# Modern clinical microbiology: new challenges and solutions

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Abstract | In the twenty-first century, the clinical microbiology laboratory plays a central part in optimizing the management of infectious diseases and surveying local and global epidemiology. This pivotal role is made possible by the adoption of rational sampling, point-of-care tests, extended automation and new technologies, including mass spectrometry for colony identification, real-time genomics for isolate characterization, and versatile and permissive culture systems. When balanced with cost, these developments can improve the workflow and output of clinical microbiology laboratories and, by identifying and characterizing microbial pathogens, provide significant input to scientific discovery.

**MALDI–TOF mass spectrometry**
A soft ionization technique that enables analysis of biomolecules using mass spectrometry.

The roles of clinical microbiologists include the identification of bacterial, viral, fungal and parasitic agents that cause human disease, providing diagnostic and therapeutic support for the clinical management of patients, and preventing the transmission of infectious diseases in both the health care system and the community. The number of identified emerging infectious diseases has steadily climbed since the 1940s[1], and in the early twenty-first century, tools such as PCR, high-throughput sequencing[2] and MALDI–TOF mass spectrometry (MS), along with new sampling and culture strategies, are changing clinical microbiology. This technological progress, which began in environmental microbiology[3,4], has revealed a much larger microbial world than was believed to exist a few years ago. In 1980, only 1,800 validated bacterial species had been published, whereas more than 500 new species are now described annually[5]. Thus, over the past 4 years, more new bacterial species have been described than were described in the period up to 1980 (FIG. 1). For example, important human pathogens such as *Helicobacter pylori*, which can cause gastric ulcers and cancer, and *Tropheryma whipplei*, the causative agent of Whipple's disease, were first isolated in 1985 and 2001, respectively. For viruses, the progress has been comparable; severe acute respiratory syndrome (SARS)-associated coronavirus[6] and the arenavirus Lujo virus, which causes haemorrhagic fever[7], were identified in 2003 and 2009, respectively.

Moreover, our view of microbial pathogenesis, in which one given microorganism causes one given disease — a view that was inherited from Pasteur and Koch — began to change when new tools allowed us to observe the diversity of microorganisms and the impact that this diversity has on human diseases and their treatment[8]. For example, we now know that nine *Mycobacterium* species can cause tuberculosis, some of which, such as *Mycobacterium bovis*, require specific antibiotic treatments.

In this Review, we detail the main changes that have recently occurred in the technologies and techniques available to clinical microbiology laboratories (CMLs), including syndrome- and disease-based sampling kits, point-of-care (POC) testing, and isolate typing and characterization by MALDI–TOF MS or by next-generation sequencing (NGS). We also try to predict the most important developments for the future.

## Developments in sampling

*Syndrome- and disease-based kits.* The conventional diagnosis of infectious diseases usually relies on a stepwise approach in which the physician examines the patient, diagnoses a clinical syndrome and then tests for pathogens that are potentially responsible for that syndrome, until a diagnosis is made. However, the growing number of emerging pathogens makes it difficult for physicians to memorize the actual list of pathogens for each infectious disease and thus prescribe all the appropriate diagnostic microbiology tests. To reduce the delays associated with resampling or retesting, sampling can be carried out using diagnostic kits that are standardized according to the syndrome or disease. Such a strategy enables optimization of the type and number of specimens collected from the patient, simplification of the laboratory test prescription

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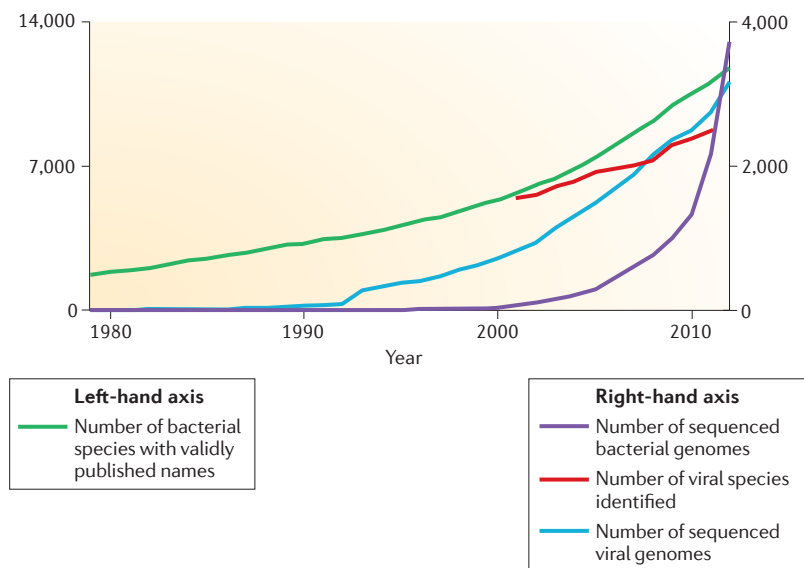
Figure 1 | **The number of identified microbial species from 1979 to 2012.** The development of new technologies has had a substantial impact on the number of microbial species that are identified each year.

**Fastidious**
Pertaining to a microorganism: having complex nutritional requirements and unable to grow under routine conditions used in clinical microbiology laboratories.

**Axenically**
Pertaining to culture of a microorganism: without a living support; for example, culture on agar medium.

**Culturomics**
The diversification of culture conditions for the isolation of fastidious microorganisms.

for the physician, and the creation of a scheduled workflow for the nurse and the doctor, and it also allows the clinical laboratory to easily trace samples. To date, such diagnostic kits have been developed for endocarditis, pericarditis, diarrhoea, osteitis, meningitis, encephalitis, uveitis and keratitis[9–11], and their design has been based on the repertoire of pathogens responsible for the syndrome or disease, and on the optimal methods to achieve the direct or indirect detection and identification of these pathogens. Further syndrome-based kits can be designed for specific groups of febrile patients, such as those presenting to the emergency room for POC testing, travellers, pilgrims to Mecca (Saudi Arabia), the homeless, or patients with cystic fibrosis and respiratory tract infections[12]. In addition to the broad syndrome- or disease-based kits, pathogen-specific kits can be used, as for pulmonary tuberculosis[13].

Compared to a more traditional approach, in which the most common agents of an infectious syndrome are tested first, followed by testing for the rarer agents, a broad syndrome- or disease-based strategy might seem to be economically disadvantageous. But it is possible that the increased laboratory costs will be balanced by shorter hospitalization times owing to an earlier diagnosis and an earlier initiation of the appropriate antibiotic therapy, and by avoiding unnecessary treatment in the case of viral infections[14].

In addition, specimens recovered from such kits can be preserved in biobanks for retrospective testing when new emerging pathogens are described, and data on kit usage can be used for epidemiological analyses such as seasonal changes in sampling or to evaluate the cost-effectiveness of the kits. To date, only a few CMLs have adopted syndrome- and disease-based kits; in France, for example, they are used in 25 university hospitals.

*Strain collections.* Currently, reference microorganisms are preserved in international collections such as the American Type Culture Collection, the Deutsche Sammlung von Mikroorganismen und Zellkulturen and the Japan Collection of Microorganisms. However, because of their number, most clinical isolates routinely cultivated in CMLs are neither conserved nor referred to these collections. The conservation of clinical isolates by CMLs might enable tracing of outbreaks and the characterization of emerging pathogens, and could thus be valuable for both public health (disease surveillance and prevention) and scientific purposes. Notably, bacterial strains cultivated from normally sterile organs and body fluids, such as blood and cerebrospinal fluid, might be conserved. However, because of the cost, few reference laboratories are currently equipped for long-term storage of samples in such collections.

### Processing of clinical samples

*Direct examination.* Gram staining, used mostly for the direct examination of uncultured clinical samples, remains an essential step in detecting microorganisms and guiding empirical antibiotic therapy, although the adoption of MALDI–TOF MS to identify cultured colonies might restrict the use of this technique[15]. However, Gram staining can be automated — for example, by a robot such as the PREVI Colour Gram (BioMérieux), which can stain up to 300 slides per hour and reduces the chemical hazard for technicians.

*Culturing clinical samples.* Culturing remains the mainstay of clinical microbiology[16]. Several approaches, many of which were first implemented by environmental and then clinical microbiologists, have been used in recent years to improve the isolation of fastidious bacteria from human specimens[17,18]. The first approach, which remains historically the most efficient, is based on empiricism and uses existing media or media with enrichment components added. For instance, *Borrelia recurrentis* was reputed to be non-cultivable axenically until the blood of a patient with louse-borne relapsing borreliosis was inoculated on media dedicated to *Borrelia burgdorferi*[19]. In the case of *Mycobacterium tuberculosis*, the use of blood-enriched medium enabled the improved and accelerated recovery of colonies[20]. Recently, we developed a strategy named culturomics, which proved to be very successful in isolating previously unknown or uncultivated bacteria (BOX 1). This empirical approach has the advantage of using common media, but is poorly adapted to the detection of emerging fastidious bacteria.

The second, non-empirical approach is to use genome sequence data to develop media that are specifically adapted to a given fastidious pathogen[18]. For example, axenic culture of *T. whipplei* strains obtained by cell culture or directly from clinical samples was achieved using standard cell culture medium that had been supplemented with those amino acids for which there were no corresponding metabolism genes in the genomes of the cultured strains[21]. A comparable approach was recently initiated for the culture of two other strictly intracellular bacteria, *Coxiella burnetii* and

Box 1 | **Culturomics: the diversification of culture methods for microbial isolation**

A major challenge in the twenty-first century is the need for a comprehensive characterization of both the human microbiome and its relationships with health and disease[108]. Recent genomic and metagenomic studies have demonstrated that approximately 80% of bacterial species detected in the human gut are either uncultured so far or even unculturable[109]. Therefore, in the omics era, culturing became outdated for the study of complex microbial communities. However, several discrepancies have been observed among metagenomic studies, apparently reflecting biases of the techniques used and the fact that clinically relevant minor populations were not considered. For example, potentially pathogenic bacteria (such as *Salmonella enterica* subsp. *enterica* serovar Typhi, *Tropheryma whipplei* and *Yersinia enterocolitica*) that can be present in faeces at concentrations of <$10^5$ colony-forming units per ml, were ignored. Thus, we designed a new culture strategy that we named culturomics, which uses diversified culture conditions (medium, temperature and atmosphere) to complement culture-independent approaches and to complete our knowledge of the human microbiota[110].

In the first culturomics study, which used 212 distinct culture conditions, 341 bacterial species were cultured from Senegalese patients and classified as belonging to seven phyla and 117 genera[110]. In a second study of a patient receiving massive antibiotic drug therapy for multidrug-resistant tuberculosis, 70 distinct culture conditions enabled the isolation of 39 bacterial species, including one new species and three species that had not previously been observed in the human gut[111]. To date, culturomics has yielded 175 bacterial species never previously found in the human gut, and 32 new bacterial genera and/or species, including *Microvirga massiliensis*, which has the largest genome of the known human-associated bacteria. Metagenomic analysis of the same specimens produced 698 phylotypes, including 282 known species, 51 of which overlapped with the microbiome identified by culturomics. However, despite these promising results and although culturomics might complement metagenomics, notably by overcoming the bias inherent to metagenomic approaches of ignoring minor microbial populations, this new technique is a time-consuming, expensive strategy that, as yet, has not been adapted to routine microbiology.

*Chlamydia trachomatis*[22,23], and might be implemented for other fastidious pathogens such as *Mycobacterium leprae* and *Treponema pallidum*[24].

A third approach (often occurring by accident) is to extend the incubation time of fastidious extracellular pathogens, such as the extracellular forms of the facultative intracellular *Bartonella* spp.[25], and of intracellular pathogens, such as *Bartonella* spp., *T. whipplei* and mycobacteria[25–27]. A fourth approach is the use of cell culture, especially the shell vial assay. Briefly, in this assay, ground clinical specimens are inoculated by centrifugation into a layer of eukaryotic cells grown on a 1 cm$^2$ cover slip at the bottom of a small tube[28]. This method has been shown to be highly efficient for the isolation of fastidious agents, even those that are not strictly intracellular[28]. The versatility of the technique is related to the nearly unlimited choice of cells that can be used for culture, thus widening the spectrum of culture conditions and isolated microorganisms. For example, *Rickettsia felis*[29] can be grown only in XTC-2 cells, which require a low temperature, and *Legionella* spp.[30] can be grown only in amoebae. However, these novel cell culture approaches are restricted to the few laboratories that are equipped with specific facilities and well-trained personnel.

**Identification and resistance testing of pathogens**
*Identification of bacteria, fungi and viruses.* Phenotypic identification of bacterial isolates has long relied on a combination of biochemical properties such as oxygen requirement, Gram staining, carbohydrate metabolism and the presence of specific enzymes. However, phenotypic identification systems, such as miniaturized strips, are costly and time-consuming, even when automated, and thus over the past decade have been superseded by MALDI–TOF MS. Worldwide, many large CMLs have adopted MALDI–TOF MS owing to the increased workflow efficiency and associated cost reduction when using

this approach[31] (FIG. 2). MALDI–TOF MS can identify bacterial isolates in a few minutes (6–10 minutes) and for low costs (€1.43 per strain, compared with €2.2–€8.23 per strain using API strips or Vitek automates, both of which are commonly used phenotypic assays, and €137 per strain for 16S rRNA sequencing)[31]. Moreover, the approach can also efficiently identify clinical isolates of fungi[32], as well as bacteria in blood culture vials[33].

The identification of microbial isolates using MALDI–TOF MS relies on a comparison between the mass spectrum of the isolate and mass spectra in available databases. In addition to the rapidity and low cost of MALDI–TOF MS, other advantages include the precise identification of the isolate at the species level and, in some species, at the subspecies and strain levels. Currently, bacterial species from many genera have been identified by MALDI–TOF MS, including fastidious microorganisms (reviewed in REF. 34). However, the discriminatory power of the method varies depending on the species and the exhaustiveness of the database used. Notably, some bacterial taxa are under-represented in databases, and technical problems (such as variations in culture conditions, sample preparation and the spectrometer used) further affect the discriminatory power of this technique[35]. In addition, streptococci, non-fermenting Gram-negative bacteria and anaerobic bacteria are generally harder to discriminate than other bacteria, although this weakness can be partially corrected by enriching the available databases with spectra from more strains[31]. MALDI–TOF MS can be used for typing community-acquired pathogens, such as *Listeria monocytogenes*, *Legionella pneumophila* and *Yersinia enterocolitica*; health care-associated pathogens, such as *Corynebacterium striatum*[36] and *Candida parapsilosis*[37]; and both community- and health care-associated pathogens, such as *Staphylococcus aureus*. Finally, MALDI–TOF MS is now routinely used in several large CMLs for
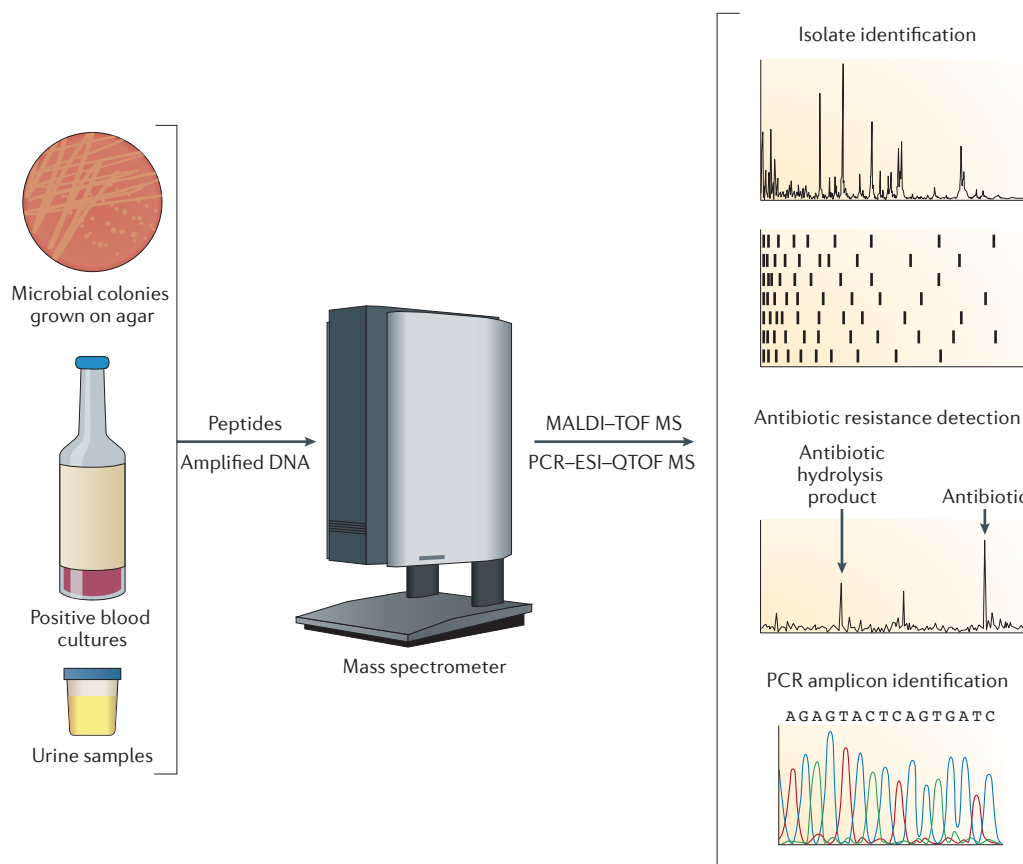
Figure 2 | **Current applications of MALDI–TOF mass spectrometry in the clinical microbiology laboratory.**
MALDI–TOF mass spectrometry (MS) allows the identification and antibiotic-susceptibility testing of microbial pathogens in urine samples or cultivated on agar or in blood culture bottles. Alternatively, strain identification can be carried out by PCR–ESI–QTOF MS (PCR followed by electrospray ionization–quadrupole TOF MS) characterization of PCR amplicons from the microbial DNA.

the direct identification of bacteria in blood cultures[33]. Although MALDI–TOF MS can identify various viral species[38], commercially available MS databases do not contain viral spectra; therefore, MALDI–TOF MS does not yet enable the identification of viruses in routine microbiology.

The recent development of high-throughput phenotypic microarrays, such as the Biologue system (Biologue, Inc.), which combines up to 2,000 phenotypic tests, might challenge the leading position of MALDI–TOF MS. Microarrays can be used for several applications, such as the determination of bacterial metabolic and chemical properties, the formal description of new species, and the development and optimization of culture media. The Biologue system has been demonstrated to be highly efficient for phenotypic identification of organisms, although it is less discriminatory than16S rRNA sequencing[39] and slower than MALDI–TOF MS[31].

In the past 10 years, Raman spectroscopy has been demonstrated to accurately and rapidly identify bacterial, fungal and yeast isolates at the species and subspecies levels[40,41]. In this method, light scattering from a laser-illuminated bacterial colony is analysed and transformed into a spectrum that is compared to a specific database.

Although this method is inexpensive, reagent-free and as rapid as MS, Raman spectroscopy is not currently widely used in CMLs owing to technical hurdles such as low signal strength, and perhaps also owing to the initial cost of a new instrument. In addition, as for MALDI–TOF MS, the reliability of Raman spectroscopy depends on the quality and coverage of the reference databases.

*Detection of antibiotic resistance.* Classical methods, such as phenotypic antibiotic-susceptibility testing of a clinical isolate based on minimum inhibitory concentrations (MICs) and clinical breakpoints, remain the gold standard for *in vitro* prediction of antibiotic resistance, but many factors can influence the results obtained for a given isolate and can lead to a false positive or false negative result. MIC is defined as the lowest concentration of antibiotic able to inhibit the growth of a bacterial isolate *in vitro*, whereas breakpoints are used to predict the clinical outcome of an antibiotic treatment *in vivo*. Some new and/or emerging resistance mechanisms might be missed because they are not easily detected phenotypically *in vitro*. Instead, they require additional molecular testing. Moreover, results from the classical resistance tests might not be rapidly available, especially for

fastidious bacteria such as *M. tuberculosis*[42]. The application of real-time PCR (RT-PCR) to the rapid screening and detection of common antibiotic resistance genes such as *mecA* or *ndm1* (which provide resistance to methicillin in *S. aureus*[43] and carbapenem in bacteria of the family Enterobacteriaceae[44], respectively) was revolutionary. In a similar manner, non-fluorescent DNA microarrays[45] and pyrosequencing[46] can detect gene mutations or SNPs that are known to be associated with *M. tuberculosis* resistance to antituberculous compounds. DNA microarrays are another new technology that can detect, in a single step, many resistance genes in Gram-negative and Gram-positive bacteria[47]. However, microarrays are expensive (~300€ per Mb for microarrays versus 15€ per Mb for high-throughput genome sequencing) and can detect known genes only. Recently, MALDI–TOF MS has also been used clinically for the rapid phenotypic detection of antibiotic resistance, especially of β-lactamase activity[48]. For example, it is possible to detect carbapenemase activity in bacteria of the family Enterobacteriaceae, in *Pseudomonas aeruginosa* and in *Acinetobacter baumannii* through the identification of carbapenem compounds and their degradation products by MALDI–TOF MS in only 1–4 hours and with high sensitivity and specificity[49–51] (FIG. 2). One of the main advantages of this technique is that any enzymatic activity associated with antibiotic resistance can be detected, even if the causative enzyme is unknown, and this could lead to the discovery of new antibiotic resistance determinants.

In addition, antibiotic resistance diagnostics can be further improved to detect and interpret resistance patterns using automated intra-laboratory surveys of antibiograms. This allows the detection of unusual phenotypes or emerging clusters of strains with a similar profile in clinical isolates. We recently developed a computer tool for real-time surveillance of resistance[52,53]. On a weekly basis, this program automatically and systematically compares the resistance patterns obtained from clinical samples in our routine microbiology laboratory to a Microsoft Excel database of resistance patterns based on results from 12,000 clinical isolates tested in our laboratory in 2012. The aim is to detect any atypical, abnormal or rare patterns of resistance or susceptibility. Such a tool might improve the diagnosis, monitoring and characterization of antibiotic resistance.

## Sequencing of microbial genomes

Genomic sequence information from cultivated microorganisms is widely used for epidemiological studies (TABLE 1). In clinical microbiology, applications of genome sequencing include the development of detection, identification and genotyping tools, the design of culture media and the assessment of antibiotic resistance or virulence repertoires[42,54,55]. Recently, thanks to NGS technologies such as the MiSeq (Illumina), Ion Torrent Personal Genome Machine (PGM) (Life Technologies) and 454 GS Junior (Roche) bench-top sequencers, bacterial genome sequencing has become fast (only a few hours) and cheap (only a few hundred US dollars)[56], making whole-genome sequencing compatible with the routine clinical microbiology workflow[42,55,57,58]. This

strategy might offer rapid and exhaustive access to the virulence determinants[59], antibiotic resistance markers[60] or genotypes[61] of unusual or difficult-to-grow bacterial strains isolated from clinical specimens. Recent examples of the use of whole-genome sequencing in clinical microbiology include the investigations of hospital outbreaks of *A. baumannii*, *S. aureus* and *Clostridium difficile* infections[62,63], and the identification of the virulence determinants of a *Staphylococcus epidermidis* strain that was the aetiological agent of native valve endocarditis[64]. In addition, as demonstrated recently, NGS might also enable complete genome sequencing of a pathogen directly from a clinical specimen[65].

However, despite the undeniable advantages, NGS requires extensive bioinformatics for sequence analysis, from assembly to annotation, and this remains a significant problem for the routine application of genomics to clinical microbiology. The development of automated tools for the analysis of genomic sequences and the compilation of databases containing whole genome sequences together with specific genetic criteria, such as virulence factors and resistance markers, might solve this problem in the near future and enable NGS to be used in routine practice[55].

In addition, NGS has been used to begin to decipher complex human microbial communities in metagenomic studies[66]. Furthermore, a recent analysis of the gut flora provided hints that might enable the prediction of infection susceptibility or antibiotic resistance, or the assessment of the risk of developing diseases such as obesity[67].

*Genotyping.* Genotyping consists of tracing clones by identifying sequence-specific signatures. Various genotyping methods have been developed, including several methods based on DNA banding patterns, such as pulsed-field gel electrophoresis[68], PCR–restriction fragment-length polymorphism (RFLP)[69], multiple locus variable number of tandem repeats analysis (MLVA)[70], random amplification using arbitrary primers (RAPD)[71] and amplified fragment-length polymorphism (AFLP)[72]. However, these methods often lack reproducibility within and between laboratories. DNA hybridization-based methods using nucleotide probes, such as DNA macroarrays[73] and DNA microarrays[74], are expensive, can identify only the genetic markers present on the arrays and thus might underestimate the genetic diversity among bacterial populations. DNA sequence-based methods such as multilocus sequence typing (MLST)[54], multispacer sequence typing[2] and genome sequencing[62,63] are discriminatory and reproducible, but are time-consuming and can be expensive. Although genotyping all the pathogenic isolates from all cultures would probably have a significant impact on infection control policies and their execution, most genotyping techniques are not carried out routinely, except for the detection of specific clones of certain pathogens. For example, *M. tuberculosis* str. Beijing can be detected within 2 hours using specific RT-PCR[75], enabling patient isolation, contact tracing and extended antituberculous treatment.

Metagenomic studies
Studies of the genetic material obtained from complex microbial communities.

Table 1 | **Examples of infectious disease outbreaks that were investigated using next-generation sequencing**

| Microorganism | Location | Year | Reference |
|---|---|---|---|
| Carbapenem-resistant *Klebsiella pneumoniae* | USA | 2011 | 112 |
| *Clostridium difficile* | Worldwide | 2013 | 113 |
| *Escherichia coli* O104:H4 | Germany | 2011 | 114,115 |
| *Legionella pneumophila* serogroup 1 | United Kingdom | 2013 | 116 |
| Methicillin-resistant *Staphylococcus aureus* (MRSA) | United Kingdom | 2009 | 117 |
| *Mycobacterium tuberculosis* | Canada | 2006–2008 | 118 |
| *Vibrio cholerae* O1 biovar El Tor | Haiti | 2010–2011 | 119 |
| Arenavirus | Australia | 2008 | 120 |
| Bas-Congo virus | Democratic Republic of the Congo | 2009 | 121 |
| Influenza A virus H1N1 | Worldwide | 2009 | 122 |

## Direct microbial identification in specimens

*Mass spectrometry.* In 2009, for the first time, MALDI–TOF MS was reported to efficiently identify bacteria directly from blood collected in culture bottles, with results obtained less than 2 hours after the blood culture vial was determined to be positive, and with a 97.5% success rate[76] (FIG. 2). However, the accuracy of bacterial identification might be influenced by unstandardized sample preparation, differences in bacterial concentrations, pre-incubation, prolonged incubation and the blood culture system used[35]. Commercially available extraction kits[77] and the implementation of a minimum concentration of $10^6$–$10^8$ colony-forming units (CFU) per ml (REF. 78) might resolve some discrepancies. In 2010, MALDI–TOF MS was also used for the direct identification of bacteria in urine samples, with an accuracy rate of 91.8% and with bacterial concentrations as low as $10^3$ CFU per ml (REF. 78). Finally, in addition to identifying bacteria, MALDI–TOF MS has been shown to be capable of identifying fungi in blood cultures and nail specimens[79]. However, MALDI–TOF MS itself has certain limitations, including the inability to identify species among mixed populations or directly in solid clinical specimens, and poor discriminatory power among corynebacteria and streptococci[31].

*Molecular detection.* Molecular detection methods — notably, bacterial PCR combined with sequencing — have played a major part in clinical microbiology and enabled the discovery of several non-cultivable pathogens, such as *Bartonella quintana* and *T. whipplei*[80,81]. Among molecular diagnostic methods, RT-PCR has proved highly valuable, as it enables diagnoses to be obtained in less than 5 hours, whereas traditional microbiological methods, including conventional molecular diagnostics such as broad PCR followed by sequencing, take a minimum of 1 day. RT-PCR also enables the quantification of pathogens[82] and is routinely used to quantify the viral load in patients infected with HIV. RT-PCR assays can target either specific pathogens and resistance- or virulence-encoding genes, or universal targets, such as 16S rDNA for bacteria and rDNA internal transcribed spacers for fungi. In addition, RT-PCR multiplex assays can simultaneously target several DNA fragments, thus enabling the detection of a whole panel of pathogens that are implicated in particular syndromes, such as bloodstream infections[83], community-acquired pneumonia, meningitis[84], sexually transmitted diseases[85] or urinary tract infections[86].

Although RT-PCR-mediated detection of specific agents avoids sequencing, conventional PCR using broad-range targets combined with sequencing remains an irreplaceable tool for the identification of new pathogens in clinical specimens. Over the past decade, PCR amplicon sequencing has been increasingly implemented by CMLs for the identification or genotyping of microbial pathogens[87].

Alternatively, PCR–ESI–QTOF MS (PCR followed by electrospray ionization–quadrupole TOF MS), is a recent application of mass spectrometry that enables bacterial identification following PCR amplification of species-specific DNA fragments[88]. This commercially available technology (in the form of the PLEX-ID machinery from Abbott) can identify important human pathogenic bacteria, including *Bacillus anthracis*, *Francisella tularensis*, *Yersinia pestis*, *Burkholderia mallei*, *Burkholderia pseudomallei*, *Brucella* spp. and *C. burnetii*, along with influenza A virus and influenza B virus. However, further studies are needed to validate the usefulness of PCR–ESI–QTOF MS in routine microbiology.

*Electronic nose.* By identifying the specific combination of volatile organic compounds produced by microorganisms within a clinical specimen, an electronic nose is able to detect the presence and type of bacteria responsible for infectious diseases such as urinary tract and pulmonary infections before other methods, and this approach thus allows early initiation of appropriate antibiotic therapy[89,90]. A limit of the electronic nose is that it cannot quantify microorganisms within a specimen and therefore cannot discriminate between infection and colonization.

## The clinical microbiology laboratory

*Core laboratories.* In several countries, the concentration of CMLs into large core laboratories aims to lower the management costs while increasing the number of routine and specialized tests available for various diseases[91]. However, this organization distances patients
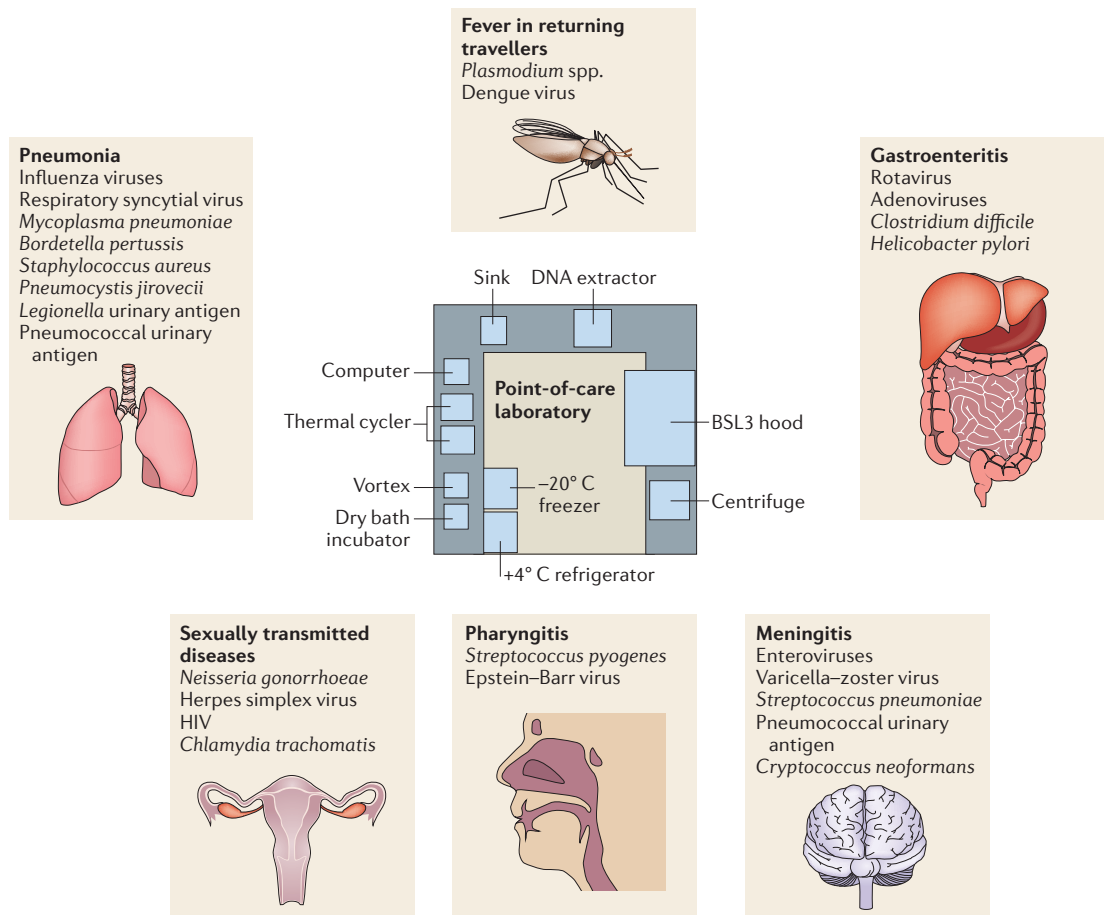
**Figure 3 | Diagnosis of infectious diseases using point-of-care assays.** Specimens referred to the point-of-care (POC) laboratory are tested according to the clinical picture and the physicians' queries. POC tests that will detect the most likely causative pathogen or a pathogen-associated antigen are carried out according to the clinical syndrome and the patient history. BSL3, biosafety level 3.

and doctors from the laboratory, and this might result in delayed reporting of test results and inappropriate management of patients. In 2011, an outbreak of Shiga toxin-producing *Escherichia coli* O104:H4 in Germany, a country in which core CMLs have been created, highlighted this risk, as there was delayed reporting of clinical cases[92].

*Point-of-care laboratories.* The need for automation and workflow improvement made it necessary to centralize biomedical analyses, but the increased distance from the laboratory to the site of patient care, as well as the batch processing of clinical specimens, has resulted in delayed delivery of results. As a consequence, core CMLs have been unable to contribute to timely decision-making for most infectious diseases. By contrast, POC laboratories (POCLs) are on-site laboratories that carry out rapid diagnostic tests within 4 hours (compared with >12 hours for culture methods or serology assays) and operate around the clock, and these laboratories are perfectly adapted to provide results in the early stages of patient care and can help with making appropriate hospitalization, isolation and therapy decisions (FIG. 3).

Initially, most POC tests relied on immunochromatographic or agglutination assays; however, the latest automated DNA extraction and RT-PCR devices using simplified procedures have made molecular detection assays compatible with POC objectives.

To date, several POC tests, such as the immunochromatographic detection of *Streptococcus pyogenes* antigen in the throat and *Plasmodium falciparum* in the blood, have demonstrated their usefulness in physicians' offices and on board ships visiting malaria-endemic countries (D.R., unpublished observations). POCLs should provide an accurate and rapid answer to a limited number of clinical microbiology questions and have a clear impact on patient management[14]. POC assays address the requirements to hospitalize patients, to isolate contagious individuals, and to initiate and focus anti-infective therapy. Assays routinely used in POCLs include those developed to detect bacteria (for example, *Bordetella pertussis*, *C. difficile*, *Neisseria meningitidis*, *Streptococcus pneumoniae* and *S. pyogenes*), viruses (including adenoviruses, dengue virus, enteroviruses and influenza viruses) and parasites (such as *P. falciparum*). Early studies have demonstrated that on-site POCLs met physicians' needs and have

influenced the management of up to 8% of the patients who presented to emergency wards[14]. This strategy might represent a major evolution of decision-making regarding the management of infectious diseases and patient care. In particular, it has been demonstrated that the POCL strategy enables the rapid adaptation of antibiotic therapy for patients with bacterial meningitis and reduces the length of the hospital stay for patients with viral meningitis[14]. Typically, POCLs feature operator-independent tests, including RT-PCR and immunochromatographic assays, most of which are cleared by the US Food and Drug Administration and the European Commission. Compared with standard microbiological procedures, most POC tests have a high positive predictive value, but some of them lack sensitivity[93,94].

Syndrome-based kits can also be used in POCLs for the rapid diagnosis of respiratory tract infections[95], meningitis[96] and sexually transmitted diseases[94]. The results are immediately transmitted to physicians by SMS and to the hospital information system. POCLs can be easily implemented in any type of environment, including remote areas, as demonstrated recently with the development of a POCL in rural Senegal[97]. Furthermore, test panels can even be adapted to the local epidemiology.

*Automation.* Most CMLs face common constraints, including the need to process increasing numbers of samples and increasing numbers of assays per sample. They need to maintain a continuous workflow, sometimes even under the pressure to reduce human resources. As many of the assays are characterized by repetitive manual operations, partial or full automation reduces human intervention and increases the quality of results by reducing human error, thus improving the workflow and output. For years, CMLs have automated various tasks, such as blood culture monitoring, biochemical phenotypic identification of bacteria and yeasts, antibiotic-susceptibility testing, Gram staining, DNA extraction and PCR amplification. The automated early detection of positive blood cultures and the identification of the pathogens and their antimicrobial susceptibilities can significantly reduce mortality in patients with bacteraemia and are among the most important functions of CMLs[98]. However, it is likely that MALDI–TOF MS automation will progressively replace phenotypic microbial identification[15].

One of the most recent advances in automation is the development of plate streakers that couple a bi-directional interface with a laboratory information system, and the possibility of inoculating different plates with a unique sample. To date, the three available automated systems — PREVI Isola (BioMérieux), WASP (Walk-Away Specimen Processor; Copan) and InoqulA (Kiestra) — all allow better CFU recovery than manual inoculation[99]. However, most specimens (except urine specimens) require time-consuming pretreatment, such as fluid sputum sample preparation or solid specimen grinding, or the use of specific and expensive swabs. Concomitantly, there is an increasing need to freeze and store samples in biobanks after inoculation or DNA extraction. Therefore, the future generation of automated plate streakers should incorporate functionalities such as aliquoting or DNA extraction or should connect to automated DNA extraction and/or amplification systems, such as the GeneXpert system (Cepheid). Finally, with the aim of complete automation, these systems should be coupled with automated urinary flow cytometry[100] and automated incubation in adapted atmospheres. Currently, only the fully automated Kiestra platform partially allows this.

In addition, the steps following incubation — that is, plate sorting according to positivity, colony picking for MALDI–TOF MS identification, and antimicrobial-susceptibility testing — should also be automated. Automatic incubators and plate sorters are currently under development, but are not yet available. A system that can discard (at the minimum) negative samples, similar to blood culture automated systems, would allow significant time savings. In our laboratory, 52% of the 161,240 samples inoculated annually are sterile. Automated colony picking that enables both MALDI–TOF MS identification and antimicrobial-susceptibility testing is already available using the Kiestra platform[101].

*Quality assurance and quality control.* CMLs should implement a system of quality management that monitors all aspects of the pre- and post-analytical service provided by the CML, from the receipt of patient samples to the reporting of results[102], ideally in accordance with the common International Standard Organization (ISO 9001) framework. In addition, all organizational and technical operating procedures should be standardized, made permanently available to the laboratory personnel and updated on a quarterly basis. Moreover, laboratories should participate in external and internal quality-assurance schemes.

### Result reporting

Result interpretation and reporting is essential in the management of infectious diseases at both the patient and community levels and thus should be a constant priority of CMLs and health authorities.

*Interpretation.* For patient management, it is essential that the laboratory provide reliable results which the clinician can trust. Therefore, the correct interpretation of microbiology results is crucial. Interpretation can be improved by automated interpretation software that reports any unknown or impossible phenotype or genotype. Automated systems such as VITEK2 (BioMérieux), Phoenix (Becton, Dickinson and Co.) and EPIMIC[52] enable the detection of bacterial isolates that are either taxonomically unusual in a certain clinical syndrome or specimen type, or exhibit a new or rare antibiotic resistance profile. CMLs either use commercially available MALDI–TOF MS spectrum databases (such as those from Bruker Daltonik, Shimadzu or BioMérieux) for identifying bacterial isolates, or develop their own databases[15]. In addition, results from genotyping methods can be compared to online databases[69]. Databases from these three surveillance systems can be updated with the results from the laboratory. In our laboratory, a medical microbiologist validates each result with the help of these
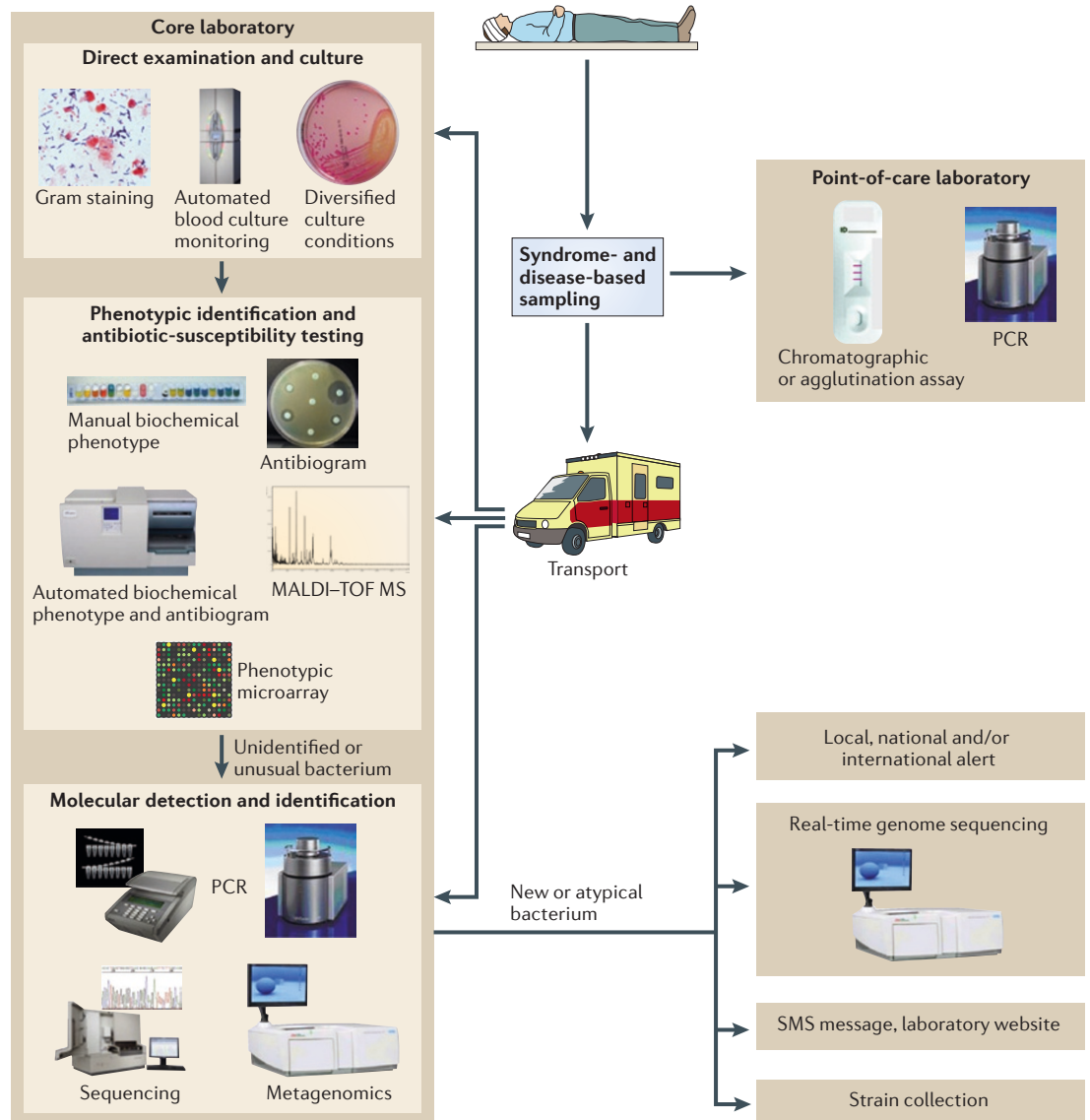
Figure 4 | **Overview of new methods in the workflow of clinical microbiology laboratories.** The figure describes the processing of specimens from patients who are thought to have an infectious disease. Sampling is carried out using syndrome- and disease-based sampling kits, and the resulting specimens are tested using point-of-care assays, when available. Culturing, strain characterization (phenotypic or molecular identification, and antibiotic-susceptibility testing) and molecular detection in clinical samples are carried out in the core laboratory. Unusual pathogens, conserved by the laboratory, are reported to the physician in charge and possibly to the sanitary authorities, and are further characterized by genome sequencing. MS, mass spectrometry.

tools and a survey of the international scientific literature before the result is transmitted to clinicians.

*Reporting.* Early reporting of microbiology results should be a priority of all CMLs, in particular for critical results (a positive direct examination, culture, or PCR from blood, cerebrospinal fluid or tissue). Faster reporting of identification and antimicrobial-susceptibility results can significantly reduce the length of hospital stay and the overall costs[103]. Of course, faster reporting implies that CMLs should follow technological progress and be equipped with the fastest microbiological methods[104]. Reporting can be carried out using laboratory information systems, electronic dashboards or mobile phones (for oral or SMS communications), thereby enabling physicians to receive timely alerts. In our centre, following the development of POCLs, we implemented SMS-based transmission of results to inform physicians as quickly as possible. Physicians might also use personal information systems to access other laboratory or clinical-patient data, or local epidemiological data.

Another important aspect of reporting is warning the local, national and international medical communities (the CDC, the European Centre for Disease Prevention and Control (ECDC) and the WHO) in case of the emergence of unusual infectious diseases with epidemic potential.

**Pathotypes**
Classification of microorganism below the subspecies level according to their pathogenicity for certain hosts.

Alert websites, such as the Program for Monitoring Emerging Diseases (ProMED-mail) and the European Travel Medicine Network (EuroTravNet), or journals such as *Eurosurveillance* are suitable channels for such alerts.

## Conclusions and perspectives

With the introduction of omics technologies (genomics, proteomics, culturomics, transcriptomics and metabolomics), CMLs face new challenges, such as obtaining a diagnosis at the time of care (FIG. 4). For example, until recently, the usefulness of blood cultures in the emergency room was limited, as the results of identification and antibiotic-susceptibility testing were only available 72 hours after sampling[105]. Under these conditions, either the empirically prescribed antibiotic treatment was effective, or it had to be changed to another treatment, the worst-case scenario being the patient's death before the diagnosis was established. Clearly, CMLs can only have a major impact on early patient management when diagnostic speed enables the appropriate medical decisions to be made rapidly. In terms of treatment, rapid pathogen identification combined with knowledge about where the patient contracted the infection (for example, whether it was hospital acquired or community acquired) enables the presumptive deduction of antimicrobial susceptibility, and such antibiotic stewardship based on rapid diagnostics can reduce hospitalization costs[106]. Another crucial challenge for clinical microbiology, at a time when the need for cost reduction favours laboratory concentration, is to avoid a disconnection between core CMLs and clinical settings. Preserving permanent interactions between clinical microbiologists and primary care physicians ensures timely reporting, proper result interpretation and optimal therapy management.

In the case of outbreaks, CMLs play an important part in warning the medical authorities, which then can corroborate the results across a particular region, country or continent. These laboratories can detect the emergence of unknown species, particular pathotypes and antibiotic resistance, and thus should detect the development of outbreaks rapidly. Early information sharing is mandatory for outbreak control, as demonstrated by a global outbreak of cotrimoxazole-resistant *E. coli*, the source of which remains mysterious, although links with poultry have been proposed[107]. CMLs also have a role in syndromic surveillance (that is, monitoring the type of specimens and the associated syndromes) and alerting, which can lead to the discovery of unexplained illnesses. As an example, an increase in the number of cerebrospinal fluid samples referred to the laboratory in the absence of an aetiologically labelled outbreak of meningitis should alert laboratory technicians and prompt the search for a new pathogen. Likewise, the emergence of an atypical organism must be reported through warning systems such as ProMED-mail or EuroTravNet, but probably also at the national and European levels. To date, Europe lacks such a centrally organized warning system.

In addition, large laboratories should act as strain repositories. Although European, American and Japanese reference strain collections exist, the collection of pathogenic microorganisms detected in CMLs should be extended to conserve clinical strains of interest. Moreover, large CMLs have an educational role and should offer training and up-to-date courses on currently used and evolving technologies. Finally, as CMLs identify and characterize microbial pathogens, sequence genomes and are involved in scientific discoveries in general, they can also have an important impact on the scientific literature.

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**Competing interests statement**
The authors declare no competing financial interests.

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