

Early detection of gram-negative bacteria using metagenomic next-generation sequencing in acute respiratory distress syndrome: A case report

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Abstract. Metagenomic next-generation sequencing (mNGS) is an effective method that can be used for the identification of early pathogens in patients with suspected severe pneumonia. However, the potential of mNGS for evaluating the prognosis of acute respiratory distress syndrome (ARDS) in patients with severe pneumonia remains unclear. In the present report, hospital-acquired gram-negative bacteria infections were detected in a case using metagenomic next-generation sequencing (mNGS) in a sample of bronchoalveolar fluid. This was obtained from a 58-year-old male patient with traumatic wet lung after a neurosurgery. According to the results, of which the profiles of the resistance genes were detected by mNGS, drugs designed to control infection were adjusted, namely to polymyxin B (500,000 U/12 h), azithromycin (0.5 g/24 h) and ganciclovir (0.25 g/12 h). Following adjusting treatment for 8 days, the symptoms of lung infection and hypoxemia were markedly improved, resulting in the patient being transferred out of the intensive care unit 15 days after treatment. To conclude, observations from the present report suggest that mNGS is a useful method for the early identification of pathogens in patients with pneumonia caused by ARDS. However, further studies are required to identify the complementary role of mNGS in supporting conventional microbiological methods in routine clinical practice.

Introduction

Road traffic accidents are one of the major causes of severe multiple injuries around the world (1,2). The proportion of

patients with chest trauma among patients that were admitted to German hospitals with multiple injuries stood at 44.8% in 2019 (3). Despite improvements in emergency systems and clinical research, treatment protocols for severe acute trauma has not achieved therapeutic success (4). Although the mortality rate of patients with trauma has gradually decreased in the early stages, the mortality rate of patients with post-traumatic complications remain unacceptably high (5). Chest trauma can cause lung impairment, leading to oxygen deficiency, pleural effusion and increased inflammatory response (6). Pneumonia and acute respiratory distress syndrome (ARDS) are common pulmonary complications that are caused by thoracic trauma following prolonged mechanical ventilation (7). The estimated mortality rate of these pulmonary events can reach 24-40% (8). In addition, ~8.3% patients are afflicted with ARDS of unknown causes (9,10). The current recommendation for ARDS treatment is invasive ventilation, but other treatment strategies have been tried with varying degrees of efficacy (11,12). Ventilator-associated pneumonia was previously found to be a risk factor for nosocomial infection, which can lead to ARDS in patients with trauma (12,13). Therefore, early detection of high-risk pneumonia caused by ARDS would assist in guiding the design of treatment protocols for patients (14). The standard detection method for bacterial pathogens in patients with pneumonia is the culture of bacteria from respiratory specimens (15). However, the time of culture required is >3 days (15). In addition, previous studies have been shown that the predictive value of this traditional culture protocol in bronchoalveolar fluid is frequently low (16), especially in patients treated with antibiotics (17). PCR is also a method that can be used for detecting microbial pathogens, but the detection pool of the types of strains is limited to the finite number of targeted regions on the genome sequence (18). This is especially the case when detecting target genes that have mutated, meaning that the existing pathogen-specific PCR can no longer detect this target gene, leading to false negative results (19). Metagenome next-generation sequencing (mNGS) is a technique that involves the whole genome sequencing of the pathogen. This method breaks the genomic DNA into small DNA fragments, which are then amplified and measured (20). Furthermore, mNGS is a powerful method for detecting pathogens, which can rapidly detect all forms of microbial

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nucleic acids from various types of biological samples in one test, including blood, respiratory tract fluid and cerebrospinal fluid (21). Previous studies indicate that mNGS is effective for diagnosing of pneumonia and has been successfully used for the rapid identification of early pathogens that can cause severe pneumonia (16,22-24). Since infection-induced pneumonia is a leading cause of ARDS (25), mNGS has potential diagnostic value for ARDS caused by unknown pathogen infections. In the present report, samples were isolated from a patient with ARDS, which were then analyzed using mNGS to identify the pathogen causing this particular multidrug-resistant microbial pulmonary infection. This provided valuable evidence of the applicability of mNGS for early pathogen identification in the setting of patients with severe pneumonia caused by ARDS. In addition, mNGS may benefit patients with ARDS of unknown etiology in clinical practice.

Case report

History of the present illness and treatments. A 58-year-old male was admitted to Union Jiangbei hospital with multiple injuries following a road traffic collision, including a penetrative head injury (right frontal bone fracture, scalp laceration, facial injury and nasal skin avulsion), neck trauma, blunt thoracic trauma (multiple rib fractures on the right side and lung contusion) and right thigh lacerations. Following neurosurgery, he was transferred to the intensive care unit (ICU) on October 5, 2021. He was suspected of being afflicted with traumatic wet lung, pneumonia and persistent hypoxemia.

On the first day of ICU, the patient had a blood pressure of 132/64 mmHg, body temperature of 36.9°C, heart rate of 81 bpm and transient hypoxemia with 35% oxygen saturation. He immediately received oxygen which increased the oxygen saturation to 98% (Fig. 1; Table I). The lung breathing sounds were coarse but without rales. Blood test results revealed a white blood cell count of $15.06 \times 10^9/l$, a neutrophil ratio of 83.1%, a C-reactive protein (CRP) levels <5 mg/l, procalcitonin (PCT) levels of 0.05 ng/ml (Fig. 1; Table I).

Chest computerized tomography (CT) revealed increased interstitial marking in both lungs on day 1. Areas of ground-glass opacity were also observed in the middle and upper sections of the right lung in addition to the lower lobes of the bilateral lungs. There were small quantities of fluid in the pleural cavities of the bilateral lungs. In addition, the attenuation of the anterior superior mediastinum was not homogeneous. There were signs of air accumulation, where an iso-density strip was seen in this area. A right lung contusion and pleural effusion at the lower right lung bilateral cavities were observed (Fig. 2A). On day 2, the amount of pleural fluid in the lungs decreased compared with that on day 1 according to the CT scan images (Fig. 2B).

On day 1 of ICU admission, the patient received supportive care, such as mechanical ventilation, symptom management, circulatory and nutrition support. He was also administered piperacillin sodium and sulbactam sodium intravenously (4.5 g every 8 h). On day 2, CRP measured 176 ng/l, PCT measured 0.18 ng/ml, white blood cell ($8.63 \times 10^9/l$) was lower compared with that on day 1. However, the temperature ~39.1°C was higher compared with that on day 1. Moist rales could be heard in the breathing sounds using a stethoscope when the

patient inhaled and exhaled. During routine clinical practice, the blood cultures all returned negative results. However, CT scan images showed bilateral pulmonary infections and a small amount of pleural fluid buildup in both lungs on day 2 (Fig. 2B). On day 3, the body temperature remained at 39°C, and the peripheral blood (5 ml), urine (10 ml) and bronchoalveolar fluid (3 ml) samples were all subsequently collected for mNGS detection and bacterial culture. The samples were transferred to Shanghai Topgen Biomedical Technology Co., Ltd. for pathogenic microorganism detection using mNGS at 8 a.m. of day 3. On day 4, a positive result according to mNGS was confirmed in the bronchoalveolar fluid and the urine, whereas a negative result was reported by mNGS in the blood samples. There was the presence of *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae*, *Streptomonas maltophilia* in the bronchoalveolar fluid. By contrast, the presence of John Cunningham polyoma virus and *Ureaplasma urealyticum* were detected in the urine (Tables II and SI). Genes associated with multidrug resistance, including *MEX*, *Omp*, *OxA*, *abe* and *Emr*, were found, which were resistant to Penicillenes, Penicillins, Fluoroquinolones, Aminoglycosides, Aminocoumarin, Carbapenems, Sulfonamides, Tetracyclines, Diaminopyrimidine, Monocyclic lactams, Triclosan and Cephalosporins (Table SII).

Therefore, based on the positive results of mNGS, an adjustment was made to the antibiotic therapy regimen, such that the patient received polymyxin B (500,000 U/12 h), azithromycin (0.5 g/24 h) and ganciclovir (0.25 g/12 h). In total, 5 days after admission into the ICU, the body temperature was decreased to 37.9°C. However, ventilator support continued until day 8 and the temperature was effectively controlled on day 13 (Fig. 1). On day 15, gas accumulation and fluid accumulation in the lungs were markedly decreased, the inflammation indicators decreased significantly, the body temperature was controlled back into the normal range and the patient was transferred out of the ICU (Figs. 1 and 2C; Table I).

Ethics statement. The present study was reviewed and approved by the Union Jiangbei hospital, Huazhong University of Science and Technology (approval no. 2021-09-15; Wuhan, China). Written informed consent was obtained from the patient and all procedures were conducted in accordance with the Declaration of Helsinki. Shanghai Topgen Biomedical Technology Co., Ltd. had a College of American Pathologists certificate (approval no. 8561525-01) for assessing human samples using NGS genetic testing.

mNGS. Plasma (1 ml), urine (1 ml) and bronchoalveolar fluid (600 μ l) samples were mixed with proteinase kinase enzyme (cat. no. DP316; Tiangen Biotech, Co., Ltd.) and glass beads (0.5 mm diameter; zirconia/silica cat. no. 11079105z; Thistle Scientific), before being vortexed at 3,000 rpm for 30 min at 4°C. TIANamp Micro DNA kit (cat. no. DP316; Tiangen Biotech, Co., Ltd.) was used for extracting the total DNA. The DNA extraction and library construction were performed using an NGS automatic DNA library system (cat. no. MAR002; MatriDx Biotech Corp.) and a total DNA library preparation kit (cat. no. MD001T; MatriDx Biotech Corp.). Libraries were then quantified by quantitative PCR using (KAPA Library Quantification

Table I. The time course of oxygen saturation, C-Reactive Protein (CRP), procalcitonin (PCT), blood pressure and the arterial blood gas parameters.

Indexes ^a	Unit	Day 1	Day 2	Day 8	Day 9	Day 10	Day 11	Day 13	Day 14	Day 15
Oxygen saturation	%	98%	98%	100%	100%	100%	100%	100%	100%	100%
CRP (0-10)	mg/l	4	3	4.6	1.9	2.2	3.2	4.3	5.5	6.3
PCT (0-0.5)	ng/l	<0.05	0.18	0.44	0.51	0.10	0.58	<0.05	<0.05	<0.05
Blood pressure	mmHg	132/64	130/68	125/65	128/65	128/65	129/64	129/64	129/64	129/64
pH (7.35-7.45)		7.31	7.46	7.42	7.45	7.45	7.44	7.45	7.44	7.46
PO ₂ (80-100)	mmHg	141.17	101.34	123.91	80.17	131.97	128.1	103.83	128.1	200.34
PCO ₂ (35-45)	mmHg	48.64	34.72	38.85	37.1	41.76	46.19	47.45	46.19	38.23
Total hemoglobin (11-17.4)	g/dl	10.81	7.82	8.24	7.85	8.02	8.58	9.29	8.58	9.61
K (3.2-4.5)	mmol/l	3.92	3.21	3.5	3.54	3.57	4.26	4.02	4.26	3.48
Na (135-148)	mmol/l	137.38	139.85	141.81	154.2	148.35	148.21	147.15	148.21	143.71
Cl (97-107)	mmol/l	110.48	111.71	113.96	115.93	114.52	114.19	111.68	114.19	110.8
Ca (1.12-1.42)	mmol/l	1.06	1	1.06	1.02	0.97	1.07	1.03	1.07	1.04
Hematocrit (35-55)	%	30.7	22.61	22.6	20.85	21.13	23.72	26.59	32.72	28.28
Lactic acid (1.1-7)	mmol/l	3.15	1.71	1.35	1.42	1.05	1.17	1.1	1.17	1.53
Concentration HCO ₃ (22-27)	mmol/l	24	24.3	24.7	24.9	28.4	30.9	32.5	39.9	26.5
Concentration HCO ₃ standard (45-54)	mmol/l	22.1	24.9	24.6	25.1	27.7	29.4	30.8	29.4	26.5
Buffered base (-3-3)	mmol/l	43.8	45.5	45.5	45.9	49.1	51.5	53.3	51.5	48.3
Base excess (-3-3)	mmol/l	-2.46	0.64	0.29	0.87	4.06	6.16	7.72	6.16	2.61
BE _{ecf} (-3-3)	mmol/l	-2.28	0.54	0.26	0.85	4.42	6.83	8.61	6.83	2.7
BE _{act} (-3-3)	mmol/l	-2.3	1.29	0.83	1.47	4.68	6.75	8.32	6.75	3.26
Anion gap (12-20)	mmol/l	6.8	7	6.6	7.9	9	7.04	7.6	7.4	9.9
Concentration H ⁺		48.9	34.4	37.9	35.9	35.4	36	35.2	36	34.7
Osmolality (270-300)	mOs	278	282	289	296	297	299	297	299	290
pH standard (7.35-7.45)		7.37	7.42	7.41	7.42	7.47	7.49	7.49	7.49	7.45
Normalized Ca ²⁺ (1.15-1.33)	mmol/l	1.01	1.03	1.07	1.05	1	1.1	1.06	1.1	1.07
Fraction of inspired oxygen (0.21-1)	%	0.4	9.4	0.4	0.7	0.4	0.35	0.35	0.35	0.5
Concentration O ₂ (19-21)	ml/dL	15.6	10.89	11.57	10.87	11.32	12.4	12.85	12.04	13.74
Concentration CO ₂ (22-28)	mmol/l	25.5	25.4	25.9	26.1	29.7	32.3	34	32.3	27.7
Respiratory index (10-37)	%	62	143	95	473	81	54	90	54	57
PF index (PaO ₂ /FiO ₂ ratio) (400-500)	mmHg	352.93	253	309.77	114.53	329.94	365.73	296.65	365.73	400.68
Partial arterial O ₂	mmHg	229	246	241.6	459.1	238	197.6	197.4	197.6	315.4
Alveolar arterial O ₂ (75-100)	%	61.5	41.2	51.3	17.5	55.4	64.8	52.6	64.8	63.5
Qs/Qt (3-5)	%	5.99	9.44	7.16	19.93	6.56	4.07	7.28	4.7	6.57
p50 (25-29)	mmHg	19.4	18.4	15.1	15.1	16.3	0	19.6	0	7.6
MetHb (0-1.5)	%	0.8	0.68	0.66	0.84	0.58	0.28	0.65	0.28	0.71
CoHb (0-3)	%	1.48	1.29	1.5	0.98	1.39	2.12	1.55	2.12	1.14
HHb (0-2.9)	%	0.33	0.75	0.24	0.83	0.24	0	0.84	0	0.01
O ₂ Hb (94-98)	%	97.38	97.28	97.6	97.35	97.78	97.6	96.97	97.6	98.14
FO ₂ Hb (90-95)	%	0.97	0.97	0.98	0.97	0.98	0.89	0.97	0.98	0.98
MCHC (320-360)	g/l	35.2	34.6	36.4	37.6	38	36.2	34.9	36.2	34

^aValues in brackets are the normal ranges. CRP, C-reactive protein; PCT, procalcitonin; Qs/Qt, pulmonary shunt fraction; MCHC, mean corpuscular hemoglobin concentration; BE-ecf, base excess of the extracellular fluid; BE-act, actual base excess.

Kits (cat. no. KK4828-07960166001; Kapa Biosystems). All reactions were detected under the following conditions: Pre-denaturation at 95°C for 3 min, followed by 40 cycles

of denaturation at 95°C for 10 sec and extension/annealing at 60°C for 1 min. Subsequently, the samples were pooled and sequenced on an Illumina Next Seq 500 platform using

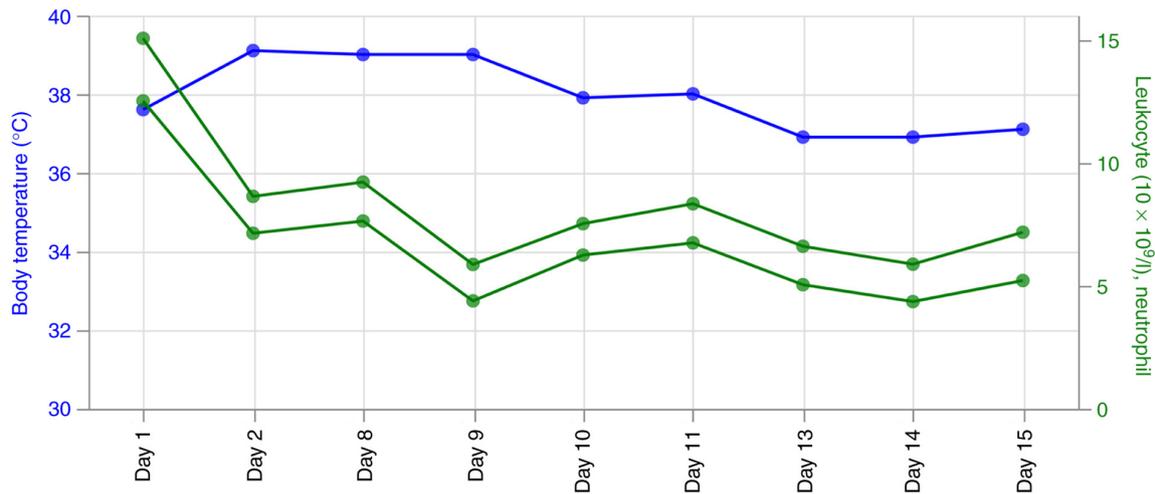


Figure 1. Time course of body temperature, leukocyte and neutrophil counts. The upper green line and the lower green line show the leukocyte counts and neutrophil counts in the peripheral blood, respectively. The blue line on the top shows the body temperature values.

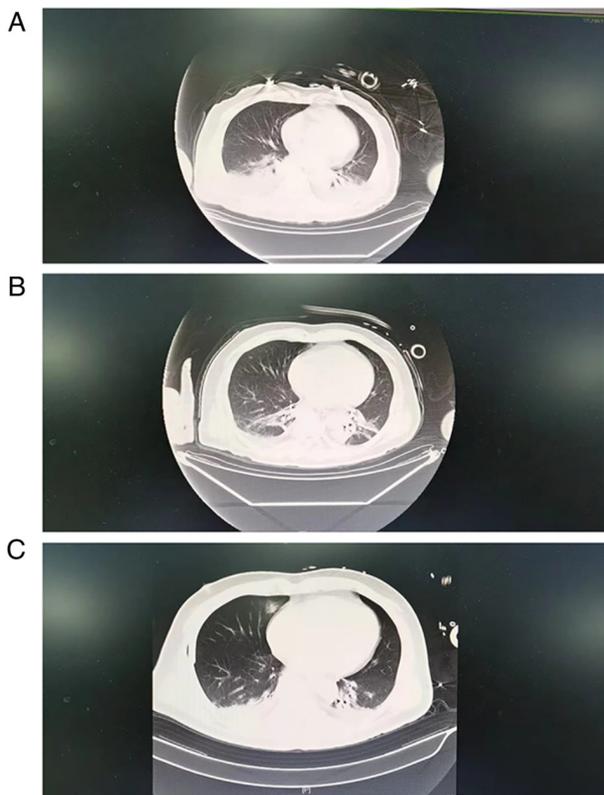


Figure 2. CT scan images of the patient. CT scan images on (A) day 1, (B) day 2 and (C) day 15 of intensive care unit. CT, computed tomography.

a 75-cycle sequencing kit (cat. no. 20024906; Illumina, Inc.). The library concentration had to pass the quality control cut-off (>50 pmol l^{-1}). A total of 10–20 million 50 bp single-end reads were obtained for each library.

All raw reads with high-quality data were obtained from the machine and low quality and low-complexity reads were removed. Next, the clean reads were mapped onto the UCSC human hg 19 reference database and excluded. The remaining reads were then aligned with the current bacterial, virus, fungal and parasite databases (NCBI; <http://ftp.ncbi.nlm.nih.gov/genomes>). Data were

then classified and arranged by the microbial RefSeq database with bowtie2 (26) and BLAST (version 2.9.0+; <https://blast.ncbi.nlm.nih.gov>) was used to verify candidate reads. The database used in this study included 1,428 bacterial species, 1,130 viral species, 73 fungal species and 48 parasite species related to human diseases. For each sequencing run, a negative control [culture medium containing 10^4 Jurkat cells/ml (cat. no. TIB-152; American Type Culture Collection)] was included.

Discussion

Mortality as a result of chest trauma accounts for 20–25% of all types of trauma-related deaths, ranks it third in the leading causes of death in patients with multiple injuries (27). Occurrence of pneumonia following chest trauma in patients with severe injury range from 13.2 to 45% (3). Thoracic trauma is a risk factor for the development of ARDS and earlier onset of ARDS in patients with multiple injuries (28). In addition, ARDS is associated with pneumonia in patients following thoracic trauma caused by severe illness (29).

The present report described a patient with thoracic trauma, who underwent neurosurgery. Afterwards, the patient was transferred to the ICU on October 5, 2021 due to suspected traumatic wet lung infections and persistent low blood oxygen concentration. Greater of injury severity is associated with the higher risk of infections (30). A previous study of 5,500 patients following trauma revealed that the injury severity score in the infected group was significantly higher compared with that of the non-infected group (31). In addition, the risk factors of hospital-acquired infections have been associated with longer duration of mechanical ventilation and hospitalization time (13). Although mNGS is regularly used in clinical practice, its role in pneumonia caused by ARDS of unknown causes remains unclear. Determining the cause would be useful for designating the appropriate treatment strategy and for improving the outcomes of patients with ARDS (10). For patients with ARDS, early determination of the pathogen is an important step for impeding the spread of infection (8). Culturing of bacteria in the respiratory samples is recognized as the gold standard for the diagnosis of infectious agents in

Table II. Results of metagenome next-generation sequencing.

Sample	Genus name	Sequence reads	Relative abundance (%)	Species name	Sequence reads	Relative abundance (%)
Bronchoalveolar fluid	<i>Pseudomonas</i>	58766	21.15	<i>Pseudomonas aeruginosa</i>	26021	9.37
	<i>Acinetobacter</i>	21008	7.56	<i>Acinetobacter baumannii</i>	13941	5.02
	<i>Klebsiella</i>	1834	0.66	<i>Klebsiella pneumoniae</i>	1321	0.48
	<i>Stenotrophomonas</i>	776	0.28	<i>Stenotrophomonas maltophilia</i>	731	0.26
	<i>Betapolyoma virus</i>	86875	85.2	<i>Human polyomavirus 2</i>	86692	84.84
Urine	<i>Ureaplasma</i>	1710	1.67	<i>Ureaplasma urealyticum</i>	1554	1.52
Blood	Negative					

Table III. Previous successful applications of metagenome next-generation sequencing for optimizing the treatment strategy of patients.

Author, year	mNGS result	Treatment	Changes in treatment strategies	(Refs.)
Yan <i>et al</i> , 2022	Pseudorabies virus	Antibiotic treatment for upper respiratory tract infection	Phosphonoformate combined with Acyclovir	(32)
Liu <i>et al</i> , 2021	<i>Mucormycosis</i>	Antibiotics	Amphotericin B liposomes	(33)
Wang <i>et al</i> , 2020	<i>Ureaplasma urealyticum</i>	Intravenous oxacillin (50 mg/kg/dose, q8h) and sulperazon	Vancomycin and meropenem	(34)
Zhang <i>et al</i> , 2021	Adenovirus type 7	Cephalosporin, oseltamivir and moxifloxacin	Arbidol	(35)
Wang <i>et al</i> , 2021	<i>Chlamydia psittaci</i>	Antibiotics	Meropenem and ganciclovir	(36)
Wu <i>et al</i> , 2021	<i>Gardnerella vaginalis</i>	Ceftazidime, levofloxacin	Ornidazole	(37)
Zhang <i>et al</i> , 2020	<i>Fulminant psittacosis</i>	Imipenem/cilastatin, combined with linezolid and oseltamivir	Doxycycline in combination with ceftazidime	(38)
Zhan <i>et al</i> , 2022	<i>A. flavus/A. oryzae</i> and <i>Epstein-Barr virus</i>	Antibiotics and antivirals	Cefoperazone sodium and tazobactam sodium, ganciclovir and voriconazole	(39)
Duan <i>et al</i> , 2022	<i>Ureaplasma parvum</i>		Erythromycin and ciprofloxacin	(40)
Zhang <i>et al</i> , 2021	<i>Enterococcus faecium</i> , <i>Enterococcus hirae</i> , <i>Pseudomonas aeruginosa</i> , <i>Pseudomonas denitrificans</i> and <i>Candida albicans</i>	Cefodizime and fluconazole	Linezolid, meropenem and fluconazole	(41)

patients with pneumonia (42). However, the predictive power of this type of bacterial culture technique is low, ranging from 40-60%. In addition, the culture duration requires >3 days (43).

A previous study has reported that the detection rate of bacteria using mNGS is 96.4%, which is higher compared with that of traditional culture (40.7%) (44). In the present report, the negative results shown by bacterial culture in the bronchoalveolar fluid, blood and urine samples were obtained after the mNGS results.

Gram-negative bacterial pathogens account for >30% of all nosocomial infections and are responsible for 47% of all cases of multidrug resistance in ventilator-associated pneumonia infections (45). A previous analysis of 296 patients following trauma with nosocomial infections in different parts of the body isolated 432 strains of bacteria, of which gram-negative bacteria accounted

for 62.90% whereas gram-positive bacteria only accounted for 37.0% (46). By contrast, no fungal infections could be detected. The most common of negative bacteria were *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Escherichia coli*, *Klebsiella* and *Enterobacter*. In the present report, *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Streptomonas maltophilia* were found to be infectious factors in patients with ARDS caused by pneumonia.

The rapid identification of pathogenic bacteria and their susceptibility to antibiotics are important steps for the treatment of patients with ARDS caused by pneumonia. This would reduce ICU hospitalization time, mechanical ventilation time and ICU hospitalization fees. Supporting this, previous studies found that the NGS findings were helpful for optimizing treatment strategy for patients (Table III). To conclude, findings

from the present report suggested that mNGS was a useful method for the early identification of pathogens that may cause ARDS. However, further studies are required to identify the complementary role of mNGS to conventional microbiological methods in routine clinical practice.

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Availability of data and materials

The raw data of mNGS can be accessed via accession number PRJNA836298 in SRA of NCBI (<https://www.ncbi.nlm.nih.gov/sra>).

Authors' contributions

RW was responsible for the conceptualization of the present study and writing the manuscript. RF, CX, FR, PL and JG acquired the majority of the data, analyzed the data, performed literature research and prepared the original draft. RW and JG confirmed the authenticity of all the raw data. JG was responsible for editing and performing critical review of the manuscript. All authors read and approved the final manuscript.

Ethics approval and consent to participate

The study involving a human participant was reviewed and approved by the Union Jiangbei Hospital, Huazhong University of Science and Technology (approval no. 2021-09-15; Wuhan, China). Written informed consent was obtained from the patient and all procedures were conducted in accordance with the Declaration of Helsinki. Shanghai Topgen Biomedical Technology Co., Ltd. had a College of American Pathologists certificate (approval no. 8561525-01) for assessing human samples using NGS genetic testing.

Patient consent for publication

The patient provided consent for publication.

Competing interests

The authors declare that they have no competing interests.

References

- Baru A, Azazh A and Beza L: Injury severity levels and associated factors among road traffic collision victims referred to emergency departments of selected public hospitals in Addis Ababa, Ethiopia: The study based on the Haddon matrix. *BMC Emerg Med* 19: 2, 2019.
- Gopalakrishnan S: A public health perspective of road traffic accidents. *J Family Med Prim Care* 1: 144-150, 2012.
- Wutzler S, Bläsius FM, Störmann P, Lustenberger T, Frink M, Maegele M, Weuster M, Bayer J, Caspers M, Seekamp A, *et al*: Pneumonia in severely injured patients with thoracic trauma: Results of a retrospective observational multi-centre study. *Scand J Trauma Resusc Emerg Med* 27: 31, 2019.
- Markowitz S and Fanselow M: Exposure Therapy for Post-Traumatic Stress Disorder: Factors of Limited Success and Possible Alternative Treatment. *Brain sci* 10: 167, 2020.
- Chhabra HS, Sharawat R and Vishwakarma G: In-hospital mortality in people with complete acute traumatic spinal cord injury at a tertiary care center in India—a retrospective analysis. *Spinal Cord* 60: 210-215, 2022.
- Rendeki S and Molnár TF: Pulmonary contusion. *J Thorac Dis* 11 (Suppl 2): S141-S151, 2019.
- Bakowitz M, Bruns B and McCunn M: Acute lung injury and the acute respiratory distress syndrome in the injured patient. *Scand J Trauma Resusc Emerg Med* 20: 54, 2012.
- Matthay MA, Zemans RL, Zimmerman GA, Arabi YM, Beitler JR, Mercat A, Herridge M, Randolph AG and Calfee CS: Acute respiratory distress syndrome. *Nat Rev Dis Primers* 5: 18, 2019.
- Wu C, Chen X, Cai Y, Xia J, Zhou X, Xu S, Huang H, Zhang L, Zhou X, Du C, *et al*: Risk factors associated with acute respiratory distress syndrome and death in patients with coronavirus disease 2019 pneumonia in Wuhan, China. *JAMA Intern Med* 180: 934-943, 2020.
- de Prost N, Pham T, Carreaux G, Mekontso Dessap A, Brun-Buisson C, Fan E, Bellani G, Laffey J, Mercat A, Brochard L, *et al*: Etiologies, diagnostic work-up and outcomes of acute respiratory distress syndrome with no common risk factor: A prospective multicenter study. *Ann Intensive Care* 7: 69, 2017.
- Fernando SM, Ferreyro BL, Urner M, Munshi L and Fan E: Diagnosis and management of acute respiratory distress syndrome. *CMAJ* 193: E761-E768, 2021.
- Beitler JR, Schoenfeld DA and Thompson BT: Preventing ARDS: Progress, promise, and pitfalls. *Chest* 146: 1102-1113, 2014.
- Wu D, Wu C, Zhang S and Zhong Y: Risk factors of ventilator-associated pneumonia in critically ill patients. *Front Pharmacol* 10: 482, 2019.
- Luo J, Yu H, Hu YH, Liu D, Wang YW, Wang MY, Liang BM and Liang ZA: Early identification of patients at risk for acute respiratory distress syndrome among severe pneumonia: A retrospective cohort study. *J Thorac Dis* 9: 3979-3995, 2017.
- Gadsby NJ, Russell CD, McHugh MP, Mark H, Conway Morris A, Laurenson IF, Hill AT and Templeton KE: Comprehensive molecular testing for respiratory pathogens in community-acquired pneumonia. *Clin Infect Dis* 62: 817-823, 2016.
- Teng XQ, Gong WC, Qi TT, Li GH, Qu Q, Lu Q and Qu J: Clinical analysis of metagenomic next-generation sequencing confirmed chlamydia psittaci pneumonia: A case series and literature review. *Infect Drug Resist* 14: 1481-1492, 2021.
- Chen X, Ding S, Lei C, Qin J, Guo T, Yang D, Yang M, Qing J, He W, Song M, *et al*: Blood and bronchoalveolar lavage fluid metagenomic next-generation sequencing in pneumonia. *Can J Infect Dis Med Microbiol* 2020: 6839103, 2020.
- Ho CC, Wu AK, Tse CW, Yuen KY, Lau SK and Woo PC: Automated pangenomic analysis in target selection for PCR detection and identification of bacteria by use of ssGeneFinder Webserver and its application to *Salmonella enterica* serovar Typhi. *J Clin Microbiol* 50: 1905-1911, 2012.
- Wang CX, Huang Z, Fang X, Li W, Yang B and Zhang W: Comparison of broad-range polymerase chain reaction and metagenomic next-generation sequencing for the diagnosis of prosthetic joint infection. *Int J Infect Dis* 95: 8-12, 2020.
- Huang J, Jiang E, Yang D, Wei J, Zhao M, Feng J and Cao J: Metagenomic next-generation sequencing versus traditional pathogen detection in the diagnosis of peripheral pulmonary infectious lesions. *Infect Drug Resist* 13: 567-576, 2020.
- Zheng Y, Qiu X, Wang T and Zhang J: The diagnostic value of metagenomic next-generation sequencing in lower respiratory tract infection. *Front Cell Infect Microbiol* 11: 694756, 2021.
- Wu HH, Feng LF and Fang SY: Application of metagenomic next-generation sequencing in the diagnosis of severe pneumonia caused by *Chlamydia psittaci*. *BMC Pulm Med* 21: 300, 2021.
- Li H, Gao H, Meng H, Wang Q, Li S, Chen H, Li Y and Wang H: Detection of pulmonary infectious pathogens from lung biopsy tissues by metagenomic next-generation sequencing. *Front Cell Infect Microbiol* 8: 205, 2018.
- Shi CL, Han P, Tang PJ, Chen MM, Ye ZJ, Wu MY, Shen J, Wu HY, Tan ZQ, Yu X, *et al*: Clinical metagenomic sequencing for diagnosis of pulmonary tuberculosis. *J Infect* 81: 567-574, 2020.

25. Wu Z, Zhang R, Liu D, Liu X, Zhang J, Zhang Z, Chen S, He W, Li Y, Xu Y and Liu X: Acute respiratory distress syndrome caused by human adenovirus in adults: A prospective observational study in Guangdong, China. *Front Med (Lausanne)* 8: 791163, 2022.
26. Langmead B and Salzberg SL: Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9: 357-359, 2012.
27. Beshay M, Mertzluft F, Kottkamp HW, Reymond M, Schmid RA, Branscheid D and Vordemvenne T: Analysis of risk factors in thoracic trauma patients with a comparison of a modern trauma centre: A mono-centre study. *World J Emerg Surg* 15: 45, 2020.
28. Haider T, Halat G, Heinz T, Hajdu S and Negrin LL: Thoracic trauma and acute respiratory distress syndrome in polytraumatized patients: A retrospective analysis. *Minerva Anestesiol* 83: 1026-1033, 2017.
29. Bauer TT, Ewig S, Rodloff AC and Müller EE: Acute respiratory distress syndrome and pneumonia: A comprehensive review of clinical data. *Clin Infect Dis* 43: 748-756, 2006.
30. Komori A, Iriyama H, Kainoh T, Aoki M, Naito T and Abe T: The impact of infection complications after trauma differs according to trauma severity. *Sci Rep* 11: 13803, 2021.
31. Lazarus HM, Fox J, Lloyd JF, Evans RS, Abouzelof R, Taylor C, Pombo DJ, Stevens MH, Mehta R and Burke JP: A six-year descriptive study of hospital-associated infection in trauma patients: Demographics, injury features, and infection patterns. *Surg Infect (Larchmt)* 8: 463-473, 2007.
32. Yan W, Hu Z, Zhang Y, Wu X and Zhang H: Case report: Metagenomic next-generation sequencing for diagnosis of human encephalitis and endophthalmitis caused by pseudorabies virus. *Front Med (Lausanne)* 8: 753988, 2022.
33. Liu Y, Zhang J, Han B, Du L, Shi Z, Wang C, Xu M and Luo Y: Case report: Diagnostic value of metagenomics next generation sequencing in intracranial infection caused by mucor. *Front Med (Lausanne)* 8: 682758, 2021.
34. Wang Q, Wang K, Zhang Y, Lu C, Yan Y, Huang X, Zhou J, Chen L and Wang D: Neonatal *Ureaplasma parvum* meningitis: A case report and literature review. *Transl Pediatr* 9: 174-179, 2020.
35. Zhang XJ, Zheng JY, Li X, Liang YJ and Zhang ZD: Usefulness of metagenomic next-generation sequencing in adenovirus 7-induced acute respiratory distress syndrome: A case report. *World J Clin Cases* 9: 6067-6072, 2021.
36. Wang L, Shi Z, Chen W, Du X and Zhan L: Extracorporeal membrane oxygenation in severe acute respiratory distress syndrome caused by chlamydia psittaci: A case report and review of the literature. *Front Med (Lausanne)* 8: 731047, 2021.
37. Wu S, Hu W, Xiao W, Li Y, Huang Y and Zhang X: Metagenomic next-generation sequencing assists in the diagnosis of *Gardnerella vaginalis* in males with pleural effusion and lung infection: A case report and literature review. *Infect Drug Resist* 14: 5253-5259, 2021.
38. Zhang H, Zhan D, Chen D, Huang W, Yu M, Li Q, Marcos PJ, Tattevin P, Wu D and Wang L: Next-generation sequencing diagnosis of severe pneumonia from fulminant psittacosis with multiple organ failure: A case report and literature review. *Ann Transl Med* 8: 401, 2020.
39. Zhan L, Huang K, Xia W, Chen J, Wang L, Lu J, Wang J, Lin J and Wu W: The diagnosis of severe fever with thrombocytopenia syndrome using metagenomic next-generation sequencing: Case report and literature review. *Infect Drug Resist* 15: 83-89, 2022.
40. Duan J, Zhang C, Wang J, Fu J, Song P, Pang F, Zhao Q and You Z: Diagnosis of *Ureaplasma parvum* meningitis by mNGS in an extremely low birth weight infant with multi-system lesions. *Indian J Med Microbiol*: May 24, 2022 (Epub ahead of print).
41. Zhang M, Wang W, Li X, Zhang X and Yang D: Fast and precise pathogen detection and identification of overlapping infection in patients with CUTI based on metagenomic next-generation sequencing: A case report. *Medicine (Baltimore)* 100: e27902, 2021.
42. Kolenda C, Ranc AG, Boisset S, Caspar Y, Carricajo A, Souche A, Dauwalder O, Verhoeven PO, Vandenesch F and Laurent F: Assessment of respiratory bacterial coinfections among severe acute respiratory syndrome coronavirus 2-positive patients hospitalized in intensive care units using conventional culture and BioFire, FilmArray pneumonia panel plus assay. *Open Forum Infect Dis* 7: ofaa484, 2020.
43. Giuliano C, Patel CR and Kale-Pradhan PB: A guide to bacterial culture identification and results interpretation. *P T* 44: 192-200, 2019.
44. Su SS, Chen XB, Zhou LP, Lin PC, Chen JJ, Chen CS, Wu Q, Ye JR and Li YP: Diagnostic performance of the metagenomic next-generation sequencing in lung biopsy tissues in patients suspected of having a local pulmonary infection. *BMC Pulm Med* 22: 112, 2022.
45. Peleg AY and Hooper DC: Hospital-acquired infections due to gram-negative bacteria. *N Engl J Med* 362: 1804-1813, 2010.
46. Luyt CE, Hekimian G, Koulenti D and Chastre J: Microbial cause of ICU-acquired pneumonia: Hospital-acquired pneumonia versus ventilator-associated pneumonia. *Curr Opin Crit Care* 24: 332-338, 2018.



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