Critical Care

REVIEW

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The role of rapid multiplex molecular syndromic panels in the clinical management of infections in critically ill patients: an experts-opinion document

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Abstract

Rapid multiplex molecular syndromic panels (RMMSP) (3 or more pathogens and time-to-results < 6 h) allow simultaneous detection of multiple pathogens and genotypic resistance markers. Their implementation has revolutionized the clinical landscape by significantly enhancing diagnostic accuracy and reducing time-to-results in different critical conditions. The current revision is a comprehensive but not systematic review of the literature. We conducted electronic searches of the PubMed, Medline, Embase, and Google Scholar databases to identify studies assessing the clinical performance of RMMSP in critically ill patients until July 30, 2024. A multidisciplinary group of 11 Spanish specialists developed clinical questions pertaining to the indications and limitations of these diagnostic tools in daily practice in different clinical scenarios. The topics covered included pneumonia, sepsis/septic shock, candidemia, meningitis/encephalitis, and off-label uses of these RMMSP. These tools reduced the time-to-diagnosis (and therefore the time-to-appropriate treatment), reduced inappropriate empiric treatment and the length of antibiotic therapy (which has a positive impact on antimicrobial stewardship and might be associated with lower in-hospital mortality), may reduce the length of hospital stay, which could potentially lead to cost savings. Despite their advantages, these RMMSP have limitations that should be known, including limited availability, missed diagnoses if the causative agent or resistance determinants are not included in the panel, false positives, and codetections. Overall, the implementation of RMMSP represents a significant advancement in infectious disease diagnostics, enabling more precise and timely interventions. This document addresses relevant issues related to the use of RMMSP on different critically ill patient profiles, to standardize procedures, assist in making management decisions and help specialists to obtain optimal outcomes.

Keywords Infection, Critically ill patient, Syndromic molecular diagnosis, Multidrug resistant pathogens, Septic shock, sepsis, bloodstream infection

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Introduction

Inadequate empirical antimicrobial therapy in critically ill patients has been associated with worse outcomes, including increased in-hospital mortality and morbidity, prolonged hospital stays, and higher healthcare costs [1, 2]. Co-morbidities and the infecting pathogens significantly impact infection severity, leading to severe outcomes [1]. Optimal antibiotic use in ICUs is essential, as 30–60% of antibiotics prescribed are unnecessary or inappropriate [2], and at least 20% of patients experience adverse effects [3]. While timely treatment is vital for sepsis survival, bacterial resistance complicates effectiveness, contributing to higher mortality [4, 5]. The rise of multi- and pan-drug-resistant pathogens complicates empirical antibiotic selection, often leading to combinations that increase toxicity, costs, and resistance [6, 7]. Since the FDA approved the first respiratory syndromic panel in 2011 [8], molecular syndromic panels have been used to improve diagnosis and reduce unnecessary antibiotic use by guiding targeted therapy [9, 10]. Although molecular testing can improve pathogen identification and treatment tailoring and occasionally may replace conventional culture procedures in gastrointestinal infections, it must not replace conventional culture procedures in other scenarios, particularly when phenotypic susceptibility testing is needed. Interpreting molecular syndromic panel results can be complex, as detecting a target does not confirm it as the causative agent, and genotypic resistance markers may not always reflect in vivo expression, causing discrepancies with phenotypic susceptibility [11, 12]. The SARS-CoV-2 pandemic led to widespread use of these panels in clinical microbiology, especially for critically ill patients, where their use should be justified. This document reviews commercially available FDA and/or CE-marked Rapid Multiplex Molecular Syndromic Panels (RMMSP), capable of detecting multiple pathogens (at least five), including bacteria, viruses, fungi, and/or parasites most frequently associated with specific clinical syndromes (e.g., respiratory infections, meningitis, or gastrointestinal diseases), and for some platforms, genotypic markers of antimicrobial resistance with results provided in less than 6 h [13]. In addition, due to the general self-limiting nature of infectious gastrointestinal disease in the majority of cases, these were not included in our evaluation. The goal was to provide updated, practical information on their use, diagnostic timing, and management of infections in critically ill patients, including pneumonia, bacteremia, meningitis, and off-label applications of syndromic panels.

Methods

On January 29, 2024, an expert-panel meeting was held to discuss the need for a comprehensive review on the use of RMMSP in diagnosing infectious diseases in critically ill patients. Panel members were selected based on their expertise from the Study Group on Infection in the Critically Ill Patient (GEIPC-SEIMC) and the Infectious Diseases and Sepsis Task Force (GTEIS-SEMICYUC) of the Spanish Society of Clinical Microbiology and Infectious Diseases and the Spanish Society of Critical Intensive Care Medicine and Coronary Care Units. The panel identified key topics concerning the indications and limitations of RMMSP in daily clinical practice.

Search strategy

A systematic literature review was conducted on July 30, 2024, utilizing databases such as PubMed, EMBASE, Web of Science, and the Cochrane Library. The search included the keywords ["Molecular syndromic platforms" OR "molecular syndromic testing" OR "Syndromic panels"] AND ["Critically ill patients" OR "Severe infections" OR "Multidrug resistance" OR "septic shock" OR "sepsis"]. Filters were applied to include studies on humans and publications in English, French, Italian, Portuguese, or Spanish, and to limit the review to articles published within the past 10 years. The search was limited to studies published in the past 10 years, as most commercially available rapid tests began to emerge around 2012 [14]. However, some older references were also included due to their significance in the initial development and implementation of this technology.

To ensure a comprehensive review, reference lists from the included studies were manually searched for additional relevant publications not captured in the initial search. The selection process involved two phases: screening titles and abstracts, followed by a full-text review to confirm eligibility. Studies that assessed the use of molecular syndromic platforms in diagnosing severe infections, particularly focusing on multidrug resistance, sepsis, or septic shock in critically ill patients, were included. Animal models, in vitro studies, editorials, and articles without clinical data applicable to patient care were excluded. The study selection was conducted by two independent reviewers to minimize bias, with disagreements resolved by consensus. Covidence software was used for deduplication, organization, and management of the articles [15].

Results

Molecular syndromic panels to diagnose infectious diseases in critically ill patients: general considerations

Time until microbiological diagnosis and minimum diagnostic standards for therapeutic adequacy.

The key point of a rapid diagnosis is administering the correct antibiotic treatment at the right time, through the appropriate route, and with the necessary dose to control the infection and symptoms. Additionally, it involves adjusting or discontinuing treatment when it is no longer needed [8].

While traditional culture-based techniques remain the standard, molecular methods, such as nucleic acid amplification, are rapid and can provide results in a few hours [16–19]. However, its implementation requires adequate technical and organizational resources.

Although clinical microbiology laboratories have maintained their fundamental mission, they have undergone significant transformation, largely driven by molecular diagnostics [20, 21]. Indeed, RMMSP have revolutionized the management of infectious diseases, expanding their impact from routine cases in everyday practice to rare conditions handled by specialists [22–24].

Syndromic molecular panels

Introduction of RMMSP in daily practice capability facilitates timely clinical management decisions, such as hospital admission, isolation, and initiation or avoidance of antimicrobial treatment [25–27].

These panels are tailored to screen for the most common microorganisms associated with specific clinical syndromes such as bloodstream infection, meningitis/encephalitis, gastrointestinal infection, respiratory tract infection, joint infection, urinary tract infection, and sexually transmitted diseases [26–29].

Some RMMSP, like those for bloodstream infection and lower respiratory tract infection, have the capability to detect genotypic markers of β-lactam resistance, such as genes encoding main carbapenemases (*bla*KPC, *bla*NDM, *bla*VIM, *bla*OXA-48), extended spectrum β-lactamases (ESBL) from the $bla_{\text{CTX-M}}$ group, methicillin resistance in *Staphylococcus aureus* (*mecA*), or glycopeptide resistance in *Enterococcus* spp. (*van A/vanB*) [30, 31].

Commercially available RMMSP typically offer turnaround times of 1–4.5 h. Overall, they enhance microbial detection compared to standard procedures like bacterial culture, potentially speeding up pathogen identification and treatment initiation [8, 20, 21, 26–29]. RMMSP, when combined with antimicrobial stewardship efforts, play a crucial role in clinical decision-making, however, although some recent publications have shown promising results, their impact on patient clinical and economical outcomes requires further investigation through studies with large sample sizes and preferably clinical trial designs [21, 32].

RMMSP offer mainly qualitative results, though some provide (semi)quantitative values for specific bacterial targets, and some are suitable for point-of-care testing [33].

Proper interpretation of PCR detection results is challenging due to the test's ultra sensitivity. This precision enables detection of pathogens or resistance mechanisms, even when present only as colonizers, which may lead to unnecessary treatments. Literature reports overdiagnosis rates of 49% for the *mecA* gene, 27% for ESBL, and 15–38% for carbapenem resistance genes unrelated to clinical conditions [18].

Careful interpretation of positive results from RMMSP is crucial, as they may detect colonization or molecular remnants rather than active infection, particularly in non-sterile fluids. This highlights the importance of diagnostic stewardship to differentiate between infection and colonization [31–33]. Commercial RMMSPs are standardized, encompassing nucleic acid extraction, amplification, detection, and reporting, which minimizes the need for direct specimen handling [8, 20, 21, 26–29, 31–33].

Effective use of these results demands specialized training, encompassing test interpretation, result integration, and patient management. Collaborative protocols and strong partnerships between microbiologists and intensivists are essential. Crucially, PCR findings must be contextualized with pretest probabilities, culture results, and the patient's clinical status.

Table 1 outlines clinical situations where the interpretation of molecular test results may be limited due to insufficient data or clinical biases.

Currently available European Community (EC)-marketed RMMSP and their most relevant characteristics are displayed in Table 2.

Microbiological diagnostic stewardship concept and complement to classical microbiological tests

Microbiological diagnostic stewardship programs aim to optimize diagnostic techniques, supporting appropriate, cost-effective clinical, therapeutic, and preventive decision-making [38–40]. Their implementation can reduce overdiagnosis, promote correct antimicrobial use, and enhance patient safety and care [41–43]. Effective stewardship requires actions such as developing a service portfolio, establishing multidisciplinary committees, utilizing a laboratory information system, implementing quality assurance, conducting cost-effectiveness assessments, providing education programs for nurses and technicians, and continuously evaluating the program [44].

Table 1 Clinical situations in which the rapid multiplex molecular syndromic panels (RMMSP) result may not be interpreted as significant due to technical limitations, insufficient data, or clinical bias. [34] Modified from Walker et al.

Challenging clinical situations limiting RMMSP interpretation
Immunosuppressed patients (in this group of patients, there may be other clinically significant pathogens not included in the panel)
Poor quality sample (BAS G5 or BAL would be recommended) [35, 36]
Absence of clinical context justifying the test (tracheobronchitis, absence of IDSA criteria for pneumonia requiring admission to ICU) [37]
Patient with long stay in ICU, suffering worsening [18]*

* The pretest predictive value could be high and detect false positives in resistance mechanisms of bacteria colonizing the bronchial tree. Indication of test, interpretation of results, and therapeutic attitude should be individualized, in consensus between the microbiologist and the intensivist, including surveillance microbiological studies and local ecology

BAS Bronchioaspirate, BAS G5: < 10 buccal squamous epithelial cells and > 25 leukocytes/field 100 × magnification; IDSA: Infectious Diseases Society of America; ICU: Intensive care unit

Advantages and disadvantages of implementing a molecular platform for diagnosis of critically ill patients.

Multiplex testing, which enables simultaneous detection of multiple pathogens and antimicrobial resistance (AMR) genes, has become integral to routine diagnostics, offering rapid results to guide patient management [27–29, 45, 46]. However, these panels are more costly than single-plex tests, and the clinical relevance of some targets has been questioned [11, 47, 48]. There are concerns about broad screening, especially for patients with community-acquired pneumonia, where the relevance of molecular techniques and prognosis remains unclear [49, 50]. While multiplex panels offer high sensitivity, specificity, and negative predictive value, they should not replace blood cultures and conventional testing [51–53]. Their limitations include the inability to detect all pathogens, incomplete antibiotic susceptibility data, high costs, and potential false results, with debates about their impact on clinical outcomes [37, 54, 55]. Although they can improve diagnostic efficiency, their cost-effectiveness remains uncertain [56], highlighting the need for careful evaluation before integration into clinical workflows.

The integration of molecular diagnostic platforms for critically ill patients offers notable advantages, particularly in achieving rapid and precise pathogen identification. However, their diagnostic impact is subject to variability influenced by factors such as local prescribing practices, clinical guidelines, and regional epidemiological trends. Furthermore, considerations such as associated costs, technical complexities, and inherent limitations of these platforms must be critically evaluated to ensure optimal implementation and utility [57, 58].

The Table 3 summarizes the main advantages and disadvantages of the RMMSP.

Table 2 Relevant characteristics of European Community (EC)-marketed rapid multiplex molecular syndromic panels (RMMSP)

NAAT platform	*Characteristics*
BD MAX system (BD Diagnostics)	An automated real-time PCR platform utilizing TaqMan hydrolysis probes for pathogen detection
	The BD MAX instrument integrates extraction reagents and a real-time microfluidic cartridge, automating all sample handling and PCR processes
	Results are generated within a 3-h turnaround time
ePLex (GenMark Diagnostics)	A fully automated system that employs eSensor technology, utilizing electrochemical detection of ferrocene-labeled PCR amplicons via capture probes immobilized on gold-plated electrodes
	Results are available within 30 to 90 min, depending on the specific system (ePLex, ePlex NP, or eSensor XT-8)
Biofire Filmarray system (BioFire Diagnostics)	Fully automated platform performing nucleic acid extraction, real-time PCR detection and high-resolution melting for target identification
	Turnaround time less than 2 h
Unyvero System (Curetis USA)	Fully automated platform that includes a sample lysis device and a PCR panel analyzer
	The turnaround time is 4–5 h
VERIGENE system (Luminex Corporation)	Fully automated for nucleic acid extraction, purification, target amplification and hybridization of the target amplicons to a glass detection array in the test cartridge
	NanoGrid technology is used to identify target molecules. Turnaround time of around 3 h
xTAG technology (Luminex Corporation)	Multiplexed PCR coupled to bead-hybrization with a bead-specific fluorescent reporters that require offline nucleic acid extraction
	Turnaround time of 5 h

NAAT Nucleic acid amplification tests; *PCR* Polymerase chain reaction

Table 3 Overview of the main advantages and disadvantages of implementing a molecular platform for diagnosis of critically ill patients

Main advantages and disadvantages of Implementing RMMSP	
Advantages	**Disadvantages**
Rapid Results	*Cost*
Molecular platforms can provide fast turnaround times, allowing for timely diagnosis and treatment initiation in critically ill patients	Molecular platforms can be expensive to purchase and maintain, requiring significant initial investment and ongoing expenses
High Sensitivity and Specificity	*Complexity*
Molecular tests typically exhibit high sensitivity and specificity, enabling accurate detection of pathogens even at low concentrations	Molecular testing may require specialized equipment and trained personnel, increasing complexity compared to traditional methods
Multiplexing Capability	*Technical Challenges*
Many molecular platforms offer multiplexing, allowing for simultaneous detection of multiple pathogens and antimicrobial resistance genes in a single test, which is beneficial for critically ill patients with complex infections	Molecular tests may be susceptible to technical issues such as sample contamination or inhibition, missing detections due to genetic variants, or lack of correlation between genotype and phenotype for the AMR genes, which can affect test accuracy
Reduced Hands-on Time	*Limited Coverage*
Automation in molecular platforms reduces hands-on time for laboratory staff, freeing up time for other tasks	While molecular platforms offer broad pathogen coverage, they may not detect all pathogens relevant to a specific clinical scenario, potentially leading to missed diagnoses
Potential for Point-of-Care Testing	*Interpretation Challenges*
Some molecular platforms are suitable for point-of-care testing, enabling rapid diagnosis at the bedside, which is advantageous for critically ill patients requiring immediate intervention	Molecular test results may be complex to interpret, especially in cases of co-infections or detection of commensal organisms, requiring careful clinical correlation

RMMSP Rapid multiplex molecular syndromic panels; AMR: Antimicrobial resistance

Syndromic Polymerase chain reaction (PCR) panels for the diagnosis and management of multidrug-resistant bacteria (MDR) high-risk severe community acquired pneumonia, hospital-acquired pneumonia and ventilator associated pneumonia

Two CE-marked molecular syndromic panels, BioFire® FilmArray® Pneumonia (PN) panel (FA-PNp) and the Unyvero Hospitalised Pneumonia Panel (HPN), are designed for diagnosing hospital-acquired (HAP), ventilator-associated (VAP), and severe community-acquired (CAP) pneumonia in high-risk patients for MDR bacteria [59–73] (Table 4).

The F A-PNp panel displays a positive percentage agreement of 96.2% and a negative percentage agreement of 98.1% for identifying bacterial targets compared to routine culture [72]. Sensitivity varies depending on specimen type, with lower sensitivity for sputum-like specimens. For deep respiratory samples, sensitivity and specificity are high [74].

Enne et al. [75] evaluated, by Bayesian latent class analysis, the clinical efficacy of FA-PNp, HPN, and standard microbiological techniques in 652 lower respiratory tract samples from critically ill patients. Compared to traditional methods, RMMSP detected pathogens in a significantly higher proportion of samples (74.2% for FA-PNp and 60.4% for HPN). For common HAP/VAP pathogens, FA-PNp demonstrated a sensitivity of 91.7–100.0% and a specificity of 87.5–99.5%, while HPN exhibited a sensitivity of 50.0–100.0% and a specificity of 89.4–99.0%. Conversely, conventional methods showed low sensitivity, ranging from 27.0 to 69.4%, in comparison to RMMSP. The INHALE WP1 study had several limitations. Its findings may not have been applicable to all healthcare settings due to variations in diagnostic infrastructure and patient populations. The study compared PCR results with routine microbiology, which has lower sensitivity, potentially overstating the benefits of PCR. Although PCR improved pathogen detection, its clinical impact on patient outcomes and antibiotic stewardship remained uncertain, and the study lacked a cost-effectiveness analysis. Additionally, differences in sensitivity and specificity between the PCR platforms tested may have complicated their routine clinical adoption.

There appears to be certain discrepancy between the results of conventional methods and RMMSP, particularly in cases where antimicrobial therapy exposure is present. [74, 76]. Regarding the HPN panel, studies have reported varying sensitivity and specificity for different targets, with antibiotic resistance marker positive predictive values (PPVs) ranging from 79.7 to 100% [63, 64, 66].

Potential clinical impact of the use of syndromic pneumonia molecular panels on patients with HAP/VAP and MDR-CAP patients

Several observational studies suggested that the use of syndromic pneumonia molecular panels in patients with

Table 4 BioFire® FilmArray® Pneumonia Panel *plus* multiplex (FDA-approved and CE-Marked) and the Unyvero pneumonia panel (CE-Marked) multiplex PCR platforms. The information to build this table has been extracted from references [59–73]

Characteristic	BioFire® FilmArray® Pneumonia Panel *plus*	Curetis Unyvero Hospitalised Pneumonia Panel (HPN)
Number of targets	34	36
Analytical design	Automated sample preparation and and nucleic acid extraction and nested PC (melting curve analysis)	Automated sample preparation and nucleic acid extraction and multiplex PCR and microarray detection of targets
Turnaround time	Around 1.5 h	Around 5 h
Results reporting	Quantitative (binned values: 10^4, 10^5, 10^6, $\geq 10^7$) for bacterial targets, excluding atypical bacteria	Semiquantitative (+/+ +/+ + +) for bacterial and fungal targets
Bacterial targets	*Acinetobacter baumannii* complex *Citrobacter freundii* *Enterobacter cloacae* complex *Escherichia coli* *Haemophilus influenzae* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Klebsiella variicola* *Moraxella catarrhalis* *Morganella morganii* *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens* *Stenotrophomonas maltophilia* *Streptococcus pneumoniae* *Chlamydophila pneumoniae* *Legionella pneumophila* *Mycoplasma pneumoniae*	*Acinetobacter calcoaceticus/baumannii* complex *Enterobacter cloacae* complex *Escherichia coli* *Haemophilus influenzae* *Klebsiella aerogenes* *Klebsiella oxytoca* *Klebsiella pneumoniae* *Moraxella catarrhalis* *Proteus* spp. *Pseudomonas aeruginosa* *Serratia marcescens* *Streptococcus agalactiae* *Streptococcus pneumoniae* *Streptococcus pyogenes* *Chlamydophila pneumoniae* *Legionella pneumophila* *Mycoplasma pneumoniae*
Virus targets	Adenovirus Coronaviruses OD43, NL63, HKU1 and 229E Coronavirus del síndrome respiratorio de Oriente Medio (MERS-CoV) Human metapneumovirus Human rhinovirus/ enterovirus Influenza A Influenza B Parainfluenza virus Respiratory syncytial virus	None
Fungal targets	None	*Pneumocystis jirovecii*
Antimicrobial resistance genes	bla_{KPC} bla_{NDM} $bla_{OXA\text{-}48\ like}$ bla_{VIM} bla_{IMP} $bla_{CTX\text{-}M}$ *mecA/C* and MREJ	*ermB* *mecA* *mecC* bla_{TEM} bla_{SHV} $blaI_{MP}$ bla_{KPC} bla_{NDM} $bla_{OXA\text{-}23}$ $bla_{OXA\text{-}24/40}$ $bla_{OXA\text{-}48}$ $bla_{OXA\text{-}58}$ bla_{VIM} sul1 gyrA83 gyrA87
Results of the AMR targets unavailable if bacterial targets are below the limit of detection	Yes	Yes
PPV	63.0–96.2%	71.6–100.0%
NPV	92.0–98.1%	97.9–99.8%
A similar panel, featuring a reduced number of antimicrobial resistance genes, has US FDA clearance		

Table 4 (continued)

AMR Antimicrobial resistance; *FDA* Food and Drud Administration; *PCR* Polymerase chain reaction; *PPV* Positive predictive value; *NPV* negative predictive value

HAP/VAP and MDR organisms-CAP increased the diagnostic yield, led to a reduction in the time to appropriate antimicrobial therapy and decreases antibiotic consumption [62, 68, 69, 71]. Currently available clinical trials have supported this assumption. Poole et al. [77] showed that time to results-directed therapy was 2.3 h in the FA-FNp (mPOCT) group and 46.1 h in the control group (standard microbiological procedures). Similarly, Firezein et al. in ventilated pediatric intensive care unit patients, observed that the length of the time to identification of organism was significantly shortened from 67 to 5 h after implementing the use of FA-FNp [77]. Regarding the duration of antibiotic therapy, Poole et al. [77] reported that 42% of patients in mPOCT group had antibiotics safely de-escalated compared with 8 (8%) of 98 in the control group. Nevertheless, there was no major difference in antibiotic duration or in clinical or safety outcomes between the two groups [77]. Additionally,

Firezein et al. found a significant reduction in the length of antibiotic therapy from 9 to 5 days after introducing the FA-FNp [78].

Darie et al. [79] showed that multiplex bacterial PCR examination of bronchoalveolar lavage (by HPN) decreases the duration of inappropriate antibiotic therapy of patients admitted to hospital with pneumonia and at risk of Gram-negative rod infection. The study had several limitations. Its findings, based on data obtained at two Swiss tertiary centers, may not be generalizable to other healthcare settings. The lack of physician blinding introduced potential bias in antibiotic decisions, while the small sample size (208 patients) limited the strength of subgroup analyses. Additionally, the focus on Gram-negative bacteria excluded other significant pathogens, emphasizing the need for further research to validate its relevance across diverse clinical contexts [79].

Moreover, Markussen et al. [80], in a randomized clinical trial, demonstrated that molecular testing by the FA-PNp significantly increased the proportion of hospitalized patients with suspected CAP who received pathogen-directed treatment and reduced the median time to pathogen-directed treatment by 9.4 h compared with the standard of care.

It has been recently published a single-center, open-label randomized controlled trial that assessed the impact of the BioFire FilmArray pneumonia panel on antibiotic management in hospitalized patients with suspected pneumonia [81]. Participants were randomized to receive diagnostics with the BioFire panel plus conventional culture or conventional culture alone. Among 1152 patients analyzed, the intervention group showed significantly reduced median times to antibiotic escalation for Gram-positive (10.3 vs. 24.6 h, $p=0.044$) and Gram-negative organisms (17.3 vs. 27.2 h, $p=0.010$). Median time to Gram-positive antibiotic de-escalation was also shorter (20.7 vs. 27.8 h, $p=0.015$). However, the authors recommended the need of further research for optimizing Gram-negative antibiotic de-escalation in lower respiratory infections.

What patient profile would benefit from being tested by a pneumonia syndromic PCR Platform including genotypic resistance marker targets?

RMMSP may facilitate early adjustment of empirical antimicrobial therapy (EAT) or targeted antimicrobial therapy. In severe CAP patients at high risk for MDR bacteria involvement or with shock, syndromic panels should be requested to guide antimicrobial therapy [21, 82].

For patients with HAP or VAP, testing with syndromic panels is recommended regardless of the risk for MDR bacteria involvement or shock risk factors (Fig. 1). Broad-spectrum empirical treatment should be initiated and adjusted based on panel results [18, 52, 83–86]. Early treatment algorithms may be proposed for CAP and HAP among patients with MDR risk factors. These strategies should be guided by the clinical presentation, regional epidemiological trends, and prevailing patterns of pathogen resistance [23]. Final adjustments to antimicrobial therapy should be made based on standard culture and antimicrobial susceptibility testing results.

The challenges in analyzing colonization without limiting the scope to diagnosed patients are exemplified by RMMSP panels' high sensitivity (98.55%) but lower specificity (69%), which detects organisms missed by culture methods. Discordances often reflect early infection or host reaction, as evidenced by increased white blood cells and neutrophil counts in PCR-positive, culture-negative samples. Biomarkers like C-reactive protein and procalcitonin may aid clinical interpretation, akin to optimizing *C. difficile* PCR in high pre-test probability cases [34].

- **Experts' opinion 1:** Molecular syndromic panels may be considered as valuable tools for the diagnosis and therapeutic management of HAP, VAP, and CAP when MDR bacteria are suspected. These panels can aid in guiding the adjustment of empirical antimicrobial therapy and facilitating quasi-targeted antimicrobial interventions.

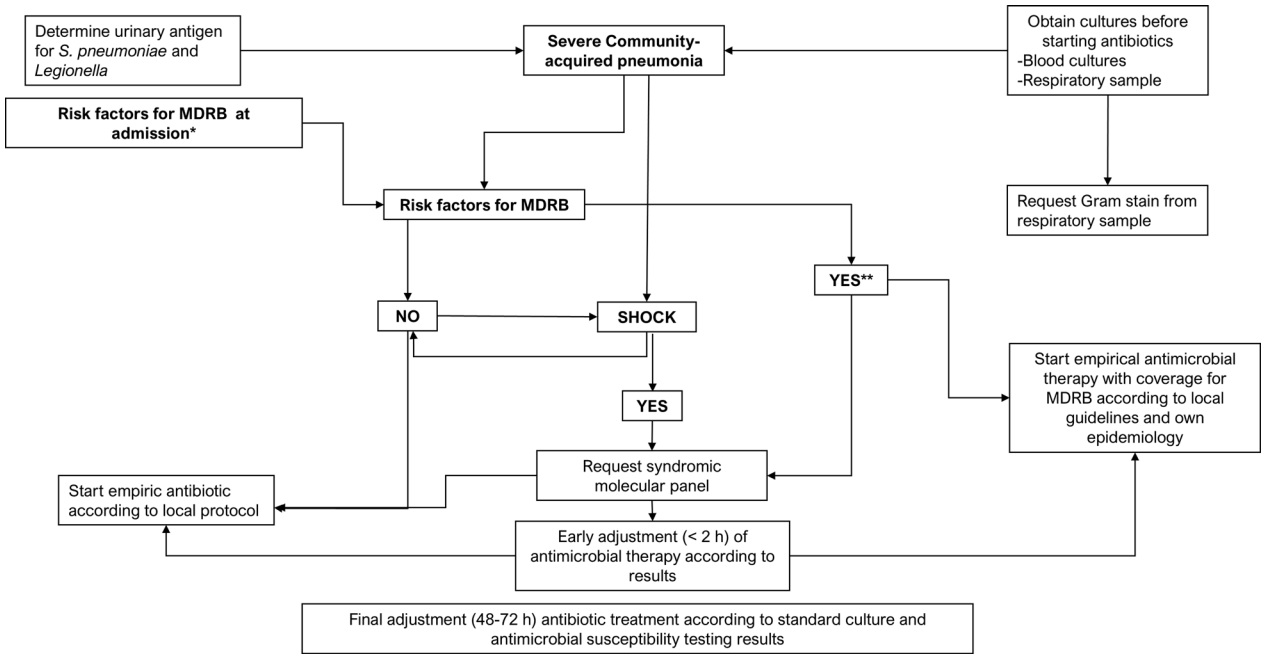

Fig. 1 Severe Community-acquired pneumonia. Adapted from Martin-Loeches et al. [87]. *Risk factors for Multiresistant drug bacteria (MDRB) at admission: Prior contact or hospital admission; stay in chronic health center; Chronic obstructive pulmonary disease; Cystic fibrosis; Bronchiectasis; Immuno-depression; Previous antibiotic treatment; Underweight; High severity illness; Endemic areas; Diabetes mellitus; Chronic alcoholism; Prior hospitalization; Prior colonization; Intravenous drug use; Post influenza or Severe-acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection. **With or without shock. MDRB Multiresistant drug bacteria

May antimicrobial therapy be withheld until the results of the syndromic PCR panel are available (early targeted antimicrobial therapy)?

In cases where shock is absent, physicians may opt for front-line targeted antimicrobial therapy based on syndromic panel results. The high negative predictive value of RMMSP for all panel targets enables the avoidance of antimicrobials directed at undetectable targets (e.g., linezolid in the absence of methicillin-resistant *Staphylococcus aureus* [MRSA]).

- **Experts' opinion 2:** In patients with hemodynamic stability (no shock), antimicrobial therapy could be delayed until molecular syndromic panel results are available (no longer than 3 h thereafter).

What specimens should be run on pneumonia syndromic PCR panels?

Both, bronchoalveolar lavages (BAL) and high-quality (as evaluated microscopically) sputum-like specimens (sputa and endotracheal aspirates) may be used.

- **Experts' opinion 3:** High quality lower respiratory tract specimens, as assessed microscopically, should be used for testing.

How should the results of the syndromic PCR panel be informed (qualitative vs. quantitative for the filmarray panel)?

When feasible (use of the FA-PNp) results should be reported in a quantitative fashion.

- **Experts' opinion 4:** Molecular syndromic panel results should be reported quantitatively, when possible. Quantitative results (in copies/ml) should be interpreted on an individual basis considering factors such as patient clinical condition, receipt of antimicrobials at the time of sampling, bacterial load, and number of detected targets.

How should results be interpreted?

The FA-PNp provides quantitative results (genomic copies/ml) in binned values ($\log_{10}$ increments from 10^4 to $\geq 10^7$ genomic copies/ml) for 15 bacterial targets, excluding atypical bacteria. High bacterial burdens (10^6 or $\geq 10^7$) generally indicate causality. However, lower genomic copies/ml (10^4 or 10^5) may also indicate causality, particularly for certain microorganisms as *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and MRSA, especially in patients receiving appropriate antimicrobial therapy.

Interpreting the detection of more than two targets should be approached cautiously, especially when only qualitative results are available. However, high bacterial burdens (10^6 or $\geq 10^7$), regardless of the number of co-detected microorganisms, may indicate.

The results of genotypic resistance markers offer valuable insights, particularly for Enterobacterales and MRSA. However, it is crucial to interpret these markers in conjunction with the clinical context, local resistance patterns, and antimicrobial stewardship guidelines.

Should pneumonia syndromic PCR Panels replace standard microbiological procedures?

The use of Pneumonia Syndromic molecular panels must not replace conventional methods, but rather serve as a complementary tool for improving the management of patients and decrease the selection of MDR bacteria.

Regarding FA-PNp assay, it demonstrated strong concordance with standard-of-care diagnostics for included species, with no false positives compared to clinical symptoms or cultures. FA-PNp assay reduced the median time to clinical interpretation, potentially improving patient outcomes. However, the assay missed clinically important pathogens, including *H. parainfluenza* and fungal species such as *Aspergillus*. While the assay may expedite diagnosis and treatment for certain bacterial and viral infections, it cannot replace standard culture techniques, especially in lung transplant recipients [88].

For fungal species detection, combining RMMSP with next-generation sequencing (NGS) offers the potential for enhanced accuracy, thereby improving its overall clinical utility [89].

- **Experts' opinion 5:** Molecular syndromic panel must not replace conventional microbiological procedures.

Summary

The use of syndromic pneumonia molecular panels enhances diagnostic yield, reduces the time to appropriate antimicrobial therapy, and may decrease antibiotic consumption [59–73]. However, challenges remain, including the need for further studies on clinical outcomes, cost-effectiveness, and the involvement of antimicrobial stewardship groups. While molecular diagnostics offer promising benefits for antibiotic stewardship, concerns persist about the necessity of initiating broad-spectrum antibiotics to protect unstable patients [90]. Staying updated with guidelines and ensuring multidisciplinary collaboration are crucial for the effective implementation and cost-effectiveness of these technologies in patient care.

Molecular syndromic panels for the diagnosis and management of sepsis and bloodstream infections

Molecular syndromic panels for the diagnosis and management of bloodstream infections

Bloodstream bacterial and fungal infections are common and lead to significant morbidity and mortality in critically ill patients [91, 92]. Rapid initiation of appropriate therapy is crucial, as delays increase mortality by approximately 10% per hour [93–96]. RMMSP testing enables quicker identification of pathogens and distinguishes between infectious agents and contaminants, aiding in the reduction of unnecessary antibiotic use [97, 98]. The Surviving Sepsis Campaign recommends initiating adequate antimicrobial therapy within the first hour for patients in shock and within three hours for patients with sepsis [99].

While blood cultures remain the standard diagnostic method, they are limited by slow growth, particularly in candidemia, and inefficiency in patients on antibiotics or those with fungal or slow-growing infections [91, 92]. RMMSP testing enhances pathogen detection and improves treatment timing [100–103]. Studies show that inappropriate empirical antibiotic therapy increases mortality in sepsis and septic shock, while timely appropriate therapy improves survival rates [3–5, 104–108].

The EUROBACT-2 study identified common ICU pathogens, including carbapenem-resistant strains, and found that only 51.5% of patients received adequate antimicrobial therapy within 24 h [109]. Delayed antibiotic administration beyond 6 h increases mortality, but immediate initiation is not necessary in all cases, especially for stable surgical patients [93, 110–114].

Invasive fungal infections, including candidemia, are rising in ICU, oncology, and transplant patients due to factors like complex surgeries and prolonged antibiotic use [115–118]. *Candida spp.* are responsible for up to 10% of hospital bloodstream infections [109]. Tools like *Candida* scores and beta-delta-glucan tests help rule out invasive candidiasis, and timely antifungal treatment, including echinocandins, improves outcomes [119–123].

Can we recommend the use of syndromic panels directly from blood? Which patients could benefit from their use?

Different RMMSP are available for diagnosing and phenotyping bacteremia, sepsis, or candidemia in critically ill patients (see Table 5).

RMMSP, directly analyzing blood samples, when applied to sepsis and candidemia, reduce the time to reach an etiological diagnosis and often enhance the sensitivity of conventional blood cultures.

T2 assays, utilizing miniaturized magnetic resonance technology, enable the identification of microorganisms

Table 5 Overview of the rapid multiplex molecular syndromic platforms that can be used in bacteremia, candidemia or sepsis. Sensitivity–specificity and predictive values

Name	Bacteria	Fungi	Resistance determinants	Performance	Sample	Time to results	Tecnology	Reference
Eazyplex BloodScreen GN CE-IVD	*E.coli* *K.pneumoniae* *K.oxytoca* *P.mirabilis* *P.aeruginosa*	None	*bla*CTX-M-1, *bla*CTX-9	Se: 95.5–100% Sp: 99.8–100% PPV: 84.6–100% PNV: 99.5–100%	Positive Blood culture	< 20 min	LAMP	[124]
Eazyplex BloodScreen GP	*E.faecalis* *Enterococcus* spp *S.pneumoniae* *Streptococcus* spp	None	*van*A *van*B	Se: 95.2–100% Sp: 93–98% PPV: 92.3–100% PNV: 96.4–100%	Positive Blood culture	< 20 min	LAMP	[125]
Eazyplex superbug CRE	None	None	*bla*CTX-M-1, *bla*CTX-M-9 VIM, NDM, OXA-48, KPC	S: 95–100% E: 97.9%	Positive Blood culture Urine	< 20 min	LAMP	[126]
ePlex BCID GN	21 Gram-negative bacterial genera or species Pan grampositive Pan *Candida*	None	*bla*CTX-M, *bla*OXA, *bla*VIM, *bla*NDM, *bla*KPC, *bla*IMP	Se: 75–99% Sp: 97–100% PPV: 96%	Positive Blood culture	90 min	Multiplex real-time PCR	[127–129]
ePlex BCID GP	20 Gram-positive bacterial genera or species Pan gramnegative Pan *Candida*	None	*mec*A, *mec*C, vanA, *van*B	Se: 97–100% Sp: 97–100% PPV: 99%	Positive Blood culture	90 min	Multiplex real-time PCR	[127, 128, 130]
ePlex BCID FD	None	15 fungal genera or species	None	Se: 100% Sp: 100%	Positive Blood culture	90 min	Multiplex real-time PCR	[127]
Biofire BCID 2	26 Gram-positive and Gram-negative genera or species (including "enterobacterales")	*Candida albicans, C. auris, C. glabrata, C. krusei, C. parapsilosis, C. tropicalis, Cryptococcus (C. neoformans/C. gattii)*	*mec*A/C *mec*A/C and MREJ (MRSA) *van*A/*van*B *bla*KPC, *bla*IMP, *bla*NDM, *bla*OXA-48-like, *bla*VIM *mcr*−1 *bla*CTX-M	Grampositives: Se: 97% Sp: 99% Gramnegatives: Se: 100% Sp: 100% Fungi: Se: 99% Sp: 100%	Positive Blood culture	About 1 h	Multiplex real-time PCR	[131, 132]
VERIGENE® Gram-Positive Blood Culture Test (BC-GP)	13 Gram-positive bacterial genera or species	None	*mec*A, *van*A, *van*B	PPA (positive percent agreement): 85.7- > 93.1% NPA (negative percent agreement: 81.5- > 98.2%	Positive Blood culture	2,5 h	PCR + gold nanoparticle probe chemistry (NanoGrid)	[133]

Table 5 (continued)

Name	Bacteria	Fungi	Resistance determinants	Performance	Sample	Time to results	Tecnology	Reference
VERIGENE® Gram-Negative Blood Culture Test (BC-GN)	9 Gram-negative bacterial genera or species	None	*bla*KPC, *bla*IMP, *bla*NDM, *bla*OXA, *bla*VIM *bla*CTX-M	PPA: 92.9–100% NPA: 99.5–99.9%	Positive Blood culture	2 h	PCR + gold nanoparticle probe chemistry (NanoGrid)	[133]
Sepsis Flow Chip	12 Gram-positive bacterial genera or species	*Candida albicans*	*mec*A, *van*A/B, *bla*CTX, *bla*SHV, *bla*SME, *bla*KPC, *bla*NMC/IMI, *bla*GES, *bla*IMP, *bla*GIM, *bla*VIM, *bla*SPM, *bla*SIM, *bla*NDM, *bla*OXA-23, *bla*OXA-24, *bla*OXA-48, *bla*OXA-51 and *bla*OXA-58	For bacterial identification: S: 93.3% Sp: 100% For resistance markers: S: 93.6% Sp: 100%	Positive Blood culture	4 h	Multiplex real-time PCR	[134]
T2Bacteria® panel	*Enterococcus faecium, Staphylococcus aureus, Klebsiella pneumoniae, Acinetobacter baumannii, Pseudomonas aeruginosa, Escherichia coli*	None	None	Se: 90% Sp: 96–98% PNV: 91–99.7% PPV: 48.9%	Blood	4–7 h	PCR + miniaturized magnetic resonance	[135, 136]
T2Candida® panel	None	*C. albicans/C. tropicalis, C. glabrata/C. krusei, C.parapsilosis*	None	Se: 78–91% Sp: 98% VPP: 50% (67% ICU patients) VPN: 99%	Blood	3–5 h	PCR + miniaturized magnetic resonance	[136, 137]
T2Resistance® panel	None	None	*bla*CTX-M 14, *bla*CTX-M 15, *bla*CMY, *bla*DHA, *bla*KPC, *bla*OXA-48, *bla*NDM, *bla*VIM, *bla*IMP, *van*A\B, *mec*A\C	PPA:80% NPA:69.2% PPV:42.9% NPV:92.3%	Blood	3–5 h	PCR + miniaturized magnetic resonance	[135]

Table 5 (continued)

Name	Bacteria	Fungi	Resistance determinants	Performance	Sample	Time to results	Tecnology	Reference
Fungiplex® Candida	None	*Candida* spp. *(C. albicans, C. parapsilosis, C. dubliniensis, C. tropicalis), C. glabrata, C. krusei*		Se: 100% Sp: 94.1%	Blood	3 h	Multiplex real-time PCR	[138]
Yeast Traffic Light PNA FISH®	None	*C. albicans/C. parapsilosis, C. tropicalis, C. glabrata/C. krusei*		Se: 92.3% (C.g/C.k) −100% Sp: 94.8% (C.g/C.k) −100%	Positive Blood culture	90 min	FISH	[139]
Magicplex™ Sepsis	73 Gram positives, 12 Gram negatives	*C.albicans C. tropicalis C. parapsilosis C. glabrata C. krusei A. fumigatus*	*mec*A, *van* A/B	S: 29–47% Sp: 66–95% PPV: 23% NPV: 87%	Blood	3–5 h	Multiplex real-time PCR	[140, 141]
Micro-Dx	200 bacterial genera	65 fungal genera	None	-	Blood	7 h	Targeted metagenomics (PCR 16S/18S)	[142]
Hybcell Pathogens DNA	56 species and 11 genera	19 species and 5 genera	*van*A/B, *mec*A/C, *bla*CTX-M, *bla*KPC, *bla*OXA-48, *bla*NDM, *bla*IMP	S: 63% Sp: 83%	Blood	3 h	Targeted metagenomics (PCR 16S/18S)	[143]

directly in blood samples. These diagnostic panels are not universally accessible and are currently limited in their ability to detect a narrow spectrum of bacterial and fungal pathogens. A critical challenge lies in identifying appropriate patient populations for testing to optimize cost-effectiveness and maximize clinical utility. They demonstrate higher sensitivity than blood culture in detecting pathogens, especially in patients with ongoing antimicrobial treatment or localized infections causing intermittent bacteremia [144–146]. this system enables the detection of intact cells rather than free- deoxyribonucleic acid (DNA) [144].

Persistent positivity on this platform indicates persistent infection, aiding in identifying patients at risk of poor control or metastatic infection. While it has a high negative predictive value for the microorganisms included in the trial, its coverage is limited [135, 144–147].

T2 panels are also effective in diagnosing candidemia and can predict complicated cases when positivity persists on the 5th day [137, 148, 149]. Combining them with classic markers like [1, 3]-β-D-glucan improves diagnosis, particularly in non-hematological critical patients in the ICU [149].

Can we recommend the use of syndromic panels in grown blood cultures? Which patients could benefit from their use? How and when to use syndromic panels in grown blood cultures based on the characteristics of each hospital?

Most commercialized systems for detecting microbial genomes in positive blood cultures showed high sensitivity and specificity for the included targets [124–143]. New diagnostic techniques, such as mass spectrometry (MALDI TOF), rapid tests for resistance determinants, and RMMSP, significantly enhanced diagnostic accuracy [150]. RMMSP, in particular, provided rapid and precise identification of common pathogens and multidrug-resistant bacteria, supporting treatment decisions. However, their clinical utility heavily depended on optimizing the entire diagnostic process. This included ensuring proper pre-analytical procedures (e.g., collecting adequate blood volumes and preventing contamination), conducting analytical processes in a 24/7 microbiology system, and ensuring that results promptly reached clinicians for treatment adjustments [102, 151, 152].

Rapid identification of bacteremia and candidemia etiology, along with key resistance determinants, remained crucial due to the severity of these infections. RMMSP proved especially useful when MALDI-TOF was unavailable or inconclusive, such as in cases of polymicrobial bacteremia, Gram-positive microorganisms, or yeast infections. Additionally, RMMSP was beneficial when rapid resistance gene detection methods were lacking. The protocols are detailed in Figs. 2 and 3.

Experts' opinion 6: Diagnostic tools that allow rapid identification of the microorganism causing bacteriemia/candidemia, as well as the main determinants of resistance, should always be used in critically ill patients. RMMSP on grown blood cultures would be recommended, but they should be used when other procedures are not available, due to their high cost.

Limitations of syndromic panels and considerations regarding the determination of resistance genes. May antimicrobial therapy be withheld until the results of the RMMSP panel are available (early targeted antimicrobial therapy)?

Molecular diagnostic techniques, while offering benefits such as quicker treatment initiation, have certain limitations [124–143]. They may not identify all microorganisms in polymicrobial bacteremia or rare pathogens. Detection of anaerobic bacteria is limited, and they mainly identify beta-lactam drug resistance mechanisms due to beta-lactamases. Presence of a resistance gene does not always imply its expression, causing genotype–phenotype discrepancies during susceptibility testing [124–143]. Specific limitations in detecting determinants of resistance in gram-positive and gram-negative microorganisms are detailed in Table S1.

Despite limited evidence, these techniques generally reduce the time to appropriate treatment, though they do not significantly impact mortality rates, largely due to the complexity of managing critically ill patients [153–155]. Empirical treatments remain vital, and knowledge of local epidemiology, particularly regarding multidrug-resistant bacteria, is crucial [156–158]. Future integration of clinical and microbiological data, along with advancements in artificial intelligence and next-generation sequencing, is expected to improve infection management further [159–161].

- **Experts' opinion 7:** RMMSP allow a rapid initiation of targeted antibiotic therapy. However, some limitations must be considered, mainly referring to the targets included, both in identification and in resistance genes. Therefore, knowledge of local epidemiology is essential so that their impact on decision-making is optimal.

Rapid multiplex molecular syndromic panels in meningitis and encephalitis

What are the rapid multiplex molecular syndromic panels available in meningitis and encephalitis?

RMMSP have recently been introduced for diagnosing meningitis and encephalitis. The most widely used panels, FilmArray ME and QIAstat-Dx Meningitis/Encephalitis,

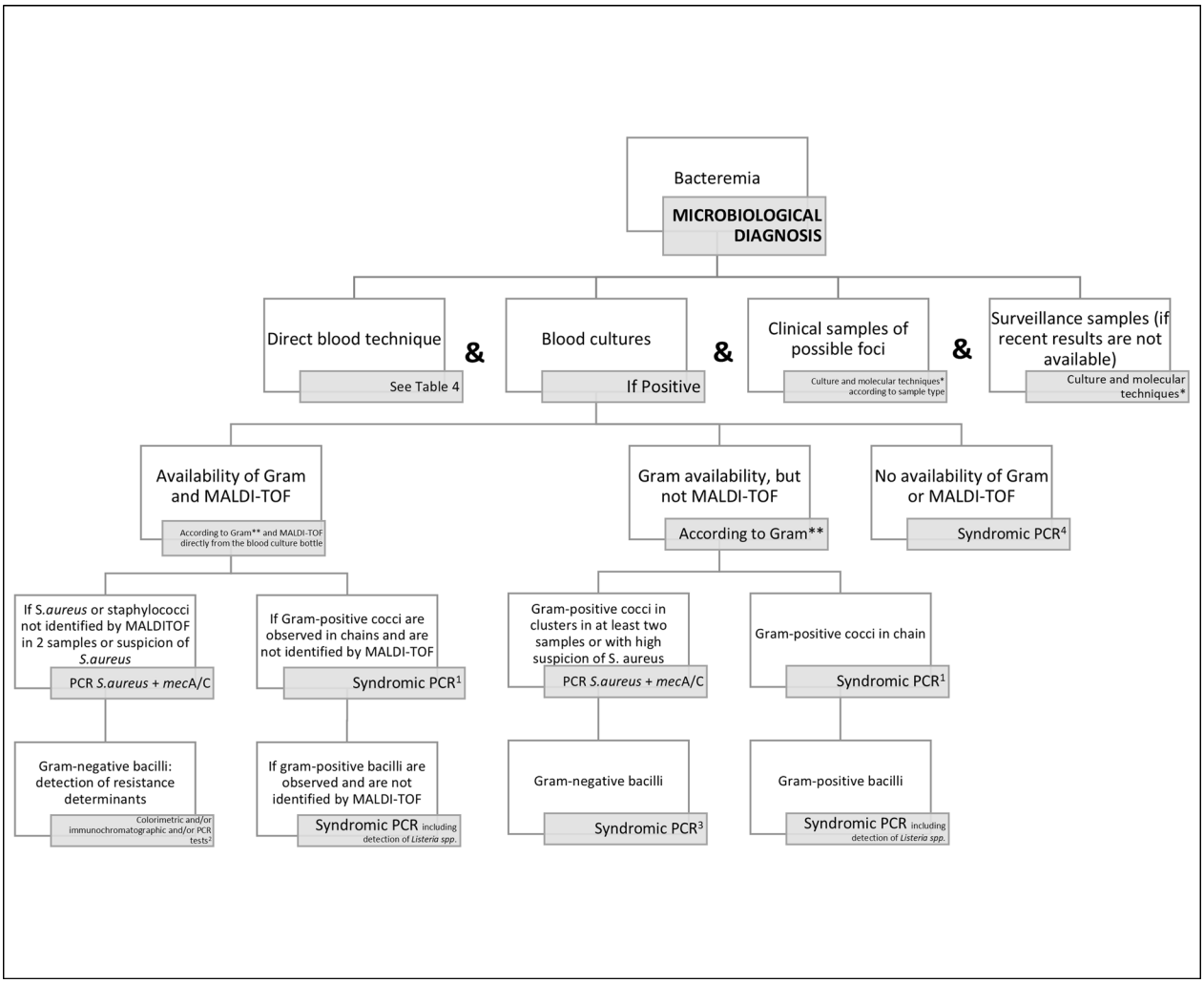

Fig. 2 Algorithm for the use of the rapid multiplex molecular syndromic platforms in bacteremia. [1]Syndromic PCR panels that detects at least: *Streptococcus pyogenes, Streptococcus pneumoniae, Genus Streptococcus, Enterococcus faecalis, Enterococcus faecium, vanA/vanB.* [2]Perform directly from the blood culture sample. Assess techniques to be carried out according to microorganism and local epidemiology. As a guide, the following instructions can be followed: *Escherichia coli:* Colorimetric test for the detection of 3rd generation cephalosporin hydrolyzing enzymes. If it is negative and there is clinical suspicion of infection with an extended-spectrum beta-lactamase-producing strain or the patient is very serious/vulnerable: immunochromatography or PCR to detect CTX-M production/gene. *Klebsiella spp:* Colorimetric test for the detection of enzymes hydrolyzing 3rd generation cephalosporins and carbapenemases. If they are negative and there is clinical suspicion of multidrug-resistant strain infection or the patient is very serious/vulnerable: immunochromatography or PCR for detection of CTX-M production/gene and immunochromatography or PCR for detection/production of genes VIM, NDM, OXA-48, and KPC. *Proteus spp:* Colorimetric test for the detection of 3rd generation cephalosporin hydrolyzing enzymes. If it is negative and there is clinical suspicion of infection with an extended-spectrum beta-lactamase-producing strain or the patient is very serious/vulnerable: immunochromatography or PCR to detect CTX-M production/gene. *Enterobacter spp:* Colorimetric test for the detection of carbapenemases. If they are negative and there is clinical suspicion of infection with a multidrug-resistant strain or the patient is very serious/vulnerable: immunochromatography or PCR for detection of production/genes of VIM, NDM, OXA-48 and KPC. *Pseudomonas aeruginosa:* According to local epidemiology, colorimetric test for the detection of carbapenemases, immunochromatography or PCR for detection of VIM and NDM production/genes. *Acinetobacter baumanii:* According to local epidemiology, colorimetric test for detection of carbapenemases, immunochromatography or PCR for detection of VIM and NDM production/genes (ideally include OXA-23, OXA-24 and OXA-58). [3]Syndromic PCR panels that detects at least: Species-level identification of *Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa and Acinetobacter baumanii* and the resistance genes *blaCTX-M, blaVIM, blaNDM, blaOXA-48 and blaKPC*. [4]Syndromic PCR panles that has targets to detect at least: *Staphylococcus aureus, Streptococcus pyogenes, Streptococcus pneumoniae, Genus Streptococcus, Enterococcus faecalis, Enterococcus faecium, Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumanii, Listeria spp, mecA/C, vanA/vanB, blaCTX-M, blaVIM, blaNDM, blaOXA −48 and blaKPC*. *See corresponding sections of the current Experts' opinion Document. **If polymicrobial infection: directly perform syndromic PCR

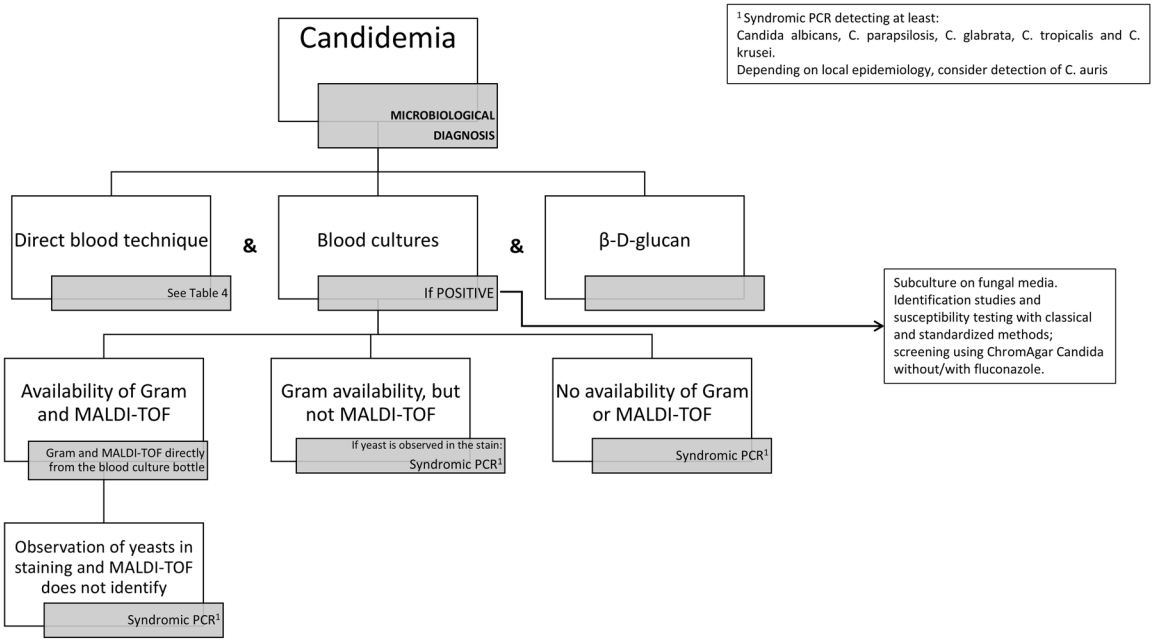

Fig. 3 Algorithm for the use of the rapid multiplex molecular syndromic platforms in *Candida* infection. [1]Syndromic PCR that detects at least: *Candida albicans, C. parapsilosis, C. glabrata, C. tropicalis and C. krusei.* According to local epidemiology, consider *C. auris* detection

require a small sample volume (200 µl) and provide rapid results (around one hour). They detect various pathogens, including bacteria like *E. coli K1, H. influenzae, L. monocytogenes, N. meningitidis, S. agalactiae, S. pneumoniae*, several viruses and *Cryptococcus neoformans/C.gattii* [162, 163]. While both panels detect almost the same pathogens, QIAstat-Dx Meningitis/Encephalitis includes additional pathogens like *M. pneumoniae* and *S. pyogenes* but do not include cytomegalovirus [164, 165]. Both systems can provide information on the threshold cycle (Ct) value and amplification curves, in the case of FilmArray ME through an additional application (Fireworks) [166].

What is the accuracy of the available rapid multiplex molecular syndromic panels in meningitis/encephalitis?

The FilmArray ME panel has been evaluated in several studies. Sensitivity and specificity were high, although they varied among targets. Some viruses, such as enterovirus and herpesvirus, exhibited lower sensitivity [162, 164, 165, 167]. In cases of negative results with high clinical suspicion, it has been recommended to perform monoplex PCR [167]. Interpretation of results for herpesvirus 6 should be cautious due to possibility of its DNA being integrated into chromosomes (1% of human population): in this sense, performing a viral load in peripheral blood can help the interpretation of the results and diagnosis. In bacteria, there were high sensitivity and specificity rates, although false positives were observed for certain species due to contamination. Correct material handling is crucial [168, 169].

A meta-analysis evaluating the clinical performance of the FilmArray ME panel for all bacterial targets found combined sensitivity/specificity ranging from 89.5–92.1% to 97.4–99.2%, respectively, depending on the reference test [154]. A higher proportion of false positives has been observed for *S. pneumoniae*, *H. influenzae* and *S. agalactiae* [163, 170], possibly due to contamination during the procedure [163], emphasizing the importance of proper material handling and cleaning. Both false positives and false negatives have been reported for *S. agalactiae* [162].

Cryptococcal meningitis diagnosis requires cerebrospinal fluid (CSF) culture and cryptococcal antigen detection, along with syndromic panels. A multicenter study found FilmArray ME panel sensitivity/specificity at 96.4%/99.6% versus culture and 83.8%/99.9% versus antigen [171]. Recent comparisons of FilmArray ME and QIAstat-Dx Meningitis/Encephalitis showed similar diagnostic values [163, 172]. Nosocomial central nervous system (CNS) infections, like those from ventricular catheters, may demand alternative strategies beyond these panels (see 4.4.)

Which patient profile would benefit from the use of syndromic panels in meningitis/encephalitis?

Meningoencephalitis is a severe neurologic syndrome associated with high mortality and disability rate. One in two will require admittance in an intensive care unit. Early antimicrobial therapy is likely to improve the prognosis [173]. RMMSP are of particular interest to antimicrobial stewardship programs, with the aim of placing patients on optimized therapy as soon as possible and discontinuing unnecessary antimicrobials [174]. Diagnosis is based on blood cultures, CSF analysis, Gram stain, culture, molecular tests (PCR) and, in special cases, latex agglutination test (Fig. 4) [175]. However, diagnosis by culture can be delayed and give false negatives. In viral infections, traditional protocols may not be available in laboratories with a smaller number of samples or only with a morning sift schedule [176].

In this context, if the etiology is not clear in the first microbiological evaluation, a syndromic panel should be performed in all patients if meningitis or encephalitis is threatened. RMMSP does not replace the other standard microbiological diagnostics and attempt to identify the pathogen in culture and determination of antibiotic susceptibility [175].

- **Experts' opinion 8:** We suggest performing a RMMSP in all patients when meningitis or encephalitis is suspected, particularly in those patients with admittance criteria in an intensive care unit.

Rapid multiplex molecular syndromic panels: other applications and "Off-label" use

What are the most relevant off-label applications of syndromic panels so far?

In critically ill patients, early diagnosis of intra-abdominal infections is essential. Syndromic panels, such as T2Bacteria and T2Candida, can significantly improve the diagnostic process in just 3–5 h, identifying microorganisms that are often not detected in blood cultures [177, 178]. Additionally, other samples, such as peritoneal fluid and bile, can be used in conjunction with molecular techniques like syndromic panels for a more rapid diagnosis. This strategy offers new possibilities, as discussed as follows.

RMMSP, although licensed for specific clinical uses, show off-label applications documented in the literature, particularly FilmArray™ Blood Culture Identification Panel (BCID). Its detection limit can vary, being lower than other panels, because it is designed for use with positive blood cultures [179–181]. The reliability of the "off-label" result varies depending on the series and type of sample (Table 6). A negative result does not rule out infection by the microorganisms included (or detected) in/by the panel.

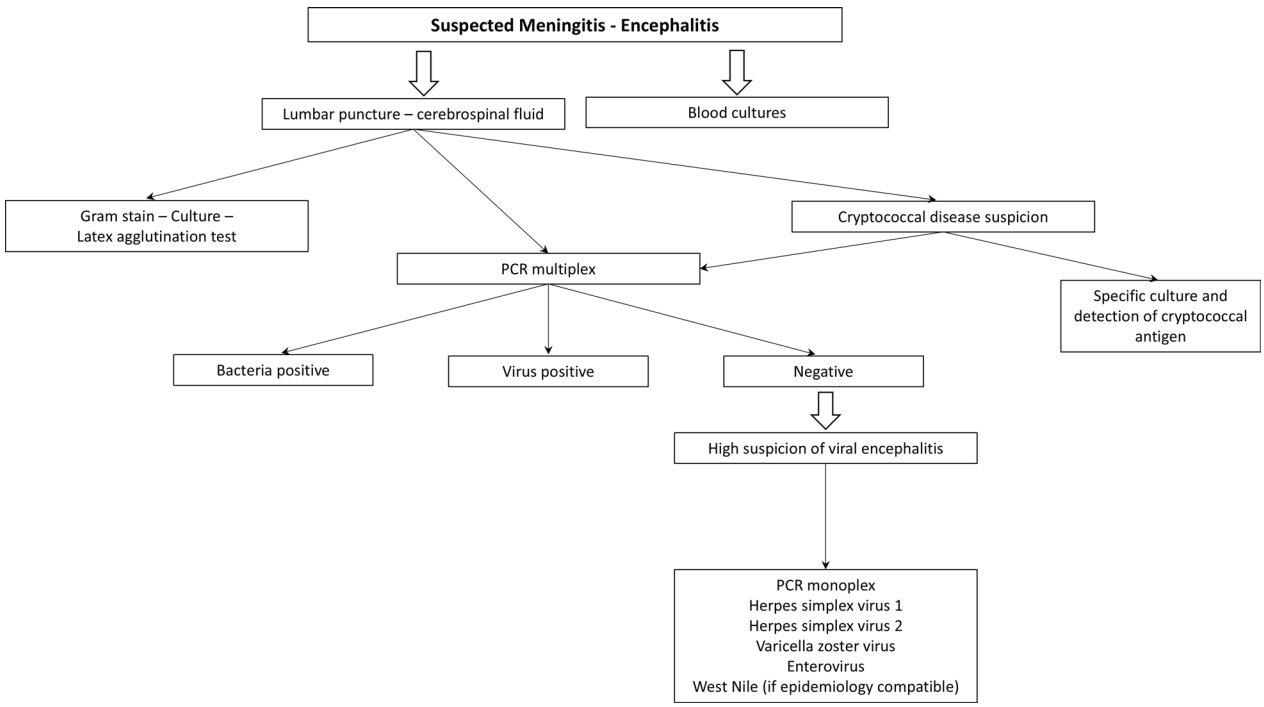

Fig. 4 Diagnostic algorithm for meningoencephalitis. *PCR* Polymerase chain reaction

Table 6 Rapid multiplex molecular syndromic panels used in different microbiological samples (off-label uses)

Samples	System	Comparator	Results	Reference
Ascitic/peritoneal fluid intra-abdominal abscess	FilmArray™ BCID	Culture, metagenomics	Sens: 25–90.5% Spec: 100%	[178, 182]
Bile fluid	Verigene system assays	Culture	Sens: 35.7%	[183]
Pleural fluid	FilmArray™ BCID, FilmArray ME	Culture	Sens: 25% Spec: 100%	[169, 184, 185]
Joint fluid	FilmArray™ BCID Biofire Joint infection panel	Culture	Se: 57–90%	[169, 186–188]
CSF (Nosocomial CNS infections)	FilmArray™ BCID	Culture	Sens: 77.4% Spec: 100% With melting curve analysis: Sens: 83.9%, Spec: 98.3%	[180, 184]
CSF (community and shunt-associated meningitis)	FilmArray™ BCID	Culture	Sens: 50% Spec: 91.7% PPV: 80% NPV: 73.3%	[179]
CSF (community meningitis)	FilmArray™ BCID	Culture	Sens: 73% Spec: 100%	[169]
Aspirates from abscesses and cellulitis	FilmArray™ BCID	Culture	Sens: 89% Spec: 100%	[169, 184]
Bone and tissue biopsies	FilmArray™ BCID	Culture	Concordance 95–100%	[184]
Lymph node aspirates	FilmArray™ BCID	Culture	Concordance 100%	[184]

Sens Sensitivity; *Spec* Specificity; *CSF* Cerebrospinal fluid; *CNS* Central Nervous System; *PPV* positive predictive value; *NPV* negative predictive value

RMMSP, which now include resistance markers, are being also used to study rectal colonization in septic patients without an etiological diagnosis [189–191]. Rectal cultures, biomarkers, and risk scales have proven effective in guiding empirical therapy. RMMSP now offer rapid results within an hour, a significant improvement over the 24–48 h required for conventional surveillance cultures [189–191]. However, its high cost and lack of detection of some resistance mechanisms are challenges [192, 193].

New applications of syndromic panels are being explored, such as the detection of *Cryptosporidium* spp. in respiratory samples and adenovirus in serum using panels designed for other samples [186, 194].

- **Experts' opinion 9:** It is not possible to establish a general opinion on the off-label use of syndromic panels. However, their use in critically ill patients could be considered in the clinical settings described above to obtain indicative microbiological information while awaiting the results of conventional methods.

Conclusions

An overview of the main subjects and essential conceptual insights is shown in Table 7.

Inadequate antibiotic therapy can lead to treatment failure, prolonged illness, and the spread of antibiotic-resistant bacteria. When antibiotics are prescribed without identifying the pathogen or selecting ineffective drugs, infections may persist and facilitate the emergence of MDR bacteria. Therefore, accurate diagnosis and proper antibiotic selection are crucial for managing critically ill patients and controlling antibiotic resistance.

RMMSP offer a promising approach to expedite the diagnosis of infectious diseases by simultaneously testing for multiple pathogens associated with a particular clinical syndrome. These panels utilize molecular techniques, such as PCR, to detect the presence of bacterial, viral, and fungal pathogens directly from clinical specimens like blood, urine, respiratory secretions, or sterile fluids.

RMMSP provide rapid results and comprehensive pathogen detection, potentially supporting precision medicine, improving patient outcomes, and contributing to public health surveillance. However, their clinical performance in specific scenarios and their impact on antibiotic use remain areas of ongoing debate. Therefore, they should always be used from a clinical perspective and within the appropriate clinical context.

Furthermore, RMMSP, combined with NGS, have revolutionized diagnostic workflows by providing precise pathogen identification and resistance profiling. This integration significantly reduces turnaround times, enhancing clinical decision-making and patient

Table 7 Concluding directives: synopsis of principal matters and essential conceptual insights

Key-points	Essential conceptual insights
Item 1	These new diagnostic tools (including RMMSP) do not substitute or replace traditional microbiology; instead, they complement and reinforce it
Item 2	The value, qualification, translation, and impact of the results of the "expert system" must be validated and endorsed by a microbiologist with experience and clinical judgment
Item 3	The principles of precise, accurate, reliable, agile, and rapid diagnosis, based on their predictive values, should guide the indication of new diagnostic tools to minimize errors, inadequacy, or ineffectiveness of empirical antibiotic treatment in an MDR context
Item 4	It is not advisable to periodically repeat these diagnostic procedures (without a justified cause) in those patients who do not experience significant clinical changes over the follow-up, which justifies or makes necessary their use
Item 5	These diagnostic tools are not indicated for patients who have recently had a positive and clinically value microbiological test, simply for ruling out other potential pathogens
Item 6	The results of these diagnostic tools should be binding (unless the patient has been diagnosed with septic shock). Tests results should impact on antimicrobial optimization and decision-making, either escalation, de-escalation, or stop the antibiotic regime
Item 7	Although in real-clinical practice these diagnostic tools have been used in "off-label" clinical scenarios, their use will always be customized (following a patient tailored approach) according to microbiologist criteria and agreed with the clinician. Their results cannot be extrapolated to other patients
Item 8	It should be noted that the projection and use of these diagnostic tools may be subject to different nuances and implications in critically ill patients, compared to other complex but not severely infected patients
Item 9	All these microbiological diagnostic tools need to be progressively evaluated in terms of cost–benefit, influence on morbidity and mortality, and health-related outcomes, in order to select the best diagnostic panel for each patient
Item 10	All these considerations and recommendations must be considered within a dynamic context and should be continually reassessed to implement emerging discoveries and future evidence as they become available

RMMSP rapid multiplex molecular syndromic panels; *MDR* multi-drug resistant

outcomes, particularly in time-sensitive scenarios such as respiratory and central nervous system infectious diseases.

In conclusion, while syndromic panels provide rapid, comprehensive diagnostics for infectious diseases, their use must be balanced with clinical judgment and antibiotic stewardship to optimize care and reduce risks like inadequate therapy and antibiotic resistance. Future advancements may include integrating RMMSP with predictive artificial intelligence, further enhancing diagnostic accuracy and refining the management of critically ill, infected patients.

Abbreviations

AMR	Antimicrobial resistance
AWMF	Association of the scientific medical societies
BAL	Bronchoalveolar lavages
BCID	Blood culture identification panel
CAP	Community-acquired
CFU	Colony-forming unit
CNS	Central nervous system
CSF	Cerebrospinal fluid
DNA	Deoxyribonucleic acid
DTR	Difficult-to-treat resistance
EAT	Empirical antimicrobial therapy
EC	European community
ESBL	Extended spectrum β-lactamases
FA-PNp	BioFire® FilmArray® Pneumonia (PN) panel
FDA	Food and drug administration
GEIPC-SEIMC	Spanish society of clinical microbiology and infectious diseases
GTEIS-SEMICYUC	Spanish society of critical intensive care medicine and coronary care units
HAP	Hospital-acquired
HPN	Unyvero hospitalized pneumonia panel
ICU	Intensive care unit
MALDI TOF	Matrix-assisted laser desorption/ionization time-of-flight
MDR	Multi-drug resistant
MRSA	Methicillin-resistant *Staphylococcus aureus*
PCR	Polymerase chain reaction
PPV	Positive predictive value
RMMSP	Rapid multiplex molecular syndromic panels.
SARS-CoV-2	Severe acute respiratory syndrome-coronavirus type 2.
VAP	Ventilator-associated

Supplementary Information

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Additional file 1

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