European Journal of Clinical Microbiology & Infectious Diseases
https://doi.org/10.1007/s10096-021-04259-6

ORIGINAL ARTICLE

An evaluation of the Unyvero pneumonia system for rapid detection of microorganisms and resistance markers of lower respiratory infections—a multicenter prospective study on ICU patients

Lingxiao Sun[1,2] · Lijuan Li[1,2] · Sisi Du[1,2] · Yingmei Liu[1,2] · Bin Cao[1,2,3]

Received: 14 February 2021 / Accepted: 19 April 2021
© The Author(s), under exclusive licence to Springer-Verlag GmbH Germany, part of Springer Nature 2021

Abstract

Rapid diagnosis of microorganisms and antibiotic resistance is vital for the appropriate treatment of patients with lower respiratory infections, especially for patients in Intensive Care Unit. We conducted a multicenter prospective study to evaluate the ability of the Unyvero pneumonia system for rapid detection from bronchoalveolar lavage fluid (BALF) in China. Eighty-four patients with lower respiratory infections were enrolled, and their BALF samples were collected, and Unyvero, a rapid molecular diagnostic sample-to-answer solution based on multiple PCRs, was applied to detect 21 types of pathogens and 19 types of resistance markers, compared to a routine bacterial culture method. The overall concordance of Unyvero and routine culture was 69/84 (82.1%). Unyvero detected more microorganisms than routine culture (38.1% vs 27.4%, $P<0.05$) and reported multi-pathogens in more patients than routine culture (10.7% vs 2.4%, $P=0.01$). The overall sensitivity and specificity of Unyvero for bacteria detection were 84.0% and 98.0%. Besides, Unyvero showed a good performance for antibiotic-resistant bacteria, except *Pseudomonas aeruginosa*. The concordance was 87.5–100% for methicillin-resistant *Staphylococcus aureus* and carbapenem-resistant isolates but was only 20–33.3% for *Pseudomonas aeruginosa*. The high-level semi-quantitative signal intensity of microorganisms detected positive by Unyvero correlates well with positive bacterial cultures. For specimens that were exposed to antibiotic treatment, the Unyvero pneumonia system showed a high concordance with routine bacterial culture and performs well for the detection of antibiotic-resistant bacteria, especially, carbapenem-resistant *Klebsiella pneumoniae*. It shows promise in guiding the clinical use of antibiotics, such as ceftazidime/avibactam. However, the system needs improvement in detecting resistance markers of *Pseudomonas aeruginosa.*

Keywords Multiplex PCR · Lower respiratory infections · BALF · Microorganisms · Antibiotic resistance

Introduction

According to the World Health Organization, lower respiratory infections were the world's most deadly communicable disease, ranked the fourth among the top 10 causes of death in 2019 [1]. Despite improved diagnosis and treatment, pneumonia remains a common cause of hospitalization and death [2, 3]. Infections are common in patients in Intensive Care Units, and patients are at increased risk of infection during Intensive Care Unit (ICU) stay [4]. More than 50% of severe patients will receive at least one antibiotic treatment at ICU, and pneumonia is the main cause of antibiotic treatment [5].

Inappropriate antibiotic treatment may increase the risk of death [6]. Reasonable and effective antibiotic treatment depends on the detection of pathogens [7]. Etiological diagnosis of respiratory tract specimens using traditional bacterial culture methods takes 24 to 48 h or longer to obtain results, and determining the antimicrobial susceptibilities of the causative pathogens also takes time [8]. When results are pending, the treatment is empirical, which may cause drug-resistant bacteria [9]. It is important to shorten the time for pathogen detection.

✉ Bin Cao
caobin_ben@163.com

1 National Clinical Research Centre for Respiratory Diseases, China-Japan Friendship Hospital, Capital Medical University, Beijing, China

2 Department of Pulmonary and Critical Care Medicine, Centre for Respiratory Diseases, China-Japan Friendship Hospital, Beijing, China

3 Tsinghua University-Peking University Joint Centre for Life Sciences, Beijing, China

Published online: 01 May 2021

In recent years, the development of multiple PCR technology has speeded up pathogen diagnosis and is used not only in the diagnosis of bloodstream infection and infection after bone and joint replacement [10–12] but also in the diagnosis of pulmonary infection [13–15]. At present, rapid molecular detection methods are mostly for respiratory viruses [16, 17] but rarely for bacteria.

The Unyvero pneumonia diagnostic system is a rapid molecular diagnostic sample-to-answer solution based on multiple PCRs and can simultaneously detect common pathogens of lower respiratory tract infection and drug-resistance genes. Some studies showed that the Unyvero pneumonia diagnostic system could shorten the time of pathogen detection down to about 5 h [13, 18–20], while the cartridge versions of Unyvero used in these studies varied, which include P50, P55, and HPN cartridge.

In this prospective multicenter study, we evaluated the ability of Unyvero pneumonia panels for the rapid molecular diagnosis of the microorganisms and resistance markers in BALF from patients with lower respiratory tract infection, especially after antibiotic treatment. The semi-quantitative signal intensity of microorganisms in Unyvero was also estimated.

Materials and methods

Study design

This is a prospective multicenter study, performed from August 2016 to November 2017, on respiratory ICU patients with lower respiratory tract infection. Seventeen hospitals in 4 provinces in China participated in this study. Bronchoalveolar lavage fluids (BALF) were collected within 2 days after the onset of infection using bronchoscopy and immediately transported to the central laboratory, preserved at 4°C. The Unyvero pneumonia system was applied to BALF to detect microorganisms and resistance markers, and the results were compared with a routine bacterial culture as the gold standard. This study was approved by the Ethics Committee of China-Japan Friendship Hospital (2016-34). Informed consent was signed by all participating patients or their authorized clients.

Patients

Inclusion and exclusion criteria are listed below, and the patient demographic data, clinical symptoms, laboratory examination results, and imaging data were collected.

Inclusion criteria: (1) Age, 18 years old ≤ age ≤80 years old; (2) pneumonia diagnoses were based on the American Thoracic Society and Infectious Disease Society of America's (ATS/IDSA) guidelines [21, 22]; (3) indications for bronchoalveolar lavage examination; and (4) ICU inpatients.

Exclusion criteria: (1) Fever and lung shadows were caused by known noninfectious pulmonary diseases, such as lung tumors, interstitial lung diseases, pulmonary embolism, and other non-infectious lung infiltration; (2) contraindication of bronchial examination; and (3) other conditions in which TB infection has been clearly diagnosed or is highly suspected or in the case that the patient sample was of poor quality.

Routine bacterial culture

All BALF specimens underwent a routine bacterial culture. Isolates were identified by using matrix-assisted laser desorption/ionization mass spectrometry (Bruker Daltonics, Billerica, MA, USA), and antimicrobial susceptibility testing was performed by a VITEK-2 compact system (bioMerieux, Marcy-l'Etoile, France).

Unyvero pneumonia panels

The Unyvero system (the Unyvero P55 or the Unyvero HPN Application) was performed to detect microorganisms and resistance markers. The Unyvero HPN Application is an upgraded version of P55, with the incorporation of *Chlamydophila pneumoniae*. The Unyvero HPN Application detects 21 types of microorganisms (*Staphylococcus aureus*, *Streptococcus pneumoniae*, *Citrobacter freundii*, *Escherichia coli*, *Enterobacter cloacae* complex, *Enterobacter aerogenes*, *Proteus spp.*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Klebsiella variicola*, *Serratia marcescens*, *Morganella morganii*, *Moraxella catarrhalis*, *Pseudomonas aeruginosa*, *Acinetobacter baumannii* complex, *Stenotrophomonas maltophilia*, *Legionella pneumophila*, *Pneumocystis jirovecii*, *Haemophilus influenzae*, *Chlamydia pneumoniae*, *Mycoplasma pneumoniae*) and 19 different resistance markers (*tem*, *shv*, *oxa*-23, *oxa*-24, *oxa*-48, *oxa*-58, *vim*, *imp*, *kpc*, *ndm*, *ctx*-M, *sul*1, *mec*A, *mec*C, *erm*B, *gyr*A83, and *gyr*A87 for *E. coli* and *P. aeruginosa*). The first 16 specimens were detected by the Unyvero P55, and the remaining 68 were detected by the Unyvero HPN Application after an upgrade of the system.

BALF specimens for Unyvero detection were stored at −80°C and thawed in batches for detection. Primarily, 180uL BALF specimens were lysed in the Unyvero Lysator. The cartridge was then fitted with the Unyvero Sample Tube and Master Mix Tube for fully automated subsequent processing in the analyzer. All microorganisms, antibiotic resistance markers, and semi-quantitative microorganism signal intensity detected by Unyvero were recorded. The semi-quantitative signal intensity of microorganisms from Unyvero was compared with bacterial culture results.

Statistical methods

Only the microorganisms listed in the Unyvero pneumonia panels were included in the analysis. Enumeration data were expressed as frequency and percentage. The chi-square test was used to compare the Unyvero pneumonia panels and routine bacterial culture results. Concordance was calculated for Unyvero pneumonia panels and routine bacterial culture. Negative concordance means both Unyvero pneumonia panels and routine bacterial culture were negative, and positive concordance means identical microorganism species with or without additional organism(s) were detected by both tests. Routine bacterial culture was considered as the gold standard, and the true positive, true negative, false positive, and false negative were computed based on it. The *t* test was used to compare the semi-quantitative Unyvero signals between the positive culture and negative culture specimen groups. Statistical analyses were performed using SPSS 21.0 software and GraphPad Prism 9. P <0.05 was considered statistically significant.

Results

Patient characteristics

A total of 87 ICU patients with lower respiratory tract infection were enrolled, 3 patients were excluded based on inclusion criteria of age, and the data of 84 patients were used for the analyses. Fifty-seven patients were (67.9%) males, and the median age of all patients was 62 years old (age range 51–71). Fourteen (16.7%) patients had pneumonia within a year. The first four primary diseases were hypertension (41.7%), diabetes (19.0%), chronic obstructive pulmonary disease (13.1%), and coronary heart disease (11.9%). All patients received antibiotics prior to bronchoscopy (Table 1). Cough was the most common symptom (71.4%), moist rales were audible in 77.4% of patients, and an oxygenation index of < 300mmHg was observed for 81.1% of patients (Table 2).

Table 1 Demographic characteristics and basic diseases

Subject	Cases (%)
Male	57 (67.9)
Age (years, median, quartile)	63 (52–71)
18–64	46 (54.8)
65–74	24 (28.5)
75–79	14 (16.7)
Antibiotic exposure before bronchoscopy	84 (100)
History of pneumonia within 1 year	14 (16.7)
Basic diseases	
Hypertension	35 (41.7)
Diabetes	16 (19.0)
COPD	11 (13.1)
Coronary heart disease	10 (11.9))
Chronic renal disease	7 (8.3)
Malignant tumor (including leukemia, lymphoma)	7 (8.3)
Chronic bronchitis	5 (6.0)
Bronchiectasis	4 (4.8)
Interstitial lung disease	3 (3.6)
Bronchial asthma	2 (2.4)
Smoking	
Smoking at present	29 (34.5)
Smoking in the past	4 (4.8)
Drinking	8 (9.5)

COPD chronic obstructive pulmonary disease

Concordance

All 84 specimens were tested both by routine bacterial culture and Unyvero. For bacteria that could be detected by both methods, 32 cases tested positive for Unyvero, while 23 cases were tested positive by routine bacterial culture (38.1% vs 27.4%, P < 0.05). Overall concordance of Unyvero and routine bacterial culture was 82.1%. Of these, negative results were detected by both tests in 50 (59.5%) patients. Identical microorganism species with or without additional organism(s) were detected by both tests in 19 (22.6%) patients. In 2 cases, bacteria were detected routine culture positive and Unyvero negative. And in 11 cases, bacteria were only detected Unyvero positive and routine culture negative. In addition, for two cases, pathogens were detected by both routine bacterial culture and Unyvero pneumonia cartridges, but the pathogens detected by two methods were different. Besides, microorganisms that were undetectable by routine bacterial culture, like *Legionella pneumophila*, *Pneumocystis jirovecii*, and *Mycoplasma pneumoniae*, were detected positive in 13 cases by Unyvero. *Chlamydia pneumoniae* was not detected by Unyvero HPN Application in all specimens, and the results from Unyvero P55 and Unyvero HPN Application were combined in the analyses.

Pathogen identification

Detection rates of *Acinetobacter baumannii* complex, *Pseudomonas aeruginosa*, *Stenotrophomonas maltophilia*, and *Staphylococcus aureus* by Unyvero were higher than by routine bacterial culture (Table 3). Three cases of *Escherichia coli*, two cases of *Streptococcus pneumoniae*, and one case of *Haemophilus influenza* were detected by Unyvero pneumonia cartridges only. Unyvero pneumonia cartridges detected 2 fewer *Klebsiella pneumoniae* cases than conventional bacterial culture. Neither of the two methods detected *Enterobacter cloacae* complex,

Table 2 Clinical features and laboratory examination within 48 h before bronchoscopy

Subject	Cases (%)
Cough	60 (71.4)
Dyspnea	49 (58.3)
Axillary temperature ≥38°C	32 (38.1)
Disturbance of consciousness	27 (32.1)
Cyanosis	17 (20.2)
Thoracalgia	5 (6.0)
Moist rale	65 (77.4)
Dry rale	13 (15.5)
SBP<90mmHg	1 (1.2)
WBC ($\times 10^9$/L, *n*=82)	
>10.0	39 (47.6)
<4.0	7 (8.5)
4.0–10.0	36 (43.9)
BUN>7.0mmol/L (*n*=70)	29 (41.4)
PH<7.30 (*n*=74)	6 (8.1)
PaO_2/FiO_2<300mmHg (*n*=74)	60 (81.1)
PCT (mg/mL, *n*=78)	
PCT≤0.25	21 (26.9)
0.25<PCT<1	22 (28.2)
1<PCT<2	6 (7.7)
PCT≥2	29 (37.2)

SBP systolic blood pressure, *WBC* white blood cell, *BUN* blood urea nitrogen, *PH* pondus hydrogenii, *PaO2/FiO2* oxygenation index, *PCT* procalcitonin

Enterobacter aerogenes, *Proteus* spp., *Morganella morganii*, *Citrobacter freundii*, *Klebsiella oxytoca*, *Klebsiella variicola*, and *Moraxella catarrhalis* (Table 3). For the pathogens that were undetectable by routine bacterial culture, Unyvero pneumonia cartridges detected *Pneumocystis jirovecii* in 7 cases, *Legionella pneumophila* in 3 cases, *Mycoplasma pneumoniae* in 3 cases, and *Chlamydia pneumoniae* was not detected in any of the cases (Table 3). The sensitivity, specificity, positive predictive value, and negative predictive value of Unyvero for bacteria were calculated (Table 3). The overall sensitivity and specificity of Unyvero for bacteria detection were 84.0% and 98.0%, respectively.

The number of microorganisms detected per specimen varied. The Unyvero pneumonia cartridges reported more than one pathogen in 9 cases, while the routine bacterial culture reported multi-pathogens in only 2 cases (9/84 vs 2/84, *P*=0.01) (Fig. 1).

Drug-resistant bacteria detection

Routine bacterial culture detected drug-resistance bacteria in 17 (20.2%) cases, and bacteria with resistance markers were positive in 21 (25%) cases by Unyvero pneumonia cartridges. Besides, *ermB*, *Pneumocystis jirovecii* with *mecA*, and *Mycoplasma pneumoniae* with *ermB* were detected in 3 cases by Unyvero pneumonia cartridges.

Considering the clinical significance of carbapenem and methicillin resistance, carbapenem-resistant bacteria and methicillin-resistant *Staphylococcus aureus* (MRSA) were tested. Besides, the fluoroquinolone resistance (FQR) among *Escherichia coli* and *Pseudomonas aeruginosa* were also tested. Unfortunately, two cases should be excluded for antibiotic resistance analysis, because the antimicrobial susceptibility testing results of two cultured isolates, including 1 *Pseudomonas aeruginosa* and 1 *Acinetobacter baumannii*, were lost.

MRSA was detected in two cases by routine bacterial culture, and Unyvero detected *Staphylococcus aureus* with *mec*A resistance gene for these two cases. Concordance of Unyvero and routine bacterial culture in MRSA detection was 100%. Besides, methicillin-susceptible *Staphylococcus aureus* (MSSA) was detected in one case by routine bacterial culture, and Unyvero also detected *Staphylococcus aureus* and neither *mec*A nor *mec*C in that case. In addition, another MSSA was reported by Unyvero. Carbapenem-resistant *Pseudomonas aeruginosa* isolates were detected by routine bacterial culture in 4 cases and by Unyvero in 2 cases, with one concordant case (20%). Carbapenem-resistant *Acinetobacter baumannii* isolates were detected by routine bacterial culture in 8 cases and by Unyvero in 7 cases, all of which were concordant (87.5%). Carbapenem-resistant *Klebsiella pneumoniae* were detected by routine bacterial culture and the Unyvero in the same case (100% concordant). And in another case, carbapenem susceptible *Klebsiella pneumoniae* were detected by two methods. Quinolone-resistant *Pseudomonas aeruginosa* isolates were detected by routine bacterial culture in 3 cases, one of which was detected by Unyvero, giving a concordant rate of 33.3% (Table 4). Quinolone-resistant *Escherichia coli* was not detected by either method.

The signal intensity of Unyvero

The signal intensity of microorganisms is reported semi-quantitatively by Unyvero. According to the results of routine bacterial culture, cases with a positive pathogen detection by Unyvero were separated into two groups, the positive culture group and the negative culture group. The signal intensity of microorganisms detected by Unyvero in the positive culture group was higher than that in the negative culture group (*P*=0.013) (Fig. 2).

Table 3 Cases of bacteria detected by Unyvero and routine bacterial culture

Pathogen	Routine bacterial culture	Unyvero	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV
Staphylococcus aureus	3	4	3	80	1	0	100%	98.77%	75%	100%
Klebsiella pneumoniae	4	2	2	80	0	2	50%	100%	100%	97.56%
Pseudomonas aeruginosa	7	9	5	73	4	2	71.43%	94.81%	55.56%	97.33%
Acinetobacter baumannii complex	9	15	9	69	6	0	100%	92%	60 %	100%
Stenotrophomonas maltophilia	2	10	2	74	8	0	100%	90.24%	20%	100%
Escherichia coli	0	3	0	81	3	0	-	96.43%	-	100%
Streptococcus pneumoniae	0	2	0	82	2	0	-	97.62%	-	100%
Haemophilus influenza	0	1	0	83	1	0	-	98.81%	-	100%
Enterobacter cloacae complex	0	0	0	84	0	0	-	100%	-	100%
Enterobacter aerogenes	0	0	0	84	0	0	-	100%	-	100%
Proteus spp.	0	0	0	84	0	0	-	100%	-	100%
Morganella morganii	0	0	0	84	0	0	-	100%	-	100%
Citrobacter freundii	0	0	0	84	0	0	-	100%	-	100%
Klebsiella oxytoca	0	0	0	84	0	0	-	100%	-	100%
Klebsiella variicola	0	0	0	84	0	0	-	100%	-	100%
Moraxella catarrhalis	0	0	0	84	0	0	-	100%	-	100%
Pneumocystis jirovecii	NA	7	NA	NA	NA	NA	NA	NA	NA	NA
Legionella pneumophila	NA	3	NA	NA	NA	NA	NA	NA	NA	NA
Mycoplasma pneumoniae,	NA	3	NA	NA	NA	NA	NA	NA	NA	NA
Chlamydia pneumoniae	NA	0	NA	NA	NA	NA	NA	NA	NA	NA
Overall							84.0 %	98.0%		

TP true positive: cases of bacteria detected by both routine bacterial culture and Unyvero

TN true negative: cases of bacteria not detected by either routine bacterial culture or Unyvero

FP false positive: cases of bacteria detected by Unyvero, but not detected by routine bacterial culture

FN false negative: cases of bacteria detected by routine bacterial culture, but not detected by Unyvero

PPV positive predictive value, *NPV* negative predictive value, *NA* not available

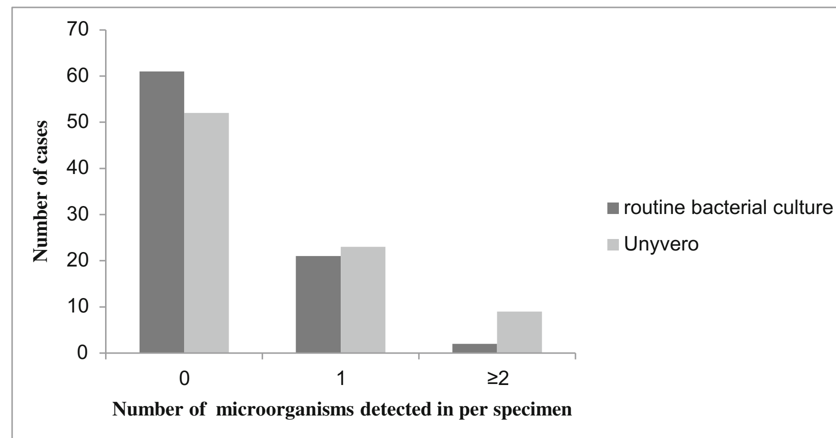

Fig. 1 Detection of multiple bacteria in a single specimen. Routine bacterial culture: *Klebsiella pneumoniae* and *Acinetobacter baumannii* complex were detected in 1 case; *Acinetobacter baumannii* complex and *Pseudomonas aeruginosa* were detected in 1 case. Unyvero: *Acinetobacter baumannii* complex and *Stenotrophomonas maltophilia* were detected in 2 cases; *Acinetobacter baumannii* complex and *Pseudomonas aeruginosa* were detected in 2 cases; *Pseudomonas aeruginosa* and *Stenotrophomonas maltophilia* were detected in 1 case; *Acinetobacter baumannii* complex, *Pseudomonas aeruginosa*, and *Stenotrophomonas maltophilia* were detected in 1 case; *Acinetobacter baumannii* complex, *Stenotrophomonas maltophilia*, and *Staphylococcus aureus* were detected in 1 case; *Acinetobacter baumannii* complex, *Staphylococcus aureus*, and *Streptococcus pneumoniae* were detected in 1 case; *Acinetobacter baumannii* complex, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Escherichia coli* were detected in 1 case

Table 4 The concordance of Unyvero and routine bacterial culture for antibiotic-resistant isolates detection

	MRSA	CRAB	CRKP	CRPA	Quinolone-resistant *Pseudomonas aeruginosa*
Drug-resistant bacteria detected by routine bacterial culture	2	8	1	4	2
Resistance genes detected by Unyvero	2	7	1	2	2
Consistency	2/2	7/8*	1/1	1/5**	1/3***

MRSA methicillin-resistant *Staphylococcus aureus*, *CRAB* carbapenem-resistant *Acinetobacter baumannii*, *CRKP* carbapenem-resistant *Klebsiella pneumoniae*, *CRPA* carbapenem-resistant *Pseudomonas aeruginosa*

* CRAB were detected in 8 and 7 samples by culture and Unyvero pneumonia system. Seven samples were coincident, and culture detected CRAB in another 1 sample. The consistency for CRAB was 7/8

** CRPA were detected in 4 and 2 samples by culture and Unyvero pneumonia system. One sample was coincident, culture detected CRPA in the other 3 samples, and Unyvero detected CRPA in another 1 sample. The consistency for CRAB was 1/5

*** Quinolone-resistant *Pseudomonas aeruginosa* were detected in 2 and 2 samples by culture and Unyvero pneumonia system. One sample was coincident, culture detected Quinolone-resistant *Pseudomonas aeruginosa* in another 1 sample, and Unyvero detected CRPA in another 1 sample. The consistency for Quinolone-resistant *Pseudomonas aeruginosa* was 1/3

Discussion

This study is the first prospective study conducted in China to evaluate the rapid molecular diagnosis of microorganisms and antibiotic resistance markers in BALF sample collected from patients with lower respiratory tracts infections using Unyvero pneumonia system. Despite that antibiotics were used before specimens were obtained, the Unyvero pneumonia system showed an overall concordance of 82.1% with routine bacterial culture and a good prediction for resistant bacteria, except *P. aeruginosa*. The overall sensitivity and specificity of Unyvero pneumonia cartridges for bacteria detection were 84.0% and 98.0%. The high-level bacteria signal of the Unyvero pneumonia cartridges was associated with positive culture results.

The rapid detection of microorganisms and drug resistance is vital for patients in ICUs. Several studies have investigated the performance of the Unyvero for etiological exploration, but results varied with different study designs and versions of Unyvero. P50, the earliest version of the Unyvero system, was used in 5 studies [7, 8, 13, 23, 24], showing the sensitivity of 70.6–95.7%, and specificity of 32.6–97.8%. The Unyvero

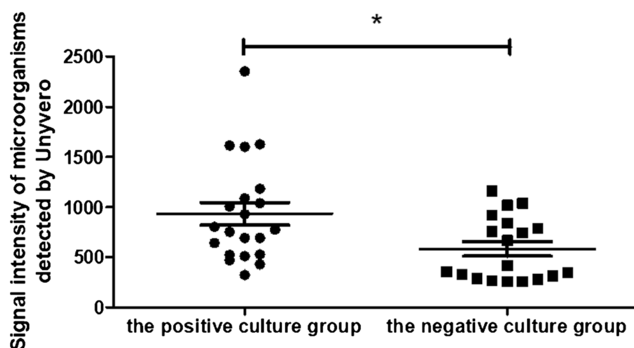

Fig. 2 The signal intensity of microorganisms detected by Unyvero

HPN Application is an upgraded version of P55, with the incorporation of *Chlamydophila pneumoniae* detection. Six studies evaluated P55 and HPN panels [18–20, 25–27] and reported sensitivity of 56.9–97% and specificity of 17.3–99.9%. Since the results of the P55 and HPN cartridge did not differ [20], we analyzed them together in our study. Our results showed that the performance for bacteria detection was similar to the above studies, with the sensitivity and specificity of 84.0% and 98.0%, respectively.

Our study was conducted on specimens from patients who were exposed to antibiotic treatment, which is the most important difference from other studies. In the absence of robust diagnostic tests, most of early treatments are empirical for patients with suspected pneumonia, especially in ICU patients. Though collecting respiratory specimens prior to antibiotic administration could significantly improve the detection rate of bacteria [28], it is difficult to get the BALF samples from patients before the antibiotic treatment in the real world. Driscoll et al. reported that antibiotics were associated with approximately a 20% reduction in yield from induced sputum culture, while there was only a 7% reduction of bacterial detection by PCR [29]. Their result suggested that antibiotic use has less effect on pathogen detection with PCR than with routine bacterial culture. Unyvero is a detection method based on multiple PCRs, which may be the reason why the positive detection rate of Unyvero is higher than that of routine bacterial culture.

The positive predictive value of Unyvero was 52.5 %, and *Stenotrophomonas maltophilia* was detected in 8 more cases by Unyvero. This is due to the fact that the Unyvero detected typically more organisms than culture, and the prior antibiotic treatment may also contribute to low detection of *Stenotrophomonas maltophilia* by culture. The negative predictive value of Unyvero was over 97%, which suggests that the negative results by Unyvero may therefore be used for

early antibiotic de-escalation. We detected 4 false negatives (2 *Klebsiella pneumoniae* and 2 *Pseudomonas aeruginosa*) in our studies. The false negatives were common for Unyvero. Collins et al. [26] detected 6 false negatives among 175 samples. The false negatives were also reported by Personne [24], Ozongwu [18], and Peiffer-Smadja [27]. Especially, Peiffer-Smadja reported 3 false negatives of *K. pneumonia.* One of the advantages of Unyvero is their semi-quantification, with results being positive only when the sample's bacterial burden is sufficiently high [20]. In our study, Unyvero failed to detect the corresponding pathogen for 4 samples: for one sample, the growth level was low (1000 cfu/mL); for the other three samples, the growth levels were significant (> 10,000 cfu/mL). A similar result was reported by Luyt et al., which showed Unyvero failed to detect a pathogen for 11 episodes, despite their significant growth levels (> 10,000 cfu/mL) [20]. The relationship of growth level and Unyvero performance should be further studied.

For specimens exposed to antibiotics, Unyvero showed a good performance for antibiotic-resistant bacteria, except *Pseudomonas aeruginosa*. The resistance mechanism discordance of *Pseudomonas aeruginosa* was also reported by Ozongwu [18] and Luyt[20]. The resistance profile of *Pseudomonas aeruginosa* often changes over time, which may cause difficulty for resistance marker detection. Besides, resistance can be conferred by a wide variety of genes or even other mechanisms like membrane pumps [30] that are not addressed by the Unyvero panel. Previous studies reported that the antibiotic resistance concordance of Unyvero and routine bacterial culture results were 66.6–77.8% [20, 26], and in our study, the concordances for MRSA (2/2), CRAB (6/9), and CRKP (1/1) were relatively high.

Carbapenem-resistant Enterobacterales (CRE), especially carbapenem-resistant *Klebsiella pneumoniae* (CRKP), have emerged as a major public health concern worldwide [31]. Ceftazidime/avibactam (CZA) is a β-lactam/β-lactamase combination antibiotic with activity against CRE that produces *Klebsiella pneumoniae* carbapenemases (KPC). However, CZA is resistant to isolates with Metallo-β-lactamases such as New Delhi Metallo-β-lactamases (NDM) [32]. The Unyvero covers these resistance markers. We detected *K. pneumonia* with *kpc* by Unyvero in one case, and CRKP was cultured from the same specimen, which can indicate CZA treatment. Peiffer-Smadja [27] detected 3 *ndm* out of 3 carbapenemase-producing Enterobacteriaceae by Unyvero, with 100% concordance, which may indicate CZA is not suitable for these cases. Though antimicrobial susceptibility testing of CZA was not tested in these isolates, Unyvero shows potential in early detection for infections caused by CRE and provides guidance for the usage of CZA.

The signal intensity of the microorganisms detected by Unyvero represents the semi-quantitative level of pathogen detected by this method. Papan [7] found that for BALF samples whose signal strength from Unyvero P50 was higher than 1500, there was a certain correlation between the signal strength from Unyvero and the number of detected bacteria in conventional bacterial culture, and the lower the signal strength of Unyvero, the weaker the correlation. In this study, we found that the signal intensity of Unyvero in the positive culture group was higher than that in the negative culture group, which indicates that the high level signal intensity of Unyvero was related to the positive culture of bacteria. The higher the signal intensity of Unyvero, the more reliable the positive results were. Our results may be useful for the interpretation of Unyvero HPN Application results in the future.

One limitation of this study was that all patients had received antibiotic treatment before BALF sample collections, which may have reduced the positivity rate of routine bacterial culture. In addition, results from Unyvero testing had not been communicated to the clinicians in a timely manner, and thus, it was not possible to analyze the impact on clinical treatment and prognosis.

The randomized control trials should be implemented in the future to investigate the effect of Unyvero on medication treatment and the prognosis of patients. Besides, Unyvero shows potential for multi-pathogen detection, so evaluations of patients with immunosuppression should be performed.

In conclusion, as a rapid molecular diagnostic technique, Unyvero detects higher numbers of microorganisms in BALF from ICU patients with a lower respiratory tract infection than routine bacterial culture. On samples with antibiotic exposures, the Unyvero pneumonia system shows an overall concordance of 82.1% with routine bacterial culture, and a good prediction for resistant bacteria, except *Pseudomonas aeruginosa*. High-level signal intensities of Unyvero pneumonia panels correlate well with positive bacterial cultures.

Acknowledgements We thank Binin Li, Chunlei Wang, Zhanwei Wang, Xiaohong Wang, Qingyuan Zhan, Xu Huang, Yadong Yuan, Zheng Duan, Yali Ren, Jinying Shi, Chuan Wang, Jinxiang Wang, Suiyang Zhang, Bo Zhang, Ying Liu, Zuoqing Zhang, Lisi Song, Jiangbo Liu, Zhiyong Wang, Qiuming Deng, Lin Zhang, Ling Jiang, Yan Zhang, Jiguang Meng, Huimin Liu, Shufeng Xu, Qi Tian, Feifei Liu, Rui Tang, and Xuecong Ning for their help.

Funding This study was supported by the National Science Grant for Distinguished Young Scholars (81425001/H0104) (Bin Cao).

Data availability Data is available from the corresponding author on reasonable request.

Declarations

Ethics approval This study was approved by the Ethics Committee of China-Japan Friendship Hospital (2016-34).

Consent to participate Informed consent was signed by all participating patients or their authorized clients.

Consent for publication This manuscript has been approved by each author.

Conflict of interest The Unyvero panel reagents and instruments were provided by Curetis GmbH.

References

1. WHO (2020) The top 10 causes of death. https://www.hoint/news-room/fact-sheets/detail/the-top-10-causes-of-death
2. Buzzo AR, Roberts C, Mollinedo LG, Quevedo JM, Casas GL, Soldevilla JM (2013) Morbidity and mortality of pneumonia in adults in six Latin American countries. Int J Infect Dis 17(9): e673–e677
3. Corrado RE, Lee D, Lucero DE, Varma JK, Vora NM (2017) Burden of adult community-acquired, health-care-associated, hospital-acquired, and ventilator-associated pneumonia: New York City, 2010 to 2014. Chest 152(5):930–942
4. Vincent JLRJ, Marshall J, Silva E, Anzueto A, Martin CD, Moreno R, Lipman J, Gomersall C, Sakr Y, Reinhart K (2009) International study of the prevalence and outcomes of infection in intensive care units. JAMA 302:2323–2329
5. Rello MU J, Lisboa T (2011) Determinants of prescription and choice of empirical therapy for hospital-acquired and ventilator-associated pneumonia. Eur Respir J 37:1332–1339
6. Iregui M, Ward S, Sherman G, Fraser VJ, Kollef MH (2002) Clinical importance of delays in the initiation of appropriate antibiotic treatment for ventilator-associated pneumonia. Chest 122(1): 262–268
7. Papan C, Meyer-Buehn M, Laniado G, Nicolai T, Griese M, Huebner J (2018) Assessment of the multiplex PCR-based assay Unyvero pneumonia application for detection of bacterial pathogens and antibiotic resistance genes in children and neonates. Infection 46(2):189–196
8. Jamal W, Al Roomi E, AbdulAziz LR, Rotimi VO (2014) Evaluation of Curetis Unyvero, a multiplex PCR-based testing system, for rapid detection of bacteria and antibiotic resistance and impact of the assay on management of severe nosocomial pneumonia. J Clin Microbiol 52(7):2487–2492
9. Timsit JF, Bassetti M, Cremer O, Daikos G, de Waele J, Kallil A, Kipnis E, Kollef M, Laupland K, Paiva JA, Rodriguez-Bano J, Ruppe E, Salluh J, Taccone FS, Weiss E, Barbier F (2019) Rationalizing antimicrobial therapy in the ICU: a narrative review. Intensive Care Med 45(2):172–189
10. Tat Trung N, Van Tong H, Lien TT, Van Son T, Thanh Huyen TT, Quyen DT, Hoan PQ, Meyer CG, Song LH (2018) Clinical utility of an optimised multiplex real-time PCR assay for the identification of pathogens causing sepsis in Vietnamese patients. Int J Infect Dis 67:122–128
11. Villa F, Toscano M, De Vecchi E, Bortolin M, Drago L (2017) Reliability of a multiplex PCR system for diagnosis of early and late prosthetic joint infections before and after broth enrichment. Int J Med Microbiol 307(6):363–370
12. Borde JP, Hacker GA, Guschl S, Serr A, Danner T, Hubner J, Burrack-Lange S, Ludke G, Helwig P, Hauschild O, Kern WV (2015) Diagnosis of prosthetic joint infections using UMD-Universal Kit and the automated multiplex-PCR Unyvero i60 ITI((R)) cartridge system: a pilot study. Infection 43(5):551–560
13. Kunze N, Moerer O, Steinmetz N, Schulze MH, Quintel M, Perl T (2015) Point-of-care multiplex PCR promises short turnaround times for microbial testing in hospital-acquired pneumonia–an observational pilot study in critical ill patients. Ann Clin Microbiol Antimicrob 14:33
14. Rogers BB, Shankar P, Jerris RC, Kotzbauer D, Anderson EJ, Watson JR, O'Brien LA, Uwindatwa F, McNamara K, Bost JE (2015) Impact of a rapid respiratory panel test on patient outcomes. Arch Pathol Lab Med 139(5):636–641
15. Semret M, Schiller I, Jardin BA, Frenette C, Loo VG, Papenburg J, McNeil SA, Dendukuri N (2017) Multiplex respiratory virus testing for antimicrobial stewardship: a prospective assessment of antimicrobial use and clinical outcomes among hospitalized adults. J Infect Dis 216(8):936–944
16. Shengchen D, Gu X, Fan G, Sun R, Wang Y, Yu D, Li H, Zhou F, Xiong Z, Lu B, Zhu G, Cao B (2019) Evaluation of a molecular point-of-care testing for viral and atypical pathogens on intravenous antibiotic duration in hospitalized adults with lower respiratory tract infection: a randomized clinical trial. Clin Microbiol Infect 25(11): 1415–1421
17. Chen JHK, Lam HY, Yip CCY, Wong SCY, Chan JFW, Ma ESK, Cheng VCC, Tang BSF, Yuen KY (2016) Clinical Evaluation of the new high-throughput Luminex NxTAG Respiratory Pathogen Panel Assay for multiplex respiratory pathogen detection. J Clin Microbiol 54(7):1820–1825
18. Ozongwu C, Personne Y, Platt G, Jeanes C, Aydin S, Kozato N, Gant V, O'Grady J, Enne VI (2017) The Unyvero P55 'sample-in, answer-out' pneumonia assay: a performance evaluation. Biomol Detect Quantif 13:1–6
19. Gadsby NJ, McHugh MP, Forbes C, MacKenzie L, Hamilton SKD, Griffith DM, Templeton KE (2019) Comparison of Unyvero P55 Pneumonia Cartridge, in-house PCR and culture for the identification of respiratory pathogens and antibiotic resistance in bronchoalveolar lavage fluids in the critical care setting. Eur J Clin Microbiol Infect Dis 38(6):1171–1178
20. Luyt CE, Hekimian G, Bonnet I, Brechot N, Schmidt M, Robert J, Combes A, Aubry A (2020) Usefulness of point-of-care multiplex PCR to rapidly identify pathogens responsible for ventilator-associated pneumonia and their resistance to antibiotics: an observational study. Crit Care 24(1):378
21. Mandell LA, Wunderink RG, Anzueto A, Bartlett JG, Campbell GD, Dean NC, Dowell SF, File TM, Jr., Musher DM, Niederman MS, Torres A, Whitney CG (2007) Infectious Diseases Society of America/American Thoracic Society consensus guidelines on the management of community-acquired pneumonia in adults. Clin Infect Dis 44 Suppl 2:S27–S72
22. American Thoracic Society, Infectious Diseases Society of America (2005) Guidelines for the management of adults with hospital-acquired, ventilator-associated, and healthcare-associated pneumonia. Am J Respir Crit Care Med 171(4):388–416
23. Schulte B, Eickmeyer H, Heininger A, Juretzek S, Karrasch M, Denis O, Roisin S, Pletz MW, Klein M, Barth S, Ludke GH, Thews A, Torres A, Cilloniz C, Straube E, Autenrieth IB, Keller PM (2014) Detection of pneumonia associated pathogens using a prototype multiplexed pneumonia test in hospitalized patients with severe pneumonia. PLoS One 9(11):e110566
24. Personne Y, Ozongwu C, Platt G, Basurto-Lozada P, Shamin M, Gant VA, Zumla A, Enne VI (2016) 'Sample-in, answer-out'? Evaluation and comprehensive analysis of the Unyvero P50 pneumonia assay. Diagn Microbiol Infect Dis 86(1):5–10
25. Franchetti L, Schumann DM, Tamm M, Jahn K, Stolz D (2020) Multiplex bacterial polymerase chain reaction in a cohort of patients with pleural effusion. BMC Infect Dis 20(1):99
26. Collins ME, Popowitch EB, Miller MB (2020) Evaluation of a novel multiplex PCR panel compared to quantitative bacterial culture for diagnosis of lower respiratory tract infections. J Clin Microbiol 58(5)
27. Peiffer-Smadja N, Bouadma L, Mathy V, Allouche K, Patrier J, Reboul M, Montravers P, Timsit JF, Armand-Lefevre L (2020) Performance and impact of a multiplex PCR in ICU patients with

ventilator-associated pneumonia or ventilated hospital-acquired pneumonia. Crit Care 24(1):366
28. Harris AM, Bramley AM, Jain S, Arnold SR, Ampofo K, Self WH, Williams DJ, Anderson EJ, Grijalva CG, McCullers JA, Pavia AT, Wunderink RG, Edwards KM, Winchell JM, Hicks LA (2017) Influence of antibiotics on the detection of bacteria by culture-based and culture-independent diagnostic tests in patients hospitalized with community-acquired pneumonia. Open Forum Infect Dis 4 (1):ofx014
29. Driscoll AJ, Deloria Knoll M, Hammitt LL, Baggett HC, Brooks WA, Feikin DR, Kotloff KL, Levine OS, Madhi SA, O'Brien KL, Scott JAG, Thea DM, Howie SRC, Adrian PV, Ahmed D, DeLuca AN, Ebruke BE, Gitahi C, Higdon MM, Kaewpan A, Karani A, Karron RA, Mazumder R, McLellan J, Moore DP, Mwananyanda L, Park DE, Prosperi C, Rhodes J, Saifullah M, Seidenberg P, Sow SO, Tamboura B, Zeger SL, Murdoch DR, for the PERCH Study Group (2017) The effect of antibiotic exposure and specimen volume on the detection of bacterial pathogens in children with pneumonia. Clin Infect Dis 64 (suppl_3):S368–S377
30. Azam MW, Khan AU (2019) Updates on the pathogenicity status of Pseudomonas aeruginosa. Drug Discov Today 24(1):350–359
31. Bonomo RA, Burd EM, Conly J, Limbago BM, Poirel L, Segre JA, Westblade LF (2018) Carbapenemase-Producing Organisms: A Global Scourge. Clin Infect Dis 66(8):1290–1297
32. Zhanel GG, Lawson CD, Adam H, Schweizer F, Zelenitsky S, Lagace-Wiens PR, Denisuik A, Rubinstein E, Gin AS, Hoban DJ, Lynch JP 3rd, Karlowsky JA (2013) Ceftazidime-avibactam: a novel cephalosporin/beta-lactamase inhibitor combination. Drugs 73(2):159–177

Publisher's note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.