European Journal of Clinical Microbiology & Infectious Diseases (2019) 38:427–448
https://doi.org/10.1007/s10096-018-3431-3

REVIEW

Fourier transform infrared spectroscopy: unlocking fundamentals and prospects for bacterial strain typing

Ângela Novais[1] · Ana R. Freitas[1] · Carla Rodrigues[1] · Luísa Peixe[1]

Received: 13 September 2018 / Accepted: 11 November 2018 / Published online: 27 November 2018

Abstract

The need to identify highly related bacterial strains is ancient in clinical, industrial, or environmental microbiology. Strategies based on different phenotypic and genotypic principles have been used since the early 1930s with variable outcomes and performances, accompanying the evolution of bacterial features' knowledge as well as technologies, instruments, and data analysis tools. Today, more than ever, the implementation of bacterial typing methods that combine a high reliability and accuracy with a rapid, low-cost, and user-friendly performance is highly desirable, especially for clinical microbiology. FT-IR developments for bacterial discrimination at the infra-species level settled on the identification of bacterial groups previously defined by phenotypic or genotypic typing methods. Therefore, this review provides a brief historical overview of main bacterial strain typing methods, and a comprehensive analysis of the fundamentals and applications of Fourier transform infrared spectroscopy, a phenotypic-based method with potential for routine strain typing. The different studies on FT-IR-based strain typing of diverse Gram-negative and Gram-positive bacterial species are discussed in light of genotypic, phenotypic, and biochemical aspects, in order to definitively give this methodology credit to be widely accepted by microbiologists. Importantly, the discriminatory biochemical fingerprints observed on FT-IR spectra have been consistently correlated with sugar-based coating structures that besides reflecting strain variation are also of high relevance for the specificity in pathogen-host interactions. Thus, FT-IR-based bacterial typing might not only be useful for quick and reliable strain typing but also to help understanding the diversity, evolution, and host adaptation factors of key bacterial pathogens or subpopulations.

Keywords Bacterial typing · Strain differentiation · FT-IR spectroscopy · Capsular types · Serotypes · Serogroups · Surface polysaccharides · Multivariate data analysis

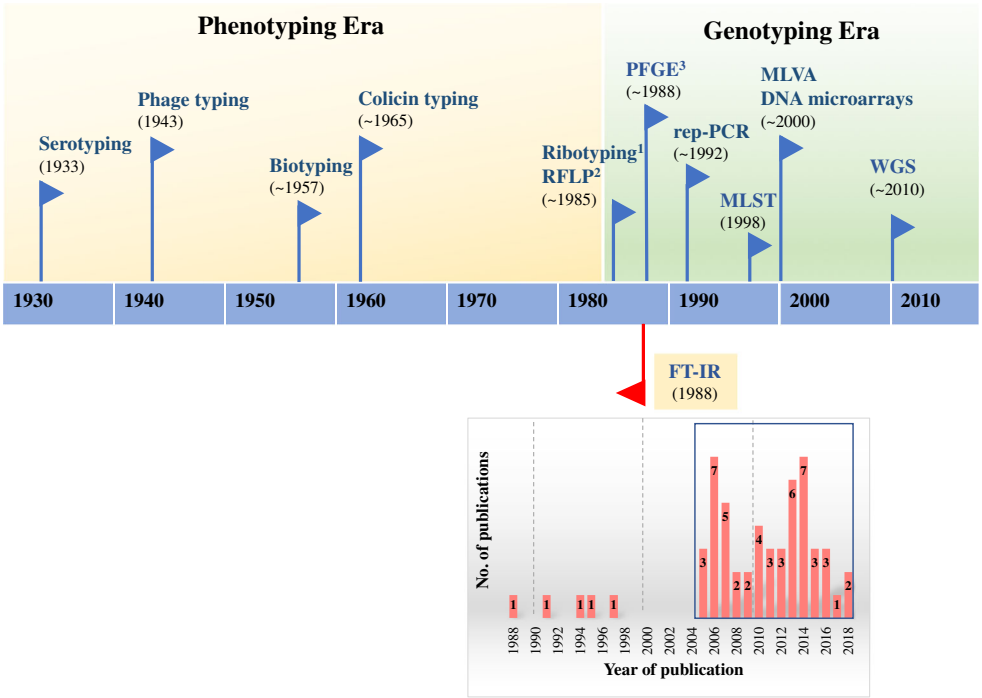
Evolution of bacterial typing methods

Bacterial typing is the process of generating discriminatory strain-specific fingerprints or datasets within isolates from a given bacterial species that is useful to infer phylogenetic descents in clinical, industry, or environmental contexts. Ideally, a typing method needs to provide a reliable and accurate bacterial type, at the highest speed and lower cost possible [1]. In addition, stability of results over time, portability, and appropriate software for both data storage and automated interpretation are a plus to guarantee standardized data and international coverage [2, 3]. The development of typing methods has been intimately linked to the increasing knowledge on bacterial features alongside with the evolution of instruments, technologies, and software for analysis of biological data (bioinformatics tools). A temporal perspective on the evolution of bacterial typing methods contextualized with their fundamentals, advantages, and disadvantages is provided below.

Phenotyping methods were fundamental between 1940 and the 1980s to aid our understanding of bacterial intraspecies diversity and the epidemiology of bacterial infections, and also to distinguish pathogenic from non-pathogenic strains (Fig. 1). Serotyping was one of the most important and it is still applied today in health- or food-associated laboratories for several Gram-negative and Gram-positive bacterial species (e.g., *Escherichia coli*, *Salmonella enterica*, *Streptococcus pneumoniae*, *Listeria monocytogenes*). Because it is labor- and time-consuming, difficult to interpret, and has a high cost associated with the purchase and maintenance of reagents,

✉ Luísa Peixe
lpeixe@ff.up.pt; lpeixe@gmail.com

[1] UCIBIO@REQUIMTE, Laboratório de Microbiologia, Faculdade de Farmácia, Universidade do Porto, Rua Jorge de Viterbo Ferreira, n. 228, 4050-313 Porto, Portugal



The year of first description is indicated in parenthesis. [1] PCR-ribotyping developed in 1993–1999 is a reference method for C. difficile typing; [2] RFLP-IS*6110* developed in 1993 is a reference method for M. tuberculosis typing; [3] Developed in 1984 by Schwartz and Cantor but applied to bacterial typing only at the end of the 1980s.

Fig. 1 Temporal evolution of the development of main phenotypic and genotypic typing methods

traditional serotyping is progressively being replaced by molecular methods [4–7] or in silico serotyping tools based on whole genome sequencing (WGS) data [8–12] (see below).

Established as early as 1930s, traditional serotyping is based on the use of antisera against surface polysaccharides with high specificity such as the somatic (O), flagellar (H), and/or capsular (K) antigens. The antigen formula (O:H:K) is often used as a strain signature for outbreak investigation and identification of particular pathogenic strains (e.g., the enterohemorrhagic *E. coli* O157:H7) [13–18]. Besides some structural differences between Gram-positive and Gram-negative bacteria, the type, composition, and relative abundance of polysaccharide antigens vary between and within species [19, 20]. Gram-negative bacteria possess an outer membrane, where the lipopolysaccharide (LPS) composed of the variable O-antigen (a core polysaccharide) and the lipid A is anchored [21]. On the other hand, Gram-positive strains possess teichoic acids (and less frequently teicuronic acids) covalently bound to the peptidoglycan, which composition seems to vary in their side chains. Some bacteria may also additionally possess capsule (CPS), a hydrated polysaccharide gel that determines the K-antigen. Several of these surface structures are composed of variable types and combinations of saccharide units that determine a specific composition and structure of oligo and polysaccharide chains. Whereas well-characterized for some bacterial pathogens, the diversity and evolution of surface antigens is still largely understudied for some bacterial species [20].

In the late 1970s, the surge of molecular biology tools for the detailed analysis of nucleic acid sequences supported the development of genotyping methods that assess variation in the composition, structure, and sequence of genetic material (in most cases, a fraction of it), the fundamentals of which have been reviewed elsewhere [1, 2]. In Table 1, we present a detailed comparison of the coverage and performance of different genotyping methods with historical importance, some of which are discussed below. The coverage, discriminatory power, and simplicity of these methods are highly variable and, though some of them are semi-automated, for many of them the high cost and length of analysis impair their routine implementation.

Methods with a high discriminatory power such as rep-PCR and especially the gold standard pulsed-field gel electrophoresis (PFGE) have been extremely useful for surveillance and outbreak management in a broad range of bacterial species [23, 24, 48, 49]. More recently, multilocus sequence typing (MLST) provided a standardized protocol and nomenclature that was fundamental to document large-scale dissemination events of particular bacterial clones [50–52], and also foodborne transmission of multidrug-resistant bacterial pathogens [53–55]. However, many MLST schemes currently in use (in some cases more than one *per* species) have a suboptimal discriminatory power and are not able to distinguish

Table 1 Performance of main phenotypic and genotypic methods for bacterial typing

Type	Methods	Species[a]	Discriminatory power[b,c]	Typeability[c,d]	Reproducibility[c,e]	Labor intensity[c]	Time of analysis (days)	Cost[c]	Automation (Y/N/S)[f]	Reference(s)
Phenotypic	Serotyping[g]	Gram− and Gram+	++++	+++	+++	++++	2–7	+++	N	[22]
Genotypic	Amplification and/or restriction-based methods									
	PFGE	Gram− and Gram+	++++	++++	+++	++++	3–5	++	N	[23, 24]
	PCR-RFLP	*Mycobacterium tuberculosis* complex (IS*6110*-RFLP)	++++	+++	++++	+++	1–5	+++	S[h] (RiboPrinter™ System)	[25–27]
	PCR-Ribotyping	*Clostridium difficile*	++++	+++	+++	+++	1–2	+++	S[h] (RiboPrinter™ System)	[28–30]
	AFLP	Gram− and Gram+	+++	+++	+++	+++	1–2	++	N	[25, 31]
	Rep-PCR	*Acinetobacter* spp. *Klebsiella* spp. *Streptococcus pneumoniae* *Listeria monocytogenes*	++	+++	++	++	1–2	+++	S[h] (DiversiLab®)	[25, 32–35]
	DNA microarrays	*Campylobacter* *M. tuberculosis* *C. difficile* *Salmonella* spp. *Staphylococcus aureus* *Escherichia coli*	++++	+++	++++	+++	1–2	++++	S (xMAP®)	[7, 25, 36–39]
	Sequencing-based methods									
	Gene-specific target	Gram− and Gram+	+++	++++	++++	+	1–5	++	N	[4, 27, 40, 41] https://www2a.cdc.gov/ncidod/biotech/strepblast.asp
	MLST	Gram− and Gram+	+++	++++	++++	++	1–5	++	N	https://pubmlst.org/databases/
	MLVA	*S. aureus* *Bordetella pertussis* *S. pneumoniae* *Haemophilus influenza* *Neisseria meningitis* *C. difficile*	++++	+++	+++	+	2–3	++	N	[25, 27, 29] https://www.cdc.gov/pulsenet/pathogens/mlva.html https://www.mlva.net

Table 1 (continued)

Type	Methods	Species[a]	Discriminatory power[b,c]	Typeability[c,d]	Reproducibility[c,e]	Labor intensity[c]	Time of analysis (days)	Cost[c]	Automation (Y/N/S)[f]	Reference(s)
		Gram− foodborne pathogens								
Genotypic	CRISPRs typing	*M. tuberculosis* complex (spoligotyping) *Salmonella* spp.	++++	+++	++++	++	1–2	++	N	[37, 42] http://www.pasteur-guadeloupe.fr:8081/SITVIT_ONLINE/; https://github.com/C3BI-pasteur-fr/Salmonella-CRISPR-Typing
	WGS	Gram− and Gram+	+++++	++++	++++	+++	1–30	++++	N	[3, 43]
Spectroscopic	MALDI-TOF MS	Gram− and Gram+	+++	+++	+++	+	1	+	S (MALDI Biotyper®, VITEK®MS)	[44]
	FT-IR	Gram− and Gram+	++++	++++	+++	+	1	+	S (IR-Biotyper®)	[45, 46]
	Raman	Gram− and Gram+	++	++	++	+	1–2	+	S (SpectraCell RA®)	[47]

PFGE pulsed-field gel electrophoresis, *PCR* polymerase chain reaction, *RFLP* restriction fragment length polymorphism, *AFLP* amplification of fragment length polymorphism, *rep-PCR* repetitive sequencing-based PCR, *CRISPRs* clustered regularly interspaced short palindromic repeats, *MLST* multilocus sequence typing, *MLVA*, multiple locus variant analysis, *WGS* whole genome sequencing, *MALDI-TOF MS* matrix-assisted laser desorption ionization-time of flight mass spectrometry, *FT-IR* Fourier transform infrared spectroscopy

[a] Species for which a method is considered gold standard are indicated, as well as the corresponding performance

[b] Discriminatory power is the ability of a given typing method to differentiate strains sampled randomly from the population of a given species

[c] Characteristics ranged from +, which is poor/simple/low-cost, to ++++, which is excellent/intensive/high-cost according to the characteristic evaluated

[d] Typeability indicates the proportion of strains for which a type can be generated by a given typing method

[e] Reproducibility refers to the capability of a given typing method to generate the same result when tested repeatedly

[f] *Y* yes, *N* no, *S* Semi-automated

[g] Only performed at reference centers by highly skilled personnel

[h] Automated procedures associated with higher costs but with lower labor intensity

particular bacterial lineages [56–59]. Although these methods revolutionized our understanding on the population structure of main (but not all) bacterial pathogens, they had neglected the phenotypic variation of certain genetic changes, and especially genotypic or phenotypic features related with key macromolecules at the cell surface. The assessment of the genetic variation using WGS provides increased genetic information, and efforts are being made to reach a universal approach for real-time strain typing applications [3, 43, 60]. Also, they allowed an increased knowledge on the genetic variation of surface polysaccharides biosynthetic locus and the development of in silico serotyping tools, but these are available only for a small number of bacterial species [8, 11, 61, 62]. Besides, the phenotype prediction based on genetic data is not always straightforward and there is a gap between genetically or serologically defined serotypes [20, 61].

Beyond DNA-based approaches, matrix-assisted laser desorption ionization time of flight mass spectrometry (MALDI-TOF MS) has very recently shown promising results for typing of certain bacterial groups within Gram-positive and Gram-negative bacteria [44, 63, 64]. The low cost and time of analysis and the simplicity of the experimental procedure, together with the availability of instruments in many microbiology laboratories, makes it an attractive option for routine strain typing (Table 1). However, inconsistencies between adopted experimental or data analysis protocols hindered to achieve the desired level of reproducibility, standardization, and portability for clinical microbiology routine laboratories, and validation of target discriminatory biomarkers is still incipient [44, 65]. Considering that MALDI-TOF MS is based on the identification of the molecular masses of many peptides and small proteins (most of which are ribosomal), it is possible that the proteins analyzed might not be reliable evolutionary markers for strain typing at least for some bacterial species [66–68].

Vibrational spectroscopy methods such as infrared (IR) and Raman spectroscopy provide a very attractive performance due to their high-throughput, speed, low cost, and simplicity, though they are less popular bacterial typing methods (Table 1) [69, 70]. They are complementary phenotypic techniques (based on absorption or scattering of the light, respectively) providing biochemical fingerprints of the bacterial cells. Essential developments in instrumentation, experimental procedures, and data analysis occurring during the 1990s boosted a number of publications on Fourier transform infrared (FT-IR) spectroscopy technique for bacterial discrimination (Fig. 1) [71, 72]. In fact, the discriminatory power obtained is comparable to that of gold standard genotyping methods including WGS, rendering it very attractive properties for routine strain typing (Table 1, further details in the next section). Raman spectroscopy and especially its derivatives such as surface-enhanced Raman scattering (SERS) for improvement of signal intensity, resonance-Raman, or micro-Raman have been considered useful even for detection of outbreaks, but they seem to present comparatively lower sensitivity, reproducibility, and discriminatory power at a higher cost, and the basis for bacterial discrimination are poorly understood [71, 73–77]. The lack of consistent databases and protocols for vibrational spectroscopy techniques and the development of the genotypic methods at the beginning of the 2000s motivated their growing abandonment (Fig. 1).

In the next section, we will discuss the current state-of-the-art regarding the potential application of FT-IR for bacterial typing. Our aim is to provide a comprehensive interpretation of existing data in the light of the most recent knowledge on population structure and surface structures for different bacterial species, and contribute to definitively give this methodology credit to be widely accepted by microbiologists as an alternative bacterial typing tool.

Fourier-transform infrared spectroscopy for bacterial typing: a historical perspective

FT-IR spectroscopy is a phenotypic method traditionally used in chemistry to determine the molecular composition of a wide range of sample types. It is a rapid, non-destructive, simple, inexpensive, and high-throughput analytical tool, based on the differential vibrational modes of distinct chemical bonds when exposed to an infrared beam [78]. The first experiments of IR spectroscopy and microorganisms date back to the 1950s [79, 80] but it was during the 1990s that Naumann and collaborators established the principles of the technique for microbiological applications, setting up the experimental conditions for sample preparation and data analysis [72, 81]. It was possible after the development of modern interferometers and Fourier transform techniques (improved time of analysis, reproducibility, and sensitivity) along with potent hardware and software analysis tools. These and many subsequent studies have paved the way the utilization of this methodology for discrimination, classification, and identification of bacteria at different taxonomic levels (genera, species, serotype/serogroup, or even at the strain level), a topic that has been subject of different review papers in the past 10 years [45, 46, 71, 82–84]. All of them agree in recognizing the potential of the methodology as a quick, inexpensive, and high-throughput tool for bacterial typing. However, the molecular basis for that discrimination has been poorly understood till very recently, when it was substantiated with variation on surface bacterial structures in different bacterial species [85–89]. Throughout the years, associations between phenotypic (FT-IR) and genotypic features were established blindly with a high diversity of genotyping methods with variable DNA targets and resolution [87, 88, 90–93]. But considering (i) that many of these

methods do not correlate genomic evolution with the phenotypic behavior or key surface cell structures and (ii) the limitations of the sample in many studies, it is possible that some of the associations established might result from pure coincidences or, conversely, it is not surprising the lack of correlation occasionally observed.

Besides bacterial typing, FT-IR has also been used for other purposes such as to evaluate stress response, the physiological state (viable, non-viable), host-pathogen interactions, to detect dipicolinic acid in bacterial endospores (e.g., *Bacillus*), or the accumulation of polymeric storage like polyhydroxyalkanoates (e.g., PHB) under different nutritional conditions amongst other biological processes; some of these applications have been reviewed elsewhere [94–101].

Fundamentals of the technique

The principle of the technique is that the absorption of the infrared (IR) radiation by a given sample type (bacterial cells in this case) causes excitation and vibration of the different chemical compounds of the cell. Different functional groups absorb radiation at different wavenumber ranges and thus originate characteristic spectral peaks. Thus, an IR spectrum of a bacterial cell provides a specific fingerprint that reflects its composition in nucleic acids, proteins, lipids, and carbohydrates, and thus each microorganism has highly specific infrared absorption signatures, correlated with genetic information (Fig. 2) [46, 72]. IR spectra from intact bacterial cells are complex and peaks represent a superposition of contributions from all its biomolecules. The tentative assignment of IR absorption bands to each of these main building blocks can be performed using established correlations between band frequencies (peak positions cm^{-1}, peak intensities, and peak width) and known biochemical structures (functional groups) of the most important biological organic macromolecules [102]. These correlations can be found in a number of publications [71, 94, 103], but the absence of specific information on discriminatory biomarkers is a well-recognized limitation of the method [46].

The wavenumbers commonly used for acquisition of bacterial FT-IR spectra (and also for other FT-IR applications) is the 4000–400 cm^{-1} region that corresponds to the mid-infrared region of the electromagnetic spectrum. The classical studies of Naumann and collaborators [81] determined five spectral windows corresponding to the absorption expressed in wavenumbers (cm^{-1}) of (i) lipids (window 1, 3000–2800 cm^{-1} dominated by vibrations of functional groups usually present in fatty acids); (ii) proteins and peptides (window 2, 1800–1500 cm^{-1}, dominated by vibrations of amide I and amide II bands); (iii) a mixed region (window 3, 1500–1200 cm^{-1}, with information of proteins, fatty acids, and phosphate-carrying compounds); (iv) polysaccharides (window 4, 1200–900 cm^{-1}, dominated by absorption bands of the carbohydrates present within the cell wall); and (v) a fingerprint region (window 5, 900–700 cm^{-1}, showing some remarkably specific spectral patterns, not yet assigned to cellular components or functional groups) (Fig. 2). Windows 3 and 4 are consistently the most discriminatory and powerful for bacterial identification [81, 88, 104, 105].

The experimental conditions to use FT-IR to analyze biological samples have been described in detail by Baker et al. [106], being essential steps sample preparation, spectra acquisition, and data analysis. Sample preparation is minimal (a pure bacterial colony or suspension) and non-destructive. It has been assumed that variations in culturing conditions (especially the culture media and the incubation time) can greatly affect reproducibility between spectra, at least when the whole spectra is considered [107]. However, preliminary data from our group support previous observations [108, 109] that small variations in the incubation time (± 4 h) and growth in different non-selective culture media are not detrimental to reproducibility, when evaluated against spectra databases obtained in similar conditions (data not shown). One plausible explanation for this is that the discriminatory region used for bacterial typing (1200–900 cm^{-1} dominated by polysaccharides) is less affected by changes in culturing conditions.

The most common spectra acquisition modes for bacterial characterization are transmittance, diffuse reflectance, and attenuated total reflectance (ATR) (~ 1 min per spectra), that will be discussed briefly. The transmission mode consists on the application of a bacterial suspension to water-insoluble optical plates (frequently from ZnSe), that is dried at 50–60 °C or under vacuum before measurement. It results in an increased signal-to-noise ratio but the increased time of sample preparation and the spectra variability due to differences in sample amount or thickness are commonly recognized disadvantages. The diffuse reflectance mode is used to analyze low amounts of solid, powder, or freeze-dried samples, and because of its higher cost, it has been barely applied to bacterial cells [110]. In the ATR mode, the sample (one colony or a small fraction of a bacterial suspension) is placed directly on an optically dense crystal, the IR beam creates an evanescent wave, and the reflected radiation is detected by the detector. The greater simplicity (little or no sample preparation) and the lower cost of analysis together with the best reproducibility possible makes it a very attractive option for bacterial typing. The low sample throughput (one sample per measurement) is however an important caveat, possibly circumvented by the development of an ATR-accessory for multiple sample analysis (https://irubis.com/products/) [111]. Excellent technical reviews provide further details on experimental conditions, instruments and technical specifications, and data analysis tools [46, 83, 112].

Data analysis comprises pre-treatment of spectra (to correct variation related with spectra acquisition) and further comparison by multivariate data analysis tools (to interpret complex chemical data). Unsupervised or supervised methods are used to generate models that can be used for classification and/or identification of bacterial isolates [45, 103, 113]. Isolates relationships' are

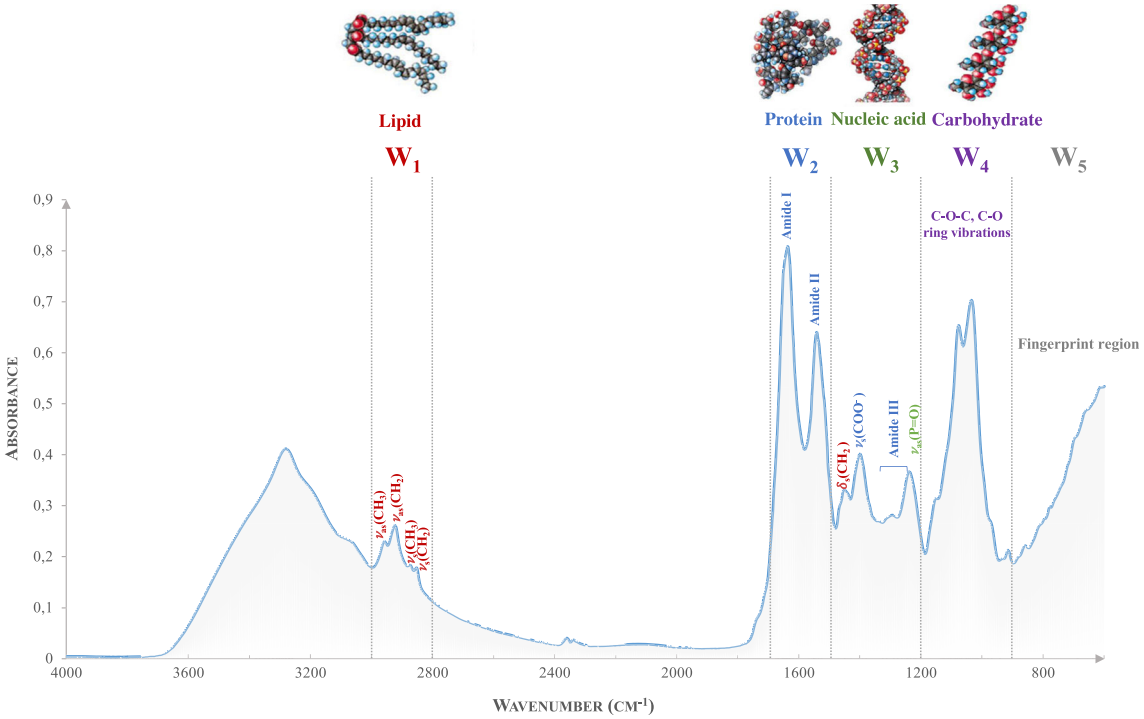


Fig. 2 Typical bacterial FT-IR spectrum showing the spectral windows and main band assignments. The spectrum is from a *Klebsiella pneumoniae* isolate acquired using a Perkin Elmer Spectrum BX FT-IR System spectrophotometer in the ATR mode with a PIKE Technologies Gladi ATR accessory from 4000 to 600 cm^{-1}, a resolution of 4 cm^{-1} and 32 scan co-additions. *ν*, stretching vibrations, *δ* bending vibrations, *s* symmetric vibrations, *as* asymmetric vibrations

established according to the similarity between spectra using pattern recognition techniques for classification of groups of related isolates that might not necessarily rely on existing spectral databases. On the other hand, identification of unknown isolates depends on the comparison of spectra with those from a reference database, in a manner similar to that used for MALDI-TOF MS-based microbial identification. Methods based on machine learning techniques such as partial least squares discriminant analysis (PLSDA) or artificial neural networks (ANN) are known to be powerful to extract specific spectral signatures [45, 85, 88], but they have only been tested and validated on specific and limited collections of isolates and require skills of specialized personnel. Only one commercial database created for bacterial species identification is known (Bruker Optik), and most studies have relied on in-house databases (libraries of spectra from well-characterized isolates) that have been used essentially for identification at the genera or species level [103, 108, 114–117].

Expanding the current perspective of bacterial typing at the infra-species level by FT-IR

We searched PubMed-indexed records in MEDLINE for studies including the typing of bacterial species by FT-IR using the medical subject heading (MeSH) terms "Spectroscopy, Fourier Transform Infrared" or "Spectrophotometry, Infrared" including their related subheadings (instrumentation, methods, economics) and "Bacterial Typing Techniques" or "Bacterial typing." We identified 110 entries, which resulted in 59 publications that documented bacterial typing studies at the infra-species level by FT-IR. It should be highlighted that though designed with the purpose of bacterial subtyping, some studies lack robustness (limited sample with low representativeness, lack of reproducibility, and/or comparison with reference typing methods) and hence do not support appropriately FT-IR-based assignments with bacterial groups with biological significance. For these reasons, these studies were excluded or just briefly mentioned. Additionally, studies such as those evaluating different bacterial stress responses (e.g., resistance to heat, biocides, etc.) or of specific cellular components (e.g., production of spores, identification of pigments) were excluded. Additional search outside MEDLINE retrieved ten additional entries corresponding to manuscripts with MeSH terms that are still not available or had different designations besides "bacterial typing" such as "discriminant analysis," "DNA fingerprinting," "cluster analysis," "microbiological techniques," and "serotyping." Only full-text articles with an English abstract were included (use of language filter). In total, 69 publications were included and will be discussed in detail in light of current literature. We pretend to compile and correlate main aspects from spectroscopic, biochemical, and microbiological perspectives in order to expand our knowledge on the fundamentals of the discriminatory potential of FT-IR for typing different bacterial species at the infraspecies level.

Gram-negative bacteria

The potential of FT-IR to differentiate and identify particular groups of strains within Gram-negative species has been explored particularly for *E. coli*, *S. enterica*, and *Acinetobacter baumannii* in the contexts of clinical or food microbiology. Most of the studies used transmission FT-IR, diverse sampling conditions (e.g., culture media), and much more variable data analysis workflows. It is worth noting that correlations with surface structures have been established by different researchers, but only recently these correlations were adequately supported by phenotypic (variations on the composition and structure of surface antigens) and genotypic (well-defined lineages) bacterial features, providing robustness to the FT-IR based assignments. Studies performed to differentiate serogroups, serotypes, and/or strains within different Gram-negative bacteria are summarized and discussed below.

Escherichia coli The gold standard for *E. coli* serotyping is based on the characterization of highly diverse O-polysaccharide ($n = 186$) and H-flagellar ($n = 53$) surface antigens, though the K (capsular) antigen is also usually present. Molecular serotyping methods are increasingly used [7] and molecular epidemiology studies suggest very close associations of particular serotypes and clinically relevant clones (e.g., ST131-O25b:H4, ST393-O15:H1) [118].

The ability of FT-IR to differentiate *E. coli* at the subspecies level is documented even in the classical studies by Helm et al. [81]. In these very first attempts, *E. coli* isolates were grouped according to their O-antigenic structures, and it was more recently demonstrated that FT-IR could be useful to distinguish certain genetically identical O-antigen subtypes [119], both of them considering a region dominated by polysaccharides (1200–900 cm^{-1}). Different research groups have also independently optimized FT-IR for detection and quantification of *E. coli* O157:H7 in different food matrices (ground beef, apple juice). Also, differentiation of this serotype from other closely related serotypes or other non-pathogenic *E. coli* strains [120–122], or even to discriminate diverse O157:H7 strains in concordance with MLVA typing results [123], which is of high interest for food quality control. The spectral region considered for analysis ranged from 1800–900 cm^{-1}, 1700–750 cm^{-1}, or only 1200–900 cm^{-1} and included different non-supervised [hierarchical clustering analysis (HCA) and principal component analysis (PCA)] and supervised [canonical variates analysis (CVA) and soft independent modeling of class analogies (SIMCA)] methods.

Subsequent studies pointed out strain-specific phenotypic variation [108, 124] but in these cases, only a very small number of isolates was analyzed ($n = 2–10$) and results were not compared with reference typing methods. More recently, studies performed by us and another research group demonstrated a very good reliability on the differentiation of eight typical uropathogenic *E. coli* clones using representative (though uneven) collections and distinct experimental workflows (sample preparation, acquisition modes) and methods for data analysis. FT-IR-based assignments were compared with those obtained by MLST and high prediction rates (91–100%) were obtained for the worldwide spread sequence type (ST)131 [104, 125, 126], suggesting a highly congruent phenotypic profile for the isolates tested, and also that FT-IR is a good alternative diagnostic tool for this clone. On the other hand, varying accuracy was reported for other *E. coli* clones, i.e., from 30 to 91% in the study by Dawson et al. [125] or 100% in the study by Sousa et al. [104], which might be explained by the different spectral regions analyzed (from 4000 to 700 cm^{-1} or 1500 to 900 cm^{-1}, respectively) and/or the different data analysis workflows used: (i) discriminant function analysis (DFA) and PLSDA or (ii) PLSDA/SIMCA. Alternatively, it could be explained by differences on the natural diversity of the collections used (e.g., intraclonal variation) characterized by MLST. In fact, recent WGS data uncovered a suboptimal discriminatory power of MLST and a wide diversity of genotypic and phenotypic traits (including at the O and K antigens locus) among isolates belonging to the same ST, including ST131 [8, 127–129]. Whereas all ST131 isolates included in the study by Sousa et al. [104] belonged to the O25b serogroup (data not shown); this information is missing in the study by Dawson et al. [125] and was not taken into account in both studies. It is however of remark that subsequent studies performed by our group showed that different ST131 subtypes defined by partial or whole genome typing methods can be distinguished by FT-IR, and that there is a correlation with variation on surface antigens (Novais et al. unpublished data). These data stress the need to provide significance to the bacterial groups' assignments obtained by FT-IR by a comprehensive correlation of genotypic and phenotypic data on well-characterized collections of *E. coli* isolates.

Finally, several studies attempted to differentiate the susceptibility status (susceptible or resistant) of *E. coli* to different antibiotics, most of them using the ATR mode and ANN models. However, considering the diverse antibiotic resistance mechanisms for each antibiotic and the absence of correlation with strain typing methods, it remains to be elucidated if the declared discrimination potential is related to the expression of antibiotic resistance traits or strain variation [98, 130–132].

Salmonella enterica Serotyping is still recognized as the very first approach to discriminate *S. enterica* isolates, based on agglutination reactions with specific antisera targeting the somatic (O) and the flagellar (H) antigens [14]. Currently, more than 2500 serotypes have been described, though only a few (essentially those from serogroups B, C, D, E) are linked to the majority of foodborne or nosocomial infections in industrialized countries [133, 134]. Phage typing is also widely used,

where phage specificity depends on molecular traits of the bacteriophage and receptors of the bacterial cell, especially variations at the O-polysaccharide [135].

The differentiation of *S. enterica* serogroups, serotypes, and phage types by FT-IR has been repeatedly tested by different research groups, experiments that contributed to establish the discriminatory potential and to evaluate different experimental conditions, but that were not always considered successful [136, 137]. All of them consistently indicate the polysaccharide region (1200–900 cm^{-1}), or even specific peaks (within 1000–700 cm^{-1}) as major contributors for differentiation, whereas no differences were observed in outer membrane proteins (OMP) extracts, as expected [137]. Most studies have been dedicated to assess the discrimination between *S. enterica* serotypes, using only one representative isolate of a low number of serotypes ($n = 4–6$) from serogroups B, C, D, and/or E [96, 138–141]. Even with limitations in sample (size and representativeness), these studies reported discriminatory spectra and high rates of correct predictions (100%), particularly when using LPS extracts that performed better than the whole cell [138, 140]. These results were reproducible in either non-selective (plate count agar, tryptic soy broth) or selective and differential (xylose lysine deoxycholate agar, Miller Mallinson agar) culture media, in different biological replicates, using different equipment (transmission, ATR, or microspetroscopy) and multivariate data analysis workflows (CVA or SIMCA) [139–141]. It is of remark that in some of these studies, sample preparation requires several steps of suspension, centrifugation, and washing of bacterial cells, increasing the complexity and time of analysis with no clear improvement on the discriminatory results. A more representative collection of isolates was analyzed by Preisner et al. [142] ($n = 87$ isolates from 26 different serotypes). In this study, successful discrimination was obtained and validated for serogroups B, C1, C2-C3, and D1, and specific peaks were suggested as characteristic of serogroups B (976 cm^{-1}), C1/C2/C3 (968 cm^{-1}), and D1 (986 cm^{-1}) and, in a subsequent model, C1 from C2/C3 (1010–940 cm^{-1} and 1180–1140 cm^{-1} regions). In turn, discrimination of serotypes within each of the serogroups was only liminarly possible.

The involvement of O-polysaccharide structures has been suggested in some of the aforementioned studies, but the correlation of the discriminatory results with available data on O-unit structures has only been analyzed in a recent paper from our group. In the study performed by Campos et al., a wide and well-representative collection was tested ($n = 325$ isolates from 15 serogroups and 57 serotypes) and FT-IR-based typing was validated for particular serogroups (B, C, D, E) and serotypes (*S.* Rissen, *S.* Enteritidis and *S.* Senftenberg) using a simple whole cell approach, and for the first time supported by molecular-based knowledge [88]. FT-IR-based phage differentiation among *S. enterica* serovar Enteritidis has also been tested with apparently good results but very few phage types were evaluated hindering to extract robust conclusions [143, 144].

Klebsiella pneumoniae Serotyping is still useful for epidemiological purposes and is currently performed by genotypic methods or in silico analysis of whole genome sequences, whereas classical serotyping is mainly restricted to reference centers [4, 10]. In any case, it is based on the characterization of K- and O-antigens (H-antigen is absent), having been initially recognized 77 K-types and 8 O-types for *K. pneumoniae* [145]. Considering the low variability of O antigens (O1, O2, and O3 being the most prevalent), K-typing is more generally used and genomics studies recently suggested a higher capsular diversity that has been barely correlated with phenotypic data and available sera [61]. Much less is known regarding capsular variation in other *Klebsiella* species.

Using a well-characterized collection of *K. pneumoniae* clones exhibiting variable capsular types, we tested FT-IR differentiation using the whole cell and the ATR mode. A two-step PLSDA model revealed a high accuracy (ca. 95% of correct predictions, using the carbohydrates vibration region 1200–900 cm^{-1}) in discriminating all 21 *K. pneumoniae* K-types tested, which was substantiated by differences on the composition and structures of K-types [87]. The differentiation obtained is higher than that obtained by MLST, and allows discriminating main lineages carrying specific capsular types (e.g., ST15-K24, ST147-K64, or ST258-KL106) that are of high clinical relevance in the context of clinical infections and antibiotic resistance (Rodrigues et al., unpublished results). These in-house models are being applied in real-time clinical contexts to identify outbreak isolates reliably and quickly (in less than 36 h including an overnight incubation step). These results highlight the suitability of this methodology as an alternative cost-effective typing method for identification of clinically relevant MDR *K. pneumoniae* strains, at least in short-term epidemiological contexts [117]. A very recent study evaluated FT-IR discriminatory ability of 68 *Klebsiella* sp. isolates using the commercially available IR Biotyper (Bruker Daltonics) and a semi-automated analysis workflow [146]. The authors correlated the clustering of FT-IR spectra and that obtained by single-nucleotide polymorphisms (SNP)-based comparison of whole genome sequences, but no information about surface antigens is provided that would support both correct and incorrect FT-IR-based assignments, especially considering the diversity of the collection analyzed [146].

Klebsiella oxytoca Only one study was found in the literature using FT-IR to assess the relationship between 10 *K. oxytoca* strains from contaminated liquid hand soap [92]. FT-IR spectra were acquired in the transmission mode and analyzed with the HCA algorithm (using spectral windows 900–750 cm^{-1},

1200–900 cm^{-1}, 1500–1300 cm^{-1}, and 3000–2800 cm^{-1}). Isolates were grouped in a unique homogenous cluster and the authors report a good correlation with the genotypic methods used (MLST, PFGE, and SNP-based typing from WGS data). Thus, the methodology allowed to establish a common origin for the epidemiologically related isolates, and it remains to be elucidated the discriminatory potential in a wide population of *K. oxytoca* isolates.

Acinetobacter baumannii Despite several attempts to develop a serotyping scheme for *A. baumannii* strains, the task was only successful in the late 1990s (34 serovars were defined), and the contemporaneous development of genotyping methods led to the abandonment of this typing method [147]. More recently, with the boost of WGS, the interest in the *cps* locus of *A. baumannii* strains was revived and a deeper knowledge of the structures involved and surface composition is available [148].

The suitability of FT-IR to differentiate *A. baumannii* at the clonal level was firstly described in 1995 by Seltmann et al. [149]. In this work, clinical *A. baumannii* strains involved in different nosocomial outbreaks and also sporadic isolates were typed by different reference phenotypic (biotyping, antibiotic susceptibility testing) and genotypic (PFGE, plasmid typing) methods at that time, and the results were compared with those obtained by FT-IR. FT-IR was used in the transmission mode and three spectral regions (3000–2800 cm^{-1}, 1500–1200 cm^{-1}, and 1200–900 cm^{-1}) were considered for cluster analysis using Ward's algorithm. Isolates were grouped in six clusters comprising outbreak isolates but also some sporadic strains and no correlation was established with any of the other typing methods used or even with epidemiological data [149].

Twenty years later, Sousa et al. described the ability of FT-IR to type this species at the clonal level and to correlate the results with those obtained by MLST [105, 150]. The two different works were performed in different FT-IR devices and using different acquisition modes (transmission and ATR), but the region analyzed (1500–900 cm^{-1}) and the mathematical analysis (HCA and PLSDA) were identical and yielded identical results. Three (ST98, ST103, ST208) and four (plus ST218) clones were discriminated with > 99% of accuracy [105, 150], clones that are frequently involved in nosocomial outbreaks and multidrug resistant profiles worldwide. In both studies, the ability of FT-IR to differentiate between the different clones relied on two pronounced spectral regions (1400–1350 cm^{-1} and 1200–950 cm^{-1}), although difficulties were perceptible in the discrimination of ST98 from ST103 [105, 150]. These two STs share the same *cps* locus. In fact, in a recent work performed by our group (Silva et al. unpublished results), the ability of FT-IR to discriminate *A. baumannii* clones was correlated with differences in surface capsular composition, depicting specific associations between capsular types and high-risk clones over large periods of time.

Other Gram-negative species

Legionella pneumophila Horbach, Naumann, and Fehrenbach were in 1988 pioneer in the use of a spectroscopic procedure for strain differentiation of *L. pneumophila* isolates obtained from different patients [151]. First, the authors identified the best discriminative spectral region (1200–900 cm^{-1}) using two reference strains belonging to serogroups 1 and 4. They subsequently used the same polysaccharide region to differentiate between *L. pneumophila* isolates from the same or different patients and results were compared with other reference strains from known serogroups. They estimated the serogroup of each isolate using the low "differentiation indices" values, but conclusions are limited given the lack of serotyping or alternative typing methods for comparison. The authors were also able to identify additional regions with potential for discrimination between isolates of the same patient (1400–1200 cm^{-1} and 1800–1500 cm^{-1}), though their significance was not explored. Soon after, Helm et al. demonstrated the grouping of *L. pneumophila* ($n = 15$) according to the production of poly-β-hydroxy fatty acids that are particularly evidenced in the 1200–900 cm^{-1} spectral region, though this association could not be clearly demonstrated [81].

Yersinia enterocolitica *Y. enterocolitica* can be grouped into various biotypes (by biochemical tests) and different serotypes (by immunological tests). Two studies using the same workflow (ANN) and including samples with diverse backgrounds (179 pets from different EU countries versus 123 human and foodborne animals from Switzerland) were able to differentiate isolates from main *Y. enterocolitica* biotypes (1A, 2, 3, 4, 5) and serotypes (O:3, O:5, O:9, and "non-O:3, O:5, and O:9") [152, 153]. Kuhm et al. [152] achieved correct predictions of 98.3% and 92.5% for biotypes and serotypes, respectively, and used the wave number range of 1800–500 cm^{-1}. Stamm et al. [153] considered the spectral ranges of 1400–500 cm^{-1} and 3000–2800 cm^{-1}, but the exact contribution of the different zones remains to be clarified. In both studies, the authors were able to identify one relevant pathogenicity marker, the *ail* gene codifying for an outer membrane protein that promotes attachment and invasion, in all *Y. enterocolitica* biotypes but 1A with similar rates of correct predictions (98.5%), though they did not discuss about testing variable spectral regions or the region related to the identification of *ail*. Furthermore, negative *ail* isolates were all of biotype 1A (usually less pathogenic), thus the differentiation between ail^+ and ail^- isolates could be misinterpreted by the accurate distinction of pathogenic and non-pathogenic bioserotypes.

Burkholderia cepacia Coutinho et al. [154] were able to successfully differentiate at the ribopattern level isolates from different species (> 10) from the *B. cepacia* complex. They

could discriminate between strains belonging to the same species including *B. cepacia* ($n = 84$, 5 ribopatterns), *B. cenocepacia* lineage III-A ($n = 38$, 4 ribopatterns), and *B. cenocepacia* lineage III-B ($n = 36$, 4 ribopatterns) with correct predictions of 96.1%, 97.9%, and 92.6%, respectively. The spectral region generating the best discriminatory results was that of 1500–900 cm^{-1} which is greatly characterized by polysaccharidic vibrations of C–O–C and C–O (1200–900 cm^{-1}) and asymmetric stretching vibrations of phospholipids (~ 1250 cm^{-1}).

***Campylobacter* spp.** Different strains of both *Campylobacter coli* and *Campylobacter jejuni* species were differentiated within the polysaccharide region (1200–900 cm^{-1}) and results generally agreed (98.7% correct assignments using a supervised method) with those obtained by enterobacterial repetitive intergenic consensus (ERIC)-PCR [155], a highly discriminatory genotyping method, though the robustness or fundamentals for these assignments are uncertain.

Xanthomonas oryzae Ge et al. [156] could distinguish between two pathovars of *X. oryzae* (*X. oryzae* pv. *oryzae* and *X. oryzae* pv. *oryzicola*) using several spectral regions covering nucleic acids, proteins, phospholipids, and polysaccharides. They identified specific wave numbers (from 3433 to 951 cm^{-1}) associated with different functional groups [CH_3/CH_2 (a)symmetric stretching, CH_3 scissoring and bending, CH_2 bending, amide I/II/A, amide II/COO^- stretching, N-O or C-O stretching] as distinctive of the two pathovars. Even though the analysis was performed without chemometrics, this study revealed a higher ratio of protein-to-lipid in *X. oryzae* pv. *oryzae* strains than that in *X. oryzae* pv. *oryzicola* (calculated in the 2992–2837 cm^{-1} region).

Gram-positive bacteria

The ability of FT-IR to discriminate Gram-positive species at the strain level has been greatly explored in species mostly considered of human or food safety relevance, such as *S. aureus* or *L. monocytogenes*. Most species can produce capsular polysaccharides (in some cases, not all strains within the same species), that is on the basis of serotyping and apparently on strain discrimination by FT-IR. Studies performed to differentiate serogroups, serotypes, and/or strains within different Gram-positive bacteria are analyzed below, some of them providing a precise correlation with specific surface structures (particularly on the most frequently studied *S. aureus* or *S. pneumoniae*). Most of them used transmission FT-IR and similar sampling conditions (e.g., nutritive culture medium, cell washing with water or saline) but diverse data analysis workflows (CVA, HCA, ANN).

Staphylococcus aureus Among the 13 recognized serotypes in *S. aureus*, it is known that the great majority of clinically important *S. aureus* express serotype 5 (CP5) or serotype 8 (CP8) capsular polysaccharides or non-typeable often enuncapsulated variants (e.g., serotype 336) [157]. CP5 [β(1,4)-D-ManAcA-α(1,4)-L-FucNAc(3OAc)-β(1,3)-D-FucNAc] and CP8 [β(1,3)-D-ManAcA(4OAc)-α(1,3)-L-FucNAc-α (1,3)-D-FucNAc] share the same sugar repeat units but differ in their glycosidic linkages and acetylation, and are serologically distinct [158].

A number of studies have explored the potential of FT-IR to determine the capsular polysaccharide variants of *S. aureus* strains according to their variable surface glycostructural composition [85, 90, 159, 160]. These studies were able to successfully differentiate most clinically relevant *S. aureus* capsular types (CP5, CP8, and non-typeable variants) following the workflow described by Grunert et al. [85] and showed a reliable CPS discrimination through the combination of the polysaccharide (1200–900 cm^{-1}) and fingerprint (900–800 cm^{-1}) regions. Differentiation was associated with peaks at diverse spectral wavenumbers within the W4 (1110, 1097, 1070, 1058, 1030, and 975 cm^{-1}) and W5 (834 and 823 cm^{-1}) regions, the latter putatively corresponding to structural α- and β-glycosidic linkages of *S. aureus* capsules [85, 90]. Very recently, Grunert et al. [161] demonstrated that whole cell FT-IR spectroscopy is able to detect changes in *S. aureus* wall teichoic acids (WTAs) glycoepitope composition by discriminating between α-/β-*O*-*N*-acetylglucosamine substitutions, known to be important for host cell adhesion and pathogenesis. Further studies are still needed to elucidate if FT-IR is able to accurately differentiate capsular types other than CP5 and CP8.

Attempts to differentiate between *S. aureus* subpopulations generally matched those previously recognized by PFGE, MLST, *spa* typing, and/or *dru* typing depending greatly on the correlation between a given lineage and a given capsule genotype or surface associated glycostructures [90, 116, 159, 160, 162–164]. The most relevant clones in hospital-acquired infections (CC5 and CC8) or livestock and bovine (CC398 and CC705, respectively) were tested using the spectral region 1200–800 cm^{-1} [90]. With a few exceptions (CC45 and CC705), the clonal complexes of *S. aureus* were hardly differentiated by FT-IR spectroscopy which is explainable by the fact that the same capsular (*cap*) locus can occasionally be associated with different CCs [90, 159]. Amiali and colleagues [163] had shown discriminatory potential using other spectral regions, but the workflow for spectra analysis used was also unique [single-value decomposition algorithm followed by PCA, self-organizing map (SOM), and k-nearest neighbors (KNN)]. They differentiated community (mostly USA300 and USA400) from hospital MRSA strains in the narrow spectral region of 1361–1236 cm^{-1} (differences in protein amide III and nucleic acid phosphodiester

contents) and hospital Canadian MRSA strains (5 PFGE types) in 1180–1040 cm^{-1} and 2904–2864 cm^{-1} (polysaccharide and lipids, respectively) [163].

Beyond the clonal assignments by FT-IR, other approaches included the use of this technique to distinguish between normal and small colony variants (SCV are linked to persistent/recurrent infections) of *S. aureus* on solid and broth media with prominent differences being observed in the polysaccharide and protein regions [165]. Notably, isolates with different clonal backgrounds were consistently clustered according to their normal or SCV status (the latter characterized by a dormant metabolic state). In another study, Amiali et al. [166] were able to separate vancomycin-intermediate ($n = 35$) from vancomycin-susceptible ($n = 25$) heterogeneous *S. aureus* strains, on the basis of spectral data from regions 1352–1315 cm^{-1} and 1480–1460 cm^{-1}, despite not providing additional information (e.g., clonal types). Although the mechanism for intermediate-resistance phenotypes is not perfectly understood, it is presumably associated with increased cell wall thickness, reduced peptidoglycan cross-linking, altered surface protein profile, and changes in bacterial growth, all of them metabolic changes that might be detected by FT-IR [167]. Further studies on wider and comprehensive collections of *S. aureus* strains will support the suitability of FT-IR in the rapid and reliable screening of such worrisome strains in clinical and food contexts.

Streptococcus pneumoniae More than 45 pneumococcal serogroups (e.g., 9, 23) and 90 capsule types (commonly termed serotypes; e.g., 9A, 9V, 23A, 23B, 23F) have been identified among *S. pneumoniae* based on their reactivity with reference sera [168]. The genetic loci for capsule synthesis (*cps* loci) were sequenced for all known pneumococcal capsule types in 2006, and it is common to use genetic data to infer capsule serotypes [168–170]. WGS-based comparative genomics clarified conventional serotyping and revealed that specific serotypes from different serogroups can be more related among their *cps* loci than those within the same serogroup which can be explained by the fact that serogroups were defined by common epitopes in the absence of known CPS structures or *cps* sequences at that time [169]. This is something that must be considered when relating genotypic and capsule phenotypic data obtained by FT-IR.

Pneumococcal capsular repeating units generally have two to eight saccharide residues, varying in their linkages, and often have *O*-acetyl, phosphoglycerol, and pyruvyl acetal substitutions located at various sites with various substitution rates [168, 171]. Two studies achieved the discrimination of serogroups and main serotypes covering much of invasive pneumococcal disease (2, 3, 6A, 9V, 14, 18C, 23F) [86] versus those associated with carriage (9F, 9N, 14, 19A, 19F, 23A, 23B, 23F) [172] in the carbohydrate region of 1185–900 cm^{-1}. Vaz et al. [172] attributed the discrimination of serogroups to specific vibrations in nucleic acids (P=O stretching) and other spectral differences to the differentiation of some serotypes. These included 9N and 9V (mainly 1030 cm^{-1}), 19A and 19F (1040–1020 and 990–970 cm^{-1}), and 23A, 23B, and 23F (1165 and 950 cm^{-1}). Remarkably, these differences can be corroborated by available genomic or structural CPS data such as (i) serotypes 9N and 9V differ in the repeat unit (Glc*p*NAc vs Glc*p*) and in the presence/absence of an *O*-acetyl transferase gene (*wcjD*) and an adjacent IS element [169]; (ii) serotypes 19A and 19F differ in their respective polysaccharide polymerase genes (*cps19aI* and *cps19fI*, respectively), which are predicted to form different glycosidic linkages in type 19A ($\alpha 1 \rightarrow 3$) and type 19F ($\alpha 1 \rightarrow 2$) [173]; and (iii) serotype 23A differs from 23F in the disaccharide backbone and the di-substituted β-Gal (β-Rha as a side chain), whereas the structure of the serotype 23B is the same as 23F, but without the terminal α-rhamnose [174].

Sahu et al. [86] have also distinguished capsulated and non-encapsulated *S. pneumoniae* forms by cluster analysis in the same polysaccharide region, and subsequently opaque and transparent forms within the encapsulated strains in both carbohydrate and lipids/phospholipids (1350–1480 cm^{-1}) regions. Remarkably, these and other authors [175] have quantitatively evaluated the capsular content of *S. pneumoniae* phase variants within a given serotype on the basis of their relative carbohydrate content (opaque variants had a higher content compared with their transparent counterparts).

Given the possibility of genotypic-phenotypic discrepancies and the genetic similarities of certain serotypes (not all serogroups can be resolved by genomics), a combined approach of FT-IR and full sequencing of serogroups and serotypes determinants in larger and comprehensive collections of pneumococcus could be helpful to clarify serotyping.

Listeria monocytogenes The serotyping of *L. monocytogenes* is based on the combined expression of surface O-somatic and H-flagellar antigens, with WTAs constituting the major O-antigen determinants. WTAs are cell wall-anchored anionic glycopolymers accounting for up to 60% of *Listeria* cell wall [176].

The very first attempt to differentiate *L. monocytogenes* below the species level dates from 1997 [109]. Since then, different studies succeeded in the delineation of serogroups (correct identifications up to 98.8%) and main serotypes of this species (up to 96.6%), including those greatly associated with human listeriosis (1/2a and 4b). In all cases, spectra corresponding to the different serogroups or serotypes were better differentiated in the polysaccharide region (1200–900 cm^{-1}) and the distinction between serotype 4b and serotypes 1/2a or 1/2b was much easier than between the closely related 1/2a and 1/2b [109, 177–180]. A relationship between serogroups and WTA structures is well known since 1984 [181], but available studies on FT-IR and *L. monocytogenes* evidenced

that the biochemical composition of WTAs might also contribute to the variations in FT-IR spectra of different serotypes [180]. Pronounced diversity in WTA structure and antigenicity is conferred by differences in the glycosidic substitution(s) (rhamnose in serogroup1/2 vs galactose and/or glucose in serogroup 4) of the ribitol phosphate units (polyribitol phosphate with *N*-acetylglucosamine, Glc-NAc) [182]. Thus, as each serotype of *Listeria* should possess a unique WTA structure, that seems to be corroborated by comparative genomics data of the biosynthetic pathway operon [183], it is tempting to project the future optimization of FT-IR toward the detection of each WTA signature. Much less is known about the structural basis underlying the H serotype specificity. The flagellin of *L. monocytogenes* from different serotypes (1/2a, 1/2b, and 4b) displays an identical pattern of glycosylation with β-*N*-acetylglucosamine [184] and it might not influence, as much as the surface O-somatic antigen, putative FT-IR serogroup/serotype assignments.

A few approaches have also successfully differentiated between *L. monocytogenes* strains in comparison to standard molecular methods such as amplified fragment length polymorphism (AFLP) [185], multilocus genotyping (MLGT) [180], or multivirulence-locus sequence typing (MvLT) [186]. In the first study, the polysaccharide and fingerprint regions of FT-IR spectra showed the highest discriminatory power, with two bands at 985 and 840 cm^{-1} attributed to pyranose being the most significant for strain's discrimination [185]. Grouping of *L. monocytogenes* strains according to haplotypes, as defined by MLGT and PFGE, was achieved in the "mixed region" comprising the polysaccharide and fingerprint regions (1400–750 cm^{-1}) with an accuracy of 91.7–95% [180]. Finally, *L. monocytogenes* epidemic clones ECIII (serotype 1/2a) and ECIV (serotype 4b) were discriminated with 100% accuracy with the most prominent differences being identified in amide I (1650 cm^{-1}) and amide II band of proteins (at ~ 1650 and 1540 cm^{-1}), and asymmetric and symmetric deformations of methyl and methylene of proteins (at ~ 1455 and 1398 cm^{-1}) [186]. This result is not surprising considering that each of these clones belong to different serotypes. The authors also stated they were also able to differentiate strains within the same epidemic clone (undistinguishable by MvLT) but the collection only comprised two strains from each clone, making conclusions fairly questionable. A higher number of *L. monocytogenes* isolates ($n = 46$) was tested by Wenning et al. (in the regions of 3030–2800 cm^{-1} and 1800–700 cm^{-1}) but results were compared only to random amplified polymorphic DNA [random amplified polymorphic DNA (RAPD)]-PCR patterns, that the authors considered incongruent [45].

Bacillus cereus *B. cereus* is only rarely encapsulated. Some pathogenic *B. cereus* strains can exhibit capsule that, at least in one case, seems to be structurally different from that of *B. antrachis* (poly-γ-D-glutamic acid capsule) by showing a polysaccharide structure with hyaluronic acid [187]. They can also have S-layers in their cell walls that seem to be frequent among clinical *B. cereus* strains. However, the carbohydrate components of these surface layers composed of proteins or glycoproteins subunits are still not well characterized in this species [188].

A study by Mietke et al. intended to differentiate between probiotic *B. cereus* strains and wild-type strains of the *B. cereus* group (*B. cereus*, *B. thuringiensis*, *B. mycoides*, and *B. weihenstephanensis*) in the spectral wave number 1000–940 cm^{-1}, and also 3000–2800 cm^{-1}, 1200–900 cm^{-1}, and 900–700 cm^{-1} [189]. However, considering that the taxonomy of closely related species of *Bacillus* is problematic and that it has been subjected to a significant restructuration over recent years, the claimed distinction might be dubious.

Other study was able to cluster emetic-toxin-producing isolates from non-emetic enterotoxin-producing *B. cereus* strains (food poisoning and food isolates) by FT-IR in an analogous way to molecular (RAPD) or phenetic (biochemical assays, protein profiling) methods [190]. However, the presence of capsule or other surface structures was not explored in this study, hindering the extraction of reliable conclusions.

It has been later demonstrated that closely related *B. cereus* strains have cell wall glycosyl residues related to the clade or lineage they belong as defined by MLST [191]. Strains belonging to the same lineage also vary from one another in the amount of various glycosyl residues, which indicate the presence of strain-specific cell wall carbohydrates [191], so both content and quantity of glycosyl residues can be on the basis of IR spectral *B. cereus* differences, which deserve further investigation.

Enterococcus faecium The cell wall of *E. faecium* is ornamented with a variety of polysaccharides and proteins that are mostly directly attached to the peptidoglycan by covalent linkages (polysaccharides, teichoic acids, and surface-anchored proteins), whereas lipoteichoic acid and lipoproteins are anchored to membrane lipids by covalent attachment [192]. The presence of antigenically diverse surface polysaccharides, including a putative capsule locus and/or variable *epa* (enterococcal polysaccharide antigen) loci, has been suggested. Available genomics data showed different *E. faecium* clones exhibiting variable polysaccharide clusters that resemble capsule synthetic genes genetically arranged as in *S. pneumoniae* capsule loci [193].

Preisner et al. have identified spectral ranges evidencing relevant chemical information for human clinical *E. faecium* strain differentiation according to PFGE (31 isolates belonging to 2 PFGE types) [82]. They were associated with the spectral region between 1500–850 cm^{-1} but mainly to bands assigned to P=O stretching in a selected carbohydrate region (1110–1060 cm^{-1}). It is however impossible to adequately

correlate genotypic (PFGE) and phenotypic (FT-IR) data since PFGE is not adequate for typing strains belonging to the highly recombinant *E. faecium* species unless strains are obtained from short-time frames (e.g., outbreaks), and because no additional molecular or phenotypic data from the isolates was provided to support strain differentiation.

Later, Wenning et al. [114] were able to differentiate 5 authorized *E. faecium* probiotic feed additives between them and from 15 environmental *E. faecium* strains with an accuracy of 97% and 98%, respectively, in the spectral windows between 1800–700 cm^{-1} and 3000–2800 cm^{-1}. Only API and RAPD were used to characterize the *E. faecium* isolates with results being less discriminatory than FT-IR, and hardly comparable. It remains to be elucidated to what extent does FT-IR reliably differentiates a much larger and comprehensive collection of isolates and what is the biological significance of the potential FT-IR based assignments in this bacterial species.

To give further insights, we analyzed a collection of *E. faecium* isolates ($n = 105$) obtained from diverse human, animal, and environmental sources, and corresponding to different well-characterized clonal lineages by FT-IR [194] (Freitas et al. unpublished). Our in-house workflow (spectra acquisition in the carbohydrate region of 1200–900 cm^{-1}) and multivariate data analysis (see other aforementioned studies of our group) showed an apparent high diversity among the cellular surface composition of this set of isolates (as suggested by Palmer et al. in [193]). Nevertheless, main clonal lineages causing hospital epidemics were more homogeneous and distinguished from all the other isolates, suggesting a common and specific composition in surface polysaccharides that needs to be further investigated.

Other Gram-positive species

Besides the species aforementioned, a scarcity of studies has been dedicated to explore the potential of FT-IR spectroscopy in the subtyping of strains from the Gram-positive group.

A recent study comparing *Streptococcus agalactiae* isolates from humans and animals have demonstrated a great correlation between the infrared spectra using FT-IR and capsular types [91]. Clustering of *Corynebacterium ulcerans* isolates from various sources in the course of an outbreak was also possible by FT-IR analysis (in the wave number range of 500–1400 cm^{-1} and 2800–3000 cm^{-1}) and was highly congruent with MLST [115]. A novel method using nitrocellulose membrane filter and a single reflection horizontal ATR accessory with FT-IR was developed to discriminate between *Alicyclobacillus* strains with high accuracy in the 3000–2800 cm^{-1} and 1800–700 cm^{-1} regions, and the greatest contribution to the total variance in the FT-IR spectral data came from variables between 1500 cm^{-1} and 900 cm^{-1} [195].

Acid-fast bacteria

Finally, metabolomic fingerprints of the ten major spoligotypes of *Mycobacterium bovis* obtained by FT-IR showed an excellent agreement with clustering with genetic markers [196]. In this study, data collected by transmission FT-IR illustrated better differentiation of the *M. bovis* isolates according to spoligotypes than that collected by reflectance FT-IR.

Conclusions

It is undoubtful the potential of FT-IR methodology to accurately discriminate biologically significant bacterial groups at a quick, low cost, and high-throughput rate, which constitute essential features to fulfill current demands on clinical, food, or environmental microbiological contexts. The link between FT-IR discrimination and variations in Gram-positive and Gram-negative sugar-based coating structures with relevant properties for host interaction has been consistently pointed out in different studies and microorganisms. However, only very recently it has been substantiated with a solid demonstration of key genotypic-phenotypic bacterial features that are certainly of high relevance for bacterial differentiation but also to understand the diversity, evolution, and host adaptation factors of key bacterial pathogens or subpopulations, opening new avenues on potential applications that can go far beyond strain typing.

Future perspectives

Despite the accumulated experience on FT-IR applications in microbiology for the past 30 years, this technique is still considered exotic and its potential in microbiology routines is underestimated. First, the generalized acceptance of this methodology by the microbiological community depends on a solid identification of the surface structures accounting for bacterial discrimination, as well as the comprehension of their value for bacterial typing. In the genomics era, this knowledge must be substantiated with genomic information (provided namely by WGS) using well-characterized bacterial collections representing intraspecies variability, a task that will more certainly be successful with skilled microbiologists. It is however of remark that the strain-typing ability of FT-IR will be limited to bacteria that have surface variations that can be correlated with strain evolution. Second, routine applications depend on the adaptation of the method for a non-specialist user. To achieve this, reproducibility between instruments and laboratories must be guaranteed in large-scale experiments, throughout a careful standardization of culture conditions, sample preparation, and spectra acquisition modes to

minimize spectra variability, allowing the creation of judicious spectral databases for different bacterial species that could be shared. Additionally, automation of data analysis is essential for incorporation in routine microbiology workflows. These issues were partially resolved by Bruker, that launched in 2016 a FT-IR-based equipment for strain typing (IRBiotyper®, https://www.bruker.com/applications/microbiology/strain-typing-with-ir-biotyper/overview.html), that needs to be adequately validated.

Considering the strain differentiation performance, minimal hands-on-time, high throughput, and cost-effectiveness, FT-IR could add value to the microbiology laboratory toolbox (e.g., downstream MALDI-TOF MS). Potential applications are (i) simple and quick serogroup or capsular typing, depending on the species; (ii) phenotypic validation of capsular types predicted by genomics data; (iii) early detection of outbreak strains in clinical or food contexts, that can be subsequently substantiated with data from WGS or other genotypic typing methods as required; (iv) source-tracking of bacterial pathogens or contaminants in food safety or food industry, and improvement of quality control methods; and (v) quick and low-cost monitorization of epidemiological trends in a given geographical frame, requiring knowledge on local epidemiology. The precise establishment of clone-surface antigens variation for each bacterial species in a given epidemiological context can further potentiate FT-IR outcome, hence providing presumptive clone identification.

Acknowledgments The authors received financial support from the European Union (FEDER funds POCI/01/0145/FEDER/007728) and National Funds (Fundação para a Ciência e Tecnologia and Ministério da Educação e Ciência) under the partnership agreement PT2020 UID/MULTI/04378/2013. Fellowship support was provided by Fundação para a Ciência e Tecnologia through Programa Operacional Capital Humano to Â.N. (grant SFRH/BPD/104927/2014) and A.R.F. (grant SFRH/BPD/96148/2013). The interest on the topic and part of the developments and applications described here occurred in the context of fellowships and projects designed to improve bacterial typing strategies including a Marie Curie IEF (ref. PIEF-GA-2009-255512), a project financed by FCT (EXPL-DTP-EPI/0196/2012) and an ESCMID research grant 2012 granted to Ângela Novais. The authors would like to thank the European Society of Clinical Microbiology and Infectious Diseases (ESCMID) Food- and Water-borne Infections Study Group (EFWISG).

Compliance with ethical standards

Conflicts of interest The authors declare that they have no conflict of interest.

References

1. MacCannell D (2013) Bacterial strain typing. Clin Lab Med 33:630–650. https://doi.org/10.1016/j.cll.2013.03.005
2. Sabat AJ, Budimir A, Nashev D, Sá-Leão R, van Dijl JM, Laurent F, Grundmann H, Friedrich AW (2013) Overview of molecular typing methods for outbreak detection and epidemiological surveillance. Euro Surveill 18:20380. https://doi.org/10.2807/ese.18.04.20380-en
3. Nadon C, Van Walle I, Gerner-Smidt P, Campos J, Chinen I, Concepcion-Acevedo J, Gilpin B, Smith AM, Kam KM, Perez E, Trees E, Kubota K, Takkinen J, Nielsen EM, Carleton H, Panel F-NE (2017) PulseNet International: vision for the implementation of whole genome sequencing (WGS) for global foodborne disease surveillance. Eurosurveillance 22:30544. https://doi.org/10.2807/1560-7917.ES.2017.22.23.30544
4. Brisse S, Passet V, Haugaard AB, Babosan A, Kassis-Chikhani N, Struve C, Decré D (2013) *wzi* gene sequencing, a rapid method for determination of capsular type for *Klebsiella* strains. J Clin Microbiol 51:4073–4078. https://doi.org/10.1128/JCM.01924-13
5. Wattiau P, Boland C, Bertrand S (2011) Methodologies for *Salmonella enterica* subsp. *enterica* subtyping: gold standards and alternatives. Appl Environ Microbiol 77:7877–7885. https://doi.org/10.1128/AEM.05527-11
6. Jauneikaite E, Tocheva AS, Jefferies JMC, Gladstone RA, Faust SN, Christodoulides M, Hibberd ML, Clarke SC (2015) Current methods for capsular typing of *Streptococcus pneumoniae*. J Microbiol Methods 113:41–49. https://doi.org/10.1016/j.mimet.2015.03.006
7. Fratamico PM, DebRoy C, Liu Y, Needleman DS, Baranzoni GM, Feng P (2016) Advances in molecular serotyping and subtyping of *Escherichia coli*. Front Microbiol 7:644. https://doi.org/10.3389/fmicb.2016.00644
8. Ingle DJ, Valcanis M, Kuzevski A, Tauschek M, Inouye M, Stinear T, Levine MM, Robins-Browne RM, Holt KE (2016) In silicoserotyping of *E. coli* from short read data identifies limited novel O-loci but extensive diversity of O:H serotype combinations within and between pathogenic lineages. Microb Genomics 2:e000064. https://doi.org/10.1099/mgen.0.000064
9. Joensen KG, Tetzschner AMM, Iguchi A, Aarestrup FM, Scheutz F (2015) Rapid and easy in silico serotyping of *Escherichia coli* isolates by use of whole-genome sequencing data. J Clin Microbiol 53:2410–2426. https://doi.org/10.1128/JCM.00008-15
10. Wyres KL, Wick RR, Gorrie C, Jenney A, Follador R, Thomson NR, Holt KE (2016) Identification of *Klebsiella* capsule synthesis loci from whole genome data. bioRxiv 2:071415. https://doi.org/10.1101/071415
11. Yoshida CE, Kruczkiewicz P, Laing CR, Lingohr EJ, Gannon VPJ, Nash JHE, Taboada EN (2016) The *Salmonella* in silico typing resource (SISTR): an open web-accessible tool for rapidly typing and subtyping draft Salmonella genome assemblies. PLoS One 11:e0147101. https://doi.org/10.1371/journal.pone.0147101
12. Zhang S, Yin Y, Jones MB, Zhang Z, Deatherage Kaiser BL, Dinsmore BA, Fitzgerald C, Fields PI, Deng X (2015) *Salmonella* serotype determination utilizing high-throughput genome sequencing data. J Clin Microbiol 53:1685–1692. https://doi.org/10.1128/JCM.00323-15
13. Grimont P, Weill FX (2007) Antigenic formulae of the *Salmonella* serovars. (9th ed.) Paris: WHO Collaborating Centre for Reference and Research on Salmonella
14. Ørskov I, Ørskov F (1984) 4 Serotyping of *Klebsiella*. Bergan T (ed) Methods in Microbiology. Academic Press, New York, pp 143–164
15. Ørskov F, Ørskov I (1984) 2 Serotyping of *Escherichia coli*. Bergan T (ed) Methods in Microbiology. Academic Press, New York, pp 43–112
16. Salmonella Subcommittee (1934) The genus Salmonella Lignières, 1900. J Hyg (Lond) 34:333–350
17. Lund E, Rasmussen P (1966) Omni-serum. A diagnostic Pneumococcus serum, reacting with the 82 known types of Pneumococcus. Acta Pathol Microbiol Scand 68:458–460

18. Seeliger HPR, Höhne K (1979) Chapter II serotyping of *Listeria monocytogenes* and Related species. Methods Microbiol 13:31–49. https://doi.org/10.1016/S0580-9517(08)70372-6
19. Hobot JA (2015) Chapter 2—bacterial ultrastructure. In: Sussman U (ed) Molecular Medical Microbiology (2nd edn), Academic Press, New York, pp 7–32
20. Mostowy RJ, Holt KE (2018) Diversity-generating machines: genetics of bacterial sugar-coating. Trends Microbiol xx:1–14. https://doi.org/10.1016/j.tim.2018.06.006
21. Whitfield C (1995) Biosynthesis of lipopolysaccharide O antigens. Trends Microbiol 3:178–185. https://doi.org/10.1016/S0966-842X(00)88917-9
22. Henriksen SD (1978) Serotyping of Bacteria. Bergan T, Norris JR (eds) Methods in Microbiology. Academic Press, New York, pp 1–13
23. Schwartz DC, Cantor CR (1984) Separation of yeast chromosome-sized DNAs by pulsed field gradient gel electrophoresis. Cell 37:67–75. https://doi.org/10.1016/0092-8674(84)90301-5
24. Tenover FC, Arbeit RD, Goering RV, Mickelsen PA, Murray BE, Persing DH, Swaminathan B (1995) Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. J Clin Microbiol 33:2233–2239
25. Foley SL, Lynne AM, Nayak R (2009) Molecular typing methodologies for microbial source tracking and epidemiological investigations of Gram-negative bacterial foodborne pathogens. Infect Genet Evol 9:430–440. https://doi.org/10.1016/j.meegid.2009.03.004
26. Said HM, Krishnamani K, Omar SV, Dreyer AW, Sansom B, Fallows D, Ismail NA (2016) Evaluation of semiautomated IS6110-based restriction fragment length polymorphism typing for *Mycobacterium tuberculosis* in a high-burden setting. J Clin Microbiol 54:2547–2552. https://doi.org/10.1128/JCM.00408-16
27. Miao J, Chen L, Wang J, Wang W, Chen D, Li L, Li B, Deng Y, Xu Z (2017) Current methodologies on genotyping for nosocomial pathogen methicillin-resistant *Staphylococcus aureus* (MRSA). Microb Pathog 107:17–28. https://doi.org/10.1016/j.micpath.2017.03.010
28. Bingen EH, Denamur E, Elion J (1994) Use of ribotyping in epidemiological surveillance of nosocomial outbreaks. Clin Microbiol Rev. 7:311–327. https://doi.org/10.1128/CMR.7.3.311
29. Knetsch CW, Lawley TD, Hensgens MP, Corver J, Wilcox MW, Kuijper EJ (2013) Current application and future perspectives of molecular typing methods to study *Clostridium difficile* infections. Euro Surveill 18:20381. https://doi.org/10.2807/ese.18.04.20381-en
30. Collins DA, Elliott B, Riley TV (2015) Molecular methods for detecting and typing of Clostridium difficile. Pathology 47:211–218. https://doi.org/10.1097/PAT.0000000000000238
31. Vos P, Hogers R, Bleeker M, Reijans M, van de Lee T, Hornes M, Frijters A, Pot J, Peleman J, Kuiper M (1995) AFLP: a new technique for DNA fingerprinting. Nucleic Acids Res 23:4407–4414
32. Deplano A, Denis O, Rodriguez-Villalobos H, De Ryck R, Struelens MJ, Hallin M (2011) Controlled performance evaluation of the DiversiLab repetitive-sequence-based genotyping system for typing multidrug-resistant health care-associated bacterial pathogens. J Clin Microbiol 49:3616–3620. https://doi.org/10.1128/JCM.00528-11
33. Nielsen JB, Skov MN, Jørgensen RL, Heltberg O, Hansen DS, Schønning K (2011) Identification of CTX-M15-, SHV-28-producing *Klebsiella pneumoniae* ST15 as an epidemic clone in the Copenhagen area using a semi-automated Rep-PCR typing assay. Eur J Clin Microbiol Infect Dis 30:773–778. https://doi.org/10.1007/s10096-011-1153-x
34. Grisold AJ, Zarfel G, Strenger V, Feierl G, Leitner E, Masoud L, Hoenigl M, Raggam RB, Dosch V, Marth E (2010) Use of automated repetitive-sequence-based PCR for rapid laboratory confirmation of nosocomial outbreaks. J Infect 60:44–51. https://doi.org/10.1016/j.jinf.2009.10.045
35. Ahmed SS, Alp E (2015) Genotyping methods for monitoring the epidemic evolution of *A. baumannii* strains. J Infect Dev Ctries 9:347–354. https://doi.org/10.3855/jidc.6201
36. Taboada EN, Clark CG, Sproston EL, Carrillo CD (2013) Current methods for molecular typing of *Campylobacter* species. J Microbiol Methods 95:24–31. https://doi.org/10.1016/j.mimet.2013.07.007
37. Jagielski T, van Ingen J, Rastogi N, Dziadek J, Mazur PK, Bielecki J (2014) Current methods in the molecular typing of *Mycobacterium tuberculosis* and other mycobacteria. Biomed Res Int 2014:1–21. doi: https://doi.org/10.1155/2014/645802
38. Gawlik D, Slickers P, Engelmann I, Müller E, Lück C, Friedrichs A, Ehricht R, Monecke S (2015) DNA-microarray-based genotyping of Clostridium difficile. BMC Microbiol 15:158. https://doi.org/10.1186/s12866-015-0489-2
39. Michael Dunne W, Pouseele H, Monecke S, Ehricht R, van Belkum A (2017) Epidemiology of transmissible diseases: array hybridization and next generation sequencing as universal nucleic acid-mediated typing tools. Infect Genet Evol. https://doi.org/10.1016/j.meegid.2017.09.019
40. Weissman SJ, Johnson JR, Tchesnokova V, Billig M, Dykhuizen D, Riddell K, Rogers P, Qin X, Butler-Wu S, Cookson BT, Fang FC, Scholes D, Chattopadhyay S, Sokurenko E (2012) High-resolution two-locus clonal typing of extraintestinal pathogenic *Escherichia coli*. Appl Environ Microbiol 78:1353–1360. https://doi.org/10.1128/AEM.06663-11
41. Pan Y-J, Lin T-L, Chen Y-H, Hsu C-R, Hsieh P-F, Wu M-C, Wang J-T (2013) Capsular types of *Klebsiella pneumoniae* revisited by *wzc* sequencing. PLoS One 8:e80670. https://doi.org/10.1371/journal.pone.0080670
42. Fabre L, Zhang J, Guigon G, Le Hello S, Guibert V, Accou-Demartin M, de Romans S, Lim C, Roux C, Passet V, Diancourt L, Guibourdenche M, Issenhuth-Jeanjean S, Achtman M, Brisse S, Sola C, Weill F-X (2012) CRISPR typing and subtyping for improved laboratory surveillance of *Salmonella* infections. PLoS One 7:e36995. https://doi.org/10.1371/journal.pone.0036995
43. Schürch AC, Arredondo-Alonso S, Willems RJL, Goering RV (2018) Whole genome sequencing options for bacterial strain typing and epidemiologic analysis based on single nucleotide polymorphism versus gene-by-gene-based approaches. Clin Microbiol Infect 24:350–354. https://doi.org/10.1016/j.cmi.2017.12.016
44. Sauget M, Valot B, Bertrand X, Hocquet D (2017) Can MALDI-TOF mass spectrometry reasonably type bacteria? Trends Microbiol 25:447–455. https://doi.org/10.1016/j.tim.2016.12.006
45. Wenning M, Scherer S (2013) Identification of microorganisms by FTIR spectroscopy: Perspectives and limitations of the method. Appl Microbiol Biotechnol 97:7111–7120. https://doi.org/10.1007/s00253-013-5087-3
46. Lasch P, Naumann D (2015) Infrared spectroscopy in microbiology. In: Encyclopedia of analytical chemistry. John Wiley & Sons, Ltd., Chichester, pp 1–32
47. Willemse-Erix DFM, Scholtes-Timmerman MJ, Jachtenberg J-W, van Leeuwen WB, Horst-Kreft D, Bakker Schut TC, Deurenberg RH, Puppels GJ, van Belkum A, Vos MC, Maquelin K (2009) Optical fingerprinting in bacterial epidemiology: Raman spectroscopy as a real-time typing method. J Clin Microbiol 47:652–659. https://doi.org/10.1128/JCM.01900-08
48. Swaminathan B, Barrett TJ, Hunter SB, Tauxe RV, PulseNet Task Force CDC (2001) PulseNet: the molecular subtyping network for foodborne bacterial disease surveillance, United States. Emerg Infect Dis 7:382–389. https://doi.org/10.3201/eid0703.010303
49. Li W, Raoult D, Fournier P-E (2009) Bacterial strain typing in the genomic era. FEMS Microbiol Rev. 33:892–916. https://doi.org/10.1111/j.1574-6976.2009.00182.x

50. Coque TM, Novais Â, Carattoli A, Poirel L, Pitout J, Peixe L, Baquero F, Cantón R, Nordmann P (2008) Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum β-lactamase CTX-M-15. Emerg Infect Dis 14:195–200. https://doi.org/10.3201/eid1402.070350
51. Breurec S, Guessennd N, Timinouni M, Le TAH, Cao V, Ngandjio A, Randrianirina F, Thiberge JM, Kinana A, Dufougeray A, Perrier-Gros-Claude JD, Boisier P, Garin B, Brisse S (2013) *Klebsiella pneumoniae* resistant to third-generation cephalosporins in five African and two Vietnamese major towns: multiclonal population structure with two major international clonal groups, CG15 and CG258. Clin Microbiol Infect 19:349–355. https://doi.org/10.1111/j.1469-0691.2012.03805.x
52. Woodford N, Turton JF, Livermore DM (2011) Multiresistant Gram-negative bacteria: the role of high-risk clones in the dissemination of antibiotic resistance. FEMS Microbiol Rev. 35:736–755. https://doi.org/10.1111/j.1574-6976.2011.00268.x
53. Vincent C, Boerlin P, Daignault D, Dozois CM, Dutil L, Galanakis C, Reid-Smith RJ, Tellier P-P, Tellis PA, Ziebell K, Manges AR (2010) Food reservoir for *Escherichia coli* causing urinary tract infections. Emerg Infect Dis 16:88–95. https://doi.org/10.3201/eid1601.091118
54. Bortolaia V, Espinosa-Gongora C, Guardabassi L (2016) Human health risks associated with antimicrobial-resistant enterococci and *Staphylococcus aureus* on poultry meat. Clin Microbiol Infect 22:130–140. https://doi.org/10.1016/j.cmi.2015.12.003
55. Davis GS, Waits K, Nordstrom L, Weaver B, Aziz M, Gauld L, Grande H, Bigler R, Horwinski J, Porter S, Stegger M, Johnson JR, Liu CM, Price LB (2015) Intermingled *Klebsiella pneumoniae* populations between retail meats and human urinary tract infections. Clin Infect Dis 61:892–899. https://doi.org/10.1093/cid/civ428
56. Rodrigues C, Fernandez Lanza V, Machado E, Peixe L, Novais Â, Coque TM (2017) High-resolution analysis of the globally disseminated multidrug-resistant *Klebsiella pneumoniae* clonal groups 14 and 15. In: 27th ECCMID, the European Congress of Clinical Microbiology and Infectious Diseases. Viena
57. Deleo FR, Chen L, Porcella SF, Martens CA, Kobayashi SD, Porter AR, Chavda KD, Jacobs MR, Mathema B, Olsen RJ, Bonomo RA, Musser JM, Kreiswirth BN (2014) Molecular dissection of the evolution of carbapenem-resistant multilocus sequence type 258 *Klebsiella pneumoniae*. Proc Natl Acad Sci USA 111:4988–4993. https://doi.org/10.1073/pnas.1321364111
58. Stoesser N, Sheppard AE, Pankhurst L, De Maio N, Moore CE, Sebra R, Turner P, Anson LW, Kasarskis A, Batty EM, Kos V, Wilson DJ, Phetsouvanh R, Wyllie D, Sokurenko E, Manges AR, Johnson TJ, Price LB, TEA P, Johnson JR, Didelot X, Walker AS, Crook DW, Modernizing Medical Microbiology Informatics Group (MMMIG) (2016) Evolutionary history of the global emergence of the *Escherichia coli* epidemic clone ST131. MBio 7: e02162–e02115. https://doi.org/10.1128/mBio.02162-15
59. Lytsy B, Engstrand L, Gustafsson Å, Kaden R (2017) Time to review the gold standard for genotyping vancomycin-resistant enterococci in epidemiology: comparing whole-genome sequencing with PFGE and MLST in three suspected outbreaks in Sweden during 2013–2015. Infect Genet Evol 54:74–80. https://doi.org/10.1016/j.meegid.2017.06.010
60. Carriço JA, Rossi M, Moran-Gilad J, Van Domselaar G, Ramirez M (2018) A primer on microbial bioinformatics for nonbioinformaticians. Clin Microbiol Infect 24:342–349. https://doi.org/10.1016/j.cmi.2017.12.015
61. Follador R, Heinz E, Wyres KL, Ellington MJ, Kowarik M, Holt KE, Thomson NR (2016) The diversity of *Klebsiella pneumoniae* surface polysaccharides. Microb Genomics. https://doi.org/10.1099/mgen.0.000073
62. Thrane SW, Taylor VL, Lund O, Lam JS, Jelsbak L (2016) Application of whole-genome sequencing data for O-specific antigen analysis and in silico serotyping of *Pseudomonas aeruginosa* isolates. J Clin Microbiol 54:1782–1788. https://doi.org/10.1128/JCM.00349-16
63. van Belkum A (2008) Newer methods for bacterial strain typing. Clin Microbiol Newsl 30:63–69. https://doi.org/10.1016/J.CLINMICNEWS.2008.04.001
64. Lartigue M-F (2013) Matrix-assisted laser desorption ionization time-of-flight mass spectrometry for bacterial strain characterization. Infect Genet Evol 13:230–235. https://doi.org/10.1016/j.meegid.2012.10.012
65. Spinali S, van Belkum A, Goering RV, Girard V, Welker M, Van Nuenen M, Pincus DH, Arsac M, Durand G (2015) Microbial typing by matrix-assisted laser desorption ionization-time of flight mass spectrometry: do we need guidance for data interpretation? J Clin Microbiol 53:760–765. https://doi.org/10.1128/JCM.01635-14
66. Rodrigues C, Novais Â, Sousa C, Ramos H, Coque TM, Cantón R, Lopes JA, Peixe L (2017) Elucidating constraints for differentiation of major human *Klebsiella pneumoniae* clones using MALDI-TOF MS. Eur J Clin Microbiol Infect Dis 36:379–386. https://doi.org/10.1007/s10096-016-2812-8
67. Antunes P, Campos J, Sousa C, Mourão J, Lopes J, Perez J, Cantón R, Peixe L (2018) Assessing the potential use of MALDI-TOF MS for discrimination of clinically relevant *Salmonella* serogroups and serotypes. In: 28th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). ESCMID, Madrid.
68. Sousa C, Botelho J, Grosso F, Silva L, Lopes J, Peixe L (2015) Unsuitability of MALDI-TOF MS to discriminate *Acinetobacter baumannii* clones under routine experimental conditions. Front Microbiol 6:481. https://doi.org/10.3389/fmicb.2015.00481
69. Ellis DI, Goodacre R (2006) Metabolic fingerprinting in disease diagnosis: biomedical applications of infrared and Raman spectroscopy. Analyst 131:875. https://doi.org/10.1039/b602376m
70. Dunn WB, Bailey NJC, Johnson HE (2005) Measuring the metabolome: current analytical technologies. Analyst 130:606. https://doi.org/10.1039/b418288j
71. Maquelin K, Kirschner C, Choo-Smith L-P, van den Braak N, Endtz HP, Naumann D, Puppels GJ (2002) Identification of medically relevant microorganisms by vibrational spectroscopy. J Microbiol Methods 51:255–271
72. Naumann D, Helm D, Labischinski H (1991) Microbiological characterizations by FT-IR spectroscopy. Nature 351:81–82. https://doi.org/10.1038/351081a0
73. Harz M, Rösch P, Popp J (2009) Vibrational spectroscopy-a powerful tool for the rapid identification of microbial cells at the single-cell level. Cytometry A 75:104–113. https://doi.org/10.1002/cyto.a.20682
74. Kusters JG, van Leeuwen WB, Maquelin K, Blok HEM, Willemse HFM, de Graaf-Miltenburg LAM, Fluit AC, Troelstra A (2016) Raman spectroscopy-based identification of nosocomial outbreaks of the clonal bacterium *Escherichia coli*. Eur J Clin Microbiol Infect Dis 35:83–87. https://doi.org/10.1007/s10096-015-2511-x
75. te Witt R, Vaessen N, Melles DC, Lekkerkerk WSN, van der Zwaan EAE, Zandijk WHA, Severin JA, Vos MC (2013) Good performance of the SpectraCellRA system for typing of methicillin-resistant *Staphylococcus aureus* isolates. J Clin Microbiol 51:1434–1438. https://doi.org/10.1128/JCM.02101-12
76. Henderson KC, Benitez AJ, Ratliff AE, Crabb DM, Sheppard ES, Winchell JM, Dluhy RA, Waites KB, Atkinson TP, Krause DC (2015) Specificity and strain-typing capabilities of nanorod array-surface enhanced raman spectroscopy for *Mycoplasma pneumoniae* detection. PLoS One 10:e0131831. https://doi.org/10.1371/journal.pone.0131831

77. Witkowska E, Korsak D, Kowalska A, Janeczek A, Kamińska A (2018) Strain-level typing and identification of bacteria—a novel approach for SERS active plasmonic nanostructures. Anal Bioanal Chem 410:5019–5031. https://doi.org/10.1007/s00216-018-1153-0
78. Griffiths PR, De Haseth JA (2007) Fourier transform infrared spectrometry. John Wiley & Sons, Inc., Hoboken, New Jersey
79. Goulden JD, Sharpe ME (1958) The infra-red absorption spectra of lactobacilli. J Gen Microbiol 19:76–86. https://doi.org/10.1099/00221287-19-1-76
80. Randall HM, Smith DW, Colm AC, Nungester WJ (1951) Correlation of biologic properties of strains of *Mycobacterium* with infra-red spectrums. I. Reproducibility of extracts of *M. tuberculosis* as determined by infra-red spectroscopy. Am Rev Tuberc 63:372–380
81. Helm D, Labischinski H, Schallehn G, Naumann D (1991) Classification and identification of bacteria by Fourier-transform infrared spectroscopy. J Gen Microbiol 137:69–79. https://doi.org/10.1099/00221287-137-1-6982.
82. Preisner O, Lopes JA, Guiomar R, Machado J, Menezes JC (2007) Fourier transform infrared (FT-IR) spectroscopy in bacteriology: towards a reference method for bacteria discrimination. Anal Bioanal Chem 387:1739–1748. https://doi.org/10.1007/s00216-006-0851-1
83. Zarnowiec P, Lechowicz Ł, Czerwonka G, Kaca W (2015) Fourier transform infrared spectroscopy (FTIR) as a tool for the identification and differentiation of pathogenic bacteria. Curr Med Chem 22:1710–1718. https://doi.org/10.2174/0929867322666150311152800
84. Quintelas C, Ferreira EC, Lopes JA, Sousa C (2018) An overview of the evolution of infrared spectroscopy applied to bacterial typing. Biotechnol J 13:1–10. https://doi.org/10.1002/biot.201700449
85. Grunert T, Wenning M, Barbagelata MS, Fricker M, Sordelli DO, Buzzola FR, Ehling-Schulz M (2013) Rapid and reliable identification of *Staphylococcus aureus* capsular serotypes by means of artificial neural network-assisted fourier transform infrared spectroscopy. J Clin Microbiol 51:2261–2266. https://doi.org/10.1128/JCM.00581-13
86. Sahu RK, Mordechai S, Pesakhov S, Dagan R, Porat N (2006) Use of FTIR spectroscopy to distinguish between capsular types and capsular quantities in *Streptococcus pneumoniae*. Biopolymers 83:434–442. https://doi.org/10.1002/bip.20576
87. Rodrigues C, Branquinho R, Sousa C, Andrade L, Machado E, Darini A, Novais Â, Peixe L (2015) Identification of diverse capsular types in multidrug-resistant (MDR) *Klebsiella pneumoniae* clones using *wzi* sequencing and FTIR. In: 6th Congress of European Microbiologists, Maastricht, The Netherlands.
88. Campos J, Sousa C, Mourão J, Lopes J, Antunes P, Peixe L (2018) Fourier transform infrared spectroscopy with attenuated total for non-typhoidal *Salmonella* clinically relevant serogroups and serotypes discrimination: a comprehensive analysis. Int J Food Microbiol 285:34–41. https://doi.org/10.1016/j.ijfoodmicro.2018.07.005
89. Bosch A, Prieto C, Serra DO, Martina P, Stämmler M, Naumann D, Schmitt J, Yantorno O (2010) Type-IV pili spectroscopic markers: applications in the quantification of piliation levels in *Moraxella bovis* cells by a FT-IR ANN-based model. J Biophotonics 3:522–533. https://doi.org/10.1002/jbio.201000027
90. Johler S, Stephan R, Althaus D, Ehling-Schulz M, Grunert T (2016) High-resolution subtyping of *Staphylococcus aureus* strains by means of Fourier-transform infrared spectroscopy. Syst Appl Microbiol 39:189–194. https://doi.org/10.1016/j.syapm.2016.03.003
91. Eisenberg T, Rau J, Westerhüs U, Knauf-Witzens T, Fawzy A, Schlez K, Zschöck M, Prenger-Berninghoff E, Heydel C, Sting R, Glaeser SP, Pulami D, van der Linden M, Ewers C (2017) *Streptococcus agalactiae* in elephants—a comparative study with isolates from human and zoo animal and livestock origin. Vet Microbiol 204:141–150. https://doi.org/10.1016/j.vetmic.2017.04.018
92. Dieckmann R, Hammerl JA, Hahmann H, Wicke A, Kleta S, Dabrowski PW, Nitsche A, Stämmler M, Al Dahouk S, Lasch P (2016) Rapid characterization of *Klebsiella oxytoca* isolates from contaminated liquid hand soap using mass spectrometry, FTIR and Raman spectroscopy. Faraday Discuss 187:353–375. https://doi.org/10.1039/c5fd00165j
93. Silva L, Rodrigues C, Grosso F, Peixe L (2018) The secret is on sugar: capsular type explains the discrimination of *Acinetobacter baumannii* clones by Fourier-transform Infrared (FT-IR). In: 28th European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). Madrid.
94. Beekes M, Lasch P, Naumann D (2007) Analytical applications of Fourier transform-infrared (FT-IR) spectroscopy in microbiology and prion research. Vet Microbiol 123:305–319. https://doi.org/10.1016/j.vetmic.2007.04.010
95. Grunert T, Monahan A, Lassnig C, Vogl C, Müller M, Ehling-Schulz M (2014) Deciphering host genotype-specific impacts on the metabolic fingerprint of *Listeria monocytogenes* by FTIR spectroscopy. PLoS One 9:e115959. https://doi.org/10.1371/journal.pone.0115959
96. Sundaram J, Park B, Hinton A, Yoon SC, Windham WR, Lawrence KC (2012) Classification and structural analysis of live and dead *Salmonella* cells using Fourier transform infrared spectroscopy and principal component analysis. J Agric Food Chem 60:991–1004. https://doi.org/10.1021/jf204081g
97. Toziou P-M, Barmpalexis P, Boukouvala P, Verghese S, Nikolakakis I (2018) Quantification of live *Lactobacillus acidophilus* in mixed populations of live and killed by application of attenuated reflection Fourier transform infrared spectroscopy combined with chemometrics. J Pharm Biomed Anal 154:16–22. https://doi.org/10.1016/j.jpba.2018.03.009
98. Adamus-Białek W, Lechowicz Ł, Kubiak-Szeligowska AB, Wawszczak M, Kamińska E, Chrapek M (2017) A new look at the drug-resistance investigation of uropathogenic *E. coli* strains. Mol Biol Rep 44:191–202. https://doi.org/10.1007/s11033-017-4099-y
99. Jin N, Zhang D, Martin FL (2017) Fingerprinting microbiomes towards screening for microbial antibiotic resistance. Integr Biol 9:406–417. https://doi.org/10.1039/C7IB00009J
100. Santos M, Gerbino E, Tymczyszyn E, Gomez-Zavaglia A (2015) Applications of infrared and Raman spectroscopies to probiotic investigation. Foods 4:283–305. https://doi.org/10.3390/foods4030283
101. Brandes Ammann A, Brandl H (2011) Detection and differentiation of bacterial spores in a mineral matrix by Fourier transform infrared spectroscopy (FTIR) and chemometrical data treatment. BMC Biophys 4(14). https://doi.org/10.1186/2046-1682-4-14
102. Coates J (2006) Interpretation of infrared spectra, a practical approach. In: Encyclopedia of Analytical Chemistry. John Wiley & Sons, Ltd, Chichester, UK
103. Davis R, Mauer LJ (2010) Fourier tansform infrared (FT-IR) spectroscopy: a rapid tool for detection and analysis of foodborne pathogenic bacteria. In: Méndez-Vilas A (ed) Current research, technology and education topics in applied microbiology and microbial biotechnology, 2nd editio. Formatex Research Center, pp 1582–1594
104. Sousa C, Novais Â, Magalhães A, Lopes J, Peixe L (2013) Diverse high-risk B2 and D Escherichia coli clones depicted by Fourier transform infrared spectroscopy. Sci Rep 3:3278. https://doi.org/10.1038/srep03278
105. Sousa C, Grosso F, Meirinhos-Soares L, Peixe L, Lopes J (2014) Identification of carbapenem-resistant *Acinetobacter baumannii*

clones using infrared spectroscopy. J Biophotonics 7:287–294. https://doi.org/10.1002/jbio.201200075
106. Baker MJ, Trevisan J, Bassan P, Bhargava R, Butler HJ, Dorling KM, Fielden PR, Fogarty SW, Fullwood NJ, Heys KA, Hughes C, Lasch P, Martin-Hirsch PL, Obinaju B, Sockalingum GD, Sulé-Suso J, Strong RJ, Walsh MJ, Wood BR, Gardner P, Martin FL (2014) Using Fourier transform IR spectroscopy to analyze biological materials. Nat Protoc 9:1771–1791. https://doi.org/10.1038/nprot.2014.110
107. Helm D, Labischinski H, Naumann D (1991) Elaboration of a procedure for identification of bacteria using Fourier-transform IR spectral libraries: a stepwise correlation approach. J Microbiol Methods 14:127–142. https://doi.org/10.1016/0167-7012(91)90042-O
108. Wenning M, Breitenwieser F, Konrad R, Huber I, Busch U, Scherer S (2014) Identification and differentiation of food-related bacteria: a comparison of FTIR spectroscopy and MALDI-TOF mass spectrometry. J Microbiol Methods 103:44–52. https://doi.org/10.1016/j.mimet.2014.05.011
109. Lefier D, Hirst D, Holt C, Williams AG (1997) Effect of sampling procedure and strain variation in *Listeria monocytogenes* on the discrimination of species in the genus *Listeria* by Fourier transform infrared spectroscopy and canonical variates analysis. FEMS Microbiol Lett 147:45–50. https://doi.org/10.1111/j.1574-6968.1997.tb10218.x
110. Winder CL, Goodacre R (2004) Comparison of diffuse-reflectance absorbance and attenuated total reflectance FT-IR for the discrimination of bacteria. Analyst 129:1118–1122. https://doi.org/10.1039/b408169b
111. Sykora L, Müller A (2017) ATR-FTIR microplate reader and micromachined ATR silicon crystals. In: 11th Workshop FT-IR spectroscopy in microbiological and medical diagnostic. Berlin.
112. Lee LC, Liong CY, Jemain AA (2017) A contemporary review on data preprocessing (DP) practice strategy in ATR-FTIR spectrum. Chemom Intell Lab Syst 163:64–75. https://doi.org/10.1016/j.chemolab.2017.02.008
113. Lasch P (2012) Spectral pre-processing for biomedical vibrational spectroscopy and microspectroscopic imaging (2012) Chemometrics and Intelligent Laboratory Systems. Chemom Intell Lab Syst 117:100–114. https://doi.org/10.1016/j.chemolab.2012.03.011
114. Wenning M, Büchl NR, Scherer S (2010) Species and strain identification of lactic acid bacteria using FTIR spectroscopy and artificial neural networks. J Biophotonics 3:493–505. https://doi.org/10.1002/jbio.201000015
115. Eisenberg T, Mauder N, Contzen M, Rau J, Ewers C, Schlez K, Althoff G, Schauerte N, Geiger C, Margos G, Konrad R, Sing A (2015) Outbreak with clonally related isolates of *Corynebacterium ulcerans* in a group of water rats. BMC Microbiol 15:42. https://doi.org/10.1186/s12866-015-0384-x
116. Fetsch A, Contzen M, Hartelt K, Kleiser A, Maassen S, Rau J, Kraushaar B, Layer F, Strommenger B (2014) *Staphylococcus aureus* food-poisoning outbreak associated with the consumption of ice-cream. Int J Food Microbiol 187:1–6. https://doi.org/10.1016/j.ijfoodmicro.2014.06.017
117. Silva L, Rodrigues C, Sousa C, Lira A, Leão M, Mota M, Lopes P, Lameirão A, Abreu G, Lopes J, Novais Â, Peixe L (2016) Real-time identification of carbapenem-resistant *Klebsiella pneumoniae* outbreak isolates by Fourier transform infrared (FTIR) spectroscopy. In: 11th International Meeting on Microbial Epidemiological Markers (IMMEM XI). Estoril, Portugal.
118. Mora A, Blanco M, López C, Mamani R, Blanco JE, Alonso MP, García-Garrote F, Dahbi G, Herrera A, Fernández A, Fernández B, Agulla A, Bou G, Blanco J (2011) Emergence of clonal groups O1:HNM-D-ST59, O15:H1-D-ST393, O20:H34/HNM-D-ST354, O25b:H4-B2-ST131 and ONT:H21,42-B1-ST101 among CTX-M-14-producing *Escherichia coli* clinical isolates in Galicia, northwest Spain. Int J Antimicrob Agents 37:16–21. https://doi.org/10.1016/j.ijantimicag.2010.09.012
119. Beutin L, Wang Q, Naumann D, Han W, Krause G, Leomil L, Wang L, Feng L (2007) Relationship between O-antigen subtypes, bacterial surface structures and O-antigen gene clusters in *Escherichia coli* O123 strains carrying genes for Shiga toxins and intimin. J Med Microbiol 56:177–184. https://doi.org/10.1099/jmm.0.46775-0
120. Al-Qadiri HM, Lin M, Cavinato AG, Rasco BA (2006) Fourier transform infrared spectroscopy, detection and identification of *Escherichia coli* O157:H7 and *Alicyclobacillus* strains in apple juice. Int J Food Microbiol 111:73–80. https://doi.org/10.1016/j.ijfoodmicro.2006.05.004
121. Kim S, Burgula Y, Ojanen-Reuhs T, Cousin MA, Reuhs BL, Mauer LJ (2006) Differentiation of crude Lipopolysaccharides from *Escherichia coli* strains using fourier transform infrared spectroscopy and chemometrics. J Food Sci 71:M57–M61. https://doi.org/10.1111/j.1365-2621.2006.tb08908.x
122. Davis R, Irudayaraj J, Reuhs BL, Mauer LJ (2010) Detection of *E. coli* O157:H7 from ground beef using Fourier transform infrared (FT-IR) spectroscopy and chemometrics. J Food Sci 75:M340–M346. https://doi.org/10.1111/j.1750-3841.2010.01686.x
123. Davis R, Paoli G, Mauer LJJ (2012) Evaluation of Fourier transform infrared (FT-IR) spectroscopy and chemometrics as a rapid approach for sub-typing Escherichia coli O157:H7 isolates. Food Microbiol 31:181–190. https://doi.org/10.1016/j.fm.2012.02.010
124. Al-Holy MA, Lin M, Al-Qadiri H, Cavinato AG, Rasco BA (2006) Classification of foodborne pathogens by Fourier transform infrared spectroscopy and pattern recognition techniques. J Rapid Methods Autom Microbiol 14:189–200. https://doi.org/10.1111/j.1745-4581.2006.00045.x
125. Dawson SE, Gibreel T, Nicolaou N, AlRabiah H, Xu Y, Goodacre R, Upton M (2014) Implementation of Fourier transform infrared spectroscopy for the rapid typing of uropathogenic *Escherichia coli*. Eur J Clin Microbiol Infect Dis 33:983–988. https://doi.org/10.1007/s10096-013-2036-0
126. AlRabiah H, Correa E, Upton M, Goodacre R (2013) High-throughput phenotyping of uropathogenic *E. coli* isolates with Fourier transform infrared spectroscopy. Analyst 138:1363. https://doi.org/10.1039/c3an36517d
127. Clark G, Paszkiewicz K, Hale J, Weston V, Constantinidou C, Penn C, Achtman M, McNally A (2012) Genomic analysis uncovers a phenotypically diverse but genetically homogeneous *Escherichia coli* ST131 clone circulating in unrelated urinary tract infections. J Antimicrob Chemother 67:868–877. https://doi.org/10.1093/jac/dkr585
128. Alqasim A, Scheutz F, Zong Z, McNally A (2014) Comparative genome analysis identifies few traits unique to the *Escherichia coli* ST131 H30Rx clade and extensive mosaicism at the capsule locus. BMC Genomics 15:830. https://doi.org/10.1186/1471-2164-15-830
129. Price LB, Johnson JR, Aziz M, Clabots C, Johnston B, Tchesnokova V, Nordstrom L, Billig M, Chattopadhyay S, Stegger M, Andersen PS, Pearson T, Riddell K, Rogers P, Scholes D, Kahl B, Keim P, Sokurenko EV (2013) The epidemic of extended-spectrum—lactamase-producing *Escherichia coli* ST131 is driven by a single highly pathogenic subclone, H30-Rx. MBio 4. https://doi.org/10.1128/mBio.00377-13
130. Sharaha U, Rodriguez-Diaz E, Riesenberg K, Bigio IJ, Huleihel M, Salman A (2017) Using infrared spectroscopy and multivariate analysis to detect antibiotics' resistant *Escherichia coli* bacteria. Anal Chem 89:8782–8790. https://doi.org/10.1021/acs.analchem.7b01025
131. Bouhedja W, Sockalingum GD, Pina P, Allouch P, Bloy C, Labia R, Millot JM, Manfait M (1997) ATR-FTIR spectroscopic

investigation of *E. coli* transconjugants beta-lactams-resistance phenotype. FEBS Lett 412:39–42. https://doi.org/10.1016/S0014-5793(97)00725-4

132. Lechowicz L, Urbaniak M, Adamus-Białek W, Kaca W (2013) The use of infrared spectroscopy and artificial neural networks for detection of uropathogenic *Escherichia coli* strains' susceptibility to cephalothin. Acta Biochim Pol 60:713–718
133. European Food Safety Authority and European Centre for Disease Prevention and Control (2017) The European Union summary report on trends and sources of zoonoses, zoonotic agents and food-borne outbreaks in 2016.
134. Centers for Disease Control and Prevention (2015) Foodborne Disease Active Surveillance Network 2015 Surveillance Report (Final Data). Atlanta, Georgia.
135. Ferrari RG, Panzenhagen PHN, Conte-Junior CA (2017) Phenotypic and genotypic eligible methods for *Salmonella Typhimurium* source tracking. Front Microbiol 8:2587. https://doi.org/10.3389/fmicb.2017.02587
136. De Lamo-Castellví S, Männing A, Rodríguez-Saona LE (2010) Fourier-transform infrared spectroscopy combined with immunomagnetic separation as a tool to discriminate *Salmonella* serovars. Analyst 135:2987–2992. https://doi.org/10.1039/c0an00497a
137. Kim S, Kim H, Reuhs BL, Mauer LJ (2006) Differentiation of outer membrane proteins from *Salmonella enterica* serotypes using Fourier transform infrared spectroscopy and chemometrics. Lett Appl Microbiol 42:229–234. https://doi.org/10.1111/j.1472-765X.2005.01828.x
138. Kim S, Reuhs BL, Mauer LJ (2005) Use of Fourier transform infrared spectra of crude bacterial lipopolysaccharides and chemometrics for differentiation of *Salmonella enterica* serotypes. J Appl Microbiol 99:411–417. https://doi.org/10.1111/j.1365-2672.2005.02621.x
139. Baldauf NA, Rodriguez-Romo LA, Yousef AE, Rodriguez-Saona LE (2006) Differentiation of selected *Salmonella enterica* serovars by Fourier transform mid-infrared spectroscopy. Appl Spectrosc 60:592–598. https://doi.org/10.1366/000370206777670521
140. Baldauf NA, Rodriguez-Romo LA, Männig A, Yousef AE, Rodriguez-Saona LE (2007) Effect of selective growth media on the differentiation of *Salmonella enterica* serovars by Fourier-Transform Mid-Infrared Spectroscopy. J Microbiol Methods 68:106–114. https://doi.org/10.1016/j.mimet.2006.06.012
141. Männig A, Baldauf NA, Rodriguez-Romo LA, Yousef AE, Rodríguez-Saona LE (2008) Differentiation of *Salmonella enterica* serovars and strains in cultures and food using infrared spectroscopic and microspectroscopic techniques combined with soft independent modeling of class analogy pattern recognition analysis. J Food Prot 71:2249–2256
142. Preisner OE, Menezes JC, Guiomar R, Machado J, Lopes JA (2012) Discrimination of *Salmonella enterica* serotypes by Fourier transform infrared spectroscopy. Food Res Int 45:1058–1064. https://doi.org/10.1016/j.foodres.2011.02.029
143. Preisner O, Guiomar R, Machado J, Menezes JC, Lopes JA (2010) Application of fourier transform infrared spectroscopy and chemometrics for differentiation of salmonella enterica serovar enteritidis phage types. Appl Environ Microbiol 76:3538–3544. https://doi.org/10.1128/AEM.01589-09
144. Seltmann G, Voigt W, Beer W (1994) Application of physico-chemical typing methods for the epidemiological analysis of *Salmonella enteritidis* strains of phage type 25/17. Epidemiol Infect 113:411–424
145. Hedderich R, Whitman WB (2006) Vol. 6: Proteobacteria: Gamma Subclass, The Genus Klebsiella. In: Dworkin M, Stanley F, Eugene R, Karl-Heinz R, Erko S (eds) The prokaryotes, third. Springer-Verlag, New York, pp 159–197
146. Dinkelacker AG, Vogt S, Oberhettinger P, Mauder N, Rau J, Kostrzewa M, Rossen JWA, Autenrieth IB, Peter S, Liese J (2018) Typing and species identification of clinical *Klebsiella* isolates by Fourier-transform infrared (FTIR) spectroscopy and matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry. J Clin Microbiol. https://doi.org/10.1128/JCM.00843-18
147. Bergogne-Bé E, Zin R, Towner AKJ (1996) *Acinetobacter* spp. as nosocomial pathogens: microbiological, clinical, and epidemiological features. Clin Microbiol Rev. 9:148–165
148. Hu D, Liu B, Dijkshoorn L, Wang L, Reeves PR (2013) Diversity in the major polysaccharide antigen of *Acinetobacter Baumannii* assessed by DNA sequencing, and development of a molecular serotyping scheme. PLoS One 8:e70329. https://doi.org/10.1371/journal.pone.0070329
149. Seltmann G, Beer W, Claus H, Seifert H (1995) Comparative classification of *Acinetobacter baumannii* strains using seven different typing methods. Zentralbl Bakteriol 282:372–383
150. Sousa C, Silva L, Grosso F, Lopes J, Peixe L (2014) Development of a FTIR-ATR based model for typing clinically relevant *Acinetobacter baumannii* clones belonging to ST98, ST103. ST208 and ST218. J Photochem Photobiol B Biol 133:108–114. https://doi.org/10.1016/j.jphotobiol.2014.02.015
151. Horbach I, Naumann D, Fehrenbach FJ (1988) Simultaneous infections with different serogroups of *Legionella pneumophila* investigated by routine methods and Fourier transform infrared spectroscopy. J Clin Microbiol 26:1106–1110
152. Kuhm AE, Suter D, Felleisen R, Rau J (2009) Identification of *Yersinia enterocolitica* at the species and subspecies levels by fourier transform infrared spectroscopy. Appl Environ Microbiol 75:5809–5813. https://doi.org/10.1128/AEM.00206-09
153. Stamm I, Hailer M, Depner B, Kopp PA, Rau J (2013) *Yersinia enterocolitica* in diagnostic fecal samples from European dogs and cats: identification by fourier transform infrared spectroscopy and matrix-assisted laser desorption ionization-time of flight mass spectrometry. J Clin Microbiol 51:887–893. https://doi.org/10.1128/JCM.02506-12
154. Coutinho CP, Sá-Correia I, Lopes JA (2009) Use of Fourier transform infrared spectroscopy and chemometrics to discriminate clinical isolates of bacteria of the *Burkholderia cepacia* complex from different species and ribopatterns. Anal Bioanal Chem 394:2161–2171. https://doi.org/10.1007/s00216-009-2908-4
155. Mouwen DJM, Weijtens MJBM, Capita R, Alonso-Calleja C, Prieto M (2005) Discrimination of enterobacterial repetitive intergenic consensus PCR types of *Campylobacter coli* and *Campylobacter jejuni* by Fourier transform infrared spectroscopy. Appl Environ Microbiol 71:4318–4324. https://doi.org/10.1128/AEM.71.8.4318-4324.2005
156. Ge M, Li B, Wang L, Tao Z, Mao S, Wang Y, Xie G, Sun G (2014) Differentiation in MALDI-TOF MS and FTIR spectra between two pathovars of *Xanthomonas oryzae*. Spectrochim Acta A Mol Biomol Spectrosc 133:730–734. https://doi.org/10.1016/j.saa.2014.06.056
157. O'Riordan K, Lee JC (2004) *Staphylococcus aureus* capsular polysaccharides. Clin Microbiol Rev. 17:218–234
158. Jones C (2005) Revised structures for the capsular polysaccharides from *Staphylococcus aureus* Types 5 and 8, components of novel glycoconjugate vaccines. Carbohydr Res 340:1097–1106. https://doi.org/10.1016/j.carres.2005.02.001
159. Schauer B, Krametter-Frötscher R, Knauer F, Ehricht R, Monecke S, Feßler AT, Schwarz S, Grunert T, Spergser J, Loncaric I (2018) Diversity of methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from Austrian ruminants and New World camelids. Vet Microbiol 215:77–82. https://doi.org/10.1016/j.vetmic.2018.01.006

160. Kümmel J, Stessl B, Gonano M, Walcher G, Bereuter O, Fricker M, Grunert T, Wagner M, Ehling-Schulz M (2016) *Staphylococcus aureus* entrance into the dairy chain: tracking *S. aureus* from dairy cow to cheese. Front Microbiol 7:1603. https://doi.org/10.3389/fmicb.2016.01603
161. Grunert T, Jovanovic D, Sirisarn W, Johler S, Weidenmaier C, Ehling-Schulz M, Xia G (2018) Analysis of *Staphylococcus aureus* wall teichoic acid glycoepitopes by Fourier transform infrared spectroscopy provides novel insights into the staphylococcal glycocode. Sci Rep 8:1889. https://doi.org/10.1038/s41598-018-20,222-6
162. Amiali NM, Mulvey MR, Sedman J, Simor AE, Ismail AA (2007) Epidemiological typing of methicillin-resistant *Staphylococcus aureus* strains by Fourier transform infrared spectroscopy. J Microbiol Methods 69:146–153. https://doi.org/10.1016/j.mimet.2006.12.022
163. Amiali NM, Golding GR, Sedman J, Simor AE, Ismail AA (2011) Rapid identification of community-associated methicillin-resistant *Staphylococcus aureus* by Fourier transform infrared spectroscopy. Diagn Microbiol Infect Dis 70:157–166. https://doi.org/10.1016/j.diagmicrobio.2010.12.016
164. Johler S, Tichaczek-Dischinger PS, Rau J, Sihto H-M, Lehner A, Adam M, Stephan R (2013) Outbreak of Staphylococcal food poisoning due to SEA-producing *Staphylococcus aureus*. Foodborne Pathog Dis 10:777–781. https://doi.org/10.1089/fpd.2013.1503
165. Becker K, Al LN, Fegeler W, Proctor RA, Peters G, von Eiff C (2006) Fourier-transform infrared spectroscopic analysis is a powerful tool for studying the dynamic changes in *Staphylococcus aureus* small-colony variants. J Clin Microbiol 44:3274–3278. https://doi.org/10.1128/JCM.00847-06
166. Amiali NM, Mulvey MR, Berger-Bächi B, Sedman J, Simor AE, Ismail AA (2008) Evaluation of Fourier transform infrared spectroscopy for the rapid identification of glycopeptide-intermediate *Staphylococcus aureus*. J Antimicrob Chemother 61:95–102. https://doi.org/10.1093/jac/dkm400
167. Gardner SG, Marshall DD, Daum RS, Powers R, Somerville GA (2018) Metabolic mitigation of *Staphylococcus aureus* vancomycin intermediate-level susceptibility. Antimicrob Agents Chemother 62:e01608–e01617. https://doi.org/10.1128/AAC.01608-17
168. Geno KA, Gilbert GL, Song JY, Skovsted IC, Klugman KP, Jones C, Konradsen HB, Nahm MH (2015) Pneumococcal capsules and their types: past, present, and Future. Clin Microbiol Rev 28:871–899. https://doi.org/10.1128/CMR.00024-15
169. Bentley SD, Aanensen DM, Mavroidi A, Saunders D, Rabbinowitsch E, Collins M, Donohoe K, Harris D, Murphy L, Quail MA, Samuel G, Skovsted IC, Kaltoft MS, Barrell B, Reeves PR, Parkhill J, Spratt BG (2006) Genetic analysis of the capsular biosynthetic locus from all 90 pneumococcal serotypes. PLoS Genet 2:e31. https://doi.org/10.1371/journal.pgen.0020031
170. Kapatai G, Sheppard CL, Al-Shahib A, Litt DJ, Underwood AP, Harrison TG, Fry NK (2016) Whole genome sequencing of *Streptococcus pneumoniae*: development, evaluation and verification of targets for serogroup and serotype prediction using an automated pipeline. PeerJ 4:e2477. https://doi.org/10.7717/peerj.2477
171. Kamerling J (1999) Pneumococcal polysaccharides: a chemical view. In: Tomasz A (ed) *Streptococcus pneumoniae*: molecular biology and mechanisms of disease. Mary Ann Liebert, Larchmont, pp 81–114
172. Vaz M, Meirinhos-Soares L, Sousa CCS, Ramirez M, Melo-Cristino J, Lopes JA (2013) Serotype discrimination of encapsulated *Streptococcus pneumoniae* strains by Fourier-transform infrared spectroscopy and chemometrics. J Microbiol Methods 93:102–107. https://doi.org/10.1016/j.mimet.2013.02.008
173. Morona JK, Morona R, Paton JC (1999) Comparative genetics of capsular polysaccharide biosynthesis in Streptococcus pneumoniae types belonging to serogroup 19. J Bacteriol 181:5355–5364
174. Ravenscroft N, Omar A, Hlozek J, Edmonds-Smith C, Follador R, Serventi F, Lipowsky G, Kuttel MM, Cescutti P, Faridmoayer A (2017) Genetic and structural elucidation of capsular polysaccharides from *Streptococcus pneumoniae* serotype 23A and 23B, and comparison to serotype 23F. Carbohydr Res 450:19–29. https://doi.org/10.1016/j.carres.2017.08.006
175. Mizrachi Nebenzahl Y, Porat N, Lifshitz S, Novick S, Levi A, Ling E, Liron O, Mordechai S, Sahu RK, Dagan R (2004) Virulence of *Streptococcus pneumoniae* may be determined independently of capsular polysaccharide. FEMS Microbiol Lett 233:147–152. https://doi.org/10.1016/j.femsle.2004.02.003
176. Shen Y, Boulos S, Sumrall E, Gerber B, Julian-Rodero A, Eugster MR, Fieseler L, Nyström L, Ebert M-O, Loessner MJ (2017) Structural and functional diversity in *Listeria* cell wall teichoic acids. J Biol Chem 292:17832–17844. https://doi.org/10.1074/jbc.M117.813964
177. Rebuffo-Scheer CA, Schmitt J, Scherer S (2007) Differentiation of *Listeria monocytogenes* serovars by using artificial neural network analysis of Fourier-transformed infrared spectra. Appl Environ Microbiol 73:1036–1040. https://doi.org/10.1128/AEM.02004-06
178. Stessl B, Fricker M, Fox E, Karpiskova R, Demnerova K, Jordan K, Ehling-Schulz M, Wagner M (2014) Collaborative survey on the colonization of different types of cheese-processing facilities with *Listeria monocytogenes*. Foodborne Pathog Dis 11:8–14. https://doi.org/10.1089/fpd.2013.1578
179. Romanolo KF, Gorski L, Wang S, Lauzon CR (2015) Rapid identification and classification of *Listeria* spp. and serotype assignment of *Listeria monocytogenes* using Fourier transform-infrared spectroscopy and artificial neural network analysis. PLoS One 10:e0143425. https://doi.org/10.1371/journal.pone.0143425
180. Davis R, Mauer LJ (2011) Subtyping of *Listeria monocytogenes* at the haplotype level by Fourier transform infrared (FT-IR) spectroscopy and multivariate statistical analysis. Int J Food Microbiol 150:140–149. https://doi.org/10.1016/j.ijfoodmicro.2011.07.024
181. Fiedler F, Seger J, Schrettenbrunner A, Seeliger HPR (1984) The biochemistry of murein and cell wall teichoic acids in the genus *Listeria*. Syst Appl Microbiol 5:360–376. https://doi.org/10.1016/S0723-2020(84)80038-7
182. Promadej N, Fiedler F, Cossart P, Dramsi S, Kathariou S (1999) Cell wall teichoic acid glycosylation in *Listeria monocytogenes* serotype 4b requires gtcA, a novel, serogroup-specific gene. J Bacteriol 181:418–425
183. Nelson KE, Fouts DE, Mongodin EF, Ravel J, DeBoy RT, Kolonay JF, Rasko DA, Angiuoli SV, Gill SR, Paulsen IT, Peterson J, White O, Nelson WC, Nierman W, Beanan MJ, Brinkac LM, Daugherty SC, Dodson RJ, Durkin AS, Madupu R, Haft DH, Selengut J, Van Aken S, Khouri H, Fedorova N, Forberger H, Tran B, Kathariou S, Wonderling LD, Uhlich GA, Bayles DO, Luchansky JB, Fraser CM (2004) Whole genome comparisons of serotype 4b and 1/2a strains of the food-borne pathogen *Listeria monocytogenes* reveal new insights into the core genome components of this species. Nucleic Acids Res 32:2386–2395. https://doi.org/10.1093/nar/gkh562
184. Schirm M, Kalmokoff M, Aubry A, Thibault P, Sandoz M, Logan SM (2004) Flagellin from *Listeria monocytogenes* is glycosylated with beta-O-linked N-acetylglucosamine. J Bacteriol 186:6721–6727. https://doi.org/10.1128/JB.186.20.6721-6727.2004
185. Oust A, Møretrø T, Naterstad K, Sockalingum GD, Adt I, Manfait M, Kohler A (2006) Fourier transform infrared and raman spectroscopy for characterization of *Listeria monocytogenes* strains. Appl Environ Microbiol 72:228–232. https://doi.org/10.1128/AEM.72.1.228-232.2006

186. Nyarko EB, Puzey KA, Donnelly CW (2014) Rapid differentiation of *Listeria monocytogenes* epidemic clones III and IV and their intact compared with heat-killed populations using fourier transform infrared spectroscopy and chemometrics. J Food Sci 79:1189–1196. https://doi.org/10.1111/1750-3841.12475
187. Sue D, Hoffmaster AR, Popovic T, Wilkins PP (2006) Capsule production in *Bacillus cereus* strains associated with severe pneumonia. J Clin Microbiol 44:3426–3428. https://doi.org/10.1128/JCM.00873-06
188. Mignot T, Denis B, Couture-Tosi E, Kolstø AB, Mock M, Fouet A (2001) Distribution of S-layers on the surface of *Bacillus cereus* strains: phylogenetic origin and ecological pressure. Environ Microbiol 3:493–501. https://doi.org/10.1046/j.1462-2920.2001.00220.x
189. Mietke H, Beer W, Schleif J, Schabert G, Reissbrodt R (2010) Differentiation between probiotic and wild-type *Bacillus cereus* isolates by antibiotic susceptibility test and Fourier transform infrared spectroscopy (FT-IR). Int J Food Microbiol 140:57–60. https://doi.org/10.1016/j.ijfoodmicro.2010.02.009
190. Ehling-Schulz M, Svensson B, Guinebretiere M-H, Lindbäck T, Andersson M, Schulz A, Fricker M, Christiansson A, Granum PE, Märtlbauer E, Nguyen-The C, Salkinoja-Salonen M, Scherer S (2005) Emetic toxin formation of *Bacillus cereus* is restricted to a single evolutionary lineage of closely related strains. Microbiology 151:183–197. https://doi.org/10.1099/mic.0.27607-0
191. Leoff C, Saile E, Sue D, Wilkins P, Quinn CP, Carlson RW, Kannenberg EL (2008) Cell wall carbohydrate compositions of strains from the *Bacillus cereus* group of species correlate with phylogenetic relatedness. J Bacteriol 190:112–121. https://doi.org/10.1128/JB.01292-07
192. Hancock LE, Murray BE, Sillanpää J (2014) Enterococcal cell wall components and structures
193. Palmer KL, Godfrey P, Griggs A, Kos VN, Zucker J, Desjardins C, Cerqueira G, Gevers D, Walker S, Wortman J, Feldgarden M, Haas B, Birren B, Gilmore MS (2012) Comparative genomics of enterococci: variation in *Enterococcus faecalis*, clade structure in *E. faecium*, and defining characteristics of *E. gallinarum* and *E. casseliflavus*. MBio 3:e00318–e00311. https://doi.org/10.1128/mBio.00318-11
194. Freitas A, Sousa C, Bavlovič J, Silva L, Novais C, Coque T, Lopes J, Peixe L (2016) Discrimination of vancomycin-resistant *Enterococcus faecium* clones by MLST (eBURST/BAPS) and spectroscopic techniques (MALDI- TOF MS and FTIR-ATR). In: 11th International Meeting on Microbial Epidemiological Markers (IMMEM XI). Estoril, Portugal.
195. Wang J, Yue T, Yuan Y, Lu X, Shin J-H, Rasco B (2011) Discrimination of *Alicyclobacillus* strains using nitrocellulose membrane filter and attenuated total reflectance fourier transform infrared spectroscopy. J Food Sci 76:M137–M142. https://doi.org/10.1111/j.1750-3841.2010.02034.x
196. Winder CL, Gordon SV, Dale J, Hewinson RG, Goodacre R (2006) Metabolic fingerprints of *Mycobacterium bovis* cluster with molecular type: implications for genotype-phenotype links. Microbiology 152:2757–2765. https://doi.org/10.1099/mic.0.28986-0