РОЗДІЛ 1. Емерджентні хвороби та Єдине здоров'я
SECTION 1. Emergent diseases and One Health

DOI: 10.31073/onehealthjournal2025-V-01

METAGENOMICS SEQUENCING IN INFECTION DIAGNOSIS: CLINICAL APPLICATIONS

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Abstract. *By performing a literature review, to identify categories of patients in whom metagenomics sequencing has demonstrated potential clinical relevance in the diagnosis of infections, highlighting the practical applications, benefits and limitations of this method in the context of different clinical scenarios for further adaptation of clinical and laboratory diagnostic protocols. The study is descriptive and analytical. A literature review was conducted, using literature from open access databases. English language papers relevant to the proposed research topic published in the last 5 years were selected according to the inclusion criteria. Metagenomics sequencing (mNGS) is an innovative technology that has revolutionized traditional clinical diagnostics on multiple levels. It can be used for the simultaneous identification of multiple pathogens such as bacteria, viruses, fungi and parasites in clinical samples. The application of metagenomics would bring considerable benefits even in cases of rare infections with unknown etiology or pathogens that are difficult to culture. This study demonstrates how this new technology can be used for meaningful clinical diagnostics in microbiology. Patients with lower respiratory tract infections represent a category of patients for whom the use of mNGS brings several benefits, this technology has an overall higher sensitivity for pathogen detection compared to the culture method and is a necessary complement to conventional microbiologic tests. Sepsis is a major public health challenge and the use of mNGS for this category of patients can shorten the total hospitalization time and reduce mortality. Metagenomics sequencing technology is also useful for patients with central nervous system infections, the use of mNSG for those patients has a higher detection rate but the highest diagnostic yield is observed when combining mNGS with conventional methods. Metagenomics offers significant benefits in the detection of pathogens also in patients with deep cervical space abscesses, with a superior detection rate and sensitivity, allowing early initiation of antimicrobial therapy, improved prognosis and reduced consumption of medical resources. Metagenomics provides a complete, rapid and accurate identification of pathogens, significantly improving treatment, mainly when traditional methods are insufficient. Based on the studies presented in this article we can conclude that the application of metagenomics for patients with lower respiratory tract infections, sepsis, central nervous system infections and cervical deep space infections has a very high potential to reduce morbidity, mortality and health care costs.*

Keywords: metagenomics, diagnosis, sepsis, lower respiratory tract infections, central nervous system infections.

Introduction. Metagenomics (mNGS) is an advanced technique that allows the simultaneous identification of multiple pathogens (bacteria, viruses, fungi, parasites) from clinical samples without relying on specific antigenic tests or culture. The etiology of infectious diseases in a very large number of patients remains unidentified, especially in immunocompromised patients with long hospitalizations, oncologic, hereditary or transplant diseases, resulting in inadequate and delayed treatment, increased length of hospitalization,

readmissions, additional health care costs, and increased morbidity and mortality. A conventional bacterial culture takes 3-5 days and has a high rate of false negative results, while some pathogenic bacteria are difficult to culture and require a longer time. Metagenomics would make it easier to diagnose more quickly and adjust antibiotic therapy as soon as possible. (d'Humières, 2021; Tan, 2021; Sun, 2022; Rajendhran, 2024; Lei, 2025).

Whereas culture-based methods have limitations due to patients' prophylactic administration of broad-spectrum antimicrobial drugs (e.g. organ transplantation) or for treatment in case of the need to identify the causative agents of secondary infections in patients already receiving treatment for an infectious disease. Other causes limiting diagnosis by culture-based methods are targeted diagnosis and samples with low microbial loads. The application of metagenomics would bring considerable benefits in cases of rare infections with unknown etiology or pathogens that are difficult to culture. (d'Humières, 2021; Tan 2021; Rajendhran, 2024).

Metagenomics is changing the way clinicians diagnose and treat infectious diseases, with applications covering a wide range of fields, including antimicrobial resistance, the microbiome, human host gene expression (transcriptomics) and oncology. (Chiu, 2019).

Aim of the study. By performing a literature review, to identify categories of patients in whom metagenomic sequencing has demonstrated or potential clinical relevance in the diagnosis of infections, highlighting the practical applications, benefits and limitations of this method in the context of different clinical scenarios for further adaptation of clinical and laboratory diagnostic protocols.

Materials and methods. The study is descriptive, analytical. A literature review was performed using open access databases. Articles were identified based on the keywords: 'metagenomics', 'diagnosis', 'sepsis', 'lower respiratory tract infections', 'central nervous system infections'. A total of 223 scientific papers were selected for the study. As a result, after a comprehensive analysis based on the selection criteria, 24 publications were included for this study.

To facilitate a thorough analysis as inclusion criteria were used: articles relevant to the topic proposed for research published in English within the last 5 years. To ensure the timeliness of the data, articles published before 2019 and not presenting a clear research methodology were excluded.

To provide a complete overview and robust data analysis the analyzed articles were categorized into: systematic reviews, prevalence studies and qualitative studies. Therefore, the content of the articles was assessed on the basis of variables such as the aim of the study, methodology, year of publication and results obtained. From the information collected, we selected the most relevant and recent studies that provide clear insights into the application of metagenomic sequencing for microbiologic diagnosis in some categories of patients of importance for clinical medicine.

Results. *Lower respiratory tract infections* (LRTI) are the fifth leading cause of death worldwide and include community-acquired pneumonia, nosocomial pneumonia, bronchitis, bronchiolitis and tracheitis. LRIs are caused by a wide range of pathogens, such as bacteria, viruses, mycoplasmas and fungi, presenting similar clinical pictures. Despite thorough diagnostic investigations, the etiology of up to 62% of community-acquired pneumonia cases remain unidentified (Diao, 2021).

Patients with *lower respiratory tract infections* (LRTIs) belong to the category of patients for whom the use of metagenomics brings benefits; this is demonstrated in several studies. The efficacy of using mNGS compared to conventional microbiological methods for identifying the causative agent of pneumonia in adult patients is brought forward in a study published in 2023 involving 205 patients with community-acquired pneumonia admitted between September 2019 and September 2021 to the intensive care unit of Quanzhou First Hospital, Quanzhou, China. As a result, it was obtained that mNGS has a higher overall sensitivity for pathogen detection than the culture method and is a necessary supplement to conventional microbiologic tests for the detection of causative agents of pulmonary infections (Lin, 2023).

The necessity of using mNGS for the diagnosis of causative agents of pneumonia in children is demonstrated in a 2024 study involving 60 children with pneumonia treated in the Department of Respiratory Medicine at a Beijing Children's Hospital. The study demonstrated that mNGS shows superior efficiency over traditional methods in the diagnosis of pneumonia in children, significantly increasing the detection rate of pathogens in pneumonia cases (Wang, 2024).

Additional studies involving the use of metagenomics and demonstrating the beneficial impact of its application for patients with lower respiratory infections have been presented in (Table 1).

Sepsis. Clinically, sepsis is a continuum ranging from systemic inflammatory response syndrome to multiple organ failure syndrome. Sepsis is a major public health challenge and was listed as a global health priority by the World Health Organization (WHO) in 2017. Approximately 1.94-31.5 million deaths occur annually globally, of which approximately 20% (5.3 million deaths) are attributed to sepsis (Tan, 2021; Sun, 2022; Nielsen, 2025).

Septic patients is another category of patients for whom mNGS would bring significant benefits. A 2024 study of 150 sepsis patients admitted to the intensive care units of four hospitals in southwest China from October 1, 2018 to October 1, 2021, showed that mNGS has high sensitivity in diagnosing sepsis patients, especially for fungal and viral infections, and that mNGS technology is useful for critically ill sepsis patients (Li, 2024). Other studies demonstrating the effectiveness of using metagenomics for patients with sepsis are presented in (Table 2).

Central nervous system infections (CNSI) are complex disease entities, associated with a high potential for morbidity and mortality or even a certain proportion of permanent neurological sequelae, depending on the type of causative pathogen. Globally, there are an estimated 10.6 million cases of meningitis and 288,000 deaths from meningitis each year, the vast majority of which are attributed to low- and middle-income countries (Saha, 2019; Piantadosi, 2021; Chen, 2024).

Recent studies describing the advantages of using metagenomics for the diagnosis of patients with central nervous system infections have been included in (Table 3).

Cervical deep space infections. Another category of patients for whom the use of metagenomics would provide significant benefit includes those with *cervical deep space infections*. This is relevant according to a study conducted in 2025 that included 106 adult patients with cervical deep space abscesses (DCSA). It concluded that mNGS has a higher detection rate and sensitivity in identifying pathogens associated with DCSA and may help to rationalize antimicrobial therapy early, thereby facilitating patient recovery, improving prognosis and reducing the burden on medical resources. (Lei, 2025).

Discussion. Based on the review of scientific publications, articles were identified that presented studies describing the application of metagenomics for microbiologic diagnosis in several categories of patients. Batool M and Galloway-Peña J (2023) found analogous to those described in the current study that mNGS has undeniable benefits over traditional testing and provides a more complete picture of the clinical condition and treatment prospects. The combination of mNGS and conventional diagnostic methods could be a superior diagnostic strategy for improving overall public health and healthcare costs. The authors of that study also described the target populations for implementing clinical metagenomics as a standard of care - patients at risk of mortality due to rare or difficult to treat pathogens, critically ill and immunocompromised patients (transplant, cancer, etc.) (Batool, 2023).

Table 1

Studies involving the use of metagenomics with impact on patients with lower respiratory infections

References	Number of patients	Patients' description	Tests performed	Conclusions
1	2	3	4	5
Charalampous, 2019	41	Patients suspected of pneumonia	mNGS and conventional microbiological tests	With metagenomics, we can quickly and accurately identify bacterial infections of the lower respiratory tract and could help reduce the use of broad-spectrum antibiotics.
Charalampous, 2024	87	128 samples from 87 patients with suspected lower respiratory tract infection	mNGS and conventional microbiological tests	The clinical usefulness of metagenomics has been demonstrated by identifying both microorganisms detected by routine methods and those that are fastidious or unculturable, while providing information for predicting antimicrobial resistance.
Tsitsiklis, 2022	397	276 children with LRTI and 121 without evidence of LRTI in critical condition	mNGS and conventional microbiological tests	mNGS allowed microbiologic diagnosis in a higher proportion of cases (90%) than routine microbiologic testing (67%).

Table 2

Studies involving the use of metagenomics with impact on patients with sepsis

References	Number of patients	Patients' description	Tests	Conclusions
1	2	3	4	5
Sun, 2022	124	Patients with severe sepsis	mNGS and conventional microbiological tests	The use of mNGS for pathogen detection in patients with severe sepsis has advantages such as a rapid and high positive detection rate compared to routine blood cultures.
Li, 2022	71	Patients with sepsis and sepsis-induced lymphopenia	mNGS and conventional microbiological tests	The mNGS tests performed showed a higher detection rate compared to conventional microbiological tests.
Qin, 2023	194	Patients with sepsis and bloodstream infections	mNGS and conventional microbiological tests	mNGS combines the advantages of identifying the pathogens that cause sepsis and bloodstream infections at a high positivity rate and in a short time. Routine blood culture supplemented with mNGS can significantly reduce mortality in these patients. Early detection using mNGS may shorten the overall hospitalization time of patients with sepsis and bloodstream infections.
Li, 2023	308	Adult patients with sepsis	mNGS and conventional microbiological tests	mNGS identified pathogens more accurately than conventional culture methods and contributed to more effective treatment of patients.

Table 3

Studies involving the use of metagenomics with impact for patients with central nervous system infections

References	Number of patients	Patients' description	Tests	Conclusions
1	2	3	4	5
Wilson, 2019	204	Patients with meningitis, encephalitis, myelitis	mNGS and conventional microbiological tests	The highest diagnostic yield resulted from a combination of CSF mNGS and conventional testing.
Piantadosi, 2021	68	44 patients diagnosed with CNS infections; 24 - suspects.	mNGS and conventional microbiological tests	Rational use of molecular diagnostic techniques such as mNGS can have a positive impact on patient care and associated costs.
Chen, 2024	89	Patients diagnosed with infectious meningitis or encephalitis	mNGS and conventional microbiological tests	mNGS tests provide additional information to conventional microbiological tests.
Wang, 2023	50	Pediatric patients suspected of viral encephalitis or meningitis	mNGS and conventional microbiological tests	mNGS had a higher detection rate compared to traditional PCR
Gámbaro, 2023	23	Patients with aseptic meningitis and no etiology identified by conventional tests	mNGS and conventional microbiological tests	mNGS can identify pathogens that are not usually identified by routine diagnostic methods

Rajendhran J. et al. (2024) similarly reported that the use of mNGS represents a revolutionary advance in the microbiologic diagnosis of infectious diseases and that it provides complete, rapid and accurate identification of pathogens, significantly improving the diagnosis of patients, mainly when traditional methods are insufficient. In addition to the current study the authors also described the need to establish a standard protocol for the diagnosis of various infectious diseases using mNGS (Rajendhran, 2024).

d'Humières, C. et al. (2021) described, similar to the current study, the use of metagenomic sequencing for the diagnosis of various infectious diseases but in their conclusions focused more on describing the need for standardization of methods and the conduct of randomized controlled trials to define the role of mNGS in the diagnostic algorithm and develop robust recommendations on key methodological issues, nucleic acid coverage and bioinformatics analysis (d'Humières, 2021).

Conclusions. Metagenomic sequencing has a higher overall sensitivity for the detection of causative agents of lower respiratory tract infections than the culture method and is a necessary complement to conventional microbiological tests. Routine blood culture supplemented with mNGS can significantly reduce mortality in patients with sepsis and bloodstream infections. The highest diagnostic yield for patients with central nervous system infections is the combination of CSF mNGS and conventional tests. mNGS has a higher detection rate and sensitivity in the identification of pathogens associated with DCSA and may help to rationalize antimicrobial therapy in a timely manner.

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МЕТАГЕНОМНЕ СЕКВЕНУВАННЯ В ДІАГНОСТИЦІ ІНФЕКЦІЙ: КЛІНІЧНЕ ЗАСТОСУВАННЯ

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Резюме. *Аналіз спеціалізованої літератури був проведений з метою визначення категорій пацієнтів, у яких метагеномне секвенування має доведену або потенційну клінічну значущість для діагностики інфекцій. Висвітлено практичне застосування, переваги та обмеження цього методу в контексті різних клінічних сценаріїв для подальшої адаптації клінічних та лабораторних діагностичних протоколів. Дослідження має описовий та аналітичний характер. Бібліографічний аналіз було проведено з використанням спеціалізованої літератури з баз даних відкритого доступу. Статті англійською мовою, що стосуються запропонованої теми дослідження, опубліковані за останні 5 років, були відібрані відповідно до критеріїв включення. Метагеномне секвенування (mNGS) – це інноваційна технологія, яка здійснила революцію в традиційній клінічній діагностиці в багатьох аспектах. Її можна використовувати для одночасної ідентифікації кількох патогенів, таких як бактерії, віруси, грибки та паразити, в клінічних зразках. Застосування метагеноміки принесе значні переваги, зокрема у випадках рідкісних інфекцій невідомої етіології або патогенів, які важко культивувати. Це дослідження демонструє, як цю нову технологію можна використовувати для змістовної клінічної діагностики в мікробіології. Пацієнти з інфекціями нижніх дихальних шляхів представляють категорію пацієнтів, для яких використання mNGS приносить більше переваг. Ця технологія має вищу загальну чутливість для виявлення патогенів порівняно з методом культивування та є необхідним доповненням до звичайних мікробіологічних тестів. Сепсис є серйозною проблемою для громадського здоров'я, і використання mNGS для цієї категорії пацієнтів може скоротити загальну тривалість госпіталізації та знизити смертність. Технологія метагеномного секвенування також корисна для пацієнтів з інфекціями центральної нервової системи, використання mNSG для них має вищий рівень виявлення, але найвища діагностична ефективність спостерігається при поєднанні mNGS спинномозкової рідини з традиційними методами. Метагеноміка також пропонує значні переваги у виявленні патогенів у пацієнтів з глибокими абсцесами шийного простору, маючи вищий рівень виявлення та чутливість, що дозволяє раніше розпочати антимікробну терапію, покращуючи прогноз та зменшуючи споживання медичних ресурсів. Метагеноміка забезпечує повну, швидку та точну ідентифікацію патогенів, значно покращуючи лікування, головним чином, коли традиційні методи недостатні. На основі досліджень, представлених у цій статті, можна зробити висновок, що застосування метагеноміки для пацієнтів з інфекціями нижніх дихальних шляхів, сепсисом, інфекціями центральної нервової системи та інфекціями глибоких шийних відділів шийного відділу хребта має дуже високий потенціал для зниження захворюваності, смертності та витрат на охорону здоров'я.*

Ключові слова: метагеноміка, діагностика, сепсис, інфекції нижніх дихальних шляхів, інфекції центральної нервової системи.

DOI: 10.31073/onehealthjournal2025-V-01