Critical Care

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Real-life impact of clinical metagenomics in the intensive care unit: a multicenter retrospective study in greater paris area hospitals

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Abstract

Background Infection are the leading cause of intensive care unit (ICU) admission, yet conventional microbiological methods frequently fail to identify the causative pathogen. Metagenomic next-generation sequencing (mNGS) is an emerging, unbiased, pan-pathogen diagnostic tool. However, its real-world microbiological and clinical impact in the ICU remains poorly characterized. This study aimed to assess the microbiological yield and clinical impact of mNGS when implemented in routine ICU practice.

Methods This retrospective multicenter study was conducted across ten tertiary-care ICUs in the Greater Paris area between January 2018 and April 2024. All patients for whom an mNGS analysis was requested by clinicians from a microbiological sample were included. Any additional pathogens identified by mNGS were independently classified as causative, possibly causative, or non-causative by two reviewers. The independent reviewers also categorised therapeutic changes attributable to mNGS as escalation, de-escalation, discontinuation, or other decision support. Discrepancies were adjudicated by a third reviewer.

Results A total of 144 mNGS analyses were performed in 132 critically ill patients (median age 55 years), 31% of whom were immunocompromised. The number of mNGS analyses requested increased each year. The most common sample types were cerebrospinal fluid (CSF) (n = 60/144, 41.7%) and pleural fluid (n = 21/144, 14.6%). Pathogens were identified by mNGS in 58 samples (40.3%), with a higher yield in pleural fluid (n = 11/21, 52.4%) than in CSF (n = 16/60, 26.6%). Of the 107 pathogens identified, 43 (40.2%) were detected exclusively by mNGS, notably anaerobic bacteria in pleural fluid and abscess samples. mNGS identified an additional pathogen in 34 cases (25.8%) of the 132 patients included, which was deemed causative in 18 cases (13.6%). mNGS findings influenced therapeutic management in

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seven patients (5.3%) including five cases of antibiotic de-escalation, one appropriate antibiotic escalation, and one case of clinical decision support.

Conclusion In this real-life ICU cohort, mNGS identified additional pathogens in 25.8% of patients, deemed causative in 18 cases (13.6%), and a direct therapeutic impact was observed in 5.3% of cases. However, the median turnaround time of 14 days likely limited its clinical impact. Further studies are needed to better define the role of mNGS in the diagnostic management of critically ill patients.

Keywords Metagenomics, Clinical metagenomics, Intensive care unit, Infections

Introduction

Infections are the leading cause of admission to the intensive care unit (ICU) [1]. Early and accurate identification of the causative pathogen(s) is essential in order to guide timely and targeted antimicrobial therapy. However, conventional microbiological techniques fail to identify the causative pathogen(s) in approximately one-third of patients with a confirmed or suspected infection [2]. More specifically, no pathogen is isolated in almost two-thirds of community-acquired pneumonia [3], and around 30% of sepsis episodes [4, 5]. This lack of microbiological documentation often leads to the continuation of empirical therapy, exposing patients to two major risks. First, the prolonged use of potentially ineffective antimicrobial treatment due to the natural or acquired resistance of the causative pathogen. Second, the unnecessary administration of broad-spectrum molecules, with well-known consequences such as resistance selection, toxicity, and increased cost [6]. Importantly, this diagnostic gap is particularly striking in conditions such as meningoencephalitis, where viral or fungal pathogens are frequent and often remain unidentified by conventional methods [7].

Several molecular diagnostic tools have been developed to improve microbiological yield in the ICU, including syndromic PCR panels targeting respiratory, gastrointestinal, and neurological pathogens [8], pathogen-specific PCRs, and broad-range 16S rRNA or ITS PCRs (for bacterial and fungal detection, respectively). However, these targeted approaches may fail cases involving novel or unexpected pathogens—particularly viruses due to the absence of universal molecular target—or in immunocompromised patients, who may present with a wide range of opportunistic infections and atypical clinical symptoms.

In this context, metagenomic next-generation sequencing (mNGS) has emerged as a novel, unbiased, pan-pathogen approach based on the sequencing of all the genetic material present in a biological sample [9]. Its diagnostic performance seems of interest in various clinical scenarios, including meningoencephalitis [10–13], blood culture-negative endocarditis [14, 15], osteoarticular infections [16], pleural infections [17], liver abscesses [18], pneumonia [19], and necrotizing soft tissue infections [20], especially in patients who have received prior antimicrobial therapy [15]. Nevertheless, the real-world microbiological and therapeutic impact and yield of mNGS when implemented in routine ICU practice remains unclear.

This study aimed to assess the microbiological yield and clinical impact of implementing mNGS in routine ICU practice.

Methods

Settings and patients

This retrospective multicenter study was conducted across ten ICUs in tertiary teaching hospitals in the Greater Paris area, including Paris and its metropolitan area, from January 2018 to April 2024. Patients were eligible for inclusion if they met all of the following criteria: age ≥ 18 years and admission to one of the participating ICUs for which an mNGS analysis was requested by the attending physician based on a microbiological specimen. Patients were identified through the metagenomics platform database by selecting samples sent from the participating ICUs during the study period. Patients who underwent mNGS testing exclusively as part of a research protocol were excluded. When multiple samples were collected from the same patient during a single ICU admission, each mNGS result was analyzed independently initially; subsequently, results were pooled to assess the overall microbiological and clinical impact at the patient level. Data regarding demographics, clinical examinations, biology, microbiology, radiology, and treatment characteristics were collected from electronic medical records.

Clinical metagenomics

Samples collected from the participating ICUs were transported at + 4 °C to the metagenomics platform at Henri Mondor Hospital (Créteil, France). Upon arrival, all samples were stored at − 20 °C until the start of the weekly sequencing run. The employed mNGS workflow was a semi-quantitative, pan-microorganism, DNA-, and RNA-based approach called MetaMIC [21]. This protocol has been independently validated [22] and its performance has been compared with that of molecular approaches in various clinical scenarios [18, 20,

21]. Briefly, samples underwent mechanical, chemical, and enzymatic lysis. Then, DNA and RNA extractions were performed using the QIAsymphony DSP DNA Midi kit protocol (Qiagen, Hilden, Germany). Next, DNA and RNA libraries were constructed using the Nextera XT kit, and the Human RiboZero TruSeq Stranded Total RNA Library Prep Kit (Illumina, San Diego, CA, USA), respectively. Sequencing was performed on a NextSeq 500 sequencer (Illumina), with a NextSeq 500/550 High Output v2.5, 2 × 150 bp tape.

Data analysis was performed using METAMIC (V2.2.1) bioinformatic pipeline [21] (Supplemental Methods). Only samples with a cumulative read count exceeding 15 million (comprising reads from both DNA and RNA libraries) were included, as per the prevailing guidelines [23, 24], which propose a threshold of 10 million reads per sample. According to our routine activity, a threshold of one read per million of total reads was retained in order to consider a viral, bacterial or fungal species. Details regarding taxonomic assignment are provided in the Supplemental Methods.

Our method is accredited according to the ISO15189 norm and used in routine practice, and the results were provided to clinicians by a team of microbiologists composed of bacteriologists, virologists and parasitologist-mycologists.

Conventional microbiological analyses

Conventional microbiological analyses included all non-mNGS diagnostic techniques performed during the infectious episode presumed to have led to the mNGS request. These analyses included standard culture techniques, molecular assays (e.g., pathogen-specific PCRs, syndromic panels, and 16S rRNA PCR), and serological tests.

Microbiological and clinical impact of mNGS

The microbiological and clinical impact of mNGS results, in addition to conventional microbiological findings, was independently assessed for each patient by two experts in infectious disease and intensive care medicine (RL and DC, respectively) who were not associated with any of the participating ICUs. In cases of disagreement between the two experts, a third investigator (GM) made the final decision. Detected microorganisms were classified into two categories: pathogens and organisms without established pathogenic potential. Pathogens were further subclassified as causative, possibly causative, or unlikely to be responsible for the clinical presentation [25]. This classification was based on literature review and concordance with the patient's clinical features. The clinical impact of mNGS results was categorized as follows: discontinuation of antimicrobial therapy, appropriate de-escalation of antimicrobial treatment, appropriate escalation of antimicrobial treatment, facilitation of therapeutic decision-making (e.g., initiation of immunosuppressive therapy), or inappropriate therapeutic management directly attributable to mNGS results.

Statistical analysis

Descriptive results are presented as means (± standard deviation) or medians (interquartile range) for continuous variables, and as counts (percentages) for categorical variables. Statistical analyses were performed using IBM SPSS Statistics v22.0 (IBM Corp., Armonk, NY), GraphPad Prism v8 (GraphPad Software, San Diego, CA, USA), and RStudio v4.2.0 (https://www.r-project.org/). The methods and results of this study are reported according to the STROBE guidelines [26].

Ethical considerations

This observational study was approved by the Ethics Committee of the French Intensive Care Society (CE SRLF 24- 036, IRB N°00014135). Patients were informed of their inclusion in the study and written consent was waived in accordance with French law.

Results

Population

From January 2018 to April 2024, a total of 144 diagnostic mNGS analyses were performed in 132 patients during their ICU stays (see flowchart eFigure 1). The median age was 56 years [44–68] (Table 1). Patients had few cardiovascular comorbidities, while 31.1% (n = 41/132) were immunocompromised, primarily due to onco-hematological malignancies. The most common reason for ICU admission was coma. During the ICU stay, 63.6% of patients (n = 84/132) required invasive mechanical ventilation (Table 1). ICU mortality was 19.7% (n = 26/132), with a median ICU length of stay of 13 days [8–24].

Characteristics of mNGS sampling

The characteristics of mNGS analyses are summarized in Table 2. The number of samples increased steadily over the years (Fig. 1A). The most frequently analysed sample types were cerebrospinal fluid (CSF) (n = 60/144, 41.7%) and pleural fluid (n = 21/144, 14.6%) (Fig. 1B), reflecting the most common suspected sites of infection at the time of sampling: neurological (n = 74/144, 51.4%) and respiratory (n = 58/144, 40.3%). Samples were collected early in the ICU stay, with a median delay of 2 days [1–9] after admission. mNGS results were returned after a median of 14 days [13–20] following sample collection (Table 2 and eFigure 2). Most samples (88.9%, n = 128/144) were collected while patients were receiving antibiotics, with a median duration of 5 days [3–9].

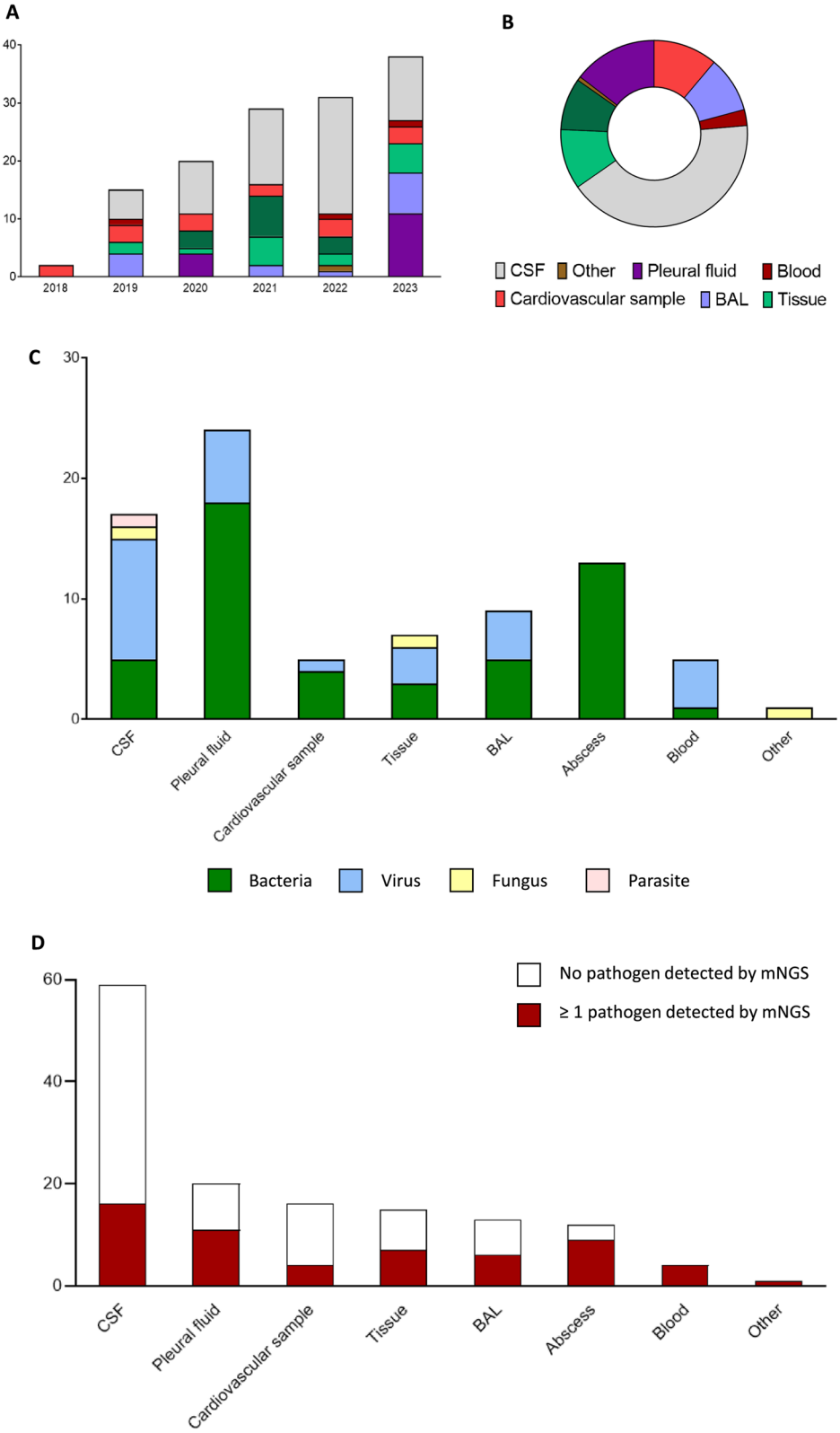

Fig 1. Characteristics of mNGS sampling and identified pathogens (**A**). annual number of NGS analyses and distribution according to sample type. the year 2024 was not shown as the inclusion period covered only january to april. (**B**). distribution of mNGS sample types in the study cohort. (**C**). distribution of pathogen types: bacteria (green), viruses (blue), fungi (yellow), parasites (pink) identified by mNGS, according to sample type. (**D**). number of samples with no or at least one pathogen identified by mNGS, stratified by sample type. abbreviations: BAL, bronchoalveolar lavage CSF, cerebrospinal fluid

Table 1 Characteristics of patients

Variable	Patients, *N*=132
Demographic and baseline characteristics of patients	
Age, years	56 [44–68]
Gender women	49 (37.1)
Body mass index, kg/m^2	24 [21–27]
Medical admission	119 (90.2)
SAPS II at ICU admission	42 [28–56]
Comorbidities	
Charlson comorbidity index	1 [0–2]
Hypertension	38 (28.8)
Diabetes	24 (18.2)
Chronic heart failure	12 (9.1)
COPD	8 (6.1)
Immunosuppressive conditions	41 (31.1)
Solid organ transplantation	10 (7.6)
Onco-hematological malignancies	20 (15.2)
Solid tumors	2 (1.6)
HIV infection	7 (5.3)
Immunosuppressive treatments[1]	9 (6.8)
Reason for admission	
Shock	24 (18.2)
Acute respiratory failure	42 (31.2)
Coma	50 (37.8)
Cardiac arrest	3 (2.3)
Scheduled surgical	2 (1.6)
Unscheduled surgical	11 (8.3)
Management and outcomes of patients	
Invasive MV	84 (64)
MV duration, days	12 [6–28]
Vasopressor support	64 (48.5)
Renal replacement therapy	22 (16.7)
In-ICU mortality	26 (19.7)
Duration of ICU stay, days	13 [8–24]
Duration of hospital stay, days	29 [19–61]

Results are N (%), means (± standard deviation) or medians (interquartile range, i.e., quartile 1; quartile 3).

[1]Includes long-term corticosteroid treatment

ICU, intensive care unit; COPD, chronic obstructive pulmonary disease; HIV, human immunodeficiency virus; MV, mechanical ventilation

Table 2 Characteristics of the 144 samples analyzed by mNGS

Variable	
Time from ICU admission to sampling, days	2 [1–9]
Time from sampling to lab reception, days	1 [0–5]
Time from sampling to mNGS results, days	14 [13–20]
Antibiotic therapy received before sampling	128 (88.8)
Number of antibiotics administered before sampling	2 [2–4]
Duration of antibiotic treatment before sampling, days	5 [3–9]
Suspected site of infection	
Bone and joint	10 (6.9)
CNS	74 (51.4)
Cardiovascular	26 (18.1)
Digestive	10 (6.9)
Disseminated disease	19 (13.2)
Genito-urinary	11 (7.6)
Hepatitis and ocular	21 (14.6)
Pulmonary	58 (40.3)
Skin and soft tissue	16 (11.1)
Type of sample	
Abscess[1]	13 (9.0)
Blood	4 (2.8)
Bronchoalveolar lavage	14 (9.7)
Cardiovascular sample[2]	16 (11.1)
Cerebrospinal fluid	60 (41.7)
Pleural fluid	21 (14.6)
Tissue[3]	15 (10.4)
Bone marrow	1 (0.7)

Results are N (%), means (± standard deviation) or medians (interquartile range, i.e., quartile 1; quartile 3)

CNS, central nervous system; ICU, intensive care unit; mNGS, metagenomic next-generation sequencing

[1]Liver abscess (*N*=7), muscular abscess (*N*=3), pulmonary abscess (*N*=1), splenic abscess (*N*=1), peri renal abscess (*N*=1)

[2]Endomyocardial biopsy (*N*=3), aortic prosthesis (*N*=1), mediastinal collection (*N*=2), periaortic collection (*N*=1), pericardial effusion (*N*=6), arterial stent (*N*=1), thrombosis (*N*=1), cardiac valve sample (*N*=1)

[3]Colonic sample (*N*=1), liver biopsy (*N*=4), lung biopsy (*N*=4), lymph node (*N*=1), Skin biopsy (*N*=3), bone biopsy (*N*=2)

mNGS findings

Among the 144 samples analyzed, NGS detected 81 pathogens and 6 microorganisms without a known pathogenic role, all corresponding to Torque Teno Virus. The 81 pathogens were identified in 58 positive samples (40.3%), including 49 bacteria, 28 viruses, 3 fungi, and 1 parasite (Fig. 1C). The distribution of microorganisms according to sample type is shown in Fig. 1D. Pathogens were detected in 52.3% of pleural fluid samples (n = 11/21) and in 26.7% of CSF samples (n = 16/60). The concordance between conventional microbiology and mNGS results is detailed in Fig. 2. In 70 samples (48.6%), no pathogen was identified by either method. Of the 107 pathogens identified overall, 43 (40.2%) were detected exclusively by mNGS, 34 (31.8%) by both mNGS and conventional microbiology, and 30 (28.0%) by conventional microbiology alone. Notably, mNGS detected several additional anaerobic bacteria (e.g., *Fusobacterium nucleatum*) particularly in pleural fluid and abscess samples. A complete list of all identified microorganisms is provided in eTable 1.

Microbiological impact of mNGS

Of the 132 patients included, mNGS identified at least one additional pathogen not detected by conventional methods in 34 patients (25.8%). Based on an independent expert assessment, these additional pathogens were classified as non-causative to the clinical presentation in 13 patients (9.8%), possibly causative in 3 patients (2.3%), and causative in 18 patients (13.6%) (Fig. 3A). Concordance between the first two experts regarding the microbiological impact was 96.9% (n = 128/132). The pathogens deemed non-causative were predominantly viruses

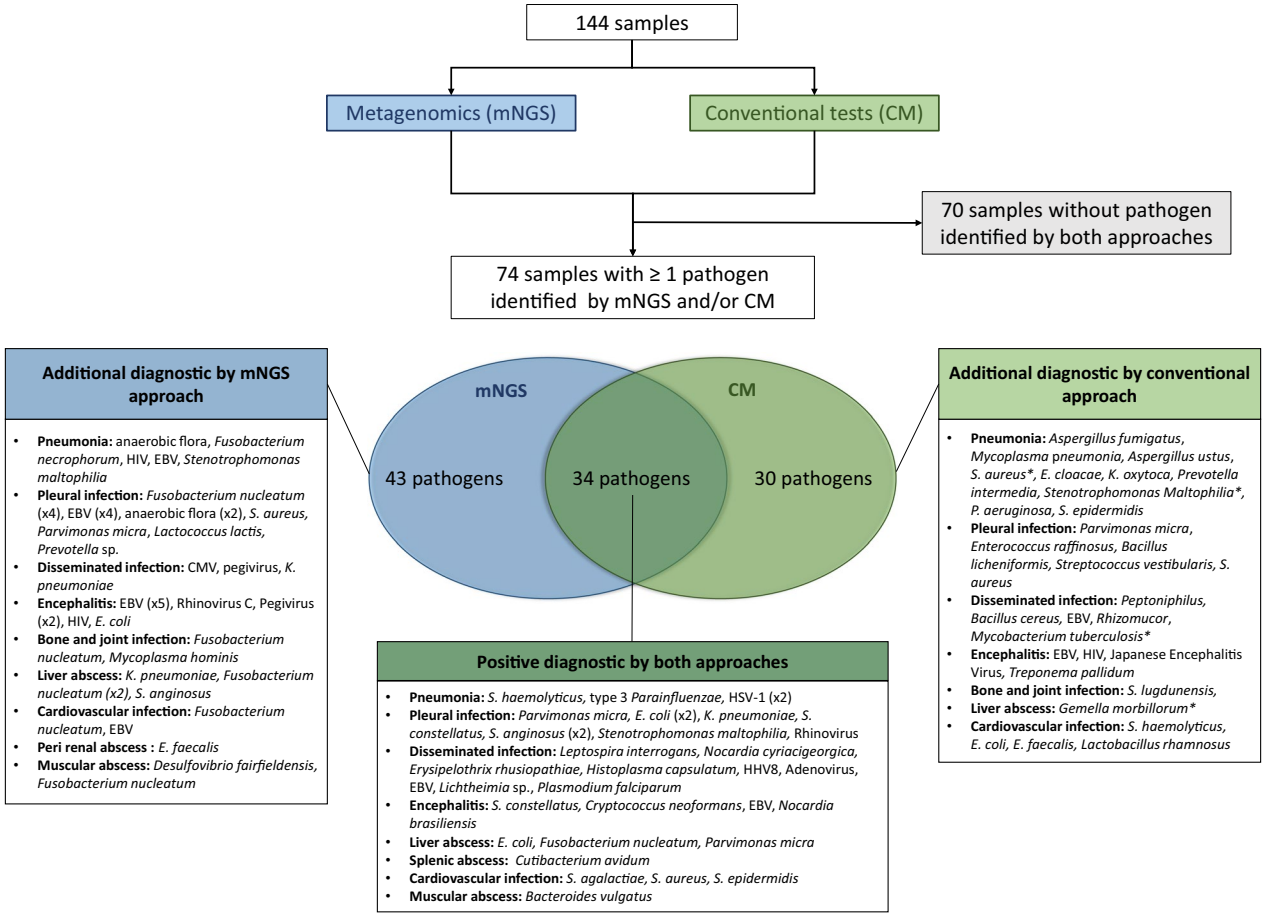

Fig 2. Concordance between mNGS and conventional microbiology. venn diagram illustrating the overlap of microorganism identification between metagenomic mNGS and conventional microbiological techniques. abbreviations: BAL, bronchoalveolar lavage CSF, cerebrospinal fluid; CM, conventional microbiology; CMV, cytomegalovirus; EBV, epstein-barr virus; HIV, human immunodeficiency virus; HHV-8, Human herpes virus 8; HSV, herpes simplex virus; mNGS, metagenomic next-generation sequencing; TTV, Torque teno virus.*four pathogens also detected by mNGS but not reported because read counts were below the reporting threshold or the organism was considered a contaminant

(Epstein Barr virus n = 8, Pegivirus n = 2, and Rhinovirus C n = 1). In contrast pathogens considered causative were mainly anaerobic bacteria (*Fusobacterium* spp. n = 9, *Parvimonas micra* n = 1, *Prevotella* spp. n = 1) and Enterobacterales (*K. pneumoniae* n = 2, *E. coli* n = 1). Detailed microbiological findings for all samples with additional mNGS-detected pathogens are provided in eTable 2. The type of sample analyzed ($p < 0.01$, Fig. 4) was significantly associated with the microbiological impact of mNGS (defined as the detection of at least one additional causative or possibly causative pathogen). Specifically, mNGS yielded a microbiological impact in 46.2% (n = 6/13) of abscess samples, 28.6% (n = 6/21) of pleural fluid samples and 26.7% (n = 4/15) of tissue samples, compared with only 5.0% (n = 3/60) of CSF samples, none (n = 0/4) of blood samples and 6.2% (n = 1/16) of cardiovascular samples. Prior antibiotic therapy ($p = 0.99$), and immunosuppression status ($p = 0.66$) showed no significant association.

Therapeutic impact of mNGS

Of the 132 patients, seven (5.3%) had mNGS results that prompted changes in therapy, as determined by independent, blinded adjudication. Concordance between the first two experts regarding the therapeutic impact was 94.7% (n = 125/132). Three patients underwent antibiotic de-escalation, two with discontinued antibiotic therapy, one with appropriate therapeutic escalation, and one benefited from clinical decision support (Fig. 3B). The presence of antibiotic therapy prior sampling ($p = 0.52$), the type of sample analyzed ($p = 0.77$), immunosuppression status ($p = 0.098$) and turnaround time ($p = 0.7$) were not significantly associated with therapeutic impact (eTable 3). The clinical scenarios in which mNGS findings influenced patient management are detailed in eTable 4. No patient had inappropriate therapeutic management directly attributable to mNGS results.

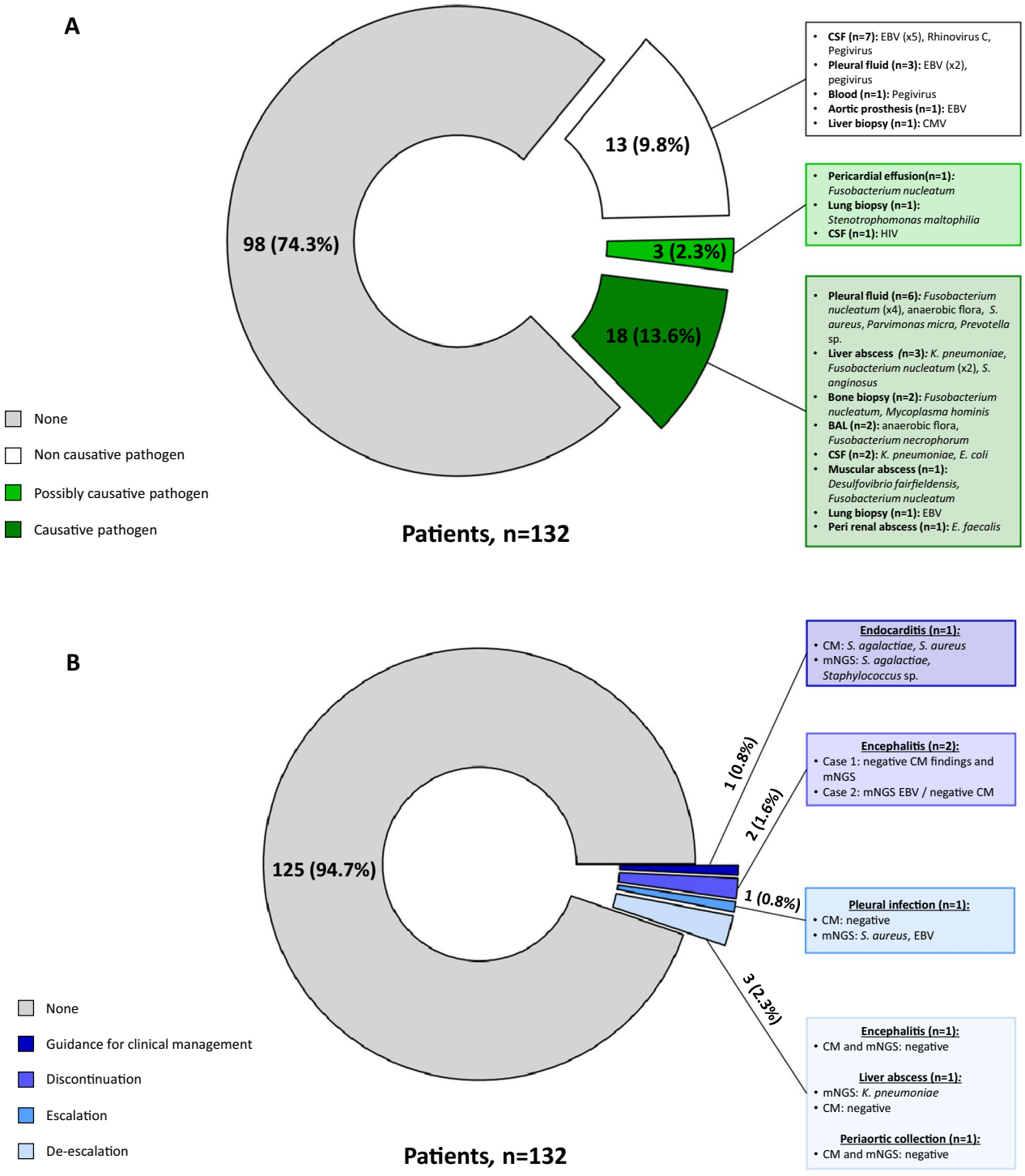

Fig 3. Microbiological and clinical impact of mNGS (**A**). microbiological impact of mNGS, defined as the identification of at least one additional pathogen not detected by conventional methods. (**B**). clinical impact of mNGS, defined as any documented change in antimicrobial management (escalation, de-escalation, discontinuation) or other therapeutic decision directly attributable to mNGS results. abbreviations: BAL, bronchoalveolar lavage CSF, cerebrospinal fluid; CMV, cytomegalovirus; EBV, Epstein-Barr virus; HIV, human immunodeficiency virus; HHV-8, Human herpes virus 8; mNGS, metagenomic next-generation sequencing

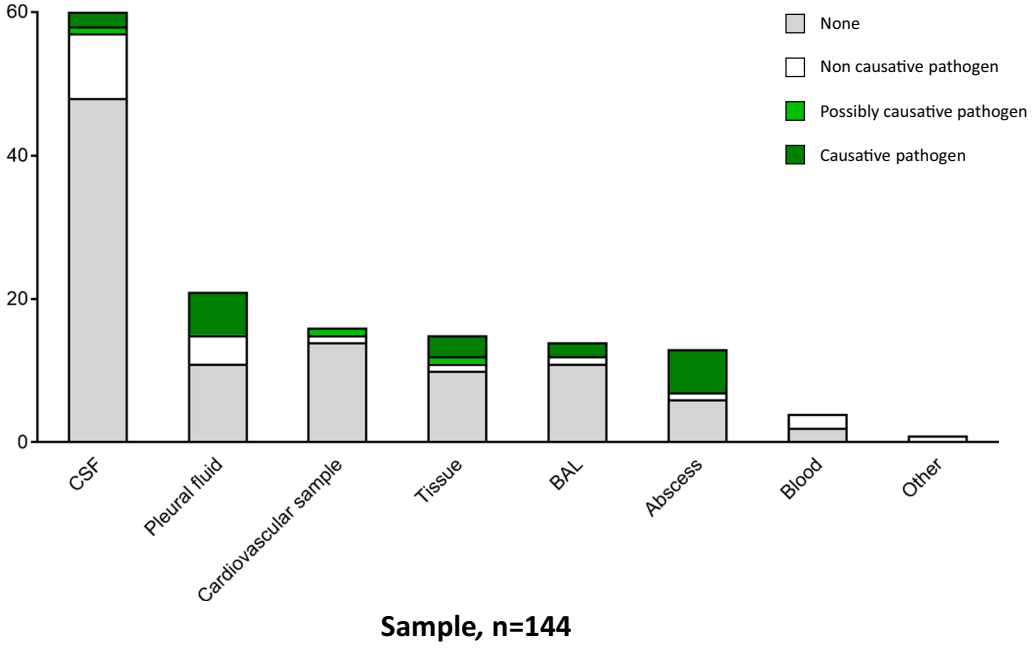

Fig 4. Microbiological impact of mNGS according to sample type. abbreviations: BAL, bronchoalveolar lavage CSF, cerebrospinal fluid

Discussion

This retrospective multicenter study involved 144 mNGS analyses performed in 132 critically ill patients. In 74 positive samples by mNGS and/or conventional microbiology, 107 pathogens were identified: 43 (40.2%) exclusively by mNGS, 34 (31.7%) by both mNGS and conventional microbiology, and 30 (28.0%) by conventional microbiology alone. Overall, mNGS identified at least one additional pathogen in 34 patients (25.7%) including 21 (15.9%) with possibly or causative pathogen according to expert assessment mostly in abscess, tissue or pleural fluid samples. mNGS directly influenced therapeutic management in seven patients (5.3%). As observed in our cohort, the use of mNGS in the ICU is steadily increasing, underscoring the need for robust real-world assessment of its clinical and microbiological impact.

The main strength of this study lies in its evaluation of mNGS in a real-life ICU clinical context, with test requests initiated by the treating physicians based on their individual clinical judgment. The multicenter design of the study further enhances the generalizability of the findings. Additionally, the microbiological and clinical impact of mNGS was assessed independently by external experts in infectiology and intensive care medicine. These methodological features strengthen the objectivity and robustness of the evaluation of this emerging diagnostic tool in routine ICU practice.

In our study, mNGS identified an additional pathogen in one-quarter of patients, including 18 cases (13.6%) where the detected pathogen was considered causative of the clinical presentation. This microbiological yield appears more limited than that reported in previous studies, which often evaluated mNGS in narrowly defined clinical settings, such as meningoencephalitis [12, 13], respiratory infections in ICU patients [19], liver abscesses [18], or pneumonia in immunocompromised hosts [27]. However, these studies frequently applied mNGS systematically within a predefined diagnostic framework, rather than based on the clinical judgment of the treating physician. This distinction makes the assessment of real-world clinical impact more challenging. Two recent studies [25, 28], have explored the microbiological contribution of mNGS in routine practice. The French study by Fourgeaud et al. [25], conducted primarily in paediatric and immunocompromised populations, integrated mNGS early in the diagnostic process. Many of the pathogens identified were specific to these population (e.g., Aichi virus, adenovirus), and causal relationship with the clinical presentation was sometimes uncertain, particularly for herpesviruses. Consistently, in our study, additional pathogens identified exclusively by mNGS and classified as non-causative were most often viruses (e.g., Pegivirus, Cytomegalovirus). Tang et al.'s study [28], conducted in China, focused on hospitalized patients and tissue samples, with lung biopsies being the most frequently collected specimens—a setting that differs substantially from ICU practice. They identified a wide range of pathogens, included anaerobic bacteria and organisms commonly involved in pneumonia (e.g., *S. aureus*, *P. aeruginosa*). Similarly, in our cohort, additional pathogens identified by mNGS and considered as causative were predominantly anaerobic bacteria, particularly in pleural

fluid and abscess samples. The superiority of mNGS over conventional microbiology in the detection of anaerobes has already been demonstrated in liver abscesses [18]. However, beyond abscess or pleural fluid samples, the added microbiological value of mNGS should be interpreted with caution. mNGS can also detect organisms of uncertain clinical relevance. This is particularly the case for viruses such as EBV or CMV, where latent carriage cannot be distinguished from active infection, or for highly prevalent viruses such as Pegivirus, whose pathogenic role remains unclear. These findings, especially in immunocompromised patients with complex presentations, complicate the clinical interpretation of results. In our cohort, CSF was the most frequently analyzed sample, reflecting the diagnostic challenges of meningoencephalitis in ICU patients which have infectious and non-infectious etiologies [29]. Of the 60 CSF samples, only two (3.3%) yielded an additional pathogen (one *K. pneumoniae* and one *E. coli*). The limited added value of mNGS in this setting contrasts with previous reports and may be explained by our study's use of mNGS as a last resort (i.e., after negative results from conventional microbiology) or by a low pre-test probability of infection (i.e., suspected autoimmune meningoencephalitis). mNGS has shown higher diagnostic performance in brain biopsy samples compared to CSF samples [30], but such invasive procedures are rarely performed in critically ill patients. In critically ill patients, our findings suggest that the microbiological yield of mNGS is highest for abscess, tissue and pleural fluid samples, while it appears lower for other specimen types, particularly blood, cardiovascular and cerebrospinal fluid samples.

In the present study, 30 of the 118 identified pathogens were detected exclusively by conventional microbiological methods. This finding underscores that metagenomics cannot replace standard diagnostic approaches but should instead be considered a complementary tool. The risk of false negatives with mNGS has been reported in several previous studies [12, 13, 19, 31], particularly in cases of invasive fungal infections. Several hypotheses may explain such detection failures: a high human DNA background in the sample [32], prior administration of antimicrobial therapy, clearance of the pathogen before sampling (with subsequent diagnosis confirmed only by serology), or detection of the causative agent in a different anatomical site than the one sampled for mNGS. Additional technical limitations may also contribute, such as low pathogen titres in the sample or DNA extraction challenges related to the structural characteristics of certain organisms, such as the thick, polysaccharide-rich cell walls of fungi like *Aspergillus spp.* [31, 33].

In our study, mNGS findings influenced therapeutic management in fewer than 10% of patients. This rate is lower than that in previous studies, where reported therapeutic impact reached up to 50%. However, those studies investigated very different settings, such as rapid-turnaround nanopore sequencing in critically-ill patients with pneumonia [19], or tissue biopsies (mainly lung biopsies) in non-ICU hospitalized patients [28]. Similarly, two recent studies have reported a high simulated therapeutic impact (20–30%) of plasma microbial cell-free DNA sequencing used as a first-line diagnostic tool in patients with a high pre-test probability of infection [34, 35]; yet, these were simulation-based analyses rather than real-world evaluations of mNGS. Conversely, the reported therapeutic impact of mNGS in meningoencephalitis by Wilson's et al. [12] was similar to that observed in our study (3.4%). Several factors may explain this limited clinical impact. First, the prolonged turnaround time reduced the clinical impact of mNGS results in many cases. Sixty-three patients in the cohort had been discharged or had died by the time the results were available. Second, the identification of an additional pathogen did not always lead to a therapeutic modification, particularly in cases such as liver hepatic abscesses or pleural infections, where the involvement of anaerobic bacteria is already well established. In other instances, the clinical relevance of the detected pathogen remained uncertain. Finally, the high performance of conventional diagnostic methods (e.g., targeted cultures, pathogen-specific PCRs, multiplex PCR panels) may have further limited the added value of mNGS in routine practice. mNGS should therefore be integrated into a thoughtful, stepwise diagnostic strategy, particularly in situations where conventional microbiological approaches have failed, and where identifying an additional pathogen is likely to directly influence clinical management. Moreover, it is important to emphasize that mNGS does not refer to a single, uniform technique but rather to a variety of approaches that differ greatly in terms of performances (extraction efficacy, depletion of human sequences, sequencing depth, …), cost and turnaround time. Optimizing mNGS for the specific needs of critically ill patients is a major challenge for the future. For instance, patients with a high pathogen load could benefit from rapid, lower-depth sequencing strategies to speed up reporting. In contrast, those with complex symptoms, particularly when rare or opportunistic pathogens such as fungi are suspected, may require deeper sequencing to maximize sensitivity. Finally, other potential applications of mNGS were not explored in this study [9]. These include the detection of antimicrobial resistance genes [36, 37], the analysis of host–pathogen interaction through transcriptomics or metatranscriptomics approaches [38], and the characterization of the microbiota and commensal organisms at the sampled site [39, 40]. Identifying clinical scenarios in which mNGS is likely to provide a meaningful clinical impact remains

challenging and requires further studies, along with continued efforts to optimise turnaround times.

Our study has several limitations. First, it is a retrospective study, which requires cautious interpretation of the results. Second, the decision to perform mNGS testing was left to the discretion of the treating physicians and was not protocolized. This approach resulted in considerable heterogeneity in clinical scenarios and pre-test probabilities of infection. In some cases, mNGS was used as a last-resort diagnostic tool after multiple conventional diagnostic tests had already been performed. Nevertheless, this reflects the real-life conditions of mNGS use in critically-ill patients. Third, the mNGS median turnaround time was longer than that previously described [13, 19, 25, 28] and may have limited its clinical impact. Several factors may explain this delay: (i) the frequent use of mNGS as a second-line test after awaiting conventional microbiology results, (ii) the weekly batch sequencing schedule used in our workflow and (iii) this workflow was designed as a "universal" sequencing pipeline, rather than being optimized specifically for critically ill patients. However, this delay allowed mNGS to be incorporated into a broader diagnostic strategy and, importantly, never led to inappropriate clinical decision-making in our cohort. Finally, mNGS analyses were performed at a single center, which may appear to limit the external validity and the cross-platform generalizability. However, this center is an accredited national reference laboratory, that regularly participates in external assessments and reproducibility of our workflow has been validated and benchmarked in published comparative studie [22, 41].

Conclusion

In this multicenter cohort of 132 critically ill patients, the real-life use of mNGS identified an additional pathogen in 25.7% of cases, of which 18 (14%) were deemed causative, and influenced therapeutic management in 5.3% of cases. However, the median turnaround time of 14 days likely limited its clinical impact. CSF was the most frequently analyzed sample type. Further prospective studies are needed to better define the role of mNGS in diagnosing critically ill patients.

Abbreviations

CSF	Cerebrospinal fluid
ICU	Intensive care unit
mNGS	Metagenomic next-generation sequencing (mNGS)

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13054-025-05764-2.

Additional file1 eFigure 1. Flow chart of the study. abbreviations: ICU, intensive care unit

Additional file2 eFigure 2. Distribution of turnaround time from sample collection to result reporting for the 144 mNGS tests

Additional file 3

Additional file 4

Additional file 5

Additional file 6

Additional file 7

Additional file 8

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None

Author contributions

PB, KR, PC, CR, CA and PLW designed the study and analysed the data. RL, DC and GM analysed the data. PB, NM, MP, GV, RS, MPdC, TU, TP, MD, FP, NdP, AMD, and KR included the patient and were responsible for clinical data collection. PB, PC, CR, PLW and KR wrote the first draft of the article. All authors revised and approved the article. The corresponding author attests that all listed authors meet authorship criteria and that no others meeting the criteria have been omitted. PB is the guarantor.

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None.

Data availability

The datasets used and/or analysed during the current study are available from the corresponding author on reasonable request.

Declarations

Ethics approval and consent to participate

This observational study was approved by the Ethics Committee of the French Intensive Care Society (CE SRLF 24- 036, IRB N°00014135). Patients were informed of their inclusion in the study and written consent was waived in accordance with French law.

Consent for publication

Not applicable.

Competing interests

KR received lecture fees from MSD, Shionogi, and a travel grant from Pfizer outside the submitted work. MD has served as a speaker for Fisher and Paykel and Isis Medical. NdP has served as an advisor or speaker for Moderna and AstraZeneca, outside of the submitted work. GV reports research grants from BioMérieux and SOS Oxygene, and support for attending meetings from Oxyvie and SOS oxygène, outside of the submitted work. CR reports having served as a speaker for Pfizer and Tecan, outside of the submitted work. AMD reports grants from Fischer Paykel, Baxter, Philips, Ferring and GSK, personal fees from Air Liquide, Baxter, Amomed, Getingue and Addmedica, outside the submitted work. All other authors declare that they have no competing interests.

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