molecular assays in 2020 is interesting. Larger prospective genotypic studies will enable more firm conclusions on emergence of new mutations and/or genotypes in the local population.

Recent studies from Italy and Taiwan revealed disparate findings with regard to AMR prevalence during the COVID-19 pandemic^{11,12} with high rates of AMR attributed in one study to increased antibiotic utilisation in COVID-19 patients.¹² In contrast to the experience in Europe and Asia, rates of SARS-CoV-2 infection were low in Australia through 2020 and early 2021, so factors relating directly to COVID-19 disease were not at play in this setting. Nonetheless, net antibiotic utilisation data from this study demonstrate a broad trend toward increased use over the period 2015–2021, and we hypothesise this is a major contributor to overall rising rates of invasive ESBL disease.

The observed rise in ESBL invasive disease suggests that local factors are key contributors to MDR Enterobacteriaceae infection in Australia. Alongside net increases in antibiotic use, other contributors may include inappropriate prescribing practices in hospital, community and agricultural settings, or prolonged carriage of MDR organisms in the gastrointestinal tract with invasive disease manifestation months to years later. This study lends weight to the important argument that considered antimicrobial stewardship is as critical in high income settings as it is in low income countries with higher baseline AMR prevalence rates.

AMR remains a critical public health issue faced locally and internationally and is a major focus of public health and research efforts. Interestingly, this study reveals that international travel is likely not the major contributor to the current ESBL epidemic, at least when viewed with a medium term lens. Rather, AMR must be owned as a local health priority driven at least in part by factors such as suboptimal antimicrobial stewardship at a community level. Future large scale studies are welcomed exploring longer term implications of travel restrictions on locally acquired versus internationally acquired ESBL infection rates, as well as ongoing surveillance for emerging mutations or genotypes.

Acknowledgements: The authors are grateful to the staff of the Nepean Hospital microbiology laboratory for their technical assistance and Mr Robert Robinson for helpful discussions.

Conflicts of interest and sources of funding: The authors state that there are no conflicts of interest to disclose.

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DOI: <https://doi.org/10.1016/j.pathol.2021.11.005>

Identification of *Mycobacterium abscessus* to subspecies level with Bruker MALDI Biotyper

To the Editor,

Mycobacterium abscessus is a rapid growing mycobacteria (RGM) which is further separated into three subspecies: *Mycobacterium abscessus* subsp. *abscessus*, *Mycobacterium abscessus* subsp. *massiliense*, and *Mycobacterium abscessus* subsp. *bolletii*. They can cause various pulmonary and non-pulmonary infections, particularly in vulnerable patients with other underlying medical conditions. Macrolides are key antibiotics used in treating these patients, and are given in combination with other companion antimicrobials.^{1,2} Resistance to macrolides correlates with poorer outcomes. The most common mechanism of resistance is due to the presence of functional *erm* (41) in *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *bolletii* which confers inducible resistance to clarithromycin.^{3,4} *Mycobacterium abscessus* subsp. *massiliense* is conversely associated with better outcomes due to truncation of *erm* (41) rendering it non-functional. Chromosomal mutations in the 23S rRNA (*rrl*) gene may result in constitutive macrolide resistance, however this is relatively uncommon.³

Although RGM such as *M. abscessus* are culturable within a relatively short time compared to other non-tuberculous mycobacteria, the time required from culture positivity to identification and susceptibility testing requires several weeks. Phenotypic susceptibility testing requires up to 2 weeks incubation time to exclude the presence of inducible clarithromycin resistance as recommended by the Clinical and Laboratory Standards Institute.⁵ While treatment may be delayed for some patients, earlier initiation of treatment may be helpful for patients with particularly serious infections. Rapid identification of *M. abscessus* to the subspecies level thus may be useful to clinicians in determining a suitable empiric antibiotic treatment regimen for these patients prior to availability of full susceptibility testing results. Moreover, it is currently recommended that identification to the subspecies level be performed as repeat positive respiratory samples with the same subspecies is required as part of diagnostic criteria of pulmonary infections.¹ Thus, we explored the utility of Bruker MALDI Biotyper (Bruker, USA) for identification of *M. abscessus* to subspecies level.

Between 2017–2019, a collection of 210 *Mycobacterium abscessus* isolates was fully identified based on whole genome sequencing.⁶ Whole genome sequencing (WGS) was performed on the NovaSeq6000 sequencing platform (Illumina, USA). An average sequencing depth of 150× was achieved for the genomes. Subspecies identification was determined based on phylogenetic analyses. The MycoEx protocol was used for inactivation and extraction prior to testing on Bruker MALDI Biotyper using the Mycobacteria Library.⁷ Bacterial suspensions were made in 300 µL of ultrapure water and inactivated by boiling for 30 min. Cold ethanol (900 µL at –18°C) was added and mixed thoroughly. The solutions were centrifuged at 13,000 rpm for 2 min and the supernatant removed. Centrifugation and removal of supernatant was repeated and allowed to air dry. Zirconia/Silica

beads (Biospec Products, USA; 0.5 mm) and acetonitrile (50 µL) were added vortexed at maximum speed for 1 min. An equal volume of 70% formic acid was then added and vortexed at maximum speed for 1 min. Centrifugation at 13,000 rpm for 2 min was performed and 1 µL of supernatant was applied onto the MALDI target plate and allowed to air dry. This was then overlaid with 1 µL MBT Galaxy α-cyano-4-hydroxycinnamic acid matrix solution (Bruker) and air dried before loading into the Bruker MALDI Biotyper for testing. The Bruker MALDI Biotyper identifications were compared against the genomic identifications as the reference standard. There were 204 isolates with MALDI score of ≥1.800 included based on previously established cut-offs for high confidence identifications in mycobacteria.⁷ The identification and highest score achieved are stratified based on subspecies identification and MALDI score in Table 1.

Overall, the results indicate that MALDI was not a reliable tool to identify *M. abscessus* to the subspecies level. There was correct identification in 86.4% of *M. abscessus* subsp. *abscessus* and 24.3% of *M. abscessus* subsp. *massiliense*. Although correct identification rates were highest in *M. abscessus* subsp. *abscessus*, this result was not reliable as *M. abscessus* subsp. *massiliense* was frequently misidentified as *M. abscessus* subsp. *abscessus* (54 *M. abscessus* subsp. *massiliense* were misidentified as *M. abscessus* subsp. *abscessus*). A MALDI result with score of ≥1.800 for *M. abscessus* subsp. *abscessus* and *M. abscessus* subsp. *massiliense* correctly identified the isolate in 50.9% (57/112) and 94.3% (33/35) of cases, respectively. This combination of results indicated that a MALDI result of *M. abscessus* subsp. *abscessus* was relatively sensitive but not specific. Conversely, a MALDI result of *M. abscessus* subsp. *massiliense* was not sensitive, but relatively specific. A MALDI result for *M. abscessus* subsp. *massiliense* was particularly reliable for scores ≥2.100 where no misidentifications

Table 1 Summary of MALDI-TOF results stratified by subspecies identification

Identification by sequencing (no. isolates)	MALDI-TOF results				
	Isolates with correct identifications, n (%)	Isolates with incorrect identifications, n (%)	Incorrect identifications		
			<i>M. abscessus</i> subsp. <i>abscessus</i>	<i>M. abscessus</i> subsp. <i>massiliense</i>	<i>M. abscessus</i> subsp. <i>bolletii</i>
MALDI score of 1.800–1.999					
<i>Mycobacterium abscessus</i> subsp. <i>abscessus</i> (18)	16 (88.9%)	2 (11.1%)	N/A	1	1
<i>Mycobacterium abscessus</i> subsp. <i>massiliense</i> (52)	9 (17.3%)	43 (82.7%)	28	N/A	15
<i>Mycobacterium abscessus</i> subsp. <i>bolletii</i> (1)	0 (0.0%)	1 (100.0%)	1	0	N/A
MALDI score of 2.000–2.099					
<i>Mycobacterium abscessus</i> subsp. <i>abscessus</i> (10)	9 (90.0%)	1 (10.0%)	N/A	1	0
<i>Mycobacterium abscessus</i> subsp. <i>massiliense</i> (37)	7 (18.9%)	30 (81.1%)	16	N/A	14
<i>Mycobacterium abscessus</i> subsp. <i>bolletii</i> (1)	1 (100.0%)	0 (0.0%)	0	0	N/A
MALDI score of 2.100–2.199					
<i>Mycobacterium abscessus</i> subsp. <i>abscessus</i> (25)	20 (80.0%)	5 (20.0%)	N/A	0	5
<i>Mycobacterium abscessus</i> subsp. <i>massiliense</i> (27)	7 (25.9%)	20 (74.1%)	7	N/A	13
<i>Mycobacterium abscessus</i> subsp. <i>bolletii</i> (0)	0 (0.0%)	0 (0.0%)	0	0	N/A
MALDI score of ≥2.200					
<i>Mycobacterium abscessus</i> subsp. <i>abscessus</i> (13)	12 (92.3%)	1 (7.7%)	N/A	0	1
<i>Mycobacterium abscessus</i> subsp. <i>massiliense</i> (20)	10 (50.0%)	10 (50.0%)	3	N/A	7
<i>Mycobacterium abscessus</i> subsp. <i>bolletii</i> (0)	0 (0.0%)	0 (0.0%)	0	0	N/A
All combined (MALDI score ≥1.800)					
<i>Mycobacterium abscessus</i> subsp. <i>abscessus</i> (66)	57 (86.4%)	9 (13.6%)	0	2	7
<i>Mycobacterium abscessus</i> subsp. <i>massiliense</i> (136)	33 (24.3%)	103 (75.7%)	54	0	49
<i>Mycobacterium abscessus</i> subsp. <i>bolletii</i> (2)	1 (50.0%)	1 (50.0%)	1	0	0

MALDI-TOF, matrix-assisted laser desorption ionisation-time of flight mass spectrometry.

occurred. When a MALDI result of *M. abscessus* subsp. *massiliense* with a high score is achieved, the isolate may be confidently identified to subspecies level, which is highly predictive of macrolide susceptibility.³

Prior to the MALDI-TOF era, some organisms were proven to be difficult to identify in routine laboratories. The usage of proteomics-based mass spectrometry has revolutionised operations and MALDI is now commonly used for identifying bacteria, fungi, and mycobacteria due to rapidity as well as accuracy. In the context of mycobacteriology, MALDI-TOF can identify commonly encountered NTM species, but is currently unable to identify closely related organisms such as *M. abscessus* to subspecies level. This is consistent with previous reports.^{8,9} However, we note that a MALDI result of *M. abscessus* subsp. *massiliense* is relatively specific, particularly with scores of ≥ 2.100 . Such a result would be highly predictive of the identification being clarithromycin-susceptible *M. abscessus* subsp. *massiliense*, and may be useful in tailoring empiric antimicrobial therapy particularly for critically ill or immunocompromised patients. Further improvements to the current proteomics-based libraries are required before more reliable subspecies identification can be performed using MALDI-TOF. Modifications to the MALDI approach include lipid-based mass spectrometry.¹⁰ Initial testing suggests that generated mass spectra can differentiate the *M. abscessus* subspecies. Although potentially promising, there remains significant validation to be performed with a larger collection of isolates to ensure reproducibility across different laboratories, and this needs to be followed by development of a publicly available database.

Further advances are required for existing MALDI-TOF based technologies before confident identifications to subspecies level can be made. Currently, molecular testing methods remain the best alternative for earlier subspecies identification. This includes polymerase chain reaction testing to identify the presence of truncated or normal length *erm* (41) genes, PCR and sequencing key genes, or using commercial molecular identification methods.

Conflicts of interest and sources of funding: Whole genome sequencing of the isolates was supported by the National Medical Research Council (NMRC, Singapore) via the Collaborative Solutions Targeting Antimicrobial Resistance Threats in Health System Antimicrobial Resistance Research Grant (CoSTAR-HS/ARGSeedGrant/2019/03). The authors state that there are no conflicts of interest to disclose.

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DOI: <https://doi.org/10.1016/j.pathol.2021.11.014>

Cadaveric donor specimens and serological testing for SARS-CoV-2

To the Editor,

The coronavirus disease 2019 (COVID-19) pandemic has raised questions around the potential risk of severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in tissue and solid organ transplant (SOT) donors and recipients. Cadavers are the major source of donor organs and tissues worldwide. There is a higher risk of severe COVID-19 outcomes related to chronic immunosuppression and associated morbidities. Testing of cadavers for SARS-CoV-2 is limited by the availability and validity of upper respiratory samples, and alternative testing is needed. Screening for SARS-CoV-2 antibodies in donor blood should be given due consideration as a simple and effective way of assessing SARS-CoV-2 infections in cadavers.¹ Clinical outcomes of tissue and organ recipients from cadaveric donors with prior SARS-CoV-2 infection remain uncertain. Approximately 70% of organs and tissues used in transplantation are obtained from deceased donors. All of these require screening for blood borne viruses. More recently with the COVID-19 pandemic, issues have arisen around reductions in donors during the pandemic and during times of increased case numbers, and how to deal with the issues of respiratory virus infection in a laboratory setting mainly directed at blood borne pathogens. Retrieval of SOT and tissues from cadavers introduces unique challenges in testing for infectious disease markers, particularly in cases of tissue donation where cadaveric blood is the only source for testing.² Therefore, adequate testing for SARS-CoV-2 antibodies in cadaveric blood is necessary in order to ensure the suitability and safety of the transplantation.