

# Ventilator-Associated Pneumonia: The Role of Emerging Diagnostic Technologies

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## Abstract

Antibiotic resistance has emerged as a key determinant of outcome in patients with serious infections along with the virulence of the underlying pathogen. Within the intensive care unit (ICU) setting, ventilator-associated pneumonia (VAP) is a common nosocomial infection that is frequently caused by multidrug-resistant bacteria. Antimicrobial resistance is a growing challenge in the care of critically ill patients. Escalating rates of antibiotic resistance add substantially to the morbidity, mortality, and cost related to infection in the ICU. Both gram-positive organisms, such as methicillin-resistant *Staphylococcus aureus* and vancomycin-intermediate *S. aureus*, and gram-negative bacteria, including *Pseudomonas aeruginosa*, *Acinetobacter* species, carbapenem-resistant *Enterobacteriaceae*, such as the *Klebsiella pneumoniae* carbapenemase-producing bacteria, and extended spectrum  $\beta$ -lactamase organisms, have contributed to the escalating rates of resistance seen in VAP and other nosocomial infections. The rising rates of antimicrobial resistance have led to the routine empiric administration of broad-spectrum antibiotics even when bacterial infection is not documented. Moreover, there are several new broader-spectrum antibiotics that have recently become available and others scheduled for approval in the near future. The challenge to ICU clinicians is how to most effectively utilize these agents to maximize patient benefits while minimizing further emergence of resistance. Use of rapid diagnostics may hold the key for achieving this important balance. There is an urgent need for integrating the administration of new and existing antibiotics with the emerging rapid diagnostic technologies in a way that is both cost-effective and sustainable for the long run.

## Keywords

- ▶ rapid diagnostics
- ▶ antibiotic resistance
- ▶ microbiology
- ▶ outcomes

Ventilator-associated pneumonia (VAP) is one of the most common infections occurring in mechanically ventilated patients and is frequently caused by antibiotic-resistant bacteria.<sup>1</sup> Mortality, hospital lengths of stay, and health care costs are typically greater among patients with respiratory failure complicated by VAP compared with patients who do not develop VAP.<sup>2</sup> Moreover, we know that the administration of inappropriate initial antibiotic therapy (IIAT) for VAP, usually attributed to multidrug-resistant (MDR) bacteria, is associated with greater hospital mortality and longer

hospital lengths of stay.<sup>3,4</sup> These outcome influencing characteristics of VAP make it an important infection for intensivists to manage in an optimal manner. The ideal management of VAP requires intensive care units (ICUs) and hospitals to have consensus-derived strategies in place for the prevention, diagnosis, and treatment of this important nosocomial infection, which unfortunately are often lacking. Moreover, the overall perceived clinical importance of VAP has diminished in the United States due to the imprecise under-coding of this nosocomial infection using

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the Centers for Disease Control and Prevention surveillance definitions.<sup>5</sup> This has resulted in the promotion of ventilator-associated events (VAEs) as a preferred surveillance tool for assessing the quality of ICU care in the United States and reducing VAP to a nonreportable condition.<sup>6</sup> This may encourage suboptimal practices for VAP treatment that could be detrimental for patient outcomes and promote further antibiotic resistance.

The clinical importance of VAP is demonstrated by recent surveillance studies showing that it is a common nosocomial infection across all continents.<sup>7-9</sup> Moreover, the emerging problem of antibiotic resistance has added a new premium to the importance of accurately diagnosing and more importantly treating VAP with appropriate initial antibiotic therapy.<sup>10,11</sup> It is also imperative to recognize that one of the major clinical issues related to the management of VAP, as well as other nosocomial infections, is the increasing prevalence of MDR or extremely drug-resistant (XDR) pathogens.<sup>12-15</sup> There appears to be a direct relationship between overall antibiotic consumption for VAP and the emergence of newly resistance bacterial strains.<sup>16,17</sup> The latest and most fearsome example of this trend, due in large part to escalating use of colistin, has been the emergence of plasmid-mediated colistin resistance.<sup>18</sup> The development of colistin resistance in carbapenem-resistant *Enterobacteriaceae*, including New Delhi metallo- $\beta$ -lactamase-1 (NDM-1) strains, brings a renewed sense of urgency to minimize any further resistance emergence and to prevent spread of these XDR bacteria.<sup>19</sup> As a result of this trend of increasing antibiotic resistance and broader spectrum empiric antibiotic treatment of suspected VAP, more precise and rapid microbiologic diagnostic approaches for the antibiotic management of suspected VAP are urgently needed.

## Diagnostic Criteria for VAP

The diagnosis of VAP is problematic because noninfectious conditions can cause pulmonary infiltrates and systemic findings such as leukocytosis, fever, and increased oxygen requirements.<sup>20</sup> Various diagnostic criteria with variable rigor have been developed to assist in the diagnosis of VAP. However, the most stringent criteria available have been associated with the greatest observed mortality and establishing the diagnosis of VAP took significantly longer when applying them compared with less stringent criteria, potentially resulting in delayed therapy.<sup>21</sup> Erring on the side of caution, most clinicians employ the finding of a new or progressive radiographic infiltrate and at least one clinical feature (fever, leukocytosis, worsening oxygenation, or purulent tracheal secretions), which has high sensitivity but low specificity for VAP.<sup>22</sup> The difficulty in relying on clinical criteria for the diagnosis of VAP is the potential for over diagnosis, resulting in the unnecessary administration of antibiotics to noninfected patients. This has the potential to promote further emergence of antibiotic resistance, especially when employed for prolonged time periods, and to dilute out the ability of clinicians to identify the beneficial impact of treating patients with appropriate initial antibiotic therapy.<sup>23,24</sup>

Owing to the lack of a proven diagnostic method, two different strategies have been used and compared using clinical or bacteriologic criteria, each associated with advantages and disadvantages.<sup>22</sup> The clinical strategy employs the abovementioned clinical and radiographic criteria in diagnosing VAP. A combination of two out of three clinical criteria and a radiographic infiltrate yielded a sensitivity of 69% and a specificity of 75% for the diagnosis of VAP in 25 mechanically ventilated patients using histology and quantitative lung tissue culture on autopsy as the reference.<sup>25</sup> Increasing the number of clinical criteria resulted in greater specificity but at the cost of lesser overall sensitivity.<sup>25</sup> In a postmortem analysis of 39 mechanically ventilated patients, clinical criteria did not provide reliable predictive accuracy for histologic pneumonia.<sup>26</sup> A semiquantitative endotracheal aspirate culture can be used to identify a causative pathogen of VAP and, if positive, has been shown to correlate with quantitative cultures of the lower respiratory tract obtained via protected specimen brush (PSB).<sup>27</sup> Additionally, a negative endotracheal aspirate culture has good negative predictive value in excluding the presence of VAP if antibiotics have not recently been started or changed.<sup>28</sup> However, semiquantitative cultures are generally not as reliable as quantitative cultures of the lower respiratory tract due to an inability to differentiate between colonization and infection.<sup>29</sup> The use of clinical criteria and a reliance on semiquantitative cultures can result in clinical false-positive results for the diagnosis of VAP resulting in unnecessary antibiotic use.

The bacteriologic strategy uses quantitative cultures obtained from the lower respiratory tract via endotracheal aspirate, PSB, or bronchoalveolar lavage (BAL) to confirm or exclude the diagnosis of nosocomial pneumonia based on thresholds of bacterial growth of  $\geq 10^5$  colony forming units (CFU)/mL for an endotracheal aspirate,  $\geq 10^4$  CFU/mL for a BAL specimen, and  $\geq 10^3$  CFU/mL for a PSB sample. Results of these procedures guide decisions such as when to initiate or stop antibiotics and which drug should be used against the offending agent. There are no definitive data to support the use of one sampling technique over another; however, the cellular analysis of BAL fluid may provide an advantage, as a sample containing less than 50% neutrophils was associated with excellent negative predictive value in one study.<sup>26</sup> Also, given the multifocal nature of VAP, even mini-BAL samples obtained blindly without the use of bronchoscopy can be effective.<sup>30-32</sup> However, other studies caution on the use of unilateral cultures even when directed to the side of the dominant radiographic abnormality.<sup>33</sup> The bacteriologic strategy has resulted in less overall prescription and more narrowed antibiotic use, an important point given the surge of antibiotic resistance in the ICU setting.<sup>34-36</sup> A major disadvantage of the bacteriologic approach is the concern for false negatives which could result in cases of nosocomial pneumonia going untreated, especially in the setting of recently introduced antibiotics.<sup>37</sup>

Multiple studies have compared the clinical and bacteriologic strategies. Only one prospective, randomized trial demonstrated a mortality benefit when using the bacteriologic strategy at 14 days.<sup>34</sup> Others have failed to reproduce these findings, including a large study conducted by the Canadian

Critical Care Trials Group and a comprehensive meta-analysis.<sup>38,39</sup> In addition, the bacteriologic strategy does not seem to reduce the duration of mechanical ventilation or ICU length of stay.<sup>39</sup> The decision to employ either the clinical or bacteriologic strategy rests with the clinician on a case-by-case basis. If bronchoscopic sampling can be performed safely and the appropriate personnel is available, it is reasonable to utilize this approach as antibiotic decisions may change based on culture results allowing for more effective antimicrobial deescalation. If the clinical strategy is used, the clinician should reevaluate the patient often for guidance on antibiotic usage. Regardless of the diagnostic strategy, an unstable patient with a high pretest probability of nosocomial pneumonia should be initiated on empiric antibiotics, as a delay in antibiotic administration leads to higher mortality.<sup>40-42</sup>

The lack of consistency in establishing a precise diagnosis of VAP has led some national guidelines to reflect on the relatively low accuracy of microbiology cultures as a diagnostic tool in VAP.<sup>22</sup> Moreover, contamination with upper respiratory tract pathogens or endotracheal tube colonizers is common and traditional microbiology laboratory flow with Gram staining, cultures, and antibiotic susceptibility testing requires at least 48 to 96 hours for information to be processed for clinical decision making. These current limitations in establishing a rapid and precise microbiologically confirmed diagnosis of VAP serve as the impetus for developing new rapid diagnostic approaches for this important infection.

## New Diagnostic Technologies

### Multiplex Real-Time Polymerase Chain Reaction

A broad range of viral and bacterial pathogens can cause acute respiratory tract infections including VAP often with similar clinical and radiographic presentations (→Fig. 1).

Rapid detection of the causative pathogen offers the potential for providing timely administration of appropriate antimicrobial therapy as well as minimizing the use of broad-spectrum antibiotics when they are not justified based on microbiologic evaluation. Multiplex real-time polymerase chain reaction (PCR) offers rapid detection of a broad array of respiratory pathogens to optimize antimicrobial treatment. The FilmArray Respiratory Panel (RP; bioMérieux BioFire, Salt Lake City, UT) assay (→Fig. 2) is the first FDA-cleared assay for the qualitative detection of nucleic acid targets from both viruses and bacteria in nasopharyngeal swab specimens.<sup>43</sup> The FilmArray RP can detect 17 viral targets and three bacterial species (*Bordetella pertussis*, *Chlamydia pneumoniae*, and *Mycoplasma pneumoniae*) more typically associated with community-acquired pneumonia with a turnaround time of approximately 1 hour and has been applied to direct respiratory specimens, including BAL specimens from mechanically ventilated patients (→Table 1).<sup>44-46</sup> More recently, the FilmArray RP has been employed to demonstrate that more than 24% of nonventilated hospital-acquired pneumonia (HAP) episodes were associated with respiratory virus infection alone or concomitant viral and bacterial infection.<sup>47</sup> This type of information could have important implications in terms of modifying or deescalating antibiotic therapy.<sup>44</sup>

A new Luminex NxTAG Respiratory Pathogen Panel (NxTAG-RPP, Austin, TX) has been introduced as a high-throughput system that can detect nucleic acid from 21 respiratory viruses, including all pathogens detected by the FilmArray RP except *B. pertussis* plus *Legionella pneumophila* and human bocavirus.<sup>48</sup> A comparison of these two technologies demonstrated complete concordance in 98.8% (318/322) of positive results (kappa = 0.92). The high sample throughput with reasonable turnaround time of these

## NON-SPECIFICITY OF THE CHEST X-RAY FOR THE ETIOLOGY OF PNEUMONIA

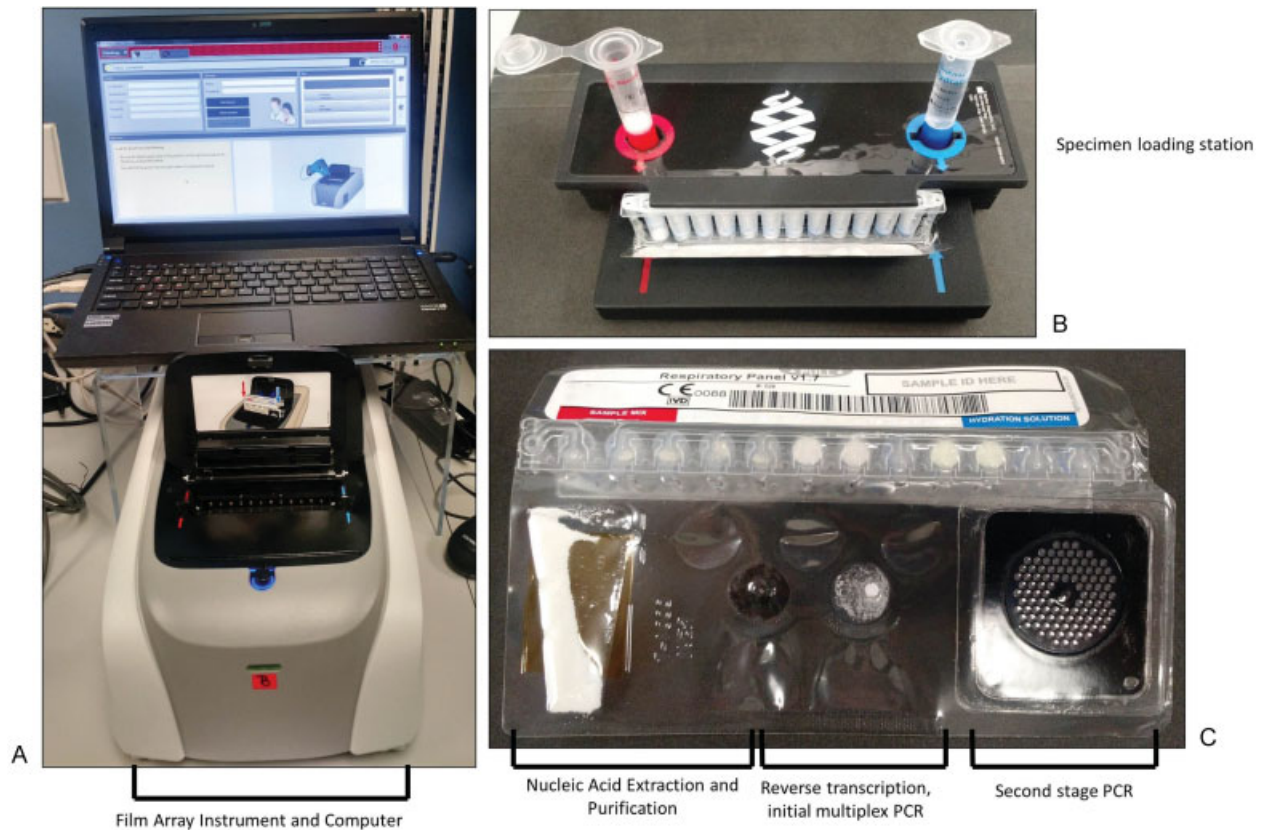


**Influenza A and MRSA**

***Legionella pneumophila***

***P. aeruginosa***

**Fig. 1** Three chest X-rays of patients with microbiologically confirmed pneumonia showing similar types of infiltrates for different pathogens. These X-rays illustrate the general nonspecificity of the radiographic findings for establishing a precise microbiologic diagnosis of pneumonia. MRSA, methicillin-resistant *Staphylococcus aureus*.



**Fig. 2** The BioFire FilmArray System. (A) The BioFire instrument and computer. Each instrument can run one FilmArray pouch at a time. (B) The specimen loading station. The FilmArray pouch is fixed in the station, and rehydrating buffer and specimen are added. (C) The FilmArray pouch. The specimen is moved through a series of reagents, including nucleic acid extraction and purification steps, a reverse transcriptase and initial PCR step, and a second-stage PCR. PCR product detection is performed in the "honeycomb" of the second-stage PCR.

assays makes them suitable multiplex platforms for routine screening of respiratory specimens in hospital-based laboratories. Moreover, the use of multiplex real-time PCR has been associated with reduced antibiotic utilization in patients evaluated for respiratory tract infections demonstrating their potential value as antibiotic stewardship adjuncts.<sup>49,50</sup> Another potential use of multiplex real-time PCR would be the addition of emerging respiratory viral pathogens to the panel, facilitating surveillance to identify patients with new, and often virulent, respiratory virus syndromes such as Middle East respiratory syndrome coronavirus infection.<sup>51</sup>

A preclinical evaluation was recently conducted to evaluate the performance of the Cepheid Xpert MRSA/SA SSTI real-time PCR assay (Cepheid, Sunnyvale, CA) on 135 lower respiratory tract secretions for detection of methicillin-resistant *Staphylococcus aureus* (MRSA) and *S. aureus*.<sup>52</sup> Compared with the gold standard quantitative culture, the sensitivity, specificity, and positive and negative predictive values were 99.0, 72.2, 90.7, and 96.3%, respectively. The same assay has been employed to exclude the presence of MRSA and *S. aureus* in VAP demonstrating negative predictive values of 99.7% (98.1–99.9%) and 99.8% (98.7–99.9%) for methicillin-susceptible *S. aureus* (MSSA) and MRSA, respectively.<sup>53</sup>

### Other Nucleic Acid Detection Techniques

New point-of-care PCR systems for rapid identification of pathogens and antibiotic resistance markers are available and show promise for the management of infections like VAP. Kunze et al evaluated point-of-care multiplex PCR (Unyvero, Curetis AG, Holzgerlingen, Germany) for patients with HAP.<sup>54</sup> Mean turnaround test result times were 6.5 hours (4.7–18.3 hours) for multiplex PCR and 71 hours (37.2–217.8 hours) for conventional microbiology. However, they found concordant results in only 45% and nonconcordant results in 45% of all patients. Only 55% of the results were concordant in patients with a clinical pulmonary infection score higher than 5, suggesting a high likelihood for the presence of HAP. These authors concluded that Unyvero allowed point-of-care microbial testing with short turnaround times, but the system performance was poor and what was needed was an improved system with more reliable performance and an extended microbial panel.

Vincent et al employed culture-independent polymerase chain reaction/electrospray ionization-mass spectrometry (PCR/ESI-MS) to test 616 bloodstream infection samples, 185 pneumonia samples, and 110 sterile fluid and tissue specimens from 529 patients.<sup>55</sup> From the 616 bloodstream samples, PCR/ESI-MS identified a pathogen in 228 cases (37%) and conventional culture methods in just 68 (11%). Conventional

**Table 1** Pathogens identified with the FilmArray panels

FilmArray respiratory panel	FilmArray blood culture ID panel
Adenovirus	<i>Staphylococcus</i> species
Coronavirus 229E	<i>Staphylococcus aureus</i>
Coronavirus HKU1	<i>Streptococcus</i> species
Coronavirus OC43	<i>Streptococcus agalactiae</i>
Coronavirus NL63	<i>Streptococcus pyogenes</i>
Human Metapneumovirus	<i>Streptococcus pneumoniae</i>
Human Rhinovirus/ Enterovirus	<i>Enterococcus</i> species
Influenza A	<i>Listeria monocytogenes</i>
Influenza A/H1	<i>Klebsiella oxytoca</i>
Influenza A/H1–2009	<i>Klebsiella pneumoniae</i>
Influenza A/H3	<i>Serratia</i> species
Influenza B	<i>Proteus</i> species
Parainfluenza 1	<i>Acinetobacter baumannii</i>
Parainfluenza 2	<i>Haemophilus influenzae</i>
Parainfluenza 3	<i>Neisseria meningitidis</i>
Parainfluenza 4	<i>Pseudomonas aeruginosa</i>
RSV	<i>Enterobacteriaceae</i>
<i>Bordetella pertussis</i>	<i>Escherichia coli</i>
<i>Chlamydomphila pneumoniae</i>	<i>Enterobacter cloacae</i> complex
<i>Mycoplasma pneumoniae</i>	<i>Candida albicans</i>
	<i>Candida glabrata</i>
	<i>Candida krusei</i>
	<i>Candida parapsilosis</i>
	<i>Candida tropicalis</i>
	mecA
	vanA/B
	<i>bla</i> <sub>KPC</sub>

cultures were positive and PCR-ESI-MS was negative in 13 cases, and both were negative in 384 cases, giving PCR/ESI-MS a sensitivity of 81%, specificity of 69%, and negative predictive value of 97% at 6 hours from sample acquisition. Similar observations were made for pneumonia and sterile fluid and tissue specimens. An independent clinical analysis of results suggested that PCR/ESI-MS technology could potentially have resulted in altered treatment in up to 57% of patients. The findings of this study were promising in suggesting that clinical decision making could potentially be influenced in a positive manner with PCR/ESI-MS by allowing more rapid and accurate modifications in antibiotic therapy.

Banerjee et al performed a randomized trial in a total of 617 patients with positive blood culture bottles (BCBs) who underwent stratified randomization into three arms: standard BCB processing (control,  $n = 207$ ), rapid multiplex PCR reported with templated comments (rmPCR,  $n = 198$ ), or rmPCR reported with templated comments and real-time

audit and feedback of antimicrobial orders by an antimicrobial stewardship team (rmPCR/AS,  $n = 212$ ).<sup>56</sup> The primary outcome was antimicrobial therapy duration. The rmPCR panel used in both intervention arms was the FilmArray Blood Culture ID Panel (BioFire Diagnostics/bioMérieux BioFire), which was performed as soon as a BCB signaled positive, 24 hours a day, 7 days a week. This assay detects the pathogens and resistance genes shown in **Table 1**. Compared with the control group, both intervention groups had decreased broad-spectrum piperacillin-tazobactam use and increased narrow-spectrum  $\beta$ -lactam antibiotic use, and fewer instances of antibiotic therapy for contaminants. Time from Gram stain to appropriate antimicrobial deescalation or escalation was shortest in the rmPCR/AS group. The aim would be to replicate these types of findings in patients with pneumonia using lower respiratory specimens.

The Verigene Nanosphere system is a multiplex nucleic acid detection assay that is being used in clinical laboratories for pathogen identification and resistance gene detection in positive blood culture broth and for respiratory pathogen detection (**Table 2**, **Fig. 3**).<sup>57–59</sup> Similar to the BioFire blood culture assay, use of the Verigene assay for bacteremic patients has been associated with reduced length of stay, reduced mortality, and improvement in time to optimization of antimicrobial therapy.<sup>57,59,60</sup> Panels directed toward lower respiratory tract pathogens are in development.

#### Matrix-Assisted Laser Desorption Ionization Time-of-Flight Mass Spectrometry

Traditionally, the identification of microbes recovered in culture has relied on microbial growth and metabolism in the presences of various biochemical substrates. In contrast, matrix-assisted laser desorption ionization time-of-flight mass spectrometry (MALDI-TOF MS) uses proteomic profiling to assign an identification; this can be applied to a variety of microbes, including bacteria, yeast, mold, and mycobacteria.<sup>61–66</sup> It is primarily ribosomal proteins that are detected using this method. The MALDI BioTyper system (Bruker Daltonics, Billerica, MA) and the VITEK MS (bioMérieux, Durham, NC) are the commercially available MALDI-TOF MS instrumentation/database platforms for microorganism identification. While MALDI-TOF MS has been used most frequently for expediting the identification of microbes recovered on solid culture media, it has also been used to identify some microbes from clinical specimens, including positive blood culture broth and urine.<sup>67–69</sup> In addition, proof-of-principle studies have demonstrated the power of this method to simultaneously identify important resistance determinants during routine organism identification, such as a vancomycin-intermediate *S. aureus* and certain KPC-containing plasmids.<sup>70,71</sup> As this technology becomes more widespread, it is likely that the rapid and accurate identification of pathogens will facilitate optimization of antimicrobial therapy in patients with all types of infection, including respiratory infection.

#### Fluorescence In-Situ Hybridization

The fluorescence in-situ hybridization (FISH) technique is based on fluorescently labeled oligonucleotide probes that

**Table 2** Pathogens detected with the Verigene panels

Verigene respiratory pathogen panel	Gram-positive blood culture test	Gram-negative blood culture test
Adenovirus Human Metapneumovirus Influenza A Influenza A (subtype H1) Influenza A (subtype H3) Influenza B Parainfluenza 1 Parainfluenza 2 Parainfluenza 3 Parainfluenza 4 Rhinovirus RSV A RSV B <i>Bordetella pertussis</i> <i>Bordetella parapertussis/B. bronchiseptica</i> <i>Bordetella holmesii</i>	<i>Staphylococcus aureus</i> <i>Staphylococcus epidermidis</i> <i>Staphylococcus lugdunensis</i> <i>Streptococcus anginosus</i> Group <i>Streptococcus agalactiae</i> <i>Streptococcus pneumoniae</i> <i>Streptococcus pyogenes</i> <i>Enterococcus faecalis</i> <i>Enterococcus faecium</i> <i>Staphylococcus</i> spp. <i>Streptococcus</i> spp. <i>Listeria</i> spp. <i>mecA</i> <i>vanA</i> <i>vanB</i>	<i>Escherichia coli</i> <i>Klebsiella pneumoniae</i> <i>Klebsiella oxytoca</i> <i>Pseudomonas aeruginosa</i> <i>Acinetobacter</i> spp. <i>Citrobacter</i> spp. <i>Enterobacter</i> spp. <i>Proteus</i> spp. CTX-M IMP KPC NDM OXA VIM



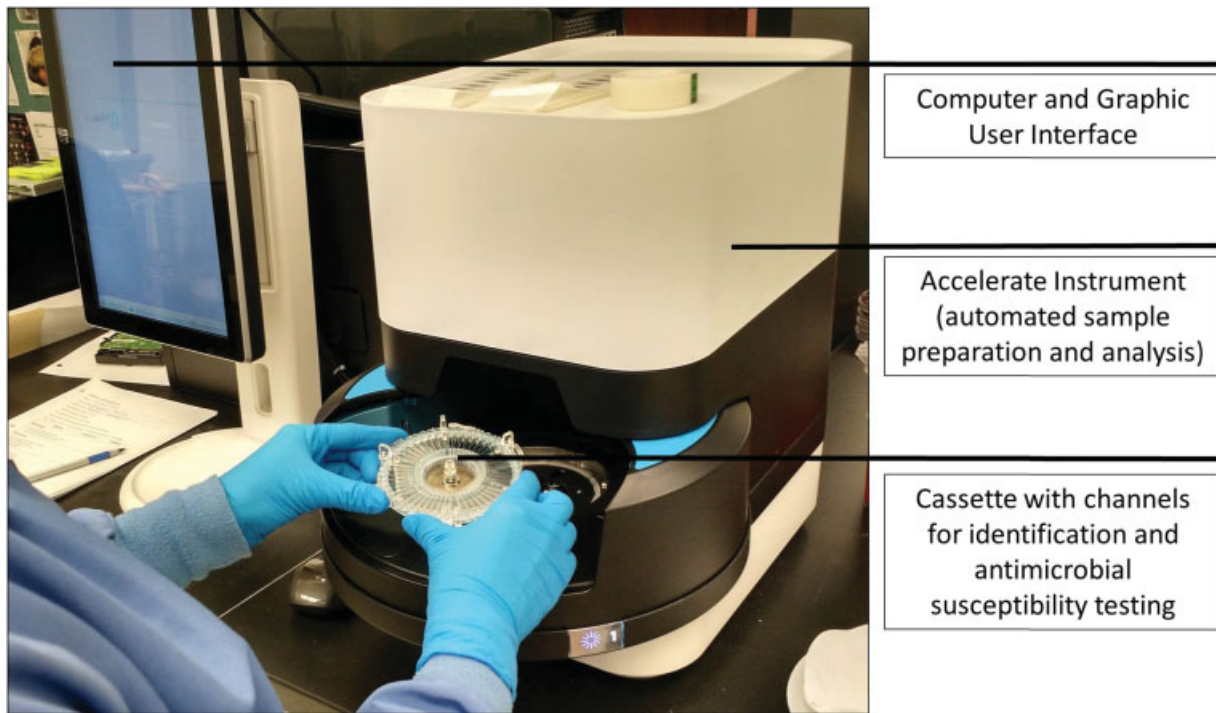
**Fig. 3** The Nanosphere Verigene System, consisting of instrumentation (A, B) and the test cartridge (C). Sample and reagents are added to the processing unit. After analysis is completed, the cartridge is moved briefly to the reading unit for interpretation.

complementarily bind to specific target ribosomal RNA sequences of bacteria, yeasts, or other microorganisms. Target sequences are naturally present in bacteria at a concentration high enough to enable visual detection of the specific fluorescent signal.<sup>72</sup> FISH can be used to detect pathogens that are difficult or time consuming to identify with traditional culture methods, especially when more than one species is present in the sample, as in the case of polymicrobial infections including VAP. RespiFISH HAP Gram (–) Panel (miacom diagnostics GmbH, Duesseldorf, Germany) is a classic FISH technology employing fluorescently labeled DNA molecular beacon probes to develop a simple procedure known as the beacon-based FISH technology.<sup>73</sup> This panel is able to detect most gram-negative bacterial pathogens and has been shown to be accurate in detecting the causative pathogens in patients with pneumonia, including VAP.<sup>74</sup>

#### Automated Microscopy

Douglas et al employed a real-time multiplexed FISH-based microscopy ID/AST system (Accelerate Diagnostics, Tucson, AZ), capable of evaluating antibiotic sensitivity and resis-

tance against live pathogenic organisms from blood cultures or respiratory samples using automated phenotypic growth pattern analysis (– Fig. 4), to study surveillance for potential preempted treatment of VAP.<sup>75</sup> Seventy-seven mini-BAL specimens were obtained in 33 patients. One patient (3%) was clinically diagnosed with VAP. Of 73 paired samples, conventional culture methods identified seven, containing pneumonia panel bacteria ( $>10^4$  colony-forming units/mL) from five patients (four *S. aureus* [three MRSA], two *Stenotrophomonas maltophilia*, one *Klebsiella pneumoniae*) and resulted in antimicrobial changes/additions to two of five of those patients. Microscopy identified seven of seven microbiologically positive organisms and 64 of 66 negative samples compared with culture. Antimicrobial changes/additions would have occurred in three of seven microscopy-positive patients had those results been clinically available in 5 hours, including one patient diagnosed later with VAP despite negative mini-BAL cultures. Overall, automated microscopy was 100% sensitive and 97% specific for high-risk pneumonia organisms compared with clinical cultures suggesting that rapid microscopy-based surveillance may be



**Fig. 4** The Accelerate System. A cassette, reagent pack, and clinical sample are loaded into the analyzer. Following automated sample preparation, organism identification and antimicrobial susceptibility testing are performed. The results are available via the graphic user interface.

informative for treatment and antimicrobial stewardship in patients at risk for VAP. In addition, this system has been demonstrated to rapidly detect carbapenem resistance in *K. pneumoniae*, and, if present, predict if the resistance can be attributed to KPC carbapenemase.<sup>76</sup>

#### **Analysis of Exhaled Breath Condensate Fluid and Volatile Organic Compounds**

May et al employed a novel strategy for the rapid diagnosis of VAP utilizing exhaled breath condensate fluid (EBCF) obtained from heat moisture exchangers to provide a substrate for testing with PCR to identify bacterial DNA.<sup>77</sup> These investigators showed in critically ill surgical patients excellent concordance between pathogen identification using PCR of EBCF and pathogens isolated from BAL fluid using conventional microbiology techniques. Additionally, they found that increasing DNA load among serial EBCF samples preceded the clinical suspicion of VAP. The potential advantages of this type of diagnostic approach include noninvasive sampling of EBCF, ease of acquiring serial samples to potentially allow preemptive or targeted preventative treatment of early VAP or tracheobronchitis, and pathogen-specific characterization. The latter could help direct antibiotic therapy limiting the unnecessary use of broad-spectrum antibiotics for pathogens that are not identified, thus promoting antibiotic stewardship principles. The main disadvantage of this type of PCR-directed diagnostic approach is that it does not provide true antimicrobial susceptibility testing of the causative pathogens.

Volatile organic compound (VOC) detection is another promising diagnostic technology with probably the greatest

applicability in VAP. Both humans and bacteria produce VOCs (volatile carbon molecules) as part of their metabolism. The VOCs vary depending on disease states, growth environment, and the presence of other bacteria. This technology is particularly appealing to lung diseases, as it can be monitored noninvasively analyzing exhaled breath (similar to EBCF). Changes in VOC patterns can trigger an early workup and also can be monitored to assess response to treatment. Mass spectrometry can swiftly identify and quantify VOCs. New technologies like electronic noses and optical spectra systems can describe the VOC patterns or fingerprints of bacteria.<sup>78,79</sup> In a study that included 38 ventilated patients, electronic nose-derived VOC fingerprints showed good correlation with clinical pneumonia scores.<sup>80</sup> A recent study monitored 45 ventilated patients thrice weekly using electronic nose technology.<sup>81</sup> The obtained VOC fingerprints were able to differentiate between infected, colonized, and noninfected patients. The potential for VOC detection in diagnosing lung infections using either few specific biomarkers or the whole VOC fingerprint is currently being actively pursued.<sup>82,83</sup>

#### **Potential Limitations and Implications of Novel Diagnostics for VAP**

As suggested earlier,<sup>56</sup> experiences with rapid diagnostics for the evaluations of blood culture specimens suggest that rapid diagnostics may play an important role in enhancing antimicrobial prescribing practices in hospitalized patients. The benefits to this can be numerous, including optimizing

clinical outcomes, reducing toxicity, and facilitating clinical trials for new anti-infective agents by stratifying patients eligible for the trial at the earliest possible opportunity. However, it is also important to understand the limitations of these new technologies including that they cannot differentiate colonization from infection, which could be highly problematic in mechanically ventilated patients, nor give us the true susceptibility patterns of the responsible pathogens. The latter is true with the exception of a few specific mechanisms of resistance provided by the previously described molecular techniques and automated microscopy which has the potential to provide real susceptibility data.

Further illustrating the potential role of rapid diagnostics in improving antimicrobial therapy and outcome when embedded in a well-organized antimicrobial stewardship program is the study by Huang et al from the University of Michigan.<sup>84</sup> These investigators performed a quasi-experimental study to analyze the impact of MALDI-TOF MS in conjunction with an antimicrobial stewardship team intervention in patients with bloodstream infections.<sup>84</sup> The antimicrobial stewardship team provided antibiotic recommendations after receiving real-time notification following blood culture Gram stain, organism identification, and antimicrobial susceptibilities using conventional microbiology methods in the before-period and MALDI-TOF MS in the after-period. Use of MALDI-TOF MS significantly decreased time to organism identification, and improved time to effective antibiotic therapy as well as optimal directed antibiotic therapy. Mortality, length of ICU stay, and recurrent bacteremia were also lower during the intervention period. Similarly, the PCR-based GeneXpert MRSA/SA diagnostic platform (Cepheid, Sunnyvale, CA) was studied at the Veterans Affairs Medical Center in Houston demonstrating that for MSSA bacteremia, the mean time to initiation of appropriate therapy was reduced from 49.8 to 5.2 hours and the duration of unnecessary MRSA drug therapy was reduced by 61 hours per patient.<sup>85</sup> It is hoped that the application of rapid diagnostic methods to respiratory specimens could have a similar impact on patients with pneumonia including VAP.

It is clear that we are entering a new era in the management and treatment of serious infections such as VAP. Spellberg et al made a recent plea to change our current patterns of managing patients with proven and presumed infections to reverse the spiraling trend of antibiotic resistance that has occurred over the last century.<sup>86</sup> Within the next 3 to 5 years, new antibiotics directed against MDR Gram-negative bacteria, in addition to the recently approved ceftolozane-tazobactam and ceftazidime-avibactam, will likely become available, including carbavance, plazomicin, eravacycline, relebactam, brilacidin, BAL30072, aztreonam-avibactam, carbapenems with ME 1071, and S-649266—a novel siderophore cephalosporin. These agents can provide enhanced activity against  $\beta$ -lactamase producers, carbapenem-resistant bacteria, and in some cases even metallo- $\beta$ -lactamase-producing bacteria.

The challenge to ICU clinicians is how to most effectively utilize these agents once they become available to maximize patient benefits while minimizing the emergence of resistance (►Table 3). This is an especially important challenge in resource-limited countries that have often been at the forefront of the emergence of novel antimicrobial resistance mechanisms due to local patterns of antibiotic use. The use of rapid diagnostics may hold the key for achieving this important balance. There is an urgent need for clinical studies aimed at understanding how to best integrate the use of these new antibiotics with the emerging rapid diagnostic technologies in a way that is cost-effective and sustainable for the long run.<sup>87</sup> In addition, the microbiology laboratory must work closely with their clinical partners to deploy these new diagnostic tools in a manner that will afford the maximum benefit of these new technologies, including incorporation of the antimicrobial stewardship team and interpretative report comments, when applicable. Clinical outcome studies demonstrating the benefit of these new technologies on patient outcomes are needed. VAP may be an ideal infection to demonstrate the impact of rapid diagnostics as a means of enhancing antimicrobial treatment and stewardship.<sup>88</sup>

**Table 3** Characteristics of diagnostic methods for ventilator-associated pneumonia

Diagnostic method	Conventional culture time (h)	Pathogen/Biochemical identification time (h)	True antibiotic susceptibility available	Antibiotic susceptibility time (h)	Total diagnostic time (h)
Conventional culture method	24–36	n/a	Yes	12–24	36–72
BioFire/Luminex	n/a	2–4 <sup>a</sup>	No	n/a	2–4
PNA-FISH	n/a	2–4 <sup>a</sup>	No	n/a	2–4
AXDX ID/AST	n/a	2–4 <sup>a</sup>	Yes	3–6	6–10
VOC fingerprints	n/a	2–4 <sup>a</sup>	No	n/a	2–4

Abbreviations: FISH, fluorescence in situ hybridization; ID/AST, identification/antibiotic susceptibility testing via automated microscopy; n/a, not applicable; VOC, volatile organic compounds.

<sup>a</sup>Assumes direct specimen inoculation from respiratory samples including endotracheal aspirates and bronchoalveolar lavage samples.



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