

## Current concepts in diagnosis of pneumonia

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# Urgent need for a rapid microbiological diagnosis in critically ill pneumonia

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### ABSTRACT

Severe lower respiratory tract infection is a common issue in Intensive Care Units that causes significant morbidity and mortality. The traditional diagnostic-therapeutic approach has been grounded on taking respiratory samples and/or blood cultures as soon as possible and starting empirical antibiotic therapy addressed to cover most likely pathogens based on the presence of the patient's risk factors for certain microorganisms, while waiting for the culture results in the following 48-72 hours to adequate the antibiotic treatment to the sensitivity profile of the isolated pathogen. Unfortunately, this strategy leads to use broad-spectrum antibiotics more times than necessary and does not prevent possible therapeutic failures. The recent development of rapid molecular diagnostic techniques, based on real time polymerase chain reaction (RT-PCR), makes it possible to determine the causative agent and its main resistance pattern between 1 and 5 hours after sampling (depending on each technique), with high precision, some of them reaching a negative predictive value greater than 98%, facilitating the very early withdrawal of unnecessary broad-spectrum antibiotics. Its high sensitivity can also detect unsuspected pathogens based on risk factors, allowing adequate treatment in the first hours of stay. This short review discusses the potential usefulness of these techniques in critically ill patients with lower respiratory tract infection and advocates their immediate implementation in clinical practice.

**Keywords:** Rapid diagnostic tests, RT-PCR, Multiplex PCR, Xpert, critically ill, Lower respiratory tract infection, Community-acquired pneumonia, Hospital-acquired pneumonia, Ventilator-associated pneumonia, Antibiotic stewardship, Empirical treatment

### INTRODUCTION

Both community-acquired pneumonia (CAP) and hospital-acquired pneumonia (HAP), including ventilator-associated pneumonia (VAP), remain one of the leading causes of intensive care admissions or prolonged hospital stay and are related with substantial mortality.

Classically, when CAP/HAP/VAP are suspected, particularly in severe cases, promptly initiation of empirical antibiotics (very often two or more), based on the most likely involved pathogens, is recommended followed by de-escalation to a narrower spectrum pathogen-directed antibiotic once the causative agent has been isolated in microbiological culture [1-3]. However, this classical approach does not guarantee giving each patient the best antibiotic from the start, while many patients result overtreated and very few times a real early de-escalating strategy is implemented because the standard cultures are very frequently negative [4]. Over the last few years different rapid diagnostic tests (RDT) based on real-time polymerase chain reaction (RT-PCR) have emerged allowing to identify, in around 60 minutes, the etiologic agent and/or its main mechanism of resistance in a respiratory sample [5-9].

SARS-CoV-2 pandemic has highlighted the importance of implementing RDT capable of detecting the virus in a nasal-pharyngeal or respiratory sample [10], avoiding unnecessary antibiotics in many cases and this will be even more important when the pandemic ends as sporadic cases will come up. In fact, the use of multiplex RDT in respiratory samples reveals a significant number of viruses as etiological agents in CAP and these RDT detect 23.6% more pathogens than traditional culture techniques [11].

The potential utility of this new technology is enormous, particularly in severe cases of HAP/VAP, where it could allow not only giving the most appropriate antibiotic from the beginning, but also withdraw unnecessary drugs, preventing late resistance and adverse events. Implementing a new strategy

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of rapid testing would be the first step for a real antimicrobial stewardship program defined as "coordinated interventions designed to improve and measure the appropriate use of antimicrobials by promoting the selection of optimal antimicrobial drug regimen, dose, duration of therapy, and route of administration".

It is worth noting the importance of knowing very well the limitations of the particular RDT that is being used because those microorganisms not included in the panel, obviously cannot be ruled out. From this perspective, RDT should be considered as a complementary tool in adjunction to standard culture and clinical judgment to allow for an earlier pathogen-directed therapy.

Because some of these RDT have been developed to identify the most frequent CAP pathogens (viruses and bacteria), while others have been designed to detect microorganisms and bacterial resistance genes more commonly involved in HAP/VAP, this brief review will discuss how the implementation of these RDT could improve the correct daily use of antibiotics, saving unnecessary drugs, and potentially the outcome of severe CAP and HAP/VAP separately.

## COMMUNITY ACQUIRED PNEUMONIA

Among European adults, CAP has an annual incidence of 1.07–1.2 per 1000 person-years, rising to 14 per 1000 person-years in those older than 65 years [12]. In USA, CAP is estimated to cause ~1.5 million hospitalizations and ~100,000 deaths each year [13]. CAP-related mortality in those patients admitted to Intensive Care Units (ICU) was approximately 30% before the SARS-CoV-2 pandemic but it has gone up to 35–50% in COVID-19 patients who require invasive mechanical ventilation [3].

Although there is increased recognition of the role of viral pathogens in CAP, currently, the empiric antibiotic therapy for severe cases, is based on international guidelines [1] which recommend using a macrolide or a respiratory fluoroquinolone in combination with a  $\beta$ -lactam to cover the most frequent pathogens such as *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, methicillin-susceptible *Staphylococcus aureus* (MSSA), *Legionella* spp., *Chlamydia pneumoniae*, and *Moraxella catarrhalis*. The coverage for PES pathogens (*Pseudomonas aeruginosa*, *Enterobacteriaceae* with extended-spectrum  $\beta$ -lactamases -ESBL-, and methicillin-resistant *S. aureus* -MRSA-) should only be initiated if "risk factors" are present because of the low prevalence of these pathogens, but this decision is not always easy because failing with the initial empiric treatment has been associated to worst outcome [14]. A Spanish retrospective study [15] with 1.597 CAP patients reported a 6% incidence of PES pathogens. Other study found that enteric Gram-negatives, such as *P. aeruginosa*, can be isolated in up to 2% of identified CAP microorganisms and are usually present in patients with prior structural lung disease, those who are on corticosteroids, have recently received antibiotic therapy or are in septic shock at admission

[16]. Regarding MRSA, a multicenter, prospective surveillance study of 2.259 adults hospitalized with CAP, identified 1% with MSSA and 0.7% with MRSA. Chronic hemodialysis was more common among patients with MRSA (20%) than pneumococcal (2.6%) CAP. Nevertheless, clinical features at admission were similar, including concurrent influenza infection, hemoptysis, multi-lobe infiltrates, and prehospital antibiotics. Patients with MRSA had higher mortality (13.3% vs 4.4%) [17]. The Global initiative for MRSA pneumonia (GLIMP) study found a prevalence of confirmed MRSA in CAP patients of up to 3%, and MRSA was isolated mainly from patients with a prior MRSA infection or colonization, recurrent skin infections, or those with severe pneumonia [18].

However, the prevalence and risk factors for CAP related to MRSA may vary widely among regions. A study performed in the Pays de la Loire region in France [19] to determine the demographic characteristics of MRSA carriers in the community and to assess their risk factors and possible past hospitalization history, found 15% incidence rate of MRSA carriers. The isolates were most frequently recovered from skin and soft tissue infections (41.2%), urine (38.3%), genital samples (8.3%) and sputum (1.9%). Other pathological samples represented 10.3%, mainly from the ear-nose-throat sphere. Among the 313 patients who answered a questionnaire, 36 (11.5%) had none of the risk factors included in the questionnaire, such as home care, hospitalization during the preceding 12 months, and the presence of chronic cutaneous lesions.

In a systematic review and meta-analysis of Asia-Pacific region [20] the ranges of prevalence and characteristics associated with CAP-MRSA carriage varied from India (16.5%–23.5%), followed by Vietnam (7.9%) and Taiwan (3.5%–3.8%).

Because of the difficulties to predict the etiologic agent in severe CAP, some scores have been proposed to guide the empiric treatment, such as the PES score [15] (Table 1).

The decision to empirically treat these pathogens should be reserved for patients at high risk (i.e., PES score  $\geq 5$  points).

Table 1	PES score
Variables	Points
Age > 65 years	1
Male	2
Previous antibiotic use	2
Chronic respiratory disorder	2
At Emergency	
Consciousness impairment or aspiration evidence	2
Fever or shivers	-1

Low risk Multi Drug Resistant (MDR) score:  $\leq 1$ ; Medium risk MDR score: 2–4; High risk MDR score:  $\geq 5$ . PES (*Pseudomonas aeruginosa*, *Enterobacteriaceae* extended spectrum  $\beta$ -lactamase-positive, and methicillin-resistant *Staphylococcus aureus*).

However, the clinician's fear of failing in the initial treatment of severe CAP, particularly in those patients in shock, leads to overuse broad-spectrum antibiotics such as antipseudomonal  $\beta$ -lactams, vancomycin or linezolid. In the aforementioned study of 2,259 hospitalized adults with CAP [17], besides the very low prevalence of MRSA (0.7%), almost a third of the patients received anti-MRSA antibiotics. Therefore, there is an urgent need to improve this strategy.

Implementing RDT in the initial approach of severe CAP could contribute to save broad spectrum antibiotics, ruling out MRSA even in those patients with high PES score where *S. pneumoniae* is a frequent causative microorganism. Conversely, a few patients in shock and multiorgan failure with low PES score might benefit from a RDT because, although very unlikely, the impact of not treating a potential MRSA within the first hours would be detrimental. This may be particularly useful in regions with high prevalence of community MRSA carriers.

Very interestingly, a study performed in 212 hospitalized adult patients with CAP in Taiwan, showed a greater number of etiological agents identified when RDT were used. Bacterial pathogens were detected in 106 (50%) patients, viruses in 77 (36.3%), and fungal pathogens in 1 patient (0.5%). The overall detection rate (culture and molecular testing method) was 70.7%. Traditional microbial culture yielded positive results only in 36.7% while molecular testing in 61.3%. The most common pathogens were influenza (16.1%), *Klebsiella pneumoniae* (14.1%), *P. aeruginosa* (13.6%), human rhinovirus (11.8%), and *S. pneumoniae* (9.9%). Multiple pathogen co-infections accounted for 28.7%, of which co-infection with *K. pneumoniae* and human rhinovirus comprised the largest proportion [11].

Several studies performed in patients with lower respiratory tract infections (LRTIs) consistently find that microbiological documentation is almost twice as high using RDT compared to the standard method due to the higher sensitivity of the RDT. This is an advantage for patients treated with antibiotics prior to sampling but it also needs a cautious interpretation because RDT might detect nucleic acids from dead pathogens not involved in the current pneumonia episode leading to an overtreatment of non-viable microorganisms. Bearing these limitations in mind, different studies, most of them observational/retrospective, show that empirical treatment when RDT are used in LRTIs might be modified more than 50% of the time, mainly to de-escalate, although there are too few studies in CAP, particularly in severe cases, to draw robust conclusions about the impact of routinely RDT use on outcome (Table 2).

Gadsby NJ et al [21] studied respiratory samples from 323 adults with radiologically confirmed CAP. Specimens were cultured as per routine practice and also tested with fast multiplex real-time PCR assays for 26 respiratory bacteria and viruses. *H. influenzae* and *S. pneumoniae* were the most frequently agents detected. Viruses were present in 30% of cases; 82% of these were codetections with bacteria. Most (85%) patients had received antimicrobials in the 72 hours before admission. Of these, 78% had a bacterial pathogen identified by PCR but only 32% were culture-positive ( $P < .0001$ ). PCR detected sig-

nificantly more *H. influenzae*, *S. pneumoniae*, *M. catarrhalis*, *S. aureus*, *E. coli*, and *K. pneumoniae* than standard culture-based methods. Molecular testing results were not released to the attending physician, but they could have had the potential to lead to de-escalation in number and/or spectrum of initial empirical antibiotic agents in 247 (77.2%) patients and to escalate in number and/or spectrum of antibiotic in 19 (5.9%) patients. The majority of the potential de-escalation events were related to switching from amoxicillin-clavulanate to narrower-spectrum agents such as amoxicillin and doxycycline in cases where *S. pneumoniae* or *H. influenzae* were detected by PCR and to withdraw clarithromycin in cases where atypical bacteria were not identified by PCR.

Quite similar results were found by Monard C et al [22]. They retrospectively studied 150 pneumonia episodes (54 CAP, 68 HAP, 37 VAP). In 37 out of 54 (69%) CAP episodes an expert committee considered the empirical treatment could change, mainly to deescalate to a narrower spectrum drug or stopping a companion antibiotic (37%) but also to escalate in 15% of the time.

Clinical metagenomics uses next generation sequencing of total nucleic acid from clinical samples to detect all the microbes simultaneously. The routinely application of this technology is still being validated but nanopore sequencing platform (Nanopore, Oxford, UK) has proven its ability to rapid LRTI pathogen detection [23]. Mu S et al [24] evaluated the clinical performance of rapid nanopore-sequencing based metagenomics test for diagnosis of bacterial pathogens in LRTIs. Among six different presentations of LRTIs, 171 bronchoalveolar lavage fluid (BAL) and 121 sputum samples were collected from 292 hospitalized patients. The turnaround time (from sample registration to result) for the rapid metagenomics test was  $6.4 \pm 1.4$  hours, compared to  $94.8 \pm 34.9$  hours for routine culture. Compared with culture and real-time PCR validation tests, rapid metagenomics achieved 96.6% sensitivity and 88% specificity and identified pathogens in 63 out of 161 (39.1%) culture-negative samples. Among those most common pathogens (*S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*), rapid metagenomics detected 37 cases while traditional methods identified only 13 cases. Interestingly, rapid metagenomics detected 38 anaerobic bacterial species in 49% samples while none of them were identified by culture techniques. Although these results must be cautiously interpreted because some of the anaerobic bacteria may be contaminants from the upper respiratory tract, correlation between enriched anaerobes and lung abscess was observed by Gene Set Enrichment Analysis. If the results of the metagenomics test had been used to guide therapy, 33 patients might have had their empiric therapy de-escalated compared to 1 using standard culture. This new technology can be very helpful in cases of aspiration pneumonia and lung abscesses, in addition to critical patients with unexplained respiratory failure or those immunocompromised patients who are infected by uncommon pathogens not covered by conventional methods.

The study published by Qian Y et al [25] tested, with the FilmArray Respiratory Panel, respiratory samples from 112 hos-

**Table 2** Potential implications of rapid diagnostic tests on the CAP empirical treatment

Author	Study type	Aim	Population	Assay	Result
Monard C et al.[22]	Retrospective multicenter, in 4 French university hospitals	Relevance of rapid multiplex PCR test to guide antimicrobial therapy.	150 pneumonia episodes (CAP = 54, HAP = 68, VAP = 37)	Rapid multiplex PCR	Proportion of potential antibiotic modifications: in VAP (87%), in HAP (79%) and CAP (69%)  Modification of the empirical treatment for CAP: de-escalation in 37%, escalation in 15%, no change in 32%, undetermined in 17%.
Shengchen D et al.[28]	Single center randomized controlled study in China	To evaluate duration of IV antibiotic, LOS, cost of hospitalization and de-escalation.	800 patients with LRTI 398 allocated to POCT and 402 to standard RT-PCR	FilmArray Respiratory Panel as POCT vs Routine RT-PCR	Reduce IV antibiotic use, LOS and costs in hospitalized patients. More patients in the intervention group achieve de-escalation.
Gadsby NJ et al.[21]	Retrospective, in two United Kingdom hospitals	Utility of comprehensive molecular diagnosis approach.	323 CAP patients	Fast multiplex PCR for 26 pathogens	De-escalation in number and/or spectrum in 77% of patients, escalation in 5.9% and no change in 16.9%.
Huang AM et al.[29]	Observational study in 8 United States hospitals	Potential impact on modifications to antimicrobial therapy.	LRTI Respiratory samples (57 BAL, 48 sputum) from unique patients	FilmArray LRTI Panel compared to SOC methods including bacterial culture and PCR based on standard laboratory	The most common type of potential intervention was antimicrobial de-escalation in > 50% of patients, using FilmArray LRTI Panel
Qian Y et al.[25]	Single center, prospective cohort in China compared with a previously not tested cohort	Clinical impact of FilmArray on unexplained pneumonia compared with conventional methods.	Unexplained pneumonia (67.4%CAP, 32.6% HAP): 112 patients prospectively tested vs 70 as control group	FilmArray Respiratory Panel	Significantly lower antibiotic/antifungal use in the intervention group.  Significant higher antiviral treatment in the interventional group.
Mu S et al.[24]	Single center, prospective cohort in China	To evaluate the clinical performance of a commercial rapid metagenomics test.	292 LRTI (51% in ICU: CAP = 83 HAP = 66 Other = 143)	Rapid nanopore-sequencing metagenomics test	Hypothetical impact of metagenomics test proposed antibiotic de-escalation
Maataoui N et al.[26]	Single center, observational and retrospective study in Paris (France)	To evaluate the performance and the impact of the BioFire FilmArray Pneumonia plus panel	112 respiratory samples from 67 COVID-19 ICU patients suspected of bacterial coinfections	BioFire FilmArray Pneumonia Panel Plus	Modification of treatment in 50%. Positive tests led to antibiotic initiation or adaptation in 15% of episodes and de-escalation in 4%. When negative, 28% of episodes remained antibiotic-free (14% no initiation, 14% withdrawal).
Verroken A et al.[27]	Single center, prospective cohort in Belgium	To investigate the respiratory co-infection rate in COVID-19 critically ill and its impact on antibiotic management.	32 ICU COVID-19 patients	FilmArray Pneumonia Panel Plus Test (FA-PNEU)	Speeded-up antibiotic modification in 46,9% of patients.

CAP: community acquired pneumonia, LRTI: lower respiratory tract infection. HAP: hospital acquired pneumonia. VAP: ventilator associated pneumonia. LOS: length of stay. IV: Intravenous. POCT: point-of-care test. RT-PCR: real-time polymerase chain reaction. ICU: Intensive Care Unit. SOC = Standard of care

pitalized patients with unexplained pneumonia (75 CAP and 37 HAP) between October 2016 and March 2018. The most frequently found pathogens were Influenza A/B (47.3%). They recorded the demographic characteristics of these patients and their clinical data and were compared with a historical control cohort of 70 patients, who were hospitalized between October

2014 and March 2016, using the same inclusion criteria. The interventional group received significantly more antiviral treatment (oseltamivir and acyclovir) and the adjustment of antibiotics was recorded more frequently (69.6% vs 5.1%) compared to control group. This study suggests again, although with important limitations as a result of its design, that the RDT may

assist in clinical decision making, reducing unnecessary antibiotic usage in the treatment of pneumonia and starting earlier specific treatment in some cases.

Finally, special mention deserves COVID-19 pandemic where an accurate use of the antibiotic treatment, particularly in critically ill patients, is challenging. In this clinical setting, RDT can help improve antibiotic stewardship programs.

A French study [26] tested 112 respiratory samples from 67 COVID-19 ICU patients suspected of having bacterial coinfections with the BioFire® FilmArray® Pneumonia plus Panel. Among the 8 suspicions of CAP, for which all patients were treated, the positive RDT result led to a de-escalation and the 7 negatives to 3 antibiotic withdrawals and 4 continuations. Regarding the 104 suspected episodes of HAP/VAP, 36 RDT results were positive and 68 were negative. Among positives, in 36% (13/36) antibiotic treatment was initiated, in 8% (3/36) antibiotic therapy was modified, and in 4 (11%) was de-escalated. In one episode, neither the pre nor the post RDT, antibiotic treatment was adequate because of the presence of an unexpected *Stenotrophomonas maltophilia* not identified by the panel. Among negatives, 24% (16/68) remained antibiotic-free and 13 (19%) led to antibiotic withdrawal. Although in 57% (39/68) episodes, antibiotics were maintained due to severe sepsis ( $n = 20$ ), infection from another site ( $n = 9$ ), continuation of previous treatment ( $n = 7$ ), or severely immunocompromised patients ( $n = 3$ ), RDT produced antibiotic changes in 38/112 (34%) episodes.

Other interesting study performed in 32 COVID-19 ICU patients identified 13 (40.6%) cases with a bacterial co-infection [27]. The most frequently identified bacteria with significant genome copies were *S aureus* (one of them MRSA), *H. influenza*, and *M. catarrhalis*. None of the 32 RDT identified atypical bacteria neither other respiratory viruses. Direct communication of RDT led to speeded-up antibiotic modifications in 15/32 (46.9%) patients. Once again, the use of RDT reveals to be a key element of the antimicrobial stewardship strategy in COVID-19 severe disease.

## HOSPITAL ACQUIRED PNEUMONIA AND VENTILATOR ASSOCIATED PNEUMONIA

Lower respiratory tract infections (LRTIs) such as HAP/VAP are associated with a significant increase in morbidity and mortality, even higher when effective antibiotic treatment is delayed [30]. Choosing the adequate empiric antibiotic for HAP/VAP is more challenging than for CAP because of the increased number of MRSA and potential difficult to treat antibiotic-resistant Gram-negative pathogens.

International guidelines advocate the empirical use of broad-spectrum antibiotics including carbapenems, when the patient has risk factors for MDR pathogens, such as previous colonization by MDR pathogens, has previously received antibiotics, or VAP develops after 5 days on mechanical ventilation. Furthermore, in those patients in ICUs where >10% of gram-negative isolates are resistant to an agent being consid-

ered for monotherapy, patients in an ICU where local antimicrobial susceptibility rates are not available, patients who are in septic shock at time of VAP, suffered ARDS preceding VAP, or they were receiving acute renal replacement therapy prior to VAP onset, should be empirically receiving 2 antipseudomonal antibiotics from different classes and anti-MRSA coverage [2]. Unfortunately, these risk factors are very common among critically ill patients and this strategy leads to overtreatment without ensuring full adequacy due to the potential MDR carbapenemase-producing pathogens.

Implementing a new strategy based on RDT might reduce the uncertainty of the empirical treatment, optimizing the antimicrobials stewardship programs in this setting (Table 3).

Interestingly, based on the high negative predictive value (NPV) of MRSA nasal colonization for developing MRSA pneumonia, some hospitals have implemented a protocol, as part of the antimicrobial stewardship program, to inform the staff about the usefulness of testing some patients for nasal MRSA before prescribing anti-MRSA in cases of pneumonia and to consider withdrawal of MRSA coverage when the RDT result is negative. In a normal basis, the RDT result is displayed in the patient's electronic medical record and then the attending physician can consider stopping anti-MRSA antimicrobials following the protocol, unless other indication to keep them exists. Furthermore, the clinical pharmacist can order MRSA nasal PCR testing without a direct physician order when a patient receives a prescription of vancomycin or linezolid for suspicion of pneumonia. Once this protocol was implemented, a retrospective study was designed to evaluate the impact of a pharmacist-initiated MRSA nasal PCR protocol (PCR group) on pneumonia therapy compared with a routine schedule (Pre-PCR group). In the Pre-PCR group, 138 patients met the inclusion criteria, while 72 patients were included in the PCR group. There were no significant differences between the 2 groups except for higher ICU admission in the Pre-PCR group and more cases of HAP in the PCR group. There were no VAP cases in either study group. All patients eligible for study received vancomycin. Compared with the Pre-PCR group, the mean duration of IV vancomycin in the PCR group was 1.1 days shorter ( $2.5 \pm 1.3$  days vs  $1.4 \pm 1.2$  days,  $P < .001$ ). Among the 72 patients in the PCR group, 45 (62.5%) MRSA nasal PCR orders were placed by a clinical pharmacist while the remainder were ordered by an attending physician. There were 63 (87.5%) patients with a negative MRSA nasal PCR result, and 56 (88.9%) patients had their vancomycin order discontinued within 24 hours of the negative result. The mean total LOS was similar between groups. No differences were observed in clinical outcomes and adverse events between groups [31].

Other retrospective study aimed to evaluate the analytical performance of the MRSA/SA SSTI assay for rapid detection of MRSA in LRT specimens and its potential role in antimicrobial stewardship [32]. They prospectively analyzed in 100 respiratory specimens, from patients with VAP, the performance of the test. Xpert MRSA/SA identified MRSA in 5 of 6 specimens positive by standard-of-care culture, (sensitivity 83.3%). The false negative was a BAL specimen. Interestingly, Xpert MRSA/SA de-

**Table 3** Potential implications of rapid diagnostic tests on the HAP/VAP empirical treatment

Author	Study type	Aim	Population	Assay	Result
Pham SN et al.[31]	Single center, retrospective, quasi-experimental (Pre-PCR vs PCR) study, in United States	To evaluate the impact of a pharmacist-initiated MRSA nasal PCR protocol on pneumonia therapy	210 patients: 138 Pre-PCR and 72 PCR, mainly in HAP	MRSA nasal PCR test	Compared with the Pre-PCR group, mean duration of IV vancomycin in the PCR group was 1.1 days shorter ( $2.5 \pm 1.3$ days vs $1.4 \pm 1.2$ days, $P < .001$ ). The median number of doses of IV vancomycin in the Pre-PCR group was 3 doses (IQR: 2-4) versus 1 dose (IQR: 1-2) in the PCR group ( $P < .001$ ).
Trevino SE et al.[32]	Single center, retrospective, in United States	To evaluate the analytical performance of the MRSA/SA SSTI assay for the rapid detection of MRSA in LRT specimens and its potential role in antimicrobial stewardship	100 specimens from VAP	GeneXpert® MRSA/SA	Potential reduction of free antibiotic days by 68.4% for vancomycin and by 83% for linezolid
Monard C et al.[22]	Observational and Retrospective Multicenter	Number of pneumonia episodes in which PCR-guided therapy differed from empirical therapy.	150 pneumonia episodes (CAP = 54, HAP = 68, VAP = 37)	BioFireFilmArray® Pneumonia plus Panel	Proportion of potential antibiotic modifications in VAP 87%, in HAP 79%
Buchan BW et al.[33]	Observational in 8 US clinical centers	To examine the potential impact of the BioFire® FilmArray Pneumonia Panel Test on antibiotic utilization.	259 samples BAL (n=237) or mini-BAL (n=22) from HAP and VAP	BioFire® FilmArray Pneumonia Panel test	Potential adjustment in 70,7% of patients, including discontinuation or de-escalation in 48,2%.
Peiffer-Smadja N et al.[34]	Prospective in 3 ICUs of one French academic hospital	We assessed the performance and the potential impact of the M-PCR on the antibiotic therapy of ICU patients.	95 clinical samples from 85 HAP or VAP patients (72 BAL and 23 PTC)	Unyvero Hospitalized Pneumonia (HPN, Curetis)	Expert panel: the RT-mPCR could have led to antibiotic changes in 66% episodes. Earlier initiation of an effective antibiotic: 21%, early de-escalation: 39%, and optimization: 3%. Among 17 empirical treatments with carbapenems, 10 could have been de-escalated
Pickens C et al.[35]	Retrospective in 4 hospitals of United States	To predict the impact of Unyvero LRT Panel results on adjustment of empiric antibiotic regimens.	659 hospitalized patients with LRTI	Unyvero Lower Respiratory Tract Panel	The LRT Panel result predicted no change in antibiotics in only 12.4%. In 65.9% of patients the results favored de-escalation (69% had unnecessary MRSA coverage and 64% had unnecessary P aeruginosa coverage).
Posteraro B et al.[36]	Prospective in a large university hospital in Italy	Changes to targeted and/or appropriate antimicrobial therapy	212 respiratory samples from 150 COVID-19 patients mechanically ventilated HAP, VAP	FilmArray® Pneumonia plus Panel	Panel results allowed initiating or changing organism-targeted antibiotics in 118 (98.3%) of 120 episodes

LRTI: lower respiratory tract infection. CAP: community acquired pneumonia. HAP: hospital acquired pneumonia. VAP: ventilator associated pneumonia. LOS: length of stay. IV: Intravenous. POCT: point-of-care test. RT-PCR: real-time polymerase chain reaction. ICU: Intensive Care Unit. SOC = Standard of care. BAL: bronchoalveolar lavage TA: tracheal aspirate, BW: bronchial washing. PTC: plugged telescoping catheter. IQR: interquartile range

tected MRSA in five specimens where MRSA was not recovered by routine culture: specificity 94.7%, PPV 50%, and NPV 98.9%. In order to study the potential impact of this findings on the antibiotic use, the clinical data of the patients were obtained from clinical data repository, including microbiological culture results as well as antimicrobials consumption. They found that 96 patients received vancomycin and/or linezolid. Those four subjects who did not receive these agents were negative for MRSA based on both Xpert MRSA/SA and culture. If the anti-MRSA agent had been discontinued one calendar day after a negative RDT result in patients without any additional culture or PCR results positive for MRSA (including surveillance swabs), the vancomycin total antibiotic-days would have decreased by 68.4% (512 days) to a mean duration of 2.7 days, and linezolid by 83% (253 days) to a mean duration of 1.9 days.

In the aforementioned retrospective study of 150 pneumonia episodes (54 CAP, 68 HAP and 37 VAP) an expert committee considered that in HAP cases antibiotics should have been de-escalated 37% and escalated 27% of the times, while in VAP even 49% might have been de-escalated and 24% should have been escalated according to the RDT results [22]. Of course, it is a retrospective study and the clinical impact of having prescribed the antibiotic according with the RDT results is unknown but it shows that it could be a very useful complementary tool for saving antibiotics.

The potential impact of the BioFire® FilmArray® Pneumonia Panel Test on antibiotic utilization has also been studied in 259 BAL samples from patients with HAP/VAP [33]. This RDT showed 96.2% positive agreement and 98.1% negative agreement for the qualitative identification of 15 bacterial targets compared to standard bacterial culture. Viral targets were identified by this RDT in 17.7% of specimens tested, of which 39.1% were detected in conjunction with a bacterial target. A review of patient medical records, including clinically prescribed antibiotics, revealed the potential for antibiotic adjustment in 70.7% of patients based on the RDT result, including discontinuation or de-escalation in 48.2% of patients, producing an average saving of 6.2 antibiotic days/ patient. It is worth noting that molecular tests for viral pathogens were clinically ordered for only 93/259 (35.9%) BAL samples submitted for bacterial culture and included primarily multiplexed respiratory panel tests. At least one viral target was detected by the RDT in 46/259 (17.7%) BAL specimens, either alone or in addition to bacterial targets. Only 11/46 (23.9%) specimens with a positive viral detection by the RDT had a clinician-ordered molecular test for viral pathogens. Although the role of viral pathogens is not well established in HAP/VAP, there were 7 BAL specimens positive for influenza A/B virus. Early identification of these agents might have led to prescribe specific antivirals that could have shortened the duration or severity of the episode.

Another study tested the usefulness of the Unyvero Hospitalized Pneumonia (HPN, Curetis) platform for potential optimization of broad-spectrum antibiotics in 95 clinical samples from 85 ventilated HAP or VAP patients [34]. This panel is another RDT able to detect 21 bacteria and 19 resistance genes on respiratory samples within 5 hours. A total of 90/112 bac-

teria were detected by this RDT with a sensitivity of 80% and specificity of 99%. The sensitivity was better for Gram-negative bacteria (90%) than for Gram-positive cocci (62%). There were 14 bacteria detected by this RDT that were not found in conventional cultures and 5/8 ESBL (CTX-M gene) and 4/4 carbapenemases genes (3 NDM, one oxa-48) were also identified. This RDT could have led to the earlier initiation of an effective antibiotic in 20/95 patients (21%) and to early de-escalation in 37 patients (39%) but could also have led to one (1%) inadequate antimicrobial therapy. Among 17 empiric antibiotic treatments with carbapenems, 10 could have been de-escalated in the following hours according to the RDT results. This RDT also identified 2 unexpected cases of severe legionellosis confirmed by culture methods. This study is another example of how RDT could make an impact in a better adequacy of antimicrobials.

This very RDT was used in another retrospective study on 659 hospitalized patients for microbiological diagnosis of suspected pneumonia [35]. Similar results to the previous study were found with an overall sensitivity of 85.7%, specificity of 98.4% and a NPV of 97.9%. According with the RDT result only 12.4% of cases did not need a change in prescribed antibiotics. Reassured by the excellent NPV of this RDT panel, the authors determined that if MRSA or *P. aeruginosa* were not detected by the panel, then anti-MRSA and/or anti-pseudomonal therapies were not indicated. Accordingly, antibiotic de-escalation was recommended in 65.9% (405/615) of patients, of whom 278/405 (69%) had unnecessary MRSA coverage and 259/405 (64%) had unnecessary *P. aeruginosa* coverage.

Finally, it is worth mentioning a prospective study performed in 150 COVID-19 patients mechanically ventilated with the Film Array Pneumonia Plus panel. A total of 212 samples were processed for standard culture and tested with the RDT from 150 patients suspected of bacterial pneumonia. The RDT results were immediately accessible to ICU clinicians for antimicrobial therapy management. Etiologically, 120 samples were positive and 90 were negative by both methods. RDT detected no culture-growing organisms (mostly *S. aureus* or *P. aeruginosa*) in 19 of 120 samples or antimicrobial resistance genes in two culture-negative samples for *S. aureus*. Fifty-nine (27.8%) of 212 samples were from empirically treated patients. Antibiotics were discontinued in 5 (33.3%) of 15 patients with RDT negative samples and were escalated/deescalated in 39 (88.6%) of 44 patients with RDT positive samples. Overall, antibiotics were initiated in 87 (72.5%) of 120 pneumonia episodes and were not administered in 80 (87.0%) of 92 no-pneumonia episodes. Antimicrobial-resistant organisms caused 78 (60.0%) of 120 episodes. Authors concluded that RDT in LRT samples may become indispensable for the clinical and therapeutic management of VAP or non-VAP episodes in ICU patients with COVID-19.

## CONCLUSIONS

In agreement with the public policy document that the Infectious Diseases Society of America (IDSA) published a few years ago, declaring that, in order for tests to have a positive

impact on patient care, new tests need to provide information about the causative organism, including antimicrobial susceptibility/resistance information, if possible, and must have rapid results, ideally within 1 h [37], we have seen how these RDT are now a real useful tool, complementary to clinical judgment in the treatment of LRTI. In the difficult decision-making process of treating a critical patient with CAP/HAP/VAP accurately and promptly, the classic strategy based on risk factors is no longer justified because it leads to the excessive use of broad-spectrum antibiotics while potential pathogens are undetected. A great educational effort must be made among intensivists and microbiologists to implement these new rapid diagnostic tests into clinical practice because the positive impact on patient care can only be achieved if physicians act quickly upon the results and start adequate or stop inadequate antibiotics in these critically ill patients with little room to fail.

It is highly important to know very well the limitations of the particular RDT that is being used because those microorganisms or mechanisms of resistance not included in the RDT cannot be ruled out.

## CONFLICTS OF INTEREST

Authors declare no conflicts of interest

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