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Research Article

Infectious Disease Diagnostics and Public Health Surveillance by Precision and Rapid Metagenomics: Systematic Review and Meta-Analysis

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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. The objective of the study is 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. A comprehensive search was carried out in mainstream bibliographic databases or Medical Subject Headings, including Science Direct, PubMed, Scopus, and ISI Web of Science. The search was applied to the articles that were published between 2002 and 2018. Needed article information was extracted from each article by: 1) direct information including journal, title, authors, abstract, full text documents of candidate studies, publishing year; 2) study period; 3) research method used; 4) type of infectious diagnostics and public health surveillance variables studied; 5) types of infectious disease studied; and 6) the conclusions made about the roles of metagenomics in the diagnosis of human infectious diseases. With strict literature search and screening processes, it yielded 20 articles from 226 articles of initial literature database. The limitations of metagenomic diagnostics is that Recognition of problem with pan-bacterial metagenomic NGS detection can be confounded as well as in PCR by differences between specimens and specimen types. 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.

Keywords: Metagenomics; Infectious Diseases; Health Surveillance

Abbreviations

AMR: Antimicrobial Resistance; CD: Celiac Disease; cDNA: Complementary Deoxyribonucleic Acid; CNS: Central Nervous System; COPD: Chronic Obstructive Pulmonary Disease; E coli: Escherichia coli; FEV1: Forced Expiratory Volume in One Second; FVC: Forced Vital Capacity; GFD: Gluten-Free Diet; NA: Not Available; NGS: Next-Generation Sequencing; PCR: Polymerase Chain Reaction; rDNA: Ribosomal Deoxyribonucleic Acid; rRNA: ribosomal Ribonucleic Acid; UK: United Kingdom.

Introduction

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. 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 an metagenomic NGS-based results [6]. Metagenomic NGS has three advantages: 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 (FEV1)/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].

Methods of the Study

Search strategy and inclusion criteria

A comprehensive search was carried out in mainstream bibliographic databases or Medical Subject Headings, including ScienDirect, PubMed, Scopus, and ISI Web of Science. The search was applied to the articles that were published between 2002 and 2018. Our first involved performing searches of article abstract/keywords/title using strings of [("metagenomics" or "metagenomic diagnostics", "infectious disease" and "health surveillance")]. After a first approach of search, published articles focusing on human infectious disease were retained and the information on infectious disease diagnostics, health surveillance and metagenomics was extracted for having a crude knowledge involving their themes. Another round of publication search was conducted for adding the missing published articles that were not identified by the first round.

All keywords combinations from infectious disease diagnostics, public health surveillance and metagenomics to bind the population of cases under consideration. Search string for disease groups include ["infectious disease" or "metagenomic diagnostics" or "health surveillance"]. The initial literature databases were further manually screened with the following rules: 1) non-human infectious disease-related articles were excluded; 2) articles that did not report a diagnosis of infectious diseases and public health surveillance related to metagenomics were not considered, such as commentary articles, or editorial; 3) non-peer reviewed articles were not considered to be of a scholarly trustworthy validity; and 4) duplicated and non-English articles were removed. The articles were carefully selected to guarantee the literature quality, which is a trade-off for quantity.

Results

With strict literature search and screening processes, it yielded 20 articles from 226 articles of initial literature database. Needed article information was extracted from each article by: 1) direct information including journal, title, authors, abstract, full text documents of candidate studies, publishing year; 2) study period; 3) research method used; 4) type of infectious diagnostics and public health surveillance variables studied; 5) types of infectious disease studied; and 6) the conclusions made about the roles of metagenomics in the diagnosis of human infectious diseases. An overview of the information required for the present analysis that was captured by those themes was shown in the Figure 1. Results from 20 yielded articles was demonstrated in the Table 1.

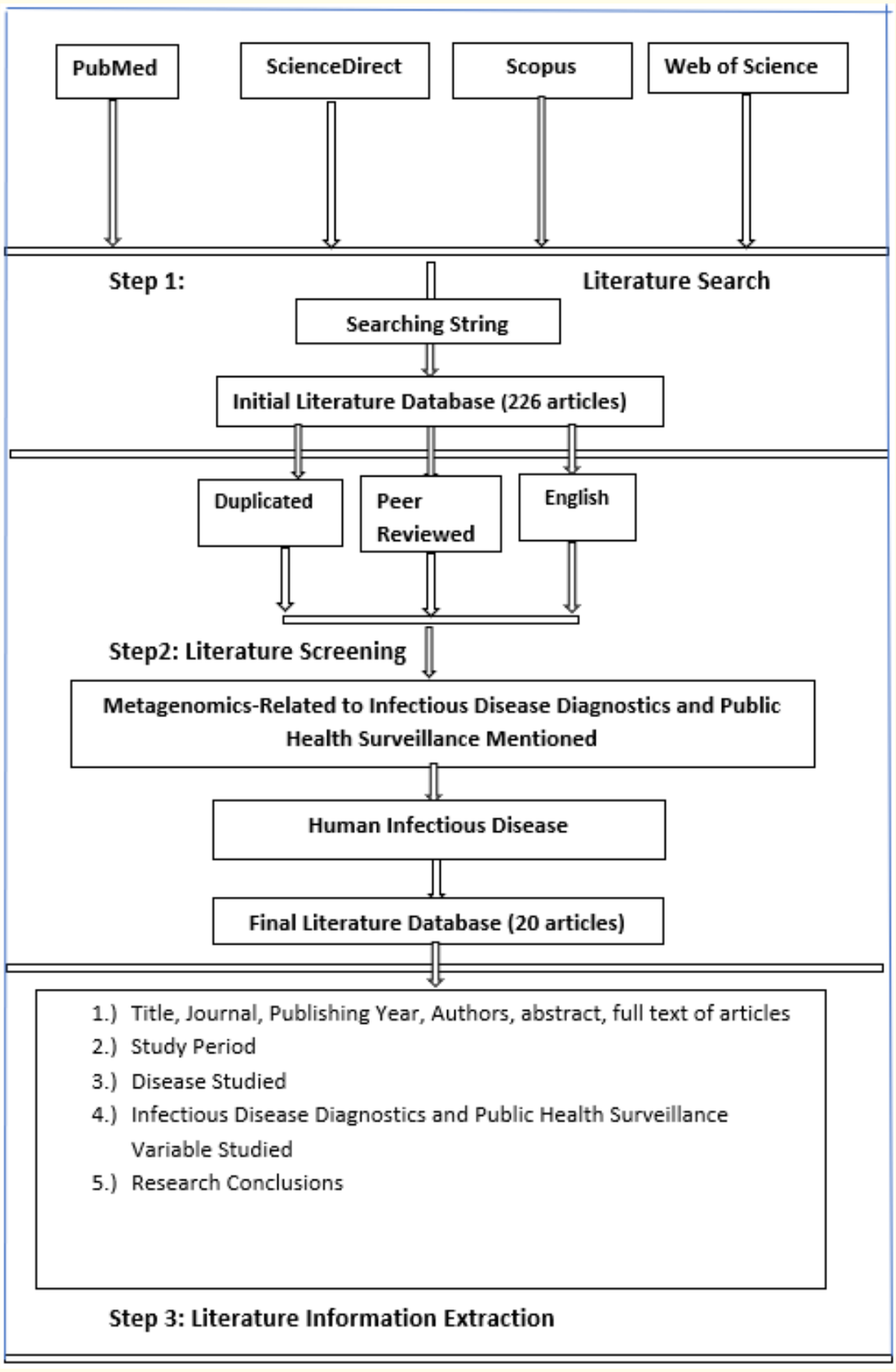

Figure 1: Literature Search and Screening Flow.

Author (s)	Results	References
Vartoukian., *et al*. 2010	Recovery of "unculturables" from soil and aquatic environment	1
Liu., *et al*. 2016	MCR-1 gene indicating polymyxin resistance was carried in *E coli* isolates collected from 15% of raw meat and 21% of animals	2
Brown., *et al*. 2018	No randomly controlled trials to be assessed the utility of NGS as a diagnostic tool from 25 identified articles reporting 44 case reports of patients with suspected encephalitis	3
Albanese., *et al*. 2017	Benefits of NGS in detecting several important bacterial species in real samples	4
Afshinnekoo., *et al*. 2017	Precision NGS can be applied and implemented across various sequencing platforms for clinical specimens	5
Wilson., *et al*. 2014	Reporting a 14-year-old boy with severe combined immunodeficiency with hydrocephalus by NGS detecting the CNS leptospira infection	6
Schubert., *et al*. 2015	Metagenomics represent promising method of research for improvement upon the diagnostic yield of current assays for infectious and autoimmune encephalitis	7
D' Argenio., *et al*. 2016	Metagenomics can detect dysbiosis in patients with celiac disease	8
Cameron., *et al*. 2016	Metagenomics can assess COPD severity	9
Kaplan., *et al*. 2011	Presenting a new way, called " time-driven activity-cased costing " to analyze cost in health care for process improvement, better organization of health care, and new reimbursement approaches	10
Patel., *et al*. 2002	Metagenomics can assess COPD exacerbation severity	11
Erb-Downward., *et al*. 2011	Pyrosequencing of 16S amplicons can analyze lung microbiome in COPD patients	12
Pragman., *et al*. 2012	Pyrosequencing of 16S amplicons can analyze lung microbiome in COPD patients	13
Sze., *et al*. 2012	Pyrosequencing of 16S amplicons can analyze lung microbiome in patients with severe COPD	14
Schlaberg., *et al*. 2017	NGS held great promise to improve infectious disease diagnostics, particularly in critically ill and immunocompromised patoients	15
Chiu., *et al*. 2017	Metagenomics can detect meningoencephalitis	16
Wilson., *et al*. 2018	Metagenomics can detect subacute or chronic meningitis	17
Harris., *et al*. 2003	Detection of bacterial infection in pediatric patients by 16S rDNA PCR	18
Chan., *et al*. 2014	NGS can detect viral infections in frozen brain tissue of patients with viral encephalitis	19
Hills., *et al*. 2009	What diagnostic tests are appropriate for confirming Japanese encephalitis infection surveillance? NGS?	20

Table 1: Results from 20 yielded articles**.**

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 [10]. 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 [11] Microbiomic changes as COPD progress have been investigated [12-14]. Some investigators are validating the use of metagenomic NGS for clinical use [15]. 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)" [6,16]. 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 [17].

Limitations of metagenomic diagnostics in detecting infectious diseases

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 [18]. 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 [19]. 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 [20]. The principal diagnosis of JEV infection is serology [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.

Bibliography

1. Vartoukian SR., *et al*. "Strategies for culture of "unculturable" bacteria". *FEMS Microbiology Letter* 309.1 (2010): 1-7.

2. Liu YY., *et al*. "Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study". *Lancet Infectious Diseases* 16.2 (2016): 161-168.

3. Brown JR., *et al*. "Encephalitis diagnosis using metagenomics: application of next- generation sequencing for undiagnosed cases". *Journal of Infection* 76.3 (2018): 225-240.

4. Albanese D., *et al*. "Strain profiling and epidemiology of bacterial species from metagenomic sequencing". *Nature Communications* 8.1 (2017): 2260.

5. Afshinnekoo E., *et al*. "Precision metagenomics: rapid metagenomic analyses for infectious disease diagnostics and public health surveillance". *Journal of Biomolecular Techniques* 28.1 (2017): 40-45.

6. Wilson MR., *et al*. "Actional diagnosis of neuroleptospirosis by next-generation sequencing". *New England Journal of Medicine* 370.25 (2014): 2408-2417.

7. Schubert RD., *et al*. "A tale of two approaches: how metagenomics and proteomics are shaping the future of encephalitis diagnostics". *Current Opinion in Neurology* 28.3 (2015): 283-287.

8. Valeria DA., *et al*. "Metagenomics reveals dysbiosis and a potentially pathogenic N. flavescens strain in duodenum of adult celiac patients". *American Journal of Gastroenterology* 111.6 (2016): 879-890.

9. Cameron SJS., *et al*. "Metagenomic sequencing of chronic obstructive pulmonary disease upper bronchial tract microbiome reveals functional changes associated with disease severity". *PLoS One* 11.2 (2016): e0149095.

10. Kaplan RS., *et al*. "How to solve the cost crisis in health care". *Harvard Business Review* (2011): 46-52.

11. Patel IS., *et al*. "Relationship between bacterial colonization and the frequency, character, and severity of COPD exacerbations". *Thorax* 57.9 (2002): 759-764.

12. Erb-Downward JR., *et al*. "Analysis of the lung microbiome in the "healthy" smoker and in COPD". *PLoS One* 6.2 (2011): e16384.

13. Pragman AA., *et al*. "The lung microbiome in moderate and severe chronic obstructive pulmonary disease". *PLoS One* 7.10 (2012): e47305.

14. Sze MA., *et al*. "The lung tissue microbiome in chronic obstructive pulmonary disease". *American Journal of Respiratory and Critical Care Medicine* 185.10 (2012): 1073-1080.

15. Schlaberg R., *et al*. "Validation of metagenomic next-generation sequencing tests for universal pathogen detection". *Archives of Pathology and Laboratory Medicine* 141.6 (2017): 776-786.

16. Chiu CY., *et al*. "Diagnosis of fatal human case of St. Louis encephalitis virus infection by metagenomic sequencing, California, 2016". *Emerging Infectious Diseases* 23.10 (2017): 1964-1968.

17. Wilson MR., *et al*. "Chronic meningitis investigated via metagenomic next-generation sequencing". *JAMA Neurology* 75.8 (2018): 947-955.

18. Harris KA., *et al*. "Development of broad-range 16S rDNA PCR for use in the routine diagnostic clinical microbiology service". *Journal of Medical Microbiology* 52.8 (2003): 685-691.

19. Chan BK., *et al*. "Deep sequencing to identify the causes of viral encephalitis". *PLoS One* 9.4 (2014): e93993.

20. Hills S., *et al*. "Evidence and rationale for the World Health Organization recommended standards for Japanese encephalitis surveillance". *BMC Infectious Diseases* 9 (2009): 214.

Volume 2 Issue 6 June 2019