therapy is often the first-line treatment, some patients are unable to tolerate it due to gastrointestinal side effects or poor absorption.³ In these cases, iron infusions may be a suitable alternative.

Iron infusion

A common iron infusion therapy in Australia is ferric carboxymaltose (FCM). FCM is a complex of iron and carbohydrate that is administered intravenously and has been shown to be safe and effective in treating iron deficiency anaemia in patients who are unable to tolerate oral iron therapy.⁴

Hypophosphatemia

However, there have been reports of symptomatic hypophosphatemia associated with FCM use. This is a rare condition where the levels of phosphate in the blood are abnormally low, which can cause a range of symptoms including weakness, fatigue, and bone pain. It is thought to occur due to the rapid increase in fibroblast growth factor 23 (FGF23) which increases phosphate secretion. Prolonged hypophosphatemia and associated secondary hyperparathyroidism may be a risk factor for metabolic bone disease, including the development of osteomalacia.

Managing the risk of hypophosphatemia

To help manage this potential risk, doctors may consider monitoring serum phosphate, calcium, vitamin D, and parathyroid hormone (PTH) levels in patients who present with symptoms following FCM infusions. If symptomatic

hypophosphatemia occurs, treatment may include phosphate and vitamin D supplementation, along with discontinuation of FCM use.

“Doctors may consider monitoring serum phosphate, calcium, vitamin D, and parathyroid hormone (PTH) levels in patients who present with symptoms of hypophosphatemia following FCM infusions.”

Despite this potential risk, iron infusions with FCM can offer significant benefits for patients who are unable to tolerate oral iron therapy. By restoring iron levels in the body, patients may experience improved energy levels and improved quality of life. Clinical review and laboratory monitoring for side effects are recommended in patients who present with concerning symptoms following iron infusion.

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MET exon 14 skipping mutation (METex14sk) in lung cancer

By Associate Professor Mirette Saad

Introduction

NSCLC comprises approximately 85%-90% of all lung cancers. The *MET* exon 14 skipping mutation (*MET* Δ ex14), also known as *MET*ex14sk, is present in ~ 3% to 5% of non-small cell lung cancer (NSCLC) cases across all histologic subtypes (incidence varies by histology). Some studies have reported the detection of *MET* Δ ex14 in squamous cell carcinoma (~2%) and large cell carcinoma (0.8%).

The *MET* gene is a proto-oncogene that encodes for a receptor tyrosine kinase protein. *MET* abnormalities have been associated with rapid tumour growth, aggressively invasive disease, and a poor prognosis. Today, approved targeted therapies are available for patients with NSCLC who are positive for oncogenic drivers, such as *EGFR*, *ALK*, *BRAF V600E*, *ROS1*, *NTRK1/2/3*, *RET*, and *MET* Δ ex14.

MET Δ ex14 are characterised by an average age of over 70 years at diagnosis, a smoking history, and a higher frequency in pleomorphic carcinoma and adenosquamous cell carcinoma than in adenocarcinoma. The correlation between the frequency of *MET* Δ ex14 and race, sex, stage, and histological grade has not yet been reported or is still controversial.

Recommended by guidelines

MET Δ ex14 skipping mutations now are incorporated into professional guidelines for the clinical management of NSCLC. This category of mutations is considered clinically actionable in NSCLC because clinical trial data showed the association of *MET* Δ ex14 alterations with therapeutic responsiveness to oral *MET* tyrosine kinase inhibitors (TKIs).

MET exon 14 skipping mutation and molecular targeted therapies

In 2020 and 2021, the approval of two *MET*-TKIs, capmatinib and tepotinib, for NSCLCs carrying *MET* Δ ex14 dawned a new era for *MET*-targeted therapy. Both *MET*-TKIs are potent and highly selective ATP competitors for *MET* in *in vitro* or *in vivo* models carrying *MET* Δ ex14. These drugs yielded progression-free survival of 5.4–12.4 months in clinical trials.

Studies reported on-target and off-target mechanisms of acquired resistance to *MET*-TKIs. NSCLCs with this mutation may harbour highly heterogeneous co-driver mutations. Therefore, it is understandable that some patients show inherent resistance to *MET*-TKIs.

In contrast with *EGFR/ALK*-positive NSCLC having zero or low *PD-L1* expression, *MET* Δ ex14 NSCLC tumours were found to express high levels of *PD-L1*. Currently, there are conflicting data regarding the impact of *MET* Δ ex14 skipping mutations on immunotherapy, and this consideration is under active investigation. In addition to immunotherapy, novel treatments, including novel *MET*-TKIs, *MET* antibodies, and novel combination therapies, are now being evaluated in clinical trials.

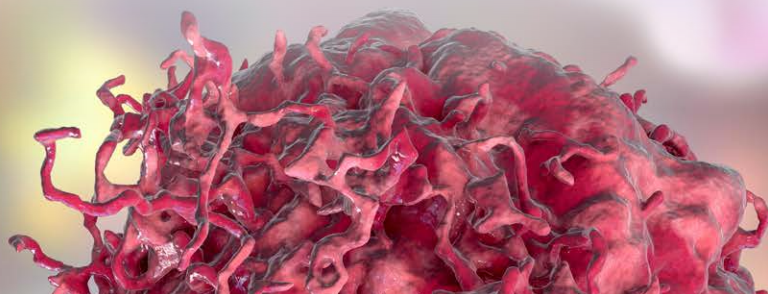
When to test?

Surgical resection is unfeasible for patients that present with advanced disease. NSCLC specimens typically are small, yet requirements for molecular biomarker analysis are ever-expanding. Single-gene testing for multiple biomarkers sequentially may result in longer turnaround times, increase the risk of tissue exhaustion, and may reduce the sensitivity to identify molecular biomarkers.

Broad molecular profiling is strongly advised by the NCCN guidelines in NSCLC given the other oncogenic biomarkers recommended for routine analysis in this tumour type using tissue biopsy specimens that often are composed of limited tissue.

Laboratory methods

There are several methods to detect *MET* Δ ex14 in NSCLCs. These include next-generation sequencing (NGS)-based panel tests, with RNA-based or DNA-based techniques that employ some degree of target enrichment. Although DNA-based assays may be used for successful detection of the underlying molecular biology of *MET* Δ ex14 skipping mutations, it has its limitations. It has been reported that the sensitivity of RNA-based tests is better than DNA-based tests. RNA-based assays, by directly detecting omission of exon 14 from the transcript, overcome many of the limitations of DNA-based analyses. However, RNA-based testing may be hampered by a higher rate of poor-quality RNA in clinical tumour samples.



Immunohistochemistry (IHC), although applicable for some biomarker analyses, has been shown to be of limited clinical utility in NSCLC for the detection of *MET* Δ *ex14* skipping mutations. It is important for clinicians to recognise that assays including *MET* in their list of covered genes may not detect all alterations that lead to *MET* Δ *ex14* skipping. Careful consideration of the limitations of the sequencing assays to guide treatment decisions is recommended.

Conclusion

MET Δ *ex14* skipping mutations are clinically relevant because they are predictive for approved targeted therapies and are deemed a part of routine biomarker analysis in professional guidelines for the clinical and laboratory management of NSCLC. Molecular profiling is strongly advised in NSCLC. Data will continue to emerge with ongoing assessment of the most recently approved targeted agents.

How to order

To request testing for *MET* Exon 14 skipping, please complete the Somatic Mutation Request Form on our website, which can be found on our Lung Cancer page at clinicallabs.com.au/cancer-services. Please ensure you select *MET* Exon 14 skipping (RNA testing) and indicate whether the Medicare criteria has been met. If you have an older request form, please add *MET* Exon 14 skipping in the Lung Panel section and also indicate whether the Medicare criteria has been met.

MET exon 14 skipping test MBS Eligibility Criteria (Item 73436)

A test of tumour tissue from a patient diagnosed with locally advanced or metastatic non-small cell lung cancer requested by, or on behalf of, a specialist or consultant physician to determine if the requirements relating to *MET* proto-oncogene, receptor tyrosine kinase (*MET*) exon 14 skipping alterations (*MET* Δ *ex14sk*) status for access to tepotinib are fulfilled under the Pharmaceutical Benefits Scheme.

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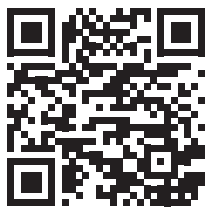


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Winter and vitamin D

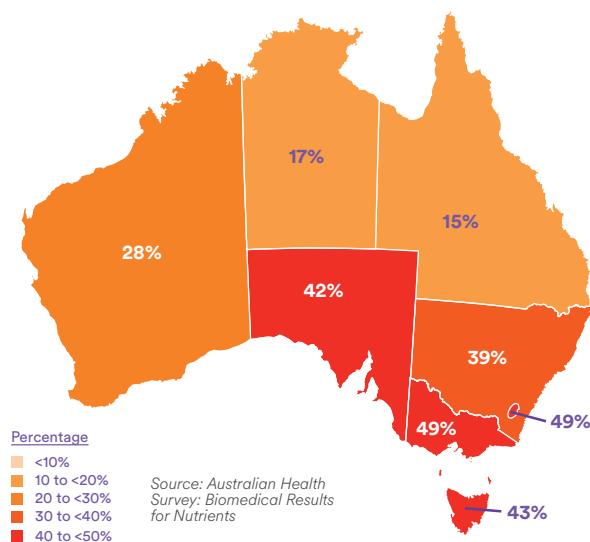
By Dr David Deam

As the weather gets colder and the amount of sunlight decreases as we head into winter, it is a good time to consider if vitamin D testing may be helpful in selected patients.

Vitamin D levels fluctuate significantly depending on the season, especially in the southern states of Australia. At the end of winter, approximately 36% of Australians are vitamin D deficient, in comparison to 14% at the end of summer.

Diagram 1 below indicates the percentage of Australians with vitamin D deficiency in winter.

Diagram 1 - Vitamin D deficiency in winter by state (2011-2012)



Target vitamin D levels

The international recommendations for adequate vitamin D levels vary, but based on a review of current literature and recently published recommendations^{1,2} experts suggest that adequate vitamin D status is a serum level equal to or greater than 50 nmol/L at the end of winter.

This level should be 10-20 nmol/L higher at the end of summer to allow for seasonal decrease.



Adequate vitamin D levels in nmol/L at the end of summer



Adequate vitamin D levels in nmol/L at the end of winter

Who to test

Vitamin D testing should be ordered for patients at risk of vitamin D deficiency.

This includes:

- Housebound people - including the sick and disabled
- Elderly in high care situations
- People with darker skin
- People who cover their skin due to religious or cultural reasons
- People who regularly avoid the sun
- People who work indoors

Also, patients with:

- Signs, symptoms, and/or planned treatment of osteoporosis or osteomalacia
- Increased alkaline phosphatase with otherwise normal LFTs
- Hyperparathyroidism, hypo- or hypercalcemia, or hypophosphatemia
- Malabsorption (i.e. CF, IBD, coeliac, etc.)
- Medications known to decrease vitamin D levels (i.e. anticonvulsants)
- Patients with chronic renal failure and transplant recipients

Associated tests

When ordering a vitamin D test for a patient, also consider ordering a serum calcium and parathyroid hormone (PTH) test, which will assist in placing the vitamin D level within the context of overall calcium homeostasis. If Osteoporosis is present, fasting blood crosslaps (CTX) will provide a way of monitoring bone turnover in response to therapy.

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