Table 10.1 Barriers to using MALDI-TOF MS exclusively as a universal typing tool

Limitations of MALDI-TOF MS	Comments
MALDI-TOF MS uses a subset of the proteome	The method selects the most abundance proteins of the cells (ribosomal proteins) - small peaks below the threshold may be unreliable and are excluded in the creation of the database.
Linear MALDI-TOF MS as used in microbiology is qualitative	MALDI-TOF MS, as currently used for species identification, is a qualitative method; mass ions from the same sample can vary in mass intensity between different analyses. However, for practical applications, MALDI- TOF MS relies on pattern matching algorithms using multiple mass ions in the spectrum and therefore acquires sufficient data for identification at the species level.
Interlaboratory reproducibility of instruments	Reports to-date utilise minor peaks in the mass spectrum for typing. The use of these ions to aid typing can be misleading because these are the most variable mass ions derived even when using the same instrument. When the same sample is analysed using a different instrument, the magnitude of variability of these minor peaks are exacerbated.
Reproducibility among technical staff	Interlaboratory reproducibility as 'ring tests' have shown successful identification to the species level but variation in identification scores (Veloo *et. al.,* 2017). If uniform identification scores are unachievable at the species level among a group of collaborators, where sample preparation and analytical parameters are rigorously standardised, it is unlikely to be achieved for typing in different laboratories.
Comparative 16S rRNA and MALDI-TOF MS	Because MALDI-TOF MS spectra comprise mainly highly conserved ribosomal protein structures, it is often assumed that the topology of dendrograms would mirror 16S rRNA trees. It was reported that if strains are >98.2%, concordance between both methods, bifurcations are shared in dendrograms (Schumann and Maier, 2014). However, we could not demonstrate this for strains of *P. aeruginosa* that were >99% similarity in 16S rRNA nor could the same authors for family Microbacteriaceae or genus *Arthrobacter* (see Section 10.6).

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Table 10.2. Summary of the current advantages of MALDI-TOF MS

Criteria	Comments
Simplicity of technique	Minimal sample preparation is required. Formic acid extraction is simple and may be automated if required. Minimal biomaterial (a few cells) is required from a primary agar plate for analysis.
Time to result	Rapid technique - time to result may be 2 -3 minutes. Analysis may be interrupted, and new samples added during processing.
Maintenance of the instrument	Extremely low running cost; eg reagents used are miniscule. Many technical problems may be resolved remotely through electronic communications with the company.
Versatility	Allows for multiple samples to be analysed with marginal cost implication. New instruments such as ASTA's Tinkerbell MS include target plates that allow parallel analysis on a Bruker MALDI-TOF MS.
Laboratory workflow	Fits in well into existing laboratory workflows and may be automated into existing IT network systems
Technical expertise	Staff training is minimal and only low level of technical expertise is required to use an instrument routinely.
Scientific credibility	Concordance with 16S rRNA and WGS are excellent - therefore strong taxonomic endorsement at the species level
Database entries	Amenable to additional diversity being added to existing databases and acquisition of data for additional analysis (see eg Vranckx *et. al.* 2017).
Blood culture	Aids early diagnosis eg. blood cultures and provides preliminary information on antibiotic resistance.
Mixed culture identification	Analysis of mixed samples are improving (see Charretier *et. al.,* 2015, Mahé *et. al.,* 2017, Yang, *et. al.,* 2018).