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Infectious Disease Diagnostics and Public Health Surveillance by Precision and Rapid Metagenomics: An Update

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Abstract

Metagenomic next-generation sequencing (NGS) can generate a single sequence from each DNA or cDNA, allowing differentiation between the origin of sequence fragments and resolution of host and microbial sequences containing in mixed specimens. By removal of any reads mapping to human genome in metagenomic NGS, all remaining nonhuman genome sequences are able to be compared to a database of known sequences to detect the unknown sequences. In comparison to polymerase chain reaction (PCR) method, metagenomic NGS needs no assumptions or prior knowledge of the type of causing pathogenic microorganisms that are needed in PCR test. Nevertheless, metagenomic NGS wastes more cost because of dominated sequencing reaction by host rather than pathogen sequences although it can identify as few as 9 in 68 million reads of pathogen sequences. In conclusion, once the sensitivity and specificity of metagenomic NGS technologies are validated and clinically available, their potential application can lower the number of undiagnosed infectious cases, improve patient care, and enlarge public health surveillance attempts.

Key Words: *Metagenomics; Infectious Diseases; Health Surveillance*

Abbreviations: AMR : Antimicrobial Resistance, CD : Celiac Disease, cDNA : complementary Deoxyribonucleic Acid, COPD : Chronic Obstructive Pulmonary Disease, FEV1 : Forced Expiratory Volume in one second, FVC : Forced Vital Capacity, GFD : Gluten-Free Diet, NGS : Next-Generation Sequencing, PCR : Polymerase Chain Reaction, rRNA : ribosomal Ribonucleic Acid, UK : United Kingdom

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Objective of the Study

The authors aim to perform a critical review and strong summary of existing utility of the next-generation sequencing (NGS) technologies in the diagnosis of infectious diseases and public health surveillance.

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Introduction

With routine culture-based methods, a large number of microorganisms are difficult to grow [1] that contribute to the misuse of antimicrobial agents in both humans and animals and finally, leading to antimicrobial resistance both in developing and developed countries [2]. As deep sequencing, metagenomic NGS generates a single sequence from each fragment of deoxyribonucleic acid (DNA) or complementary DNA (cDNA) that present in a specimen, allowing differentiation between the origin of sequence fragments, and resolution of host and microbial sequences containing in mixed specimens [3, 4]. To exclude the possibility of identified microorganism mimicking an artefact of the bioinformatics analysis, an alternative molecular method, such as polymerase chain reaction (PCR), can be used to confirm the presence of the detected microorganism [3]. Synthesis of cDNA from total RNA enables detection of viruses with RNA genomes, including the RNA transcripts of microorganisms with DNA genomes [3]. By removal of any reads mapping to human genome in metagenomic NGS, all remaining nonhuman genome sequences are compared to a database of known sequences to identify the unknown sequences [3]. No assumptions or prior knowledge of the type of causing pathogenic microorganisms is required in metagenomic NGS as needed in PCR [3]. Metagenomic NGS composes of specimen processing (nucleic acid extraction and library preparation and sequencing) and bioinformatics [5].

A recent study revealed that the diagnostic yield for metagenomics in the diagnosis of encephalitis is 50% [3]. Effecting clinical outcomes in a case of neuroleptospirosis was revealed in a metagenomic NGS-based results [6]. Metagenomic NGS has three advantage: 1) being able to identify a microbe that is known to cause a patient's disease phenotype and rarely tested for because of its low pre-test probability of being the etiologic agent, 2) being able to identify an entirely microbe for which a traditional candidate-based test does not exist, and 3) being able to identify a known microbe that is not known to cause a particular patient's disease phenotype [7]. A recent study in 3 study groups by 16S rRNA gene sequencing (20 adult patients with active celiac disease (CD), 6 CD patients on a gluten-free diet (GFD), and 15 controls) demonstrated that the active CD patients (26%) had significantly higher relative abundance of Neisseria genus, compared to either GFD patients (4%) or controls (10%) ($p = 0.03$) [8]. A recent study in eight chronic-obstructive-pulmonary-disease (COPD) patients (5 males and 3 females, each older than 40 years (mean age = 68), each at least ten pack-year smoker, and post-bronchodilator forced expiratory volume in one second (FEV_1)/forced vital capacity (FVC) < 0.07) from two United Kingdom (UK) hospitals demonstrated that eight bacterial genera were identified in all 18 sputum specimens, Staphylococcus, Haemophilus, Streptococcus, Neisseria, Pseudomonas, Lactobacillus, Veillonella, and Ochrobactrum [9].

Limitations of metagenomic diagnostics

Recognition of problem with pan-bacterial metagenomic NGS detection can be confounded as well as in PCR by differences between specimens and specimen types, such as the ratio of host: pathogenic microorganism sequences that depends on the degree of specimen multiplexing and sequencing chemistry [10]. Metagenomic NGS wastes cost due to dominated sequencing reaction by host rather than pathogen sequences. Metagenomic NGS can detect as few as 9 in 68 million reads of pathogen sequences [11]. Depletion of host DNA or RNA prior to sequencing is needed to overcome this problem [3]. In Japanese-encephalitis-virus (JEV) infection, metagenomic NGS is unable to critically improve the diagnostic yield, whereas the most sensitive RT-qPCR detects RNA in less than 10% of cases [12]. The principal diagnosis of JEV infection is serology [12].

Discussion

In regarding pathogen identity and antimicrobial resistance (AMR), a molecular diagnostic framework with accurate and rapid information would reduce the prescription of ineffective antimicrobials and reduce AMR, in addition to controlling disease outbreaks and information of the course of infection that contribute to decreasing cost of patient care and survival [13]. Regarding microbial ecology, metagenomic NGS technologies are rapidly growing to be the major source of information [4]. COPD exacerbation related to pathogenic microorganisms has been well documented [14] Microbiomic changes as COPD progress have been investigated [15-17]. Some investigators are validating the use of metagenomic NGS for clinical use [18]. Prospective studies are already underway to evaluate whether metagenomic NGS can be applied to improve costs and patient outcome, namely "The Precision Diagnosis of Acute Infectious Disease

(PDAID)" [6,19]. Statistical scoring is necessary to enhance the of metagenomic NGS to discriminate between unimportant contaminants and exact pathogenic microorganisms due to unbiased nature of metagenomic NGS making polymicrobial and complicated data sets [20].

Conclusion

In a broad range of human pathogenic microorganisms using a single diagnostic test, metagenomic NGS is an increasing rapid and low-cost test of screening human specimens. Once the sensitivity and specificity of metagenomic NGS is validated and clinically available, its potential application can improve patient care, lower the number of undiagnosed infectious cases, and enlarge public health surveillance attempts.

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Competing Interests

The authors declare that they have no actual or potential competing financial interests.

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