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Emerging Molecular and Rapid Diagnostic Approaches for Post-Neurosurgical Central Nervous System Infections: Current Evidence and Future Perspectives

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Abstract: *Complications like CNS infection develop following surgery on the CNS. CNS infection may develop as infection of surgical wound and/or CSF shunt. Infections result in delayed hospital discharge, increased cost of care, disability resulted from CNS injury, and death. Modern neurosurgery techniques and advances in infection control have not eliminated the risk of developing CNS infections due to the following common complications of neurosurgery: meningitis, ventriculitis, abscess of brain tissue, and device-related infection.[1,2] The difficulty in accurately identifying a CNS infection shortly after surgery may occur from the clinical symptoms that are common with postoperative inflammatory process as well as the fact that previous treatment with antibiotics can obstruct the ability to use standard diagnostic methods to confirm or rule out that an infection exists.[3] Other than CSF analysis, Gram stain, and culture are the most commonly used diagnostic tests, however their current limitations often result in delay in definitive identification of a pathogen and targeted treatment for a patient's CNS infection.[4] Recent advancements in PCR, multiplex molecular assays, MALDI-TOF mass spectrometry, automated culture systems, and metagenomic next-generation sequencing (mNGS) allow for timely diagnosis of pathogens associated with post-neurosurgical central nervous system (CNS) infections than traditional methods.[5,6] Emerging biomarkers and artificial intelligence-based approaches increase the potential for precise diagnosis and improved clinical decision-making.[7] The goal of this review is to discuss current and emerging diagnostic technologies in the context of their clinical utility and limitations, and explore their potential role in improving patient outcomes and supporting antimicrobial stewardship in neurosurgical practice areas.*

Keywords: CNS infections, cerebrospinal fluid, molecular diagnostics, PCR, MALDI-TOF, metagenomic sequencing, antimicrobial resistance

I. INTRODUCTION

Postoperative central nervous system (CNS) infections remain a major complication of neurosurgery worldwide, with continued high levels of morbidity and mortality.[1] Neurologists have improved surgical techniques to minimize complications, as well as perioperative care and infection prevention, but postoperative infection remains a significant issue in the postoperative patient.[2,3] Postoperative infections occur following surgical procedures involving the brain, spinal cord, and/or cerebrospinal fluid (CSF) pathways and include common types such as postoperative meningitis, ventriculitis, brain abscesses, and infections associated with implanted medical devices (ventriculoperitoneal shunts; external ventricular drains).[4] Increased use of invasive neurosurgical procedures, longer periods of postoperative hospitalization, and more implanted

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medical devices have contributed to an increase in the incidence of healthcare-associated CNS infections.[5]The organism profiles of these infections have changed in recent years; both Gram-positive and Gram-negative organisms are common causes.[6] Examples of organisms frequently identified in these infections include Acinetobacter baumannii, Klebsiella pneumoniae, Pseudomonas aeruginosa, and methicillin-resistant Staphylococcus aureus (MRSA).[7] Due to the growing incidence of multidrug-resistant organisms associated with CNS infections, timely and accurate diagnosis and administration of appropriate therapy has become even more important.[8]The challenge associated with accurately diagnosing CNS infections following neurosurgery stems from the overlap in clinical symptoms of both infections as well as inflammation, and therefore misdiagnosis can occur.[3,9] Such symptoms include fever, headache, altered mental status, stiff neck, and neurologic decline, which occur in both types of conditions and thus may not provide reliable evidence to support the clinical diagnosis.[10] Because conventional laboratory tests used to identify specific pathogens — CSF analysis, Gram staining, microbial culture, and biochemical testing — will have lower sensitivity due to previous antimicrobial therapy, as well as extended delays from time to report test results, targeting treatment may be delayed.[11] Methods including polymerase chain reaction (PCR), multiplex molecular assays, matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS), automated cultures, and metagenomic next-generation sequencing (mNGS) have all contributed to more timely and accurate detection of the pathogen(s) responsible for CNS infections.[12,13] The purpose of this review will be to summarize these recent advances in diagnostic testing methods and their potential roles in aiding clinicians with successfully managing these patients, appropriate use of antimicrobials, and ultimately improving their outcomes after neurosurgery.

II. BURDEN OF POST-NEUROSURGICAL CENTRAL NERVOUS SYSTEM INFECTIONS

Infections of the central nervous system (CNS) following neurosurgery are among the most serious complications to result from a neurosurgical procedure.[1,3] Improvements in surgical techniques, infection control, and patient care have not mitigated the increasing impact of these infections on patient morbidity, mortality, and healthcare costs.[2] Further, the increasing number of neurosurgical interventions and implanted devices including external ventricular drains (EVDs) and ventriculoperitoneal shunts (VPSs) has exacerbated the incidence of healthcare-acquired CNS infections.[4,5] The common clinical presentations of CNS infections include postoperative meningitis, ventriculitis, brain abscess, and device-related infections, all of which can adversely affect patient recovery and neurological outcomes.[6]The costs associated with these infections go beyond the physical or clinical aspects. In many cases, patients will incur extra costs due to the need for extended stays in the hospital, further diagnostic tests, multiple surgeries, and the extended use of antimicrobial medications; all of these factors contribute to a large burden placed on healthcare resources.[14] In addition, with the increasing number of multidrug-resistant organisms, the challenge of treating these infections continues to grow, highlighting the critical importance of rapid and accurate diagnosis.[8,15] The sooner a bacterial organism is identified, the sooner targeted therapy can initiate, which will result in a decrease in the incidence of complications and improve the patient's overall outcome.[11]

III. COMMON CAUSATIVE PATHOGENS AND ANTIMICROBIAL RESISTANCE

Post-surgical infections of the CNS can be caused by an array of microbes, many of which include both Gram-positive and Gram-negative bacteria.[6,7] The distribution of pathogens following CNS surgery is influenced by many different factors including the type of neurosurgical procedure performed, amount of time spent in the hospital following the procedure, types of invasive devices used during the procedure, as well as the extent of local patterns of antimicrobial resistance.[3,9] Historically, Gram-positive organisms such as S. aureus and coagulase-negative staphylococci have been classified as the most common pathogens leading to CNS infection since they can colonize the skin and contaminate either the surgical site or the implanted device.[7,15] However, recent studies indicate that an increasing number of Gram-negative bacterial species are now being identified as pathogens responsible for healthcare-associated CNS infections.[8]Gram-negative bacterial species, including A. baumannii, K. pneumoniae, and P. aeruginosa, are

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often cultured from patients following neurosurgery. These species are concerning due to their ability to persist in the hospital environment as well as their ability to develop multiple antimicrobial resistance mechanisms.[8,15] Additionally, Enterobacteriaceae such as E. coli and Enterobacter spp. have also been reported as contributors to CNS infection in the postoperative period.[16] The growing incidence of multidrug-resistant (MDR) organisms poses a significant challenge to managing infections. Reports of resistance to frequently prescribed antibiotics, such as cephalosporins, fluoroquinolones, and carbapenems, are on the rise globally.[17,18] This increased incidence of resistance restricts treatment options, extends recovery time, and results in deterioration of clinical outcome.[8]

Table 1. Common Pathogens and Antimicrobial Resistance Patterns in Post-Neurosurgical CNS Infections

Pathogen	Type	Common Resistance Pattern
Staphylococcus aureus	Gram-positive	MRSA
Coagulase-negative Staphylococci	Gram-positive	Methicillin resistance
Acinetobacter baumannii	Gram-negative	MDR/XDR
Klebsiella pneumoniae	Gram-negative	ESBL, carbapenem resistance
Pseudomonas aeruginosa	Gram-negative	MDR, carbapenem resistance

IV. LIMITATIONS OF CONVENTIONAL DIAGNOSTIC METHODS

The continued use of traditional diagnostic techniques is critical to the diagnosis of postoperative central nervous system (CNS) infections. Routine diagnostic techniques such as cerebrospinal fluid (CSF) analysis, Gram staining, microbial culture, and biochemical identification are widely employed in clinical labs because they are readily available, affordable, and widely accepted. [3,11] These traditional diagnostic tests also provide clinicians with information about the presence of a potential infection and are helpful in directing the initial clinical treatment of these patients.[4]One limitation is that these tests have a decreased sensitivity in patients who have received antibiotic therapy prior to the collection of the specimen for testing. Antimicrobial therapy can prevent the growth of bacteria, resulting in a negative culture even in the presence of an active infection.[11] In addition, Gram staining may not detect organisms present in low quantities.[19] Furthermore, postoperative inflammation can lead to the alteration of CSF parameters such as protein concentration, glucose levels, and white blood cell counts, thus making interpretation very difficult because many of these findings may be identical to those associated with infection.[20] One other major limitation is the time required for definitive results. Culture-based methods of pathogen isolation, identification, and antimicrobial susceptibility testing can take many days; in this time many clinicians will use broad-spectrum empirical antibiotic therapy, potentially leading to the development of antimicrobial resistance and unnecessarily exposing patients to drugs.[21] Another limitation is that routine laboratory methods may not detect some fastidious or slow-growing microorganisms.[1,3] These limitations indicate the need for quicker and more accurate diagnostic methods to allow for the early identification of pathogens and better clinical decision-making in neurosurgical patients.

V. RAPID MICROBIOLOGICAL DIAGNOSTIC TECHNOLOGIES

The development of rapid microbiological technologies to complement traditional laboratory methods arose from the need for earlier and better diagnosis. Generally speaking, these rapid diagnostics help to lessen the turnaround time in providing timely patient management information to clinicians.[1,12] Rapid diagnosis of infectious diseases of the CNS following neurosurgery has a critical impact on patient outcomes, which further increases the value of rapid diagnostics.[4]Automated culture systems have allowed for improved detection of microbial growth due to continuous monitoring of specimens and more rapid identification of positive cultures compared to conventional methods.[11] A major advancement in the identification of microorganisms has been the use of Matrix-Assisted Laser Desorption/Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS) technology. MALDI-TOF MS allows for rapid identification of microorganisms by their unique protein profiles, leading to rapid species identification, as well as increased laboratory efficiency.[22,23] The use of automated antimicrobial susceptibility testing systems enables faster identification of patterns of resistance, assisting clinicians in identifying the right antimicrobial treatment for an

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infection.[17] By identifying resistant pathogens earlier, providers are able to support antimicrobial stewardship programs, and limit the excessive use of broad-spectrum antibiotics.[18] While these technologies do not completely replace traditional testing methods, they have improved the turnaround time and accuracy of microorganism identification immensely.

Table 2. Comparison of Diagnostic Methods for Post-Neurosurgical CNS Infections

Method	Turnaround Time	Sensitivity	Advantages
Gram staining	Minutes	Low	Rapid and inexpensive
CSF culture	24–72 hr	Moderate	Gold standard
PCR	4–6 hr	High	Rapid and sensitive
Multiplex PCR	1–3 hr	Very high	Multiple pathogen detection
MALDI-TOF MS	Minutes	High	Fast identification
mNGS	24–48 hr	Very high	Broad pathogen detection

VI. MOLECULAR DIAGNOSTIC APPROACHES

Molecular diagnostic techniques have markedly improved the diagnosis of central nervous system infections occurring after neurosurgery. These techniques differ from standard microbiological methods in that they provide a rapid method for the direct determination of the identity of pathogens present in clinical specimens by detecting the genetic material of microorganisms, which improves the speed and sensitivity of pathogen identification.[12,19] This is especially relevant for patients who have had prior exposure to antibiotic therapy, as this may significantly decrease the chances of detecting a specific organism when using traditional culture methods.[11]The most frequently employed method of molecular diagnosis is the Polymerase Chain Reaction (PCR). PCR can identify specific microbial DNA sequences, even if there are very few organisms in the clinical specimen.[24] The advent of Real-Time PCR has further enhanced this technique, allowing for rapid detection and quantification of pathogens in the clinical specimen to take place in a matter of hours.[25] The recent development of multiplex PCR assays and syndrome-based molecular panels allows for the simultaneous identification of multiple pathogens from a single cerebrospinal fluid specimen.[26,27] These new technologies provide clinicians with rapid and comprehensive diagnostic results, decreasing the uncertainty of diagnosis and permitting the earlier commencement of targeted antimicrobial therapy.

VII. METAGENOMIC NEXT-GENERATION SEQUENCING (MNGS)

Metagenomic next-generation sequencing (mNGS) has become an extremely valuable new technology for diagnosing infectious diseases. Unlike traditional methods that test for specific microorganisms, mNGS can examine the entire genetic makeup of a clinical specimen and can therefore detect many microorganisms including viruses, bacteria, fungi and parasites all in one test.[28,29] By using an unbiased approach based on total genetic information rather than just visible organisms, mNGS has broadened the potential to diagnose complex infections, particularly where standard laboratory tests do not identify the causative microorganism.[30]In evaluating post-surgical CNS infections, mNGS has demonstrated significant value for identifying microorganisms from patients with a negative culture and for patients with rare or hard-to-culture microorganisms.[13,31] Diagnostic accuracy is enhanced when mNGS provides comprehensive identification of a pathogen and helps the physician choose the right antimicrobial treatment.[32] However, mNGS has several practical limitations, including high cost and the need for sophisticated technology. Additionally, there are difficulties associated with interpreting the data generated by mNGS and differentiating between true pathogens and contaminants.[10,33] Nevertheless, as mNGS is improved and the cost of sequencing continues to decrease, we can expect to see more clinical usage of mNGS over time.[34]

VIII. HOST BIOMARKERS IN CNS INFECTION DIAGNOSIS

There are now many host biomarkers that assist in the early diagnosis of neurologically based postoperative infections. Traditional microbiological tests take 3–10 days to provide results on microbiological status, while biomarkers enable

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rapid assessment of infection severity (e.g., CRP, PCT, CSF lactate, cytokines).[35] These biomarkers have been studied to differentiate infectious processes from non-infectious postoperative inflammatory processes.[9,20] Any one of these markers can help determine whether an infection is present and provide assistance to clinicians when there is no clear resolution from any microbiological testing.The recent trend has shifted focus toward infection-related immunological and host-response markers that represent the body's response to an infection. Such biomarkers have the potential to improve the accuracy of diagnosis when used in combination with traditional or conventional testing methods and molecular diagnostic techniques.[36] It is currently difficult to find a single laboratory test that will reliably identify all types of CNS infection; however, a combination of laboratory tests and/or clinical information and/or microbiological data may support the identification of infection in a timely way and facilitate better patient care.[2,3]

IX. ARTIFICIAL INTELLIGENCE AND DIGITAL DIAGNOSTIC TECHNOLOGIES

The use of Artificial Intelligence (AI) within healthcare continues to grow and develop in many areas, including diagnosis of infectious diseases. AI-based infection tools can help clinical providers analyze large amounts of clinical information including microbiological data, laboratory data, and more. By using AI to recognize patterns of infection, clinicians will be able to more accurately predict disease outcomes.[37]In addition to diagnosing infections based on AI analysis, new digital health technologies are changing how we manage post-neurosurgery CNS infections. These technologies include automated laboratory systems, electronic health records (EHRs), and data-driven diagnostic platforms, which can promote improved efficiencies for workflows and allow for real-time monitoring of patients.[38] Additionally, algorithms that incorporate AI can enhance image analysis done by AI-assisted imaging technologies, predict patients at high risk of developing an infection, help clinicians optimize their use of antimicrobials, and track the occurrence of infections.[37,39] While still evolving, AI-driven molecular diagnostic systems may provide improved accuracy in diagnosing infections, better patient care, and potential for more personalized methods to manage infections in the future.

X. CHALLENGES AND LIMITATIONS OF EMERGING DIAGNOSTIC TECHNOLOGIES

While emerging diagnostic technologies offer many benefits, there are numerous challenges preventing their use in routine clinical practice. Most advanced molecular technologies such as multiplex PCR and mNGS require expensive, specialized equipment, trained personnel, and dedicated laboratory facilities. The high cost of these technologies is a significant barrier to use in many low-resource healthcare systems and greatly limits access to state of the art diagnostic facilities.[10,14] Therefore, many hospitals continue to use conventional diagnostic methods despite their limitations. A second challenge is how to interpret the diagnostic results. Highly sensitive molecular tests may identify either non-viable microorganisms, colonizing organisms, or contaminants; therefore, interpretation of results can impact clinical decision-making.[33] In addition, mNGS generates a large amount of data; however, the accurate analysis of these data sets requires the expertise of bioinformatics specialists.[29] Concerns related to the standardization of testing protocols, quality control procedures, and the interpretation of test results continue to be a challenge.[30] Therefore, although there are considerable benefits to using emerging diagnostic technologies, their successful incorporation into clinical practice will require careful evaluation considering their cost, accessibility, technical expertise, and diagnostic accuracy.

XI. FUTURE PERSPECTIVES

In the future, advancements made towards diagnosing CNS infections that occur after neurosurgery are expected to focus on increasing how quickly and accurately clinicians can obtain diagnostic testing as well as increasing access to testing. One tool that may help clinicians achieve this end is point-of-care molecular testing platforms which will allow clinicians to detect pathogens at the patient's bedside within a short amount of time, thereby reducing delays in obtaining definitive diagnoses and leading to earlier treatment decisions.[40]Another area of anticipated advances is the

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creation of more affordable and accessible metagenomic sequencing technologies, which will increase the clinical utility of metagenomic testing for routine healthcare.[34] The integration of artificial intelligence, digital health technologies, and molecular diagnostics in the field of infection management is also expected to increase.[38,39] AI systems will be beneficial for assisting practicing healthcare providers with interpreting complex diagnostic information, predicting antimicrobial resistance patterns, and identifying high-risk patients.[37] Furthermore, continued research into identifying novel biomarkers and host-response fingerprints will improve the precision of diagnosing patients.[35,36]

XII. CONCLUSION

Infectious diseases affecting the central nervous system after neurosurgery still pose major problems for healthcare because of how high-intensity these types of patients are and the associated costs to treat them; therefore, prompt and accurate diagnosis is vital for proper management, but conventional diagnostic measures are typically hindered by long wait times to receive test results and low-test sensitivity.[1,3] The ability to rapidly identify infecting pathogens using new methods of rapid microbe culture and molecular diagnostics has led to improvements in both detecting and identifying the infecting pathogens, leading to timely and more effective treatment of these patients.[12,13] PCR, multiplex molecular assays, MALDI-TOF MS, and metagenomic next-generation sequencing are examples of modern technologies that have greatly changed how we will diagnose CNS infections going forward, to efficiently manage infections, reduce the development of antibiotic-resistant infections, and improve overall patient outcomes for patients who undergo neurosurgery.[22,24,28]

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