Clinical Infectious Diseases

Emerging Concepts and Strategies in Clinical Microbiology and Infectious Diseases

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Cover Image: View of the new Méditerranée Infection building in Marseille, France. Photograph by Oleg Mediannikov © Oleg Mediannikov.

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SUPPLEMENT ARTICLE

Rewiring Microbiology and Infection

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Keywords. microbiological research; modern tools; systematic questioning; University Hospital Institute (IHU) Méditerranée Infection; single site.

The emergence of microbiology in the 19th century was made possible by the use of new tools: first, the popularization of the microscope, then the development of culture techniques, then the discovery that some bacteria could not be grown in presence of oxygen, and, finally, the development of the techniques of staining and gelification of culture media. Microbiology was initially intended to understand the processes of fermentation by Pasteur [1] and then, microbial diseases of humans, animals, and plants. To demonstrate that *Bacillus anthracis* and *Mycobacterium tuberculosis* were indeed infectious causative agents of anthrax and tuberculosis, respectively, Koch proposed criteria that were used throughout the 20th century, even if they were incomplete [2]. Pasteur immediately thought about translational research by filing patents to control microbiological processes and by developing products to protect humans from microbes, such as vaccination, which was first tested with a culture-modified microorganism [1].

However, the considerable increase in knowledge in microbiology and infectious diseases was partly due to the creation of the Pasteur Institute, which brought together, on a single site, teams working toward the same objective. They wanted to cooperate within a frame of multidisciplinary research, addressing the great problems of mankind [3]. The Pasteur Institute was born in this way. It included a hospital and services that quickly became essential in the development of knowledge on the epidemiology of infectious diseases in France and worldwide and their diagnosis, and also with the sustainability and creation of products based on research with the development of reagents [3] and culture media allowing the cultivation of microbes, but also with the commercial production of vaccines and the development of therapeutics (as serotherapy for the treatment of diphtheria). This burst of novelty and development was associated with great freedom for these early researchers in microbiology. The creation of a single site, which was then implemented in all French-speaking countries, in association with the Army Health Service, played an essential role in the fight against infectious and parasitic diseases, both in the developed world and shortly after in the southern and eastern underdeveloped colonial regions and countries.

The success of this form of research was made possible by the integration of all elements acting as a source of significant knowledge, ranging from epidemiology and surveillance, to basic microbiology, clinical care, and innovative therapeutic and prevention strategies, and was frequently associated with questions reflecting exclusively the personal curiosity of researchers. The progressive subspecialization, in Pasteur Institute as elsewhere, within microbiological sciences, and the increasing dominance of immunology, biochemistry, and molecular genetics along the second half of the 20th century inevitably eroded the above-mentioned pristine strategy. The "curiosity" for understanding infectious diseases and the biology of microbes was inevitably replaced by a more "scientific" approach focused on the dissection (based on hypothesis) of molecular mechanisms and processes. Indeed, that provided significant advances in the knowledge of the factors influencing bacterial physiology and pathogenesis, but something was lost. All that was possible because of the progressive substitution of the individual investigator (with a considerable degree of freedom) by teams, and later by networks of teams, necessarily reducing the fertile input of the individual "curiosity," leading frequently to unexpected significant novelties [4]. As the scientists became increasingly specialized in small areas of research, integration of knowledge in the same individual collapsed—for instance, the old Pasteurian sense of integration of "societal needs" and "solutions from microbiology." Much of the research for applications to practical problems of basic knowledge has disappeared, occasionally driven by the lack of real success in the race for potential vaccines in bacteriology.

Microbiological research in the last third of the 20th century has disregarded Pasteur's exemplary epistemological heritage, "great discoveries are made by chance," that obliged researchers

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not only to maintain the fire of curiosity, but also to be prepared for understanding the sense (frequently the social sense) of complex phenomena in microbiology and infectious diseases. Paradoxically, the Pasteur Institute's most famous piece of research over the last 40 years has been the discovery of the human immunodeficiency virus [5], carried out by research that would probably never have been funded by a research grant, because it did not seem logical to look for the virus causing AIDS in lymph nodes rather than blood.

Research funding structures have also been focused, in recent years, mainly toward research projects based on hypotheses about mechanisms and processes, frequently requiring "last-generation technology." The epistemology of this type of research is problematic. On the one hand, it should be based on a "logical appearance" (ie, are based on already known facts, as such eliminating unexpected research trajectories); on the other hand, the delay between the conception of a innovative idea and a project preparation, organization, submission, acceptance, and funding is too long in the current competition structure of research. We believe that the management of modern science should not underestimate the importance of recreating conditions in which the curiosity of researchers (at least those that have demonstrated skills for identification of novelties), most tempted by discovery, is financed without prejudging the potential results. Inevitably our personal experience influences these thoughts; in the case of one of us (D. R.), its 10 most quoted original publications were the result of a discovery by chance, not within the framework of a hypothesis formulated specifically in a grant.

The research structure of the University Hospital Institute (IHU) Méditerranée Infection is based on this strategy. As identified recently in an editorial comment in the journal *Nature*, the discoveries come essentially from 2 mechanisms [6]. The first mechanism is the use of specifically modern tools, which have not been used before in the field studied. As far as we are concerned, it is microbiology. Our institute has always been at the forefront of the technology arms race. Thus, the first automatic sequencer bought by the IHU ancestor organization was purchased in 1992 [7], as well as the first sequencer of new-generation sequencing bought in Europe. The first worldwide systematic use of matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) [8], to identify bacteria, was also reported by our IHU, which is currently equipped with 8 MALDI-TOF instruments [9]. All other available modern microscopy, molecular biology, and NSB3 security laboratory equipment (1200 m^2) is available in the building. The development and the use of new tools makes it possible to describe aspects that are unknown, each new tool revealing a part of a world that is hidden from us.

The second great source of discovery is the systematic questioning ("mise en question") of the dominant explanatory theories, which is also very developed, intellectually and practically, by the researchers of the Institute, leading to the weakening of a certain number of dogmas with great fertility [10]. Finally, the induction necessary to excite curiosity must hold, as far as we are concerned, with observation. Thus, the presence of 75 hospital beds for infectious diseases, under the safest conditions to avoid contagion; the establishment of a complete traceability of all care, to identify further unknown circuits to the transmissions of infectious diseases intrahospital; and the permanent presence of practitioners in infectiology, at the very center of the research, are some of the essential elements to connect the observed reality with the curiosity. We developed a systematic surveillance strategy of all the causes of infections in the region (90% of infectious disease diagnoses are analyzed weekly in a region of >5 million inhabitants) [11]. This allows to detect abnormal events, and possibly to identify situations requiring the investigation of epidemics linked to known microorganisms, new situations, necessitating the discovery of emerging pathogens. Moreover, the existence of a veterinary center allows us to have access to a large number of samples of animal origin [12]. Finally, an extremely important network, the International Research Group in Africa on Emergence (GIRAFE), extended in West Africa with the teams of Ogobara Doumbo and Cheikh Sohkna, allows an assessment of the risk of infection in these areas, the establishment of rapid diagnostic techniques (point-of-care testing [13] and MALDI-TOF), and the implementation of therapeutic prevention strategies. The association of a team of pharmacists helps us to think about the strategy to implement standardized therapeutics for common diseases (pneumopathies, endocarditis), but also on standardized therapeutics for diseases of which we have become specialists (Q fever [14], Whipple disease [15]), and finally to propose therapeutic solutions for infections with multiresistant bacteria, including fecal graft. The microbiota is the subject of a very particular approach in the IHU, based on culture. The IHU is responsible for nearly 40% of the bacteria isolated at least once in humans, and is the source of the discovery of giant viruses [16, 17] as we begin to understand the role in pathology. This approach, together with hospital services, allows cohorts of patients to be based on their syndromes, and thus to test emerging pathogens and evaluate therapeutic strategies. As for the study of the microbiota, a considerable effort has been made to identify the repertoire of bacteria associated with humans or how to obtain bacteria that can be used as probiotics. One of the major objectives of the IHU is the development of this type of new drug. Finally, to complete the transactional model, 8 start-ups were created in the IHU, focusing on the diagnosis and treatment of infectious diseases. Finally, the principle of the IHU is technological capacity, also in terms of human resources and applications, autonomy, and speed of decisions that allow the discovery. This model is very different from the model based on hypotheses and from the network model that is currently favored by research management structures. We believe that a number of these sites bringing together all the forces and conducting internal cooperation as a

priority will lead to a different evolution of research in microbiology that, we hope, might provide unique opportunities for new discoveries in microbiology and infectious diseases. The aim of this supplement is to report on the one hand a synthesis of the work carried out in the IHU, coordinated by its director, Didier Raoult; and on the other hand, an opinion on the evolution of science and the perspective of the IHU in this field, realized by the members of the scientific council of the IHU, presided by Fernando Baquero. These presentations were made at the end of October 2016.

The Fondation Méditerranée Infection was created in several stages: In 2008, a first step was the creation of a foundation called Infectiopôle Sud, which is in liaison with the other university towns on the French Mediterranean coast, providing training and funding of science students from the South (African continent). Since then, 30 students are funded for their doctoral study and 20 for postdoctoral studies, all from the developing countries. In 2011, the Foundation was transformed into a Fondation Méditerranée Infection after a government call for tender for the creation of a university hospital institute. Six institutes were selected, including the Fondation Méditerranée Infection, which received the largest financial grant of €73 million, and the funding of other partners to reach a total of €100 million. In addition, €25 million was obtained for equipment, before and after this operation, in correlation with the European union. This resulted in the construction of a 27 000-m^2 building that includes 75 hospital beds (single rooms and negative pressure), 25 of which are likely to be converted into NSB3; 5 large NSB3 laboratories for a total area of 1200 m^2; a building with 4 floors of diagnostic laboratories, research, and technological platforms; and a building allowing the institutional reception of students and researchers and host start-ups. From 2001 to 2016, 745 students or visitors were welcomed: 298 were French, and 447 were foreigners, of whom 338 were from French-speaking countries. The meeting, on a single site, of all the forces involved, in association with networks set up in Africa, will hopefully bring about a significant change in research.

Internally, projects are funded based on the potential of applicants, in an extremely quick way, and evaluation is done on written scientific production a posteriori. Each year, a dozen or so awards are distributed to the best publications and to those with the most visible scientific output. In conclusion, we have recreated a model integrating all aspects of microbiology with infectious diseases, the results of which, in the next 10 years, will show whether this model can be duplicated in other countries and other continents, or if it is a unique adventure.

Notes

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SUPPLEMENT ARTICLE

Building an Intelligent Hospital to Fight Contagion

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The idea of building hospitals to fight contagion was born with the *lazarettos*. At the time when the microorganisms were not yet known, the mechanisms of transmission of contagion were already well apprehended. Based on the same knowledge but thanks to new technologies, such hospitals have now been built downtown, next to the most highly performing technological plateau. Regrouping patient care, diagnostics, research, and development, the University Hospital Institute Méditerranée Infection building offers a wonderful tool to contain and understand contagion, in a well-designed setting, creating excellent working conditions that are attractive for interested scientists.

Keywords. hospital-acquired infection; hospital architecture and building; outbreak; infection control; biocontainment.

Years before germ theory, humans knew that some diseases were transmissible from human to human. Syphilis is generally believed to have come originally from the New World, imported into Europe by Christopher Columbus's sailors after their famous voyage in 1492, and the sexual nature of syphilis transmission and its contagiousness was noticed from the beginning. The contagiousness of leprosy, cholera, and plague was immediately recognized from as early as 1377, when the rector of the seaport of Ragusa (Dubrovnik) made mandatory for the first time a *trentina* (30-day isolation period) for ships coming from areas suspected of plague [2]. Furthermore, sick patients were cared for in places established outside the city walls [3]. Soon after in 1423, Venice set up the first *lazaretto*, a quarantine station (with a 40-day isolation period) dedicated to people coming from infected areas, which became a model for European countries, especially in Marseille [3]. The discovery of germ theory [4], the availability of diagnostic microbiology laboratories, the availability of vaccines, and the discovery of antibiotics led physicians and hospital policy makers to lose their interest in classic transmissible infectious diseases, believing that they had won the war against germs. Unfortunately, in the early 20th century, hospital-acquired infections and increased antibiotic resistance [5] were quickly followed by worldwide outbreaks of viral diseases such as influenza, severe acute respiratory syndrome (SARS), Middle East respiratory syndrome coronavirus (MERS-CoV), and viral hemorrhagic fevers; microbiology laboratory accidents; and hospital-acquired outbreaks of *Clostridium difficile* colitis [6]. The revived infection controls in hospitals and microbiology laboratories led to new questions and new scientific knowledge. Laboratory biosecurity rules were made mandatory and infection control in hospitals was reinforced with emphasis on preparedness to future threats. Single bedrooms were shown as a means to avoid influenza transmission in hospitals [7], whereas hand hygiene [8] and catheter control have been proven efficient in the fight of frequent hospital-acquired infection [9, 10]. Fast microbiological identification [11], such as diagnosis at the point of care (POC), has also been reported as an efficient means to control contagion [12]; in addition, microbiologic and epidemiological surveys should help to anticipate sanitary crises. The SARS lessons lead to the revival of patient biocontainment, suggesting the creation of high-level isolation units (HLIUs) across the word [13, 14] and updating healthcare protocols for secure care of very highly contagious patients [15], which have been proven efficient in the care of Ebola virus disease in Europe and the United States [16].

Based on past experience [17], Marseille's University Hospital Institute (IHU) architecture was designed to achieve these objectives in combining in one building care and research on contagious patients, epidemiological surveys, advanced microbiology and POC diagnostics, human and social sciences approaches of contagion, innovative technologies to monitor hand hygiene and catheter management, remote control fever detection, a biosafety level 3 (BSL3) laboratory and healthcare unit, and an optimized circuit for diagnostic care and research on contagious patients [18].

CONTAINMENT OF CONTAGION IN MARSEILLE IN THE PAST

Marseille, which was founded on a rocky landscape facing the sun and the sea, continues to play its role of European–African–Middle East gateway in the Mediterranean region. The reception

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and transit of diverse goods, food, and travelers, and with them microorganisms (causing plague, cholera, etc) has always been a major issue for the city. Since the 12th century, quarantine and isolation of patients has been a structuring public health issue. Marseille in 1120 used the Fort Saint-Jean as a containment area and created the first lazaret in 1526 (under Aragon Spanish Kingdom rule), then built the first hospital Hotel Dieu in 1643 and the Lazaret of Saint Martin of Arenc installed in the northern part of the city in 1814. All of these quarantine stations were situated out of town but on the coast of Marseille. In 1821, a large yellow fever outbreak in Africa reached Spain, indicating that Europe was not protected against this disease. Finally, it was on the Frioul Island, at the forefront of Marseille, that was erected in 1828 the Caroline hospital designed by the architect M. R. Penchaud (1772–1833), able to assure the containment of 48 patients and 24 convalescent patients. Penchaud chose this place essentially because of the main direction of the wind (mistral), expecting to dilute the presumed contagious entities (miasmas). It was first used for the quarantine of soldiers evacuated from African areas with yellow fever. It was then rehabilitated in 1850

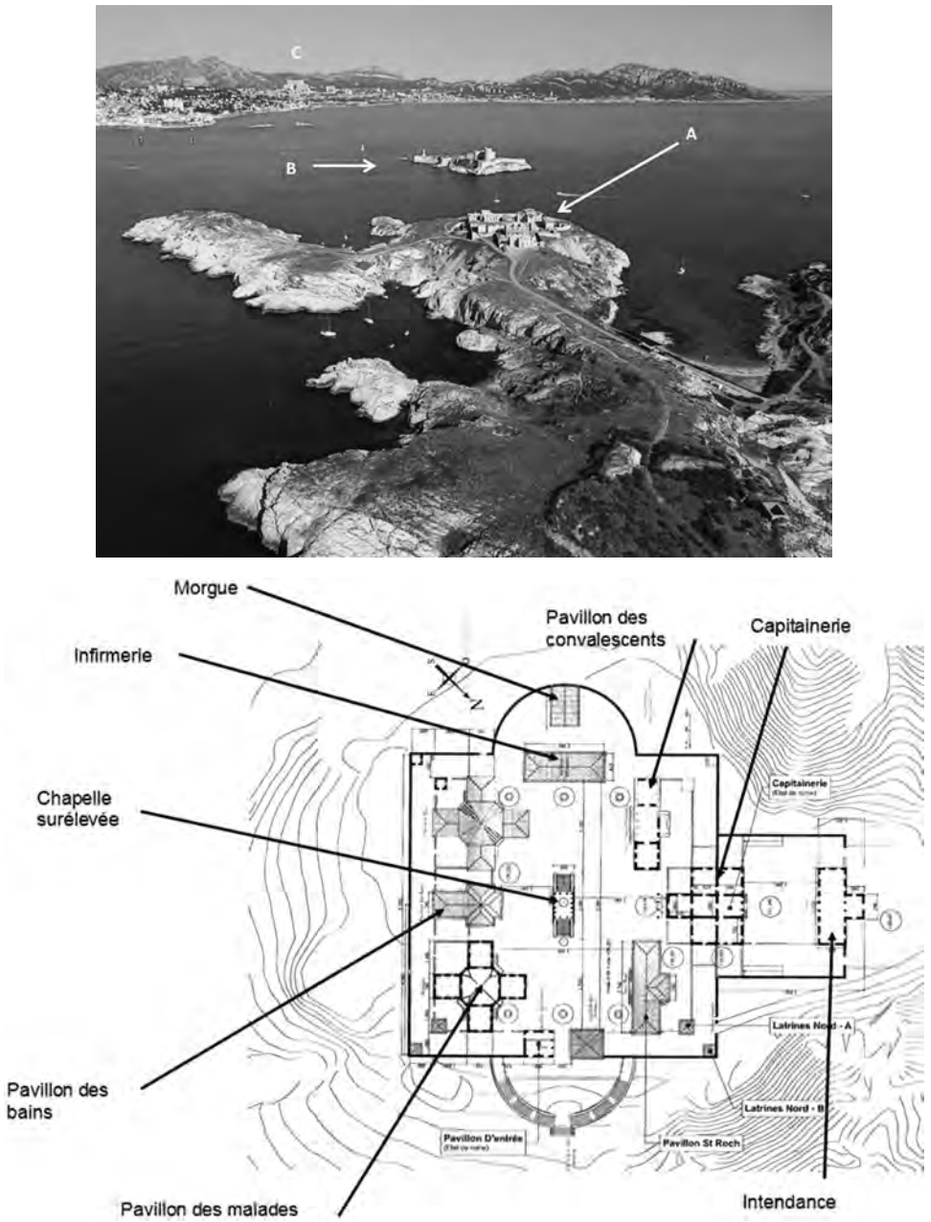

Figure 1. *A*, Ruins of the Caroline hospital (Ratonneau) and outbuildings on Frioul Island, the largest lazaret of the Mediterranean in Marseille: Caroline hospital (A), Chateau d'If (B), Marseilles (C). *B*, Architecture and organization of care of contagious patients in the Caroline hospital. Clockwise from top: Convalescent pavilion, harbor master's office, supply corps, contagious patient's pavilion, bath pavilion, chapel, sick bay, morgue.

by the health architect Jean-Marc Samuel Vaucher (1798–1877) to replace the Arenc Lazaret. The Caroline hospital was renamed "Ratonneau" after the name of the island and would become the Lazaret Island, the largest quarantine station and isolation hospital of the Mediterranean region [19]. At this time, 2 theories were erected concerning contagion: that transmission was due to contact between humans, justifying quarantine; and that of Lazaret and others, who considered transmission to be due to miasmas that were carried by the air and trapped in contaminated areas. In the Caroline Hospital, the 2 theories were combined: isolation of patients and natural air circulation. Contagious patients were cared for in a specially isolated area next to the entry of the hospital, avoiding unnecessary circulation within the building, while the convalescent patients were placed next to intendancy and administration, the nurses being located in between (Figure 1A and 1B). It is particularly interesting to note that these principles of protection implemented at the time remain valid: isolation, containment, air as a vector of asepsis, and easy cleaning of the walls to evacuate the miasmas.

GENERAL ARCHITECTURE OF THE MARSEILLE'S UNIVERSITY HOSPITAL INSTITUTE MÉDITERRANÉE INFECTION

A Building in the City

The technology available today, notably for air filtration and recycling, no longer necessitates a specific "windy" location. The evolution of knowledge about the transmission of pathogens and the technical ability to confine the sectors at risk by sealed protective enclosures with the master of air ventilation allows us to control contagion. The IHU Méditerranée Infection was thus settled downtown, between the University Hospital la Timone and the Faculty of Medicine, to combine research, teaching, and an outstanding clinical investigation platform providing a modern medical service to patients.

Abstraction as an Expression of Timelessness

The architecture sealed a pact of timelessness with the site. Its façade consists of horizontal sunbreaks made of high-performance fiber, giving a mineral expression with balanced proportions. It rises above the public entrance on boulevard Jean Moulin, magnified by a very large staircase and ramp (Figure 2A). Refined, it goes straight to the point by protecting its interior spaces from the wind and the eyes from the sun. The main ceiling of the hall that connects the University and the Hospital is painted with the deep blue hue of the Mediterranean Sea (Figure 2B).

A BUILDING COMBINING INFECTIOUS DISEASE RESEARCH AND CARE FOR PATIENTS

IHU MI was built to gather on the same place the care for infectious and contagious patients, advanced diagnostics in microbiology including parasitology and mycology, epidemiological surveys, traveler counseling, and multifaceted integrative research. It brings together researchers, students, academics, and civilian and military infectious diseases practitioners; clinical microbiology specialists; and patients and families needing a calm framework for research and high-quality care (Figure 3).

Five major sectors were identified and were distributed in 4 blocks for a total of 27 000 m^2. A first triangular block of 3000 m^2 connects the patient healthcare section of the IHU with the technological plateau (radiology and intensive care) and the emergency department and includes on the first floor the outpatient care and council, including day hospital (13 beds and 8 chairs) and the travel clinic, full hospital with 2 units of 25 single "intelligent" bedrooms at each floor (first and second), and 1 specialized unit with 25 single intelligent bedrooms in 3 BSL3 modules dedicated to contagious patients on floor 3 (see below).

A second triangular block is dedicated to modular and open space offices, bringing together clinicians, biologists, engineers, students, and researchers in microbiology and in human and social sciences with the challenging objective to "break the frontiers."

A third block, now rectangular, is dedicated to advanced diagnostic laboratories, located in 3 floors (basement, first and second floors), sharing platforms devoted to high-technology diagnostics, including automated antibody testing, genomics, culturomics, proteomics, or microscopic imaging, and a 3000 m^2 BLS3 laboratory complex divided into 5 modules with anterooms including a specifically preequipped BSL3 room for autopsy [15] on floor 3, at the same level as the BSL3 clinical care unit. The last block includes a 150-seat amphitheater and several classrooms with a total capacity of 300 persons for meetings, and is oriented toward the Faculty of Medicine and devoted to teaching (Figure 2B).

Blocks are separated by faults that mark the boundary between the different functions and facilitate the control of access from one sector to the other. Regarding risk management, a hierarchy was installed from the lowest risk on the first floor to the highest (BSL3) at floor 3 where the most contagious patients are cared for. A cascade of pressure and supply and exhaust HEPA filtration system ensures air quality according to technical recommendations [17, 20]. At the floor below, the risk is lower; the BSL2 laboratory space is organized on the same principle but with a lower airflow rate. On the first floor, accommodation and diagnostic laboratories are equipped with the common and mandatory means. While laboratories are located in the storefront on boulevard Jean Moulin, the bedrooms are quiet as they are located between the hospital and the faculty campus. Finally, a floor at level 4 on the roof is dedicated to management, and 1000 m^2 is dedicated to IHU MI's startup allowing for a perfect symbiosis between patient–diagnosis–research and development, fulfilling the mission of

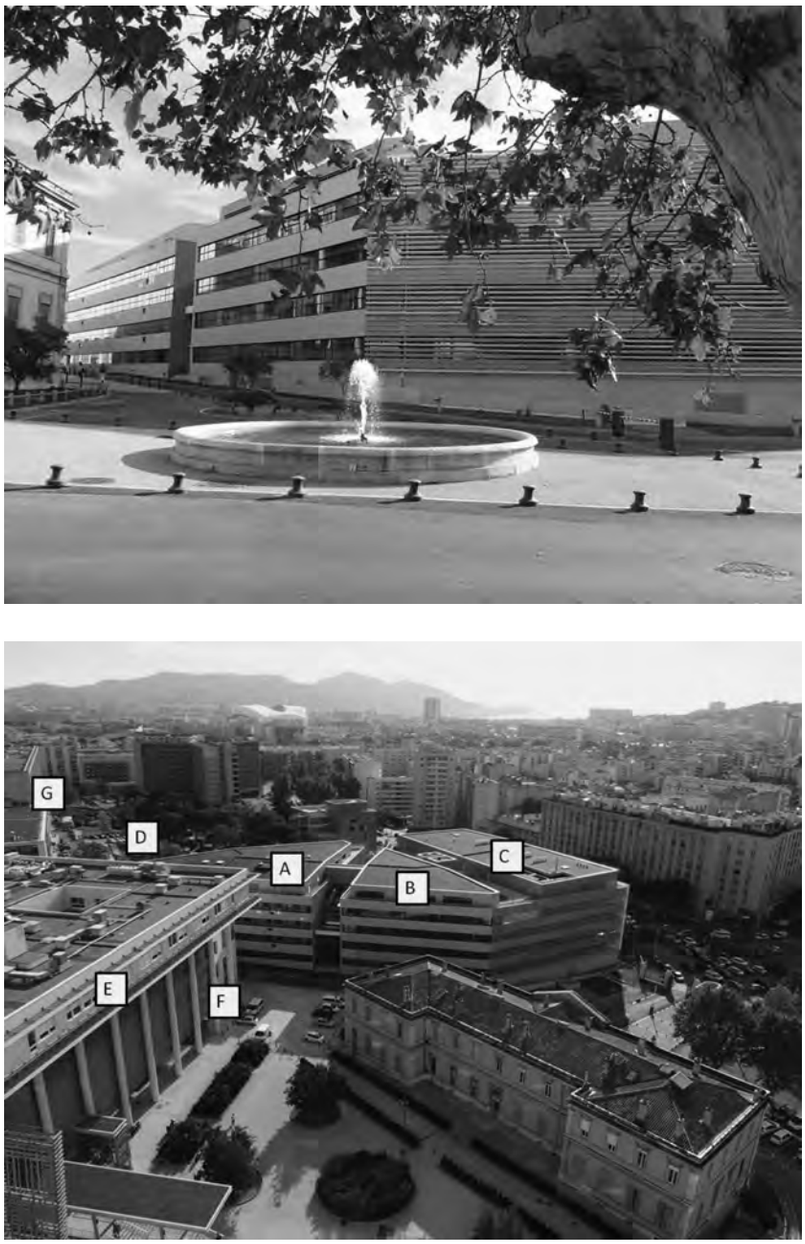

Figure 2. *A*, Façade and horizontal sunbreaks of the new University Hospital Institute Méditerranée Infection (IHU MI) building. *B*, Architectural organization: infectious disease healthcare block (A), open space offices (B), diagnostic and research laboratories (C), meeting and classrooms and amphitheater (D). IHU MI is surrounded by the intensive care unit and radiology (E) and emergency department (F) of the main university hospital, and by Faculty of Medicine (G). The central fault splitting the research and the healthcare sections is shown in blue.

a research hospital. Research and innovation are at the center of this place.

AN INTELLIGENT HOSPITAL HARBORING NEW TECHNOLOGIES

To reach the above objective, the building is equipped with outstanding technology to ensure the automatic remote control detection of body temperature at the general entrance, allowing access to a specific circulation pathway and healthcare (see below). All doors of the building including the patient's bedrooms are secured by an individual badge, allowing a specific control of access for IHU employees but also for visitors and for the patient's family. For example, access cards for visitors allow them to enter their patient's room only and in between 1 to 8 PM. The access control also permits the management of the entrance of isolation rooms. As for example, when in airborne precaution, visitors can access a patient's room only with a specific card given with protective mask; in the case of highly

Figure 3. The main entrance, Grand Hall.

contagious disease or, more frequently, in cases of extensively drug-resistant tuberculosis (TB), no access is allowed but intercom is available to ensure visitor exchange with their patient. Intelligent bedrooms include automatic continuous bedside monitoring system for hand hygiene [21], bedside traceability of care by patient smart reader, a gown and glove distributor, and a house-made door notice enabling an enhanced security of care and reinforcing infection control for each patient [1]. Moreover, a specific biometric control allows access to the BSL3 laboratory complex.

INFECTION CONTROL AND SECURITY CIRCUIT

Single bedrooms have been shown to decrease the incidence of resistant *Staphylococcus*, notably in the intensive care unit, as well as *C. difficile* enterocolitis. Influenza incidence is 2.67 times higher in double-room occupancy [7]. Single bedroom occupancy reduces airborne as well as cross-contamination of pathogens between patients [22]. Multidrug-resistant (MDR) airborne pathogens such as TB should be cared for in negative air pressure bedrooms [17]. As a consequence, infectious diseases wards in IHU have been built as a suite of concentric circles with the health care worker (HCW) at the center, around the HWC pathway, around the patients in single rooms, and surrounding the unit, a visitor corridor with access limited to the patients if not in isolation (Figure 4). In front of each bedroom are masks, gloves, and coats in patented dispenser boxes. The entrance doors of the building have been equipped with infrared cameras to detect febrile persons and guide them to a specific zone of the consultation area to be cared for, allowing for a secure isolation in a dedicated part of the outpatient clinic for quick clinical investigation and diagnostics at the POC and eventually healthcare at the level 3 floor if contagious [23] (Figure 5). As an example of prevention of fomite transmission, smartphones as well as stethoscopes are covered by a protective film [24].

A HIGH-LEVEL ISOLATION (BIOCONTAINMENT) UNIT TO CARE FOR HIGHLY CONTAGIOUS PATIENTS AND OUTSTANDING BIOSAFETY LEVEL 3 LABORATORY

The university hospital in Marseilles is one of the 12 French referral centers for bioterrorism and prevention of emerging infections and outbreaks. The IHU MI laboratory is authorized to test suspected anthrax spores and all other bioterrorism agents, and we admitted patients with MDR-TB and extensively drug-resistant TB and suspected SARS, MERS-CoV, and Ebola hemorrhagic fever, as well as all victims of a yet undiscovered highly contagious emerging infectious agent. The third floor of the IHU MI building is dedicated to BSL3 activities with 1450 m^2 for BSL3 laboratories including a BSL3 necropsy room, and a 1300 m^2 BSL3 ward for healthcare including a BSL3 POC laboratory. The latter is dedicated to the care of contagious patients and has been built according to the most recent recommendations [13–15]. During routine care from a sanitary crisis, 25 single bedrooms can be used individually with negative pressure (–50 Pa) or positive pressure (+20 Pa) to care, for example, for patients with Beijing genotype or MDR-TB as well as

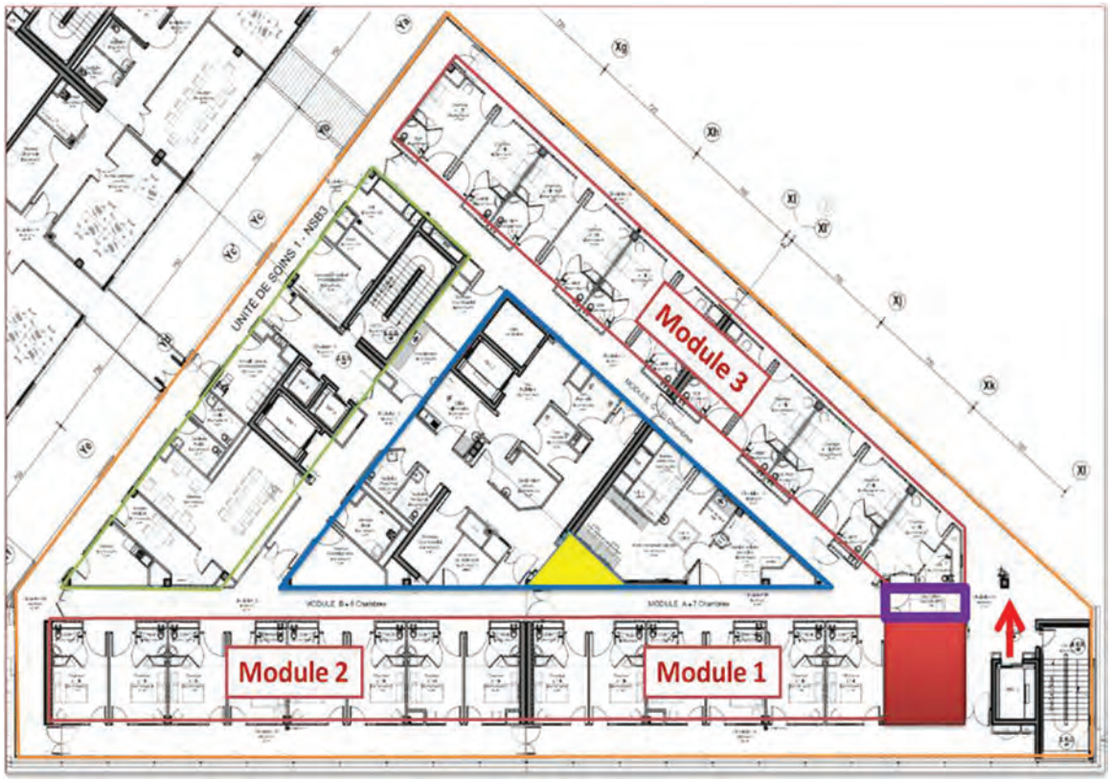

Figure 4. Biosafety level 3 (BSL3) ward at the University Hospital Institute Méditerranée Infection. The blue core is the health care worker (HCW) office and backup, the green area is for physicians and administration, and the red area represents the patients' single rooms. The blue and red areas are separated by HCW working corridors while the orange and red areas are separated by the visitor corridors. For the BSL3 ward, all rooms are independently in positive or negative pressure. When BSL3 is needed, 3 modules can be activated independently. The yellow triangle is the HCW anteroom to access modules 1, 1 and 2, or 1, 2, and 3. The red rectangle is the autoclave and the point-of-care laboratory diagnostic unit. The violet rectangle is the patients' anteroom. The red arrow represents the contagious patients' elevator exit.

immunocompromised patients with febrile neutropenia, but also to optimize the care for *C. difficile* colitis or emerging MDR bacterial infections. In case of sanitary crisis, the ward can be used as a modular BSL3 HLIU (8 + 7 + 10 beds) for patients with highly pathogenic microorganisms with secure anteroom access, a dedicated nurse room, autoclave, and POC laboratory (Figure 5). Each room is equipped as stated above and with video surveillance. The whole building is Wi-Fi equipped, allowing communication for patients in long-term isolation. Specifically set for training infectious diseases HCW in infection control in hospitals, this unit is dedicated for all contagious patients.

THE BIOBANK

This is the main patrimony of the IHU. It is currently composed of a collection of clinical specimens that includes >100 000 serum samples. The European Virus Archives collection contains 750 viral strains, among them SARS coronavirus, most of the tropical arboviruses, and their derived products and is the most important collection of arboviruses in the world. The collection of the Unité des Rickettsies (WDCM 875) was created in 2004 and includes >3500 strains and derived products, including 910 strains of intracellular bacteria (Rickettsiales, Anaplasmataceae, Bartonellae, Whipple bacillus, etc), and representing the largest collection of intracellular bacteria strains in the world. The creation of syndromic sampling kit cohorts from patients has considerably increased the need for sample storage. Currently in draft, the "human-associated bacterial community collection," whose objective is to constitute the largest collection of human-associated bacterial species, includes 779 of the 2156 currently known species (36%). To keep this collection, we have been recently funded at €5 million by Fond Europeen de Developpement Regional to host the IHU biobank, which owns 2 automated freezers at –20°C and 2 automated freezers at –80°C for a capacity of 3 million tubes and is located at floor 1 in the building.

The IHU Méditerranée Infection of Marseille is a building of exception dedicated to outstanding research on emerging infectious and tropical diseases. It is built to answer new types of conflicts, successive outbreaks of new emerging pathogens (SARS, MERS-CoV), the spread of highly pathogenic hemorrhagic fever virus outbreaks causing impressive and severe sanitary crises, and the unsolved pandemic of hospital-acquired

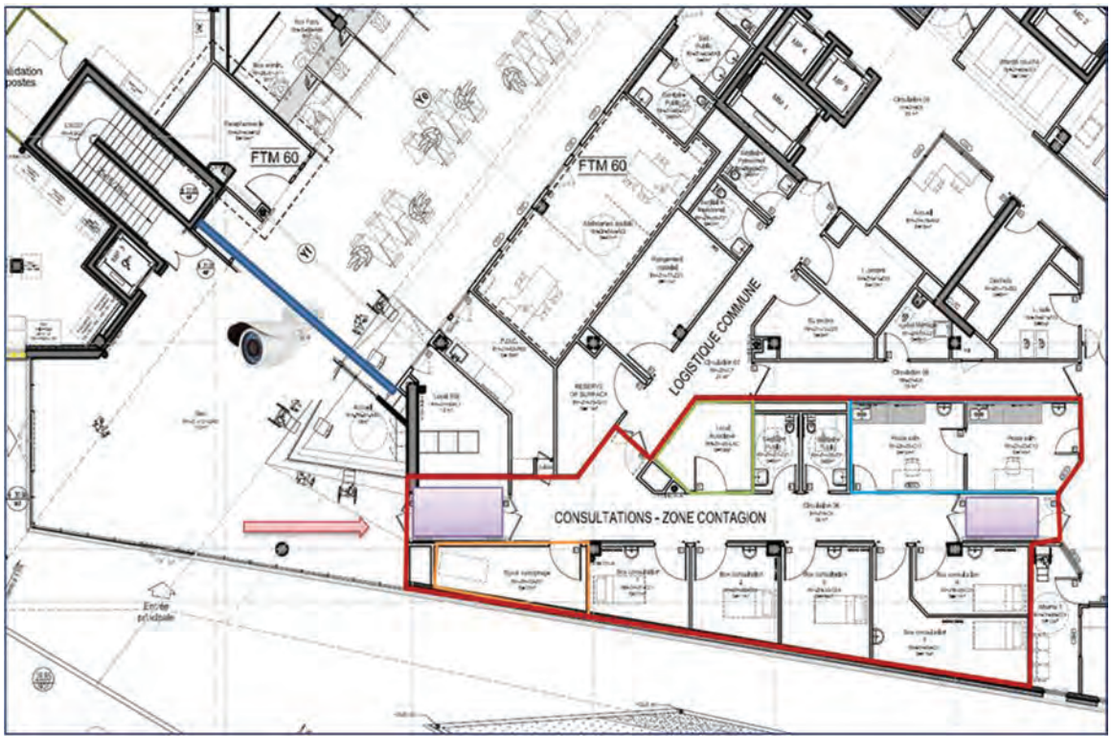

Figure 5. Isolation path for patient/visitor with fever at University Hospital Institute Méditerranée Infection. Infrared camera detects febrile individuals, which prevents the doors from opening. The person is directed to the outpatient clinic following the red arrow throughout the patient's anteroom (violet) and is cared for by an equipped nurse and doctor. The green area is the autoclave room, the orange area is the isolation stretcher backup, and the blue zone is the nurse room. If a contagious disease is diagnosed, the patient is driven through the exit anteroom (violet) to the biosafety level 3 ward on level 3.

infection in a context of unprecedented migration and intense international travel exchanges.

Marseille is an international city, shaped by the Mediterranean region. This mineral building designed as a Greek temple aims to focus on worldwide pandemics including the health link between the shores of the Mediterranean, where it also draws many skilled researchers and students. If it still holds the only protective role of the *lazaretto*, it develops an especially human and scientific approach, essentially through its vocation for research in infectious disease.

Notes

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Clinical Infectious Diseases

SUPPLEMENT ARTICLE

From Expert Protocols to Standardized Management of Infectious Diseases

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We report here 4 examples of management of infectious diseases (IDs) at the University Hospital Institute Méditerranée Infection in Marseille, France, to illustrate the value of expert protocols feeding standardized management of IDs. First, we describe our experience on Q fever and *Tropheryma whipplei* infection management based on in vitro data and clinical outcome. Second, we describe our management-based approach for the treatment of infective endocarditis, leading to a strong reduction of mortality rate. Third, we report our use of fecal microbiota transplantation to face severe *Clostridium difficile* infections and to perform decolonization of patients colonized by emerging highly resistant bacteria. Finally, we present the standardized management of the main acute infections in patients admitted in the emergency department, promoting antibiotics by oral route, checking compliance with the protocol, and avoiding the unnecessary use of intravenous and urinary tract catheters. Overall, the standardization of the management is the keystone to reduce both mortality and morbidity related to IDs.

Keywords. antibiotic stewardship; standardization; fecal microbiota transplantation.

The management of infectious diseases (IDs) remains an important challenge for clinicians. Complexity of handling IDs suggests that ID experts were better at caring for IDs than other physicians [1, 2]. Also, stringent antibiotic stewardship programs have demonstrated a significant impact on ID outcome including life-threatening infections such as endocarditis [3, 4]. The compliance of clinicians with their established treatment protocols must be evaluated before reaching the conclusion of "failure" during antibiotic therapy [5, 6], as this critical point is difficult to analyze in multicenter studies [7]. Furthermore, device-associated infections including catheter-associated bloodstream infection and catheter-associated urinary tract infections are among the most frequently encountered life-threatening healthcare infections, which requires the avoidance of unnecessary indwelling catheter devices and an appropriate strategy of oral antibiotics [8, 9].

To highlight our contribution in the rational management of IDs, we present hereby 4 exemplary cases in the management of IDs in the University Hospital Institute (IHU) Méditerranée Infection in Marseille, France. First, we report our 30 years of expertise and management regarding intracellular bacterial infections such as Q fever and *Tropheryma whipplei*, based on both in vitro data and clinical outcome. Second, we described our 15 years of experience with a management-based approach for the treatment of infective endocarditis (IE), with important reductions in the mortality rate [3]. Third, we report the protocol of using fecal microbiota transplantation to decolonize *Clostridium difficile* and emerging highly resistant bacteria [10, 11]. Finally, since 2015, we have set up an acute ID unit dedicated to the standardized management of acute infections in patients admitted through the emergency department. We established protocols under the expertise of both emergency care and ID specialists to treat the most frequently encountered infectious syndromes, and promoting the preferential use of antibiotics through the oral route. We checked compliance with this protocol, and we monitored the need for both blood and urinary tract catheters in these and other hospital-based patients [12, 13].

Q Fever and *Tropheryma* Infections

Q Fever

Since 1985 to December 2015, we tested in our function of the French National Referral Center for Q fever 286 273 samples from France and abroad for *Coxiella burnetii* [14]. Our current cohort of patients includes 1954 patient files and since 2007, 1784 are considered to have an active infection (1382 acute Q fever, 492 patients with persistent focal infection, 90 with both acute Q fever and a subsequently persistent focalized infection [15]). Infected patients were identified from all over France (including overseas territories such as La Reunion and French Guiana) and other countries (mainly Italy, the United Kingdom,

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and Israel). This allowed us to capture, mine, and analyze all the heterogeneity of clinical expressions and complications of the infection by different *C. burnetii* clones in different human populations. Our role as a reference center with a clinical personal experience of >30 years (D. R.), thorough analysis of our cohort database [15, 16], the inclusion of new technologies (specific polymerase chain reaction [PCR], ^{18}F deoxy-glucose positron emission tomography combined with computed tomography [PET/CT]) [17, 18], the study of misleading classifications by other teams [19], and the questioning of our recommendations [19] led us to establish new standardized diagnostic criteria and therapeutic protocols (Supplementary Tables 1–9) [17].

Formerly, patients infected with *C. burnetii* were classified into only 2 medical conditions: acute and chronic Q fever. Our work on *C. burnetii* infections helped to accurately define 10 medical conditions linked to *C. burnetii* infection (Supplementary Tables 1–6), classifying 98.8% (1764/1784) of our patients considered as infected. These definitions contributed to the establishment of more specific treatment protocols (Supplementary Tables 7 and 8) in the context of primary infection and 5 treatment protocols for cardiovascular infections (Supplementary Table 9). Each of them corresponds to a specific treatment or management (Supplementary Tables 7–9), in the scope of laboratory-based personalized medicine.

By clarifying the clinical management of patients infected by *C. burnetii*, we demonstrated that the "chronic Q fever" term should be banned and replaced for "persistent focalized Q-fever infection" [17]. Indeed, endocarditis could occur during acute Q fever [15, 20], requiring a carefully adapted treatment. Moreover, the "chronic Q fever" term is a mix of very different medical conditions leading to inadequate management [18]. We believe that the newly proposed diagnostic criteria and treatment protocols [17] significantly and dramatically improved the care of *C. burnetii*–infected patients. Using these criteria and protocols available online (http://en.mediterranee-infection.com/article.php?laref=157&titre=q-fever-treatment), any ID specialist (and/or medical doctor) is able to classify and treat 97% of patients infected by *C. burnetii*. Obviously, an expert opinion remains essential for patients with unfavorable serological outcome (serological failure or relapse) despite careful protocol application or for the 3% of patients with a rare presentation. Reporting such cases to our worldwide expert center (failure, relapse, rare cases) will contribute to ongoing studies on the classification and treatment of these particular situations, and will lead to the proposition of new evidence-based protocols improving the global care for *C. burnetii*–infected patients.

Tropheryma whipplei

Tropheryma whipplei is the causative agent of Whipple's disease, which is defined by the characteristic histological involvement in small-bowel biopsies (positive periodic acid-Schiff staining and or immunohistochemistry). This bacterium can caused localized chronic infections without digestive involvement, mainly endocarditis, encephalitis, uveitis, and osteoarticular infections [21]. Since the first culture of *T. whipplei* performed in our laboratory in 2000 [21], we have diagnosed >300 *T. whipplei* infections; some of these patients were referred to one of us (D. R.) for expert management. We are also sometimes contacted by patients or physicians for an opinion concerning a second-line treatment [22].

Once we made available for the first time the possibility of culturing *T. whipplei*, susceptibility tests were carried out that were able to explain why trimethoprim-sulfamethoxazole, the most frequent empiric treatment proposed, is frequently ineffective [7, 21]. First, *T. whipplei* proved to be naturally resistant to trimethoprim, and acquired resistance to sulfamethoxazole was frequent. A recent study has demonstrated that 25.9% of the *T. whipplei* strains were resistant to sulfamethoxazole [21]. In addition, the association of doxycycline and hydroxychloroquine was shown as the sole bactericidal treatment for *T. whipplei*. Second, the lifetime susceptibility of the patients with Whipple disease to *T. whipplei* highlighted reinfections caused by different genotypes, leading us to propose lifetime treatment and monitoring [23]. Indeed, we demonstrated by in vitro studies, then clinical outcomes studies, that a 1-year treatment with an association of doxycycline (200 mg per day) and hydroxychloroquine (600 mg per day), followed by lifetime treatment with doxycycline was the most appropriate treatment for patients with Whipple's disease [7]. Lifetime surveillance including antibiotic serum dosages to monitor the patient's compliance is required [7]. For endocarditis, the same lifetime treatment is required as we demonstrated that *T. whipplei* endocarditis can transform secondarily into classic Whipple's disease [24]. We propose the same management for encephalitis because of frequent relapses. For other localized chronic infections, we suggest a combination of doxycycline and hydroxychloroquine for 12–18 months' duration [7], followed by a lifetime surveillance. All these protocols are available for physicians on our website (http://www.mediterranee-infection.com/article.php?larub=65&titre=les-protocoles-therapeutiques).

INFECTIVE ENDOCARDITIS

Despite improvements in its management, IE is a deadly disease that remains associated with high mortality and severe complications. Antibiotics remain the central pillar in the treatment of IE, but it is of first importance to rely on an "endocarditis team," including cardiologists, microbiologists, ID specialists, and surgeons with a very high level of expertise [3]. This collaboration allows an early cardiologic diagnosis (clinic, echocardiography, imaging, PET scan) [3, 25], a rapid surgical decision, and an early antibiotic treatment, adapted to the clinical and microbiological situation. In our center, the microbiological investigations of patients with clinical suspicion of IE are systematically performed with a specific "diagnostic kit" for endocarditis,

including 3 sets of blood cultures and detection of specific antibodies directed against *C. burnetii*, *Bartonella* species, *Brucella* species, *Aspergillus* species, *Legionella pneumophila*, *Mycoplasma pneumoniae*, *Chlamydia pneumoniae*, a detection of the rheumatoid factor, anticardiolipin, and serum concentration of specific immunoglobulin E using pig epithelium. We systematically perform cultures, molecular detection methods, and histopathological analysis on the surgically excised valves. When results of first-rank tests are negative, we systematically perform molecular detection of *C. burnetii*, *Bartonella* species, *T. whipplei*, *Mycoplasma* species, *Streptococcus mitis*, *Streptococcus gallolyticus*, *Enterococcus faecalis*, *Enterococcus faecium*, *Staphylococcus aureus* from ethylenediaminetetraacetic acid blood, and *Bartonella* species by Western blot.

First-line empiric antibiotic protocols (Supplementary Table 10) are systematically prescribed, according to the microbiological and clinical situation: ceftriaxone and gentamicin are used in *Streptococcus* species IE, and ceftriaxone and amoxicillin are used in *Enterococcus* species IE, as recommended by the 2015 European Society of Cardiology guidelines. Clindamycin and co-trimoxazole are used for *S. aureus* IE, whatever their sensitivity to methicillin and the clinical situation (native or prosthetic valve) [26]. In IE due to coagulase-negative *Staphylococcus*, whatever their sensitivity to methicillin, we use vancomycin and gentamicin. In blood culture–negative endocarditis (BCNE) cases, an evaluation of the epidemiological factors, the history of prior infections including cardiovascular infections, exposure to antimicrobials, clinical course, severity, and extracardiac foci of infection are to be considered. However, since 2002 we have used standardized protocols: In community-acquired BCNE, we have prescribed 6 weeks of amoxicillin and 3 weeks of gentamicin, whereas in hospital-acquired BCNE, we have used 6 weeks of vancomycin and 3 weeks of gentamicin. If fever persisted after 48 hours of treatment, liposomal amphotericin B was added to the protocol.

A recent evaluation of these BCNE protocols showed an 87% adherence to the protocol (all deviations were justified) and a global fatality rate of 5.1%, which is low compared to the literature review [4]. Although they are different from the European Society of Cardiology and American Heart Association "consensual" guidelines, our "expert's recipes" have proven to be easy to apply to the vast majority of IE cases, efficient, and well-adapted to the local conditions [3, 4, 26].

FECAL MICROBIOTA TRANSPLANTATION

Clostridium difficile Infections

While the first traces of fecal transplant date from the fourth century CE in China, used to treat patients ingesting poisoned food or having severe diarrhea [27], fecal microbiota transplantation has seen an extraordinary revival following the publication of the first randomized trial demonstrating the superiority of this technique in comparison to the use of antibiotics in recurrent *C. difficile* infections [28], and then the recommendations of the European Society of Clinical Microbiology and Infectious Diseases [29]. Contrary to the idea that it is an infection with a low mortality rate, from 2012 to 2015, the lethality rate of *C. difficile* infections has been evaluated in France as ranging from 17.2% to 17.9% whatever the causative ribotype [30], corresponding to approximately 1800 deaths each year. In 2013, during a *C. difficile* hypervirulent 027 ribotype regional outbreak, the observed mortality was >50% at 1 month, and almost 75% of the deaths occurred during the first week of evolution, which made the recommended strategy totally ineffective for most of our patients [10]. This led us to propose to perform fecal transplants from the first episode, as soon as possible and in any case no later than 7 days following the infection [10]. The nasogastric route was chosen because it was the easiest, and we prescribed concomitant antibiotics to reduce the bacterial load (Supplementary Materials). The results were spectacular, with a 5-fold reduction of the number of deaths [10]. About one-third of the patients, however, needed a second fecal transplant [10]. Beyond the 027 ribotype, fecal microbiota transplantation has also demonstrated efficiency in first intention in severe infection of *C. difficile*, whatever the ribotype [31]. Indeed, we also reported fecal microbiota transplantation performed by nasogastric transplantation for 2 patients for whom colectomy was considered [31]. Our experience demonstrated the feasibility and success of fecal microbiota transplantation in early stages of severe *C. difficile* infections. At the moment we cannot provide comparative randomized studies with conventional approaches, but the difference in mortality appeared so compelling that to propose randomized studies was considered unethical, as in other life-threatening diseases [32].

Gut Decolonization of Multidrug-Resistant Bacteria

The colonization by emerging highly resistant bacteria and, in particular, by those Enterobacteriaceae producing carbapenemases is a growing public health problem in France [33]. Hospital patient care, regulated by a report of the High Council for Public Health, includes reinforced isolation measures, as well as screening and cohorting of contact subjects, which is expensive and frequently unfeasible [33]. Treating by antibiotics such colonized patients in the objective of decolonizing the multidrug-resistant strain is not only harmful but totally unnecessary. In this context, we proposed fecal microbiota transplantation in an 82-year-old woman for management of a long-term carriage of OXA-48 carbapenemase-producing *Klebsiella pneumoniae* [11]. She received a bowel lavage associated with 4 successive administrations of colimycin (2.5 MIU) and gentamicin (100 mg) according to the strain and the antibiotic susceptibility testing results to reduce the bacterial load [11]. We then performed, by nasogastric route, a fecal microbiota transplantation prepared from a healthy anonymous donor

after testing for the absence of pathogens according to French recommendations [34]. Since this case report, we have treated 4 other colonized patients by fecal microbiota transplantation. Overall, the outcome has been suitable in 4 of the 5 cases (80%), with a mean follow-up of 98 days (10–155 days) (unpublished data). Finally, 10 case reports, including our own case, were published describing the decolonization of multidrug-resistant bacteria including extended-spectrum β-lactamase–producing, carbapenemase-producing Enterobacteriaceae or vancomycin-resistant enterococci [35–37]. Large studies, including cost-effectiveness studies, are needed to definitively demonstrate the efficiency of fecal microbiota transplantation decolonization of antibiotic-resistant organisms.

Although simplified by using the nasogastric route and freezing the microbiota [38], fecal microbiota transplantation remains a complex process with limitations for some patients. Our preliminary unpublished in vitro results using optimized freeze-dried microbiota protocols are encouraging. To the best of our knowledge, only 1 case report was previously published on the efficient use of freeze-dried, capsulized fecal microbiota transplantation in a patient suffering from relapsing *C. difficile* infection [39], but this way must be the future prospect of this treatment.

STANDARDIZED MANAGEMENT OF INFECTIOUS DISEASES FROM EMERGENCY ROOMS

One of our ID units is exclusively dedicated to hospitalized patients originating from emergency rooms of our tertiary care hospital. The recruitment in this emergency acute ID unit is based on the presence of fever whatever the age, the supposed cause, and the underlying diseases. The standardized protocols were elaborated in a multidisciplinary approach following a review of evidence-based studies and using local bacterial resistance data. These protocols are available as part of the software used for drug prescription by the ID clinicians as well as by emergency room clinicians. One of our main concerns was to focus on the main infection syndromes detected in the emergency room, and also to promote a more comprehensive use of oral route of antibiotic administration rather than the intravenous route to avoid catheter-associated bloodstream infections (Supplementary Table 11). Since the beginning of 2015, 3 distinct periods have followed. Period 1 (baseline period) was a 4-month period starting at the opening of this unit until the establishment and acceptance of antibiotic protocols. Period 2 (implementation period) was an 8-month period starting from the initiation of the antibiotic protocols until the beginning of the interventional file. Finally, period 3 (interventional period) was an 8-month period starting with the creation of a compulsory document to be completed by emergency specialists and including justification for antibiotics, intravenous catheters, and urinary catheters (Supplementary Materials; Figure 1).

During our 20-month study, 1356 patients were hospitalized in our acute ID unit, including 281 patients in period 1 (21%), 544 in period 2 (40%), and 531 in period 3 (39%) with no difference in the main demographic characteristics during the 3 following periods (Table 1; Figure 1). The mortality rate ranged from 0.75% (period 3) to 1.4% (period 1) without any significant difference. Of the 1356 patients hospitalized in our ID unit, 1308 (96.4%) were hospitalized from the emergency room, 30 patients from intensive care units (2.2%), and 18 from diverse medical units. Overall, the mean duration of hospitalization was of 3.6 days (without significant difference between the 3 periods). Among the 1356 patients, 573 patients (42.2%) had pneumonia, 210 patients (15.5%) had a urinary tract infection, 153 patients (11.3%) had a soft cutaneous infection, 59 patients had meningeal syndrome (4.3%), 44 patients had febrile diarrhea (3.2%), 35 patients had febrile illness after they returned from the tropics (2.6%), 8 patients had a pharyngitis (0.6%), and 7 patients were febrile during neutropenia (0.6%) (Figure 2). Finally, 185 had a fever of unknown origin (13.6%), 36 patients had arthritis or osteitis (2.7%), 27 patients

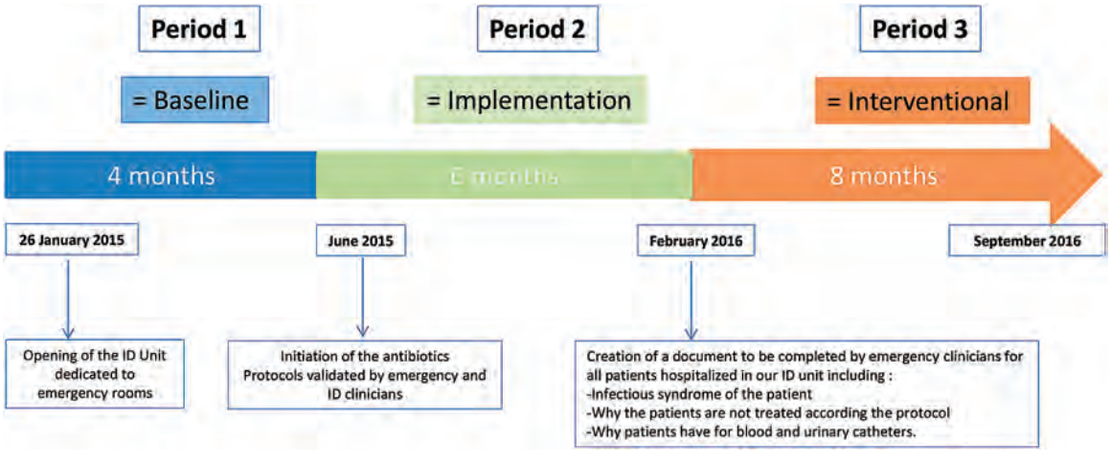

Figure 1. Baseline, implementation, and interventional periods. Abbreviation: ID, infectious diseases.

Table 1. Main Demographic Characteristics

Characteristic	Period 1 Baseline Period	Period 2 Implementation	Period 3 Interventional
Duration	4 mo	8 mo	8 mo
No. of hospitalized patients	281	544	531
No. of male/female (sex ratio)	171/110 (1.5)	306/238 (1.3)	304/227 (1.3)
Mean age, y	63	64.9	61.8
No. (%) of patients >85 y	60 (21.3)	117 (21.5)	94 (17.7)
Hospitalization duration	3.7 d	3.7 d	3.4 d
No. of deaths (mortality rate)	4 (1.4%)	6 (1.1%)	4 (0.7%)

had eruptive fever (2%), 14 patients had febrile abdominal pain (1%), and 5 patients were hospitalized for another cause (0.4%).

Peripheral Intravenous and Urinary Tract Catheters

A total of 1167 patients (86.1%) arrived in our unit with a peripheral intravenous catheter. The proportion of patients hospitalized with a peripheral intravenous catheter decreased from period 1 (267/281 [95%]) to period 2 (487/544 [89.5%]) and period 3 (413/531 [77.8%]) ($P < 10^{-6}$; (Supplementary Table 12; Figure 3). During the evaluable period (period 2 + period 3), the number of unnecessary blood catheters was 430 of 900 hospitalized patients (47.7%). The proportion of unnecessary blood catheters decreased significantly from period 2 (272/487 [55.8%]) to period 3 (158/413 [38.2%]) ($P = .001$; Supplementary Table 12). The number of unnecessary intravenous catheter was significantly higher overnight (307/609 [50.4%]) compared with patients hospitalized during the day (123/291 [42.2%]) ($P = .02$; Supplementary Table 13). Finally, among the 1167 patients with intravenous devices, peripheral intravenous catheters were removed in 659 cases (56.5%) during the first 24 hours of hospitalization (Supplementary Table 12).

Of the 1356 hospitalized patients, 235 had been given a urinary tract catheter (17.3%) (Table 3; Figure 3). The proportion of patients hospitalized with a urinary tract catheter decreased from period 1 and 2 (160/825 [19.4%]) to period 3 (75/531 [14.1%]) ($P = .01$; Supplementary Table 14). Among the 235 patients with a urinary catheter, the device was unnecessary in 52 cases (22.1%). The proportion of unnecessary urinary tract catheters decreased from period 1 and 2 (41/160 [25.6%]) to period 3 (11/75 [14.7%]) ($P = .06$) (Supplementary Table 14). The number of unnecessary urinary tract catheters was significantly higher in patients hospitalized overnight (33/144 [23%]) compared with patients hospitalized during the day (9/91 [10%]) ($P < .01$; Supplementary Table 13). Finally, urinary tract catheters were removed in 77 cases (32.7%) during the first 24 hours of hospitalization (Supplementary Table 14).

Protocol Compliance

Among the 1075 patients hospitalized during period 2 and period 3, 699 were hospitalized for one of the syndromes for which the protocols were established. Overall, the antibiotic protocols compliance in emergency rooms was observed for 403 patients (58.2%). From period 2 to period 3, the protocols were followed in 58% and 56.6%, respectively, of the cases in the emergency room. During period 3, the justifying management document was completed for 224 of 531 patients (42.2%). In our unit, the compliance was observed in 84.2% and 83.3% of the cases from period 2 to period 3, respectively (Figure 3). Among the 116 patients for whom the protocols were not followed, it was in 30 cases (25.8%) because of failure and in 29 cases (25%) because of contraindication (Supplementary Table 15).

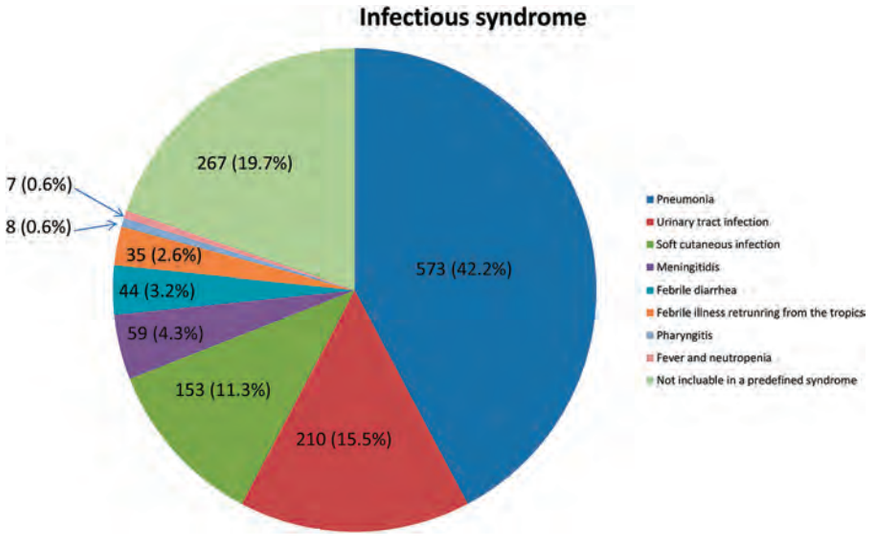

Figure 2. Infectious syndrome. Abbreviation: ID, infectious diseases.

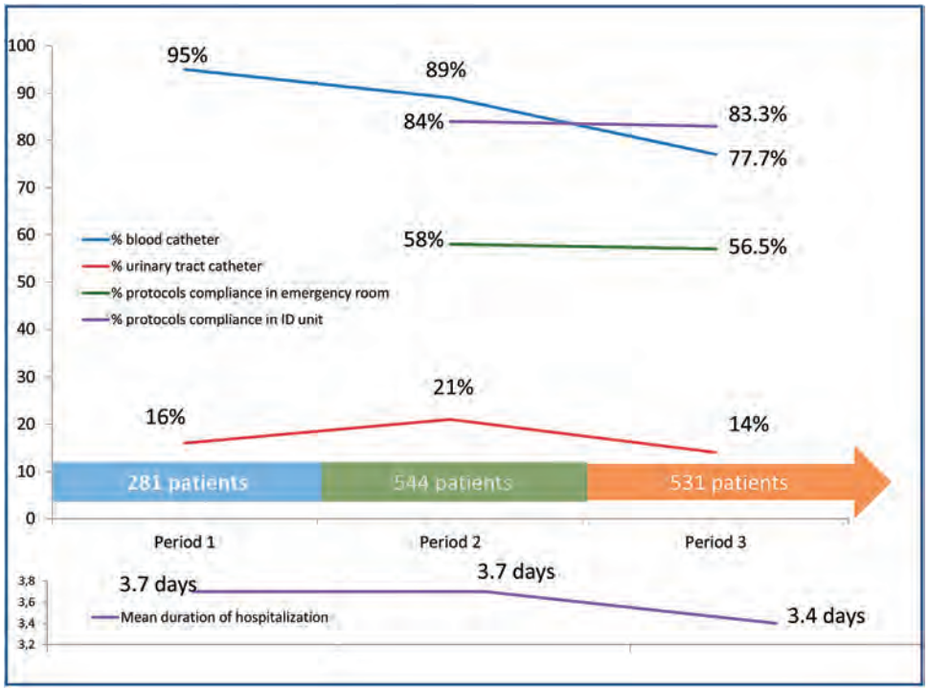

Figure 3. Patient details across the baseline, implementation, and interventional periods.

Future Perspectives

The first lesson of our common program "from emergency room to ID unit" is that 80% of the patients with fever could be included in only 9 different and simple infectious syndromes. All these syndromes were treatable using a comfortable protocol including only 12 different antibiotic drugs, most of them (66%) by oral route, as recently recommended by the implementation of antibiotic stewardship program guidelines [40, 41]. We observed 56% of unnecessary catheters in period 2, this being close to the rate of 50% observed in a Australian tertiary emergency department [42]. Considering that *S. aureus* sepsis in our hospital has a lethality rate close to 20% [30], and that venous catheters are the major causes of these bacteremia, such a high proportion of unnecessary venous catheters is intolerable. We believe that the current priority to reduce healthcare infections is to monitor the use of unnecessary catheters [43, 44], and we observed an encouraging decrease from 56% to 38% during the last period. The same trend, though not significant, was observed for urinary tract catheters, and providing regular feedback to emergency room doctors about the high rate of unnecessary devices should be continued [12].

Despite the fact that our protocol was simple and established with a multidisciplinary local consensus, the level of compliance with our guidelines was lower than expected, notably in emergency rooms [45]. Probably only a limited number of well-trained physicians will assure the success of management-based approaches [3], but in our tertiary emergency department, 130 different physicians (including residents and medical doctors with various specialties) have a rotation in the emergency room and can prescribe overnight, compared with only 25 emergency specialists (residents and medical doctors) during the day. This can explain the lower overnight compliance that we observed. A significant higher overnight percentage of intravenous ($P < 10^{-4}$) and urinary ($P = .01$) catheter prescriptions compared with the percentage by day was also observed (Supplementary Table 13). Beyond the number of prescribers, Goldstein et al have observed that the antibiotic stewardship team intervention rejection rate varied from 20% to 100% depending on the medical doctor's specialty [6]. In addition, the behavior depends on individuals because 85.6% of the rejections of the antibiotic stewardship team proposals were from only 6 medical doctors including 3 ID doctors, 2 pneumologists, and 1 internist. This should lead to personalized efforts to increase compliance [6].

Role of Clinical Microbiology Laboratories

From our point of view, the clinical microbiology laboratories play a central role in optimizing the management of IDs. We followed for many years a technology-driven approach using, for example, mass spectrometry for colony identification, optimized quantitative PCR for diagnosis, or real-time genomics for isolate characterization [46, 47]. In addition, the management of IDs in our reference center was dramatically improved with the help of our point-of care (POC) laboratory [48]. POC laboratories, operating around the clock and 7 days a week, provide rapid diagnoses of ID within 2 hours, largely based on

immunochromatography and real-time PCR tests [48]. In addition, these tests are combined into syndrome-based kits that facilitate sampling and modify rapidly the treatment management and the isolation of patients to reduce healthcare-associated infections [48]. Among the examples described above, the POC has played a central part in the management of the 027 ribotype *C. difficile* outbreak [10].

CONCLUSIONS

Through the standardization of our therapeutic protocols, our main objective for the management of infections in the IHU Méditerranée Infection is to require clinicians to follow established protocols including fighting against unnecessary catheters. From the initial expert's protocols, we would generalize our standardized approach for the management of all ID patients hospitalized in our facility. To date, we only performed limited interventions in the scheduled management of main infection syndromes detected from emergency rooms. Increasing the communication and establishing a dialogue between ID units and emergency rooms, including personalized feedback for the clinicians working in emergency rooms, appears necessary to increase the compliance and to reduce unnecessary catheter use [6, 40]. Assessment of the real necessity of catheters needs to be permanent and should include nurses [49]. Finally, we should also focus our future efforts on overnight hospitalization and among non–emergency specialist physicians.

In conclusion, we have demonstrated through the examples discussed in this review that this pragmatic approach, followed over the course of 30 years, allowed us to reduce morbidity and mortality related to IDs.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Clinical Infectious Diseases

SUPPLEMENT ARTICLE

Many More Microbes in Humans: Enlarging the Microbiome Repertoire

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The proportion of cultured microorganisms is dramatically lower than those predicted to be involved in colonization, acute, or chronic infections. We report our laboratory's contribution to promoting culture methods. As a result of using culturomics in our clinical microbiology laboratories (including amoeba co-culture and shell-vial culture) and through the use of matrix-assisted laser desorption/ionization–time-of-flight and the 16S rRNA gene for identification, we cultured 329 new bacterial species. This is also the first time that 327 of species have been isolated from humans, increasing the known human bacterial repertoire by 29%. We isolated 4 archaeal species for the first time from human, including 2 new species. Of the 100 isolates of giant viruses, we demonstrated the human pathogenicity of Mimivirus in pneumonia and Marseillevirus in diverse clinical situations. From sand flies, we isolated most of the known Phlebovirus strains that potentially cause human infections. Increasing the repertoire of human-associated microorganisms through culture will allow us to test pathogenicity models with viable microorganisms.

Keywords. human microbiota; culturomics; giant viruses; 16S rRNA amplification and sequencing; MALDI-TOF.

Only the tip of the species constituting the microbial iceberg are currently known, reflecting the fact that "we know only what we seek." Of the 10 million predicted bacterial species [1], only 14 300 have been cultured, which is quasi-similar for archaeal species, viruses, and eukaryotes [2]. If we do not expand the spectrum of the repertoire, we risk missing many species with clinical involvement, as has long been the case with rickettsial diseases [3]. Expanding the microorganism repertoire should include both commensal species and species detected in the human environment, including inanimate environments and animals, as those species may eventually become pathogenic in certain conditions. By means of example, 39 years passed between the first description of *Rickettsia parkeri* and the first human case being described in 2004 [3]. Most of the studies performed on the human microbiota repertoire have mainly focused on bacterial diversity, but the repertoire of the Eukaryota, Archaea, and viruses including giant viruses associated with humans has also dramatically increased in recent years [4]. It remains essential to present the case reports of the newly or rarely prokaryotes isolated in humans and to increment the database allowing the identification of microorganisms (16S ribosomal [rRNA] and matrix-assisted laser desorption/ionization time-of-flight [MALDI-TOF] spectra databases) to know whether the microbes isolated once in humans belong or not to the human microbiome.

To promote the rebirth of culture methods in microbiology [5, 6], in this article we propose a comprehensive review of our laboratory's contribution of the past 30 years to increasing the repertoire of human-associated microorganisms.

REPERTOIRE

Environment

Natural Environment

Our laboratory has isolated 20 new bacterial species from a variety of natural environments including 5 from hypersaline lakes, 5 from the sea, lakes, and fresh water (including 2 new species of Planctomycetes phylum), 4 new species from air samples, 3 new *Mycobacterium* species from the soil, 2 new species from salted food, and 1 new species from permafrost (Table 1; Supplementary Table 1).

A culture-based approach has been applied to characterize the fungal communities in Africa and the Mediterranean area, in the soil [7, 8] and the hospital setting [9, 10].

Amoeba

The capacity of amoeba-resistant microorganisms can be used to isolate both bacteria and viruses from the environment. We isolated giant viruses from the soil and from water (sea water, oysters, rivers, sewage water, lakes, sediments, hospital water supplies, contact lens solutions) (Table 2). We first describe the

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Table 1. Number of Bacterial Species and Subspecies Isolated at URMITE[a]

	Bacterial Species Isolated From the Environment						Bacterial Species Isolated From Humans			
	Only in Inert Environment	Only in Animals	Only Environment Using Co-culture Amoeba	Total of Environmental Bacteria Isolated by Our Laboratory	Co-culture Amoeba From Environment Then Humans	Fastidious Bacteria in Animals Then (and/or) Humans	Human (Human Gut Repertoire by Culturomics)	*Mycobacterium* spp, Fastidious, and Bacteria Isolated by Co-culture Amoeba (Only in Human Samples)	Other Species From Clinical Microbiology Laboratory	Total Human Bacteria Isolated by Our Laboratory
New bacterial species	20	24	18	62	3	18	247	18	43	329
First isolated in humans	NA	NA	NA	NA	1	1	269	5	51	327
Total	20	24	18	62	4	19	516	23	94	656

Abbreviations: NA, not available; URMITE, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes.

[a]This includes new bacterial species and subspecies isolated from the environment and from humans, and bacterial species isolated for the first time from humans (detailed list is available in Supplementary Tables 1–4)

existence of giant viruses associated with protozoa [11]. The first isolation was performed using the shell vial assay from cooling tower samples [12]. After this, we modified the protocol using microplates [11] then used a high-throughput system with amoeba monolayers on nonnutrient agar [13]. Our most recent developments using extended panels of protozoa associated with flow cell cytometry should increase the rate with which known and new giant viruses are isolated [14].

Using co-culture amoeba, we isolated 22 bacterial species (including 21 new bacterial species) from water or air-conditioning samples, including water from hospital networks comprising 6 new *Legionella* species and a new phylum (*Babela massiliensis*) (Table 1; Supplementary Tables 1 and 2). Although some giant viruses have been isolated with their protozoal hosts, such as the *Cafeteria roenbergensis* virus, we isolated an amoeba from a human sample suspected of harboring a giant virus superinfected by a virophage [15].

Plants

We cultured Pepper mild mottle virus (PMMoV) by inoculating 3 pepper-derived food items (spicy powder, pepper, and Tabasco) on *Nicotiana tabacum* plants, as demonstrated by the development of typical symptoms on tobacco leaves and viral detection in lesions [16]. In addition, tobacco mosaic virus was cultured on tobacco plants inoculated with cigarette tobacco, from 18 of the 34 (53%) samples. Our study linked the presence of PMMoV RNA in stools with a specific immune response and clinical symptoms, suggesting the possibility of a direct or indirect pathogenic role for plant viruses in humans [16].

Animals

Arthropods

We isolated 23 different rickettsial species from arthropods including from *Ixodes ovatus* (*Rickettsia asiatica*), *Ixodes persulcatus* (*Rickettsia tarasevichae*), *Amblyomma testudinarium* (*Rickettsia tamurae*), *Rhipicephalus sanguineus*, *Hyalomma* species, and *Dermacentor* species. *Bartonella massiliensis*, *Bartonella senegalensis*, and *Occidentia massiliensis* were isolated from *Ornithodoros sonrai* whereas *Rickettsia felis* was cultured from fleas (Table 1; Supplementary Tables 1 and 2).

A relative of Marseillevirus that we named Insectomime was isolated from the internal tissues of the diptera *Eristalis tenax* larva collected in Tunisia and inoculated on *Acanthamoeba polyphaga*. We also isolated a faustovirus from a batch of crushed *Culicoides* species, inoculated on *Vermamoeba vermiformis* (Table 2).

From mosquitoes, new insect-specific flaviviruses were isolated, characterized by complete genome sequencing and evolutionary studies [17–21]. Over the past decade, multidisciplinary integrated projects have isolated a large number of phleboviruses transmitted by phlebotomine sand flies from the Old World. Of the 32 strains isolated over the last decade, 23 were obtained in our laboratory: Punique, Massilia, Adana, Toros, Zerdali, Medjerda Valley, and Dashli viruses, among others. Genetic and phylogenetic analysis using such sequences have made it possible to design, evaluate, and stockpile real-time reverse-transcription polymerase chain reaction (PCR) assays targeting individual viruses or groups of viruses to be used for diagnostic purposes in clinical and research laboratories. These systems are all available through the European Virus Archive collection (www.european-virus-archive.com/) [22–27]. Interestingly, the systematic inoculation of sand flies collected in Ecuador resulted in the isolation of a novel flavivirus, which is, thus far, the only representative of its evolutionary lineage [28, 29] (Table 3).

Other Animals

We isolated 5 new bacterial species from the gorilla gut. In addition, we cultured 8 new *Bartonella* species from rodents

Table 2. Isolation of Giant Viruses in Our Laboratory

Source	Name	Classification	Sample(s), Clinical Involvement	Pathogenicity	Country	Reference, Year
Environment	Mimivirus plus ~100 other isolates	Mimiviridae	Cooling tower water, fresh water, sea water, soil		England, France, Tunisia, Brazil	[11], 2003
	Marseillevirus plus ~30 other isolates	Marseilleviridae	Cooling tower water, fresh water, sea water, soil		France, Tunisia, Brazil	[72], 2009
	Faustovirus plus 8 other isolates	Faustovirus	Sewage		France, Senegal, Lebanon	[73], 2014
	Hirudovirus	Mimiviridae	Leech, internal organs, and digestive tract		Tunisia	[74], 2013
	Insectomime virus	Marseilleviridae	*Eristalis tenax* larva obtained from stagnant water reservoirs		Tunisia	[75], 2013
Human	Lentillevirus, Sputnik 2 virophage	Mimiviridae, Lavidaviridae	Contact lens storage case liquid from a 17-year-old myopic woman with keratitis	Probable	France	[15], 2011
	Mimivirus	Mimiviridae	Serum. Pneumonia and seroconversion in a technician manipulating large amounts of mimivirus	Yes	France	[62], 2006
	LBA111 virus	Mimiviridae	Bronchoalveolar fluid from a 72-year-old Tunisian woman	Yes	Tunisia	[63], 2013
	Shan virus	Mimiviridae	Stools from a 17-year-old Tunisian girl	Yes	Tunisia	[64], 2013
	Senegalvirus	Marseilleviridae	Stools from a 20-year-old Senegalese man, living in rural Senegal	Unknown	Senegal	[31], 2012
	Giant blood Marseillevirus	Marseilleviridae	One healthy blood donor (metagenomic, PCR, FISH, lymphocyte culture, TEM, serology) + 20 blood donors (serology, PCR)	Unknown	France	[76], 2013
	Marseillevirus	Marseilleviridae	Blood donor's serum and multitransfused (serology, PCR)	Unknown	France	[77], 2013
	Marseillevirus	Marseilleviridae	Adenitis in an 11-month-old boy (lymph node + serum: serology, PCR, FISH, IF, IHC)	Primary infection (?)	France	[65], 2013
	Marseillevirus	Marseilleviridae	Hodgkin lymphoma (lymph node + plasma: serology, PCR, FISH, IF, IHC)	Yes	France	[66], 2016
	Marseillevirus	Marseilleviridae	Neurological disorders, pharynx and blood (serology, PCR in blood and pharynx)	Probable	France	[67], 2016

Abbreviations: FISH, fluorescence in situ hybridization; IF, immunofluorescence; IHC, immunohistochemistry; PCR, polymerase chain reaction; TEM, transmission electron microscopy.

and 1 from a kangaroo (*Bartonella australis*). We isolated 1 new species from a lizard (*Streptococcus varani*) and one from a lungfish (*Chryseobacterium senegalense*). Finally, we cultured *Lactobacillus ingluviei* from ostriches whose inoculation led to weight gain and liver enlargement in mice (Table 1; Supplementary Table 1).

Hirudovirus, a mimivirus strain, was isolated by inoculating the processed internal organs and digestive tract of a leech, collected in Tunisia, on a culture of *Acanthamoeba polyphaga* (*Hiruda medicinalis*) (Table 2).

Finally, a culture-based approach was used to analyze the gut mycobiota of yellow-legged gulls (*Larus michahellis*) in 5 breeding colonies spread along the Mediterranean coast in southern France, which have been studied for the first time. Seventeen distinct yeast species were identified, including antifungal-resistant opportunistic pathogenic yeast species such as *Candida albicans*, *Candida glabrata*, and *Candida krusei* [30].

Commensal Microorganisms in Humans

Bacteria

The known repertoire of human-associated bacteria is limited and includes only 2200 cultured commensal and pathogenic bacteria, of which approximately 30% were first discovered in our institute [4]. The dramatic increase in interest in the microbiota, initially based only on metagenomic studies, led

Table 3. Isolation of Viruses

Genus	Group	Virus	Isolation/ Detection	Viral Source	Country	References	Full-Length Sequence	NT Abs in Humans	Human Pathogen
Phlebovirus	Sandfly fever, Naples species	Zerdali virus	Isolation	*Phlebotomus* spp	Turkey	[23]	Yes	Unknown	Unknown
Phlebovirus	Sandfly fever, Naples species	Massilia virus	Isolation	*Phlebotomus perniciosus*	France	[27]	Yes	Yes	Unknown
Phlebovirus	Sandfly fever, Naples species	Punique virus	Isolation	*Phlebotomus papataci*	Tunisia	[26]	Yes	Yes	Unknown
Phlebovirus	Sandfly fever, Sicilian species	Toros virus	Isolation	*Phlebotomus* spp	Turkey	[23]	Yes	Unknown	Unknown
Phlebovirus	Sandfly fever, Sicilian species	Dashli virus	Isolation	*Phlebotomus* spp	Iran	Alkan et al (unpublished data)	Yes	Yes	Yes
Phlebovirus	Salehabad species	Medjerda Valley virus	Isolation	*Phlebotomus* spp	Tunisia	[24]	Yes	Yes	Unknown
Phlebovirus	Salehabad species	Balkan virus	Detection	*Phlebotomus* spp	Albania	[78]	L segment partial	Yes	Yes
Phlebovirus	Salehabad species	Adana virus	Isolation	*Phlebotomus* spp	Turkey	[25]	Yes	Yes	Unknown
Flavivirus	Ecuador Paraiso, Escondido species	Ecuador Paraiso, Escondido virus	Isolation	*Psathyromyia abonnenci*	Ecuador	[28]	Yes	Unknown	Unknown

us to develop a new strategy known as culturomics, to complete the human gut repertoire [31]. This study began in 2010 when only 690 bacterial species had been cultivated from the human gut [32]. Our strategy has consisted in multiplying culture conditions (up to 212 different ones) [31], with bacterial identification using MALDI-TOF, enabling rapid and cost-effective identification and allowing large number of colonies to be tested [33, 34] (Figure 1). Unidentified bacteria and bacteria previously unknown in humans were tested by 16S rRNA amplification and sequencing to be identified [35, 36]. The bacteria with 16SrRNA gene sequence similarity <98.7% were considered as new bacterial species and those with <95% similarity as new bacterial genera [37] (Figure 1). Overall, culturomics has enabled us to identify 247 new bacterial species from the human gut, 269 species isolated for the first time from humans, and 250 species isolated for the first time from the human gut (Figure 2). This has more than doubled the number of species cultured from the human gut [32]. We are currently performing similar culturomics studies from vaginal, urine, skin, and respiratory tract samples.

In addition, genome sequencing of the new species isolated by culturomics completed the database used for the analysis of metagenomic studies, shedding light on part of the unidentified genes usually known as "dark matter" [32, 38]. As we progressively generated several millions of spectra, the increase in the MALDI-TOF databases allowed us to easily and rapidly identify previously unknown bacterial species, which had previously been impossible to identify using classic phenotypic identification methods (Figures 1 and 2). Finally, the influence of bacteria in cancer immunotherapy effects [39], in the control of body weight, in malnutrition [40], or in obesity could be tested using models including viable bacteria rather than only bacteria deduced by their sequence.

This rapidly increasing number of new bacterial species led us to first propose a new polyphasic strategy to describe novel bacterial organisms, known as taxonogenomics, which combined phenotypic and biomolecular structural data (including MALDI-TOF spectra) and genome sequencing [37] (Figure 1). This approach has the advantage, compared with DNA-DNA hybridization and chemotaxonomic methods, of exhibiting great intra- and interlaboratory reproducibility. However, a complete description of a novel organism requires a valid publication, which can be a time-consuming process. To make the new species available as quickly as possible to the international scientific community, we then used the model of the genome announcement made to address the growing number of sequenced genomes [41] to create a format known as the "new species announcement," including brief phenotypic data, 16S rRNA GenBank accession number, and deposition number in our international collection of strains [42].

Archaea

Our protocol for culturing methanogens yielded a previously unknown methanogen, *Methanomassiliicoccus luminyensis* [43], the largest methanogen harboring the largest genome ever isolated in humans [44] and the representative of a completely new seventh order of methanogens [45]. This protocol also resulting in the isolation of all 6 methanogens currently cultured from the human gut microbiota, including 4 new isolations (*Methanobrevibacter millerae*, *M. luminyensis*, *Methanobrevibacter oralis*, *Methanobrevibacter arboriphilicus*) [46] (Table 4). In addition, the 2 sole human halophilic archaea (*Haloferax alexandrinus* and *Haloferax massiliensis*) were isolated in our laboratory [32] (Table 4). We recently performed an aerobic culture of *Methanobrevibacter smithii* and *M. oralis*, after we designed a device to simplify the culture-based detection of

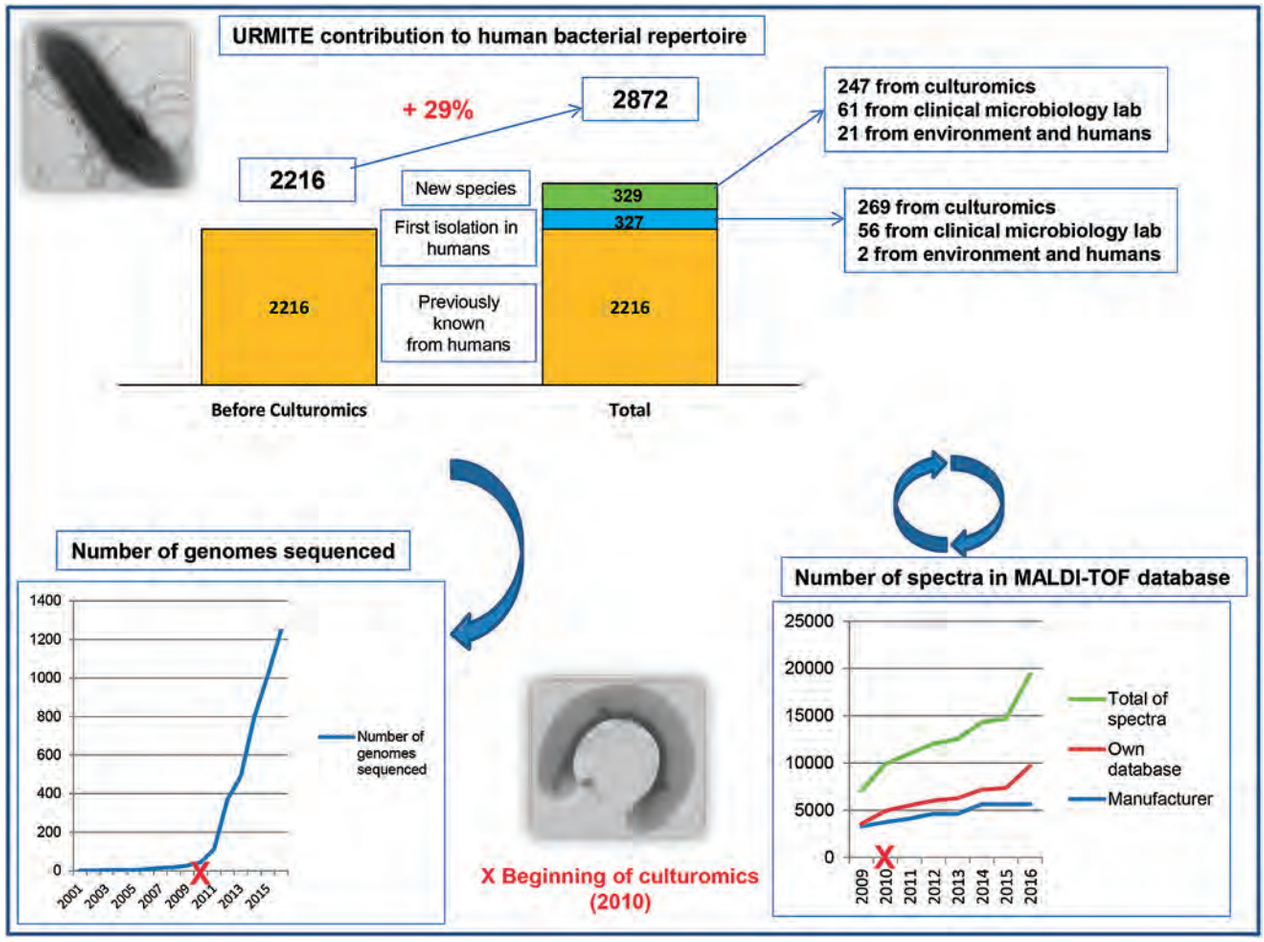

Figure 1. URMITE's contribution to increasing the human bacterial microbiota repertoire. Abbreviations: MALDI-TOF, matrix-assisted laser desorption/ionization–time of flight; URMITE, Unité de Recherche sur les Maladies Infectieuses et Tropicales Emergentes.

methanogens for every clinical microbiology laboratory with a view to expanding the limited knowledge we have of the repertoire of these archaea in microbiota and to further explore their potential as pathogens in mixed infections [47] (Table 4).

Giant Viruses

Using the culture procedures that are implemented primarily for environmental samples, giant viruses have been isolated from humans since 2012. A close relative of Marseillevirus, known as Senegalvirus, was the first giant viral strain retrieved from a human. This virus was isolated on *A. polyphaga* from the feces of a healthy young man in Senegal [31]. Another Marseillevirus, known as Giant Blood Marseillevirus, was then transiently grown by inoculating the Marseillevirus DNA–positive serum of a blood donor on Jurkat cells, an immortalized line of human T-lymphocyte cells [48] (Table 2).

Eukaryotes

Although fungi represent an important part of the eukaryotic microorganisms in the human gastrointestinal tract, few studies focused on the fungal repertoire of the human gut using culture [49, 50]. Of the 278 fungal species reported in the human gut, only 75 (27%) were detected using culture-dependent methods [51], demonstrating that a great deal of work is currently needed to improve fungal isolation from human microbiota. We conducted a first culturomics investigation of a stool sample from an obese person, which led to the isolation of 16 fungal species [50]. Of these species, 8 were not previously isolated or detected in the human gut, including 6 ascomycetes (*Aspergillus flavipes*, *Penicillium brevicompactum*, *Beauveria bassiana*, *Penicillium dipodomyicola*, *Penicillium camembertii*, and *Isaria farisona*) and 2 basidomycetes (*Climacocystis* species and *Malassezia restricta*) (Table 5). In a second work, 11 fungi were isolated from 7 fecal samples collected in 4 tropical countries [49] (Table 5). These comprised 2 ascomycetes species (*Davidiella tassiana* and *Davidiella* species) not previously described in the human gut [49]. More recently, we applied culturomics to 14 stool samples; the analysis resulted in the identification of 38 fungal species, 13 of which had never previously been found

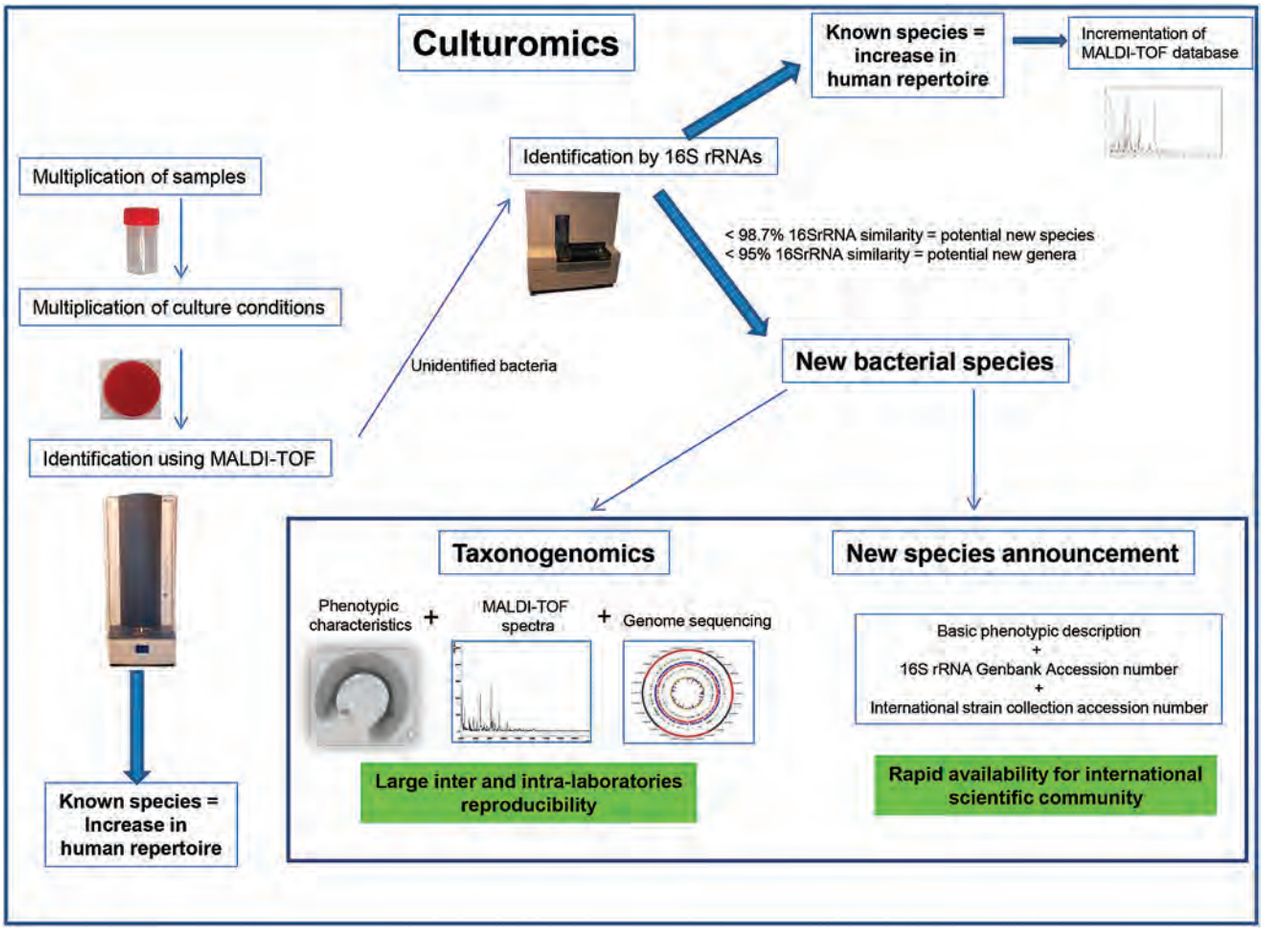

Figure 2. Principle of culturomics to exploring the human microbiota and description of taxonogenomics and new species announcement to describe new bacterial species.Abbreviations: MALDI-TOF, matrix-assisted laser desorption/ionization–time of flight; rRNA, ribosomal RNA.

in the human gut, and 2 fungal species, *Penicillium glandicola* and *Ascosphaera apis*, which had never before been isolated from clinical samples (unpublished data). Further work is required to develop new and well-adapted media to isolate fungi, especially for Basidiomycota and Zygomycota, and to standardize the fungal culturomics procedure as it has been applied for bacterial culturomics.

PATHOGENS IN HUMANS

Bacteria

Twenty-five bacterial species (including 23 new species and subspecies) were isolated from the environment and were also considered as human pathogens. This included 4 bacterial species isolated using co-culture amoeba (*Bosea massiliensis*, *Bradyrhizobium massiliensis*, *P. acanthamoeba*, and *Legionella*

Table 4. Archaeal Species Cultured in Our Unit

Group	Name	Type of Species	Reference	Pathogenicity
Methanogens archaea	*Methanomassiliicoccus lumyniensis*	New species	[43]	Unknown
	Methanobrevibacter smithii	Previously known from the human gut	[46]	Severe periodontitis [56], paravertebral abscess [57]
	Methanosphaera stadtmanae	Previously known from the human gut	[46]	Unknown
	Methanobrevibacter millerae	First isolated in the human gut	[46]	Unknown
	Methanobrevibacter arboriphilicus	First isolated in humans	[46]	Unknown
	Methanobrevibacter oralis	First isolated in the human gut	[46]	Unknown
Halophilic archaea	*Haloferax massiliensis*	New species	[32]	Unknown
	Haloferax alexandrinus	First isolated in humans	[32]	Unknown

Table 5. Fungal Species Isolated From Human Stool Samples Using Culturomics in Our Unit

Classification	Species	Description in Human Gut	Reference	Sample Country/Source
Ascomycota	*Aspergillus flavipes*	First isolation	[50]	France
Ascomycota	*Aspergillus versicolor*	Previously known	[50]	France
Ascomycota	*Beauveria bassiana*	First isolation	[50]	France
Ascomycota	*Candida albicans*	Previously known	[49]	Amazonia, Polynesia, India
Ascomycota	*Candida glabrata*	Previously known	[49]	Amazonia
Ascomycota	*Candida tropicalis*	Previously known	[50]	France
Ascomycota	*Cladosporium* sp	Previously known	[50]	France
Ascomycota	*Clavispora lusitaniae*	Previously known	[49]	India
Basidiomycota	*Climacocystis* sp	First isolation	[50]	France
Basidiomycota	*Corticiaceae* sp	First isolation	[49]	India
Ascomycota	*Davidiella tassiana*	First isolation	[49]	Amazonia
Ascomycota	*Davidiella* sp	First isolation	[49]	Polynesia
Ascomycota	*Debaryomyces hansenii*	Previously known	[49]	Polynesia
Ascomycota	*Hypocrea lixii*	Previously known	[50]	France
Ascomycota	*Isaria farinosa*	First isolation	[50]	France
Ascomycota	*Galactomyces geotrichum*	Previously known	[50]	France
Basidiomycota	*Malassezia globosa*	Previously known	[50]	France
Basidiomycota	*Malassezia restricta*	First isolation	[49, 50]	France, Polynesia, India
Basidiomycota	*Malassezia pachydermatis*	Previously known	[50]	France
Basidiomycota	*Malassezia* sp	Previously known	[49]	India
Ascomycota	*Penicillium allii*	Previously known	[50]	France
Ascomycota	*Penicillium brevicompactum*	First isolation	[50]	France
Ascomycota	*Penicillium camemberti*	First isolation	[50]	France
Ascomycota	*Penicillium dipodomyicola*	First isolation	[50]	France
Ascomycota	*Penicillium* sp	Previously known	[49]	Polynesia, India
Basidiomycota	*Trichosporon asahii*	Previously known	[49]	Polynesia, India

drancourtii) associated with pneumonia [52]. Seroconversion for *Diplorickettsia massiliensis* and quantitative PCR (qPCR) detection of *Arsenophonus nasoniae* have suggested human infections (Table 1; Supplementary Table 2). Finally, 18 rickettsial species and subspecies detected from ticks and fleas were associated with human infections (Supplementary Table 2). One of them, *R. felis*, is an emerging pathogen commonly detected in febrile patients in sub-Saharan Africa, which causes an eruptive fever known as "yaaf" [53]. In addition, we described a syndrome characterized by scalp eschars and neck lymphadenopathy following tick bites known as SENLAT and caused by *Rickettsia slovaca* and *Rickettsia raoultii* or, occasionally, by *Bartonella henselae* [54].

Our expertise, comprehensively using the shell-vial culture assay followed by a centrifugation shell-vial technique called JNSP for *je ne sais pas* ("I don't know [what I am growing]") also enabled the first culture of *Tropheryma whipplei* in 2000. Fifty different *T. whipplei* strains have now been established from diverse human fluids and tissues, increasing the understanding of *T. whipplei* infections and enabling us to propose evidence-based antibiotic protocols supported by in vitro observations and clinical outcomes [55]. We also described the fastidious culture of other causative agents of endocarditis (*Bartonella mayotimonensis*, *Bartonella alsatica*, *Bartonella vinsonii* subspecies *arupensis*, and *B. vinsonii* subspecies *berkhoffi*) (Supplementary Table 3).

Of the 11 new mycobacterial species that we isolated from humans, 5 are considered to be pathogenic for humans (*Mycobacterium barassiae*, *Mycobacterium bolletii*, *Mycobacterium conceptionense*, *Mycobacterium massiliense*, and *Mycobacterium tahitimassiliense*) (Supplementary Table 3). Finally, we reported the culture of 43 new bacterial species and 51 bacterial species first isolated from human samples and identified using both 16S rRNA amplification and sequencing and MALDI-TOF [33, 36] as bacterial identification tools (Table 1; Supplementary Tables 3 and 4). For these species of bacteria, only time will tell if they are commensal or pathogenic (Supplementary Tables 3 and 4). In terms of MALDI-TOF identifications in routine laboratory work from 2011 to 2016, 12 different species primarily isolated by culturomics were subsequently isolated in pathological circumstances, enabling the microbiological documentation of 57 cases [32].

Archaea

Likewise, a culture-based comparison of the microbiota in patients with severe periodontitis and controls yielded *M. oralis* in both groups, *M. smithii* in 2 patients, and a new species, known as *Methanobrevibacter* species strain N13, in patients with periodontitis [56] (Table 4). In addition, we recently successfully cultured *M. smithii* and *Bacteroides thetaiotaomicron* from a chronic paravertebral muscle abscess in a 41-year-old man [57].

Viruses

Although the Toscana virus is a recognized agent of meningitis, encephalitis, and peripheral neuroinvasive manifestations, the number of complete genome sequences is strikingly low. Accordingly, in collaboration with the French Reference Centre for Arboviruses, we decided to isolate and sequence all strains of the Toscana virus to better understand its genetic diversity. Thus far, all but 1 of the 15 genomes were sequenced in our laboratory [26, 58–61]. In addition, clinical material received by the Arbovirus Reference Center has been inoculated onto Vero and C6/36 cells, and isolates are being completely sequenced using a systematic approach. Since 2012, a total of 272 strains of arboviruses, mainly belonging to the *Flavivirus* and *Alphavirus* genera, have been isolated.

Giant Viruses

The first strong evidence of human infection with a giant virus was observed in a laboratory-based infection of a technician who handled large quantities of Mimivirus and who developed unexplained pneumonia with seroconversion [62]. Two mimiviruses were then isolated on *A. polyphaga* from 2 Tunisian patients presenting pneumonia of unexplained etiology. The LBA111 virus was isolated from the bronchoalveolar fluid of a 72-year-old woman [63], and Shan virus was isolated using a high-throughput culture system from the feces of a 17-year-old girl [64]. This bolstered the theory of a causal link between mimiviruses and pneumonia. In addition, a Mimivirus known as Lentille virus and its virophage (Sputnik 2) was concurrently cultured from the contact lens rinse liquid of a patient with keratitis [15].

A high immunoglobulin G titer of Marseillevirus was highlighted in serum from an 11-month-old boy with lymphadenitis. The virus was detected by PCR in his serum and by fluorescence in situ hybridization (FISH) and immunohistochemistry in his lymph node, suggesting a possible primary infection [65]. We also recently identified Marseillevirus in the lymph node of a 30-year-old woman diagnosed with Hodgkin lymphoma, by PCR, FISH, direct immunofluorescence, and immunohistochemistry [66]. Finally, we recently reported the detection of Marseillevirus in the pharynx and the blood of a 20-year-old man with neurological disorders [67].

Eukaryotes

MALDI-TOF mass spectrometry (MS) revolutionized the identification of filamentous fungi in clinical laboratories. While the accuracy of conventional morphological identification does not exceed the species complex level, the accuracy of MALDI-TOF MS–based identification is similar to the nucleotide sequence–based identification gold standard [68]. Enhanced filamentous fungi identification in the clinical laboratory, based on MALDI-TOF-MS technology coupled with an efficient reference spectrum database, is improving our understanding of the epidemiology and clinical importance of these previously unknown fungal species [69]. For example, the Agaricomycetes *Schizophyllum commune*, a common rotten wood saprophyte that was previously considered to be nonpathogenic for humans, is now a recognized chronic fungal rhinosinusitis agent that can cause invasive forms of the disease [70].

CONCLUSIONS

Overall, we isolated 329 new bacterial species from humans and 327 bacterial species that were previously known but isolated from humans for the first time (Table 1; Figure 2). These 656 bacterial species correspond to an increase of >29% of the bacterial repertoire associated with humans (Figure 2). We increased the gut archaeal human-associated repertoire, adding 4 different species, including 2 new species (Table 4). Our laboratory leads the way regarding the culture of giant viruses (with >100 different isolates), strongly suggesting their human pathogenicity (Table 2). More than 70% of the phlebovirus strains transmitted by phlebotomine sand flies that have been isolated in the last decade were isolated in our laboratory. Finally, we have extended the culture of eukaryotes associated with humans, notably from the human gut.

In conclusion, we have demonstrated that the borders between pathogenic and commensal microorganisms and between environmental and human-associated microorganisms are frequently narrow and that only greater efforts to increase the whole microorganism repertoire are needed to better understand infectious diseases [39, 71].

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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Clinical Infectious Diseases

SUPPLEMENT ARTICLE

Medical Entomology: A Reemerging Field of Research to Better Understand Vector-Borne Infectious Diseases

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In the last decade, the Chikungunya and Zika virus outbreaks have turned public attention to the possibility of the expansion of vector-borne infectious diseases worldwide. Medical entomology is focused on the study of arthropods involved in human health. We review here some of the research approaches taken by the medical entomology team of the University Hospital Institute (UHI) Méditerranée Infection of Marseille, France, with the support of recent or representative studies. We propose our approaches to technical innovations in arthropod identification and the detection of microorganisms in arthropods, the use of arthropods as epidemiological or diagnostic tools, entomological investigations around clinical cases or within specific populations, and how we have developed experimental models to decipher the interactions between arthropods, microorganisms, and humans.

Keywords. ticks; mosquitoes; fleas; lice; bedbugs.

Arthropods are invertebrate animals encompassing >1 million species and representing >80% of all living animal species [1]. Some of these, which we designate as vectors, can effect the active transmission of a pathogenic microorganism (virus, bacterium, parasite) from one vertebrate to another while taking their blood meal [2]. Mosquitoes are the main vectors of human disease and are known in tropical countries to cause hundreds of individual deaths due to malaria or dengue fever every year. Moreover, in the last decade, the Chikungunya virus (CHIKV) and Zika virus (ZIKV) outbreaks have turned public attention to some examples of the expansion of vector-borne infectious diseases throughout the globe, including parts of the New World [3, 4]. Moreover, the consequences of tick infestation are well known to veterinarians, but their medical importance has reemerged with the description of Lyme disease in the 1980s and the description of several tick-borne rickettsioses over the last 15 years [5]. Ticks are now recognized as the second main vector of human infectious diseases worldwide.

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Understanding vector biology helps anticipate the emergence of vector-borne diseases. The last CHIKV and ZIKV outbreaks are representative of this. Indeed, with such widespread anthropophilic mosquito vectors, and in the context of travelers spreading viruses, it is logical to anticipate related outbreaks. Despite our alerts [4, 6], CHIKV and ZIKV became a "public health emergency of international concern" many months or even years after our first reports [7].

The discipline of medical entomology is focused upon insects, and more globally, arthropods that impact human health. It includes many links with veterinary entomology and environmental sciences, in a "one-health" concept. Knowledge of vector biology, arthropod monitoring, and control of vector populations remains essential to preventing and surveying vector-borne infectious diseases. Research and monitoring in medical entomology are therefore essential in fighting arthropod-borne diseases.

Here, we present a number of research projects conducted by the medical entomology research teams of the University Hospital Institute Méditerranée Infection in Marseille, France. We limit this report to recent or representative studies: technological innovations for arthropod identification and the detection of the microorganisms they carry; the use of arthropods as diagnostic or epidemiological tools; entomological investigations of cases or within specific populations; and experimental models used to decipher the interactions between arthropods, pathogens, and humans.

EMERGENCE OF MALDI-TOF IN ENTOMOLOGY

Vector control remains essential in fighting transmitted diseases. The identification of vectors during entomological investigations, and a knowledge of their distribution, contribute to estimating the risk of infectious diseases in a studied area, and to planning vector control and the protection of exposed populations. In addition, regarding individual cases, the identification of an arthropod collected on a patient is also important. If a vector is recognized, this can influence the care of the patient, but can also suggest an entomological investigation of the case. For example, at our laboratory, we receive ticks collected and sent by both patients and doctors, to evaluate the risks of transmission of human pathogens [8].

Morphological identification based on dichotomic keys is today the most common method used to identify arthropods [2]. However, this approach requires both entomological expertise and comprehensive documentation. Detailed identification keys are available for some arthropods (eg, mosquitoes, ticks) and are generally organized by geographic area [9–11], but access to these documents is limited [12]. Moreover, several events can alter or compromise crucial morphological criteria, such as the deterioration of the specimen during collection or transportation, or the engorgement of the specimen. Morphological criteria can also be absent for the immature stages, but also within a species complex where the species are morphologically indistinguishable [13]. All these events can lead to a misidentification that may have an impact on the interpretation of the infectious risk. Furthermore, in the past 30 years, the number of experts in systematics has decreased, and some arthropod families have become orphans. Indeed, individual entomologists can hardly become experts on a wide range of arthropod families.

During the last 15 years, molecular biology (polymerase chain reaction [PCR], sequencing) approaches have been used for arthropod identification. These methods require molecular biology facilities and, depending on the arthropod, different genes are targeted for the identification [14–17]. Indeed, there are no ideal universal primers to identify any arthropod with certainty, and this approach is completely dependent on the comprehensiveness and reliability of the GenBank database to which the obtained sequences are compared [17].

For the last 4 years, our team has developed the use of matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) for arthropod identification. This technology is based on the thorough comparison of protein profiles of the submitted samples with a database of reference spectra, and has revolutionized the field of clinical microbiology. It is now routinely used for the rapid identification of bacteria and fungi from clinical samples [18]. The use of MALDI-TOF MS for the identification of arthropods involves several steps, including (*i*) determination of the body part of the arthropod which will be used for MS analyses (ideally the smallest, to save the remaining body parts for further analyses); (*ii*) construction of a database of reference protein profiles of definitely identified specimens; (*iii*) completion of blind tests to check if the specimens are correctly identified when compared to the database; (*iv*) continual updating of the database with new species (Figure 1) [19]. We have applied this method to the identification of several hematophagous arthropods: mosquitoes [20, 21], ticks [22], bedbugs, and triatomines (unpublished), using the spectra obtained from their legs, as well as fleas [23] and lice, using their cephalothorax. MALDI-TOF identification was recently validated on sand flies using their thorax, legs, and wings [24]. To date, our database includes reference spectra for >60 arthropod species. This technology has been transferred to our laboratory in Dakar, Senegal, where it enabled the identification of the Ceratopogonidae, which are small insects of veterinary importance whose identification can be challenging [25]. These skills have been taught to laboratory technicians who are now fully trained to identify ticks collected on patients by MALDI-TOF MS, using the legs, while the rest of the body is used for pathogen detection through molecular biology.

More recently, we showed that MALDI-TOF MS was able to distinguish *Rickettsia conorii*–infected *Rhipicephalus sanguineus* ticks and noninfected ticks [26, 27], as well as *Borrelia crocidurae*–infected *Ornithodoros sonrai* ticks [28]. Based on these promising results, MALDI-TOF MS was challenged to detect *Plasmodium* parasites directly from *Anopheles* mosquitoes. Experimentally, *Plasmodium berghei*–infected mosquitoes were successfully distinguished from noninfected mosquitoes based on the spectra from their cephalothorax [29]. Furthermore, the identification of blood meal sources of malaria vectors is key information to obtain to better understand host/vector interactions and malaria epidemiology in endemic areas. Abdomen proteins from *Anopheles gambiae* that were artificially engorged on 7 distinct types of vertebrate blood were submitted for MALDI-TOF MS, resulting in the accurate determination of feeding patterns of freshly engorged mosquitoes up to 24 hours post–blood meal [30].

THE USE OF ARTHROPODS IN CATALOGING VECTOR-BORNE INFECTIOUS DISEASES

Over the last 15 years, the development of molecular tools has enabled the use of arthropods as a tool for epidemiological and geographical monitoring of the microorganisms they carry. This method has been used to obtain specific information regarding the epidemiology of a targeted microorganism, to increase the catalogue of known infectious diseases in a geographical area of interest, and to alert clinicians and microbiologists to the presence of a pathogen in a specific area. As a reference center for rickettsiology, we have used this approach to contribute to

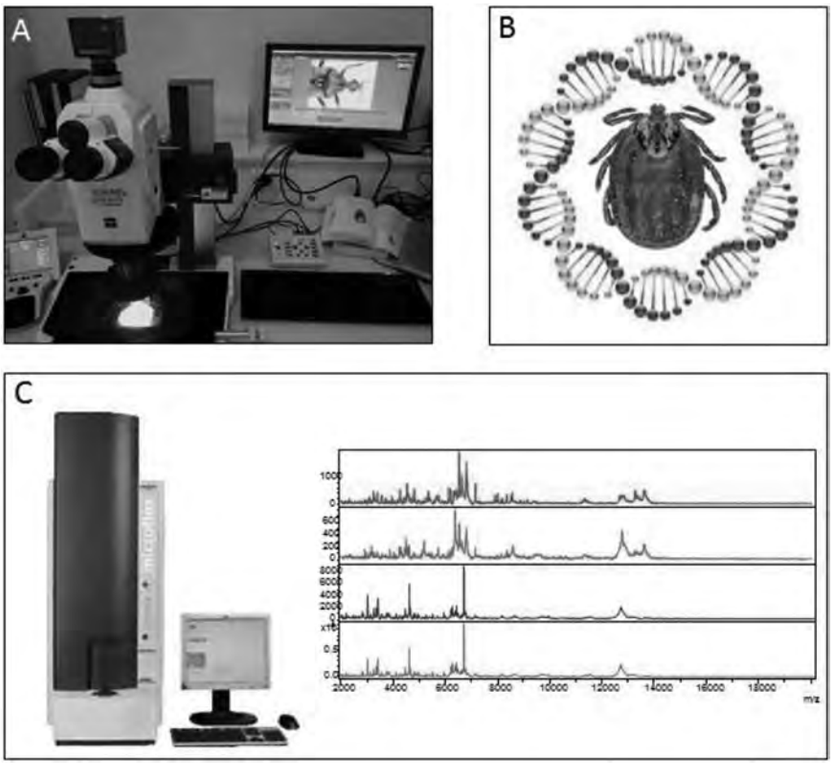

Figure 1. Standard and innovative methods for arthropod identification. *A,* Microscopy platform for morphological identification based on dichotomic keys. *B,* Molecular biology for identification of arthropod-specific genes. *C,* Development of matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry technology for arthropod identification based on protein profiling. Adapted from [19].

the knowledge of rickettsioses [5, 31]. One illustrative example is the description of African tick bite fever in the New World. Having diagnosed a spotted fever group rickettsiosis in a patient who had returned from Guadeloupe, a French Caribbean island, a literature search highlighted the presence of *Amblyomma variegatum*, an African tick introduced to the West Indies in the 18th century along with cattle from Senegal. Known as the vector of *Ehrlichia ruminantium*, the causative agent of bovine cowdriosis, this tick had recently been described as the vector of *Rickettsia africae*, the agent of the human African tick-bite fever in Sub-Saharan Africa. Specific serological analysis targeting *R. africae* confirmed that it was indeed the etiological agent of our index case [32]. An entomological survey enabled the detection and isolation of *R. africae* from *A. variegatum* ticks collected on cattle [33]. Other studies conducted by our team (and others) then confirmed the presence of *R. africae* on neighboring islands [34, 35].

Another example is the major contribution of this approach to the repertoire of rickettsioses in North Africa. In fact, until 1990, only Mediterranean spotted fever, caused by *R. conorii,* was described in this area. With the development and expansion of molecular tools such as PCR and sequencing on arthropods, we identified several new pathogens in ticks collected from North Africa, and therefore complemented the knowledge already collected on arthropod-borne pathogens circulating in Maghreb [5, 36]. We detected *Rickettsia slovaca*, the causative agent of TIBOLA (tick-borne lymphadenopathy), also known as SENLAT syndrome (scalp eschar and neck lymphadenopathy after a tick bite), and several agents of spotted fever group rickettsioses including *Rickettsia aeschlimanni*, *Rickettsia massiliae*, and *Rickettsia monacensis* [36]. When studying fleas from Africa, we also detected *Rickettsia typhi*, the agent of murine typhus and *Rickettsia felis*, an emerging pathogenic *Rickettsia* species [37]. More recently, we detected one of the causative agents of Lyme disease, *Borrelia garinii*, in *Ixodes ricinus* ticks collected in Algeria, where the epidemiology of the disease is not known [5, 38, 39].

In the field of arboviruses, we have used this technique to improve our knowledge on mosquito-borne viruses such as CHIKV and ZIKV, as well as sandfly-borne viruses. Such studies usually complete the description of cases in specific settings, as we did to describe the Toscana virus infection in Southern Europe [40–42]. We demonstrated that this virus was much more widespread than believed, with cases in all countries in North Africa and a presence beyond Europe [43–45]. These techniques also enabled us to detect and/or isolate new viruses from sandflies throughout the world [46, 47]. The latter is a good example of integrated research studies grouping entomologists,

virologists, parasitologists, ecologists, epidemiologists, and medical and veterinary doctors. Classic techniques (cell culture, electron microscopy, seroneutralization) were automated and combined with molecular techniques such as real-time PCR and next-generation sequencing [48]. Collection of phlebotomines in the field (Figure 2) [41] can be oriented from data on (*i*) *Leishmania* parasites, (*ii*) human/canine leishmaniasis cases, (*iii*) seroprevalence results for phleboviruses, and (*iv*) previous data indicative of phlebovirus isolation or detection. During the last decade, our group has discovered and isolated >50% of the newly described viruses transmitted by phlebotomines in the Old World: Punique and Medjerda Valley viruses in Tunisia [48, 49], Massilia virus in France [50], and Zerdali, Toros, and Adana viruses in Turkey [40, 47]. Similar studies have also isolated new viruses in Iran and various countries of the Balkan region (Bosnia-Herzegovina, Albania, Croatia) (unpublished data). Subsequent to the discovery of new viruses, studies aimed at providing evidence for their public health importance are increasingly being conducted; such studies have shown extremely high levels of exposure for populations living in certain regions [51–53].

All this information plays a crucial role in raising the awareness of medical doctors about the presence of pathogens and associated arthropod-borne diseases.

ENTOMOLOGICAL INVESTIGATIONS ASSOCIATED WITH CLINICAL CASES

These investigations have been conducted primarily in the field of rickettsiology, particularly in cases where unusual seasonal or ecological parameters were encountered. Such investigations may enable a better understanding of vector behaviors, as seen when we showed that the aggressiveness toward humans of the brown dog tick, *Rhipicephalus sanguineus*, was significantly greater at warmer temperatures. This may explain why cases of Mediterranean spotted fever caused by *R. conorii* and transmitted by *Rh. sanguineus* are encountered during the warmest months in the Mediterranean area [54]. Also, when investigating 2 cases of infection with *Rickettsia sibirica mongolitimonae* in the same family living in the south of France, we were able to identify *Rhipicephalus pusillus* as the potential vector, which is a tick associated with wild rabbits that can occasionally parasitize dogs and cats. These results may explain the epidemiology of

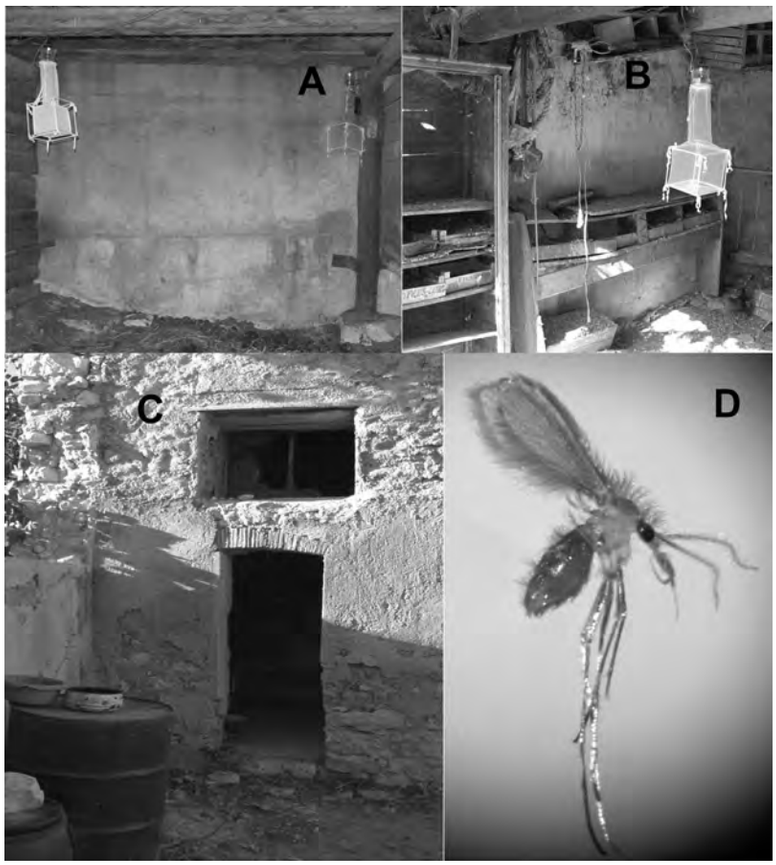

Figure 2. Centers for Disease Control and Prevention light traps, adapted for sandfly trapping. Placed in horse stables (*A*), near rabbit hutches and henhouses (*B*), and in quiet places in the shade of human habitations (*C*), where dogs sleep. *D*, Engorged female *Phlebotomus perniciosus* sandfly trapped in a horse stable. Adapted from [93].

the emerging rickettsiosis in this area, particularly its incidence is in the spring [55].

Our team is also directly solicited by patients following insect and bug bites, thanks to the expertise of one of our team members (J. M. B.). Bedbugs are frequently involved [56]. These hematophagous human parasites have indeed started reappearing since the 1990s. Host reaction to bedbug bites is highly variable, but includes dermatitis [57]. They are closely associated with human dwellings and are easily transported in luggage, allowing the infestation of hotels, boats, trains, etc [58, 59]. Their role in the transmission of vector-borne diseases in the wild is still poorly known. However, recent studies highlighted the vector competence of these bugs in the transmission of *Trypanosoma cruzi* [60] and, following our work, of *Bartonella quintana* [61]. However, investigations in patients' homes sometimes find new bugs that are poorly known both to the public and physicians. Recently, a patient was referred to us by a dermatologist for a suspected bedbug infestation. However, our investigation found straw itch mites, *Pyemotes ventricosus*. These parasites of xylophagous insects colonize wooden furniture, and their exploratory bites are painless but induce a very characteristic linear erythematous macular tract called a "comet sign," which is associated with intense pruritus [62]. We also identified another poorly known pest, the tropical rat mite (*Ornithonyssus bacoti*), as the cause of a patient's multiple bites, which were suffered while working in her office. The building recently underwent rat extermination, resulting in the *O. bacoti* feeding on unusual hosts [63].

ENTOMOLOGICAL INVESTIGATIONS AMONG SPECIFIC POPULATIONS

We have been involved for a long time in surveying and managing infections in the homeless population, particularly louse-borne diseases such as trench fever [64]. In recent years, we have investigated strategies to eradicate lice in the homeless. We demonstrated and characterized the resistance of lice to pyrethrinoids [65]. We carried out a clinical trial on infested clothes, suggesting that permethrin should no longer be used because of its strong ability to select resistance [66]. We also showed that head and body lice on homeless people have the same genotype [67]. Last, we developed molecular tools to distinguish head and body lice [68], which led us later to demonstrate that *B. quintana* is specific to the body louse [69]. Finally, the origins of body and head lice were redefined. Our involvement in tropical areas also enabled the detection of the presence of *B. quintana* DNA in black head lice collected from 3 locations in Senegal [70].

Vector-borne diseases have long been known to severely reduce the fighting capacity of armies, at times causing the suspension or cancellation of military operations. The current situation with many operations overseas increases the risk of vector-borne diseases in deployed troops [71]. We participated in monitoring the entomological status of French military bases in sub-Saharan Africa (Gabon [72] and Ivory Coast [73]) and French Guiana [74]. This monitoring was based on identifying vectors, studying behaviors, and evaluating insecticide resistance. New tools have been developed for vector trapping and the identification of pathogens in vectors, to find the best candidates for vector monitoring for pathogen transmission [75, 76]. Additionally, we participated in the evaluation of antivectorial control programs in several areas, in collaboration with overseas civilian entomology units [77–79].

We showed that the use of remotely sensed environmental and meteorological data enables the prediction of the risk of malaria transmission in Africa [80, 81] as well as dengue fever in French Guiana [82]. Tools have been also developed to evaluate the risk of exposure to vector bites in soldiers by identifying biomarkers of exposure through the analysis of mosquito salivary antigenic proteins, as well as serological responses associated with the level of exposure [83]. These tools enabled the evaluation of the effectiveness of antivectorial strategies, the estimation of the risk of disease transmission, and the monitoring of mosquito populations [84]. Additionally, genetic and environmental contributions to vector competence and viral genotypes or genetic diversity in mosquitoes have been analyzed, to evaluate the risk of emergence of arthropod-borne viruses [85].

Two entomological evaluation missions are being conducted, one in Ivory Coast and one in Gabon, where French troops are based. The main objective will be to evaluate local vector transmission. Catches will be conducted using BG-Sentinel traps and Centers for Disease Control and Prevention light traps; ticks will also be collected. Specimens will be identified using molecular biology and MALDI-TOF technology; pathogens will be sought, as well as resistance markers of the vectors. Finally, the last blood meal will be analyzed to identify the host of the concerned vector (trophic preference). This illustrates the application of all our technologies and research approaches to specific fields and populations.

EXPERIMENTAL MODELS

The molecular detection of a pathogen in a hematophagous vector does not necessarily mean that the arthropod is a competent vector of that pathogen. With the support of our insectary platform, which includes the laboratory rearing of a variety of arthropods (Figure 3), we have the ability to set up experimental models to test the vector competence of arthropods. Often, the first step has been the molecular detection of a microorganism following the cataloging strategy described above, and supported by epidemiological or clinical evidence. For example, *B. quintana*, the causative agent of trench fever, has long been known to be transmitted by body lice [86]. However, we detected this bacterium in bedbugs collected from a prison in Rwanda, raising the question of the potential vector competence of bedbugs for the transmission of *B. quintana* [87].

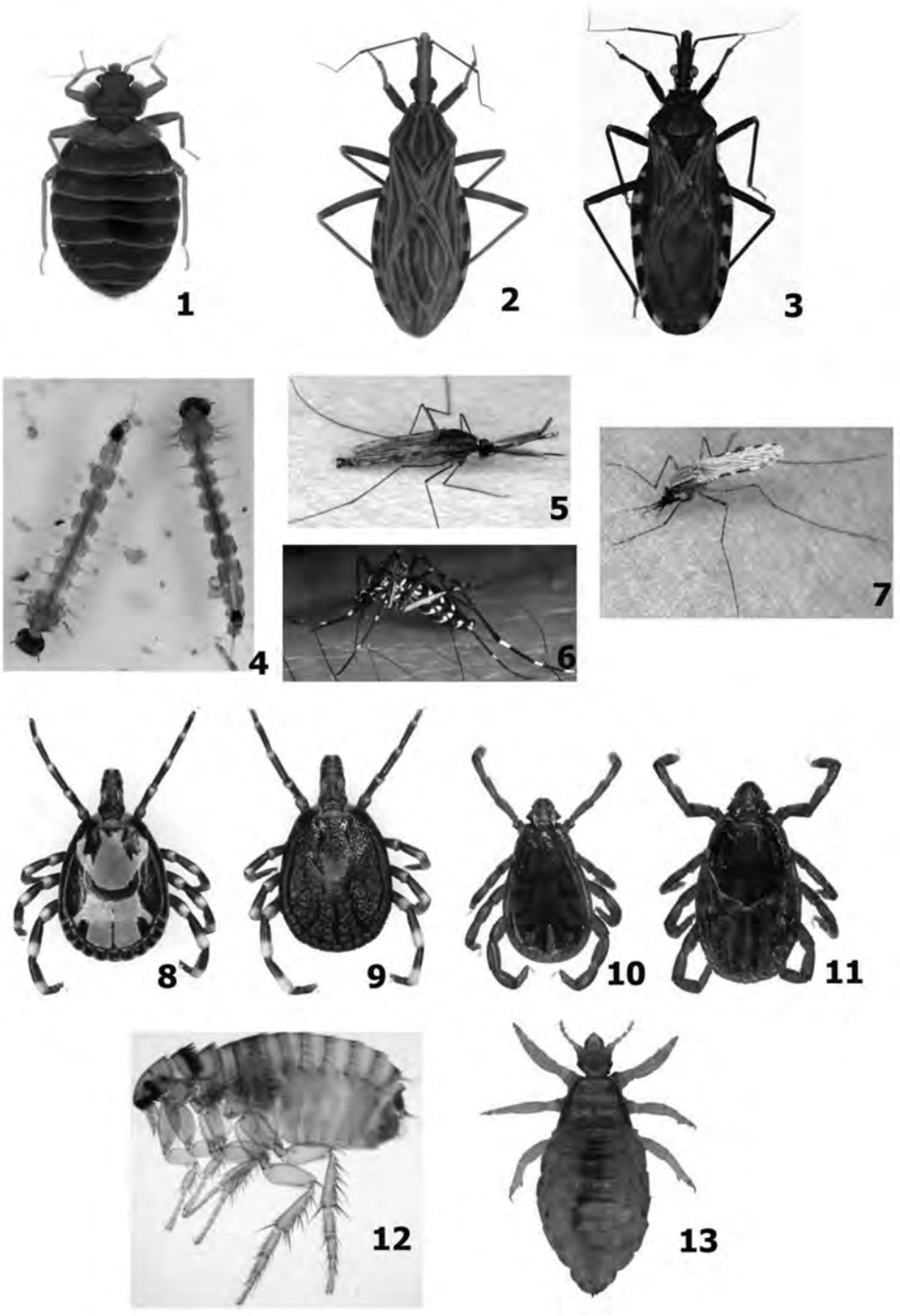

Figure 3. Pictures of arthropods of medical importance reared in the University Hospital Institute Méditerranée Infection insectary: 1. *Cimex lectularius* (bedbug); 2. *Rhodnius prolixus* (*Triatominae*); 3. *Triatoma infestans* (*Triatominae*); 4. *Aedes albopictus* larva; 5. *Anopheles gambiae*; 6. *Aedes albopictus*; 7. *Anopheles stephensi*; 8. Male *Amblyomma variegatum*; 9. Female *Amblyomma variegatum*; 10. Male *Rhipicephalus sanguineus*; 11. Female *Rhipicephalus sanguineus*; 12. *Ctenocephalides felis* (cat flea); 13. *Pediculus humanus corporis* (body louse). Adapted from [19]. See video at https://www.youtube.com/watch?v=BkpWb7CNTQs&sns=tw.

We confirmed this hypothesis with an experimental model using *Cimex lectularius* fed with *B. quintana*–infected blood through an artificial feeding device [61]. Furthermore, these data formed one of the first demonstrations of the potential vector role of bedbugs.

Another illustrative example of an experimental model contributing to knowledge of the emerging pathogen involves *R. felis*, an emerging pathogen described in 2002 [88]. In recent years, a growing number of cases have been reported around the world, and *R. felis* has been detected in several arthropods,

particularly in fleas. However, the only long-established vector has been the cat flea, *Ctenocephalides felis*. In 2012, *R. felis* was detected in mosquitoes in Africa, including *Anopheles* malaria vectors [89]. Moreover, the distribution of fevers of unknown origin associated with *R. felis* (up to 15% of cases) overlapped with malaria-endemic areas [90]. We artificially fed *An. gambiae* mosquitoes with blood or culture medium infected with *R. felis.* The bacterium was later detected in the mosquitoes, particularly in their saliva and salivary glands. Molecular detection of the bacterium's DNA in mice following exposure to *R. felis*–infected *An. gambiae* bites revealed the ability of this mosquito to transmit this *Rickettsia* from one vertebrate to another. This work was the first evidence of transmission of human pathogenic bacteria by mosquitoes and introduced *An. gambiae* as a potential vector of *R. felis* in Africa [91].

Last, an experimental model of infection of *Phlebotomus perniciosus* with bioluminescent *Leishmania infantum* parasites highlighted the variability in infection intensity due to several factors such as the vector and the parasite species, but also the infection method. Artificial feeding was described as the most efficient approach to obtain high parasite loads in the exposed flies [92].

Experimental models also enabled us to assess the powerful antifeeding and insecticidal efficiency of the dinotefuran-permethrin-pyriproxyfen ectoparasiticide on *Triatoma infestans.* Indeed, *T. infestans* bugs are vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease, for which dogs are reservoir hosts. The prevention of domestic infection of dogs is a crucial step in the protection of domestic animals and humans (unpublished data).

PERSPECTIVES

The medical entomology studies conducted by our team have enabled us to decipher many aspects of vector-borne human diseases. The vast majority of our master's and doctoral students involved in these studies come from developing countries, predominantly Africa. Most of them are financially supported by the Méditerranée Infection Foundation, and plan to create research units in their countries after being trained in Marseille.

The performance of our insectarium is based on the large diversity of arthropods we breed. It enables quick answers to epidemiological and clinical questions involving known and emerging pathogens or arthropod vectors, by developing experimental models and entomological surveys. The MALDI-TOF tool is continually challenged with identifying new arthropods as well as with the additional detection of their associated microorganisms. Indeed, we recently developed the MALDI-TOF identification of triatomines, the giant bugs responsible for the transmission of *T. cruzi*, the etiological agent of Chagas disease in the Americas. We also aim to assess the performance of MALDI-TOF in the concomitant identification of 2 vector/pathogen couples, mosquitoes/*Dirofilaria* species and *Ct. felis* fleas/*Bartonella* species. Moreover, to improve our knowledge of vector-borne diseases, we are still conducting experimental models of infection. We have shown that *An. gambiae* was able to transmit *R. felis,* but this bacterium was detected in *Aedes albopictus* from Gabon by quantitative PCR, and *Ae. albopictus* cells support *R. felis* growth. These elements raise the question of the ability of *Ae. albopictus* mosquitoes to transmit *R. felis* [7]. This question will be answered by an experimental model of infection where *Ae. albopictus* mosquitoes will be put in a situation to reveal the acquisition and transmission of *R. felis.* Finally, in the national context of the French plan for a better understanding and knowledge of Lyme disease and other tick-borne diseases, we plan to create a so-called tick clinic, where patients bitten by ticks and suspected of having been infected by a tick-borne agent will have access to a doctor, microbiologists, and entomologists to investigate their condition.

Notes

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Clinical Infectious Diseases

SUPPLEMENT ARTICLE

New Laboratory Tools for Emerging Bacterial Challenges

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Since its creation, the Méditerranée-Infection foundation has aimed at optimizing the management of infectious diseases and surveying the local and global epidemiology. This pivotal role was permitted by the development of rational sampling, point-of-care tests, and extended automation as well as new technologies, including mass spectrometry for colony identification, real-time genomics for isolate characterization, and the development of versatile and permissive culture systems. By identifying and characterizing emerging microbial pathogens, these developments provided significant breakthroughs in infectious diseases.

Keywords. clinical microbiology; syndrome-based sampling; mass spectrometry; real-time genomics; point-of-care.

The main aim of clinical microbiology is the identification of bacterial, viral, fungal, and parasitic agents that cause human diseases, to enable an optimized clinical management of patients as well as to prevent infectious disease transmission [1]. Over the past 30 years, in addition to culture that remains irreplaceable in routine clinical microbiology, we have implemented many new tools, including improved sampling and culture strategies, molecular methods (polymerase chain reaction [PCR], high-throughput genome sequencing) [2], and matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry (MALDI-TOF MS) to improve our diagnostic output. These technological advances have unveiled a much larger human-associated microbiota than was expected. Herein, we review the main progress that we have developed and/or adapted to the diagnosis of infectious diseases in our clinical microbiology laboratory, notably in the fields of syndrome- and disease-based sampling kits, new culture approaches, direct pathogen detection, point-of-care (POC) testing, and clinical isolate identification. We also propose our view of the clinical microbiology laboratory (CML) of the future.

SYNDROME-BASED SAMPLING

The number of recognized pathogens that might be producing causal effects in many types of infections have abruptly increased over the past decades. The diversity and heterogeneity of these pathogens would require multiple or extensive sampling procedures, thus reducing the time-effectiveness for diagnosis. To avoid this risk, we developed standardized kits that enabled optimized sampling and testing of panels of the most likely microorganisms. The design of each test panel is based on the prevalence of the pathogens involved in the syndrome and the use of the most efficient techniques for their identification (Figure 1). In addition, each kit contains the tubes and/or vials required to collect the various types of specimens needed both for direct detection of pathogens by culture, molecular assays, and/or pathological examinations, and for indirect detection, mainly represented by serological testing. To date, we have developed syndrome-based diagnostic kits for endocarditis, pericarditis, pneumonia, diarrhea, osteomyelitis, meningitis, encephalitis, uveitis, and keratitis [3–6]. By significantly reducing the delay until the final diagnosis and enabling an early adequate antimicrobial therapy, shorter hospital stay, and suppression of unjustified treatments in cases of viral infections, syndrome-based sampling is cost-effective.

CULTUROMICS AND THE RENEWAL OF CULTURE

The implementation of molecular detection methods in CMLs resulted in the idea that culture would soon be useless. However, the culture of microorganisms remains crucial for fully characterizing pathogenic isolates (also essential for epidemiological studies) and evaluating their antimicrobial susceptibility. Since 2012, we developed a culture strategy based on the diversification of all culture conditions (medium, temperature, incubation time, and atmosphere) [7, 8]. This strategy, which we named culturomics and is detailed in more depth in another article of this supplement [8], enabled the isolation of 1057 bacterial species from the human gut using up to 212 distinct culture conditions [9]. Among these species, 299 were new species, including 67 new genera (Table 1). Another benefit from culturomics was the demonstration of a faster growth of *Mycobacterium tuberculosis* on blood-enriched [10] and ascorbic acid–enriched media

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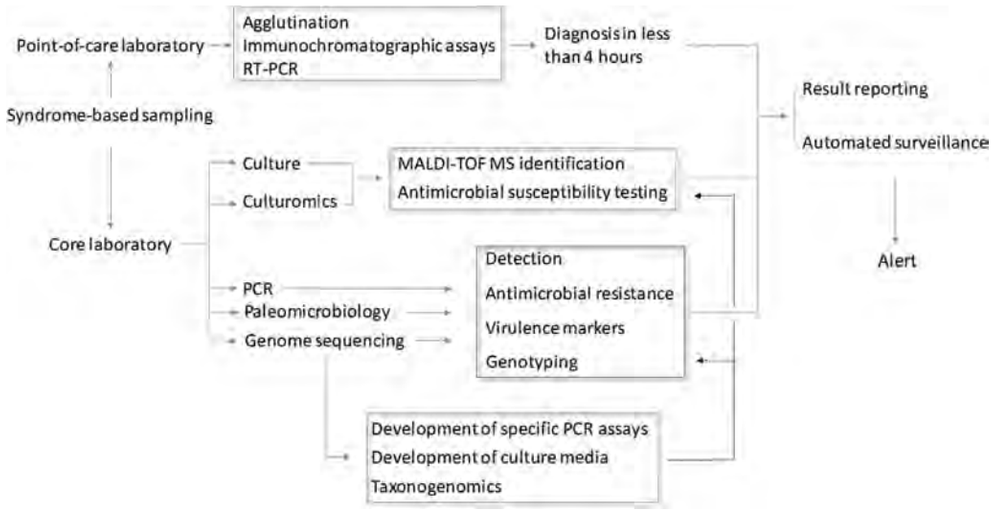

Figure 1. Clinical microbiology laboratory workflow in the Fondation Méditerranée Infection. Abbreviations: MALDI-TOF MS, matrix-assisted laser desorption/ionization–time-of-flight mass spectrometry; PCR, polymerase chain reaction; RT-PCR, reverse-transcription polymerase chain reaction.

[11] and the culture on antioxidant-supplemented agar (ascorbic acid, glutathione, and uric acid) of anaerobic bacteria in aerobic atmosphere [12]. These culture media were standardized and implemented in the routine workflow.

IDENTIFICATION OF PAST AND PRESENT MICROORGANISMS

Classic identification methods for bacterial and fungal isolates using the classic biochemical and serological properties are time-consuming and costly. As early as in 2009, we have progressively replaced them by MALDI-TOF MS [13]. We have demonstrated that MALDI-TOF MS, by being cost-effective, reproducible, and easily integrated in the CML workflow [14], was a major step forward in routine clinical microbiology. With free-access, downloadable databases from institutions such as ours (http://www.mediterranee-infection.com/article.php?laref=256&titre=urms-database) progressively completing commercially available ones, MALDI-TOF MS is currently able to identify most human-associated bacteria and fungi as well as archaea [15–17]. With its rapidity of identification (6–10 minutes) enabling earlier decision making regarding the prescription of antimicrobial treatment for a cost of less than €1 per isolate, MALDI-TOF MS has been adopted by many large CMLs worldwide.

There is an increasing interest in identifying past pathogens and past microbiotas [18] in the perspective of understanding microbial coevolution with human populations [19], factors shaping the human microbiota, emergence and evolution of pathogens, and the various patterns of epidemics. Among the methods currently used for the routine detection of microbes, several have been used for the identification of past microbes, including molecular methods, culture, immunohistochemistry, and detection of specific antibodies [18]. Using the below-described "suicide PCR," and as proofs of concept, we were able to detect *Bartonella quintana* from human teeth from a time-remote patient (4000-years), *Yersinia pestis* from a medieval human tooth, and *Tropheryma whipplei* from Dr Whipple's 1907 patient [20–22].

DETECTION OF ANTIBIOTIC RESISTANCE

Conventional in vitro antimicrobial susceptibility assays are time-consuming and may not be compatible with the time-optimality required for acute patient care. To accelerate this process, we have demonstrated that the PREVI Isola automated seeder system (bioMérieux, Marcy-l'Etoile, France) enables standardizing and speeding up seeding [23], and that real-time video imaging improves reading zone diameters around antibiotic discs in terms of rapidity, sensitivity, and specificity [24]. We have also developed an online database named ARG-ANNOT that contains most relevant antibiotic resistance-coding genes [25], and real-time PCR (RT-PCR) assays targeting the most common antibiotic resistance genes of clinical relevance such as OXA-48 [26] that codes carbapenem resistance in Enterobacteriaceae. Furthermore, we have also demonstrated the antiquity of some antibiotic-resistant genes and mechanisms, showing that antibiotic resistance is partially independent from antibiotic prescription [18]. In parallel, we have adapted MALDI-TOF MS to the detection of β-lactamases and aminoglycoside-modifying enzymes such as extended-spectrum β-lactamases and ArmA methyltransferase [27].

Table 1. List of New Bacterial Species Isolated From Human Specimens Using the Culturomics Strategy

Species Name	CSUR No.	DSMZ Reference	16S rRNA Accession Number	Genome Accession Number	PMID	Validation List No.
Acidaminococcus massiliensis	P2828	DSM103158	LT576399	FMIW01000000		
Acidaminococcus timonensis	P2764	Ongoing deposit	LT598562	Genome in progress		
Actinobaculum massiliense	P1982	DSM100580	LN870313	CYUL01000000	12409355	
Actinomyces bouchesdurhonensis	P2825	DSM103075	LT576385	Genome in progress		
Actinomyces grossensis	P242	DSM25500	JN837492	CAGY00000000		
Actinomyces ihumii	P2006	DSM100538	LN866997	CZPX00000000	27408732	
Actinomyces massiliensis	P18	CCUG53522	EF558367	AKIO00000000	19244437	
Actinomyces phoceensis	P1984	DSM100385	LN833867	CCXH01000000	27200177	
Actinomyces polynesiensis	P658	DSM27066	HF952919	CCXH00000000	27200177	
Actinomyces provencensis	P2166	DSM101041	LN881591	Genome in progress		
Actinomyces timonensis	P35	CCUG55928	EU484334	AKGF00000000	19684313	
Actinomyces urinae	P2225	DSM100700	LN870295	Genome in progress	27358739	
Aeromicrobium massiliense	P158	DSM25782	NR_125588	CAHG00000000	23408663	155
Afipia birgiae		CCUG43108	AF288304	CAJQ02000065	12361286	
Africanella massiliensis	P2538	DSM102984	LT223700	FLKL00000000	27408735	
Alistipes ihumii	P204	DSM26107	JX101692	CAPH00000000	25197494	
Alistipes jeddahensis	P1209	DSM100720	LK021116	CCXE00000000		
Alistipes massilioanorexius	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Alistipes obesihominis	P186	DSM25724	NR_133025	CAHA00000000	24019990	
Alistipes phoceensis	P2431	Ongoing deposit	LN827529	Genome in progress		
Alistipes provencensis	P2431	DSM102308	LT223566	FKYL00000000		
Alistipes senegalensis	P150	DSM25460	NR_118219	CAHI00000000	23407265	
Alistipes timonensis	P148	DSM25383	NR_125589	CAEG00000000	23408657	155
Anaerococcus jeddahensis	P1914	DSM100537	LN866994	CWHU00000000		
Anaerococcus obesihominis	P252	DSM25446	JN837490	CAGV00000000	23407456	
Anaerococcus pacaensis	P122	DSM26346	HM587325	CAJJ020000000	24501638	
Anaerococcus provenciensis	P121	DSM26345	HM587323	CAJU020000000	25197492	
Anaerococcus rubiinfantis	P2032	DSM101186	LN881592	FAVH00000000	27328611	
Anaerococcus senegalensis	P156	DSM25366	NR_118220	CAEK00000000	22675604	155
Anaerococcus urinomassiliensis	P2143		LN898272		27408746	
Anaerofustis massiliensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Anaeromassilibacillus senegalensis	P1511	DSM102954	LN866991	CCDM000000000	27330815	
Anaerosalibacter massiliensis	P762	DSM27308	HG315673	CCEZ00000000	26937282	
Anaerosalibacter timonensis	P3206	Ongoing deposit	LT598565	Genome in progress		
Anaerotruncus massiliensis	P2007	DSM100567	LN866995	Genome in progress		
Anaerotruncus rubiinfantis	P2276	DSM101192	LN881593	FKLA00000000		
Arabia massiliensis	P3078	Ongoing deposit	LT598545	Genome in progress		
Babela massiliensis	Ongoing deposit	Ongoing deposit	GQ495224	HG793133	25884386	
Bacillus andreraoultii	P1162	DSM29078	LK021120	CCFJ00000000	27257486	
Bacillus dielmonensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Bacillus jeddahensis	P732	DSM28281	HG931339	CCAS000000000	26380635	
Bacillus jeddahtimonensis	P977	DSM28582	HG964478	CCXR00000000		
Bacillus massilioalgeriensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Bacillus massilioamazoniensis	P1359	DSM45997	LK021124	CVRB00000000		
Bacillus massilioanorexius	P201	DSM26092	JX101689	CAPG00000000	24501631	
Bacillus massiliogabonensis	P2639	Ongoing deposit	LT598571	Genome in progress		
Bacillus massilionigeriensis	P2348	DSM102112	LT161887	Genome in progress		
Bacillus massiliosenegalensis	P151	DSM25957	NR_125590	CAHJ00000000	23991258	168
Bacillus massilliogorillae	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Bacillus mediterraneensis	P2366	DSM102091	LT161888	Genome in progress	27330818	
Bacillus ndiopicus	P3025	DSM27837	HG315675	CCAP000000000	27257496	
Bacillus niameyensis	P1266	DSM29725	LK985389	CTDY00000000	27076913	
Bacillus phoceensis	P2184	Ongoing deposit	LN881595	FBXX00000000		
Bacillus rubiinfantis	P1141	DSM28615	LK021113	CCFE00000000	27076912	
Bacillus saudii	Ongoing deposit	Ongoing deposit	HG726037	CCYL00000000		
Bacillus testis	P1492	DSM101190	LN827531	CVQX00000000	27222713	

Table 1. Continued

Species Name	CSUR No.	DSMZ Reference	16S rRNA Accession Number	Genome Accession Number	PMID	Validation List No.
Bacillus timonensis	P162	DSM25372	JF824810	CAET00000000	23408487	
Bacillus touaregensis	P2489	Ongoing deposit	LT223701	Genome in progress		
Bacteroides bouchedurhonensis	P2653	DSM103120	LT558803	Genome in progress		
Bacteroides congolensis	P3132	Ongoing deposit	LT598566	Genome in progress		
Bacteroides mediterraneensis	P2644	DSM103033	LT558804	Genome in progress	27408743	
Bacteroides neonati	P1500	DSM26805	HG315678	CBVB00000000	25197464	
Bacteroides phoceensis	P2244	Ongoing deposit	LN998056	Genome in progress		
Bacteroides timonensis	P194	DSM26083	JX041639	CBVI000000000	25197491	
Bariatricus massiliensis	P2179	DSM101783	LN898273	FLKG00000000	27222720	
Bartonella florenciae	B627	DSM23735	HM622139	CALU00000000	24501655	
Bartonella senegalensis	B623	DSM23168	HM636442	CALV00000000	23991259	
Beduinella massiliensis	P2846		LT576387	Genome in progress	27621822	
Beduini massiliensis	P1440	DSM100188	LN713275	CDPP00000000	26669711	
Bittarella massiliensis	P2149	DSM101081	LN881596	FAUJ00000000		
Blastococcus massiliensis	P579	DSM45753	JX101684	CBYP000000000		
Blautia massiliensis	P2132	DSM101187	LN890282	FAVI000000000		
Blautia phoceensis	P328	DSM 102113	LT223571	FLKC00000000		
Blautia timonensis	P2398	DSM102045	LT161889	FJVH00000000		
Brachybacterium massiliense	P2240	DSM101766	LN906631	Deposit in progress		
Brevibacillus massiliensis	P721	DSM25447	JN837488	CAGW00000000	23961307	153
Brevibacterium massiliense	P26	CCUG53855	EU868814	CAJD00000000	19567570	153
Brevibacterium phoceense	P2230	Ongoing deposit	LN998064	Genome in progress		
Brevibacterium senegalense	P155	DSM25783	NR_118221	CAHK00000000	23408786	
Butyricimonas massiliensis	Ongoing deposit	Ongoing deposit	JX424765	Genome in progress		
Butyricimonas phoceensis	P2478	DSM100838	LN881597	FBYB00000000	27668083	
Butyricimonas timonensis	P2440	DSM102307	LT223567	FLSM00000000		
Caecumella massiliensis	P2974	In progress	LT576402	FMIV01000000		
Cellulomonas massiliensis	P898	DSM25695	NR_125601	CAHD00000000	23408774	165
Cellulomonas timonensis	P2058	DSM100699	LN870311	FCOT00000000		
Christensenella massiliensis	P2438	DSM102344	LT161898	Genome in progress	27330817	
Christensenella timonensis	P2437	DSM102800	LT223568	FLKP00000000	In press	
Chryseobacterium oranimense	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Chryseobacterium senegalense	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Clostridium amazonitimonense	P1445	DSM28600	LK021125	CCNN00000000		
Clostridium beduini	P2776	Ongoing deposit	LT598567	Genome in progress		
Clostridium bouchedurhonense	P2181	DSM100751	LN881614	FCEY00000000		
Clostridium culturomicsense	P1184	DSM100507	LK021117	CCXK00000000		
Clostridium dakarense	P243	DSM27086	KC517358	CBTZ000000000	24501642	
Clostridium ihumi	P198	DSM26098	JX101686	CCAT000000000	26388967	
Clostridium jeddahense	P1860	DSM27834	HG726040	CBYL000000000	25197479	170
Clostridium jeddahtimonense	P1230	DSM28716	LK021118	CCNP00000000		
Clostridium marseillense	P2262	DSM20024	LN998060	Genome in progress		
Clostridium massilioamazoniense	P1360	DSM27309	HG315672	CYSO000000000		
Clostridium massiliodielmonense	P2255	Ongoing deposit	LN998063	Genome in progress	27366325	
Clostridium massiliosenegalense	P299	DSM102084	LT161890	FJVE00000000		
Clostridium mediterraneense	P2434	DSM102883	LT161897	FLSO00000000		
Clostridium niameyense	P1468	DSM100441	LN827532	CVPI00000000		
Clostridium nigeriense	P2414	DSM102218	LT161894	FLKB00000000		
Clostridium phoceense	P1929	DSM100334	LN846907	CVUG00000000		
Clostridium polynesiense	P630	DSM27072	HF952918	CCXI00000000	26485191	
Clostridium saudiense	P697	DSM27835	HG726039	CBYM0000000000	25780501	170
Clostridium senegalense	P152	DSM25507	NR_125591	CAEV00000000	23408737	165
Clostridium touaregense	P2415	DSM102219	LT161895	Genome in progress		
Colicadextra massiliensis	P3083	Ongoing deposit	LT598546	Genome in progress		
Collinsella ihumii	P2019	DSM101062	LN881598	FCOU00000000		
Collinsella massiliensis	P902	DSM26110	JX424766	CAPI00000000	25197489	170
Collinsella massilioamazoniensis	P1227	DSM26813	JF824802	CDSD00000000		

Table 1. Continued

Species Name	CSUR No.	DSMZ Reference	16S rRNA Accession Number	Genome Accession Number	PMID	Validation List No.
Colonella massiliensis	P2911	DSM103304	LT576403	FMIY01000000		
Corynebacterium bouchedurhonense	P2067	DSM100846	LN881599	FJVG00000000		
Corynebacterium ihumii	P902	DSM45751	JX424769	CAVS020000000	25197488	
Corynebacterium jeddahense	P0778	DSM45997	HG726038	CBYN000000000	25197478	
Corynebacterium karolinskerse	P1472	DSM100665	LN827533	Genome in progress		
Corynebacterium pacaense	P2417	Ongoing deposit	LT223574	Genome in progress		
Corynebacterium provencense	P2161	DSM101074	LN890283	FIZC00000000		
Corynebacterium timonense	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Culturomica massiliensis	P2698	DSM103121	LT558805	FLSN00000000	27437116	
Dakarella massiliensis	P1938	DSM100447	LK054638	CVTY00000000		
Dermabacter indicis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Desnuesiella massiliensis	P1919	DSM101500	LN846906	CYSK00000000	27158511	
Desulfomassilia massiliensis	P2429	Ongoing deposit	LT223575	Genome in progress		
Diaminobutyricimonas massiliensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Dielma fastidiosa	P149	DSM26099	NR_125593	CAEN00000000	23991263	170
Dorea massiliensis	P199	DSM26146	JX101687	CXYY00000000		
Drancourtella massiliensis	P1506	DSM100357	LN828944	CVPG000000	27257490	
Drancourtella timonensis	P2008	Ongoing deposit	LN870296	Genome in progress		
Duodena massiliensis	P2968	Ongoing deposit	LT576405	Genome in progress		
Eggerthella timonensis	P3135	DSM103474	LT598568	Genome in progress		
Eisenbergiella massiliensis	P2120	DSM101499	LN881600	Genome in progress	27358742	
Emergencia timonensis	P2260	DSM101844	LN998061	FLKM00000000	In press	
Enorma massiliensis	P183	DSM25476	JN837493	CAGZ00000000	23991260	168
Enorma timonensis	P3242	DSM26111	JX424767	CAPF00000000	25197477	170
Enterobacter massiliensis	P906	DSM26120	KR612017	CAEO00000000	24019988	155
Enterobacter timonensis	P2201	DSM101775	LN906632	FCOP00000000		
Enterococcus massiliensis	P1928	DSM100308	LN833866	CVRN00000000	27330820	
Eubacterium massiliense	P1926	DSM100743	LN850732	CVTZ00000000		
Fenollaria massiliensis	P127	DSM26367	HM587321	CALI02000000	25197455	
Flaviflexus massiliensis	P1300	DSM29058	LK985390	CSSZ00000000	27621821	
Fournierella massiliensis	P2014	DSM100451	LN846908	FAUK00000000		
Fusobacterium massiliense	P2749	DSM 20024	LN998061	FMJA01000000		
Gabonia massiliensis	P1910	DSM100571	LN849789	CYPV00000000	26862432	
Gabonibacter massiliensis	P2336	DSM101039	LN881588	FAVK00000000	27628331	
Gemmata massiliana	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Gorbachella massiliensis	P2021	DSM101082	LN870316	FBXY00000000		
Gordonibacter massiliensis	P2775	Ongoing deposit	LT558845	Genome in progress		
Gorillibacterium massiliense	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Gorillibacterium timonense	P2011	DSM100698	LN870297	CYUM00000000		
Gracilibacillus massiliensis	P1441	DSM29726	LN626645	CZRP00000000		
Gracilibacillus timonensis	P2481	DSM103076	LT223702	FLKH00000000		
Guyana massiliensis	P1169	Ongoing deposit	LK021123	CCMM00000000		
Haemophilus massiliensis	P859	DSM 28247	HG931334	CCFL00000000	27081435	
Haloferax massiliensis	P974	Ongoing deposit	HG964472	CSTE00000000	27408734	
Halomonas massiliensis	P2426	Ongoing deposit	LT223576	Genome in progress	27621824	
Halopiger djelfamassiliensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Halopiger goleamassiliensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Herbaspirillum massiliense	P895	DSM25712	NR_125602	CAHF00000000	23407294	155
Holdemania massiliensis	P195	DSM26143	JX101683	CALK00000000	24976895	
Holdemania timonensis	P2844	In progress	LT576390	Genome in progress		170
Hugonella massiliensis	P2118	DSM101782	LN881601	FAUL00000000		
Ihubacter massiliensis	P2843	Ongoing deposit	LT576391	Genome in progress	27579171	
Ihuella massiliensis	P1486	Ongoing deposit	LN827534	CYPT00000000		
Ihuprevotella massiliensis	P2826	Ongoing deposit	LT576392	FMIU01000000	27621825	
Ileobacterium massiliense	P3115	DSM103486	Deposited	Genome in progress		
Intestinimonas gabonensis	P2072	Ongoing deposit	LN876649	FBXW00000000		
Intestinimonas massiliensis	P1930	DSM100417	LN866996	CWJP00000000		

Table 1. Continued

Species Name	CSUR No.	DSMZ Reference	16S rRNA Accession Number	Genome Accession Number	PMID	Validation List No.
Intestinimonas phoceensis	P3064	Ongoing deposit	LT598548	Genome in progress		
Intestinimonas timonensis	P2010	DSM100769	LN870298	Genome in progress		
Jeddahella massiliensis	P1190	DSM100450	LK021119	CCNO00000000		
Kallipyga gabonensis	P1915	DSM100575	LN849790	CXYV00000000	26862430	
Kallipyga massiliensis	P241	DSM26229	JN837487	CAHC00000000	24501634	170
Khelaifiabacterium massiliense	P1935	DSM100591	LN850733	Genome in progress		
Kurthia massiliensis	P140	DSM24639	NR_118218	CAEU00000000	25197500	154
Kurthia senegalensis	P138	DSM24641	JF824796	Genome in progress	25197500	
Kurthia timonensis	P1394	Ongoing deposit	JF824797	CAEP00000000		
Lachnoclostridium bouchedurhonense	P2181					
Lachnoclostridium timonense	P3122	Ongoing deposit	LT576406	Genome in progress		
Lagierella massiliensis	P2012	DSM100854	LN870299	CYUO00000000		
Lascolabacillus massiliensis	P1560	DSM100190	LN827535	CTEJ00000000	27158512	
Lascolabacter vaginalis	P109	DSM101754				
Legionella massiliensis	P146	DSM24804		CCVW01000001	25323728	
Legionella saoudiniensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Legionella tunisiensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Lentibacillus massiliensis	P3089	Ongoing deposit	LT598552	Genome in progress		
Malnutritionisia massiliensis	P1907	DSM100590	LN850734	CXYX00000000		
Mannheimia massilioguelmaensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Marseilla massiliensis	P2745	DSM 103035	LT558846	Genome in progress	27408742	
Marseillobacter massiliensis	P2840	Ongoing deposit	LT576393	Genome in progress		
Marseillococcus timonensis	P2399	Ongoing deposit	LT161891	Genome in progress		
Massilibacterium senegalense	P1510	DSM100455	LN828943	CTRN00000000	26933503	
Massilioamazonia massiliensis	P1110	Ongoing deposit	LK021114	CCNL00000000		
Massiliobacillus massiliensis	P2411	DSM102838	LT161896	FLKF00000000		
Massilioculturomica massiliensis	P2935	DSM103347	LT576407	Genome in progress		
Massiliomaliae massiliensis	P2963	Ongoing deposit	LT576408	Genome in progress		
Massiliomicrobiota timonensis	P2264	DSM 101840	LN998062	Genome in progress	27358745	
Massilioprevotella massiliensis	P2439	Ongoing deposit	LT223577	Genome in progress		
Mediannikovella massiliensis	P1934	DSM100589	LN849776	CXYU00000000		
Mediterranea massiliensis	P2645	DSM 103034	LT558847	Genome in progress	27408745	
Megasphaera massiliensis	P245	DSM26228	JX424772	CAVO0000000000	24501636	168
Metaprevotella massiliensis	P3114	DSM103534	LT598559	Genome in progress		
Methanomassiliicoccus luminyensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Microbacterium gorillae	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Micromassilia timonensis	P2133	DSM101080	LN881613	CZPW00000000		
Microvirga massiliensis	P153	DSM26813	JF824802	CDSD00000000	26749561	170
Mobilicoccus massiliensis	P1306	DSM29065	LK985391	CDGT00000000		
Mobilobacterium massiliense	P2510	Ongoing deposit	LT223569	Genome in progress		
Murdochiella massiliensis	P1987	DSM100630	LN866998	FIZW00000000	27660714	
Ndiopella massiliensis	P1917	DSM100643	LN866993	CYUK00000000		
Necropsobacter massiliensis	P3511	DSM27814	HG428679	CDON00000000	26587237	
Negativicoccus massiliensis	P2082	DSM100853	LN876651	Genome in progress	27408741	
Neglecta timonensis	P2265	DSM102082	LN998059	FIZH00000000	27358741	
Neofamilia massiliensis	P1998	DSM100639	LN866999	CYUJ00000000		
Nesterenkonia massiliensis	P244	DSM26221	JX424770	CBLL000000000	25197469	170
Niameyia massiliensis	P1909	DSM100592	LN850735	CXYW00000000		
Nigerium massiliense	P1302	DSM29084	LK985392	CCYM00000000		
Nocardioides massiliensis	P894	DSM28216	HF952922	CCXJ00000000	27257488	
Nomabacter massiliense	P1305	DSM29571	LK985385	CTDZ00000000	27354918	
Nosocomiicoccus massiliensis	P246	DSM26222	JX424771	CAVG00000000	24501657	168
Oceanobacillus jeddahensis	P1091	DSM28586	HG931338	CCDM000000000	27093109	
Oceanobacillus massiliensis	P132	DSM26444	NR_133033	CAER00000000	24976893	
Olegusella massiliensis	P2268	DSM101849	LN998058		27330814	
Olsenella massiliensis	P1476	DSM100642	LN827536	CZPU00000000		
Olsenella phoceensis	P2936	DSM103159	LT576409	Genome in progress		

Table 1. Continued

Species Name	CSUR No.	DSMZ Reference	16S rRNA Accession Number	Genome Accession Number	PMID	Validation List No.
Olsenella provencensis	P2912	DSM103045	LT576410	Genome in progress		
Olsenella timonensis	P2300	DSM 102072	LT161892	Genome in progress		
Oscilibacter massiliensis	P2778	Ongoing deposit	LT558848	Genome in progress		
Pacaella massiliensis	P2770	DSM103390	LT598569	Genome in progress		
Paenibacillus antibioticophila	P1358	DSM28228	KC158472	CBLK000000000	27257493	
Paenibacillus bouchesdurhonensis	P3071	Ongoing deposit	LT598550	Genome in progress		
Paenibacillus camerounensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Paenibacillus dakarensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Paenibacillus gorillae	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Paenibacillus ihumii	P1981	DSM100664	LN881615	CYXK00000000	26958346	
Paenibacillus marasmiensis	P3069	Ongoing deposit	LT598570	Genome in progress		
Paenibacillus numidis	P1475	DSM100384	LN827528	CTEK00000000		
Paenibacillus phoceensis	P2238	DSM101777	LN998053	FCOQ00000000		
Paenibacillus reamassiliensis	P892	DSM45751T	JX424768	CTED00000000		
Paenibacillus rubiinfantis	P2076	DSM101191	LN881603	FAUQ00000000		
Paenibacillus senegalensis	P897	DSM25958	NR_125594	CAES00000000	23459006	165
Paenibacillus senegalomassiliensis	P2144	Ongoing deposit	LN890284	FAUP00000000		
Paenibacillus touaregensis	P2472	DSM102801	LT223571	FLKE00000000		
Parabacteroides massiliensis	P2231	DSM101860	LN899828	Genome in progress		
Paraliobacillus massiliensis	Ongoing deposit	Ongoing deposit	HG931929	CTEI00000000		
Paucisalibacillus algeriensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Peptoniphilus duodeni	P2932	DSM103346	LT576413	Genome in progress		
Peptoniphilus grossensis	P184	DSM25745	JN837491	CAGX00000000	23408485	
Peptoniphilus obesihominis	P187	DSM25489	JN837495	CAHB00000000	24019985	
Peptoniphilus pacaensis	P2270					
Peptoniphilus phoceensis	P2183	Ongoing deposit	LN881605	FCEX00000000	27222719	
Peptoniphilus raoultii	P110					
Peptoniphilus senegalensis	P154	DSM25694	NR_125592	CAEL00000000	24019986	170
Peptoniphilus timonensis	P165	DSM25367	NR_118307	CAHE00000000	23449949	165
Phocaeicola abscessus	P22	DSM21584	EU694176		19620382	
Phocea massiliensis	P2769	DSM103073	LT558849	Genome in progress	27482388	
Planococcus massiliensis	P1103	DSM28915	LK021122	CCXS00000000	27257487	
Polynesia massiliensis	P1280	DSM28707	HF952920	CCYG00000000		
Prevotella caccae	P2931	Ongoing deposit	LT598564	Genome in progress		
Prevotella phoceensis	P2259	DSM103364	LN998069	FIZG00000000		
Prevotella timonensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Prevotellamassilia timonensis	P2831	Ongoing deposit	LT576394	Genome in progress	In press	
Provencella massiliensis	P2780	Ongoing deposit	LT558850	Genome in progress		
Pseudomonas massiliensis	P1334	DSM29075	LK985396	CCYK00000000		
Raoultibacter massiliensis	P2849	DSM103407	LT576395	Genome in progress	27595003	
Reyranella massiliensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Risungbinella massiliensis	P1082	DSM46691	HF952921	CECI00000000	26984352	171
Romboutsia timonensis	P326	Ongoing deposit	LN998074	Genome in progress	27200178	
Rubeoparvulum massiliense	P1473	DSM100479	LN828926	CVPE00000000		
Rubidus massiliensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	CCSC01000000	27014641	
Rubiinfantum massiliense	P2452	DSM29059	LK985393	CTDX00000000		
Ruminiclostridium massiliense	P2976	DSM103344	LT598551	FMIZ01000000		
Ruminococcus massiliensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Ruminococcus phoceensis	P2086	DSM100837	LN881607	FAVJ00000000		
Senegalia massiliensis	P2130	Ongoing deposit	LN881608	Genome in progress	27330819	
Senegalimassilia anaerobia	P147	DSM25959	NR_125595	CAEM00000000	24019984	155
Soleaferrea massiliensis	P200	DSM26100	JX101688	CCYH00000000		
Staphylococcus massiliensis	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Stoquefichus jeddahensis	P1494	DSM100389	LN850736	CVRO00000000		
Stoquefichus massiliensis	P202	DSM26112	JX101690	CBLM000000000		
Streptococcus timonensis	P2915	DSM103349	LT576411	FMIX01000000		

Table 1. Continued

Species Name	CSUR No.	DSMZ Reference	16S rRNA Accession Number	Genome Accession Number	PMID	Validation List No.
Streptococcus varani	Ongoing deposit	Ongoing deposit	Ongoing deposit	Genome in progress		
Streptomyces massiliensis	P203	DSM42077	JX101691	CCXT00000000		
Sutterella massiliensis	P2435	Ongoing deposit	LT223579	Genome in progress		
Tessaracoccus massiliensis	P1301	DSM29060	LK985394	CCYJ00000000	27358740	
Thalassobacillus massiliensis	Ongoing deposit	Ongoing deposit	HG931930	CTEA00000000		
Tidjanibacter massiliensis	P3084	Ongoing deposit	LT598563	Genome in progress		
Timonella senegalensis	P909	DSM25696	NR_125603	CAHH00000000	23991262	170
Tyzzerella massiliensis	P3062	Ongoing deposit	LT598553	Genome in progress		
Urmitella massiliensis	P3072	Ongoing deposit	LN828927	Genome in progress		
Urmitella timonensis	P2918	Ongoing deposit	LT598554	Genome in progress		
Varibaculum massiliense	P2802	DSM103074				
Virgibacillus massiliensis	P2480	DSM28587	HG931931	CCDP00000000	26649181	
Virgibacillus senegalensis	P1101	DSM28587	LK021111	CCDP00000000	26693281	
Vitreoscilla massiliensis	P2036	DSM100958	LN870312	CZPV00000000		
Weeksella massiliensis	P860	DSM28259	HG931340	CCMH00000000	26649182	
Xanthomonas massiliensis	P2129	DSM100900	LN881611	FCOY00000000		

Species in bold text have standing in nomenclature.

Abbreviations: CSUR, Collection de souches de l'unité des Rickettsies; DSMZ, Deutsche Sammlung von Mikroorganismen und Zellkulturen; PMID, PubMed identifier; rRNA, ribosomal RNA.

IN SITU DETECTION IDENTIFICATION OF PATHOGENS

Mass Spectrometry

In addition to the primary use in the identification of bacteria from colonies, we have discovered that MALDI-TOF MS was able to detect pathogens directly within clinical samples, notably blood culture vials [28]. In the first study that we conducted on bacteria grown from blood collected in culture bottles, we were able to identify blood-borne bacteria in <2 hours with a success rate of 97.5% [28]. We and another team later adapted MALDI-TOF MS to the identification of bacteria in urine specimens [29].

Molecular Detection

Rapid detection and identification of infectious agents in clinical specimens are mandatory to implement appropriate therapeutic measures. Having implemented the broad-range 16S ribosomal RNA (rRNA)–based PCR assay in routine diagnosis as early as 1992 [30], we took advantage of the genome sequences that are increasingly available to develop, over the years, many species-specific PCR and RT-PCR assays that are either used individually or on a syndrome-based basis [6], including in POC laboratories [31]. We demonstrated that targeted RT-PCR and conventional broad-range 16S rRNA PCR were complementary in the syndrome-driven diagnosis of infectious diseases [32].

In addition, we designed PCR assays with increased sensitivity, either by selecting a gene or fragment of noncoding DNA present as several copies in the genome [33] or by designing nested PCR assays targeting previously unused genomic fragments [34]. Fenollar et al identified a 7-copy fragment in the genome of *T. whipplei* and demonstrated that a RT-PCR assay targeting this repeated fragment was significantly more sensitive than assays targeting a single-copy fragment [33]. Targeting multicopy fragments was later demonstrated to be also highly sensitive in the detection of brucellosis, Q fever, and infections caused by *Mycoplasma pneumoniae* or *Neisseria meningitidis*. In contrast, Drancourt et al developed a strategy named "suicide PCR" that is based on nested-PCR assays targeting genome fragments that had never been used as PCR targets previously and that will be targeted only once with single-use primers [34]. These authors showed that their method was significantly more sensitive than regular PCR to detect *Rickettsia* species in various arthropod-borne diseases and *Y. pestis* in dental samples from ancient plague outbreaks [34, 35].

Fluorescence In Situ Hybridization

As examples of fluorescence in situ hybridization (FISH) performance in our laboratory, we demonstrated the presence of *Akkermansia muciniphila* as part of the intestinal flora of patients without any gastrointestinal disorder [36], the role of a new, Marseillevirus-like virus in the blood of blood donors [37], the causative role of Marseillevirus in a lymphadenitis of a child [38] and of a woman with Hodgkin lymphoma [39], and of *Coxiella burnetii* in B-cell non-Hodgkin lymphoma [40]. Such observations suggest that FISH should be considered among the diagnostic tools of advanced clinical microbiology laboratories.

GENOTYPING

Understanding precisely the source and spread of microorganisms is required in the study of outbreaks and endemic infections, and when facing the emergence of a novel pathogen or the transmission and circulation of a hypervirulent strain. Rather

than using multilocus sequence typing (MLST), commonly used to characterize strains using a combination of housekeeping genes [41], we designed the multispacer typing (MST) that combines sequences from the most variable intergenic spacers between aligned genomes of bacterial strains instead of genes [42]. First developed for *Y. pestis* [42], MST was also efficient for type strains from various bacteria including *C. burnetii*, *Rickettsia conorii*, *Rickettsia prowazekii*, and *T. whipplei* [43–46]. MST was demonstrated to be more discriminatory than MLST for *R. conorii* strains [44]. However, bacterial whole-genome sequencing using next-generation sequencing (NGS), by giving access to the whole genetic content of a strain, is the ultimate discriminatory sequence-based genotyping method and has already demonstrated its usefulness in epidemiological investigations, showing the rapid global transmission of infectious diseases [47].We have used rapid genome sequencing, also named real-time genome sequencing, to investigate unusual cases of *Staphylococcus epidermidis* community-acquired endocarditis and *Listeria ivanovii* aortic infection [48, 49].

GENOMICS

The development of NGS bench-top sequencers such as the MiSeq (Illumina) and Ion Torrent Personal Genome Sequencer (Life Technologies) has made genome sequencing compatible with the routine CML workflow [50]. Genome sequencing may have a number of applications including the development of molecular detection, identification and genotyping tools, of specific culture media, serological tools, monoclonal antibodies, and vaccine design [51]. In addition, within a few hours and for less than €100, exhaustive access to the deep characterization and phylogeny, virulence markers, and antibiotic resistance repertoire may be obtained for a strain of interest [51]. Using NGS, we have developed novel molecular detection, identification, and genotyping tools and investigated unusual clinical isolates as described above. In addition, we have tailored specific culture media for fastidious bacteria such as *T. whipplei* and for anaerobes [12, 52]. This was achieved by supplementing culture media with components that the studied bacteria were unable to synthesize due to missing or incomplete metabolic pathways, as identified in their genomes [12, 52]. Finally, we have designed a new taxonomic strategy to describe novel human-associated bacterial species isolated using culturomics. The strategy includes the systematic genome sequencing of the type strains of all new species [53]. To date, we have sequenced the genomes of the 299 new bacterial species that we have isolated.

POINT-OF-CARE LABORATORIES

Working around the clock, POC laboratories considerably reduced the turnaround time by performing mostly agglutination or immunochromatographic assays for which the results are available in <1 hour [54], as well as RT-PCR assays that made possible the rapid molecular detection of many pathogens in emergency circumstances such as meningitis. POC assays are selected to provide an answer to the following questions having a clear impact on patient management: Is hospitalization, isolation in case of contagious risk, or onset of specific anti-infective therapy necessary? A large variety of agents can be tested, including bacteria, parasites, or viruses [54]. In a recent study, Sokhna et al described the use of a syndrome-driven strategy for the POC diagnosis of febrile illness [55]. This type of diagnostic method has the advantage of testing, in a short time and for a limited number of specimens, the most common causative agents of a given syndrome and may be especially valuable, for example, for the diagnosis of meningitis, pneumonia, endocarditis, pericarditis or sexually transmitted diseases. Finally, POC laboratories are easily implementable in any environment and may thus be established in remote areas, as we have done in rural Senegal [55] or on a commercial ship [56].

AUTOMATED SURVEILLANCE

At the source of microbial diagnosis-produced information, CMLs have the possibility to survey the emergence of unusual phenomena such as clinical syndromes or specific microorganisms on the basis of an abnormal increase in received specimens (cerebrospinal fluid, stool, oropharyngeal swabs, etc) and/or identified pathogenic species [57], In our laboratory, we have automated this surveillance through the creation of several software programs including EPIMIC (Epidemiological surveillance and alert based on microbiological data) [58], BALYSES (Bacterial real-time laboratory-based surveillance system) [59], and MARSS (Marseille antibiotic resistance surveillance system) [59] for the real-time systematic automated surveillance of infectious diseases, the number of infected patients with bacterial species isolated at least once in our CML, and resistance to antibiotics of the 15 selected bacterial species, respectively. These surveillance systems have detected an increase in urinary tract infections caused by intrinsically colistin-resistant bacteria [60] and a significant decrease in *Streptococcus pneumoniae* infections [61] in Marseille public hospitals, as well as rare cases of *Flacklamia hominis*, *Vagococcus lutrae*, and *Sporolactobacillus laevolacticus* infections [62–64]. Our system has also been able to alert regional health authorities of the early detection of a *Clostridium difficile* 027 outbreak in Marseille, France, allowing the implementation of specific control and therapeutic measures [65, 66].

CONCLUSIONS

The development of -omics technologies and the centralization of biomedical analysis led to a complete reorganization of CMLs to improve the laboratory workflow and reduce the delay in diagnosis. Consequently, CMLs have become major actors in the optimization of patient management, contributing to

reduce hospitalization costs [59]. In addition, CMLs are able to play a key role in detecting emerging infections, in syndromic surveillance, or in outbreak detection and warning of medical authorities. Future directions of CML development include increased automation of laboratory processes, accelerated result reporting (cell phones) to clinicians in charge, and constitution of large collections of clinical specimens and strains that can be used prospectively and retrospectively to investigate emerging infections.

Notes

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Clinical Infectious Diseases

SUPPLEMENT ARTICLE

New Approaches to Prevent Healthcare-Associated Infection

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Healthcare-associated infection (HCAI) in hospitals mainly results from unsolved but well-identified causes such as hand hygiene, overuse of catheters, and to a lesser extent, the airborne transmission of infectious agents caused by the misuse of respiratory precautions. The aims of the Institut Hospitalo-Universitaire Méditerranée Infection are to develop new approaches to fight HCAIs. Among them, new technologies that allow for the traceability of care and good practices reminders have been developed concomitantly to an anthropological approach, facilitating acceptability by healthcare workers. While the automated continuous monitoring system is validated and commercially available, some other technologies are still under clinical evaluation or in the early development phase. Quorum sensing–based biotechnologies are developed with the aims to fight against wound colonization.

Keywords. heath care associated infection; technological devices; infection control; anthropological approaches; biotechnology.

Healthcare-associated infection (HCAI) affects approximately 5 million people each year in Europe with an estimated cost of €13–€24 billion, and an attributable mortality varying from 50 000 to 135 000 cases [1]. In the United States, the annual rate of HCAI was estimated at 1.7 million cases, with 99 000 deaths and an economic impact of approximately US$6.5 billion. The estimated prevalence rate varies across the globe, from 5%–10% of hospital-admitted persons to 20%–30% of patients admitted in intensive care [2]. HCAI infection is the largest epidemic of infectious disease that has ever happened on Earth, with an estimated number of deaths of at least 250 000 cases per year just in the United States and Europe, with a cost of €30 billion yearly. Aiming at a reduction of 10% of total HCAI, the saved lives and money would be estimated at 2500 and €3 billion per year, respectively.

Due to the fact that most HCAIs are the results of microorganism transmission by hand, hand disinfection is regarded as key to fight against this epidemic. Cleansing hands with alcohol-based hand rub is a simple and undemanding procedure that requires only a few seconds and has been proven to be highly efficient [3]. Even if the relative risk level of different care activities and how to best define key moments for hand hygiene action are still debated among infection control experts, the "5 moments for hand hygiene" are generally admitted as key moments for efficient hand hygiene [4]. However, these 5 moments can be classified into 2 simple key actions: hand disinfection before contact with the patient and hand disinfection before leaving the contaminated healthcare zone (patient's bedroom). The observed adherence rates among healthcare workers (HCWs) have been regarded by public health authorities as unacceptably poor. There is no standard for measuring adherence to hand hygiene practices; however, directly observing adherence to hand hygiene recommendations is the method used in the majority of studies [2]. Direct observational surveys suffer from several limitations; they are time-consuming and costly, they do not allow for continuous monitoring, and they only provide information on a small sample of all hand hygiene opportunities. More importantly, staff members change their behavior when they know that they are being observed; this has been called the "Hawthorne effect" [5].

Bloodstream infection (BSI) is a major cause of morbidity and mortality throughout the world. The estimated number of hospital-acquired BSIs in 2002 in the United States was 215 000 cases, with a calculated incidence of 2.2 cases per 100 admissions (0.6 in all hospitalized patients and 9.7/100 admissions in the intensive care unit) [6]. Even if many studies have been conducted to prevent nosocomial BSI, some with success [7], in a recent matched case-control study of 830 hospital-acquired BSIs by the elderly, the mortality attributable to the hospital-acquired BSI was 2 times that of the controls, the added length of stay was of 10 days, and the total added cost was US$43 208 per patient [8]. Although peripheral venous catheters (PVCs) are the most frequently used invasive devices in hospitals with an estimated incidence varying from 30% to 80% of hospitalized patients, problems caused by PVCs are largely underevaluated [9]. Studies reporting infectious complications of such catheters

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are rare. It has been estimated recently that *Staphylococcus aureus*–associated PVC bacteremia would occur in up to 10 000 patients yearly in the United States [10]. The rate of unnecessary PVC use reaches 50% in the literature [11] and, in our own experience, 80% of patients admitted though the emergency department [12], explaining why fighting PVC has become a major aim for the Institut Hospitalo-Universitaire (IHU).

Finally, although the impact in term of incidence, mortality, and cost is much lower, hospital-acquired respiratory infections such as influenza, syncytial respiratory virus, measles, tuberculosis, Legionnaire's disease, herpes zoster virus, and others require paying a particular attention in taking care of these contagious patients. Highly contagious patients such as those with viral hemorrhagic fever or with severe acute respiratory syndrome or Middle East respiratory syndrome coronaviruses need the expertise of a skilled team in infection control [13].

NEW APPROACHES TO PREVENT CONTAGIOUS DISEASES AT THE INSTITUT HOSPITALO-UNIVERSITAIRE

Innovative Technological Approaches

Electronic hand hygiene monitoring systems have emerged not only to record compliance but also to promote it. These systems are designed to ensure that HCWs perform hand hygiene before approaching the patient's bedside and issue an alert to do so. They can use sensors that detect alcohol vapors [14] or radiofrequency identification to determine when hand hygiene has occurred [15]. Among systems that are more widespread, including for a use in medical equipment, there are Wi-Fi (wireless system based on Institute of Electrical and Electronics Engineers [IEEE] 802.11 standards) and Zigbee (wireless communication protocols based on IEEE 802.15.3 standards) [16]. Both receivers are low-cost, are easy to maintain, and can be portable. The disadvantage of Zigbee and Wi-Fi is that an accurate location may require multiple beacons in an area, or combination with another technology; some systems may record 2 HCWs with hand hygiene even if the HCWs are very close in proximity [15]. The remote video monitoring of hand hygiene with real-time feedback to HCWs was responsible for a significant increase in hand hygiene compliance [17]. The main disadvantage of the video is that it is linked to human interpretation, time-consuming, and not in real time for the feedback and analysis. The main gap with these technologies is that they are currently unable to define the exposure to transmission (contact with the patient or with contaminated fomites); even if some technology claims that they can do it, few have been evaluated for accuracy, sensitivity, and specificity [18, 19].

The IHU Méditerranée Infection has favored the emergence of technical innovation by making it possible to share scientific ideas with industrial development. The technology developed by our consortium and called here MediHandTrace (French patent register 12/60453) was born from an informal discussion between a small research and development (R&D) office (MicroBE), a bedside hand hygiene sanitizer distributor (Hygienic System), and a counseling office (EphygieHand). Our technology is based on radio-frequency identification (RFID). In brief, the system was based on the "iCode RFID 15693" tag technology using the frequency band of 13.56 MHz. Originally, each bedroom was equipped with 4 floor-level antennas used to read tags inserted in the shoes of each HCW (Figure 1A). One antenna was located just outside the room's door under the alcohol dispenser, the second antenna was located at the door entrance, the third was within the room under another alcohol dispenser, and the last antenna was located around the bed and defined a secure zone (ie, contaminated healthcare zone, the zone for which alcohol disinfection should have been performed before entering). Sensors were placed on both alcohol dispensers, measuring the use of hydroalcoholic solution inside and outside the room by indicating the number of sprays and the volume dispensed. One reader coordinates the antennas to read the HCWs' shoe-inserted tags and the dispenser's sensors, and transfers the information to the main server via an Ethernet connection. The intelligence of the system lies in the server, which manages, interprets, and provides results in real time. A new mobile system has just been released with only one antenna and one hydroalcoholic solution dispenser at the bedside. Information on entrance and exit of the HCW is given to the system by a signal transmitted to the antenna by one tag located under the door (Figure 1B). All of our innovations are protected either by patent or by copyright. MediHandTrace has been evaluated against video recording with a sensitivity of 95.65 %, specificity of 100%, and accuracy of 99.02% [20]. The consortium created ex-nihilo a startup named MediHandTrace SAS (125 000 €), which engaged in new R&D. The product is now commercially available in any place in the world (http://www.medihandtrace.com/en/home-1.html). In parallel with R&D, our research group investigated the impact of the developed technique. In a recently submitted article, we reported that we were able to record HCW paths and hand hygiene opportunities 247 and their appropriate hand hygiene for up to 5 months. We showed after a multilevel linear regression adjustment that HCWs initially did not disinfect their hands before contact with the patient in 77.4% of hand hygiene opportunities, indicating a hand hygiene adherence rate of 22.6%, with 6% only performing hand hygiene just before contact (Figure 1A, sequence 3–4) [21]. Interestingly, this was associated with a use of 7.48-fold the European recommended hand rub solution volume, indicating that the level of hand rub solution of 21 mL/day/patient for medicine wards currently recommended was too low [22]. This study raised an important point: The fact that hand hygiene is HCW dependent and that such technology records a very large number of events indicates that the number of hand hygiene opportunities (related to workflow) of one particular HCW with his or her own hand hygiene practices overweights this HCW's features, needing adjustment (multilevel

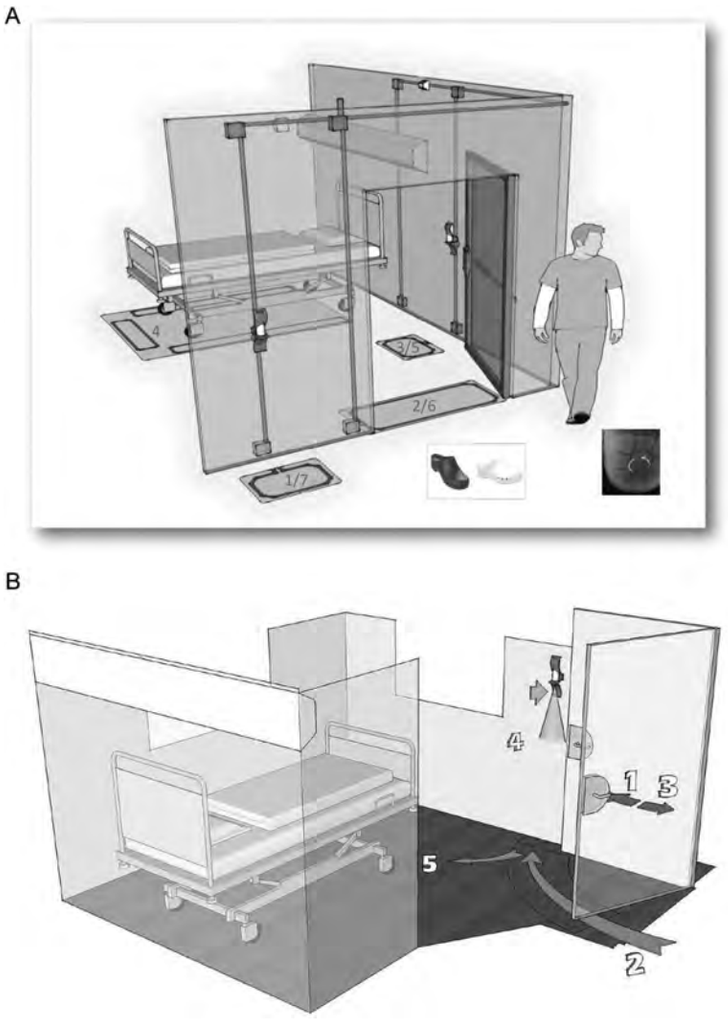

Figure 1. *A,* MediHandTrace 1.0 Hand Hygiene continuous monitoring system. Microchips are introduced in the healthcare worker's (HCW) shoes (inset). The signal is transmitted to the database Meditrace: S1 when the HCW is on the first antenna; S2 when he is on the door antenna; S3 when he is on the inside dispenser antenna; S4 when he is in contact with the bed antenna; S5 when he returns to the inside dispenser antenna after S4; S6 when he is in contact with the door antenna after S4, S3, or S5; and S7 when he is in contact with the outside antenna. A signal is sent to the database when alcohol solution is taken either outside or inside the dispenser. Example: the sequence "1, 2, 3, 4, 5, 6, 7" with alcohol taken at 3 and 5 is a perfect path; the sequence "1, 2, 4, 5, 6, 7" with alcohol taken at 1 and 7 is acceptable; the sequence "1, 2, 4 …" is an inacceptable path. *B,* In this new version of MediHandTrace, the tag 1 is located under the door and targets the signal when the door opens over the unique antenna. Here, signal S1 indicates the entry; S2 identifies the HCW; S3 indicates that the door was closed; S4 indicates that the HCW takes alcohol hand rub; S5 indicates the HCW leaves the antenna. Note that here the "care zone" has been enlarged to the whole bedroom, whereas in the first version the care zone was restricted to the bed zone.

analysis) to attenuate this overexpression [23]. Among others, the shorter the duration of the path, the worse the bedside hand hygiene, indicating that the organization of care is a key factor to improving hand hygiene [21]. Since that time, the system continuously recorded HCW hand hygiene opportunities. With the use of MediHandTrace, in the aims to improve hand hygiene, we have monitored the HCW hand hygiene practice changes after sending small text message feedbacks. The participants in our study received one of 2 types of text message every Monday morning. The first type is a felicitation message: "From date X to date Y, you have improved your hand hygiene. Congratulations, and keep up the good work." The second type is an encouragement message: "From date X to Y, your hand hygiene did not improve, and we encourage you to be more vigilant." We have recorded 15 723 paths all along our study (10 months) done by 18 HCWs. After a multilevel logistic regression model to integrate the individual effect on compliance to hand hygiene, HCWs receiving text messages improved their hand hygiene by almost two-fold (odds ratio, 1.68 [95% confidence interval, 1.45–1.79]; $P = .001$) [24]. Studies are ongoing to evaluate the impact of reorganization of care (nursing kit) on hand hygiene adherence.

Video Monitoring of Routine Healthcare

Despite the fact that video monitoring is time consuming and does not give real-time feedback, it was proven useful in enhancing hand hygiene [17]. Video recording and feedback are commonly used in sport coaching and improving practices, and video recording has been associated with MediHandTrace to evaluate HCW behavior in routine care, highlighting the complexity of hand disinfection and glove wearing [25, 26].

Peripheral Venous Catheter Traceability

Excepted care bundle interventions, very few studies have been performed to reduce unnecessary insertion or as soon as possible (ASAP) ablation of PVC, most studies focusing on central venous catheters [27]. We innovated with 2 different technologies in an attempt to stop exceeding insertion and allowing an ASAP removal of unnecessary PVCs. The patient smart reader (PSR) is a bar code personal digital assistant capable of scanning the catheter, the nurse, and the patient's bar code identification, allowing the optimization of PVC traceability. It also can inform, for every 8-hour shift, the nurse in charge to obtain the medical renewal of the catheter prescription and, if not, to remove the catheter. PSR also informed and traced each 8-hour shift for catheter maintenance. When compared to other standardized process of traceability, the PSR allowed for an increase from 58.3% to 100% ($P < .05$) in the traceability of catheter insertion and removal (O. Florea et al, unpublished data). Further studies are ongoing to evaluate the impact on PVC line infection. The second innovation is an intelligent PVC (KTtrace), which, when inserted into the patient's arm, continuously informs the HCW of its presence, and asks the HCW to regularly evaluate its need for an ASAP removal. This process is patented and under ongoing clinical evaluation.

Anthropological Approaches

New technologies introduced to monitor hand hygiene have been a matter of debate among HCWs. These technologies are better accepted by HCWs in leadership positions than among others. Among the questions raised by HCWs is the accuracy level of the data produced by systems to monitor hand hygiene and the inability of the technology to assess the situational context of hand hygiene opportunities, as well as the punitive use of data produced [28]. Our project was accompanied by human and social science evaluation. Scientific research in our infectious disease ward was presented by "reformers" (researchers) as a mandatory way to explore cause and consequence and improve the knowledge on HCAI to further adapt the intervention and reduce risk of HCAIs. This means legitimizing the introduction of new technologies and neutralizing the fears of HCWs. However, the HCWs' opinion is more nuanced; if they value scientific research in its goal to reduce HCAIs, they worry that these new technologies will not be without consequences for themselves and their profession. (C. Tarantini, manuscript in preparation).

Communication to Healthcare Workers

While signs are commonly used to remind physicians and nurses to perform hand hygiene, recent randomly assigned studies showed that signs did not significantly improve hand hygiene compared to baseline signs [29, 30]. It is not clear if this is because signs do not work or because current signs are not optimally designed. It has been shown that various evidence-based components are essential for the design of efficient signs, including gain-framed messages, alerting signal words, appeal to personal responsibility, appeal to patient consequences, a specific activity required from the reader, attention-getting features, and appropriate design features such as color and letter size. We are currently investigating this question with our own homemade signs.

Biotechnological Application of Quorum Quenching Enzymes

Quorum quenching (QQ) is a strategy using enzymes issued from quorum-sensing bacteria to fight biofilm formation and virulence factor secretion. The IHU has recently invested in the use of quorum-sensing bacteria by the creation of the Gene & GreenTK company. These approaches are appealing because they do not directly challenge bacterial survival and, consequently, selection pressure may be low, yielding a lower occurrence of resistance. QQ enzymes are particularly promising because they act extracellularly to degrade autoinducers and can be used in catalytic quantities. A recent review draws an overview of QQ enzyme–related applications, in particular, topical and dressing perspectives for treatment or decolonization of infected/colonized wounds [31].

CONCLUSIONS

Preventing transmission when caring for contagious patients is a complete approach, including the use of dedicated circuits, single room isolation, and rapid diagnostic tests with early and adapted treatment; enhancing hand disinfection and respiratory protection; avoiding unnecessary catheter use; improving the traceability of care; and reducing colonization by multidrug-resistant, gram-negative bacteria. New technologies or biotechnologies are likely to be of great help in such a challenge. HCWs should be enlisted to make these changes a success. These are the aims of the IHU.

Notes

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SUPPLEMENT ARTICLE

A Hospital-Based Committee of Moral Philosophy to Revive Ethics

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The Méditerranée Infection Foundation's primary goal is supporting a research hospital for the treatment of infectious diseases in Marseille. The main objective of this innovative center is to understand the mechanisms of contagion and face them. The Foundation will include a committee on moral philosophy that will accompany and supervise biomedical research. This is not a conventional ethics committee, frequently giving rise to a board's bureaucratic excesses, which might slow down creative biomedical clinical research without necessarily restricting abuses. Moral philosophy, however, can handle contemporary biomedical issues. In all its diversity, this discipline is able to enrich the debate on medical issues, thanks to many philosophical currents such as deontological ethics and consequentialism. The purpose of this committee is therefore to advance reflection on the bioethical issues encountered in biomedical research in infectious diseases, while respecting the precepts of moral philosophy.

Keywords. moral philosophy; infection; ethics; committee; bureaucracy.

Compared with the attention focused on abortion, euthanasia, assisted reproduction, and genetics, ethical issues involving infectious diseases are underrepresented. Nevertheless, epidemics such as the Black Death, smallpox, and 1918 influenza [1] are among the most fatal and catastrophic events that medicine has ever faced. Recently, emergent or reemergent epidemics (human immunodeficiency virus, severe acute respiratory syndrome, tuberculosis, Ebola) have been raising awareness of the current infectious risks among human beings [2]. The need and desire to insert ethics and moral philosophy into research on infectious diseases remains relatively recent. Recently, one article in the *New England Journal of Medicine* [3] was dedicated to Henry Beecher, who 15 years ago reported the presence of "unethical or questionably ethical" publications in a prestigious journal of clinical research [4]. It illustrated how difficult the scientific community's adhesion to moral approaches to clinical research actually was. Another example, this time in basic microbiology of infectious diseases, is the necessary "gain-of-function" ethical debate about biosafety risks threatening public health, concerning experiments that create novel strains of microbes expected to be virulent and transmissible in humans, so-called potential pandemic pathogens [5, 6]. To this respect, the Nuremberg Code, the main text in clinical research ethics, mandates in 10 ethical principles that (clinical or basic) experiments that pose a risk to human life should be undertaken only if they provide humanitarian benefits that sufficiently offset the risks and if these benefits are unachievable by safer means. Even though the "humanitarian background" of this text is untouchable, the conceptualization of the Code goes back to 1947 (and was based on the Guidelines for Human Experimentation of 1931) and certainly requires revision [7].

Certainly, research on infectious diseases must now necessarily have an ethical and moral approach, as it aims to protect individuals, or society, both from infections and their spread, using means ranging from vaccination to quarantine procedures [8], which eventually might restrict individual freedom for the good of the society. This is why a committee on moral philosophy was established at the "Méditerranée Infection" Foundation, to represent more fairly the interests of both patients and research, trying to solve any kind of ethical conflicts [9].

Different currents will be represented, for example, utilitarianism, which maximizes pleasures, avoids penalties, and calculates the good of an action based on its consequences [10] (also called "consequentialism"), and deontological ethics, which focus on the action itself, and on its compliance with the duties of every human being. In this committee, the Kantian precepts (which had inspired deontological ethics) will serve as a starting point for any reflection. The bases of its moral philosophy are grounded on Kant's categorical imperative as an objective. It is formed as a rationally necessary and unconditional principle that we (human beings) must always follow, as a "duty," despite any natural desires or inclinations we may have to the contrary. The sentence "Act only in accordance with that maxim through

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which you can at the same time will that it become a universal law" [11] will always be the committee's guideline from a theoretical point of view.

But moral philosophy is also about solving practical problems to ensure a form of justice [12], for example, by questioning the freedom of our actions, our free will, or our capacity to make hard choices. These are common problems in moral philosophy of scientific research [13] that we can apply to biomedical ethics, such as experimenting with new treatments on human persons (obviously well informed about short- and long-term possible risks and benefits, and with written acceptance), or in cases in which the personal free will should be suspended in favor of the human community. In these circumstances, an exhaustive scientific documentation of the case (including the personal profile of patient) should be required, as well as the written ethical judgement of moral philosophers serving in the committee, which should be themselves submitted to a constant ethical evaluation of their personal integrity by independent bodies, eventually of national range.

A major objective of the "Méditerranée Infection" Foundation is the management of contagion. The philosophical and ethical questions raised by infectious diseases are illustrated by the recent Ebola outbreak [14]. In addition, when people are confronted with life-threatening disease without specific treatments or preventive measures, one must ask whether it is ethical to propose interventions with greater risks and which are of unproven efficacy [15]. This example illustrates how clinical trials constitute an issue that involves moral consideration. Among clinical trials, noninferiority (NI) trials deserve specific attention. They were introduced in the mid-1990s, and their use is still being debated. They are designed to show that a new treatment is equal to, or not different from, the standard treatment, and that the new treatment is "not unacceptably worse" than the therapeutic gold standard [16]. This step is critical because it defines what is clinically acceptable. There is an imbalance between a stringent margin that restricts the chances of obtaining NI results, and a liberal margin that does exactly the reverse. This is why the interpretation of NI clinical trials is difficult and their ethical nature debated [17]. The discussion about the ethical dimensions of NI clinical trials in fact drove Garattini to propose banning NI and equivalence clinical trials from clinical practice. His point is that NI clinical trials expose patients to clinical experiments without sufficient evaluation of the risks they face [18].

The imbalance between patients' interests and commercial interests is not always assured and controlled in a number of clinical trials. Here, the intelligibility of the informed consent document becomes critical, and this is why one of the objectives of the Foundation, and particularly of the philosophers in it, will be to work on that point. The consent concept has to be extremely clear for research to respect contemporary ethical principles, such as self-control [19] and the respect of autonomy [20].

This ethical approach is therefore needed, to avoid the risk of reproducing the excesses that have occurred in the past, as we have seen in Beecher's article, and, although the excesses are not the same, they remain very much present. Conventional medical ethics is frequently suffering in our days from a focus on bureaucratic minutiae that might restrict the development of advanced clinical studies [21]. Such a drift narrows the potential of this new institute dedicated to research and treatment in infectious diseases to answer critical questions, limiting the possibilities of exploring a field in which many new issues, including new concepts, have yet to be discovered for the benefit of the humanity.

Nowadays, biomedical research cannot be productive with a form of bureaucracy that has often replaced philosophy in ethics committees. Ethics has felt an influence from legalistic considerations that have rendered it meaningless on the philosophical front, to the point of forgetting why it was actually created. A certain degree of equilibrium should be reached. Of course a "healthy" bureaucracy should continue in terms of "assuring proper documentation" of cases, and determination of researchers' obligations and responsibilities, but not at the expenses of replacing ethics. The "Méditerranée Infection" Foundation wants to reinsert philosophy into ethics, to overcome the paradox of a dehumanized ethics that resembles something more like a legal proceeding than a think tank on contemporary issues in biomedical research.

Notes

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SUPPLEMENT ARTICLE

Evaluating the Clinical Burden and Mortality Attributable to Antibiotic Resistance: The Disparity of Empirical Data and Simple Model Estimations

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Given the proliferation of cataclysmic predictions about antibiotic resistance, cases of which are estimated to amount to 12 500 per year in France, we herein decided to compare the empirical clinical microbiology data from our institution with estimates and predictions from 10 major international scientific articles and reports. The analysis of 7 years of antibiotic resistance data from 10 bacterial species and genera of clinical interest from our institution identified no deaths that were directly attributable to extremely drug-resistant bacteria. By comparing our observations to the 10 articles and reports studied herein, we concluded that their results lack empirical data. Interventions are urgently needed to significantly reduce both mortality and the healthcare costs associated with bacterial infections, including the implementation of local and national laboratory data–based surveillance systems for the routine surveillance of antibiotic resistance that would be helpful for a better understanding of how to manage antibiotic-resistant bacteria in the future.

Keywords. antibiotic resistance; extra deaths; multidrug resistant bacteria; old antimicrobial drugs.

In recent decades, major technological improvements have emerged around the world, particularly through the massive spread of the internet. It was recently estimated that of the 7.3 billion human beings worldwide as of June 2016, >3.6 billion (49.5% of the world's population) have access to and actively use the internet (http://www.internetworldstats.com/stats.htm). This impressive development accelerates the sharing of a diverse range of information, including health-related information. One of the main problems associated with this sharing is that individuals can distort or skew the information based on their perceptions and preconceived ideas about a given event, which can create panic among the population [1]. Therefore, the way information is communicated to the public is of critical importance.

In recent years, we have seen the proliferation of cataclysmic predictions about human health that, fortunately, have never fully materialized. These include, in particular, bioterrorism, Creutzfeldt-Jakob disease, severe acute respiratory syndrome (SARS), the H5N1 and H7N9 influenza viruses, Middle East respiratory syndrome (MERS), and Ebola. These infectious threats have killed hundreds or, in some cases, thousands of people, but the death toll remains far from the million deaths per year due to tuberculosis (1.1 million in 2015) [2], and far from the 29 000 and 15 000 estimated annual deaths due to *Clostridium difficile* in the United States and Europe, respectively [3, 4]. This disproportional reaction is particularly observable in Table 1.

The new fear that is currently taking control around the world is antibiotic resistance, and its purportedly huge impact on humans. Over the last decade, resistance to antibiotics has become a new fear, both within the scientific community and in the media, leading to several alarmist reports of the potential inability to treat patients in the future [5–14] (Table 2), and as many as 10 million extra human deaths per year by 2050 [9]. This raises the question of whether we urgently need to develop new antibiotics to maintain the efficacy in the treatment of infectious diseases [15] and/or whether these alarmist reports represent the reality of clinical practice.

Given the importance of the issue, the Institut Hospitalo-Universitaire Méditerranée Infection (IHU) has implemented its own empirical bacterial antibiotic-resistance surveillance systems [16, 17] to provide a strong foundation for discussions on the subject, rather than being based on predictions deriving from mathematical models that have yet to succeed in predicting the evolution of any other infectious risks [18–20]. In this investigation, we compare the empirical data available from the IHU bacterial antibiotic-resistance surveillance network with the estimates and predictions from various articles and reports [5–14] (Table 2).

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Table 1. Number of Articles Published in PubMed, Number of Deaths, and Number of Deaths/Number of Publications Ratios for Bioterrorism, Bovine Spongiform Encephalopathy, Avian Flu H5N1 and H7N9, Severe Acute Respiratory Syndrome, Middle East Respiratory Syndrome, Ebola, Tuberculosis, and *Clostridium difficile*

Keywords for PubMed Research	No. of Articles Published in PubMed[a]	Peak of Publication (Year)	No. of Deaths	Deaths/Publications Ratio[b]	References
Bioterrorism	5808	889 (2002)	5c	0.001	Jernigan et al, 2002; Gursky et al, 2003 [33, 34]
Bovine spongiform encephalopathy	3559	278 (2001)	224	0.1	http://www.who.int/mediacentre/factsheets/fs180/en/
H5N1	6858	754 (2009)	362	0.1	McConnell and Raoult, 2014 [35]
H7N9	7898	789 (2014)	44	0.01	McConnell and Raoult, 2014 [35]
SARS	7811	1157 (2003)	774	0.1	McConnell and Raoult, 2014 [35]
MERS	13328	1496 (2003)	60	0.005	McConnell and Raoult, 2014 [35]
Ebola	5902	1904 (2015)	11315	1.9	http://apps.who.int/ebola/current-situation/ebola-situation-report-6-january-2016
Tuberculosis	231313 (6723 in 2016)	7599 (2015)	1.1 million per year	163.6	GBD 2015 Mortality and Causes of Death Collaborators, 2016
Clostridium difficile	11720 (1070 in 2016)	1082 (2015)	At least 44000 per year	41.1	Barbut et al, 2013; Lessa et al, 2015 [3, 4]

Abbreviations: GBD, Global Burden of Disease Study; MERS, Middle East respiratory syndrome; SARS, severe acute respiratory syndrome.

[a]Number of articles published in PubMed up to 18 November 2016.

[b]Ratios calculated by dividing the number of deaths due to each infectious disease of interest to the total number of publications available in PubMed. Only publications related to bioterrorism events involving infectious agents were included in the calculation. For tuberculosis and *C. difficile*, we used the number of publications of 2016 to estimate the deaths/publications ratio.

[c]Human deaths due to anthrax.

EMPIRICAL OBSERVATIONS

The Global Burden of Disease study group recently published a systematic analysis of the number of deaths due to 249 causes of death, in which they identified that the worldwide number of human deaths due to infectious diseases has dramatically decreased from 10.7 million deaths in 2005 to 8.6 million in 2015 [2]. This is in stark contrast to the global fear about the resurgence of infectious diseases. In particular, this study underlines that the number of global deaths due to lower respiratory infections, which were classified as the leading cause of human death in 1990, and which were the third leading cause of human death in 2015, has decreased from 2.8 million deaths in 2005 to 2.7 million in 2015—that is, a 3.6% decrease over the last 10 years [2]. The decrease is even more striking in children <5 years of age, for whom a 36.9% decrease was observed over the same period [2]. Such observations can be partly explained by improved management of the patients in hospital settings, particularly by the screening and isolation strategies that were developed to control specific pathogens such as methicillin-resistant *Staphylococcus aureus* (MRSA) [21]. However, as previously discussed [21], other factors have to be considered, in particular the discovery and worldwide spread of antibiotic compounds [15], which continues to grow, with a 36% increase in the worldwide human consumption of antibiotics between 2000 and 2010 [12]. Thus, one of the best-known positive impacts of antibiotics on human health is the introduction in the 1940s of antituberculosis medicines, which contributed to the dramatic decrease in worldwide prevalence and mortality of tuberculosis [22]. This decrease is still observable today, with a drop from 1.3 million deaths in 2005 to 1.1 million deaths in 2015—that is, an impressive 15.4% decrease over the last 10 years [2]—and an estimated 47% decrease between 1990 and 2015 (http://www.who.int/gho/tb/epidemic/cases_deaths/en/). Another example is the impact of penicillin on pneumonia, which was highlighted by a dramatic decrease from 20%–40% to 5% in the worldwide mortality rate for pneumococcal pneumonia over the last 8 decades [12].

OBSERVATIONS FROM MARSEILLE

The IHU surveillance system network includes 4 automated laboratory data–based surveillance systems: epidemiological surveillance and alert based on microbiological data (EPIMIC), established in 2002 [16], and the bacterial real-time laboratory-based surveillance system (BALYSES), the Marseille antibiotic resistance surveillance system (MARSS), and the Provence-Alpes-Côte d'Azur surveillance epidemiologic system (PACASurvE), established in 2013 [17, 23]. EPIMIC, BALYSES, and MARSS analyze data produced by the Timone hospital clinical microbiology laboratory on a weekly basis, and PACASurvE analyzes data produced by 214 microbiology laboratories from France's Provence-Alpes-Côte d'Azur (PACA) region on a weekly basis. In combination, these surveillance systems make it possible to monitor the weekly number of patients where >650 bacterial species were isolated from clinical human samples collected and cultured in the 4 Assistance Publique–Hôpitaux de Marseille public hospitals.

Of these surveillance systems, EPIMIC and MARSS monitor weekly bacterial antibiotic resistance profiles (39 antibiotic

Table 2. Sources of Data Compared in Our Study

Source of Data	Description of the Analysis	Studied Pathogens/Antibiotic Profiles	Number of Extra Deaths (Region)[a]	References
Institut Hospitalo-Universitaire Méditerranée Infection	Retrospective analysis of 27 681 nonredundant bacterial infections using real clinical microbiology data	*Staphylococcus aureus*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Enterobacter cloacae*, *Enterobacter aerogenes*, *Burkholderia cepacia*, *Stenotrophomonas maltophilia*, *Proteus* spp, *Serratia* spp, and *Morganella morganii*	0 (Marseille, France)	Unpublished data
World Health Organization report	Estimate based on the analysis of 221 studies	MRSA, 3GC, and fluoroquinolone-resistant *E. coli*, and 3GC and CR *K. pneumoniae*	NC	World Health Organization, 2014
European Centre for Disease Prevention and Control report	Estimates based on data from the European Antimicrobial Resistance Surveillance System	MRSA, VISA/VRSA, VRE, *Streptococcus pneumoniae* resistant to penicillin, 3GC and CR *E. coli*, 3GC and CR *K. pneumoniae*, and CR *P. aeruginosa*	25 000 (Europe)	ECDC/EMEA Joint Technical Report, 2009
Centers for Disease Control and Prevention	Estimates based on 20 main sources of data	*Clostridium difficile*, CR Enterobacteriaceae, *Neisseria gonorrhoeae*, MDR *Acinetobacter baumannii*, *Campylobacter* spp, fluconazole-resistant *Candida* spp, ESBL-producing Enterobacteriaceae, VRE, MDR *P. aeruginosa*, *Salmonella* spp, *Shigella* spp, MRSA, *S. pneumoniae*, *Mycobacterium tuberculosis*, VRSA, erythromycin-resistant group A streptococci, and clindamycin-resistant group B streptococci	23 000 (United States)	Centers for Disease Control and Prevention, 2013
Burden study report	Estimates based on data from the European Antimicrobial Resistance Surveillance System	MRSA, glycopeptide-resistant *Enterococcus* spp, 3GC *E. coli*, 3GC *K. pneumoniae*, CR *P. aeruginosa*, CR *K. pneumoniae*, and CR *A. baumannii*	12 500 (France)	Colomb-Cotinat et al, 2015
Antimicrobial Resistance Review	Prediction of the number of deaths due to 6 public health issues using several sources of data	*E. coli*, *K. pneumoniae*, *S. aureus*, HIV, malaria, and tuberculosis	10 million in 2050 (worldwide)	O'Neill, 2014
Burden study group	Estimates based on data from the European Antimicrobial Resistance Surveillance System	Bloodstream infections due to MRSA and 3GC *E. coli*	8000 (Europe)	De Kraker et al, 2011
Laxminarayan et al, 2016	Analysis using estimates produced by Liu et al, 2015 [36] focusing on neonatal sepsis	Unspecified resistant pathogens responsible for neonatal sepsis	214 000 (worldwide)	Laxminarayan et al, 2016
European Society of Clinical Microbiology and Infectious Diseases	No methodology available	No methodology available	1 million in 2025 (Europe) 10 000 (United Kingdom)	ESCMID, 2015
Centers for Disease Control and Prevention	Estimates based on data from the EIP-ABCs population-based surveillance system	Invasive MRSA	>11 000 (United States)	Dantes et al, 2013
Laxminarayan et al, 2013	Estimates based on risk factor analysis produced by Kayange et al, 2010 [37] focusing on neonatal deaths	ESBL Gram-negative bacteria and MRSA	>58 000 (India)	Laxminarayan et al, 2013

Abbreviations: 3GC, third-generation cephalosporin resistant; CR, carbapenem resistant; ECDC, European Centre for Disease Prevention and Control; EIP-ABC, Emerging Infections Program–Active Bacterial Core; EMEA, European Medicines Agency; ESBL, extended-spectrum β-lactamase; ESCMID, European Society of Clinical Microbiology and Infectious Diseases; HIV, human immunodeficiency virus; MDR, multidrug-resistant; MRSA, methicillin-resistant *Staphylococcus aureus*; NC, not calculated; VISA, vancomycin-intermediate *Staphylococcus aureus*; VRE, vancomycin-resistant *Enterococcus*; VRSA, vancomycin-resistant *Staphylococcus aureus*.

[a]Number of extra deaths due to antibiotic and/or antimicrobial resistance.

resistance patterns for EPIMIC and 65 antibiotic-resistance profiles for 15 bacterial species of interest to MARSS) [16, 17]. Since 2010, they have monitored the results of >140 000 bacterial antibiotic resistance tests—that is, the results of >25 000 antibiotic resistance test per year. This 15-year experiment has tracked intriguing epidemiological events. For example, we identified that the level of MRSA strains routinely isolated in hospitals in Marseille dramatically decreased over the years, particularly invasive strains, for which the level of MRSA decreased from 27.4% in 2010 to 12.8% in 2015, for unexplained reasons [21]. A comparison of our level of MRSA to European data from the European Antimicrobial Resistance Surveillance Network and available international literature shows that this phenomenon is, in fact, a worldwide unexplained epidemiological event [21], emphasizing that the bacterial epidemiology is evolving, particularly through the continuous appearance and disappearance of bacterial clones that are more competitive. This phenomenon has been observed several times in the past, and was recently observed with the appearance and worldwide spread of the *Escherichia coli* sequence type 131 clone [24]. Our monitoring system has also enabled us to see that the level of resistance to some critical antibiotics (including vancomycin and imipenem) of invasive strains belonging to 11 bacterial species of particular clinical interest (*Enterobacter cloacae*, *Escherichia coli*,

Enterococcus faecalis, *Enterococcus faecium*, *Klebsiella pneumoniae*, *Proteus mirabilis*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Streptococcus agalactiae*, and *Streptococcus pneumoniae*) did not globally change or even decrease from 2001 to 2015 [15]. All these examples from the surveillance of antibiotic resistance on the local scale clearly demonstrate that the prevalence of resistance of bacteria to any given antibiotic is difficult to predict, as it depends on a multiplicity of biological, genetic, and ecological factors, creating a complex frame with high stochasticity [25]; therefore, estimates of extra deaths based on such data are highly speculative.

EXTENSIVELY DRUG-RESISTANT BACTERIA AND HUMAN EXTRA DEATHS

Recently, we decided to collect and analyze the results of 7 years of bacterial antibiotic resistance test performed in our clinical microbiology laboratory, to determine the true impact of extensively drug-resistant (XDR) bacterial strains (ie, bacterial strains susceptible to <2 generic antibiotic compounds) on the mortality of patients hospitalized in our facility (representing 1% of patients hospitalized in France). We focused our analysis on 10 bacterial species and genera of particular interest. Overall, we identified that 27 681 nonredundant bacterial infections due to the bacterial species of interest occurred over the study period, with 37 bacterial strains that were truly XDR. We then checked whether the 37 patients infected by the 37 XDR strains died in the 30 days following the infection. From this, we identified only 4 patients who met the criteria of inclusion. Looking their medical history in detail, we observed that all were admitted in intensive care units with comorbidities (1 for a hemorrhagic cerebellar stroke, 1 for septic shock, 1 for severe aortic valve endocarditis due to MRSA, and 1 for febrile respiratory distress worsened by cystic fibrosis) and that their deaths were not necessarily attributable to XDR bacterial infections. Although this analysis did not include case-controlled studies, which is a major limitation of our analysis, it allows us to conclude that, in our clinical experience, only 1 death was observed due to an XDR bacterial strain, which occurred in 2002 with an XDR *Enterobacter aerogenes* that became successively resistant to imipenem then colimycin, but which was still susceptible to gentamicin [26, 27]. The patient eventually died after a 10-week stay in the intensive care unit. The real role of the bacteria in the death of this patient is uncertain, but we certainly reached a therapeutic impasse of sorts and the patient died. In the literature, studies describing human deaths due to therapeutic impasses are scarce. Moreover, even in these cases, it is not clear whether or not the clinicians tested second-line antibiotics to treat the bacterial infections, as several useful antibiotics are no longer available [28], although they are effective against multidrug-resistant (MDR) bacteria [29]. One of the most striking examples of such efficacy is the successful use of antileprosy drugs in combination with other drugs to treat patients with pulmonary tuberculosis due to an XDR *Mycobacterium tuberculosis* strain [30]. Taken together, these observations clearly demonstrate that the relationship between XDR bacterial infections and mortality is difficult to determine, and that further analysis is needed to clarify this relationship.

WHAT SHOULD WE MAKE OF THE ALARMING ESTIMATES AND PREDICTIONS ABOUT ANTIBIOTIC RESISTANCE?

Comparing our observations to the estimates and predictions from the articles and reports mentioned in Table 2 [5–14], it is clear that authors are not analyzing true clinical microbiology data. Thus, estimates of the number of extra deaths due to MDR bacterial infections are based solely on mathematical models and formulae. In any case, the real number of deaths attributable to MDR bacteria can be counted, as such data could easily be retrieved from hospital settings. Moreover, each of these reports uses a different definition of MDR bacteria, and the attributability of deaths to those bacteria were not analyzed. Thus, the alarmist messages presented in these reports usually concern 1 bacterial species for a given antibiotic (eg, resistance to carbapenems or colistin in gram-negative bacteria), rather than resistance to a panel of antibiotics from different classes [5–14].

CONCLUSIONS AND PERSPECTIVES

Although clinical microbiology data and the results obtained from our analysis can be biased for several reasons, including the way data are extracted and analyzed and the fact that the bacterial epidemiology may vary from one place to another place, the local antibiotic resistance surveillance systems we have implemented in our institution, combined with our strategy to test a large panel of antibiotics, including older antibiotics against MDR bacteria, and the count of deaths associated with XDR, have enabled us to demonstrate that the current fears and alarmist reports both in the scientific community and in the media might be far removed from reality. The difference between our data and the estimates and predictions from the studied articles and reports [5–14] (Table 2) clearly raises the question of the usefulness of these predictive mathematical models and the quality of data that have been used to build them, as compared to empirical counting and observation of the realities on the ground. Indeed, modeling extra deaths using deductive models without actually counting the real number of deaths attributable to MDR bacteria does not reflect reality, and cannot be used as a standard, because there are many counfounding factors, such as emergence of new bacterial clones, comorbidities, local epidemiology, the availability of drugs, etc. Thus, it is impossible to predict the number of extra deaths based on exceedingly simple mathematical models when there are so many uncontrolled factors [31]. This is illustrated fairly well by Alice's living croquet theory [32], borrowed from Lewis

Carroll's game of "living croquet," where the mallets are live pink flamingoes and the balls are live hedgehogs who behave unpredictably, making it impossible to predict the outcome of the game. It is the same with predicting extra deaths attributable to bacterial infections due to MDR bacteria, because trends of resistance to antibiotics are unpredictable, and many additional factors are currently unknown or misunderstood.

This raises the question of what can be done. In the past, some antibiotics, such as imipenem, were considered to be a "magic bullet," explaining their overuse for decades. However, the recent emergence of carbapenemase-producing bacteria should prompt us to reconsider old drugs. Considering that it is still unclear how antibiotic-resistant bacterial infection impacts hospitalized patients, we believe that it is reasonable to recommend (1) the adaptation of empirical therapeutic treatments to local antibiotic-resistance epidemiology; (2) the implementation of antibiotic stewardship to guarantee best therapeutic usage; (3) the revival of our historical armamentarium of antibiotics, including those that are no longer distributed by drug companies; (4) the management of patients with bacterial infections in infectious diseases units in coordination with clinical microbiologists; and (5) the implementation of laboratory data–based surveillance systems for the routine surveillance of antibiotic resistance. Such approaches have already been implemented in our institute in its role as an experimental research hospital to fight bacterial infections, including infections due to MDR bacteria. The results from these ongoing approaches to patients with MDR bacterial infections will help us better understand how to manage such patients in the future.

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Clinical Infectious Diseases

SUPPLEMENT ARTICLE

Developing Research in Infectious and Tropical Diseases in Africa: The Paradigm of Senegal

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Infectious diseases represent one of the greatest potential barriers to achievement of the third Sustainable Development Goals in African countries and around the world because they continue to pose major public health challenges. The surveillance of infectious diseases has recently assumed greater importance in most African countries, both because of the emergence of infectious diseases and because strains of pathogens that cause tuberculosis, malaria, cholera, dysentery, and pneumonia have developed resistance to common and inexpensive antimicrobial drugs. However, data on the pathogen-specific causes of infectious diseases are limited. Developing research in infectious and tropical diseases in Africa is urgently needed to better describe the distribution of pathogen-borne diseases and to know which pathogens actually cause fever. This research is critical for guiding treatment and policies in Africa. More effective diagnostics are also needed for these diseases, which often are misdiagnosed or diagnosed too late. A comprehensive review of this type of research is presented here.

Keywords. rural dispensaries; point of care; emerging pathogens; MALDI-TOF; Senegal.

Infectious diseases continue to pose major public health challenges in African countries and around the world. Infectious diseases represent one of the greatest potential barriers to achievement of the third Sustainable Development Goals because they collectively account for 20% of mortality in all age groups (and 33% of mortality in the least developed countries) and 50% of child mortality [1]. The "big three" killer infectious diseases are malaria, human immunodeficiency virus (HIV), and tuberculosis, which together cause more than 6 million deaths per year [2]. In the past 20 years, major increases in funding have enabled significant progress to be made in the fight against these diseases, although optimism must be tempered [2]. Most frequently, this increased knowledge has resulted from large, worldwide, randomized trials [3–5]. In parallel to these multicentric studies, collaborative observational and technology-driven research has been developed, particularly to explore nonmalarial causes of fever in western Africa [6]. Here, we propose a comprehensive review of the type of research that is emerging on infections and tropical diseases in Africa.

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MALARIA

Malaria remains the most prevalent vector-borne infectious disease and has the highest rates of morbidity and mortality. However, over the last decade, important changes have occurred in how malaria is managed in Africa. These changes include a shift from chloroquine- to artemisinin-based combination therapies as the first line of treatment and the mass distribution and use of insecticide-treated bed nets [7]. Together, these strategies have led to a dramatic reduction in the prevalence, morbidity, and mortality of malaria in most African countries [8]. More generally, disease-specific national plans for controlling disease have been implemented for malaria, tuberculosis, and HIV, which have succeeded in decreasing the incidence and the mortality of these diseases.

The reduction in morbidity due to malaria reveals a significant need to assess the considerable rate of other fever-causing agents, especially in dispensaries that provide front-line healthcare. In the absence of appropriate diagnostic tools, this situation raises challenges for physicians and nurses who lack the ability to treat these patients who are now the majority of febrile patients. The development and implementation of rapid diagnostic tests (RDTs) for malaria in dispensaries also revealed that the diagnosis of malaria could be excluded in a number of cases. However, it was also reported in Ghana in 2016 that 62% of febrile patients with negative RDTs still received antimalarial treatment contrary to guidelines [9]. Indeed, health workers still tend to rely on a clinically presumptive diagnosis of malaria rather than on RDTs results because of the fear of false-negative results [9]. This practice is dangerous because it may increase

mortality due to infectious diseases in sub-Saharan Africa. It has already been demonstrated in Tanzania that mortality for malaria in smear-negative patients was higher (292/2412, 12.1%) than in the positive patients (142/2062, 6.9%, $P < .001$) (2). Only about 66% of the smear-negative patients had received antibiotic therapy [10]. Thus, the repertoire of other microorganisms involved in febrile episode must be characterized.

SENEGALESE NETWORK FOR THE EXPLORATION OF NONMALARIAL CAUSES OF FEVER

In Senegal, the proportion of morbidity due to malaria fell from 33.57% in 2006 to 3.1% in 2009. The surveillance network that was developed a few years ago includes various geographic study areas (Figure 1). Dielmo and Ndiop are 2 villages located approximately 280 km southeast of Dakar, near the Gambian border in an area of Sudan-type savanna. The Niakhar study area covers 30 villages, 120 km east of Dakar, with a population of 45 000 and a density of approximately 152 inhabitants/km^2. Mlomp includes a group of 11 villages located in Casamance, southwestern Senegal, within the Guinea savanna and mangrove ecological zone. The Bandafassi study area (Sudano-Guinean savanna ecological zone) is located in eastern Senegal, near the border between Senegal, Mali, and Guinea. Finally, Keur Momar Sarr is a village situated at the southern end of Lake Guiers (Figure 1).

Repertoire of Nonmalarial Causes of Fever

The first step was to define the prevalence of the different nonmalarial causes of fever in these 5 study regions and to establish a repertoire, which revealed the following results. Every year in the rural village of Dielmo, between 5% and 25% of inhabitants present with tick-borne borreliosis caused by *Borrelia crocidurae* without acquiring specific immunity [11]. In the Niakhar area, the prevalence of borreliosis is currently the leading cause of fever before malaria and has been estimated by some healthcare dispensaries (unpublished data) as causing up to 20% of fevers. These results support the claim that borreliosis is one of the most common causes of healthcare consultations for fever in all age groups in rural western Africa [12, 13]. Q fever is frequently encountered in Senegal, and the seroprevalence of *Coxiella burnetii* is up to 24% in Dielmo [14]. *Tropheryma whipplei* has

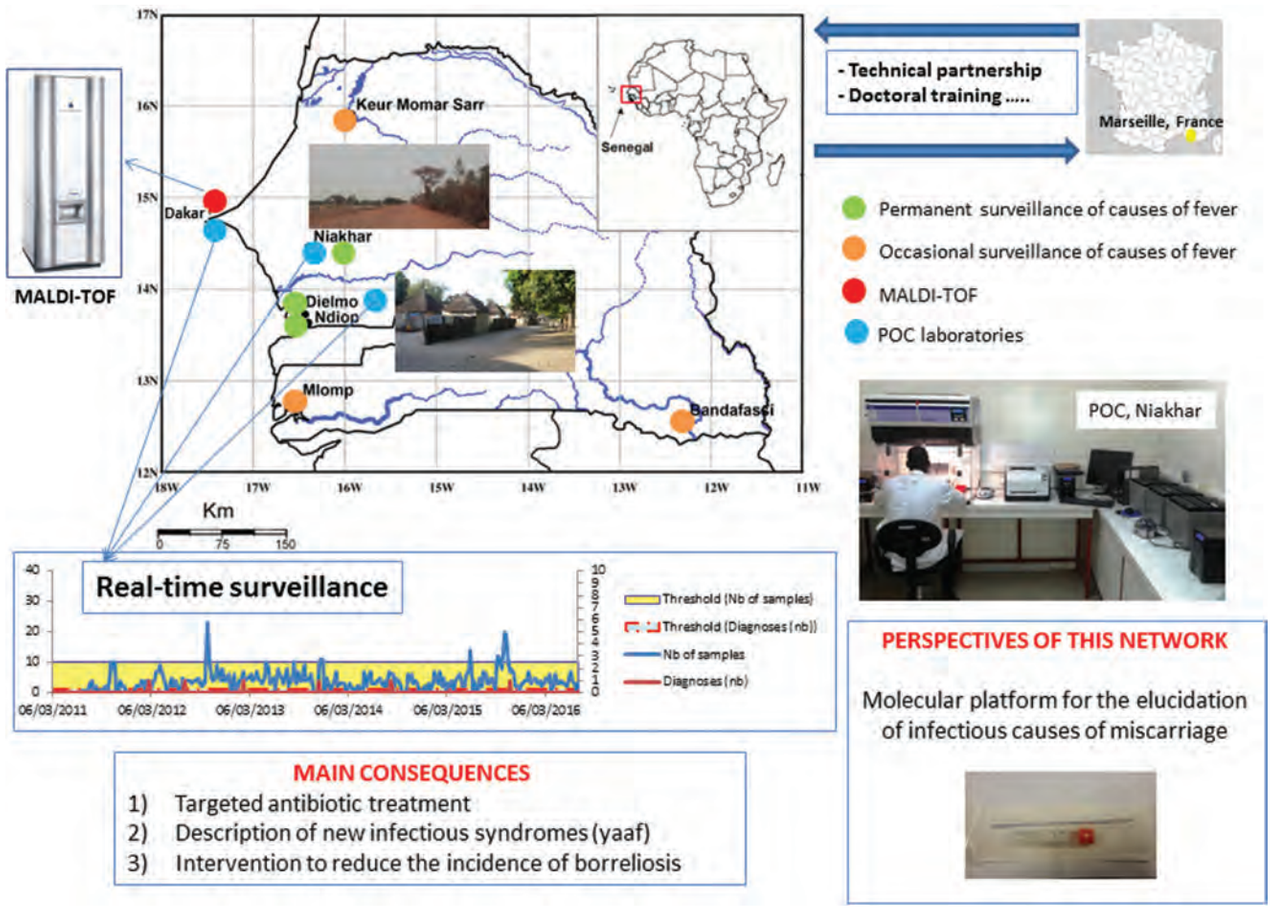

Figure 1. Network of exploration of infectious diseases in Senegal, western Africa.

Table 1. Prevalence of Pathogens Responsible of Fever Diagnosed in 2 Point-of-Care Laboratories Established in Dielmo (2010–2016) and Niakhar (2016), Rural Senegal

	Year	*Rickettsia felis*	Other *Rickettsia*	*Borrelia* spp.	*Tropheryma whipplei*	*Bartonella* spp.	*Bartonella quintana*	*Coxiella burnetii*	*Streptococcus pneumoniae*	*Staphylococcus aureus*	*Salmonella* sp.	*Plasmodium falciparum*	Total Positives	Total
Dielmo														
Total POC	2010–2016	70	5	185	17	29	16	7	6	0	0	95	430	3307
Prevalences (%)	2010–2016	2.12	0.15	5.59	0.51	0.88	0.48	0.21	0.18	0.00	0.00	2.87	13.00	…
Niakhar														
Total POC	2016	0	0	91	0	0	0	0	26	6	0	18	141	810
Prevalences (%)	2016	0.00	0.00	11.23	0.00	0.00	0.00	0.00	3.21	0.74	0.00	2.22	17.41	…

Abbreviation: POC, point-of-care.

been identified in 6.4% of blood samples of febrile patients who tested negative for malaria by RDT, with most patients having a concomitant cough [15]. *Tropheryma whipplei* is also a cause of epidemic fever in Senegal [16]. *Bartonella quintana* DNA was detected in the blood of 2% of patients with fever and head lice [17]. At least 5 named *Rickettsia* species have been identified in Senegal, including *Rickettsia conorii*, *Rickettsia africae*, *Rickettsia aeschlimannii*, *Rickettsia massiliae*, and *Rickettsia felis* [18, 19]. After this had been identified, a quick and simple diagnostic platform was installed locally.

Point-of-Care Laboratories

The point-of-care (POC) laboratories operate 24 hours a day and 7 days a week to provide rapid diagnoses, largely based on immunochromatography and real-time polymerase chain reaction (PCR) tests [20]. Based on the previous analysis of the local repertoire, a POC laboratory was established in Dielmo in 2010 [21] and, in addition to testing for dengue and malaria, it tested the pathogens highlighted during the repertoire establishment phase (Table 1). In the first year after the POC laboratory was established, 440 blood samples were collected from febrile patients, and a pathogen was identified in 127 cases (28.9%), including malaria in 54 cases, *B. crocidurae* in 35 cases, *R. felis* in 30 cases, *Bartonella* spp. in 23 cases, *C. burnetii* in 1 cases, and *T. whipplei* in 1 case, with some patients presenting with a coinfection. No cases of dengue were diagnosed. Various technical difficulties have since been overcome and due to the fact that they are thermostable, easy to transport, and protected against contamination, lyophilized, ready-to-use mixes for individual tests have been introduced to POC laboratories in Dielmo [21]. A second POC laboratory was established in January 2016 in the Niakhar area.

The main bacterial organisms that are identified can be successfully treated with doxycycline, but many of them are not sensitive to amoxicillin and/or cotrimoxazole, which are usually the empirically recommended antibiotic treatment. These observations may change the treatment strategy for acute unexplained fevers in West Africa, in the context of a decline in malaria in many parts of sub-Saharan Africa.

Description of New Syndromes

Observational research coupled with technology may also enable new clinical involvements to be described. In Ndiop, in November 2011, an 8-month-old girl suffered from fever accompanied by severe cutaneous eruptions that evolved from small vesicles to ulcers up to 5 cm in diameter. Blood samples tested in the POC laboratory for the pathogens described above were negative, but quantitative PCR specific for *R. felis* was positive for the cutaneous lesion swab. The patient recovered after 5 days of treatment with doxycycline. The Wolof word "yaaf" was proposed to identify this clinical entity, which corresponded to a primary infection with *R. felis* [22]. Only the

ability to diagnose a spectrum of infectious diseases in a rural front-line dispensary directly after the admission of the patient enabled this clinical picture to be described.

The Role of Matrix-Assisted Laser Desorption/Ionization Time-of-Flight Mass Spectrometry

Culture remains essential in clinical microbiology, particularly for patients hospitalized in teaching hospitals [23], notably in order to determine antibiotic susceptibility [24]. However, identification methods are often costly. Matrix-assisted laser desorption/ionization time-of-flight (MALDI-TOF) mass spectrometry (MS) has become the gold standard for bacterial identification in laboratories in developing countries [25]. Alongside our molecular platform, in 2012, a MALDI-TOF MS was introduced to the clinical microbiology laboratory in the Hôpital Principal de Dakar (Senegal) and was used for the routine identification of infectious agents, making it possible to identify 2082 bacteria of the 2429 bacteria (85.7%) tested [26] at the species level. The robustness of the identification results performed using MALDI-TOF MS in Dakar was confirmed by comparison with results obtained in Marseille, France [27]. The 10 most common bacteria represented 94.2% of all bacteria routinely identified in the laboratory in Dakar (*Escherichia coli*, *Klebsiella pneumoniae*, *Streptococcus agalactiae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Staphylococcus haemolyticus*, *Enterobacter cloacae*, *Enterococcus faecalis*, and *Staphylococcus epidermidis*) [25]. The correct identification of the *Candida* species was also demonstrated for more than 98% of the strains tested [28], as well as other applications such as the identification of biting midges of the genus *Culicoides* [29].

From Diagnosis to Intervention and Prevention

As tick-borne relapsing fevers were a neglected public health problem and a major cause of fever in rural Senegal, as demonstrated by our results [11–13], we implemented a borreliosis preventive control that included awareness-raising among local residents and cementing floors in bedrooms and outbuildings in order to avoid contact between the inhabitants and tick vectors. This led to a significant reduction in the incidence of borreliosis, from 10.55 to 2.63 cases per 100 person-years in Dielmo and from 3.79 to 1.39 per 100 person-years in Ndiop [28]. Public health authorities should adopt this effective tool for promoting rural health through national prevention programs.

PARASITOLOGY

Schistosomiasis

Schistosomiasis continues to be a public health problem in Africa. Urinary schistosomiasis is the most common form in West Africa. In Senegal, transmission takes place continuously along the Senegal River Basin [30] and occurs seasonally in most parts of the country. Several studies have been conducted to understand the epidemiology of urinary schistosomiasis in the district of Niakhar, an area of seasonal transmission in Senegal, and to show whether, with repeated mass treatment, it is possible to eliminate urinary schistosomiasis. The studies show that the district of Niakhar is endemic for urinary schistosomiasis, and overall prevalence has been significantly reduced from 57.7% to 10.1%. Repeated annual treatments are suggested to have had a considerable impact on the transmission dynamics of *Schistosoma haematobium* in Niakhar due to the nature of the epidemiological system with seasonal transmission [31, 32].

Leishmaniases

Leishmaniases are a group of diseases caused by protozoan parasites from more than 20 *Leishmania* species that are transmitted to humans through the bites of infected female phlebotomine sandflies. There are 3 main forms of the disease: cutaneous leishmaniasis, visceral leishmaniasis, or kala-azar, and mucocutaneous leishmaniasis. Recurrent epidemics of visceral leishmaniasis in east Africa (Ethiopia, Kenya, South Sudan, and Sudan) have caused high morbidity and mortality in affected communities [33].

Mansonellosis

Large parts of African countries are colonized by *Mansonella*, a very common but poorly described filarial nematode. The prevalence of this nematode is often very high in endemic areas, even in children, and increases with age. *Mansonella perstans*, *Mansonella streptocerca*, *Mansonella ozzardi*, and *Mansonella rodhaini* are the 3 parasites responsible for human mansonellosis in Africa. *Mansonella perstans* is a human filarial nematode that is highly prevalent in some areas across sub-Saharan Africa and South America. It is a little known but widespread human filarial parasite, more than 100 million people may be infected, and approximately 600 million people living in the 33 countries are at high risk for *M. perstans* infection in Africa alone.

Some studies have been carried out on its epidemiology and the associated health consequences in endemic populations, and no simple and effective drug therapy for treatment and control of the infection has been identified. Data recently obtained by our team from Senegal [34] showed virtual absence of microfilariae in 1159 *Culicoides* collected in a region highly endemic for mansonellosis. This may mean the presence of another vector, at least in Senegal. In equatorial Africa (in Gabon, particularly) another species of *Mansonella* may cause human mansonellosis [35].

CONCLUSIONS AND PERSPECTIVES

Knowledge of the local repertoire of pathogens responsible for fever is critical for the appropriate treatment and prevention of infectious diseases. The establishment of this sentinel network system for the molecular detection of emerging pathogens in patients with fever in rural dispensaries in Senegal

adds significant value to our epidemiological understanding of the causes of fever in rural Senegalese populations. The network also improves the quality of diagnosis and treatment for the research program that is working to improve quality of life in the study population and may serve as a catalyst for further research in this community.

The continuous evolution of microorganisms and changes in the environment and in human habits cannot be modeled [36]. The recent example of the Ebola virus outbreak has demonstrated the need for molecular biology platforms, sometimes even mobile platforms [37], in low-income countries. For studies on infectious diseases, technology-driven research is preferable to hypothesis-driven research [6], as recently highlighted by Quick et al. who demonstrated the value of real-time portable genome sequencing for Ebola surveillance. The authors sequenced 142 Ebola virus samples in Guinea, generating results less than 24 hours after receipt of the positive samples [38]. We believe that the rapid and effective identification of microorganisms as performed by POC laboratories and the ability to easily and quickly diagnose most emerging infections [20], including in rural Africa [21], remains the key to understanding infectious diseases and to improving the management of pathogens that are currently underdiagnosed.

In addition, the development of real-time surveillance systems that enable rapid and flexible responses to be made will be a significant improvement for the future of low-income countries [39]. Finally, after the elucidation of nonmalarial causes of fever, a future challenge will be to understand maternal and perinatal fever and the burden of stillbirth, which remain considerable challenges for public health in low-income countries [40]. We are convinced that, having elucidated the causes of nonmalarial fever, such molecular platforms could help to prevent death and complications during pregnancy, childbirth, and in newborns (Figure 1).

Notes

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Clinical Infectious Diseases

SUPPLEMENT ARTICLE

Place of International Congresses in the Diffusion of Knowledge in Infectious Diseases

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Through digital resources, physicians, microbiologists, and researchers around the world can stay up-to-date with the newest developments in their field and are therefore less dependent on medical congresses as a provider of knowledge and education. The role of the medical congress in spreading knowledge in the face of this changing environment needs to be reexamined. The result is a new paradigm that thinks about the dissemination of medical knowledge and discovery as ongoing conversations between professionals and their extended networks, rather than activities that happen only during the congress. Even though the tools we use to deliver information and knowledge are rapidly evolving, there is confidence in the lasting value of meetings for medical professionals. Medical congresses are environments uniquely conducive to generating new ideas and solutions to problems. As organizers explore new ways of sharing knowledge globally, it is crucial that the high quality of medical congresses be maintained.

Keywords. medical congress; medical meeting; medical education.

In an ever-shrinking world, where infectious diseases spread to far-away regions faster than ever, international scientific exchange and cooperation remain important pillars to managing and controlling infectious diseases. The global spread of antibiotic resistance, emerging and reemerging pathogens, and the need to rapidly diagnose and prevent the spread of infectious diseases further emphasize the importance of international diffusion of understanding, knowledge, and discovery in the field.

The rise of international medical meetings coincided with international travel becoming commercially available. In the past, medical congresses were important places to keep informed about scientific discovery, keep up with advances in clinical management, and to meet colleagues from around the world. Industry partners became involved to showcase their products and interact with participants, providing the financial basis of growing the size and reach of congresses. Societies treated their annual meeting as the year's most important, and oftentimes their only, point of engagement with members.

With the advent of the internet, new formats for information and knowledge dissemination became available. Online publications and communication tools; resources such as UpToDate, an evidence-based, online, clinical decision-making tool that is continuously updated [1]; an explosion of open access journals of varying quality; and the use of blogs, online courses, and virtual journal clubs make the presence of new knowledge omnipresent on a global level [2–5]. In addition, there is a push for quicker dissemination of medical advances, evidenced by decreasing submission-to-publication times of articles in medical journals and the use of social media to communicate study results. The push of making scientific discovery and medical education content freely accessible comes with the need for robust peer review process and other quality control measures.

As national and regional societies increased in number, new medical meetings were established around the world, a trend which continues today. At the same time, the financial stability of medical meetings has been threatened by complex regulatory issues and changes in industry sponsorship practices. The purpose of the medical congress in the face of this changing environment needs to be assessed and the role of the international medical meeting in disseminating knowledge needs to be reexamined.

THE REACH OF THE MEDICAL CONGRESS IN THE 21ST CENTURY

The European Society for Clinical Microbiology and Infectious Diseases (ESCMID) and the International Society for Infectious Diseases (ISID) are 2 societies in the field of infectious diseases with different programs and constituents. Since its founding in 1983, ESCMID has evolved to become Europe's leading society in clinical microbiology and infectious diseases, with members from all European countries and all continents and increasing numbers of attendees from outside Europe at its annual meeting, the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID; Figure 1). ECCMID now attracts >11 000 attendees with representation from all regions of the world. In

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addition, the society offers decentralized educational postgraduate courses and workshops and regional conferences targeting needs and knowledge gaps in and outside Europe.

In contrast, ISID has always had a global constituency. The society's governing board and council, comprised of members from all World Health Organization regions, guide its programs, congress development, and other educational initiatives. ISID's mission is to support health professionals around the world in their work to prevent, investigate, and manage infectious diseases and outbreaks when and wherever they occur. ISID's focus on countries that disproportionally bear the burden of infectious diseases is reflected in the international makeup of its membership, its congress attendees, and the speakers at the biennial ISID. International meetings need to recognize that clinical settings, resources, and priority pathogens differ around the world and must respond to the various needs and knowledge gaps by involving organizing committee members, abstract reviewers, speakers, and session chairs from all regions of the world (Figure 2).

With growing numbers of international members, the sharing of information and knowledge with colleagues who are not able to attend the congress is critical. Advances in technology have made virtual participation in congresses a reality. Talks can be live-streamed and conference content can oftentimes be made available online shortly after the meeting, allowing nonattendees to benefit virtually from the scientific exchange. Social media is contributing to reshaping the reach of congresses by making conference content immediately available, generating international engagement and global reach. Twitter has the power to amplify the content of scientific meetings with increasing numbers of congress-related tweets over the last years [6–8].

The quality and direction of the conversation at medical meetings is also changing. Historically, medical information was delivered "top-down," from leaders in the field to the audience. This paradigm shifted with the advance of social media and other forms of digital communication. Medical meetings now include the possibility of "upward" communication with experts in the field, for example, by asking questions directly on Twitter and pushing "outward" communication to peers and the public. Social media will continue to expand the reach of medical conferences by allowing followers to be part of the discussion and engage with speakers and attendees.

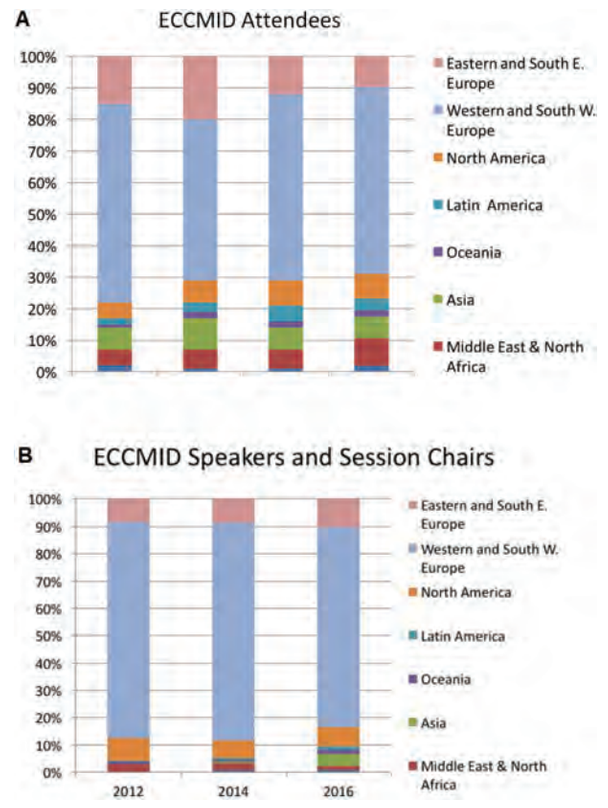

Figure 1. *A*, Country of residence of attendees at the European Congress of Clinical Microbiology and Infectious Diseases (ECCMID). *B*, Country of residence of speakers and session chairs at ECCMID.

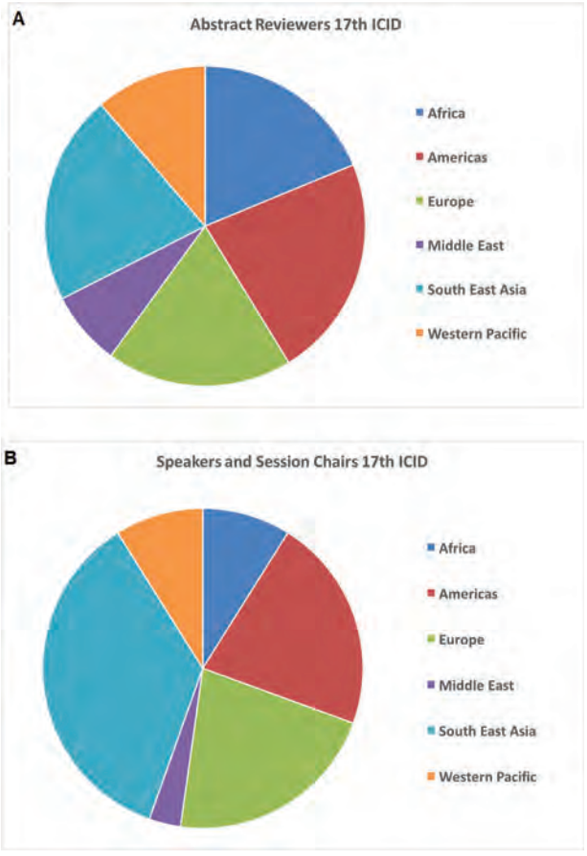

Figure 2. *A*, Abstract reviewers (n = 80) at the 17th International Congress on Infectious Diseases (ICID), according to World Health Organization (WHO) region. *B*, Country of residence of speakers and session chairs (n = 157) at the 17th ICID, according to WHO region. The ICID is a biennial meeting organized by the International Society for Infectious Diseases. It moves around the globe to allow accessibility to all of its members. The 17th ICID took place in Hyderabad, India, in 2016.

With more international, regional, and national meetings than ever, limited time off, and stricter industry regulations regarding travel support, attendees must pick and choose which event to attend. Medical congress organizers have to recognize that people want to spend their time wisely and efficiently. Less brand focused than in the past, industry participation in meetings has become more focused on science and education.

THE MEDICAL CONGRESS—THE BIGGER PICTURE

Increasingly, medical meetings are seen as a reflection of a society's other activities and not as a stand-alone event. Medical congresses are no longer the only way to keep up with scientific innovations and advances in clinical practice. Through digital resources, doctors, microbiologists, and researchers can stay up to date with the newest developments in their field and are less dependent on medical congresses as a provider of knowledge and education. Initiatives to keep members engaged and deliver and share knowledge continuously are sought.

With the advance of the internet and new forms of communication, medical meetings are no longer isolated events. Efforts to engage the scientific and medical community are continuous—before, during, and after the congress. Additional offerings to attract membership and followers are increasingly important and professionals want to stay involved and updated throughout the year.

An example of continuous member engagement is ProMED, the Program for Monitoring Emerging Diseases, which is run by ISID (www.promedmail.org). What started as a physician-to-physician network with the advent of the internet has become one of the largest publicly available outbreak reporting systems in the world [9, 10]. Constantly pushing the borders of innovative disease surveillance, ProMED's network of subject-matter experts provides curated, trusted content to its >82 000 members 24 hours per day, 7 days per week (Figure 3). To provide a forum for ProMED's followers and to advance innovation and discovery in the field, the International Meeting for Emerging Diseases and Surveillance was created in 2007. The 2016 meeting incorporated novel, participatory programs such as a hackathon—an invention marathon—at the intersection of emerging diseases and technology (hackathon.isid.org) and a workshop to train professionals from around the world in innovative disease surveillance and the use of EpiCore, a crowd sourcing platform to find and verify outbreaks faster (https://epicore.org).

THE ROLE OF THE INTERNATIONAL CONGRESS IN A CHANGING WORLD

The goals of meetings today are to spread knowledge, share information instead of presenting it top down, stimulate discussion and scientific exchange, and provide a networking platform (Table 1). With the vast amount of information available

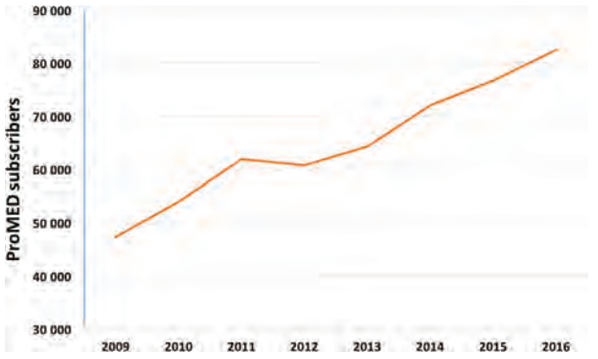

Figure 3. Number of ProMED (Program for Monitoring Emerging Diseases) subscribers, January 2009 through October 2016. ProMED is a publicly available outbreak alert system that reports on new and emerging disease threats in humans, animals, and plants 24 hours per day, 7 days per week. ProMED is a program of the International Society for Infectious Diseases.

in the digital age, it remains crucial to have trusted sources that can highlight relevant studies and publications, endorse and discuss guidelines, and debate the pros and cons of clinical and scientific controversies. Presenting information that is readily found online should be replaced by presenting and discussing emerging technologies and cutting-edge research and providing expert opinion and discussion on new discoveries and controversial topics. Input from attendees in building collaborative conference agendas is desirable.

Will the medical congress evolve to become a knowledge exchange point, virtual and on site where everyone can get and offer information? Technologic innovations are poised to change the way information is shared and education is delivered, but it remains to be determined how to best use these tools to engage people and advance global communication and scientific exchange. New technologies will also allow for changed approaches to abstract submission, late-breaking information, and virtual participation of speakers from around the world to discuss hot topics.

In a shrinking world with greater subspecialization where the need for collaboration is bigger than ever, it is imperative for societies to collaborate and have joint initiatives. Working in teams and combining multiple backgrounds is also becoming increasingly important. Professionals still operate in information silos that need to be broken down. Involvement of

Table 1. The Role of the International Congress in a Changing World

International congresses provide a platform to:
• Exchange knowledge
• Discuss hot topics
• Network with colleagues, make personal connections, and initiate international collaborations
• Orient and advance one's career
• Seek and offer mentorship
• Highlight relevant studies and publications, endorse and discuss guidelines, and debate pros and cons of clinical and scientific controversies
• Obtain continuing medical education (CME) credits

presenters and attendees from other disciplines in medical congresses will lead to the development of new and innovative ideas and novel approaches to the challenges in the area of global infectious diseases.

Patients have more information about their health than ever and also wish to contribute to the discussion. They are assuming a more central role in determining how their care is delivered and when they interact with the healthcare system. Patients take advantage of digital information resources and participation in social communities that enable them to gather advice or self-diagnose before visiting a medical professional. Public lectures to inform the public and the press about relevant health topics have been successfully integrated into medical congresses and have provided the basis for delivering unbiased information and debunking myths and misconceptions.

In a time where open access to data information is important and industry support for meetings is declining, new ways to sustain the medical meeting need to be found. The new formula must be scientifically sound and economically valid. Industry will no doubt adapt to the new information dissemination era and experiment with new ways of reaching doctors virtually and providing open engagement. As the industry continues to change, the challenges facing medical meeting planners are likely to increase, along with opportunities for creativity and innovation. The size of big congresses and meetings mandates that they be professionally managed and financially sustainable.

CONCLUSIONS

New technologies offer an array of opportunities that will allow medical congresses to be reshaped based on the evolving needs of attendees. Societies need to be brave enough to experiment with new approaches to provide knowledge and up-to-date content to their membership. However, as they explore new ways of sharing knowledge globally, it is crucial that the high quality of medical congresses be maintained.

The result is a new paradigm that thinks about the dissemination of medical information, the exchange of knowledge, and scientific discovery as ongoing conversations between professionals and their extended networks, rather than activities that happen only during the medical congress. Even though the tools we use to deliver information and knowledge are rapidly evolving, there is confidence in the lasting value of meetings for medical professionals. Medical congresses are environments uniquely conducive to generating new ideas and solutions to problems. Conversations with colleagues facilitate insight and creativity in a way nothing else can. As powerful as new technologies are, they will never substitute for the face-to-face discussions and collaborations that develop throughout congresses.

Notes

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Clinical Infectious Diseases

SUPPLEMENT ARTICLE

Viruses in the 21st Century: From the Curiosity-Driven Discovery of Giant Viruses to New Concepts and Definition of Life

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The curiosity-driven discovery of giant DNA viruses infecting amoebas has triggered an intense debate about the origin, nature, and definition of viruses. This discovery was delayed by the current paradigm confusing viruses with small virions. Several new definitions and concepts have been proposed either to reconcile the unique features of giant viruses with previous paradigms or to propose a completely new vision of the living world. I briefly review here how several other lines of research in virology converged during the last 2 decades with the discovery of giant viruses to change our traditional perception of the viral world. This story emphasizes the power of multidisciplinary curiosity-driven research, from the hospital to the field and the laboratory. Notably, some philosophers have now also joined biologists in their quest to make sense of the abundance and diversity of viruses and related capsidless mobile elements in the biosphere.

Keywords. giant viruses, virion, phage, virocell, life definition.

For years, most biologists considered viruses as byproducts of biological evolution that could only play a minor role in the history of life. This has gradually changed recently as a result of several advances in different fields of biology. The molecular ecologists focusing on "viromes" have highlighted the extraordinary abundance of viral particles and viral genes in the environment, including our own bodies ([1, 2 and references therein). We are hosts of a myriad of viruses infecting our eukaryotic cells but also of viruses (phages) that coevolve with our microbiota (Figure 1) [2]. More generally, one can conclude that most genetic information on our planet originated in viruses and related capsidless elements [3, 4]. Structural biologists have shown unexpected kinship between viruses infecting organisms that belong to different cellular domains (Archaea, Bacteria, or Eukarya) by identifying homologous traits in the structure of proteins forming the viral capsids [5], strongly suggesting that viruses were present on our planet long before the last universal common/cellular ancestor [6]. At the same time, the study of archaeal viruses revealed a fascinating world of different viruses previously unknown in bacteria and eukaryotes, revealing that each of the 3 domains of life overlaps with a different part of the virosphere [7]. To top it all, the discovery of giant viruses in the laboratory of Didier Raoult in Marseille has caught the imagination of the scientific community by revealing the existence of viruses whose genomes are greater than those of many bacteria and archaea [8]. The Phocean city is now the mecca of giant virology, with the continuous discovery of viruses with still bigger and bigger genomes by the laboratories of Didier Raoult, Jean-Michel Claverie, and Chantal Abergel (for recent reviews, see [9, 10]). Some of these viruses, such as *Mimivirus*, *Pandoravirus*, and *Pithovirus*, produce virions that are bigger than some small archaeal or bacterial cells (Figure 2) and are still visible with an optical microscope. When first observed following hospital infection (for *Mimivirus*) or in thin sections of eukaryotic cells by protistologists (for *Pandoravirus*), they were first confused with small bacterial (*Mimivirus* staining gram positive) or even with small eukaryotic cells. It became difficult to consider that these viruses—mimicking microbes—were not living organisms. Finally, some of these viruses can be infected by their own viruses, the virophages, raising the question of whether it is possible for a virus (the virophage) to infect a nonliving organism. All of these findings have revived interest in viruses and rested the issue of their definition, their nature—living or not—and the definition of life itself [11, 12].

THE TRADITIONAL VIEW OF VIRUSES

Traditionally, viruses, the ultimate parasites, have been considered to be at the border between living and nonliving. Many authors have concluded that viruses are not living

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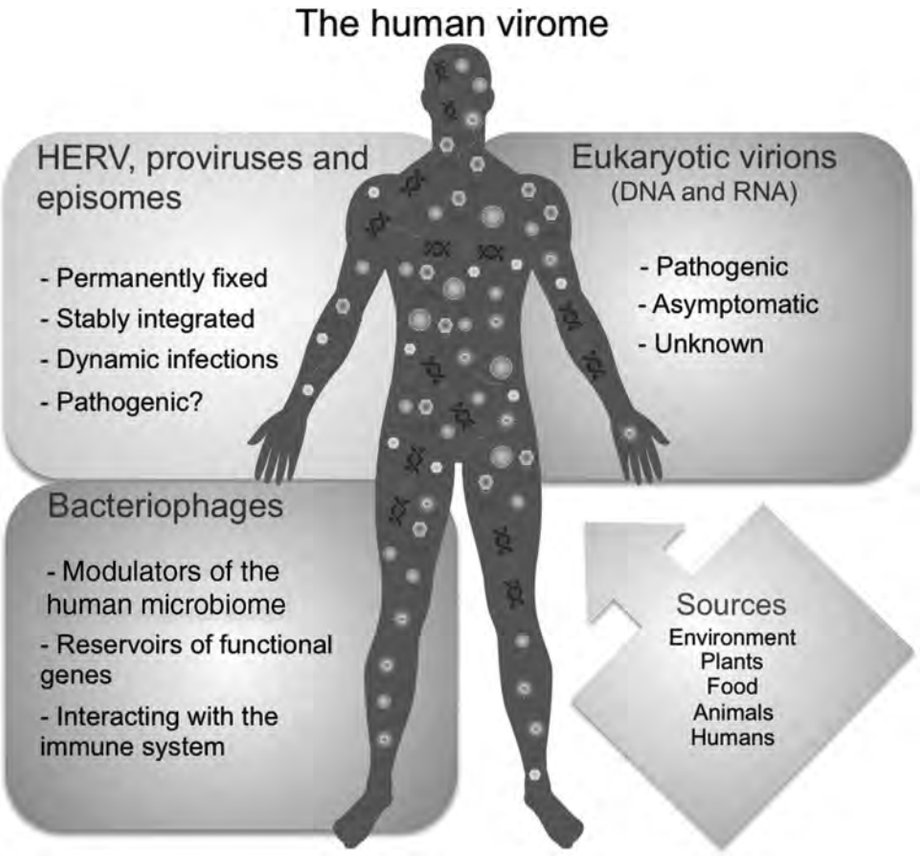

Figure 1. The human virome. Our body hosts a myriad of viruses acquired from different sources. It includes viruses infecting eukaryotic cells that circulate as virions in the human body. Symptoms from these viral infections range from imperceptible to severe and even fatal disease. It is estimated that a significant proportion of these viruses remains to be discovered and their role elucidated. Viruses are also found latent inside human cells (as human endogenous retroviruses [HERVs], proviruses, or episomes). Some have lost the ability to reactivate (eg, certain HERVs), some can be reactivated but remain as proviruses for long periods of time, and others display dynamic turnovers (frequent infections with regular virion production). Bacteriophages (ie, Bacterioviruses), on the other hand, can only infect the bacterial communities that inhabit our body, but have a relevant role as modulators of the human microbiome, as reservoirs of bacterial genes involved in different metabolic processes, and contribute to the maturation of the immune system.

because they lack autonomy and metabolism ([13 and references therein). This conclusion was even officially endorsed by the International Committee on Taxonomy of Viruses. This led most evolutionists to consider viruses as byproducts of biological evolution that could not have played an important role in the history of life. For instance, Moreira and López-García wrote that "viruses have only played a minor role in shaping the gene content of cells" [13]. As a consequence, viruses are still often missing in textbooks devoted to the origin and evolution of life and in scenarios describing the major steps of life history on our planet. Hence, Koonin and Wolf recently correctly noticed that "viruses are no part of the modern synthesis or more generally the traditional narrative of evolutionary biology" [14]. A damaging consequence of this view is that several evolutionists failed to recognize that viruses can be the cradle of new genes and new functions. Viruses are often viewed as passive entities that are used to rob cellular genes but cannot provide themselves new genes to cellular organisms. For instance, Moreira and Brochier-Armanet concluded from phylogenetic analyses focusing on *Mimivirus* genes with cellular homologs that this giant virus is a chimera that acquired most of these genes by horizontal gene transfer, either from its eukaryotic hosts or from bacteria sharing the same hosts [15] (see [16] for a critical analysis of their phylogenies and interpretation). In my opinion, this misleading conclusion (viruses are in fact responsible for the origin of most genes in nature, including cellular genes; see [3] and discussion below) originated from the classical assimilation of the viruses to their virions (viral particles). For instance, a well-known definition of viruses posits that viruses, unlike cells, only contain one type of nucleic acid (RNA or DNA) [17], forgetting that DNA viruses have both DNA genome and messenger RNA. Virions are inert structures that lack metabolism and more closely resemble cellular organelles, justifying the current claim that viruses, being likened to their virions, cannot be alive.

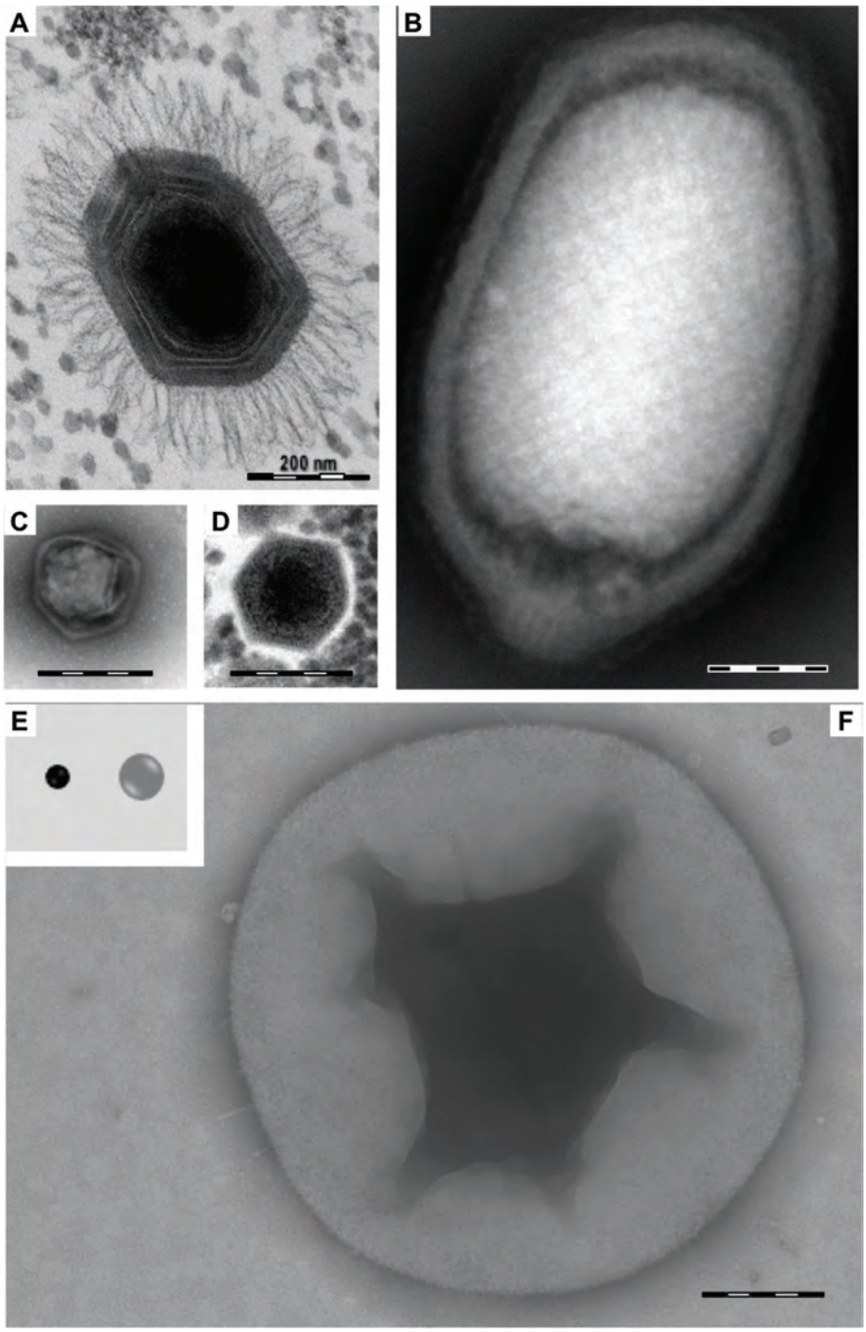

Figure 2. Electron microscopic images showing giant amoeba virion sizes compared to those of a bacterium. *A, Mimivirus. B, Pithovirus massiliensis. C, Marseillevirus. D, Faustovirus. E*, Diameters of virions for a norovirus (black disk) and human immunodeficiency virus (gray disk). *F, Parachlamydia.* All scale bars indicate 200 nm.

THE VIROCELL CONCEPT

Several authors in the past have pointed out that viruses should not be confused with their virions and that viruses can be considered as "living" during the intracellular stage of their reproduction (life) cycle [18–20]. I recently proposed the concept of "virocell" to focus on the cellular step of the viral cycle that involves the transformation of all or part of the infected host into a viral "living organism" ([11, 21] and references therein). In the virocell, the virus expresses its own metabolism and autonomy as the "aim" of the infected cell is no longer to give 2 daughter cells but to produce as many virions as possible to allow the reproduction and multiplication of the viral information. The virocell concept ends up considering viruses as cellular organisms for part of their reproduction cycle. Being

organisms, they can be strict parasites but also live in symbiosis with the infected cell. This can produce various forms of equilibrium between the virus and its cellular host, the ribocell (a cell encoding ribosomes). Coexistence of the viruses and the infected organism (a bacterium, an archaeon, or an eukaryote) in the same "ribovirocell" can be short lived or very persistent, leading to various forms of long-term symbioses [22–24]. These relationships are especially complex in the case of RNA or DNA viruses infecting complex eukaryotic cells. It is fascinating that even small RNA viruses encoding only a few genes can completely reorganize the behavior of giant eukaryotic cells. These viruses can produce complex viral factories by manipulating various types of cell membrane [25] and reorganize the cellular metabolism and the expression of hundreds or thousands of genes with only a handful of expressed viral proteins [26].

VIRUSES AND RELATED CAPSIDLESS MOBILE ELEMENTS AS CRADLES OF NEW GENES

In my view, the main merit of the virocell concept is to highlight the fact that viruses can be the cradles of new genes produced during the intracellular step of viral genome replication. Comparative genomic analyses of closely related yeast and *Drosophila* strains have recently revealed how new genes are formed during genome evolution [27, 28]. Most genes seem to originate from "protogenes" corresponding to short piece of intergenic DNA that are randomly transcribed and translated. These protogenes appear and disappear continuously as long as they are not useful for the organism. However, if a peptide so produced turns out to be beneficial for the fitness of the organism, the protogene encoding it will be stabilized and progressively transformed into a gene. There is no reason not to believe that this mechanism, which has been observed in cellular genomes, did not occur in viral genomes. This would explain why the genomes of viruses and related capsidless mobile elements contain many small genes that have often no homolog, even in the genomes of closely related viruses. The multiple rounds of viral genome replication that take place in virocells and the stunning abundance of viral genomes in the biosphere thus explain well why viral genes are so diverse and numerous, representing the vast majority of the genosphere. In turn, this viral genosphere represents an unlimited reservoir of new genes—thus putative new functions—for the cellular world itself as viral genomes continuously integrate themselves in cellular genomes. The exaptation of viral proteins is thus a major source of new functions in the cellular world. A dramatic example of such domestication was provided by the discovery that major proteins involved in the formation of the placenta in mammals originated from retroviral proteins (for review, see [29]). The syncytins that are involved in cell fusion for placenta formation are indeed derived from retroviral *env* genes and exhibit anti-immunosuppressive properties that evolved during the previous arms race between animal cells and retroviruses. Beside syncytins, another protein of retroviral origin, Peg10, derived from a *gag* gene, apparently plays a critical role in placenta development [29]. From the virus viewpoint, Villarreal suggested that the complex genetic network involved in the placenta formation originated from the manipulation by retroviruses (for their own benefit) of mammal regulatory networks in the framework of long-term persistent infection [30].

The coevolution of viruses and cells that started billions years ago and is still going on today in our microbiota and everywhere in the biosphere thus most likely shaped the history of life more than any other major evolutionary force [3, 4, 29]. Several hypotheses have been put forward that suggest a viral (virocell) origin for proteins responsible for major evolutionary transitions such as the origin of DNA [31, 32], the origin of the prokaryotic and eukaryotic immune systems [33], or the origin of the eukaryotic nucleus [34] (and references therein).

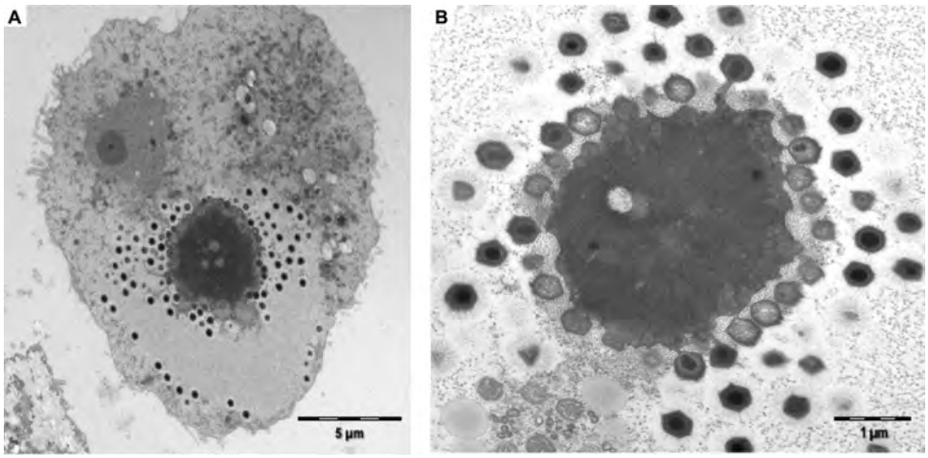

Figure 3. Electron microscopic images showing *Mimivirus*-infected *Acanthamoeba* species. *A, Mimivirus*-infected *Acanthamoeba* species with a *Mimivirus* factory in the center of the host cytoplasm, surrounded by newly produced virions; scale bar indicates 5 µm. *B, Mimivirus* factory at a greater magnification; scale bar indicates 1 µm.

In particular, the complex viral factory produced by *Mimivirus* in the cytoplasm of the infected cell (Figure 3) makes it reasonable to suggest an evolutionary link between the cellular nucleus (a chromosome factory) and the viral factories of giant viruses. It now seems likely that many aspects of eukaryotic cellular and molecular biology evolved as byproducts of the continuous interaction between evolving protoeukaryotic cells and the ancestors of modern giant viruses infecting them [34]. The well-known impact of integrated viral genomes on the infectivity of bacterial pathogens and the control of the microbiota by viruses and related capsidless mobile elements are another critical aspect of this long-term coevolution between viruses and cells [35]. The deciphering of this coevolution will most likely be critical in understanding the origin and evolution of many infectious diseases.

A PHILOSOPHICAL TOUCH

The virocell concept a priori removes the traditional obstacles to consider viruses as living, as the virocell expresses a specific viral metabolism and a high degree of autonomy [11, 36]. The virocells are subject to selection pressure, which justifies considering that viruses are not evolved by cells (ribocells stricto sensu) as previously suggested [13], but that viruses are evolved by virocells [11]. Interestingly, considering that viruses are alive raises new problems that are worth considering in philosophical terms, using biological examples as a case study. Viruses have been defined as capsid-encoding organisms (or virion-producing organisms) by opposition to cells defined as ribosome-encoding organisms [37]. This definition allows for discrimination between viruses and other mobile capsidless elements, such as plasmids, that do not encode for capsid proteins. It has been noticed that the smallest known virus, whose genome encodes only 2 genes—one encoding a replication protein, the other a capsid protein—can be distinguished from the smallest plasmid with a single gene, encoding a replication protein, precisely by the presence in the viral genome of the gene encoding a capsid protein [38]. If one considers that a virus is living whereas a plasmid is not (something widely assumed by most biologists), this means that the presence or absence of a single gene (in that case, the gene encoding the capsid protein) is sufficient to shift from living to nonliving, from organism to macromolecule. This is of course absurd. A similar reasoning can be made in the case of the difference between an intracellular bacterium (definitely living for all biologists) and an intracellular organelle such as a mitochondrion or a chloroplast (definitely not living for most biologists). It is impossible (and absurd) to determine at which moment, during the evolution that led from an intracellular bacterium to a mitochondria or a chloroplast, the living organism became a nonliving organelle [11].

WHAT IS LIFE? AN EXTENDED VIEW

The impossibility to rigorously define a living organism vs a cellular organelle can be viewed as a serious philosophical impasse. At that stage, one should be reminded that the concept of life has been originally associated with human life (by opposition to death) and endorses a high dose of anthropomorphism and sanctity. In biology, the concept of life has been from the very beginning tainted of vitalism and we realize now, especially in studying the world of microorganisms, that using the terms "life" and "living organisms" in a scientific framework is not such an easy thing (for an interesting report on this issue, see [39]). From a materialistic viewpoint, I recently proposed, in a somewhat provocative way, to consider all living biological entities (proteins, chromosomes, plasmids) that participate in a living process to be alive [11]. In that case, it remains to define what is meant by "biological entities" and "living process." In philosophical terms, a biological entity could be equated to a biological "individual," an individual being an entity "which is separable, countable, has acceptably clear-cut spatial boundaries, and … the capacity to remain the same while changing through time" [40]. This would avoid the claim that a gene is living because, unlike, for instance, chromosomes, genes are not "individuals" but human concepts that can be defined in different ways, especially in the case of eukaryotes. Similarly, a protein could be living but a protein "domain" could not. Defining a living process could be done tentatively by opposing life and death, in fact coming back to the original definition of life. For instance, one could consider that a protein is living as long as it can be functional in a living cell, but dead if it is irreversibly denatured. In that definition of living entities, a virion, not only a virocell, can be considered as living as long as it can potentially infect successfully a host cell. After ultraviolet treatment, the virion can be "dead" if its genome is irreversibly damaged. The merits of such a broad definition of living entities are 2-fold. On the one hand, it allows using the concept of "life" and "living" in biology, bypassing the impossibility to define "living" vs "nonliving" biological entities in the traditional definition of life based on autonomy. On the other hand, it clearly eliminates all "sanctified" or "vitalistic" aspect of the terms "life" and "living." Life and living processes are simply names for complex evolving forms of matter that are now present on our planet.

Notes

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Clinical Infectious Diseases

SUPPLEMENT ARTICLE

Basic Sciences Fertilizing Clinical Microbiology and Infection Management

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Basic sciences constitute the most abundant sources of creativity and innovation, as they are based on the passion of knowing. Basic knowledge, in close and fertile contact with medical and public health needs, produces distinct advancements in applied sciences. Basic sciences play the role of stem cells, providing material and semantics to construct differentiated tissues and organisms and enabling specialized functions and applications. However, eventually processes of "practice deconstruction" might reveal basic questions, as in de-differentiation of tissue cells. Basic sciences, microbiology, infectious diseases, and public health constitute an epistemological gradient that should also be an investigational continuum. The coexistence of all these interests and their cross-fertilization should be favored by interdisciplinary, integrative research organizations working simultaneously in the analytical and synthetic dimensions of scientific knowledge.

Keywords. basic sciences; microbiology; infection; innovation; experimental organizations.

Microorganisms constitute the simpler expression of cellular life, and therefore any new knowledge about microorganisms is a contribution to the understanding of life in general. Microbiology is therefore not only the science of microbes, but micro-biology—biology of the fundamental biological entities. But some microbes (a huge minority) are also causing infectious diseases, and many others (a huge majority) influence individual, public, and environmental health. Therefore, microbiology constitutes a crossroads where fundamental biology and health interests necessarily converge.

Slightly more than 150 years ago, on 7 April 1864, Louis Pasteur presented in one of the "Soirées Scientifiques de la Sorbonne" [1] his seminal conclusions about a fundamental (maybe the more fundamental) topic of biology, spontaneous generation: "The spontaneous generation of microscopic beings is a mere chimera …" This is a founder statement of basic science, as what is true for a microbial cell is true for all type of cells and forms of life. But Pasteur was fully conscious that microbes were not only experimental objects to understand life in general, but *agents* to explain the constant transformation of the Earth: "This is the role of those tiny beings which serve as agents of fermentation, putrefaction, and disorganization of everything on the surface of this globe … this role is immense, marvelous, positively moving" and—much more widely known—causative agents of infectious diseases.

DISCOVERY, THE DEEP TASK OF SCIENCE

The term "science" derives from very old Indo-European roots (the Indo-European *skei*; *skhízo* in the Greek, and *scindo* or *scindere* in Latin derivatives), meaning to split or to cleave something with a knife. The sense is to reveal what is hidden below the surface, below the external appearance, to remove what is covering the reality, to discover. It is not by chance that the origin of modern medical sciences in the Renaissance times is tightly linked to anatomical dissection, the scalpel serving as an early scientific tool facilitating discoveries inside human bodies. Indeed, the first scalpel of microbiology was the microscope, but many other technologies of physical, chemical, and, more recently, of computational nature have allowed along the last 2 centuries to go deeper in the discovery of microbes. Note that frequently these tools used in microbiology or infectious diseases are imported from other areas of scientific knowledge, and a critical part of the success in scientific discovery is depending on the creative importation and application of novel tools to use them as updated scalpels. But it should be stressed that to "discover" is primarily unlinked with any applied activity; only the structure and function of the microbiological matter is revealed. Only in a second step, such findings might be used to imagine ways to influence the biological behavior, giving rise to secondary or applied sciences.

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BASIC SCIENCES AND INNOVATION: TRIGGERING FORCES AND OBSTACLES

Basic sciences are those which are devoted to fundamental theoretical or experimental investigative research to advance knowledge without a specifically envisaged or immediately practical application, in the quest for new knowledge and the exploration of the unknown [2]. Thus, the main epistemological force triggering basic sciences is the curiosity, immediately followed by creativity, to find the way to enter and explore the unknown spaces [3]. Because of that, progress occurs more rapidly when investigators are allowed to pursue their passions [4]. In a certain sense, basic sciences only look forward, ignoring the consequences of the new elements of knowledge. Basic sciences play the role of stem cells providing material and semantics to develop functional (applied) tissues and organisms. Differentiated tissues are certainly much less creative (innovative), but this Lamarckian view can be mitigated by the occasional possibility of de-differentiation of specialized into stem-like cellular entities. An intelligent deconstruction of applied themes might also reveal unanswered basic questions (reverse translational research).

Of course, the net result of basic science is knowledge innovation. Basic science is a bedrock of progress [4], but the current dominance (not only in the industry, but also in public agencies, and even in academic research institutes) of clerical "science managers" orientates research to rapid obtention of funds, patents, and rapid return of investments. The success of science is frequently oriented as gaining success for management, which orientates the otherwise naive scientists to take the right path of societal progress (frequently disguised as public health needs or interests), just measured in the framework of monetary units. As it was stated by Francis S. Collins, "when everybody gets to one side of the boat, it usually tips over"—meaning that if all investments are located on one part of the research continuum, the business-oriented research, the entire enterprise of discovery, innovation, and progress may sink [5].

In fact the requirements of the clerical "management of science" evaluation, funding, and communication structures rarely selects creativity and innovation, and certainly fosters vulgarity and sterile repetitiveness in science. Repetitiveness provides a certain flavor of truth, so that any creative novelty is taken as suspicious of scientific weakness. The result is a rampant plethora of journals publishing avalanches of manuscripts, with the consequence of an "inflation" in the intrinsic value of knowledge; eventually important findings might remain buried forever among tons of irrelevant reports. An important secondary effect of this inflationary process is the perception that almost everything has been already published, and that the only possibility of contributing is at the expense of minor variations of what is already known, reducing the creative excitement of passion associated with creativity and creativity recognition and condemning the scientist to an increasingly clerical type of work. The increasing fashionable trend of network research, which is certainly of interest in applied sciences, certainly does not contribute equivalently to creative discovery, which is more based on individuals than groups.

BASIC SCIENCES LAYING THE FOUNDATIONS OF CLINICAL MICROBIOLOGY AND INFECTION MANAGEMENT

An operative-descriptive definition of basic sciences fundamenting research in clinical microbiology and infection management is proposed here. These sciences are devoted to the understanding of unknown mechanisms and processes involved in cellular physiology (cell biology, biophysics, biochemistry), genetics (vertical and horizontal transmission of genes, and the rising of phenotypes), cellular adaptive biology (variation and selection), microbial multilevel population biology (from genes to mobile genetic elements, clones, and microbiotas), interactions biology (as host–bacterial interactions, including those of entomological bases; immunobiology, immunogenetics, and microbiota biology), pharmacodynamics (drug–microbe interactions), ecobiology (microbe–environment interactions, including transmission biology and basic epidemiology), and evolutionary biology (microbial variation and selection along time, evolutionary trajectories). These basic sciences constitute the source of translational research, "the process of applying ideas, insights and discoveries generated through basic scientific inquiry to the treatment or prevention of human (infectious, in our case) disease" as defined by the National Institutes of Health (NIH) [6].

Note that the process of scientific knowledge "lost on translation" [7, 8]—that is, unsuccessful translation of basic insights into medical practice—has somewhat disaccredited in recent years the role of fundamental sciences. As in professional language translation technologies, the efficiency of translation depends mainly on the current scarcity of good "translators" (understanding both basic and practical languages) combined with the number and complexity of languages in the increasingly diversified field of sciences. The NIH-based National Centre for Advancing Translational Sciences initiative (https://ncats.nih.gov/) is a promising organizational tool [9], but as Fang and Casadevall stated, "history has taught us that the path from basic discoveries to scientific and technological applications is seldom a straight line" [8]. In other words, we should consider that there is no dictionary providing translation of a "basic" word to an "applied" word; in fact each word-to-word translation is the result of a scientific inquiry by itself. Note that understanding a translation requires the recognition (even in an obscure way) of something common between the "basic" and "applied" expression, and that occurs probably across a trial-and-error process. Future advances in knowledge engineering and semantics, eventually combined with educational programs [10, 11], might facilitate such complex tasks. But an essential message is that the existence of a bottleneck (the translation)

should not impede the development of basic sciences, as basic "words" will be able to find by themselves a way of being translated into applied meanings, also in microbiology and infectious diseases. These opportunities for translational activity might paradoxically be more fruitful in nonprofit groups, where scientists have the real scientific freedom required for discovery, where individual volunteering or interning experience are vital to meet innovative approaches [12]. In fact no systematic relationship between the "basic" versus "applied" research focus of a grant and its propensity to be cited by a patent has been found in recent studies [13].

CLINICAL INFECTIOUS DISEASES: FROM PRACTICE TO SCIENCE

The behavior of clinicians is frequently based on practice, but this practice is not always rooted in solid science. Of course, diagnostic algorithms or therapeutic protocols are based on the analysis of collections of observations. But on too many occasions the results of a relatively short number of cases along clinical trials are converted into an "established knowledge" that is difficult to modify in the future. When this knowledge is turned into frequent practice, emerge in the guidelines, or reach the medicine schools and hospital residency training programs, science has not much more to say.

Common sense might suggest that we need a consistent way of turning practice into science. Consider, for instance, the millions of patients suffering bacterial infectious diseases that are treated with antibiotics; in hundreds of thousands of them, we know the offending organism and its susceptibility to antimicrobial agents. Now, try to find in the medical literature how the in vitro level of susceptibility correlates with the degree of clinical success. You will generally find little more than the data (many of them historical) during clinical trials, which are carried out on limited populations of patients that do not represent the richness in diversity of patients in real-world medical scenarios. And that will correspond only to a few particular dosages of the drug, based on limited preliminary pharmacokinetic/pharmacodynamic data; in turn, these data are based on minimum inhibitory concentrations, which frequently are the only pharmacokinetic parameter used, disregarding such critical factors as the biology (ecobiology) of cells in the site of infection, or the role of innate immunity. How will the drug behave in different type of infections caused by a given organism? Which type of response can be expected from different types of patients of different ages, normal or impaired immune response, with different underlying diseases, or with different concomitant therapies? In fact, after almost a century since the start of chemotherapy, we are unable to know in a quantitative way the risks and benefits of the use of antimicrobial agents [14]. And regarding clinical responses, why can we not "put numbers" in the different degrees of clinical response, developing solid clinimetrics procedures? All these are legitimate questions that remain without a scientific answer, just because they are depending on an extremely large number of observations.

BASIC SCIENTIFIC METHODS FOR CLINICAL SCIENCES

Science is frequently based on comprehensive collections of relevant data and observations. It is critical not to lose the great deal of information contained in usual medical practices. The observations required for doing science that were mentioned in the last paragraph are frequently available, but we have disregarded the tools and methodology of capturing and analyzing them in a precise, cumulative, continuous (online) way. However, we really need these data to root our practice in scientific data. For instance, if clinicians in a particular place were made aware online of the frequencies of resistance to a particular antibiotic, and prescribe accordingly with this updated knowledge, mathematical models predict that bacterial resistance will not continue to increase, but rather will be leveling off, reaching, and maintaining a stable internal equilibrium [15].

Beyond these examples concerning antibiotic therapy, much more precise information is critical to shape appropriate interventions in many other areas of infectious diseases management, including the effects of diagnosis, therapy, and hospital infection control. Hospitals are continuously running, and events constantly occur that are neither detected nor systematically recorded, preventing also here reaching an organized ensemble of significant data. Certainly a cohorting policy needs to be put in place both in hospitals and the community to create homogeneous cumulative ensembles of patients making possible studies to reach scientific conclusions. These will eventually serve to develop targeted interventions, but also innovative products, processes, or services—new solutions to be developed by ad hoc start-ups.

BASIC SCIENCES AS EDUCATION FOR OBJECTIVITY

Medical practice is frequently flawed with "empirical" and "traditional" considerations, not always well proved. Indeed, both clinical and basic sciences might have the temptations of apophenia (when we believe in our detection of patterns in random data), confirmation bias (when we focus our attention toward the data confirming our expectations) [16], and hindsight bias (when we tend to see an event as predictable only after it has happened), therefore reducing the reproducibility, the efficiency, and consequently the credibility of science [17]. We advocated in former paragraphs in favor of the "passion of knowing" as a source of innovative thinking and creativity, as a condition for the progress in science. But passionate researchers should be aware (more than any other) of the temptations of passion, as false assumptions might result in false trajectories in science, extremely difficult to correct once installed in practice. Basic scientists are frequently more aware of the constant need

of appropriate controls, and generally have accessible tools to observe from different perspectives of a single event. Certainly basic sciences have a heuristic potential for the training of medical sciences.

EXPERIMENTAL ORGANIZATION OF BASIC-APPLIED SCIENCE TRANSITION AND MANAGEMENT IN MICROBIOLOGY AND INFECTIOUS DISEASES

Among the key tools assuring the progress of the transition between basic and applied sciences, we should highlight scientific organizations. Scientific organizations are themselves the object of science—that is, innovative organizational hypotheses should be constructed and tested for efficient behavior in progressing toward novel and significant knowledge [18]. The interest of putting together in the same hospital organization basic and clinical microbiology with infectious diseases, but maintaining its own specificities, was proposed and successfully tested decades ago [19, 20]. The university hospital–based Méditerranée Infection Foundation in La Timone, Marseille (France) is an example of such experimental organization, ambitiously merging discovery-driven basic microbiology with research in novel analytical tools, experimental pathogenesis research, diagnostic, clinical, therapeutic, preventive procedures (encompassing the individual, hospital, and community), and epidemiology-ecology, facing the urgent challenge of public health microbiology and public health infectious diseases perspectives [21, 22], and, in general, global health. Global health in fact is a transnational, interdisciplinary effort synthesizing population-based prevention with individual-level clinical care [23]. This organization aims to act also as a knowledge center, fostering cognitive capability, skills, training, and learning in novice scientists including from less developed countries, and intending to promote skilled scientists at both sides of the necessary exchange bridge between basic and applied sciences. In this particular organization, the fact that this advanced research institute, Méditerranée Infection, is placed in proximity to a school of medicine offers an opportunity for approaching basic science to clinicians. As William Osler said at the opening ceremony of the Wistar Institute for Anatomy and Biology in Philadelphia, 21 May 1894, "Particularly for a medical doctor, to be learned in a scientific discipline is an essential gift that ferments all his life." That is exactly what we need for the progress of medical sciences, and in particular for the progress of microbiology and infectious diseases.

Notes

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Clinical Infectious Diseases

SUPPLEMENT ARTICLE

Data Science Priorities for a University Hospital–Based Institute of Infectious Diseases: A Viewpoint

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Automation of laboratory tests, bioinformatic analysis of biological sequences, and professional data management are used routinely in a modern university hospital–based infectious diseases institute. This dates back to at least the 1980s. However, the scientific methods of this 21st century are changing with the increased power and speed of computers, with the "big data" revolution having already happened in genomics and environment, and eventually arriving in medical informatics. The research will be increasingly "data driven," and the powerful machine learning methods whose efficiency is demonstrated in daily life will also revolutionize medical research. A university-based institute of infectious diseases must therefore not only gather excellent computer scientists and statisticians (as in the past, and as in any medical discipline), but also fully integrate the biologists and clinicians with these computer scientists, statisticians, and mathematical modelers having a broad culture in machine learning, knowledge representation, and knowledge discovery.

Keywords. surveillance; epidemiology; modeling; epidemics.

What should be the data science priorities of a competitive "dream" 21st-century university hospital–based research institute on infectious diseases integrating microbiology, clinical care of infectious diseases patients, and reference centers for discovery and classification of new microorganisms, and carrying out epidemiological, clinical, and fundamental research?

The fight against infectious diseases requires a multiplicity of players who use biostatistics, medical informatics, mathematical modeling, and information sciences in general. This concerns, within the hospital, laboratories of microbiology, dedicated clinical infectious wards, and almost all other clinical specialties; and, outside the hospital, system biology, ecology, veterinary science, public health, and even nonmedical disciplines such as sociology and demography. I will not take up space to detail the needs that everybody agrees upon, such as to have engineers to handle the automation of laboratory processes and bioinformaticians to exploit the genomic data. Rather, I will describe some features of the present age of information which, from my point of view, should impact the definition of the data science workforce to implement.

FROM BIG DATA TO MACHINE LEARNING

The internet of things comes among one of the most well-known trends of "big data"; e-advertising aggregates myriads of pieces of information about our habits, localizations, and internet search queries, all in order to target advertising to us. Booking a room in a large city, taking a taxi, finding one's way when driving is no longer done as it was 10 years ago—one can safely predict that biomedical research, clinical care, and public health will be similarly revolutionized; one can also observe that it has not yet been done.

Indeed, blood specimens of a patient, once analyzed with automated platforms, will provide at a low cost between hundreds and millions of pieces of information if analyzed with techniques such as next-generation sequencing. In the hospital, a patient gets a series of biomedical images that generate gigabytes or terabytes of data. His clinical information is abstracted in an electronic health record. His physical activity is recorded on his mobile phone, and downloaded to servers. The characteristics of his environment are available by mapping the lifeline of his localizations (registered by his smartphone) with public environmental databases that, for example, characterize the land cover around him, with a precision of 200 meters thanks to satellite imaging [1]. These data possess the 3 characteristics of big data: their huge volume, their variety, and the velocity with which they are acquired. This has been referenced as the 3Vs [2].

At the beginning, the advent of big data in biomedical research was just seen as a change of scale for the biostatisticians—for example, statistical testing of an observed correlation could not be done with the standard 5% level of significance. New statistical methods were designed to limit the false discovery rate due to multiple testing. But it is now clear that, with the new big data technologies rising, a total change of paradigm is

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occurring: In the past, the research strategy in biomedicine was "hypothesis driven"; it will now become "data driven" [3].

The tremendous amount of data that we have to face are currently analyzed with "machine learning" methods. Machine learning stands at the intersection of artificial intelligence and statistics. It was increasingly used in the last decade in various domains from games (with the examples of Chess and Go), to speech recognition. There are already some applications of machine learning in infectious diseases—for example, for studying multidrug resistance [4], or host–pathogen interactions [5]. The machine learning methods can be broadly separated into 2 types: "unsupervised" and "supervised." In unsupervised methods, the goal is to identify objectively "different" groups of "similar" objects; for example, clusters of patients in an n-dimensional space, or clusters of microorganisms. The hierarchical classifications (which are represented by trees and dendrograms) are another kind of unsupervised method widely used by bioinformaticians to produce phylogenies of sequences. These unsupervised methods have many variants (in cluster analysis, biostatisticians can set a priori the maximum number of clusters they want to identify; classification trees can be built top down or bottom up, etc). Here, defining a good distance between the items under study (eg, DNA sequences or patients defined by a clinical profile) is critical, and unfortunately is in general done arbitrarily. The second category of methods (supervised classification) is used when the goal is to predict a variable of interest (eg, dead from sepsis/ survived) from the set of (big) data available.

There are a dozen different algorithms for machine learning, but regardless of the specific method used, the interaction between biostatisticians and the biomedical community will be deeply changed. Take the example of the prognosis of patients in intensive care. In the past (and still now), logistic multiple regression was the classical method used to derive a prognosis score and identify the predictors among a set of bioclinical variables of interest and of possibly confounding variables (eg, severity score, social variables). The output of the model was easily understood by the clinician (eg, he or she could find that one variable remained predictive, even after controlling for all other variables, because its coefficient in the regression equation was significantly different from zero). By contrast, machine learning methods are in general black boxes, and the clinician will ignore which variables are used to make the prediction. He will know that "it works," not *how* it works. These complicated empirical predictive models can be judged only on their performance. This is why developing quality validation methods of predictive models has become an essential part of the work of statisticians, and constitutes an important body of mathematical work. The key principle to assess the quality of a prediction is to generate the predictive model using a first set of objects (the "training" sample) and test it on a second, independent sample (the "validation sample"). Special methods, such as cross-validation, have been devised for the frequent situations where the number of available samples is too small to permit splitting the study population into training and validation subsets. This article does not have the ambition to review the innumerable methodological problems posed in the analysis of big data, and the details above were given only to convince the reader that the biostatisticians needed by the hypothetical institute of infectious disease must (1) be scientists aware of the rapid development of new methods of analysis, and (2) be fully integrated in the institute because the choice of the best methods for a given problem requires an in-depth understanding of the data.

Reproducible Research

For big data analysis using cryptic algorithms of machine learning, as well as for the most classical biostatistics methods, an already important medical research trend is the call for "reproducible research" [6]. Indeed, journals increasingly ask the researcher to demonstrate that the results of his manuscript are reproducible. The best way to demonstrate this is to make public together both the computer code used and the datasets (under the restriction of respecting patient privacy, and obtaining informed consent). New tools facilitate this ambition, such as R-Markdown (http://rmarkdown.rstudio.com/), which enables writing notebooks integrating the text of the article with the data and methods of analysis, and making it possible for any reader to redo all tables and figures, and to perform any sensitivity analysis that he or she believes important but was not originally reported by the author.

MEDICAL INFORMATICS

Clinical Microbiology Informatics

Clinical microbiology informatics is a vast domain [7]. Laboratory automation, image analysis, and telemicrobiology usually come with ready-to-use software, manuals, and tutorials that can be used directly by the biologists. By contrast, bioinformatics requires specialized on-site dedicated scientists (bioinformaticians) with an in-depth knowledge of existing databases and of the new tools of sequence analysis.

An important subdomain of clinical microbiology informatics is medical informatics. The definition and use of appropriate standards for data exchange between laboratories and between laboratories and clinics, the choices of terminologies, the workable integration of the different sources of information—in the microbiology laboratory, in the infectious ward, in the rest the hospital (if the patient has or had another pathology than infectious), and outside the hospital—are not simple problems. They have to be handled by specialists (medical informaticians) knowledgeable in infectious diseases.

Public Health Informatics

In many programs, the microbiological data acquired in the institute gain value if they can be linked to public health databases. Examples of such databases are, in France, the death certificates database (CepiDC) and the French social security system database (SNIIRAM, which collects data from 1.2 billion care sheets per year). This implies a staff with informaticians able to match the biological samples with the patient information, and an important and time-consuming administrative work (to fulfill the legal and ethical constraints and regulations that guarantee the consent of the patients involved and the confidentiality of their data).

The environmental databases can be of particular interest as they enable, for example, to relate a microorganism with the ecology of a vector or with the past environment of a patient. For example, a geographic information system (GIS) was used to identify the zoonotic origin of community-acquired pneumopathies [8]. More generally, GIS enables the discovery of the time-space signature of an epidemic, which is information still lacking for most infectious diseases (it has been noted that of the 174 infectious diseases that have a strong rationale for mapping, only 7 had been comprehensively mapped) [9].

Knowledge Representation

"Knowledge" has been described by information scientists as "data plus interpretation of the data" [10]. The data are booming, and in parallel, the possible interpretations of the data. This explains the importance taken by the field of "knowledge management" whose first task is knowledge representation (what is, for example, the best way to abstract the characteristics of a collection of microorganisms?). A series of subspecialties in informatics tackles this problem, such as the semantic web and ontologies. An ontology is a representation of a domain that fully describes its components (objects and concepts), the individual properties of theses components, and the relationships that link them together. Unique identifiers that are associated to the objects and concepts of the domain can then be used to query molecular databases, and bioontologies serve to describe complex biological features [11]. Examples of ontologies in the field of microbiology are the agriculture-oriented microbial taxonomy ontology [12] and the Antibiotic Resistance Ontology, which is at the base of the Comprehensive Antibiotic Resistance Database [13].

It is inconceivable that a laboratory of microbiology would constitute a collection of microorganisms using state-of-the-art biological tools, but would lack at the end the computer science expertise to derive the proper representation needed to further classify them, retrieve them, and update the relevant global databases. On the flip side, how can the most gifted computer scientist build the "knowledge representation" of a domain in infectious diseases without a close, daily, interaction with the biologists? This is why, in this critical domain as well, the computer scientists in charge of knowledge representation must be fully integrated in the institute of infectious diseases.

Knowledge Discovery

One step beyond knowledge representation is the domain of knowledge discovery. Here, the final ambition is that of artificial intelligence resources applied to the systematic analysis of existing data and publications being used to make discoveries in biology. Is that totally unrealistic? Think to automatic translation from language A to language B: In the recent past, the approach was to model the grammars of language A and B, and try to map one on the other. It did not work well. Now, everybody can find online good automatic translations of texts in almost any existing language on Earth. This was achieved by the combination of 3 features: (1) the collection of a huge number of translated texts that provided the "training samples"; (2) the use of machine learning algorithms trained on these already translated texts, and then used to search close similarities with the text to translate; and (3) the power and speed of the computers that increased dramatically over the last decade. This enabled, for example, the use of artificial neural networks that were described long ago, in the 1980s, but could not at that time be implemented at a sufficient size and speed on the existing computers. Is it impossible to anticipate that the same kind of approaches will be used to infer the best possible treatment for a patient from the analyses of millions of life trajectories where biology, genetics, imaging, environment, treatments, and outcomes will be available? Or that the automatic analysis of thousands of publications will make results emerge that were not seen with the human eyes and brains of the few (if any) researchers that read them all? The Knowledge Integration Toolkit (KnIT) is an example of a prototype knowledge discovery tool that was developed by IBM and the Baylor College of Medicine. The authors gave a proof of concept with the example of the tumor suppressor P53 for which some 70 000 articles were published (up to now 50 000 000 articles have been published); by mining the whole literature with their tool, they could automatically find new protein kinases phosphorylating p53 [14].

DATA SCIENCE IN EPIDEMIOLOGY OF INFECTIOUS DISEASES

Clinical and Population Epidemiology

An institute of infectious diseases located in a university hospital necessarily has clinical epidemiology and population epidemiology research programs. In clinical epidemiology, defined as the epidemiology done at the bed of the inpatient, this institute has vocation to be the local leader for hospital infection control programs, and for the design and conduct of clinical trials of treatments against infectious diseases. Often, other projects arise in collaboration with other disciplines—for example to search infectious cofactors in the etiology or prognosis of noninfectious diseases. The lead and the conduct

of these projects depend on the specific organization of the hospital.

Because the microbiology laboratory diagnoses thousands of specimens coming from inside and outside the hospital, there is a great temptation to do population epidemiology grafted on these data treasures. However, a mass of data, samples, and patients does not make an epidemiological program. In epidemiological designs, data are acquired along a timeline, from cause to effect (cohort) or from effect to cause (case-control). A huge collection of patients for which one blood sample has been tested has in general little epidemiological value, as the time factor is lacking. All efforts must be devoted to construct real cohorts with a sufficient follow-up, guarantees of absence of missing follow-ups, and high data quality for the microbiological values and for the clinical endpoints. A staff with competences in epidemiological design, data curation, biostatistics, medical, population, and environmental information systems is necessary to give the proper epidemiological answer to the questions posed by the infectious diseases community.

Mathematical Modeling of Epidemics

The more visible part of mathematical models of epidemics [15] by the public is their use to predict the future sizes of emerging, or remerging, diseases. It was used for AIDS, prion diseases, influenza pandemics, Ebola, and so forth. Some of these predictions were later verified; others were contradicted by the facts. However, the important uses of mathematical modeling are rather to estimate disease parameters that are not accessible to direct observation (eg, incubation time, latent and infectious periods) and to test in silico hypothetical mitigation strategies of epidemics under well-specified hypotheses. Mathematical modeling of epidemics relies on a variety of data sometimes coming from outside the biomedical field (eg, sociologists to characterize the interactions within and between populations, anthropologists, social media specialists [16]). Mathematical modeling of epidemics has become a scientific domain by itself, with specialized journal and conferences (Epidemics). An infectious diseases university hospital may therefore choose to rely on external collaborations, rather than building its own group of critical size. Its role will then be to provide to the modeler expertise and data to build the models: For example, it has been shown, quite expectedly, that a key model parameter for the control of an infectious disease was the proportion of presymptomatic (or asymptomatic) transmission [17]. This necessitates a careful microbiological and clinical follow-up of cohorts of newly infected subjects, which can only be done in institutes of infectious diseases. The recent Ebola outbreaks remind us how difficult (and important) it can be to get a precise quantitative image of the natural history of emergent/reemergent infectious diseases.

CONCLUSIONS

The central message of this article is that biostatisticians, epidemiologists, and computer scientists must be integrated with microbiologists and clinicians to effectively fight infectious diseases. This is because the "big data" era inextricably mixes data, knowledge, and support to action. It looks impossible to model the knowledge in infectious diseases without being in situ, working hand in hand with the experimentalists and all those who work to discover new pathogens and treatments. Then, the computing personnel must also be real researchers in their field, not just excellent technicians. This is because the methods and the algorithms that will aid discovery in infectious diseases are perpetually evolving and may well be those algorithms that presently succeed in linguistics, or in a remote domain. A dialogue is 2-way: reversely, biologists and physicians of the institute must be computer literate; this means efforts to train them when they are students and, later, in continuing education programs.

Finally, it cannot be denied that all the numerous tasks and subspecialities identified in this article call for a vibrant workforce. At the moment of hiring the best data scientists to serve the cause of the fight of infectious diseases, a serious difficulty to be accounted for is competition with the private sector, which is well aware of the priorities listed in this article and offers much higher salaries to these specialists than public institutions can do. The hope is that the cause of fighting infectious diseases will be sufficiently attractive to overcome this difficulty.

Notes

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