carcasses, animal food products and wastewater, highlighting the importance of this resistance gene in a One Health context.[9–11]

In our collection, *optrA* was predominantly chromosomal and the gene was carried on the Tn*6674* transposon in conjunction with *fexA*, *erm(A)* and *ant(9)-Ia*. Tn6674 has previously been detected in circular intermediate forms,[4] suggesting chromosomally encoded *optrA* is transposable, which could explain the presence of Tn*6674* in the genetically diverse *E. faecalis* isolates in our study. Tn*6674* was always identified within the *radC* gene which is considered a hotspot for the chromosomal integration of *optrA*-carrying genetic elements. In addition to hospitalised patients, the Tn*6674* transposon has previously been identified in healthy humans and food-producing animals across different continents,[12] highlighting the importance of monitoring this mobile multi-resistance genetic element.

In conclusion, LNZ resistance in our collection was predominantly mediated by the presence of *optrA*. The *optrA* gene clusters harboured other resistance genes such as *fexA* and *erm(A)*, and in the case of chromosomally encoded *optrA*, also harboured *ant(9)-Ia* (or *spc*). However, the isolate with the highest LNZ MIC was *optrA*-negative and resistance was due to the G2576T mutation in three of its four 23S rRNA gene copies. Although *optrA* can be detected using *de novo* draft assemblies, mutations in 23S must be determined by analysing the raw sequence reads using a tool such as LRE-Finder because consensus sequences produced by assemblers ignore less abundant mutations. Based on our findings, similar genomic characterisation studies on LREfs in other parts of Australia should be conducted. Furthermore, as LNZ resistance has been associated with food-producing animals in other countries, a One Health surveillance approach is warranted.

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Macrolide resistant *Mycoplasma pneumoniae* in Auckland, New Zealand

To the Editor,

Mycoplasma pneumoniae is an important cause of community acquired pneumonia and other respiratory infections, particularly in school-aged children and young adults.[1] *Mycoplasma pneumoniae* respiratory disease typically has a mild presentation, although severe and fatal cases also occur.[1] It is a fastidious and slow growing organism, so the primary method for diagnosing acute infection is through nucleic acid amplification tests on respiratory specimens.[1] Macrolide antimicrobials are typically the treatment of choice, having the greatest *in vitro* potency and the ability to be safely used in young children.[1,2] However, treatment with macrolides has been complicated by the emergence of resistance, which occurs in association with mutations in the peptidyltransferase loop of Mycoplasma 23S rRNA, primarily at positions 2063 and 2064.[1,2] Worldwide, the prevalence of macrolide resistance displays significant geographic variation, with rates up to 90% in Asia, typically less than 10% in Europe, 7.5% in the United States (with substantial regional differences), while a study in South Australia detected no resistance.[1–4] Current data suggest macrolide resistance is associated with a prolonged duration of fever and hospitalisation in children, although an increase in severe complications or mortality has not been described.[1,5,6] In New Zealand, macrolides are frequently used in the empiric antimicrobial treatment of community acquired pneumonia and for the specific treatment of *M. pneumoniae*. However, there has been an absence of local data on macrolide susceptibility. To help guide therapy and provide a baseline resistance estimate, this study determined the prevalence of macrolide resistance associated mutations in *M. pneumoniae* in the Auckland region of New Zealand.

This was a retrospective descriptive study using stored DNA extracts from respiratory specimens that tested positive for *M. pneumoniae* using a real-time PCR assay, targeting an 89bp fragment of the repMp1 gene of *M. pneumoniae*, at the Auckland City Hospital laboratory, Auckland, New Zealand, between September 2016 and April 2020. Cases were identified from laboratory records and basic demographic and epidemiological data were extracted from electronic health records. Only cases from persons in the Auckland region were included. The presence of macrolide resistance associated mutations was assessed by PCR amplification of the peptidyltransferase (domain V) region of the 23S rRNA gene using primers described by Wolff *et al.* (5′-3, forward AACTATAACGGTCCTAAGGTAGCG; reverse GCTCCTACCTATTCTCTACATGAT) followed by sequencing of the amplicon using an Applied Biosystems 3130x sequencer.[7] Sequences were aligned against *M. pneumoniae* M129 reference genome (NCBI accession number NC_000912.1) using SeqMan II bioinformatic software (DNAStar, USA), with 23S rRNA mutations described using *M. pneumoniae* numbering. Study approval was obtained from the New Zealand Health and Disability Commission Ethics Committee (reference number 21/CEN/100). Funding was provided by the A+ Trust Microbiology Education and Research fund.

Ninety *M. pneumoniae* cases from the Auckland region were identified for possible inclusion in this study. Seventy-nine of these cases had stored DNA extracts available for further testing; these included 39 from 2016−2018 and 40 from 2019−2020. These 79 cases had a median age of 22 years (range 0.5−92.5) and 42 (53%) were female. In 74 (94%) of the cases the specimen was collected in the inpatient or emergency department setting. Mutations in the 23S rRNA gene were detected in seven (9%) of 79 strains. All seven (100%) had the single-nucleotide polymorphism A2063G. One (3%) of 39 strains from 2016−2018 had a resistance associated mutation compared to six (15%) of 40 from 2019−2020. Four (57%) of the seven resistant strains were in persons ≤15 years of age. Six (86%) of the seven were in cases tested in the inpatient or emergency department setting.

This study is the first to describe the occurrence of macrolide resistant *M. pneumoniae* in New Zealand, with the common resistance associated mutation A2063G identified in 9% of strains evaluated, comparable to rates of resistance that have been reported in Europe and the United States.[1,2,4] As this study was geographically restricted to the Auckland region and consists primarily of hospitalised cases, the rate of resistance may not be generalisable across the country or to the infected population as a whole. Nevertheless, it illustrates the presence of macrolide resistant *M. pneumoniae* in New Zealand, the expanding global distribution of macrolide resistance, and the local need to consider the role of resistance and alternative antimicrobials in cases responding poorly to empiric macrolide therapy.

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Interference of methylene blue therapy on methaemoglobin measurement

To the Editor,
Methylene blue (MB) is a classical antidote for treating methaemoglobinaemia. Conventional co-oximetry utilises multi-wavelength spectrophotometer for methaemoglobin (MetHb) determination, which shares a similar absorption spectrum to MB.[1] Previous studies demonstrated significant analytical interference of MB on MetHb measurement, but the studies were conducted more than 20 years ago, and in one study the MB concentrations investigated were at supra-therapeutic range.[2,3] With the emergence of newer co-oximetry models and better pharmacokinetic profiling of MB, this study aims to re-investigate the effect of MB interference on MetHb measurement, by spiking clinically relevant MB concentrations to heparinised whole blood samples at varying MetHb concentrations.

In the current study, MetHb concentration in heparinised whole blood sample was measured with ABL800 FLEX co-oximetry (Radiometer, Denmark), which utilised a 128-wavelength spectrophotometer, at a measurement range of 478−672 nm.[4] The analytical coefficient of variation (CV_A) of the two co-oximetry analysers used in this study was estimated to be 1.6−2.1% at MetHb concentrations of 11.5−28.5% unit.

MetHb samples were prepared in-house by treating heparinised whole blood samples with sodium nitrite solution as described by Shihana *et al.*[5] and Lim *et al.*[6] In brief, sodium nitrite solutions were prepared by dissolving sodium nitrite standard (BDH, Prod 102564W, Lot A608266 617; VWR International Ltd, USA) in normal saline (0.9% sodium chloride) to a final concentration of 100, 500 and 2000 mg/L. Two hundred µL of one of these sodium nitrite solutions was added to 1 mL of heparinised whole blood sample collected from healthy volunteers. The spiked blood samples were mixed and incubated for 5−10 min at room temperature before