Supplementary file Figure 1. Targeted sequencing report for multiple respiratory pathogens

Name：***
Hospital：Longquan People's Hospital
Sample Type：BALF
Report date：2023-10-28 14:25:29

金域医学
KingMed Diagnostics

HZ0031VHR1YFAZE

Targeted sequencing report for multiple respiratory pathogens

Patient Information

Patient information	Name: ***	Sex: Male	Age: 80 years old
	Inpatient/Outpatient Number: ****128		
Sample information	Sample ID: 0548734790		
	Sampling date: 2023-10-27 11:00:00	Experiment number: T05198	Bed number: 7-53
	Clinical diagnosis: LRTI	date of acceptance: 2023-10-27 20:02:28	
			Sample type: BALF
Detection project information: Targeted sequencing of multiple respiratory pathogens			
	Detection method: Multi targeted amplification high-throughput sequencing method		

Detection results of pathogenic microorganisms

Microbial type	Genus name	Microbial name	Number of homogenized sequences	Microbial estimated concentration (copies/mL)	Pathogenicity classification
1.List of Special Pathogens (Mycobacterium, Mycoplasma, Chlamydia, etc.)					
Not detected					
2.Bacterial List					
G⁻	*Haemophilus*	*Haemophilus influenz*	37384	>1.0x10^6	A
G⁻	*Fusobacterium*	*Fusobacterium nucleatum*	1124	1.2x10^4	C
G⁺	*Streptococcus*	*Streptococcus anginosus group*	423	<1.0x10^3	C
3.List of Fungi					
Not detected					
4.Virus List					
DNA virus	*Lymphocryptovirus*	*Epstein-Barr virus(EBV)*	23	<1.0x10^3	C类

Number of homogenized sequences: The number of sequences containing the microorganism in every 100K original sequence.
Microbial estimation concentration (copies/mL): The microbial content in the sample is calculated using bioinformatics methods.
Pathogenicity Class A: It is a specific pathogenic pathogen in respiratory specimens or a common pathogenic pathogen in clinical practice.
Pathogenicity Class B: It is an opportunistic (conditional) pathogenic pathogen in respiratory specimens, and infection may occur in patients with systemic or local immune deficiency/damage/deficiency, respiratory barrier dysfunction, or lower respiratory microbiota imbalance.
Pathogenic Class C: It is a normal microbial community in the respiratory tract that generally does not cause infection, but there is a possibility of aspiration leading to lung abscess.
Note: The above microbial classification is for clinical reference only, and the final definition of microorganisms shall be subject to clinical practice.

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Detection of resistance genes

Classification	Drug resistance gene family	Sequence number	Suggested analysis	Suspected associated bacteria
Not detected				

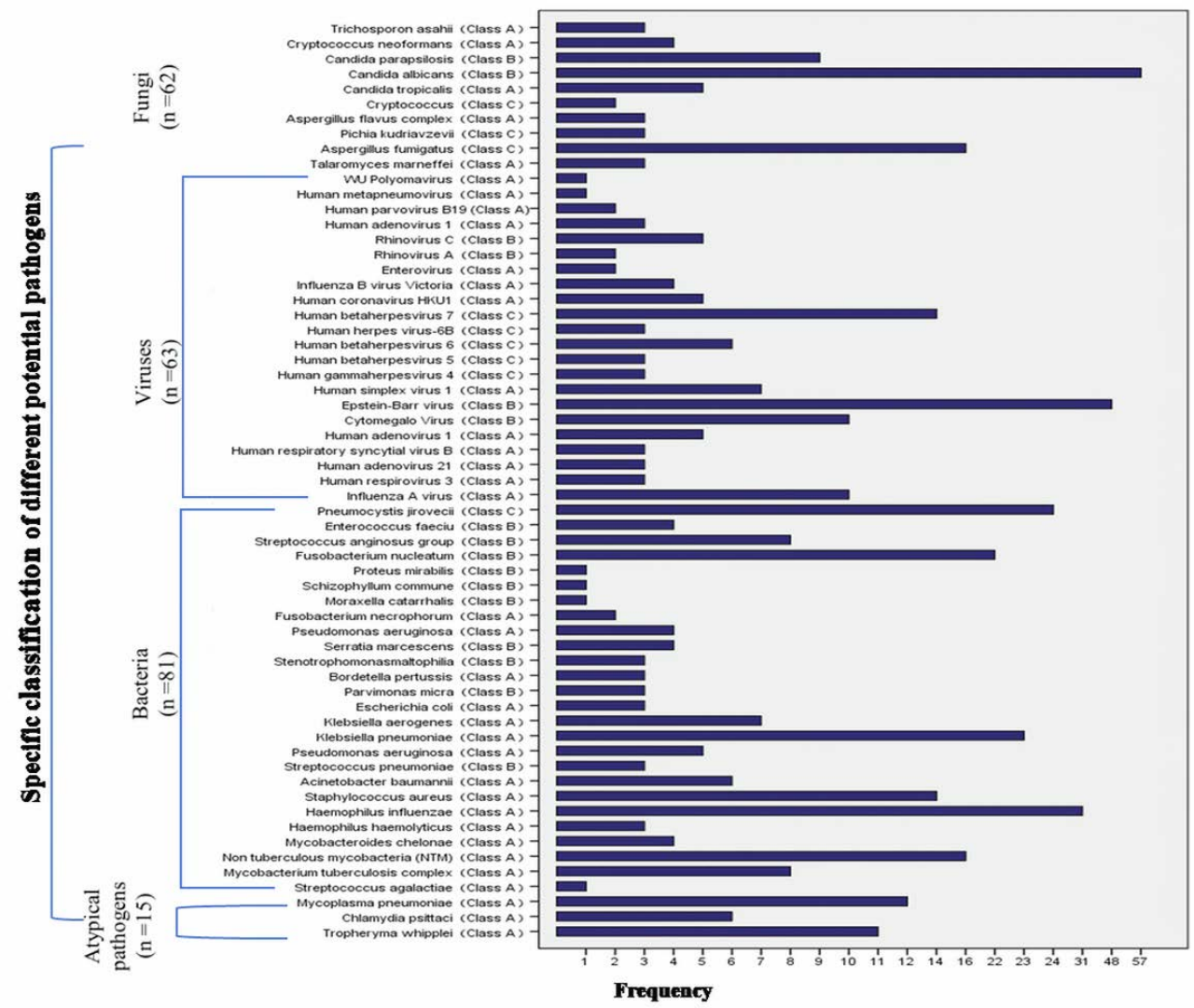
Suspected associated bacteria: The detection of resistance genes does not necessarily indicate the presence of associated pathogens, nor does it necessarily mean that associated pathogens will develop resistance.

Expected resistance information

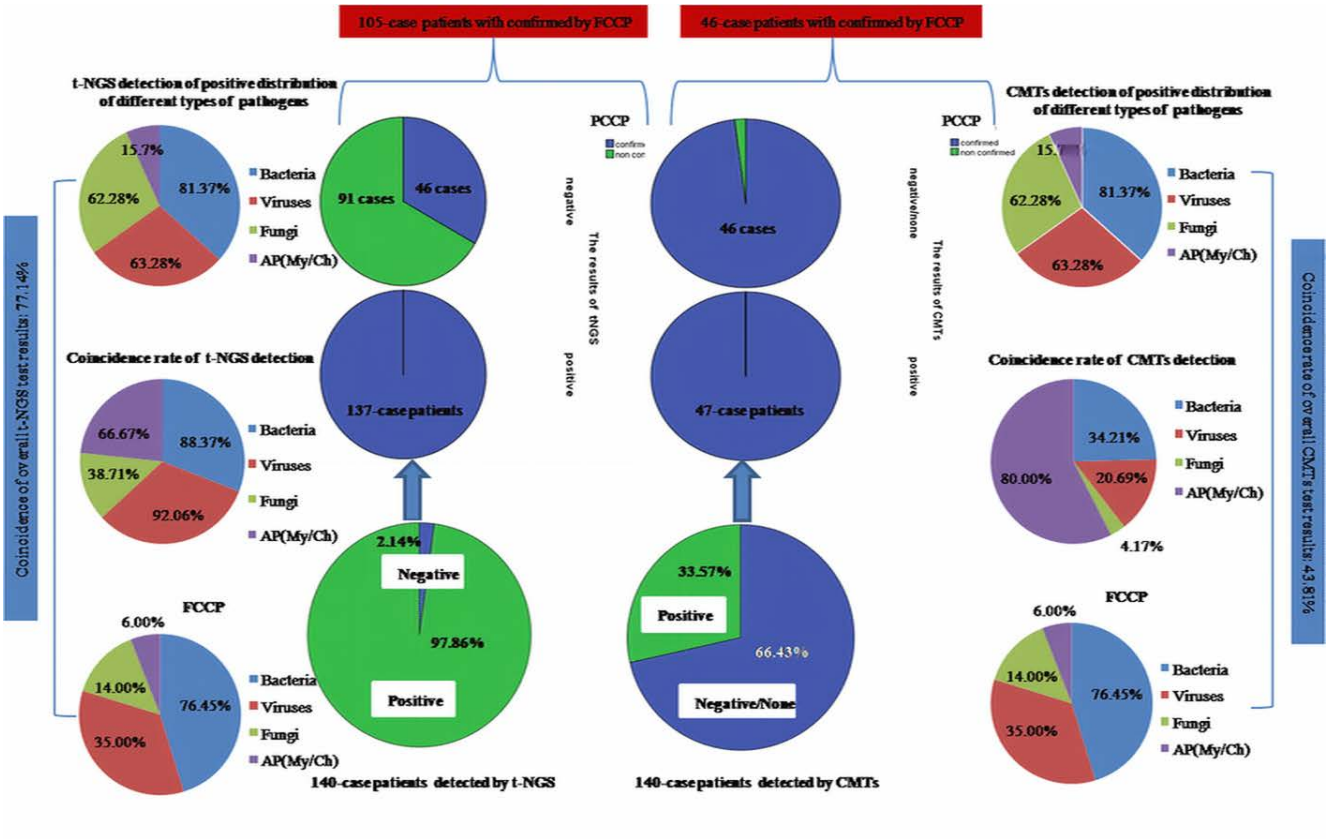
Pathogenic microorganism	Expected resistance information
Not detected	

Expected resistance information: Reference material "CLSI M100 Antimicrobial Susceptibility Test Execution Standard"

Supplementary file Figure 2. Distribution of potential pathogens in the enrolled 140-case patients through tNGS for different pathogens detection



Supplementary file Figure 3. Comparison of consistency of FCCP between and the results of different detection methods by CMTs and t-NGS



STROBE Statement—checklist of items that should be included in reports of observational studies

	Item No	Recommendation	Page
Title and abstract	1	(*a*) Indicate the study's design with a commonly used term in the title or the abstract	**1**
		(*b*) Provide in the abstract an informative and balanced summary of what was done and what was found	**1**
Introduction			
Background/rationale	2	Explain the scientific background and rationale for the investigation being reported	**1-2**
Objectives	3	State specific objectives, including any prespecified hypotheses	**2**
Methods			
Study design	4	Present key elements of study design early in the paper	**2**
Setting	5	Describe the setting, locations, and relevant dates, including periods of recruitment, exposure, follow-up, and data collection	**2-3**
Participants	6	(*a*) *Cohort study*—Give the eligibility criteria, and the sources and methods of selection of participants. Describe methods of follow-up *Case-control study*—Give the eligibility criteria, and the sources and methods of case ascertainment and control selection. Give the rationale for the choice of cases and controls *Cross-sectional study*—Give the eligibility criteria, and the sources and methods of selection of participants	**3**
		(*b*) *Cohort study*—For matched studies, give matching criteria and number of exposed and unexposed *Case-control study*—For matched studies, give matching criteria and the number of controls per case	
Variables	7	Clearly define all outcomes, exposures, predictors, potential confounders, and effect modifiers. Give diagnostic criteria, if applicable	**3**
Data sources/ measurement	8*	For each variable of interest, give sources of data and details of methods of assessment (measurement). Describe comparability of assessment methods if there is more than one group	**3**
Bias	9	Describe any efforts to address potential sources of bias	**3**
Study size	10	Explain how the study size was arrived at	**3**
Quantitative variables	11	Explain how quantitative variables were handled in the analyses. If applicable, describe which groupings were chosen and why	**3**
Statistical methods	12	(*a*) Describe all statistical methods, including those used to control for confounding	**4**
		(*b*) Describe any methods used to examine subgroups and interactions	
		(*c*) Explain how missing data were addressed	
		(*d*) *Cohort study*—If applicable, explain how loss to follow-up was addressed *Case-control study*—If applicable, explain how matching of cases and controls was addressed *Cross-sectional study*—If applicable, describe analytical methods taking account of sampling strategy	
		(*e*) Describe any sensitivity analyses	**not applicable**

Continued on next page

Results			**4-6**
Participants	13*	(a) Report numbers of individuals at each stage of study—eg numbers potentially eligible, examined for eligibility, confirmed eligible, included in the study, completing follow-up, and analysed	
		(b) Give reasons for non-participation at each stage	
		(c) Consider use of a flow diagram	
Descriptive data	14*	(a) Give characteristics of study participants (eg demographic, clinical, social) and information on exposures and potential confounders	
		(b) Indicate number of participants with missing data for each variable of interest	
		(c) *Cohort study*—Summarise follow-up time (eg, average and total amount)	
Outcome data	15*	*Cohort study*—Report numbers of outcome events or summary measures over time	
		Case-control study—Report numbers in each exposure category, or summary measures of exposure	
		Cross-sectional study—Report numbers of outcome events or summary measures	
Main results	16	(*a*) Give unadjusted estimates and, if applicable, confounder-adjusted estimates and their precision (eg, 95% confidence interval). Make clear which confounders were adjusted for and why they were included	
		(*b*) Report category boundaries when continuous variables were categorized	
		(*c*) If relevant, consider translating estimates of relative risk into absolute risk for a meaningful time period	**6**
Other analyses	17	Report other analyses done—eg analyses of subgroups and interactions, and sensitivity analyses	**5**
Discussion			**6-9**
Key results	18	Summarise key results with reference to study objectives	
Limitations	19	Discuss limitations of the study, taking into account sources of potential bias or imprecision. Discuss both direction and magnitude of any potential bias	
Interpretation	20	Give a cautious overall interpretation of results considering objectives, limitations, multiplicity of analyses, results from similar studies, and other relevant evidence	
Generalisability	21	Discuss the generalisability (external validity) of the study results	
Other information			
Funding	22	Give the source of funding and the role of the funders for the present study and, if applicable, for the original study on which the present article is based	**9**

*Give information separately for cases and controls in case-control studies and, if applicable, for exposed and unexposed groups in cohort and cross-sectional studies.

Note: An Explanation and Elaboration article discusses each checklist item and gives methodological background and published examples of transparent reporting. The STROBE checklist is best used in conjunction with this article (freely available on the Web sites of PLoS Medicine at http://www.plosmedicine.org/, Annals of Internal Medicine at http://www.annals.org/, and Epidemiology at http://www.epidem.com/). Information on the STROBE Initiative is available at www.strobe-statement.org.