Specific protein profiles can be generated by analyzing intact bacteria by MALDI-TOF MS (Holland et al. 1996). Although MALDI MS had already been applied to bacterial protein fractions, this method successfully detected identity-specific biomarkers directly from intact bacteria. This technique demonstrated remarkable success for rapid identification because it reproducibly sampled a specific protein fraction in a mass region where time-of-flight mass spectrometers perform well (5–20 kDa), making possible the development of large digital libraries to which unknown spectra could be compared. There have been a few reports of successful applications using MALDI-TOF MS to show antibiotic resistance; for example, the detection of hydrolyzed β-lactams shows the effect of β-lactamases (Sparbier et al. 2012). Cell surface proteins have been used to differentiate MRSA from methicillin-susceptible *S. aureus* (Edwards-Jones et al. 2000). Likewise, with known strains of the *Enterobacteriaceae*, it was possible to differentiate isolates that were resistant from those that were susceptible to imipenem/avibactam (Oviaño and Bou 2017). However, the last two examples do not reflect a general capability. By themselves, the proteins observed in whole-cell MALDI-TOF MS do not reveal antibiotic susceptibility. Therefore, this approach has had to be greatly modified to enhance resistance detection while maintaining its utility for identification (Hrabak et al. 2016).

Generally speaking, MALDI-TOF MS is not a quantitative technique. The signal level depends on the number of laser shots fired, the "quality" of the spot the laser is striking, and the homogeneity of the sample. It has been proposed (Albrethsen 2007; Lange et al. 2014) to use an internal standard in MALDI experiments to estimate the quantity of protein present. If cells were cultured on media with and without antibiotics, the quantity of protein could be used to assess susceptibility, and the overall MS profile could be used for identification. This was demonstrated with a large number of *Klebsiella* strains, which gave results comparable to the Etest (Lange et al. 2014), but this approach has proved difficult in other cases.

A simpler method for measuring protein levels in cells incubated with and without antibiotics makes use of *stable isotope tags*, such as ^{13}C or ^{15}N (Stump et al. 2003). Growing bacteria incorporate the isotope profile of the medium into their macromolecules, including proteins. If replicate cultures are inoculated with and without an antibiotic present, any changes in the isotope profile may reflect the ability of the cells to grow in the presence of the antibiotic (Demirev et al. 2013). This has been demonstrated only with *E. coli* so far (Demirev 2016), but is in principle a universal approach that could be used for identification and resistance determination. Changes in stable isotope profiles can also be monitored in proteins of bacteriophages infecting a target bacterium and used to show bacterial growth and phage replication in the presence of an antibiotic (Rees and Barr 2017).