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Metagenomic and clinical microbiology

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ABSTRACT

As a result of Next Generation Sequencing methods, metagenomic studies have become increasingly widespread. After being first applied to microbiome description, metagenomics is currently proposed as a diagnostic tool in clinical microbiology, although this application remains confined to the field of research. In this review, we will discuss the application of metagenomics to the detection of bacterial pathogens and demonstrate that the interpretation of the metagenomic results may fluctuate depending on the type of sample analyzed. However, we propose a view of metagenomic application to the evaluation of antimicrobial resistance, epidemic investigations and forensic medicine. Secondly, we present the many limits of metagenomic interpretation and application in routine clinical microbiology. From our perspective, metagenomics is not yet reliable enough for general use in clinical microbiology.

1. Introduction

Clinical microbiology has dramatically evolved since the end of the 20th century with the appearance of OMICS technologies. The multiplication of molecular tools has enabled rapid diagnosis without any culture step and has changed the way that some infections (such as infectious sexual diseases caused by *Chlamydia trachomatis*) are detected [1]. Simultaneously, the identification of cultured microorganisms has been facilitated as a result of MALDI-TOF mass spectrometry and its application to clinical microbiology [2]. Currently, microbial antibiotic sensibility testing remains the lengthiest part of the diagnosis process [1]. Classical clinical microbiology methods rely on the Koch postulate, which highlights the importance of a pure culture of a microorganism to prove its pathogenicity. However, since the advent of molecular tools and metagenomic analyses, culture sometimes appears to have been dropped [3]. Nevertheless, attempts by some authors to predict that molecular tools would replace pure culture were rapidly abandoned [3].

Metagenomics, the principle of which relies on the genomic analysis of a sample from a complex environment containing more than one microorganism, provides a view of the composition of this sample. Metagenomic studies became increasingly accessible with the advent of Next Generation Sequencing (NGS) [4]. For metagenomic analysis, NGS can be used with two different approaches: targeted metagenomics on a specific chosen amplified region (such as the 16S region) or shotgun metagenomics, which relies on the amplification of all the sequences in a sample without hypothesizing about its content [4].

In this review, we proposed exploring the current applications of metagenomics in clinical microbiology, focusing on the risks of these methods and difficulties with their interpretation. We propose determining whether metagenomics could be currently used as a diagnostic tool.

2. Current applications of metagenomics to clinical microbiology

2.1. Detection of pathogens

Metagenomics has been used for the detection of pathogens in clinical samples. We will describe its application to bacterial microbiology. Most studies report the evaluation of metagenomics compared to traditional culture method. However, in some cases, metagenomic analysis has made diagnosis, which was not previously performed by culture or standard molecular tools (Table 1).

Few studies have reported on the use of metagenomics as a tool for prospective diagnosis. Wilson et al. first reported a case of encephalitis which was solved by shotgun metagenomics. Despite extensive explorations including 16S rRNA amplification and sequencing on cerebrospinal fluid, no etiology had been found. *Leptospirosis* was identified in the cerebrospinal fluid (CSF) by metagenomics and was not detected in a control sample. The diagnosis was further confirmed by specific molecular tools and serology [5]. Confirmation of the diagnosis by both *Leptospira* specific PCR and serology could lead us to think that these examinations could have be performed prior to NGS, however, the very atypical clinical manifestations demonstrate the contribution which

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Table 1
Diagnosis by metagenomic analysis.

Studies	Sampling	Cases	Metagenomic analysis	Diagnosis
Nakamura, 2008 [50]	Stool	34 y.o. man, diarrhea and fever after eating chicken. Standard culture negative, PCR for Norovirus negative.	Retrospective analysis of stool sample with shotgun pyrosequencing (Roche 454) Negative control: not mentioned	*Clostridium jejuni* Confirmation by *Campylobacter* specific PCR and culture on specific media
Wilson, 2014 [5]	CSF	14 y.o. man, SCID post HSCT, meningoencephalitis and status epilepticus. Diagnostic workup including brain biopsy and 16S PCR on CSF(x2) was unrevealing.	**Prospective** analysis of CSF by shotgun sequencing(Illumina). Negative control: serum from unrelated patient.	*Leptospira* Confirmation by *Leptospira* specific PCR on CSF and serology
Mongkolrattanothai, 2017 [51]	CSF	11 y.o. girl, fever, headache, nausea, shivering for four weeks Standard culture and mycobacterial culture negative, no 16S PCR performed on CSF. Neurotuberculosis suspected	**Prospective** analysis of CSF by shotgun sequencing (Illumina) Negative control: elution buffer	*Brucella* Confirmation by *Brucella* specific PCR on CSF and serology
Salzberg, 2016 [17]	Brain biopsy	67 y.o. women, osteomyelitis, lung disease, multifocal brain and spinal lesion. Standard analysis didn't led to a diagnosis before brain biopsy. Quantiferon test showed indeterminate results (×4)	**Prospective** analysis of brain biopsy by shotgun sequencing (Illumina) Negative control: not mentioned	*M. tuberculosis* Confirmation by anatomopathology (granuloma), brain cultures stay negative.
Yao, 2016 [52]	CSF	3 cases of suspected encephalitis caused by *L. monocytogenes* CSF Cultures were negative.	**Prospective** analysis of CSF by shotgun sequencing (Ion Torrent) Negative control: 3 CSFs from patients with autoimmune encephalitis.	*L. monocytogenes* Confirmation by L. monocytogenes specific PCR on CSF.Cases 1 and 3: blood culture positive for *L. monocytogenes*, case 2: blood culture positive for gram-positive bacteria.
Thoendel, 2017 [8]	Articular fluid	52 y.o. man, hypogammaglobulinemia post B cell lymphoma, late left TKA infection with no documentation (bacterial, fungal, mycobacterial culture of articular fluid and per operative samples negative, *Mycoplasma hominis* specific PCR on articular fluid negative, no 16S PCR conducted).	Retrospective analysis of sonicate fluid from arthroplasty extraction by shotgun sequencing (Illumina) Negative control: not mentioned	*Mycoplasma salivarium* Failure of specific PCR on anatomopathology slides. Failure of sonicate fluid culture on specific media
Fukui, 2015 [6]	Cardiac valve	35 y.o. man, Infective endocarditis requiring valve replacement. Hemoculture and valve culture remain negative (16S PCR on the valve not mentioned)	**Prospective** analysis of the valve by shotgun sequencing (Illumina) Negative control: not mentioned	*Abiotrophia defective* Confirmation: not mentioned
Abril, 2016 [16]	Plasma	60 y.o. man with septic shock and acute respiratory distress. Gram smear: gram negative bacilli	**Prospective** analysis of plasma by shotgun sequencing (Illumina) Negative control: used	*Capnocytophaga canimorsus* (result on day 5) Confirmation by culture and MADI-TOF identification (result on day 5) Failure of two 16S amplification by PCR on the bacterial road, success on the third attempt at day 55.
Ye, 2015 [53]	Blood	2 y.o. boy, alloHSCT for leukemia, fever and rash No diagnosis, no 16S PCR performed, failure of probabilistic treatments	**Prospective** analysis of blood by shotgun sequencing (Ion) Negative control: blood from another patient of the same ward (although positive for *P. acnes*)	*Propionibacterium acnes* Confirmation by *P. acnes* specific PCR on blood: no statistical difference between case and control.
Pendleton, 2017 [15]	BAL	41 y.o. woman with connective tissue and interstitial lung diseases with hypoxic respiratory failure 59 y.o. man with abdominal sepsis and hypoxic respiratory failure	**Prospective** analysis of BAL by shotgun sequencing (Ion) Negative control: not mentioned	*Pseudomonas aeruginosa* (results at H9) Confirmed by culture (results at H23) *Staphylococcus aureus* Confirmed by culture the next day