Scientific Research Publishing

Advances in Microbiology, 2021, 11, 157-164
https://www.scirp.org/journal/aim
ISSN Online: 2165-3410
ISSN Print: 2165-3402

Application of 16S rDNA Sequencing Technology in the Analysis of Pathogenic Bacteria in Sputum of Severe Pneumonia

Jun Zheng[1], Juan Zhu[1], Bin Chen[1], Lingxiu Chen[1], Tian Gao[1], Xinping Chen[2], Feiyan Li[2*]

[1]The Fourth People's Hospital of Haikou, Haikou, China
[2]Hainan General Hospital, Haikou, China
Email: *g2002m@163.com

How to cite this paper: Zheng, J., Zhu, J., Chen, B., Chen, L.X., Gao, T., Chen, X.P. and Li, F.Y. (2021) Application of 16S rDNA Sequencing Technology in the Analysis of Pathogenic Bacteria in Sputum of Severe Pneumonia. *Advances in Microbiology*, **11**, 157-164.
https://doi.org/10.4236/aim.2021.113011

Received: January 20, 2021
Accepted: March 5, 2021
Published: March 8, 2021

Abstract

The diagnosis of pathogenic bacteria in severe pneumonia is difficult and the prognosis is poor. Its outcome is closely related to bacterial pathogenicity and the timeliness and pertinence of antibiotic treatment. Therefore, early diagnosis is of great significance to the prognosis of patients. Sputum examination and culture is the gold standard for the diagnosis of pathogens of severe pneumonia. However, due to the long time of bacterial culture, the early use of antibiotics, the change of bacteria species, mixed infection and other problems, the results of bacterial culture in sputum are often false negative. With the continuous application of new molecular biology techniques in clinical detection, the classification of bacteria and microorganisms has deepened from the identification of phenotypic characteristics to the classification of gene characteristics. Sequencing analysis with 16S rDNA sequencing technology has the characteristics of high sequencing flux, large amount of data obtained, short cycle, and can more comprehensively reflect the species composition of microbial community, real species distribution and abundance information. In this paper, 16S rDNA sequencing technology was used to analyze the bacterial population composition in the sputum of severe pneumonia, and to explore a new method of etiological diagnosis.

Keywords

Bacterial Severe Pneumonia, Sputum, The Pathogenic Bacterium, 16S rDNA Sequencing

1. Introduction

Pneumonia is one of the clinical very common respiratory infectious diseases,

due to the lack of typical symptoms and signs in the early diagnosis of experience after antibiotic treatment inappropriate often makes pneumonia in delay no more waiting for a reason, and gradually develops severe pneumonia (severe root, SP), also called toxic pneumonia or explosive pneumonia, which is the highest rates of pneumonia types [1]. The diagnosis of pathogenic bacteria in severe pneumonia is difficult and the prognosis is poor. Its outcome is closely related to bacterial pathogenicity and the timeliness and pertinence of antibiotic treatment, so early diagnosis is of great significance to the prognosis of patients [2]. Therefore, there is an urgent need for new clinical detection techniques in early diagnosis of pneumonia to change the status quo. 16S rDNA is the gene encoding the small subunit rRNA (16S rRNA) of the prokaryotic ribosome. The length of 16S rRNA is about 1500 bp, and it is often used as the basis for bacterial taxonomy studies. 16S rDNA sequencing analysis is characterized by high sequencing flux, large amount of data obtained, short cycle, and more comprehensive reflection of microbial community species composition, real species distribution and abundance information [3]. 16S rDNA sequencing technology is a bright spot in the development of modern molecular biology technology. In this paper, 16S rDNA sequencing technology was used to analyze the bacterial population composition in the sputum of patients with severe pneumonia. The sputum samples with positive 16S rDNA sequence were classified and identified by high-throughput sequencing technology, so as to find the common pathogenic bacteria of clinical infection of severe pneumonia and explore the new thinking of etiological diagnosis of severe pneumonia.

2. 16S rDNA Amplification and Sequencing Technology

Bacteria detection includes traditional morphological examination, isolation and culture, biochemical identification, immunoanalysis and other methods, among which the isolation and culture of bacteria is the most important, but the isolation and culture of caustic bacteria and slow-growing bacteria can achieve ideal detection effect. In recent years, a variety of gene diagnosis technologies have been developed and used in bacterial detection, especially the polymerase chain reaction (PCR) based gene diagnosis technology has played an increasingly important role.

Amplifier sequencing, PCR amplification involving a specific sequence site, usually 16S/18S rDNA. Metagenomic species classification is generally represented by OUT (operational taxonomic unit). 16S rDNA is usually used to measure prokaryotes. Its sequencing analysis has the characteristics of high sequencing flux, large amount of data obtained, short cycle, and can more comprehensively reflect the species composition of microbial community, real species distribution and abundance information. The purpose of this project is to use 16S RNA gene sequencing technology of bacterial ribosome small subunit (16S rDNA) to identify and classify bacteria in pneumonic sputum, and to explore a new method of etiological diagnosis.

16S rDNA is the gene encoding 16S rRNA of prokaryotes, with a length of

about 1500 bp. It is found in the genomes of all prokaryotes such as bacteria, chlamydia, mycoplasma, rickettsia, spirochetes and actinomycetes, and is composed of multiple Conserved regions and Variable regions intersperse with them. The conserved zone is common to all bacteria, and there is no significant difference among bacteria [4]. The variable region varies among different bacteria to a certain extent and is specific for bacteria genus or species. The use of PCR technology to amplify 16S rDNA contains two meanings: the design of universal PCR primer in the conserved region, which can theoretically amplify all kinds of bacteria existing in the sample to be tested; If the variable region is selected to design PCR-specific primers, the bacteria in the samples can be identified to the level of genus, species and even strain. Therefore, 16S rDNA has become an ideal target sequence for bacterial identification and classification research [5].

Bacterial 16S rDNA contains 9 variable regions, named V1-V9 regions [6]. Identifying bacterial species-specific sequences in a variable region may lead to useful targets for bacterial laboratory diagnosis. Except for regions V2, V3 and V4, which are slightly longer, the sequence of other hypervariable regions is very short, and no hypervariable region can distinguish all bacteria [7]. Related studies have reported that region V1 can distinguish *Staphylococcus aureus* from plasma coagulase-negative *Staphylococcus aureus*. Regions V2 and V3 can identify all bacteria except Enterobacteriaceae to the genus level. Region V2 is particularly suitable for the identification of Mycobacterium species. Region V3 can well distinguish all bacteria in Haemophilus genus. Most species except Enterobacteriaceae can be distinguished by V6 region, and Bacillus anthracis can be distinguished from Bacillus cereus with similar biological characters by single nucleotide polymorphism (SNP) analysis. However, it is not possible to identify bacterial genus or species by V4, V5, V7 and V8 region sequences [8].

3. Applications Based on Partial or Full-Length 16S rDNA Sequences

The development of 16S rDNA PCR based broad-spectrum bacterial detection technology has brought the study of microbial molecular biology to a new level. Bacterial species-specific PCR can only use a specific bacterial primer to detect specific bacteria, while 16S rDNA PCR uses 16S rDNA primer for all bacteria to efficiently and unbiased simultaneously analyze all bacterial species in the sample flora [9] At present, this technology has been applied in many aspects of clinical classification detection:

(Recommending primer sequence description): High variability region sequencing technology of 16S rDNA was used to add sample Tag sequence (Barcode) to PCR products for mixed sequencing, and species classification, operational taxonomic unit (OTU) analysis, diversity analysis, etc., were performed according to gene expression Tag (TAG). The commonly used sequencing strategy is 454 pyrosequencing of V3-V5 region.

3.1. Identification of Difficult-to-Culture Bacteria

In the early 1990s, against bacillus sexual hemangioma (bacillary angiomatosis) of bacterial culture and identification have been unsuccessful, Relman, etc. [10] with polymerase chain reaction (PCR) amplification in tissue samples from patients bacterial 16S rDNA fragment, and by sequencing and comparative analysis, the first successful identified the hemangioma of the pathogen to FuErXi rickettsia (*Rochalimaea quintana*). In the bacteriological diagnosis of endocarditis, the positive rate of traditional blood culture or heart valve culture is very low. Based on 16S rDNA technology, 177 specimens of heart valve for bacterial 16 s rDNA universal primers quantitative PCR amplification and sequencing, the results show that quantitative PCR detection sensitivity, specific, negative and positive predicted value expected value reached 96%, 95.3%, 98.4% and 88.5% respectively, and the method for the timely processing of endocarditis and antibiotics provided a reference for the rational selection of [11].

3.2. Distinguishment of Closely Evolutionarily Related Bacteria

The genetic sequences of Bacillus anthracis, Bacillus cereus and Bacillus thuringiensis are 99% homologous, which makes it difficult to identify each other. It is called Bacillus cereus group, and some scholars even believe that the three belong to the same species. The 16S rDNA sequencing method can not only accurately distinguish the three kinds of similar Bacillus, but also directly detect clinical specimens, which is suitable for rapid identification of *Bacillus anthracis* in bioterrorism events [12] [13].

3.3. Identification of Bacteria in Blood Culture

The 16S rDNA sequencing method has the advantages of rapid and accurate in pathogen diagnosis and antibiotic selection. As reported in related studies [14], the blood culture of a patient identified by the RapID automatic bacterial identification instrument for the second hospitalization was all Bacillus pallus, but the Vitek automatic bacterial identification instrument was used to test, and the results showed that the patient was Brucella aminii. The full-length 16S rDNA was amplified and sequenced, and the sequence was 100% homologous to 25 Brucella sequences in the SmartGene database. The serotype was identified as Brucella suis by the U.S. Centers for Disease Control and Prevention. The Maastricht university medical center in the research of real-time quantitative 16S rDNA PCR amplification system, using the general probe plus species or genus specific probe more probe method (multiprobe assay), Several common bacteria in blood infections, including Gram-negative Pseudomonas and Escherichia coli and Gram-positive Staphylococcus, Enterococcus and Streptococcus, can be quickly identified within 2 h [15].

3.4. New Species of Bacteria Were Discovered

Clinically, it is easy to encounter some new bacteria, and it is difficult to evaluate

their potential pathogenicity because the species is not clear. Generally, if the homology of full length 16S rDNA between two strains is ≥ 99%, they are considered to be the same bacteria. If it is 97% ~ 98.9%, it is generally considered to be a different species of the same genus. If < 95%, it is considered to be a different genus. Studies have reported that from 2001 to 2007, up to 215 new species of bacteria have been found by 16S rDNA sequencing globally, belonging to 29 bacterial genera, 15 of which are new bacterial genera, including 100 new species [16]. In terms of source, the new species were found in oral and dental specimens and gastrointestinal specimens.

In the Human Microbiome Project (HMP) led by the National Institutes of Health (NIH) in 2007, 16S rDNA sequencing is one of the commonly used methods. By analyzing the microbial communities in different parts of the oral cavity, nose cavity, digestive tract, skin and genitourine tract of volunteers. The research team has published 5177 16S rDNA sequences obtained from 242 adult volunteers. As a systematic method for bacterial classification, 16S rDNA sequencing plays an important role in the HMP program.

3.5. The Microsep 500 16S rDNA Bacterial Identification System

The system developed by American Applied Biosystems, is based on the amplification of 527 bp fragment of end of bacterial 16S rDNA. The primer de sign is targeted at all bacteria, and it is a universal bacterial identification system. After amplification and sequencing, 16S rDNA of bacteria in the samples can be compared with the database of the system, which can realize the rapid identification of bacteria such as Mycobacterium, Corynebacterium and Nocardia. Besides the system database MicroSeq ID, bacterial 16 s rDNA sequence database also includes RDP-II, RIDOM and SmartGene IDNS.

4. 16S rDNA Sequencing and Denaturing Gradient Gel Electrophoresis PCR-DGGE

Conventional polyacrylamide gel electrophoresis can only separate different DNA fragments by different fragment sizes and different electrophoretic mobility on the same concentration of glue, but it cannot effectively separate DNA fragments with similar or the same fragment sizes. DGGE (denaturing gradient gel electrophoresis) refers to denaturing gradient gel electrophoresis, which changes the electrophoretic mobility due to the different chain unwinding behaviors of DNA in denaturing agents with different concentrations, so as to separate DNA fragments with the same size but different base composition.

PCR-DGGE can well identify complex 16S rDNA samples. Usually, a single band is an OUT (operational taxonomic unit). The number of bands can reflect the diversity of bacterial community, and the signal strength of bands can reflect the relative number. Therefore, the relationship of microbial OUT and quantity in sputum of patients with different severe pneumonia can be determined according to the stripe information, and the bacterial diversity information can be

obtained, and the bacterial population closely related to severe pneumonia can be further analyzed.

Limitations should be described: the 16S rDNA clone library method used in the study of Pandey *et al.* may have certain limitations in the study of bacterial diversity due to insufficient number of clone selections or selection bias [17]. The introduction of molecular biological methods has created a new situation for the pathogen diagnosis of sepsis, which has made the pathogen diagnosis of sepsis more sensitive, efficient and convenient, and solved some difficult problems in blood culture. However, molecular biological methods still have some limitations, which need to be further optimized and improved in the practical application. Traditional blood culture methods may be more sensitive to detect certain bacteria that require less growth conditions: in theory, only one living bacterium can be observed in the culture. However, molecular biological methods may not perform well in detecting small blood samples due to problems such as the efficiency of bacterial DNA extraction methods and exogenous bacterial DNA contamination. Although molecular methods can guide sepsis treatment by simultaneously detecting bacterial resistance genes, blood culture methods are more suitable for practical clinical needs by combining simultaneous drug sensitivity tests in culture [18].

5. Analysis of Clinical and Prognostic Factors for Severe Pneumonia

Severe pneumonia is characterized by rapid onset, multiple complications and multiple organ damage, which promote the rapid deterioration of the disease and even directly lead to the death of patients. Therefore, the diagnosis and correct treatment of severe pneumonia is still one of the difficult problems in clinical treatment. Severe pneumonia can be divided into severe community acquired pneumonia (SCAP) and severe hospital acquired pneumonia (SHAP) according to different acquired environment. Both SCAP and SHAP have high morbidity and fatality rates. According to the epidemiological survey, the fatality rate of SCAP patients can be as high as 50%, while HAP can reach 70% [19] [20] [21].

6. Conclusions

In recent years, due to the increase of high-risk host infection, the phenomenon of bacterial resistance and even multi-drug resistance is becoming more and more serious. Despite the continuous emergence of new antimicrobial agents, severe pneumonia is still an infectious disease with difficult diagnosis and treatment and poor prognosis. One of the key factors is the rational use of antibiotics.

The rational use of antibiotics for severe pneumonia should follow the "3R" principle:

1) Right time, *i.e.* the Right time (early treatment and appropriate course of treatment);

2) The Right drug;

3) The patient is Right.

To achieve the 3R principle, first of all, we should master the drug resistance of the main pathogenic bacteria infected in the local area and the hospital. Secondly, it is necessary to understand the activity, pharmacokinetic characteristics and adverse reactions of various antibacterial drugs, so as to maximize the effect of antibacterial drugs. Finally, to severe pneumonia should distinguish serious degree. In this project, the 16S rDNA PCR-DGGE technology was used to monitor and analyze the bacterial population in the sputum of patients with severe pneumonia, to explore the differences of bacterial species in patients with severe pneumonia of different degrees, and to provide an important reference for the diagnosis, precise treatment and prognosis of patients.

Foundation Project

Supported by Hainan Natural Science Foundation (Project No. 819MS143).

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

References

[1] He, L.X. (2001) Progress in Diagnosis and Treatment of Severe Pneumonia. *Contemporary Medicine*, **7**, 26-30.

[2] Cook, D. (2000) Ventilator Associated Pneumonia: Perspectives on the Burden of Illness. *Intensive Care Medicine*, **26**, 31-37. https://doi.org/10.1007/s001340051116

[3] Ma, Z.L., Tang, S.M. and Lou, X.B. (2011) The Application of 16SrRNA in the Identification of Medical Microorganism. *Jilin Medical Journal*, **32**, 2291-2292.

[4] Barth Reller, L., Weinstein, M.P. and Petti, C.A. (2007) Detection and Identification of Microorganisms by Gene Amplification and Sequencing. *Clinical Infectious Diseases*, **44**, 1108-1114. https://doi.org/10.1086/512818

[5] Sontakke, S., Cadenas, M.B., Maggi, R.G., Diniz, P.P.V.P. and Breitschwerdt, E.B. (2009) Use of Broad Range 16S rDNA PCR in Clinical Microbiology. *Journal of Microbiological Methods*, **76**, 217-225. https://doi.org/10.1016/j.mimet.2008.11.002

[6] Jumpstart Consortium Human Microbiome Project Data Generation Working Group (2012) Evaluation of 16S rDNA-Based Community Profiling for Human Microbiome Research. *PLoS ONE*, **7**, e39315. https://doi.org/10.1371/journal.pone.0039315

[7] Baker, G.C., Smith, J.J. and Cowan, D.A. (2003) Review and Re-Analysis of Domain-Specific 16S Primers. *Journal of Microbiological Methods*, **55**, 541-555. https://doi.org/10.1016/j.mimet.2003.08.009

[8] Chakravorty, S., Helb, D., Burday, M., Connell, N. and Alland, D. (2007) A Detailed Analysis of 16S Ribosomal Gene Segments for the Diagnosis of Pathogenic Bacteria. *Journal of Microbiological Methods*, **69**, 330-339. https://doi.org/10.1016/j.mimet.2007.02.005

[9] Yang, X.J., Wang, X.H., Liang, Z.J., Zhang, X.Y., Wang, Y.B. and Wang, Z.H. (2014) Application of 16S rDNA Sputum Bacterial Sequencing in Diversity Analysis of

Ventilator Associated Pneumonia. *Chinese Critical Care Medicine*, **26**, 294-299.

[10] Relman, D.A., Loutit, J.S., Schmidt, T.M., Falkow, S. and Tompkins, L.S. (1990) The Agent of Bacillary Angiomatosis—An Approach to the Identification of Uncultured Pathogens. *The New England Journal of Medicine*, **323**, 1573-1580. https://doi.org/10.1056/NEJM199012063232301

[11] Marín, M., Munoz, P., Sánchez, M., del Rosal, M., Alcalá, L., Rodríguez-Créixems, M., *et al.* (2007) Molecular Diagnosis of Infective Endocarditis by Real-Time Broad-Range Polymerase Chain Reaction (PCR) and Sequencing Directly from Heart Valve Tissue. *Medicine*, **86**, 195-202. https://doi.org/10.1097/MD.0b013e31811f44ec

[12] Helgason, E., Okstad, O.A., Caugant, D.A., Johansen, H.A., Fouet, A., Mock, M., *et al.* (2000) *Bacillus anthracis*, *Bacillus Cereus*, and *Bacillus thuringiensis*—One Species on the Basis of Genetic Evidence. *Applied and Environmental Microbiology*, **66**, 2627-2630. https://doi.org/10.1128/AEM.66.6.2627-2630.2000

[13] Sacchi, C.T., Whitney, A.M., Mayer, L.W., Morey, R., Steigerwalt, A., Boras, A., *et al.* (2002) Sequencing of 16s rRNA Gene: A Rapid Tool for Identification of *Bacillus anthracis*. *Emerging Infectious Diseases*, **8**, 1117-1123. https://doi.org/10.3201/eid0810.020391

[14] Horvat, R.T., El Atrouni, W., Hammoud, K., Hawkinson, D. and Cowden, S. (2011) Ribosomal RNA Sequence Analysis of *Brucella* Infection Misidentified as *Ochrobactrum anthropi* Infection. *Journal of Clinical Microbiology*, **49**, 1165-1168. https://doi.org/10.1128/JCM.01131-10

[15] Hansen, W.L., Beuving, J., Bruggeman, C.A. and Wolffs, P.F.G. (2010) Molecular Probes for the Diagnosis of Clinically Relevant Bacterial Infections in Blood Cultures. *Journal of Clinical Microbiology*, **48**, 4432-4438. https://doi.org/10.1128/JCM.00562-10

[16] Woo, P.C., Lau, S.K., Teng, J.L., Tse, H. and Yuen, K.-Y. (2008) Then and Now: Use of 16S rDNA Gene Sequencing for Bacterial Identification and Discovery of Novel Bacteria in Clinical Microbiology Laboratories. *Clinical Microbiology and Infection*, **14**, 908-934. https://doi.org/10.1111/j.1469-0691.2008.02070.x

[17] Pandey, P.K., Verma, P., Kumar, H., Bavdekar, A., Patole, M.S. and Shouche, Y.S. (2012) Comparative Analysis of Fecal Microflora of Healthy Full-Term Indian Infants Born with Different Methods of Delivery (Vaginal vs Cesarean): *Acinetobacter* sp. Prevalence in Vaginally Born Infants. *Journal of Biosciences*, **37**, 989-998. https://doi.org/10.1007/s12038-012-9268-5

[18] Waldeisen, J.R., Wang, T., Mitra, D. and Lee, L.P. (2011) A Real-Time PCR Antibiogram for Drug-Resistant Sepsis. *PLoS ONE*, **6**, e28528. https://doi.org/10.1371/journal.pone.0028528

[19] The Human Microbiome Project Consortium (2012) A Framework for Human Microbiome Research. *Nature*, **486**, 215-221. https://doi.org/10.1038/nature11209

[20] American Thoracic Society (2001) Guidelines for the Management of Adults with Community Acquired Pneumonia. *American Journal of Respiratory and Critical Care Medicine*, **163**, 1730-1754. https://doi.org/10.1164/ajrccm.163.7.at1010

[21] American Thoracic Society (2005) Guidelines for the Management of Adults with Hospital-Acquired, Ventilator-Associated, and Healthcare-Associated Pneumonia. *American Journal of Respiratory and Critical Care Medicine*, **171**, 388-416. https://doi.org/10.1164/rccm.200405-644ST