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Usefulness of point-of-care multiplex PCR to rapidly identify pathogens responsible for ventilator-associated pneumonia and their resistance to antibiotics: an observational study

Charles-Edouard Luyt 1,2*, Guillaume Hékimian 1, Isabelle Bonnet 3,4, Nicolas Bréchot 1,2, Matthieu Schmidt 1,2, Jérôme Robert 3,4, Alain Combes 1,2 and Alexandra Aubry 3,4

Abstract

Background: The use of multiplex PCR to shorten time to identification of pathogens and their resistance mechanisms for patients with ventilator-associated pneumonia (VAP) is attractive, but poorly studied. The multiplex PCR–based Unyvero pneumonia cartridge assay can directly identify 20 bacteria and one fungus, amongst the most frequently causing VAP, and 19 of their resistance markers in clinical specimens (bronchoalveolar lavage or tracheal aspirate), with a turnaround time of 4–5 h. We performed this study to evaluate the concordance between the multiplex PCR–based Unyvero pneumonia cartridge assay and conventional microbiological techniques to identify pathogens and their resistance mechanisms in patients with VAP.

Methods: All patients suspected of having VAP (January 2016 to January 2019), who underwent fiberoptic bronchoscopy with bronchoalveolar lavage fluid (BALF) and whose BALF microscopy examination revealed intracellular bacteria, were included. BALF conventional cultures (gold standard), antimicrobial susceptibility testing and processing for the Unyvero pneumonia cartridge were done. Culture and Unyvero results were compared.

Results: Compared to cultures of the 93 samples processed for both techniques, Unyvero correctly identified pathogens in 68 (73%) proven VAP episodes, was discordant for 25 (27%), detected no pathogen in 11 and overdetected a not otherwise found pathogen in six. For the eight remaining discordant results, the pathogen responsible for VAP was not included in the Unyvero cartridge panel or it grew at a non-significant level in culture. Amongst the 31 (33%) resistance mechanism discordances observed, 22 were resistance detection failures and 24 concerned Pseudomonas aeruginosa.

(Continued on next page)
Background

Rapid identification of pathogens responsible for ventilator-associated pneumonia (VAP) and their resistance mechanisms is a challenge in the intensive care unit (ICU). Indeed, conventional microbiological cultures (CMCs) require \( \geq 48 \) h to grow the causative pathogens and determine their antimicrobial susceptibilities. While awaiting those results, empirical broad-spectrum antibiotics are prescribed [1–3]. A key issue in antimicrobial stewardship is decreasing consumption of broad-spectrum antibiotics [3]; shortening their empirical use may be a way to achieve that goal. Notably, molecular methods have been developed to supplement time-consuming CMCs, e.g. polymerase chain reaction (PCR) detection of bacterial DNA has been evaluated to shorten the time to diagnosis, but was restricted to specific pathogens and resistance mechanisms (e.g. mecA methicillin resistance in *Staphylococcus aureus* strains) [4]. Moreover, PCR is not available for pathogens usually causing VAP [5], or resistance mechanism identification requires a positive culture [4].

Recently, new multiplex PCRs (mPCRs) directly testing fresh samples have been developed to diagnose infections, including pneumonia. They target a panel of prevalent pneumonia-causing microorganisms, and some kits are commercially available. One of them, the mPCR Unyvero system pneumonia cartridge (Curetis GmbH, Holzgerlingen, Germany; henceforth Unyvero) can directly identify 20 bacteria and one fungus, amongst the most frequently causing VAP, and 19 of their resistance markers in clinical specimens, with a turnaround time of 4–5 h [6, 7]. Some published studies evaluated Unyvero, but their designs and test versions differed [6–11].

We undertook this prospective, observational study to evaluate the ability of the Unyvero pneumonia cartridge to diagnose VAP in ICU patients strongly suspected of being affected, i.e. with light microscopy visualisation of intracellular bacteria in bronchoalveolar lavage fluid (BALF).

Methods

Patients

Our ICU patients suspected of having VAP underwent fiberoptic bronchoscopy with BAL [8]. Half of BALF was sent to the bacteriology laboratory for CMM, and the rest was processed in ICU, as previously described [8, 9]: briefly, after cytocentrifugation and Diff-Quick staining, ICU physicians directly examine BALF by light microscopy for intracellular bacteria in neutrophils, thereby allowing 24/7 adaptation of empirical antimicrobials to the type of pathogens (i.e. bacilli, cocci or both) [8].

Between January 2016 and January 2019, all directly examined positive BALF (i.e. containing microscopy-detected intracellular bacteria) during office hours were included prospectively. VAP was diagnosed when all the following criteria were met: (1) clinically suspected VAP, defined as a new and persistent pulmonary infiltrate on chest radiograph associated with at least one of the following: temperature \( \geq 38 ^\circ C \), white blood cell (WBC) count \( \geq 10 \) Giga/L and/or purulent tracheal secretions (for patients with acute respiratory distress syndrome, for whom demonstration of radiological deterioration was difficult, at least one of the three preceding criteria sufficed); (2) significant quantitative CMC growth (\( \geq 10^4 \) cfu/mL) of distal BALF samples [12, 13].

Gold standard: CMCs

The bacteriology laboratory processed BALF for CMCs. Antibiotic susceptibility was determined with the disc diffusion method, as recommended by the Antiibiogram Committee of the French Society for Microbiology (CA-SFM), and the Alere® PBP2a rapid test (Abbott, Rungis, France) to identify methicillin resistance in *Staphylococcus aureus*.

Unyvero P55 and HPN cartridges

Processing of BALF for the Unyvero P55 or the hospitalised pneumonia (HPN) cartridge was performed in the ICU following the manufacturer’s recommendations. At study onset, pneumonia cartridge P55 was used. During the study (September 2017), Curetis discontinued P55 and commercialised the upgraded HPN cartridge, incorporating *Chlamydia pneumoniae* into the previous pathogen panel. The first 51 VAP episodes were tested with the P55 cartridge, and the remaining 42 with HPN.

Specimens were processed one or two at a time, either immediately after obtaining BALF or after storage at 4°C for < 12 h, depending on the sampling time of the day. Briefly, 180 µL of the patient’s native sample underwent processing in a lysator for \( \sim 30 \) min; then, the lysis
product and a master mix were loaded into a self-contained cartridge containing PCR primers (Curetis GmbH*) and placed in the analyser, where DNA was extracted, purified, amplified and specifically identified, generating complete diagnostic information within 4 h. To detect many analytes, eight mPCRs were run in parallel to detect panel-specific microarrays. The total time from obtaining BALF to results is a minimum of 4.5 h \[6, 10\]. The P55- and HPN-detected pathogens and resistance mechanisms are given in Supplementary Appendix Tables S1 and S2.

Unyvero results were collected and entered into the database but were not used to initiate or modify antimicrobial regimens.

Data collection and analysis
The following data were prospectively collected: age, sex, Simplified Acute Physiology Score (SAPS) II, McCabe and Jackson Score for comorbidities, primary reason for ICU admission, date and reason for mechanical ventilation, antimicrobials received before VAP onset, clinical data at VAP onset and antimicrobial regimen for the VAP episode (including empirical and definite treatment).

CMCs served as the gold standard for the comparison between techniques, considering a test result: (1) a true positive, when CMC and Unyvero identified the same organism (CMC+, Unyvero+); (2) a false positive, when Unyvero but not CMC detected an organism (CMC−, Unyvero+); (3) a true negative, when neither method detected an organism (CMC−, Unyvero−); and (4) a false negative, when CMC but not Unyvero detected an organism (CMC+, Unyvero−). Sensitivity, specificity and positive and negative predictive values were calculated using those findings. The 95% confidence interval (95% CI) for test characteristics was calculated with Wilson’s method. We excluded resistance gene detection from this analysis because of too few data.

Data are expressed as median [interquartile range, IQR] or n (%). Analyses were computed using the StatView 5.0 (SAS Institute Inc., Cary, NC) software, with \( p < 0.05 \) defining significance.

Ethics
In accordance with French law in January 2016 and our hospitals’ ethical committee recommendation (Committee for the Protection of Human Subjects Ile de France VI, Pitié-Salpêtrière Hospital), informed consent was not obtained because this observational study did not modify existing diagnostic or therapeutic strategies. However, patients and/or their relatives were informed about the anonymous data collection and were told that they could decline inclusion. This database is registered at the National Commission for Informatics and Liberties (CNIL registration no.: 1950673).

Results
Ninety-three suspected VAP episodes in 83 patients were evaluated prospectively. Table 1 reports the baseline characteristics of the 83 patients, and Table 2 gives the characteristics of their 93 suspected VAP episodes.

CMC and Unyvero concordance
Pathogen identification
Amongst the 93 suspected VAP episodes, Unyvero agreed with CMCs for 68 (73%) of them and differed for 25 (27%). Unyvero correctly detected the pathogens in two episodes, but their growth was non-significant (< 10^4 cfu/mL). These discords are detailed in Table 3.

Discordance patterns were classified as false positive for six of the 25 episodes and false negative for the other 19. Amongst the latter, discordance patterns varied. A VAP causative pathogen was not included in the Unyvero panel for five episodes (Enterococcus faecium for two; Achromobacter xylosoxidans, Klyvera ascorbata or Raoultella ornithinolytica for each one). Unyvero failed to detect a pathogen for 11 episodes, despite their significant growth levels (> 10^5 cfu/mL): for five Unyvero− results, a pathogen was retrieved from CMCs; the two methods detected the same pathogens for six episodes, but CMCs grew a second pathogen not detected by Unyvero. For three episodes, CMCs grew “oropharyngeal flora” with no single causative pathogen, with Unyvero− for all three.

| Characteristic Patients | Patients |
|-------------------------|----------|
| Age, years              | 58 (43–64) |
| Male sex                | 63 (76)   |
| Admission SAPS II       | 66 (52–76) |
| McCabe and Jackson Score for comorbidities ≥ 2 | 28 (34) |
| Primary reason for ICU admission |          |
| Medical                 | 61 (74)   |
| Emergency surgery       | 20 (24)   |
| Planned surgery         | 2 (2)     |
| Reason for mechanical ventilation |      |
| Shock                   | 37 (45)   |
| Acute respiratory failure| 24 (29)   |
| Postoperative respiratory failure | 19 (23)   |
| Coma                    | 2 (2)     |
| Others                  | 1 (1)     |
| Immunodepression         | 16 (19)   |
| Chronic treatment with steroids | 15 (18)   |
| Risk factor for MDR bacteria | 39 (47)   |
| ICU mortality           | 42 (51)   |

Results are expressed as median (IQR) or n (%), as appropriate
SAPS Simplified Acute Physiology Score, MDR multidrug-resistant
and 14.3% specificity for pathogen identification.

One each responsible for VAP--pathogens exceeds 93 because 27 patients had at least two pathogens--Escherichia coli

According to conventional microbiological cultures; the total number of pathogens exceeds 93 because 27 patients had at least two pathogens.

**Table 2 Clinical characteristics of the 93 suspected ventilator-associated pneumonia (VAP) episodes**

| Characteristic                  | Episodes |
|--------------------------------|----------|
| MV duration before VAP, days   | 9 (4–20) |
| Prior antimicrobial treatment  | 75 (81)  |
| Broad-spectrum antimicrobials  | 52 (56)  |
| Parameters at VAP onset        |          |
| Temperature, °C                | 37.2 (36.1–38.2) |
| White blood cell count, Giga/L | 15.5 (10.3–23.1) |
| Neutrophil count, Giga/L       | 13.1 (8.1–19.1) |
| PaO2/FiO2 ratio, mmHg          | 130 (84–179) |
| mCPIS                          | 5 (4–7)  |

Pathogen responsible for VAP<sup>a</sup>

| Pathogen                        | Episodes |
|---------------------------------|----------|
| Pseudomonas aeruginosa          | 46 (49)  |
| Other non-fermenting GNB        | 9 (10)   |
| Enterobacteriaceae              | 53 (57)  |
| Escherichia coli                | 17 (18)  |
| Enterobacter spp.               | 1 (1)    |
| Klebsiella pneumoniae           | 12 (13)  |
| Klebsiella oxytoca              | 2 (2)    |
| Klebsiella variicola            | 1 (1)    |
| Proteus mirabilis               | 6 (6)    |
| Morganella morganii             | 1 (1)    |
| Serratia marcescens             | 1 (1)    |
| Citrobacter freundii            | 1 (1)    |
| Proteus vulgaris                | 1 (1)    |
| Staphylococcus aureus           | 4 (4)    |
| Haemophilus influenzae          | 3 (3)    |
| Enterococcus spp.               | 2 (2)    |
| Polymicrobial oropharyngeal flora| 3 (3)  |
| Miscellaneous<sup>b</sup>       | 3 (3)    |
| Negative BAL                    | 2 (2)    |
| Positive blood culture          | 5 (5)    |
| Days of antimicrobial treatment | 8 (6–8)  |

Results are expressed as median (IQR) or n (%)

MV mechanical ventilation, VAP ventilator-associated pneumonia, mCPIS Modified Clinical Pulmonary Infection Score [14], BALF bronchoalveolar lavage fluid

<sup>a</sup>According to conventional microbiological cultures; the total number of pathogens exceeds 93 because 27 patients had at least two pathogens responsible for VAP

<sup>b</sup>Achromobacter xylosoxidans, Kluvyera ascorbata or Raoultella ornithinolytica, one each

Unyvero yielded six false-positive results for *Stenotrophomonas maltophilia*, in addition to other bacteria in four episodes; *S. aureus* in one episode that was CMC--; and *Escherichia coli* in addition to other bacteria in the last episode.

Compared to CMCs, Unyvero had 77.4% sensitivity and 14.3% specificity for pathogen identification (Table 4). When analysed separately, P55 and HPN cartridge results did not differ. Moreover, the analysis yielded similar concordance rates of patients with or without previous antimicrobial treatment (Table 4).

**Antimicrobial resistance detection**

Resistance mechanisms were in concordance for 62 episodes (Table 4), while Unyvero and CMCs differed for 31 (33%) episodes, mostly when *Pseudomonas aeruginosa* recovered (*n* = 24, 77%). Moreover, for most episodes, discordance was primarily attributable to Unyvero’s failure to detect resistance (71%).

Discordance patterns for *P. aeruginosa* were as follows: false resistance to fluoroquinolones for seven episodes and carbapenem or third-generation cephalosporin resistance not found in five and 12 episodes, respectively.

Excluding *P. aeruginosa*, seven resistance mechanism discordances were observed between Unyvero and CMCs: false resistance to fluoroquinolones for two episodes (*E. coli* recovered from both), penicillin resistance not detected in one (*Proteus mirabilis*-infected patient) and third-generation cephalosporin not identified in four episodes (Unyvero failed to detect extended-spectrum beta-lactamase (ESBL)–producing *Klebsiella pneumoniae*, with Unyvero accurately detecting ESBL-carrying *E. coli* in two episodes or *K. pneumoniae* in one, but not its resistance mechanism). For the latter four episodes, the patients’ ESBL-producing Enterobacteriaceae colonisation was known.

**Discussion**

Herein, the Unyvero point-of-care tool correctly detected the VAP-causing pathogen for 73% of the episodes and identified the correct resistance mechanism in 67% of them. Intriguingly, the resistance mechanism discordance rate differed when *P. aeruginosa* was the causative agent, compared to other microorganisms.

RAPID detection of the pathogens responsible for VAP and their resistant mechanisms is a critical issue for ICU patients. To date, six published studies investigated the usefulness of the mPCR-based Unyvero to achieve this goal; their concordance results between the cartridge and CMCs were heterogeneous (Table 5) [6–11]. However, most had used the older test version; only Gadsby et al. [13] evaluated the P55 cartridge, like us, and we were the only ones to assess the most recent HPN cartridge. Results differed across studies, with recent versions better identifying the pathogens. The four studies that examined CMC–Unyvero resistance concordance found similar rates of ~ 70% (Table 5).

Our study differs from the others in several ways. First, we exclusively studied patients with suspected VAP, not hospital-acquired or community-acquired pneumonia. Second, we used the most recent version (but switched
from the discontinued P55 to HPN cartridges during the study). Third, we focused on patients strongly suspected of having VAP, primarily to avoid expenditures for unnecessary tests in a context of low pretest pneumonia probability and also to assess Unyvero’s usefulness within our care organisation. These latter points could explain the very low specificity observed.

Our results showed the usual limitations of PCR-based tools for detecting pathogens in respiratory samples. The first is the overdetection (false positive), i.e. pathogen detection without pneumonia, seen in six episodes. Such overdetection may indicate nucleic acids of non-viable organisms or bacterial presence without reaching a pathogenic threshold (colonisation). One of the advantages of Unyvero’s P55 and HPN cartridges is their semi-quantification, with results being positive only when the sample’s bacterial burden is sufficiently high. Unfortunately, this system does not allow having a true quantification of the bacterial burden. The second limitation of this kind of test is underdetection (false negative), i.e. no pathogen detected despite significant pathogen growth in CMCs, as observed for 19 episodes. One explanation for underdetection was the absence of the VAP causative pathogen in the test panel. Notably, resistance mechanism discordances were more frequent when \( P. \) aeruginosa, rather than another microorganism, was the responsible agent, perhaps explained \( P. \) aeruginosa’s changing resistance profile over time and that the PCR recognises only 17 resistance markers against Gram-negative bacilli (mostly carbapenem resistance; Supplementary Appendix Table S2) [15, 16]. We observed that Unyvero did not identify ESBL in four episodes of ESBL-producing Enterobacteriaceae VAP. Although ESBL enzymes which were present in those patients are unknown (Supplementary Appendix Table S2), Unyvero does not include all ESBL in its assay panel. Importantly, all four patients with false-negative results were known to be colonised by ESBL-

| Discordance | Conventional cultures | Unyvero* |
|-------------|-----------------------|----------|
| 1           | Klebsiella pneumoniae | Escherichia coli | Escherichia coli |
| 2           | Klebsiella pneumoniae | Stenotrophomonas maltophilia |
| 3           | Entrobacter aerogenes  | Proteus mirabilis |
| 4           | Staphylococcus aureus | Acinetobacter baumannii | Acinetobacter baumannii |
| 5           | Klebsiella pneumoniae | Escherichia coli | Escherichia coli |
| 6           | Enterobacter aerogenes | Klebsiella pneumoniae |
| 7           | Enterobacter aerogenes | – |
| 8           | Klebsiella oxytoca | – |
| 9           | Klebsiella pneumoniae | – |
| 10          | Staphylococcus aureus | – |
| 11          | Klebsiella variicola | – |
| 12          | Oropharyngeal flora | – |
| 13          | Oropharyngeal flora | – |
| 14          | Oropharyngeal flora | – |
| 15          | Kluyvera ascorbata | Enterobacter cloacae |
| 16          | Raoultella ornitholytica | Proteus mirabilis |
| 17          | Acromobacter xylosoxidans | Proteus spp. |
| 18          | Enterococcus faecium | – |
| 19          | Enterococcus faecium | – |
| 20          | Pseudomonas aeruginosa | – |
| 21          | Enterobacter cloaca | – |

Unyvero overdetected pathogens

| Discordance | Conventional cultures | Unyvero* |
|-------------|-----------------------|----------|
| 22          | Pseudomonas aeruginosa | Stenotrophomonas maltophilia |
| 23          | – | Staphylococcus aureus |
| 24          | Pseudomonas aeruginosa | Pseudomonas aeruginosa |
| 25          | Pseudomonas aeruginosa | Stenotrophomonas maltophilia |

*False results (positive or negative) are in bold type

This episode had false-negative and false-positive findings
Table 4 Comparisons of Unyvero vs conventional microbiological methods for all episodes, according to Unyvero version and to previous antibiotic use

| Finding                     | N positive | N negative | Sensitivity (%) | Specificity (%) | PPV (%) | NPV (%) |
|-----------------------------|------------|------------|----------------|----------------|---------|---------|
|                             | True       | False      | True           | False          |         |         |
| Pathogen identification     | 65         | 6          | 1              | 19             | 77.4    | 14.3    | 91.5    | 5       |
| Resistance mechanism        | 19         | 9          | 43             | 22             | 46.3    | 82.7    | 67.9    | 66.2    |
| P55 cartridge (n = 51)      | 39         | 1          | 1              | 10             | 79.6    | 50      | 97.5    | 9       |
| Pathogen identification     | 12         | 7          | 21             | 11             | 52.1    | 75      | 63.2    | 65.6    |
| Resistance mechanism        | 7          | 2          | 22             | 11             | 38.9    | 91.7    | 77.8    | 66.7    |
| Previous antimicrobial treatment (n = 75) | 54         | 6          | 1              | 14             | 79.4    | 14.2    | 90      | 6.7     |
| Pathogen identification     | 15         | 8          | 31             | 21             | 41.7    | 79.5    | 65.2    | 59.6    |
| Resistance mechanism        | 4          | 1          | 12             | 1              | 80      | 92.3    | 80      | 92.3    |
| No previous antimicrobial treatment (n = 18) | 13         | 0          | 0              | 5              | 72.2    | 0       | 100     | 0       |
| Pathogen identification     | 4          | 1          | 12             | 1              | 80      | 92.3    | 80      | 92.3    |

Conventional microbiological methods were considered as the gold standard
PPV positive predictive value, NPV negative predictive value, HPN hospitalised pneumonia

Table 5 Studies that evaluated the usefulness of the Unyvero pneumonia cartridge for patients suspected of having lower respiratory tract infections

| Author, year [ref] | Study design                  | Population                           | Test Specimen type | N specimens/ N patients | Pathogen identification | Concordance* | Se/Sp |
|---------------------|--------------------------------|--------------------------------------|--------------------|------------------------|-------------------------|--------------|-------|
| Schulte, 2014 [8]   | Prospective observational, fresh samples | Adults with suspected HAP/VAP | Unyvero P50 BALF, ETA, sputum | 739/NR | NR | 70.6%/ 95.2% | – |
| Jamal, 2014 [6]     | Prospective observational, fresh samples | Children and adults with suspected HAP/VAP | Unyvero P50 BALF, ETA, sputum | 49/49 | 23/49 (47%) | NR | NR |
| Kunze, 2015 [7]     | Prospective observational, fresh samples | Adults with suspected HAP | Unyvero P50 ETA, NPTA | 40/40 | 18/40 (45%) | 75%/ 43% | – |
| Personne, 2016 [9]  | Prospective observational, fresh samples | Adults with suspected pneumonia | Unyvero P50 NR | 90/NR | 59/90 (66%) | 95.7%/ 32.6% | 75.6% |
| Papan, 2018 [10]    | Prospective observational, fresh samples | Children and neonates with suspected pneumonia | Unyvero P50 BALF, ETA, pleural fluid | 79/79 | 48/80 (60%) | 73.1%/ 97.8% | 75% |
| Gadsby, 2019 [11]   | Prospective observational, frozen samples | Adults with suspected VAP, CAP or HAP | Unyvero P55 BALF | 74/74 | 57/99 (57.5%) | 56.9%/ 58.5% | 121/166 (72.9%) |
| This study          | Prospective observational, fresh samples | Adults with suspected VAP and bacteria in BALF | Unyvero P55 and HPN BALF | 93/83 | 71% | 77.4%/ 17.3% | 62/93 (67%) |

Se/Sp sensitivity/specificity, BALF bronchoalveolar lavage fluid, ETA endotracheal aspirate, NPTA nasopharyngeal tracheal aspirate, NR not reported, HAP hospital-acquired pneumonia, VAP ventilator-associated pneumonia, CAP community-acquired pneumonia, HPN hospitalised pneumonia

*Both concordant positive (correct pathogen identification by both methods) and concordant negative (no pathogen identification by both methods)
producing Enterobacteriaceae. Therefore, our results do not support the current routine use of this system to adapt antimicrobial treatment.

Several limitations should be underlined. First, this was a monocentric, prospective study, and despite having included a large number of patients, our results warrant further investigations. Second, the strategy chosen required fiberoptic bronchoscopy and BAL, which are not universally available and remain debated as a first-line tool for diagnosing VAP [1, 2]. However, using tracheal aspirates or sputum may generate more false positives with Unyvero. Third, although Unyvero targets the most frequent pathogens responsible for hospital-acquired pneumonia, some VAP causative microorganisms are missing from the cartridge panel. Moreover, polymicrobial VAP (e.g. oropharyngeal flora) may be missed using this system. However, to overcome these limitations, we propose an algorithm based first on direct BALF examination, then Unyvero if bacilli are found. Fourth, all samples were not processed with the same cartridge, since the manufacturer shifted from P55 to HPN during our study. Nonetheless, the two cartridges differ only by the addition of *Chlamydia pneumoniae* to the HPN cartridge; because this pathogen is not frequently responsible for VAP and was never detected in our patients, the results would have not been different if we had used the same cartridge throughout the study. Indeed, results were similar when P55 and HPN cartridges were compared. Lastly, molecular tests were not run to explore discordances between the two techniques and to characterise resistance mechanisms.

**Conclusions**

In conclusion, the Unyvero pneumonia cartridges correctly detected VAP causative pathogens for 73% of the episodes and correctly identified the resistance mechanism in 67% of them, differing according to the responsible pathogen, with *P. aeruginosa* having the highest discordance rate.

**Supplementary information**

Supplementary information accompanies this paper at https://doi.org/10.1186/s13054-020-03102-2.

**Additional file 1: Supplementary Table S1.** Pathogens detected by the Unyvero hospitalised-pneumonia (HPN) cartridge [Supplementary Table S2]. Resistance markers potentially detected by the HPN system and their target(s).

**Abbreviation**

BALF: Bronchoalveolar lavage fluid; CMC: Conventional microbiological cultures; DNA: Deoxyribonucleic acid; ESBL: Extended-spectrum beta-lactamase; HPN: Hospitalised pneumonia; ICU: Intensive care unit; IQR: Interquartile range; PCR: Polymerase chain reaction; SAPS: Simplified Acute Physiology Score; VAP: Ventilator-associated pneumonia; WBC: White blood cell

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**Authors’ contributions**

CEL drafted the study design. CEL, JR and AA analysed the results and drafted the manuscript. All authors participated in the data collection, final manuscript preparation and agreed with the latest manuscript. The authors read and approved the final manuscript.

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Curetis graciously provided the P55 and HPN cartridges that were used for the study. Curetis was not involved in the study design, the running of the study and the decision to write or to submit the manuscript.

**Availability of data and materials**

The datasets generated during the current study are available from the corresponding author on reasonable request.

**Ethics approval and consent to participate**

In accordance with French law in January 2016 and our hospitals’ ethical committee recommendation (Committee for the Protection of Human Subjects Ile de France VI, Pitié-Salpêtrière Hospital), informed consent was not obtained because this observational study did not modify existing diagnostic or therapeutic strategies. However, patients and/or their relatives were informed about the anonymous data collection and were told that they could decline inclusion. This database is registered at the National Commission for Informatics and Liberties (CNIL registration no.: 1950673).

**Consent for publication**

Not applicable

**Competing interests**

CEL reports personal fees from Merck Sharp and Dohme, Thermo Fisher, Brahms, Biomérieux, Carmat, Bayer Healthcare and Aerogen and grants from Bayer Healthcare, outside the submitted work. MS reports lecture fees from Maquet, Getinge and Fresenius, outside the submitted work. Other authors declare that they have no conflicts of interest.

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