Diagnostic tools for bacterial infections in travellers: Current and future options

Sophie Alexandra Baron, Carole Eldin, Frederique Gouriet, Gregory Dubourg

Diagnostic tools for bacterial infections in travellers: current and future options

Sophie BARON[1,2], Carole ELDIN[2,3], Frédérique GOURIET[1,2], Grégory DUBOURG[1,2#]

1. Aix Marseille Université, IRD, Assistance Publique Hôpitaux de Marseille (APHM), Microbes, Evolution, Phylogeny and Infection (MEPHI), Marseille, France
2. IHU Méditerranée Infection, Marseille, France
3. Aix Marseille Univ, IRD, AP-HM, SSA, Vecteurs, Infections Tropicales et Méditerranéennes (VITROME), IHU-Méditerranée Infection, Marseille, France

#To whom correspondence should be addressed.

Dr Grégory DUBOURG

IHU Méditerranée Infection
19-21 boulevard Jean Moulin
13385 Marseille cedex 5
France
Tel: +33 (0)4 91 32 43 75
Fax: +33 (0)4 91 38 77 72

Text word count: 4,458 words

Abstract: 170 words

Keywords: infectious diseases; travellers; bacteria; PCR; NGS; Point-of-Care

Running title: Diagnosis of bacterial infections in travellers

ABSTRACT

International travel has increased dramatically over the past 50 years, and travel destinations have diversified. Although physicians are more familiar with the panel of aetiological agents responsible for illnesses of returning travellers, thanks to regular epidemiological studies, the spectrum of pathogens potentially encountered in various travel destinations is nevertheless increasing. In addition, the wide array of approaches currently available and addressed in this paper could render the procedures for microbiological analyses increasingly complex. As the time to result is crucial to adequately manage patients, modern approaches have been developed to shorten diagnosis delays. The syndromic approach, which consists of simultaneously testing a wide panel of microorganisms, substantially increases the diagnostic yield with significant time savings, particularly when coupled with point-of-care laboratories. The tools commonly used for this purpose are immunochromatographic tests, mainly targeting bacterial antigens, and multiplex real-time PCR. The emergence of next-generation sequencing technologies, which enable random amplification of genetic material of any microbe present in a clinical specimen, provides further exciting perspectives in the diagnosis of infectious diseases.

Keywords: infectious diseases; travellers; bacteria; diagnosis; PCR; NGS; Point-of-Care

Introduction

Over the past 50 years, international travel has dramatically increased, and more than one billion travellers were recorded worldwide in 2012 (1). It is expected that this number will reach 1.8 billion in 2030 (2). Currently, Europe has the highest number of international arrivals and remains the preferred destination of travellers (3). There is, however, a growing interest in travelling to other continents, such as Asia, the Americas, and Africa (2). Indeed, the spectrum of pathogens potentially encountered in these areas, which include tropical and low-income countries, differs considerably from those usually diagnosed in the tourists' countries of origin. Physicians are therefore more aware of the panel of aetiological agents responsible for illnesses of returning travellers according to the visited areas thanks to epidemiological studies regularly conducted (4–7). The procedures for microbiological analyses for diagnosis can, however, be complex. Indeed, a wide array of methods are currently available for this purpose; the performances of these methods are heterogeneous and can even differ depending on the aetiological agent.

Herein, we propose to review the different techniques commonly used to identify bacterial pathogens from clinical specimens underlining their weaknesses and strengths. We then detail, according to specific syndromes, the appropriate specimens for sampling and accurate methods to diagnose the specific pathogens involved. We finally provide the prospects and perspectives concerning the techniques of tomorrow, especially next-generation sequencing (NGS) approaches.

Tools for the diagnosis of bacterial infections: general considerations

Direct Diagnosis

Microscopic observation

The main advantages of microscopic observation are fast results and cost-effectiveness, rendering the technique suitable for low-budget laboratories, in particular in low-income

countries despite the laborious process. When this method is combined with Gram straining, a sensitivity of 10^5 CFU/mL(8) can be reached, which has been demonstrated to be useful in the diagnosis of urinary tract infections, pneumonia, and bacteremia (9). Combined with Ziehl Neelsen or auramine staining, direct examination adds real value for detecting mycobacteria, such as tuberculosis or leprae, as their definite diagnosis can take up to several weeks. Finally, relapsing fever could be diagnosed from blood by Giemsa staining or various microscopic approaches (10,11).

Culture

As culture is a sensitive, cost-effective and open approach, its application is essential to diagnose bacterial infections. The majority of agents can be easily grown on agar plates (i.e., axenic culture) within 2 days, while culture of enteric pathogens can require 5 days. Culture of *Legionella* spp. or *Mycobacterium tuberculosis* require specific culture approaches and thus have to be specifically mentioned. Finally, culture of facultative- or obligate-intracellular bacteria (i.e., *Bartonella* spp. *Coxiella burnetii*, *Rickettsia* spp., *Chlamydia* spp., *Borrelia* spp.) is not usually a first-line diagnostic test, as cell culture methods are labour intensive and some require a biosafety level-3 (BSL-3) laboratory due to high infectivity. For an extensive review concerning the culture of fastidious microbes, see (12).

Identification of grown colonies is currently widely performed using matrix assisted laser desorption ionisation-time of flight mass spectrometry (MALDI-TOF MS). This method has been used for the past decade and relies on the recognition of protein profiles compared to a database that can be regularly updated (13). The method is reliable, cost-effective, fast (i.e., few minutes) and versatile, up to the point that it is successfully used to identify bacteria directly from clinical specimens, arthropod vectors or for the vector themselves. When identification fails, molecular approaches are commonly used to achieve

final identification (see below). Alternatively, biochemical profiles are commonly used to identify grown bacterial species (14).

Molecular approaches

Molecular approaches rely on the detection of nucleic acids belonging to bacteria. An amplification step is required to render the DNA detectable through polymerase chain reaction (PCR). The universal approach (i.e., targeting the 16S rRNA gene) has gradually been replaced by a specific approach in which a region present exclusively in the pathogen of interest is targeted. This approach is currently the most widely performed as it displays a high analytical sensitivity (15), can be applied in polymicrobial specimens (i.e., stools, sputum) and offers a very short time to result (i.e., from 1 hour to several hours). Molecular methods are particularly useful for detecting bacteria for which culture approaches are too long or fastidious (i.e., Mycobacteria, intracellular bacteria) or when antibiotic therapy has been already administered.

Antigen detection

To reduce the time to diagnose infections, a wide array of tests have been developed that detect bacterial antigens based on an antigen-antibody reaction. These tests usually require a minimal hands-on time with a time to result that does not exceed 30 min and are very easy to interpret; therefore, they are commonly performed in point-of-care laboratories (POCLs) (16) in particular in low income countries (17). These tests display, however, lower sensitivity than the gold standard methods but take advantage of their high specificity.

Serological testing

Detection of antibodies from patient sera is particularly useful when direct diagnosis of bacteria is difficult, mainly intracellular bacteria. Serology could, however, lack sensitivity, and it requires the collection of paired samples to highlight serological conversion.

Principle of the syndromic approach to diagnose infectious diseases

While empirical therapy could fail, rapid pathogen identification enables the adequate management of patients suffering infectious diseases. The syndromic approach, which consists of simultaneously testing a large panel of microorganisms, represents a significant time saving strategy. Originally, syndrome- and disease-based diagnostic kits were designed for this purpose by optimizing the number of specimens to be sampled (18–20). The abovementioned kit consists of empty vials with pre-filled prescription forms. This strategy i) ensures that all the relevant pathogens will be tested according to each syndrome, ii) avoids resampling or retesting and iii) more importantly, reduces time to result by including rapid tests in particular in point-of-care laboratories (POCLs). Notably, our laboratory has designed a specific kit for the diagnosis of traveller's fever (unpublished data), and syndromic approaches are currently used to diagnose traveller's diarrhoea (21). (22). We propose to review in the following paragraphs the current methods to identify aetiological bacterial agents according to each syndrome.

Diarrhoea

Traveller's diarrhoea is the most common health problem of international travellers, representing 20-60% depending on the destination (1,23,24). The infectious aetiology of diarrhoea may be unknown in 40-50% of cases.

Bacterial traveller's diarrhoea is mainly represented by *Campylobacter, Escherichia coli, Salmonella, Shigella,* and *Aeromonas* spp. (21). Emerging enteropathogens, such as *Arcobacter* spp. (25), *Laribacter* spp. (26) and enterotoxigenic *Bacteroides fragilis* (27) were newly described in travel-related gastroenteritis. *Tropheryma whipplei* was recently suggested as a potential cause of diarrhoea in travellers (28). Indeed, treating travellers with antibiotics is not unusual, and the involvement of *Clostridium difficile* (29) or *Klebsiella oxytoca* might not be rare (30). The presumption of *V. cholerae* diarrhoea (i.e., return from endemic area, rice-

water diarrhoea) should be mentioned because its research is not usually performed in the laboratory because it requires specific culture media.

Rectal swabs could be used, but they are generally less sensitive than fresh stool for culture (30) and must be sampled during the acute phase of the disease. Gram staining is not useful with the exception of campylobacteriosis (30). Routine faecal specimens are inoculated on agar plates using a battery of chromogenic or selective medium, and suspicious colonies are subjected to MALDI-TOF MS analysis. The latter, however, is unable to accurately distinguish *Salmonella* serotypes or to distinguish *Shigella* spp. from *E. coli* (31), thus requiring additional tests such as latex agglutinations or biochemical testing. Serogrouping and serotyping using antibodies is also required for *Vibrio cholerae* isolates (32). As culture is usually long, antigen detection tests enable the early diagnosis of diarrhoeal aetiological agents, such as toxigenic *C. difficile* (33), *Campylobacter* spp. (34), *Vibrio cholerae* (35,36) or the detection of shigatoxin production (30). Regarding *T. whipplei*, classic Whipple's disease could be considered the cause of arthralgia and diarrhoea in Caucasian men, for whom positive PCR in stool and saliva are highly predictive. However, due to the significant faecal carriage of this bacterium, the definite diagnosis is established by histological examination of small bowel biopsies. PCR on urine specimens represents a promising alternative to increase the specificity of molecular methods.

Substantial improvements have recently been made to the multiplexing of molecular tests for which panels targeting the most important enteric bacterial pathogens directly from faecal samples have been designed (37). There are valuable tools for the identification of microbiologic agents from the stool of patients with traveller's diarrhoea (21), thus allowing early diagnosis (37) and detection of co-infections (38). Syndromic panels, such as Biofire's FilmArray Gastrointestinal (GI) Panel, allow the simultaneous identification of 22 different enteric pathogens, including bacteria parasites and viruses, directly from stool specimens in

less than 1 hour (39), and other multiplex molecular assays are available (37). Metagenomic sequencing has also been evaluated to identify gastro-intestinal pathogens. While agreement with multiplex PCR is often high (40), the ability of multiplex PCR to find pathogens among conventionally negative specimens could be valuable in second line diagnosis (41). Moreover, multiplex PCR enables the detection of virulence genes and emerging pathogens and provides an opportunity for typing bacteria such as *C. difficile* or *E. coli* (41). These tests are expensive and should be reserved for patients with severe clinical presentation or for difficult cases. In addition, positive results should be carefully interpreted according to symptoms and epidemiological data (age, season, country), as coinfections are frequently detected (42) and faecal carriage could lead to erroneous diagnosis.

Cutaneous infections

Skin or soft tissue infections after superficial wounds are the most frequent dermatologic disease in travellers returning from tropical areas (43). Pyogenic bacteria are recovered by standard culture followed by identification by MALDI-TOF MS from skin biopsies, blood samples or swabs, while detection of production of Panton-Valentine leucocidin (PVL) by PCR in *S. aureus* isolates can be further performed (44).

Tick-borne diseases are frequently reported in returning travellers (45–47). Spotted fever group rickettsioses are strongly associated with arthropods (i.e., ticks or fleas, lice or mites) (48), and thus, the notion of bite revealed by the anamnesis is crucial to direct microbiological procedures. Because vectors are sometimes kept by the patient, their identification could help for the diagnosis, which could be performed by PCR or more recently by MALDI-TOF MS in record time without entomology expertise (49). The detection of pathogens in vectors as revealed by PCR or more recently by MALDI-TOF MS (50) should be interpreted with caution due to the high prevalence of several pathogens, notably in ticks (51,52). Definite diagnosis can be established from clinical specimens. Serology and targeted PCR on skin

biopsies or eschar swabs (53) represent the cornerstone of the diagnosis of rickettsiosis. Diagnosis of cutaneous infections involving Mycobacteria relies on the direct examination of skin biopsies for identifying acid-fast staining bacilli and cultures (54). Molecular tests could be helpful in particular in the case of uncultured *Mycobacterium leprae,* for which assays are compatible with point of care testing (55), which could be used either in endemic areas or in countries of returning travellers as imported leprosy is not infrequent (56,57).

When travelling to temperate areas, localized Lyme disease (*Erythema migrans*) can be observed after a tick bite. The diagnosis is mainly based on clinical history and presentation, as the serology is usually negative at this stage. However, the diagnostic can be performed by a specific PCR targeting *Borrelia burgdorferi* on a cutaneous biopsy, for which performances are poorly known (10).

Respiratory infections

Respiratory infections account for approximately 10% of traveller's infections (43,58). They are one of the most common causes of hospitalization, especially among travellers (59,60), and mortality due to travels is evaluated at 1%. If viruses are main aetiological agents, the bacteria commonly found in community-acquired pneumonia can also be involved (43) **(Table 1)**, while some more "exotic" bacteria are rarely encountered, such as *Burkholderia pseudomallei*, which can be encountered in Southern Asia in patients suffering from melioidosis (61).

Direct examination

Direct staining with fluorescent antibodies allows the detection of *Legionella* (62), while light microscopy or immunofluorescence microscopy can be used to detect *B. pseudomallei* but lacks sensitivity (61) (<50%). Indeed, direct examination is currently only relevant for the detection of *Mycobacterium tuberculosis* (MTB), for which Auramine O (more rapid) or

Ziehl-Neelsen (reference method) staining is used to rapidly identify a bacilliferous patient (63). This technique is specific but lacks sensitivity (50-60%) and requires repeated experiments on several samples. Its role in extra-pulmonary tuberculosis is limited because its sensitivity is lower than on pulmonary samples. However, direct examination is utilized if the laboratory does not have access to molecular tools (64,65). *Nocardia spp.* and other non-tuberculosis bacteria can be positively identified with direct examination, and a combination of direct examination with fluorescence in situ hybridization (FISH) using a specific probe was recently proposed to fasten the identification of MTB with enhanced specificity (66).

Culture

Sputum samples have been shown to be sufficient to recover the vast majority of easily cultivable bacteria responsible for respiratory infections by culture (67). As *Legionella* species require specific media for growing, the specific culture should be specified to the laboratory. Culture is still considered the gold standard because all the *Legionella spp.* can be detected, but it is fastidious and lacks sensitivity (62,68). Research for MTB must be specifically mentioned because this bacterium belongs to risk group 3 of pathogens and is therefore carried out in NSB3 laboratories. Culture is highly sensitive and specific but requires a long incubation period of up to 30 days (12), although recent advances have reduced the time required to obtain colonies (69). The development of colony imaging could improve the delay for solid culture (12,69). Blood culture is useful in cases of fever, particularly for invasive pneumococcal disease (IPD) (70); it is the gold standard for the recovery of *B. pseudomallei*, and other specimens, such as urine, rectal swabs could be sampled and repeated to increase the culture sensitivity (61).

Most of the cultured microorganisms can be identified using MALDI-TOF MS. However, this could fail to discriminate some species closely related to Mycobacteria (71) or to distinguish

pneumococci from oral streptococci (72), thus requiring other methods (i.e., molecular tests or phenotypic reactions, respectively). In a similar fashion, *B. pseudomallei* can be confused with *Burkholderia thailandensis* (73,74). Molecular methods may therefore be necessary for final identification, for which the *groEL* gene has been suggested as more discriminating than the 16S gene.

Molecular tests

A wide array of PCR systems is available to detect bacterial pathogens from respiratory samples. Various PCRs targeting different genes have been developed to detect *Haemophilus influenzae* (75) or *Streptococcus pneumoniae* (76) from various respiratory specimens (77). However, a positive result must be interpreted with caution because these are commensals of upper airways. Several targets exist for pneumococcus, and the *lytA* and *psaA* genes were shown to be more specific than the pneumolysin gene (*plyN*) (70). Quantitative PCR could be used to predict invasive pneumococcal diseases in influenza patients (78).

Legionella PCR is highly sensitive (97%) and specific (>98%) in respiratory samples (79) and can also detect all the serotypes (80). Concerning MTB, only the Xpert MTB/RIF assay is sensitive enough to be recommended by the WHO. The Xpert MTB/RIF assay involves a closed PCR system that directly extracts and PCR-amplifies a sample or an isolate. This system allows the diagnosis and detection of rifampicin resistance in a few hours and requires little handling. Other specific PCR methods to detect resistance to anti-tuberculosis drugs have also been commercialized (71). Other systems have been designed to detect the DNA of intracellular bacteria, such as *Bordetella*, *Chlamydophila* and *Chlamydia* species (81). Multiple systems for the diagnosis of melioidosis from clinical specimens exist, and the results can vary even if the T3SS gene cluster is targeted and seems to be the most interesting candidate. *T. whipplei* pneumonia is an emerging entity that could be diagnosed by PCR on

broncho-alveolar lavage specimens (82). Finally, as a part of the syndromic approach of the diagnosis of respiratory infections, multiplex PCR systems or DNA microarrays have been designed (83). Of these, particular attention is currently given to "sample to answer" systems that require very little handling. Extraction, DNA amplification and interpretation of the result is carried out in a closed system, with a time to result that does not exceed two hours and is compatible with POCLs (84).

Serological testing

Serological testing is gradually less employed to diagnose pulmonary bacterial infections, with the exception of *Coxiella burnetii*. For the latter, a risk factor analysis could be performed to identify patients with Q fever pneumonia (85). Serology is not useful for the diagnosis of Legionnaire's disease or diseases involving intracellular bacteria but could be useful as a screening tool for epidemiological studies (62,86–88) while the diagnosis of melioidosis could be difficult to interpret, especially in endemic countries where the prevalence of positive infection can be high (61).

Rapid tests

Two reliable rapid urine tests are widely used to fasten the detection of pneumococcal antigens from urine (70) and have good sensitivity (77-88%) and specificity (67-100%); however, these tests remain positive several weeks after the infection treatment, cross-react with some closely related streptococci and are not specific enough to be used for children (76). It is recommended to combine this antigen detection tool with another method to confirm the diagnosis of pneumococcal disease. The detection of *Legionella* antigen in urine is rapid, sensitive (96%) and highly specific (>95%), but only serotype 1 of *L. pneumophila* can be detected, and this serotype persists after the onset of the disease (80). Rapid tests detecting several *Legionella* species (including *L. longbeachae*) or combined detection of *S.*

pneumoniae and *L. pneumophila* are also currently available with a high sensitivity (89). An immunochromatographic test has also been developed to detect antibodies against melioidosis that could be used for rapid screening despite modest sensitivity to the culture (90).

Next-Generation Sequencing

The increased number of *S. pneumoniae* genomes available has made it possible, through the analysis of its pan-genome, to design new and very specific PCR targets (91). For MTB, direct genome sequencing allows accurate identification and profiling of antibiotic resistance (92), but bioinformatics tools and the resistance mutation database need to be improved. More importantly, these molecular approaches enable the detection of pathogens in situ. Since respiratory specimens are frequently contaminated with resident flora, quantitative analysis could distinguish infection from colonization. A preliminary study showed that metagenomics can detect viruses but also relevant bacteria using the Illumina sequencing approach (93). Although the time to result of this method is incompatible with emergency diagnostics, several studies have succeeded in detecting the aetiological agent of respiratory infections such as *P. aeruginosa, S. aureus* or *H. influenzae* (94) using nanopore sequencing. Finally, a study reported that the use of NGS technology in the context of severe pneumonia is associated with a reduction in mortality (95).

Meningitis

Meningeal syndrome in travellers returning from the sub-Saharan meningitis belt or after the Hajj pilgrimage is strongly associated with meningococcus. Basically, gram staining performed on cerebrospinal fluid (CSF) could provide a rapid response if the bacterial load is high, indicating characteristic gram-negative diplococci. More reliable rapid tests are

available, in particular as a part of POCLs. Antigen detection is simple and fast and makes it possible to distinguish serogroups; however, according to the studies, analytical performance may vary, particularly with regard to sensitivity (96). Real-time PCR is the most reliable tool to confirm or exclude meningococcal disease with a diagnosis time of a few hours, as it could be performed in POCLs (97). Targeting two genes is recommended (frequently *CRGA* and *CTRA* genes) because cross reactivity with other species exists (98). Standard culture could also be performed but is not compatible with emergency diagnosis. False negative could also occur as meningococcus is a fragile microbe (15). The same strategy is applicable to the diagnosis of pneumococcal meningitis. While specific antigen detection tests from CSF are available to detect *S. pneumoniae* (99), those commonly used for urine samples could also be used (100). Nevertheless, these antigenic tests remain less sensitive than real-time PCR. Real-time PCR could be multiplexed, such as in the BioFire FilmArray Meningitis/Encephalitis Panel, allowing the detection of 14 pathogens including 6 bacteria in approximately one hour (101), although some false-positive and false-negative results have been reported. Finally, NGS applied to CSF has shown promising results, such as the detection of uncultured *M. tuberculosis* or *Listeria monocytogenes,* indicating that these tools could be used in the second line if the first microbiological investigations are negative (102).

Febrile illness

Fever should in any case encourage blood cultures (BC) as they could help to quickly recover the usual bacterial agents responsible for invasive infections. Importantly, BC currently remains the most reliable approach to diagnosing typhoid fever. However, the identification could be challenging because MALDI-TOF MS does not distinguish serovars among *Salmonella* isolates, even if sophisticated approaches have been proposed for this purpose (103). The use of conventional PCR followed by sequencing nevertheless showed that BC may have under-diagnosed typhoid fever. (104). Despite the development of several molecular

tools to identify Typhi serovar directly from blood specimens (105,106), there is currently no simple marketed molecular test available. Antibody detection is an alternative, but Widal's performance is low (107), and several rapid diagnostic kits have been developed to quickly detect IgM antibodies. Some of these display fairly good analytical performances and could be used in POCLs (108).

In travellers returning from Southeast Asia, murine typhus caused by *R. typhi* (transmitted by rat fleas) and scrub typhus caused by *Orientia tsutsugamushi* (transmitted by mites) are frequent causes of fever (43). Since inoculation eschar is not constant, the diagnosis first relies on serology. Serum samples were analysed using the indirect immunofluorescence assay (IFA) against typhus group and *O. tsutsugamushi* antigens (109). In scrub typhus, when an eschar is present, real-time quantitative PCR (qPCR) specific for the periplasmic serine protease gene of *O. tsutsugamushi* can be performed (110) on a swab eschar sample.

The diagnosis of leptospirosis represents a conundrum for microbiologists as its culture requires laborious processing. Direct diagnosis currently relies on PCR detection, which can be performed on either blood, serum, urine or cerebrospinal fluid (CSF). Greater sensitivity has been suggested when molecular amplification is performed from serum (111), while DNA is detectable longer from urines (112). Housekeeping genes are usually targeted to detect all *Leptospira* species, while specific PCR allows species identification (113). The performance of PCR on blood and urines is highly variable and depends on the timing of the test (i.e., higher sensitivity in very early samples). The main advantage of molecular detection is that DNA is detectable during the early acute phase of infection. Alternatively, IgM antibodies can be detected in a few hours when ELISA assays are performed, for which a wide variety of kits are currently available. Interestingly, NGS performed on CSF has recently led to the diagnosis of a case of neuroleptospirosis (114).

Relapsing fevers caused by *Borrelia duttonii*, *Borrelia crocidurae, Borrelia recurrentis, Borrelia persica* and *Borrelia hispanica* are mainly observed in tropical and subtropical regions and are transmitted by soft ticks, or lice for *B. recurrentis* (10). These infections can lead to massive bacteraemia, so direct detection methods on blood samples are possible (10). The traditional method of detection is optical microscopy after Giemsa staining of blood smears or thick blood drops. Specific quantitative PCR is now available, allowing a better sensitivity of detection on blood samples (10). Specific serological tests for the causative agents of relapsing fever have been developed, but they can be negative during the first episodes of fever and are most used as a retrospective diagnostic tool.

Acute Q fever is a clinical entity seen in travellers infected by *C. burnetii* (48,115). Serological tests remain the easiest and most common method to detect the presence of IgM against Phase I and Phase II antigens(116), although cross reactions may occur. The PCR on serum in the acute phase could be valuable for diagnosis. Further tests are useful for the prevention of persistent endocarditis (i.e., aCL, TTE) or to diagnose acute Q fever endocarditis (117). Hot prospects currently concern the use of fluorescence in situ hybridization (FISH) to diagnose persistent infections (118).

MDR bacteria carriage

Multidrug resistant (MDR) bacteria carriage is a risk to be considered in any patient who has travelled to countries where the prevalence of resistance is high (119) (i.e., Asia and Northern Africa) (120). Hospitalization, the use of antibiotics during travel and gastro-intestinal disorders are associated with a higher carriage rate, while refugee patients could constitute a substantial reservoir of antibiotic resistance (121). There is no consensus, but the multidrug-resistant bacteria usually screened include methicillin-resistant *Staphylococcus aureus,* plasmid-mediated cephalosporin-resistant *Enterobacteriaceae*, vancomycin-resistant Enterococci, carbapenemase-producing *Acinetobacter baumannii* and *Enterobacteriaceae.*

The specimen to be sampled includes a rectal swab for *Enterobacteriaceae* and *Enterococci*, while a nasal swab is usually performed to detect the presence of methicillin-resistant *S. aureus* (MRSA). A large number of selective chromogenic media for the detection of MDR bacteria have been developed, requiring growth time (i.e., 24-48 hours) with a good negative predictive value but a lower positive predictive value. Additional testing is required to confirm the presence of MDR bacteria, including identification, antibiotic susceptibility tests (ASTs) and minimum inhibitory concentration (MIC) determination by the gradient strip test or microdilution method. As conventional complementary techniques can delay response time, several rapid phenotypic tests have been designed to identify the resistance mechanism involved, such as ESBL, carbapenemase-producers and MRSA (122–125). Resistance genes coding for carbapenemases, methicillin-resistance or vancomycin-resistant genes could be targeted by real-time PCR, in particular in a multiplexed fashion with excellent analytical performances (126). Closed systems also exist, thereby enabling a time to result of one hour without prior extraction. Because the detection could be performed directly from clinical specimens, the time saved is particularly valuable for managing outbreaks of multi-resistant bacteria. These PCRs are, however, limited to what is currently known, while NGS technology allows deciphering the entire resistome of a bacterium by sequencing its whole genome. While Illumina technology (Illumina, USA) is limited for studying whole plasmids due to the presence of transposons, Nanopore technology (Oxford Nanopore, Oxford, UK) allows the sequencing of long DNA fragments, thereby enabling the reconstitution of an entire plasmid (127). The determination of the genetic environment and other resistance genes carried by the same plasmid enables real-time monitoring of outbreaks in a clinical setting by comparing the plasmids found in patients to understand the transmission of these genes. It also allows new variants to be identified that could be negative using the usual targeted PCR. However, this approach remains costly and requires further development.

Conclusions

While a wide array of technologies is currently available to diagnose infectious diseases, intense efforts are dedicated to shortening the time from sampling to result. Multiplexed molecular tests, rapid assays and integration in POCLs are the current directions for this purpose, while further developments are needed to routinely use NGS technologies.

BOX: Key messages and novel diagnostic approaches.

- The spectrum aetiological agents responsible for illnesses of returning travellers is increasing.
- The clinical examination remains crucial to adequately guide microbiological investigations.
- The syndromic approach that consists of systematically testing from the start a wide array of microorganisms increases the diagnosis yield.
- The routine microbiological examination remains essential for the first line diagnostics in particular in low-income countries.
- Point-of-care laboratories (POCLs) enable a significant time-saving for the diagnosis by using rapid tests
- Next-generation Sequencing (NGS) approaches are promising but are currently limited to the field of research and in specialized laboratories.

FIGURE AND TABLE LEGENDS

Figure 1. Overview of the syndromic approach for the diagnosis of infectious diseases, from the specimen to the laboratory. BC, blood cultures; CSF, cerebrospinal fluid; ELISA, enzyme-linked immunosorbent assay; FISH, fluorescence in situ hybridization; IIF, indirect immunofluorescence; NGS, next-generation sequencing technologies.

Table 1. Main aetiological agents responsible for each syndrome and key tests commonly used in routine laboratories for their detection. The tests are sorted (from the left to the right) in order of increasing time to result.

Table 2. Main aetiological agents responsible for each syndrome and modern key tests available for their detection. The tests are sorted (from the left to the right) in order of increasing time to result.

FUNDING SOURCES

Funding Sources: This work has benefited from French State support, managed by the “Agence Nationale pour la Recherche”, including the "Programme d’Investissement d’avenir" under the reference Méditerranée Infection 10-IAHU-03.

Conflict of interest: none to declare.

Table 1.

Aetiological agent	Key tests		Remarks
Respiratory infections			
Streptococcus pneumoniae	Antigen detection (urine)	Culture (sputum, blood culture)	
Haemophilus influenzae	Standard culture (sputum, blood culture)		
Staphylococcus aureus	Standard culture (sputum, blood culture)		
Mycobacterium tuberculosis	Direct examination / Ziehl Neelsen (sputum)	Targeted culture (sputum)	Cultured should be carried out in BLS-3 laboratories
Legionella pneumophila	Antigen detection (urine)	Targeted culture (sputum)	
Burkholderia pseudomallei	Standard culture (sputum, blood culture)		
Bordetella pertussis	Serological testing	Targeted culture	poor performance of serological testing during acute phase, culture is laborious
Chlamydophila pneumoniae	Serological testing		poor performance during acute phase
Mycoplasma pneumoniae	Serological testing		poor performance during acute phase
Cutaneous infections			
Treponema pallidum	Serological testing		Dark field microscopy is gradually less employed
Spotted fever group *Rickettsiae*	Serological testing		
Staphylococcus aureus	Standard culture (skin biopsy/swab)		
Streptococcus pyogenes	Standard culture (skin biopsy/swab)		
Mycobacterium leprae	Direct examination / Ziehl Neelsen (skin biopsy)		

Borrelia burgdorferi sensu lato	Serological testing (ELISA + western blot)		Dark field microscopy is gradually abandoned
Diarrhea			
Aeromonas spp.	Targeted culture (stool) and biochemical identification		
Arcobacter spp.			undistinguable using biochemical identification
Clostridium difficile	Toxin detection (stool)		The toxin is targeted by immunochromatographic tests
Campylobacter spp.	Gram staining	Targeted culture (stool) and biochemical identification	
Escherichia coli (EHEC, ETEC)	Targeted culture (stool) and biochemical identification		
Laribacter spp.			undistinguable using biochemical identification
Salmonella spp. (non-typhoid)	Targeted culture (stool) and biochemical identification		
Shigella spp.	Targeted culture (stool) and biochemical identification		
Tropheryma whipplei	Microscopy following PAS and IHC staining (small bowel biopsies)		Microscopy confirms the diagnosis
Febrile illness			
Typhoid fever	Blood cultures	Serological testing (Widal)	
Rickettsia typhi, Orientia tsutsugamuchi	Serological testing		
Leptospira spp.	Serological testing		
Coxiella burnetii	Serological testing		
Brucella spp.	Serological testing	Targeted culture (blood cultures)	Cultured should be carried out in BLS-3 laboratories
Relapsing fever *Borreliae*	Microscopy (GIEMSA coloration)	Quantitative Buffy Coat	
Meningitis			

Streptococcus pneumoniae	Microscopy (gram staining)	Standard culture (CSF, blood culture)	
Neisseiria meningitidis	Microscopy (gram staining)	Standard culture (CSF, blood culture)	

BLS : Biosafety level; CSF : cerebrospinal fluid; FISH: Fluorescence in situ hybridization; PAS: Periodic acid-Schiff; POCLs: Point-of-Care Laboratories; PVL : Panton-Valentine Leucokidin

16 **Table 2.**

Aetiological agent	Key tests		Remarks
Respiratory infections			
Streptococcus pneumoniae	Quantitative PCR (sputum)		Quantitative PCR could be used to assess risk of pneumonia during flu
Haemophilus influenzae	Quantitative PCR (sputum)		
Staphylococcus aureus	PCR (sputum)		Toxin detection (i.e. PVL) may be performed in case of necrotizing pneumonia
Mycobacterium tuberculosis	PCR (sputum)		Genexpert is approved by OMS, highly sensitive and detect rifampicin resistance
Legionella pneumophila	PCR (sputum)		
Burkholderia pseudomallei	PCR (sputum, blood)		Rapid serological tests exist
Bordetella pertussis	PCR (sputum)	Multiplexed PCR (sputum)	Other methods are gradually abandoned
Chlamydophila pneumoniae	PCR (sputum)	Multiplexed PCR (sputum)	Other methods are gradually abandoned
Mycoplasma pneumoniae	PCR (sputum)	Multiplexed PCR (sputum)	Other methods are gradually abandoned
Cutaneous infections			
Treponema pallidum	PCR (skin biopsy)		Dark field microscopy is gradually less employed
Spotted fever group *Rickettsiae*	PCR (skin biopsy or eschar swab)		
Staphylococcus aureus	PCR (skin biopsy/swab)		
Streptococcus pyogenes	PCR (skin biopsy/swab)		
Mycobacterium leprae	PCR (skin biopsy)		

Borrelia burgdorferi sensu lato	PCR (skin biopsy)			Dark field microscopy is gradually abandoned
Diarrhea				
Aeromonas spp.	Multiplexed PCR (stool)	targeted culture (stool) and MALDI-TOF identification		
Arcobacter spp.	targeted culture (stool) and MALDI-TOF identification			
Clostridium difficile	PCR (stool)			Genes coding for toxin production are targeted by PCR assays
Campylobacter spp.	Multiplexed PCR (stool)	targeted culture (stool) and MALDI-TOF identification		
Escherichia coli (EHEC, ETEC)	Multiplexed PCR (stool)	targeted culture (stool) and MALDI-TOF identification	Shiga-toxin detection by immunochromatographic test (stool)	
Klebsiella oxytoca	targeted culture (stool) and MALDI-TOF identification			
Laribacter spp.	targeted culture (stool) and MALDI-TOF identification			
Salmonella spp. (non-typhoid)	Multiplexed PCR (stool)	targeted culture (stool)		
Shigella spp.	Multiplexed PCR (stool)	targeted culture (stool) and MALDI-TOF identification		Difficult to distinguish from *E. coli* using MALDI-TOF
Tropheryma whipplei	PCR (stool, small bowel biopsies)			PCR is used as a screening tool while microscopy confirms the diagnosis
Febrile illness				
Typhoid fever	Rapid Serological tests			PCR may also be used
Rickettsia typhi, Orientia tsutsugamuchi	PCR on eschar swab if present (scrub typhus)			
Leptospira spp.	PCR (urine, blood, CSF)			

Coxiella burnetii	PCR (serum, blood or other specimen if persistent infection)	FISH may be used for persistent infections
Brucella spp.	PCR (lymph node, blood)	
Relapsing fever *Borreliae*	PCR (blood)	
Meningitis		
Streptococcus pneumoniae	PCR (CSF)	
Neisseiria meningitidis	PCR (CSF)	Serotyping may be performed by PCR

BLS : Biosafety level; CSF : cerebrospinal fluid; FISH: Fluorescence in situ hybridization; PAS: Periodic acid-Schiff; POCLs: Point-of-Care Laboratories; PVL : Panton-Valentine Leucokidin

17

18

19

20

21

22

23

Aetiological agent	**Key tests**		
Respiratory infections			
Streptococcus pneumoniae	Antigen detection (urine)	Quantitative PCR (sputum)	Culture (sputum, blood culture)
Haemophilus influenzae	Quantitative PCR (sputum)	Standard culture (sputum, blood culture)	

Staphylococcus aureus	PCR (sputum)	Standard culture (sputum, blood culture)	
Mycobacterium tuberculosis	PCR (sputum)	Direct examination / Ziehl Neelsen (sputum)	Targeted culture (sputum)
Legionella pneumophila	Antigen detection (urine)	PCR (sputum)	Targeted culture (sputum)
Burkholderia pseudomallei	PCR (sputum, blood)	Standard culture (sputum, blood culture)	
Bordetella pertussis	PCR (sputum)		
Chlamydophila pneumoniae	PCR (sputum)		
Mycoplasma pneumoniae	PCR (sputum)		
Cutaneous infections			
Treponema pallidum	PCR (skin biopsy)	Serological testing	
Rickettsia spp.	PCR (skin biopsy)	Serological testing	
Staphylococcus aureus	PCR (skin biopsy/swab)	Standard culture (skin biopsy/swab)	
Streptococcus pyogenes	PCR (skin biopsy/swab)	Standard culture (skin biopsy/swab)	
Mycobacterium leprae	PCR (skin biopsy)	Direct examination / Ziehl Neelsen (skin biopsy)	
Borrelia burgdorferi	PCR (skin biopsy)	Serological testing with western blot	
Diarrhea			
Aeromonas spp.	Multiplexed PCR (stool)	targeted culture (stool)	
Arcobacter spp.	targeted culture (stool)		
Clostridium difficile	antigen detection (stool)	PCR (stool)	
Campylobacter spp.	Gram staining	Multiplexed PCR (stool)	targeted culture (stool)
Escherichia coli (EHEC, ETEC)	Multiplexed PCR (stool)	targeted culture (stool)	
Klebsiella oxytoca	targeted culture (stool)		
Laribacter spp.	targeted culture (stool)		
Salmonella spp. (non-typhoid)	Multiplexed PCR (stool)	targeted culture (stool)	
Shigella spp.	Multiplexed PCR (stool)	targeted culture (stool)	
Tropheryma whipplei	PCR (stool, small bowel biopsies)	Microscopy following PAS and IHC staining (small bowel biopsies)	

Febrile illness			
Typhoid fever	Blood cultures		
Rickettsia typhi, Orientia tsutsugamuchi	Serological testing	PCR if eschar (scrub typhus)	
Leptospira spp.	PCR (urine, blood, CSF)	Serological testing	
Coxiella burnetii	Serological testing	PCR (serum, blood or other specimen if persistent infection)	
Brucella spp.	PCR (lymh node, blood)	Targeted culture (blood cultures)	Serological testing
Borrelia spp.	Microscopy (GIEMSA coloration)	PCR (blood)	Serological testing
Meningitis			
Streptococcus pneumoniae	PCR (CSF)	Microscopy (gram staining)	standard culture (CSF, blood culture)
Neisseiria meningitidis	PCR (CSF)	Microscopy (gram staining)	standard culture (CSF, blood culture)

BLS : Biosafety level; CSF : cerebrospinal fluid; FISH: Fluorescence in situ hybridization; PAS: Periodic acid-Schiff; POCLs: Point-of-Care Laboratories; PVL : Panton-Val

24

REFERENCES

1. Angelo KM, Haulman NJ, Terry AC, Leung DT, Chen LH, Barnett ED, et al. Illness among US resident student travellers after return to the USA: a GeoSentinel analysis, 2007-17. J Travel Med. 1 janv 2018;25(1).

2. UNWTO TO. Tourism Highlights, 2014 edition. World. 2014;

3. Schlagenhauf P, Weld L, Goorhuis A, Gautret P, Weber R, von Sonnenburg F, et al. Travel-associated infection presenting in Europe (2008–12): an analysis of EuroTravNet longitudinal, surveillance data, and evaluation of the effect of the pre-travel consultation. Lancet Infect Dis. 2015;15(1):55‑64.

4. Steffen R, Amitirigala I, Mutsch M. Health risks among travelers--need for regular updates. J Travel Med. juin 2008;15(3):145‑6.

5. Griffiths KM, Savini H, Brouqui P, Simon F, Parola P, Gautret P. Surveillance of travel-associated diseases at two referral centres in Marseille, France: a 12-year survey. J Travel Med. 1 janv 2018;25(1).

6. Gautret P, Angelo KM, Asgeirsson H, Duvignaud A, van Genderen PJJ, Bottieau E, et al. International mass gatherings and travel-associated illness: A GeoSentinel cross-sectional, observational study. Travel Med Infect Dis. 9 nov 2019;101504.

7. Hagmann SHF, Han PV, Stauffer WM, Miller AO, Connor BA, Hale DC, et al. Travel-associated disease among US residents visiting US GeoSentinel clinics after return from international travel. Fam Pract. déc 2014;31(6):678‑87.

8. Wilson ML, Gaido L. Laboratory diagnosis of urinary tract infections in adult patients. Clin Infect Dis Off Publ Infect Dis Soc Am. 15 avr 2004;38(8):1150‑8.

9. Peretz A, Isakovich N, Pastukh N, Koifman A, Glyatman T, Brodsky D. Performance of Gram staining on blood cultures flagged negative by an automated blood culture system. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. août 2015;34(8):1539‑41.

10. Eldin C, Jaulhac B, Mediannikov O, Arzouni J-P, Raoult D. Values of diagnostic tests for the various species of spirochetes. Med Mal Infect. mars 2019;49(2):102‑11.

11. Elbir H, Raoult D, Drancourt M. Relapsing fever borreliae in Africa. Am J Trop Med Hyg. août 2013;89(2):288‑92.

12. Lagier J-C, Edouard S, Pagnier I, Mediannikov O, Drancourt M, Raoult D. Current and past strategies for bacterial culture in clinical microbiology. Clin Microbiol Rev. janv 2015;28(1):208‑36.

13. Seng P, Drancourt M, Gouriet F, La Scola B, Fournier P-E, Rolain JM, et al. Ongoing revolution in bacteriology: routine identification of bacteria by matrix-assisted laser desorption ionization time-of-flight mass spectrometry. Clin Infect Dis Off Publ Infect Dis Soc Am. 15 août 2009;49(4):543‑51.

14. Funke G, Monnet D, deBernardis C, von Graevenitz A, Freney J. Evaluation of the VITEK 2 system for rapid identification of medically relevant gram-negative rods. J Clin Microbiol. 1998;36(7):1948‑52.

15. Morel A-S, Dubourg G, Prudent E, Edouard S, Gouriet F, Casalta J-P, et al. Complementarity between targeted real-time specific PCR and conventional broad-range 16S rDNA PCR in the syndrome-driven diagnosis of infectious diseases. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. mars 2015;34(3):561‑70.

16. Drancourt M, Michel-Lepage A, Boyer S, Raoult D. The Point-of-Care Laboratory in Clinical Microbiology. Clin Microbiol Rev. juill 2016;29(3):429‑47.

17. Sokhna C, Mediannikov O, Fenollar F, Bassene H, Diatta G, Tall A, et al. Point-of-care laboratory of pathogen diagnosis in rural Senegal. PLoS Negl Trop Dis. 2013;7(1):e1999.

18. Fournier P-E, Thuny F, Richet H, Lepidi H, Casalta J-P, Arzouni J-P, et al. Comprehensive diagnostic strategy for blood culture-negative endocarditis: a prospective study of 819 new cases. Clin Infect Dis Off Publ Infect Dis Soc Am. 15 juill 2010;51(2):131‑40.

19. Levy PY, Gouriet F, Habib G, Bonnet JL, Raoult D. Diagnosis of Coxiella burnetii pericarditis by using a systematic prescription kit in cases of pericardial effusion: an 8-year experience. Clin Microbiol Infect Off Publ Eur Soc Clin Microbiol Infect Dis. déc 2009;15 Suppl 2:173‑5.

20. Drancourt M, Berger P, Terrada C, Bodaghi B, Conrath J, Raoult D, et al. High prevalence of fastidious bacteria in 1520 cases of uveitis of unknown etiology. Medicine (Baltimore). mai 2008;87(3):167‑76.

21. Connor BA, Rogova M, Whyte O. Use of a multiplex DNA extraction PCR in the identification of pathogens in travelers' diarrhea. J Travel Med. 1 janv 2018;25(1).

22. Karnad DR, Richards GA, Silva GS, Amin P. Tropical diseases in the ICU: A syndromic approach to diagnosis and treatment. J Crit Care. août 2018;46:119‑26.

23. Warne B, Weld LH, Cramer JP, Field VK, Grobusch MP, Caumes E, et al. Travel-related infection in European travelers, EuroTravNet 2011. J Travel Med. août 2014;21(4):248‑54.

24. Giddings SL, Stevens AM, Leung DT. Traveler's Diarrhea. Med Clin North Am. mars 2016;100(2):317‑30.

25. Barboza K, Cubillo Z, Castro E, Redondo-Solano M, Fernandez-Jaramillo H, Echandi MLA. First isolation report of Arcobacter cryaerophilus from a human diarrhea sample in Costa Rica. Rev Inst Med Trop Sao Paulo. 6 nov 2017;59:e72.

26. Engsbro AL, Nielsen KL, Hornum M, Andersen LP. Laribacter hongkongensis: clinical presentation, epidemiology and treatment. A review of the literature and report of the first case in Denmark. Infect Dis Lond Engl. juin 2018;50(6):417‑22.

27. Jiang Z-D, Dupont HL, Brown EL, Nandy RK, Ramamurthy T, Sinha A, et al. Microbial etiology of travelers' diarrhea in Mexico, Guatemala, and India: importance of enterotoxigenic Bacteroides fragilis and Arcobacter species. J Clin Microbiol. avr 2010;48(4):1417‑9.

28. Gautret P, Benkouiten S, Parola P, Brouqui P, Memish Z, Raoult D. Occurrence of Tropheryma whipplei during diarrhea in Hajj pilgrims: a PCR analysis of paired rectal swabs. Travel Med Infect Dis. oct 2014;12(5):481‑4.

29. Michal Stevens A, Esposito DH, Stoney RJ, Hamer DH, Flores-Figueroa J, Bottieau E, et al. Clostridium difficile infection in returning travellers. J Travel Med. 1 mai 2017;24(3).

30. Humphries RM, Linscott AJ. Laboratory diagnosis of bacterial gastroenteritis. Clin Microbiol Rev. janv 2015;28(1):3‑31.

31. He Y, Li H, Lu X, Stratton CW, Tang Y-W. Mass spectrometry biotyper system identifies enteric bacterial pathogens directly from colonies grown on selective stool culture media. J Clin Microbiol. nov 2010;48(11):3888‑92.

32. Qadri F, Wennerås C, Albert MJ, Hossain J, Mannoor K, Begum YA, et al. Comparison of immune responses in patients infected with Vibrio cholerae O139 and O1. Infect Immun. 1997;65(9):3571‑6.

33. Novak-Weekley SM, Marlowe EM, Miller JM, Cumpio J, Nomura JH, Vance PH, et al. Clostridium difficile testing in the clinical laboratory by use of multiple testing algorithms. J Clin Microbiol. mars 2010;48(3):889‑93.

34. Giltner CL, Saeki S, Bobenchik AM, Humphries RM. Rapid detection of Campylobacter antigen by enzyme immunoassay leads to increased positivity rates. J Clin Microbiol. févr 2013;51(2):618‑20.

35. Yam WC, Lung ML, Ng MH. Evaluation and optimization of a latex agglutination assay for detection of cholera toxin and Escherichia coli heat-labile toxin. J Clin Microbiol. 1992;30(9):2518‑20.

36. Almeida R, Hickman-Brenner F, Sowers E, Puhr N, Farmer J, Wachsmuth I. Comparison of a latex agglutination assay and an enzyme-linked immunosorbent assay for detecting cholera toxin. J Clin Microbiol. 1990;28(1):128‑30.

37. Zhang H, Morrison S, Tang Y-W. Multiplex polymerase chain reaction tests for detection of pathogens associated with gastroenteritis. Clin Lab Med. juin 2015;35(2):461‑86.

38. Huang S-H, Lin Y-F, Tsai M-H, Yang S, Liao M-L, Chao S-W, et al. Detection of common diarrhea-causing pathogens in Northern Taiwan by multiplex polymerase chain reaction. Medicine (Baltimore). juin 2018;97(23):e11006.

39. Buss SN, Leber A, Chapin K, Fey PD, Bankowski MJ, Jones MK, et al. Multicenter evaluation of the BioFire FilmArray gastrointestinal panel for etiologic diagnosis of infectious gastroenteritis. J Clin Microbiol. mars 2015;53(3):915‑25.

40. Schneeberger PHH, Becker SL, Pothier JF, Duffy B, N'Goran EK, Beuret C, et al. Metagenomic diagnostics for the simultaneous detection of multiple pathogens in human stool specimens from Cote d'Ivoire: a proof-of-concept study. Infect Genet Evol J Mol Epidemiol Evol Genet Infect Dis. juin 2016;40:389‑97.

41. Joensen KG, Engsbro ALO, Lukjancenko O, Kaas RS, Lund O, Westh H, et al. Evaluating next-generation sequencing for direct clinical diagnostics in diarrhoeal disease. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. juill 2017;36(7):1325‑38.

42. Lindsay B, Ramamurthy T, Sen Gupta S, Takeda Y, Rajendran K, Nair GB, et al. Diarrheagenic pathogens in polymicrobial infections. Emerg Infect Dis. avr 2011;17(4):606‑11.

43. Leder K, Torresi J, Libman MD, Cramer JP, Castelli F, Schlagenhauf P, et al. GeoSentinel surveillance of illness in returned travelers, 2007-2011. Ann Intern Med. 19 mars 2013;158(6):456‑68.

44. Nurjadi D, Fleck R, Lindner A, Schäfer J, Gertler M, Mueller A, et al. Import of community-associated, methicillin-resistant Staphylococcus aureus to Europe through skin and soft-tissue infection in intercontinental travellers, 2011–2016. Clin Microbiol Infect. 2018;

45. Parola P, Paddock CD. Travel and tick-borne diseases: Lyme disease and beyond. Travel Med Infect Dis. déc 2018;26:1‑2.

46. Rajoelison P, Mediannikov O, Javelle E, Raoult D, Parola P, Aoun O. Rickettsia sibirica mongolitimonae human infection: A diagnostic challenge. Travel Med Infect Dis. déc 2018;26:72‑3.

47. Paris DH, Neumayr A. Ticks and tick-borne infections in Asia: Implications for travellers. Travel Med Infect Dis. déc 2018;26:3‑4.

48. Delord M, Socolovschi C, Parola P. Rickettsioses and Q fever in travelers (2004-2013). Travel Med Infect Dis. oct 2014;12(5):443‑58.

49. Yssouf A, Almeras L, Berenger J-M, Laroche M, Raoult D, Parola P. Identification of tick species and disseminate pathogen using hemolymph by. Ticks Tick-Borne Dis. juill 2015;6(5):579‑86.

50. Yssouf A, Almeras L, Terras J, Socolovschi C, Raoult D, Parola P. Detection of Rickettsia spp in ticks by MALDI-TOF MS. PLoS Negl Trop Dis. févr 2015;9(2):e0003473.

51. Sanogo YO, Zeaiter Z, Caruso G, Merola F, Shpynov S, Brouqui P, et al. Bartonella henselae in Ixodes ricinus ticks (Acari: Ixodida) removed from humans, Belluno province, Italy. Emerg Infect Dis. mars 2003;9(3):329‑32.

52. Lafri I, El Hamzaoui B, Bitam I, Leulmi H, Lalout R, Mediannikov O, et al. Detection of relapsing fever Borrelia spp., Bartonella spp. and Anaplasmataceae bacteria in argasid ticks in Algeria. PLoS Negl Trop Dis. nov 2017;11(11):e0006064.

53. Morand A, Angelakis E, Chaabane MB, Parola P, Raoult D, Gautret P. Seek and Find! PCR analyses of skin infections in West-European travelers returning from abroad with an eschar. Travel Med Infect Dis. 2018;26:32‑6.

54. Franco-Paredes C, Marcos LA, Henao-Martinez AF, Rodriguez-Morales AJ, Villamil-Gomez WE, Gotuzzo E, et al. Cutaneous Mycobacterial Infections. Clin Microbiol Rev. janv 2018;32(1).

55. Tatipally S, Srikantam A, Kasetty S. Polymerase Chain Reaction (PCR) as a Potential Point of Care Laboratory Test for Leprosy Diagnosis-A Systematic Review. Trop Med Infect Dis. 1 oct 2018;3(4).

56. Duthie MS, Balagon MF, Maghanoy A, Orcullo FM, Cang M, Dias RF, et al. Rapid quantitative serological test for detection of infection with Mycobacterium leprae, the causative agent of leprosy. J Clin Microbiol. févr 2014;52(2):613‑9.

57. Aftab H, Nielsen SD, Bygbjerg IC. Leprosy in Denmark 1980-2010: a review of 15 cases. BMC Res Notes. 5 janv 2016;9:10.

58. Leder K, Sundararajan V, Weld L, Pandey P, Brown G, Torresi J. Respiratory tract infections in travelers: a review of the GeoSentinel surveillance network. Clin Infect Dis Off Publ Infect Dis Soc Am. 15 févr 2003;36(4):399‑406.

59. Benkouiten S, Charrel R, Belhouchat K, Drali T, Nougairede A, Salez N, et al. Respiratory viruses and bacteria among pilgrims during the 2013 Hajj. Emerg Infect Dis. 2014;20(11):1821.

60. Memish ZA, Zumla A, Alhakeem RF, Assiri A, Turkestani A, Al Harby KD, et al. Hajj: infectious disease surveillance and control. Lancet Lond Engl. 14 juin 2014;383(9934):2073‑82.

61. Wiersinga WJ, Virk HS, Torres AG, Currie BJ, Peacock SJ, Dance DAB, et al. Melioidosis. Nat Rev Dis Primer. 1 févr 2018;4:17107.

62. Murdoch DR. Diagnosis of Legionella infection. Clin Infect Dis Off Publ Infect Dis Soc Am. 1 janv 2003;36(1):64‑9.

63. Leung CC, Lange C, Zhang Y. Tuberculosis: Current state of knowledge. Respirology. 2013;18(7):1047‑55.

64. Causse M, Ruiz P, Gutierrez-Aroca JB, Casal M. Comparison of two molecular methods for rapid diagnosis of extrapulmonary tuberculosis. J Clin Microbiol. août 2011;49(8):3065‑7.

65. Lawn SD, Zumla AI. Diagnosis of extrapulmonary tuberculosis using the Xpert((R)) MTB/RIF assay. Expert Rev Anti Infect Ther. juin 2012;10(6):631‑5.

66. Loukil A, Kirtania P, Bedotto M, Drancourt M. FISHing Mycobacterium tuberculosis Complex by Use of a rpoB DNA Probe Bait. J Clin Microbiol. oct 2018;56(10).

67. Dubourg G, Abat C, Rolain J-M, Raoult D. Correlation between sputum and bronchoalveolar lavage fluid cultures. J Clin Microbiol. mars 2015;53(3):994‑6.

68. Descours G, Cassier P, Forey F, Ginevra C, Etienne J, Lina G, et al. Evaluation of BMPA, MWY, GVPC and BCYE media for the isolation of Legionella species from respiratory samples. J Microbiol Methods. mars 2014;98:119‑21.

69. Asmar S, Drancourt M. Rapid culture-based diagnosis of pulmonary tuberculosis in developed and developing countries. Front Microbiol. 2015;6:1184.

70. Blaschke AJ. Interpreting assays for the detection of Streptococcus pneumoniae. Clin Infect Dis Off Publ Infect Dis Soc Am. mai 2011;52 Suppl 4:S331-337.

71. Jagielski T, Minias A, van Ingen J, Rastogi N, Brzostek A, Zaczek A, et al. Methodological and Clinical Aspects of the Molecular Epidemiology of Mycobacterium tuberculosis and Other Mycobacteria. Clin Microbiol Rev. avr 2016;29(2):239‑90.

72. Harju I, Lange C, Kostrzewa M, Maier T, Rantakokko-Jalava K, Haanpera M. Improved Differentiation of Streptococcus pneumoniae and Other S. mitis Group Streptococci by MALDI Biotyper Using an Improved MALDI Biotyper Database Content and a Novel Result Interpretation Algorithm. J Clin Microbiol. mars 2017;55(3):914‑22.

73. Lau SKP, Sridhar S, Ho C-C, Chow W-N, Lee K-C, Lam C-W, et al. Laboratory diagnosis of melioidosis: past, present and future. Exp Biol Med Maywood NJ. juin 2015;240(6):742‑51.

74. Walewski V, Mechai F, Billard-Pomares T, Juguet W, Jaureguy F, Picard B, et al. MALDI-TOF MS contribution to diagnosis of melioidosis in a nonendemic country in three French travellers. New Microbes New Infect. juill 2016;12:31‑4.

75. Norskov-Lauritsen N. Classification, identification, and clinical significance of Haemophilus and Aggregatibacter species with host specificity for humans. Clin Microbiol Rev. avr 2014;27(2):214‑40.

76. Werno AM, Murdoch DR. Medical microbiology: laboratory diagnosis of invasive pneumococcal disease. Clin Infect Dis Off Publ Infect Dis Soc Am. 15 mars 2008;46(6):926‑32.

77. Bjarnason A, Lindh M, Westin J, Andersson L-M, Baldursson O, Kristinsson KG, et al. Utility of oropharyngeal real-time PCR for S. pneumoniae and H. influenzae for diagnosis of pneumonia in adults. Eur J Clin Microbiol Infect Dis Off Publ Eur Soc Clin Microbiol. mars 2017;36(3):529‑36.

78. Wolter N, Tempia S, Cohen C, Madhi SA, Venter M, Moyes J, et al. High nasopharyngeal pneumococcal density, increased by viral coinfection, is associated with invasive pneumococcal pneumonia. J Infect Dis. 15 nov 2014;210(10):1649‑57.

79. Avni T, Bieber A, Green H, Steinmetz T, Leibovici L, Paul M. Diagnostic Accuracy of PCR Alone and Compared to Urinary Antigen Testing for Detection of Legionella spp.: a Systematic Review. J Clin Microbiol. févr 2016;54(2):401‑11.

80. Chen DJ, Procop GW, Vogel S, Yen-Lieberman B, Richter SS. Utility of PCR, culture, and antigen detection methods for diagnosis of Legionellosis. J Clin Microbiol. 2015;53(11):3474‑7.

81. Luis BAL, Guerrero Almeida MDL, Ruiz-Palacios GM. A place for Bordetella pertussis in PCR-based diagnosis of community-acquired pneumonia. Infect Dis Lond Engl. mars 2018;50(3):232‑5.

82. Lagier J-C, Fenollar F, Raoult D. Acute infections caused by Tropheryma whipplei. Future Microbiol. mars 2017;12:247‑54.

83. Wagner K, Springer B, Imkamp F, Opota O, Greub G, Keller PM. Detection of respiratory bacterial pathogens causing atypical pneumonia by multiplex Lightmix((R)) RT-PCR. Int J Med Microbiol IJMM. avr 2018;308(3):317‑23.

84. Babady NE, England MR, Jurcic Smith KL, He T, Wijetunge DS, Tang Y-W, et al. Multicenter Evaluation of the ePlex Respiratory Pathogen Panel for the Detection of Viral and Bacterial Respiratory Tract Pathogens in Nasopharyngeal Swabs. J Clin Microbiol. févr 2018;56(2).

85. Epelboin L, Chesnais C, Boulle C, Drogoul A-S, Raoult D, Djossou F, et al. Q fever pneumonia in French Guiana: prevalence, risk factors, and prognostic score. Clin Infect Dis Off Publ Infect Dis Soc Am. juill 2012;55(1):67‑74.

86. Gaydos CA, Roblin PM, Hammerschlag MR, Hyman CL, Eiden JJ, Schachter J, et al. Diagnostic utility of PCR-enzyme immunoassay, culture, and serology for detection of Chlamydia pneumoniae in symptomatic and asymptomatic patients. J Clin Microbiol. avr 1994;32(4):903‑5.

87. Guyard C, Low DE. Legionella infections and travel associated legionellosis. Travel Med Infect Dis. juill 2011;9(4):176‑86.

88. Nieuwenhuizen AA, Dijkstra F, Notermans DW, van der Hoek W. Laboratory methods for case finding in human psittacosis outbreaks: a systematic review. BMC Infect Dis. 30 août 2018;18(1):442.

89. Jorgensen CS, Uldum SA, Sorensen JF, Skovsted IC, Otte S, Elverdal PL. Evaluation of a new lateral flow test for detection of Streptococcus pneumoniae and Legionella pneumophila urinary antigen. J Microbiol Methods. sept 2015;116:33‑6.

90. Phokrai P, Karoonboonyanan W, Thanapattarapairoj N, Promkong C, Dulsuk A, Koosakulnirand S, et al. A Rapid Immunochromatography Test Based on Hcp1 Is a Potential Point-of-Care Test for Serological Diagnosis of Melioidosis. J Clin Microbiol. août 2018;56(8).

91. Croxen MA, Lee TD, Azana R, Hoang LM. Use of genomics to design a diagnostic assay to discriminate between Streptococcus pneumoniae and Streptococcus pseudopneumoniae. Microb Genomics. juill 2018;4(7).

92. Lee RS, Pai M. Real-Time Sequencing of Mycobacterium tuberculosis: Are We There Yet? J Clin Microbiol. mai 2017;55(5):1249‑54.

93. Langelier C, Zinter MS, Kalantar K, Yanik GA, Christenson S, O'Donovan B, et al. Metagenomic Sequencing Detects Respiratory Pathogens in Hematopoietic Cellular Transplant Patients. Am J Respir Crit Care Med. 15 févr 2018;197(4):524‑8.

94. Pendleton KM, Erb-Downward JR, Bao Y, Branton WR, Falkowski NR, Newton DW, et al. Rapid Pathogen Identification in Bacterial Pneumonia Using Real-Time Metagenomics. Am J Respir Crit Care Med. 15 déc 2017;196(12):1610‑2.

95. Xie Y, Du J, Jin W, Teng X, Cheng R, Huang P, et al. Next generation sequencing for diagnosis of severe pneumonia: China, 2010-2018. J Infect. févr 2019;78(2):158‑69.

96. Uadiale K, Bestman A, Kamau C, Caugant DA, Greig J. Evaluation of Pastorex meningitis kit performance for the rapid identification of Neisseria meningitidis serogroup C in Nigeria. Trans R Soc Trop Med Hyg. juill 2016;110(7):381‑5.

97. Cohen-Bacrie S, Ninove L, Nougairède A, Charrel R, Richet H, Minodier P, et al. Revolutionizing clinical microbiology laboratory organization in hospitals with in situ point-of-care. PLoS One. 2011;6(7):e22403.

98. Hou T, Du Q, Wang L, Zhou H, Xi Y, Chen Z, et al. Cross Reactivity of Neisseria meningitidis crgA Diagnostic PCR Primers with Nontypeable Haemophilus influenzae. Clin Lab. 2014;60(9):1425‑9.

99. Boulos A, Fairley D, McKenna J, Coyle P. Evaluation of a rapid antigen test for detection of Streptococcus pneumoniae in cerebrospinal fluid. J Clin Pathol. mai 2017;70(5):448‑50.

100. Samra Z, Shmuely H, Nahum E, Paghis D, Ben-Ari J. Use of the NOW Streptococcus pneumoniae urinary antigen test in cerebrospinal fluid for rapid diagnosis of pneumococcal meningitis. Diagn Microbiol Infect Dis. avr 2003;45(4):237‑40.

101. Leber AL, Everhart K, Balada-Llasat J-M, Cullison J, Daly J, Holt S, et al. Multicenter Evaluation of BioFire FilmArray Meningitis/Encephalitis Panel for Detection of Bacteria, Viruses, and Yeast in Cerebrospinal Fluid Specimens. J Clin Microbiol. sept 2016;54(9):2251‑61.

102. Zhang J-Z, Zheng P, Sun H-M, Dong J-J, Li S-L, Fan S-Y, et al. Next-generation sequencing combined with routine methods to detect the pathogens of encephalitis/meningitis from a Chinese tertiary pediatric neurology center. J Infect. 22 févr 2019;

103. Kuhns M, Zautner AE, Rabsch W, Zimmermann O, Weig M, Bader O, et al. Rapid discrimination of Salmonella enterica serovar Typhi from other serovars by. PloS One. 2012;7(6):e40004.

104. Al-Emran HM, Hahn A, Baum J, Cruz Espinoza LM, Deerin J, Im J, et al. Diagnosing Salmonella enterica Serovar Typhi Infections by Polymerase Chain Reaction Using EDTA Blood Samples of Febrile Patients From Burkina Faso. Clin Infect Dis Off Publ Infect Dis Soc Am. 15 mars 2016;62 Suppl 1:S37-41.

105. Prabagaran SR, Kalaiselvi V, Chandramouleeswaran N, Deepthi KNG, Brahmadathan KN, Mani M. Molecular diagnosis of Salmonella typhi and its virulence in suspected typhoid blood samples through nested multiplex PCR. J Microbiol Methods. août 2017;139:150‑4.

106. Darton TC, Zhou L, Blohmke CJ, Jones C, Waddington CS, Baker S, et al. Blood culture-PCR to optimise typhoid fever diagnosis after controlled human infection identifies frequent asymptomatic cases and evidence of primary bacteraemia. J Infect. avr 2017;74(4):358‑66.

107. Wasihun AG, Wlekidan LN, Gebremariam SA, Welderufael AL, Muthupandian S, Haile TD, et al. Diagnosis and Treatment of Typhoid Fever and Associated Prevailing Drug Resistance in Northern Ethiopia. Int J Infect Dis IJID Off Publ Int Soc Infect Dis. juin 2015;35:96‑102.

108. Olsen SJ, Pruckler J, Bibb W, Nguyen TMT, Tran MT, Nguyen TM, et al. Evaluation of rapid diagnostic tests for typhoid fever. J Clin Microbiol. mai 2004;42(5):1885‑9.

109. Bhengsri S, Baggett HC, Edouard S, Dowell SF, Dasch GA, Fisk TL, et al. Sennetsu Neorickettsiosis, Spotted Fever Group, and Typhus Group Rickettsioses in Three Provinces in Thailand. Am J Trop Med Hyg. 6 juill 2016;95(1):43‑9.

110. Le Viet N, Laroche M, Thi Pham HL, Viet NL, Mediannikov O, Raoult D, et al. Use of eschar swabbing for the molecular diagnosis and genotyping of Orientia tsutsugamushi causing scrub typhus in Quang Nam province, Vietnam. PLoS Negl Trop Dis. févr 2017;11(2):e0005397.

111. Agampodi SB, Matthias MA, Moreno AC, Vinetz JM. Utility of quantitative polymerase chain reaction in leptospirosis diagnosis: association of level of leptospiremia and clinical manifestations in Sri Lanka. Clin Infect Dis Off Publ Infect Dis Soc Am. mai 2012;54(9):1249‑55.

112. Haake DA, Levett PN. Leptospirosis in humans. Curr Top Microbiol Immunol. 2015;387:65‑97.

113. Picardeau M, Bertherat E, Jancloes M, Skouloudis AN, Durski K, Hartskeerl RA. Rapid tests for diagnosis of leptospirosis: current tools and emerging technologies. Diagn Microbiol Infect Dis. janv 2014;78(1):1‑8.

114. Wilson MR, Naccache SN, Samayoa E, Biagtan M, Bashir H, Yu G, et al. Actionable diagnosis of neuroleptospirosis by next-generation sequencing. N Engl J Med. 2014;370(25):2408‑17.

115. Parola P, Paddock CD, Socolovschi C, Labruna MB, Mediannikov O, Kernif T, et al. Update on tick-borne rickettsioses around the world: a geographic approach. Clin Microbiol Rev. oct 2013;26(4):657‑702.

116. Million M, Raoult D. Recent advances in the study of Q fever epidemiology, diagnosis and management. J Infect. juin 2015;71 Suppl 1:S2-9.

117. Melenotte C, Epelboin L, Million M, Hubert S, Monsec T, Djossou F, et al. Acute Q fever endocarditis: a paradigm shift following the systematic use of transthoracic echocardiography during acute Q fever. Clin Infect Dis Off Publ Infect Dis Soc Am. 11 févr 2019;

118. Prudent E, Lepidi H, Angelakis E, Raoult D. Fluorescence In Situ Hybridization (FISH) and Peptide Nucleic Acid Probe-Based FISH for Diagnosis of Q Fever Endocarditis and Vascular Infections. J Clin Microbiol. sept 2018;56(9).

119. Hassing RJ, Alsma J, Arcilla MS, van Genderen PJ, Stricker BH, Verbon A. International travel and acquisition of multidrug-resistant Enterobacteriaceae: a systematic review. Euro Surveill Bull Eur Sur Mal Transm Eur Commun Dis Bull. 2015;20(47).

120. Ruppé E, Andremont A, Armand-Lefèvre L. Digestive tract colonization by multidrug-resistant Enterobacteriaceae in travellers: an update. Travel Med Infect Dis. 2018;21:28‑35.

121. Reinheimer C, Kempf VAJ, Jozsa K, Wichelhaus TA, Hogardt M, O'Rourke F, et al. Prevalence of multidrug-resistant organisms in refugee patients, medical tourists and domestic patients admitted to a German university hospital. BMC Infect Dis. 5 janv 2017;17(1):17.

122. Hinic V, Amrein I, Stammler S, Heckendorn J, Meinel D, Frei R, et al. Comparison of two rapid biochemical tests and four chromogenic selective media for detection of carbapenemase-producing Gram-negative bacteria. J Microbiol Methods. avr 2017;135:66‑8.

123. Al-Bayssari C, Dabboussi F, Hamze M, Rolain J-M. Detection of expanded-spectrum beta-lactamases in Gram-negative bacteria in the 21st century. Expert Rev Anti Infect Ther. 2015;13(9):1139‑58.

124. Glupczynski Y, Jousset A, Evrard S, Bonnin RA, Huang T-D, Dortet L, et al. Prospective evaluation of the OKN K-SeT assay, a new multiplex immunochromatographic test for the rapid detection of OXA-48-like, KPC and NDM carbapenemases. J Antimicrob Chemother. 1 juill 2017;72(7):1955‑60.

125. Dupieux C, Trouillet-Assant S, Tasse J, Freydière A-M, Raulin O, Roure-Sobas C, et al. Evaluation of a commercial immunochromatographic assay for rapid routine identification of PBP2a-positive Staphylococcus aureus and coagulase-negative staphylococci. Diagn Microbiol Infect Dis. 2016;86(3):262‑4.

126. Oviano M, Torres I, Gonzalez M, Bou G. Evaluation of a novel procedure for rapid detection of carbapenemase-producing Enterobacteriaceae (CPE) using the LightMix(R) modular carbapenemase kits. J Antimicrob Chemother. déc 2016;71(12):3420‑3.

127. Lemon JK, Khil PP, Frank KM, Dekker JP. Rapid Nanopore Sequencing of Plasmids and Resistance Gene Detection in Clinical Isolates. J Clin Microbiol. déc 2017;55(12):3530‑43.

Syndrome-based sampling

Respiratory infections

sputum BC urine

Skin infections

Skin biopsy Skin swab

Gastroenteritis

Rectal swab stool

+ dry blood tube

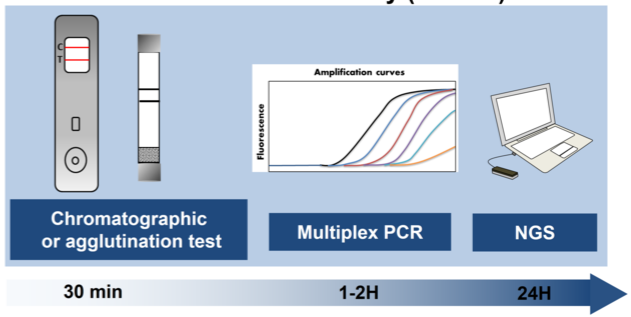
Meningitis

CSF BC

Fever of unknown origin

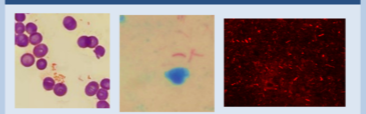
EDTA tube BC

Point-of-care laboratory (POCLs)



Core laboratory

Direct examination

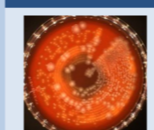


Gram staining Ziehl FISH

Serological testing

IIF, ELISA Western Blot

Culture



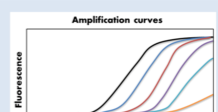
Solid culture

Blood culture

MALDI-TOF MS

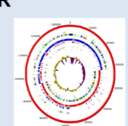
Antibiotic susceptibility testing

Molecular testing



Multiplex PCR

Metagenomics



Genome sequencing