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Methods for the discovery of emerging pathogens



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ABSTRACT

Recently, there has been a steady increase in the number of recognized pathogenic microorganisms, specifically bacteria. The development of genetic technologies, MALDI-TOF mass spectrometry and new culturing techniques has significantly widened the repertoire of known microorganisms and therefore pathogenic microorganisms. The repertoire of infectious agents has been studied in various environments including water, soil, pets, livestock, wildlife and arthropods. Using different methods, many known pathogens can be identified in these samples; therefore, the impact of emergent pathogens on humans can be examined and novel pathogens can be identified. In this special issue, we discuss the identification of emerging pathogens in the environment and animals.

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1. Introduction

There has been a recent steady increase in the number of recognized pathogenic microorganisms. The number of officially recognized bacterial species has risen from 1800 in 1980 to over 12,000 in 2013 [1]. In our laboratory, we evaluated approximately 2000 bacteria that were isolated at least once in humans as commensal or pathogenic bacteria [2]. In addition, we recently showed that over the last ten years in a hospital in Marseille, approximately 635 different pathogenic bacteria were isolated from human specimens [2]. The number of viruses isolated from humans has also increased with the recent focus on hemorrhagic fever viruses, respiratory viruses (influenza virus and coronavirus) and viruses transmitted by arthropods (for example, Toscana in Europe) [3,4]. However, the detection of new species of protists and fungi is much slower and appears to reflect lower interest in the discovery of emerging microorganisms. Finally, archaea, a group of prokaryotes different from bacteria, have not received much attention because of the difficulties associated with their culture. Therefore, these agents are not considered pathogenic but agents of the digestive microbiota that are essential for the recycling of hydrogen into methane [5]. This special issue aimed to identify the emergence of different pathogens considering that most pathogenic microorganisms exist in the environment: in animals or in the inanimate environment.

2. Repertoire

The repertoire of microorganisms in a given area is based on the need to target diagnostic and therapeutic interventions to existing pathogens in the area. Recent studies have been limited by the technical expertise of the countries that perform them [6]. In Europe or the United States, the strategies for detection of existing pathogens have been based on the circulation of microorganisms that are prevalent in these areas. Therefore, the frequency of pathogens circulating in poor areas has not been examined, and these pathogens are now considered emerging (this term reflects their recent discovery rather than their recent creation).

The repertoire of infectious agents should be evaluated in the environment, including water, soil, pets, livestock, wildlife and arthropods. Using various methods, many known pathogens can be identified in these samples; therefore, their impact on humans can be assessed and microorganisms that are not yet recognized as pathogenic can be identified. Systematic studies should be performed in humans to identify the proportion of related protists, fungi, bacteria and viruses that cause fever as well as acute and chronic infections. Such studies can yield surprising results; for instance, recent studies by different teams in different locations in Africa (Senegal and Kenya) revealed that *Rickettsia felis* was the second microorganism associated with fever after malaria [7,8]. Furthermore, microorganisms that are not considered agents of fever in Europe, such as *Borrelia recurrentis*, which causes tick-borne relapsing fever [9], and other infectious agents such as *Tropheryma whippelii* [10] or *Coxiella burnetii* [11] have a significant impact on the African population. Notably, studies testing different populations must include negative control samples from patients of the same origin without fever. The predictive value of diagnostic

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tests in the establishment of a repertoire of infectious agents depends on the number of asymptomatic carriers (without fever). In malaria-endemic areas, there can be non-febrile carriers of *Plasmodium falciparum* (in a recent unpublished study in Gabon, up to 11% of non-febrile children presenting to the emergency room in local hospitals were carriers of *P. falciparum* in the blood). In addition, *R. felis* and *T. whipplei* can also be observed in non-febrile patients examined for other reasons [7,10]. Therefore, the systematic addition of negative controls is critical to assess the causal microorganisms in the genesis of fever. It is also essential to establish a comprehensive and multiplexed pathogen repertoire, and it is not sufficient to repeatedly test for the most common pathogens. Therefore, the presence of *P. falciparum* in a febrile patient should not deter the search for other pathogens present in combination, such as *Streptococcus pneumoniae* or *Staphylococcus aureus*. Establishment of the microorganism repertoire can facilitate the development of strategies for the diagnosis of infectious agents, especially in local African areas, using the Point of Care laboratories, enabling the implementation of empirical treatment [12] (Fig. 1).

3. Techniques for the detection of emerging pathogens

Techniques for the detection of emerging pathogens are based on several strategies. Culture techniques have evolved considerably, and multiple strategies are used to produce different axenic culture media that can be easily used in local laboratories without the need for expensive equipment [13,14]. New media for the cultivation of anaerobic microorganisms without specific equipment will most likely facilitate the isolation of anaerobic bacteria that are sensitive to oxygenated derivatives [15]. The development of human cell cultures (HEL) [16] and culture in amoebae will

enable the cultivation of new pathogenic microorganisms [17]. In our laboratory in Dakar, Oleg Medianikov successfully used cell culture to isolate new species of intractable bacteria such as *Rickettsia* and *Bartonella* spp. from environmental samples [9,18,19]. New bacterial species can be easily identified using MALDI-TOF mass spectrometry [14,20]. This technology can be used to economically and rapidly identify (in a few minutes) all bacteria for which the mass spectra are present in the machine's database [21]. For bacteria that cannot be identified using MALDI-TOF mass spectrometry, 16S rDNA amplification and sequencing analysis can be used to determine whether the isolates represent novel species (less than 98.7% homology); subsequently, new mass spectra can be created for known species for later use, and the spectra of new species can be filed [14,22,23]. MALDI-TOF mass spectrometry has revolutionized microbial identification and enabled rapid bacterial identification, including in emerging countries [21]. Therefore, in collaboration with IRD and Biomérieux, we installed a MALDI-TOF mass spectrometer in Dakar, which enabled the identification previously unknown bacteria [24–27]. Furthermore, by MALDI-TOF mass spectrometric analysis of commensal bacteria, we identified more than 90 new species of bacteria, which had not been observed either in the environment or in humans [13]. In our laboratory, we systematically sequence bacterial genomes for species description in association with their MALDI-TOF mass spectra and their main morphological and biochemical characteristics [24–39] (Fig. 2).

In addition to this technique, specific molecular assays based on the universal amplification of bacteria can play an important role [40]. The most commonly used techniques are based on amplification of the universal 16S rDNA gene, which facilitates the identification of unrecognized bacteria, especially in anaerobic and multi-microbial infections [40,41]. Amplification of the *rpoB* gene

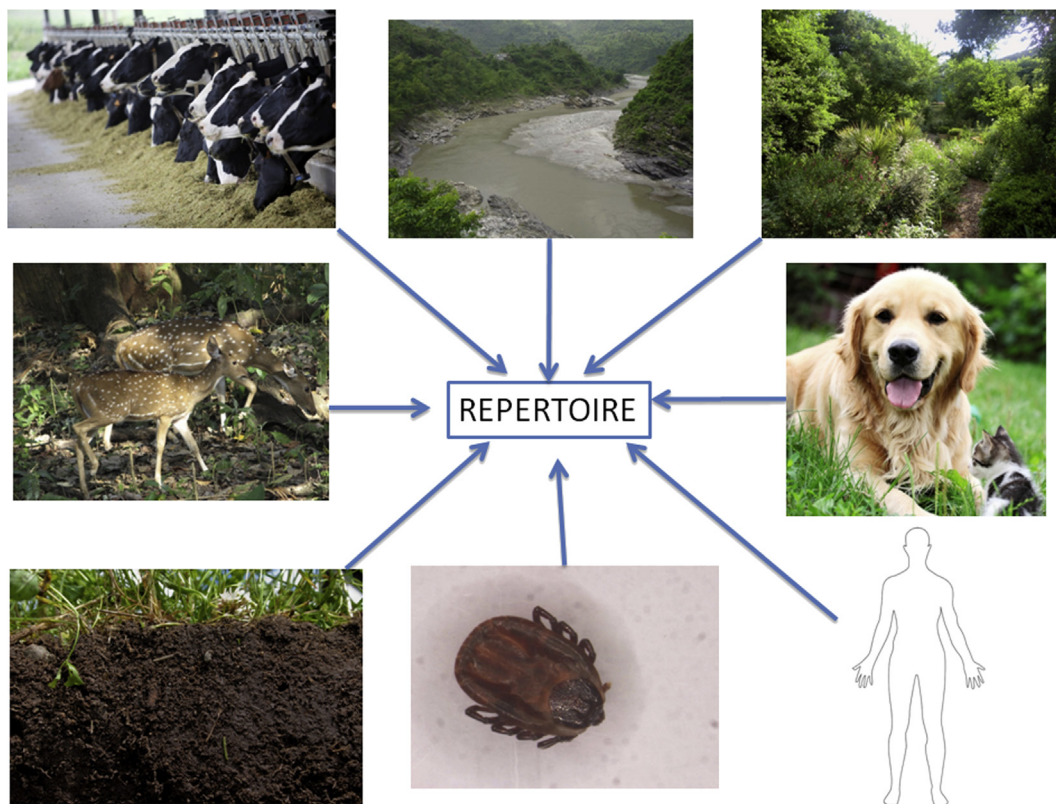


Fig. 1. Sources of the repertoire of microorganisms. The sources of the repertoire of microorganisms are mostly based on human, water, soil, pets, livestock, wildlife and arthropods samples.

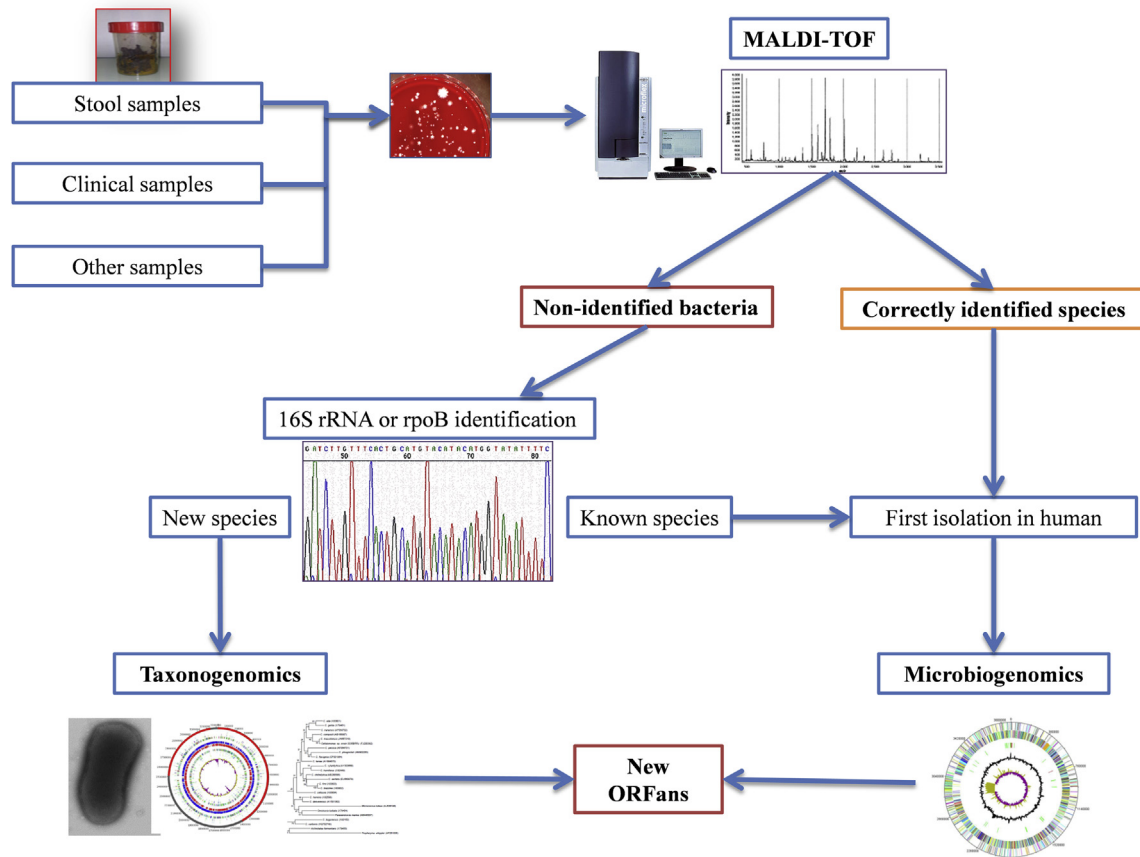


Fig. 2. Strategy for the detection of emerging pathogens based on MALDI-TOF mass spectrometer and on metagenomics analysis.

(encoding the RNA polymerase) has enabled the identification of several bacteria that were unnoticed until recently. The identification of eukaryotic microorganisms is more complex because several molecular assays amplify fungi [42], protists [43] and microscopic arthropods [44]. These techniques have broad specificity, which is reflected in the recent increase in the number of fungi identified as human commensals or pathogens (Fig. 3).

Finally, metagenomics, which is commonly defined as sequenced-based analysis of the entire collection of genomes directly isolated from a sample, can be useful. Metagenomics overcomes the key limitations of classical tools for viral detection [45]. Unlike traditional techniques for microbial and viral identification, metagenomics does not require prior isolation and clonal culturing for species characterization, and it does not rely on prior assumptions on which organisms are present or the genomic sequences to be targeted. Viral metagenomics is particularly suitable for providing a global overview of the diversity of the viral community and has functional significance [45]. The characterization of human-associated viral communities in a non-pathological state and the detection of viral pathogens during infection are essential for medical care and epidemic surveillance. These techniques are limited by the populations tested. However, these techniques have been widely used to study different microbiota and might be important to identify species related to nutritional status in Africa, especially in children suffering from kwashiorkor [46].

4. The case of giant viruses

Due to ongoing global evolution, many new microorganisms are identified in humans and in the environment. Using a combination

of different technologies, we have described giant viruses as emerging pathogens [47–51] (Fig. 3). These viruses were discovered when a “Gram-positive” bacterium isolated from an amoeba was later identified as Mimivirus [52,53]. Mimivirus has subsequently been isolated from human respiratory samples and in the stools of patients who developed pneumonia after travelling to Tunisia [54,55]. Furthermore, serological analysis revealed an increase in the prevalence of these viruses in patients with pneumonia in Canada [48]. The virophage isolated from Mimivirus [56] has also been identified by serology in two patients from Laos after consumption of raw fish from the Mekong River, which was also found to be extremely rich in virophages [57]. Finally, Marseillevirus, discovered in amoebal culture [58], was later detected by PCR-based metagenomic assays in the blood of blood donors without clinical manifestations [49,50] and by serology and hybridization analysis in the lymph-node of a child with lymph-node enlargement [51]. The discovery of giant viruses was only possible by culture techniques because viral metagenomics analysis excludes particles larger than 0.2 microns; this discovery highlighted a novel class of emerging viral pathogens [47].

5. Conclusion

In this special issue, we identified potential sources of emerging pathogenic microorganisms. The rapid development of genetic and molecular technologies, MALDI-TOF mass spectrometry and new culturing techniques has facilitated the identification of microorganisms, including those that are pathogenic [59]. It is likely that the development of diagnostic multiplex assays might generate problems in disease interpretation. Therefore, a multidisciplinary

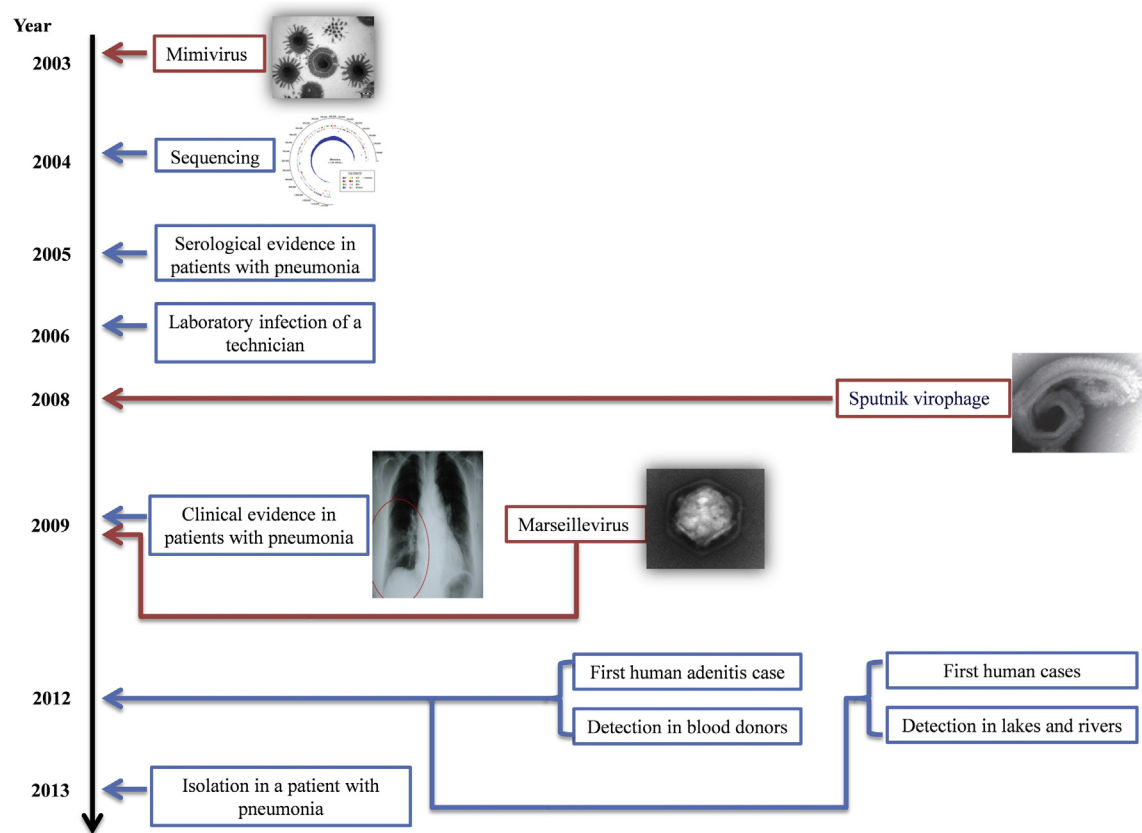


Fig. 3. Giant viruses history. A novel class of emerging viral pathogens was created since the discovery of giant virus.

approach involving culture, MALDI-TOF mass spectrometry-based identification and metagenomics analysis of environmental and human samples will generate a comprehensive repertoire of pathogenic microorganisms in humans, many of which remain to be discovered.

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