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Marcus Ståhlberg

marcus.stahlberg.2@ki.se

Karolinska Institutet

Joel Rahme

Umeå University

John Karlsson Valik

Karolinska Institutet

Christer Mehle

Umeå University

Jonas Blixt

Karolinska University Hospital

Eddie Weitzberg

Karolinska Institutet

Christian G. Giske

Karolinska Institutet

David W Nelson

Karolinska Institutet

Research Article

Keywords:

Posted Date: November 5th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-11341644/v1

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Additional Declarations: No competing interests reported.

Next-generation 16S-rRNA sequencing for early identification of external ventricular drain-related infections in a critical care setting: a retrospective cohort study with external validation

Marcus Ståhlberg[1,2*], Joel Rahme[3], John Karlsson Valik[4,5], Christer Mehle[3], Jonas Blixt[1,2], Eddie Weitzberg[1,2], Christian G. Giske[6,7], David W. Nelson[1,2]

[1*]Perioperative Medicine and Intensive Care, Karolinska University Hospital, Stockholm, Sweden.
[2]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden.
[3]Faculty of Medicine, Department of Clinical Microbiology, Infectious Diseases, Umeå University, Umeå, Sweden.
[4]Department of Medicine, Karolinska Institutet, Stockholm, Sweden.
[5]Department of Infectious Diseases, Karolinska University Hospital, Stockholm, Sweden.
[6]Division of Clinical Microbiology, Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden.
[7]Department of Clinical Microbiology, Karolinska University Hospital, Stockholm, Sweden.

*Corresponding author(s). E-mail(s): marcus.stahlberg.2@ki.se;
Contributing authors: joel.rahme@gmail.com; john.karlsson.valik@ki.se; christer.mehle@umu.se; jonas.blixt@ki.se; eddie.weitzberg@ki.se; christian.giske@ki.se; david.nelson@ki.se;

Abstract

Introduction: External ventricular drain (EVD)-associated infections (EVDIs) are difficult to distinguish from aseptic inflammation, leading to excessive broad-spectrum antibiotic use while slow culture results hinder timely decisions. Next-generation 16S-rRNA sequencing offers rapid bacterial detection with

the potential to improve surveillance and antibiotic stewardship. We aimed to evaluate the diagnostic performance of 16S-rRNA sequencing compared to conventional CSF cultures and its ability to guide EVDI-targeted antibiotic therapy. **Methods:** Adult ($\geq$ **18** years) patients treated with EVDs in the intensive care unit (ICU) at Karolinska University Hospital, Stockholm, Sweden, between November 2021 and October 2024, were included. Infection classification was based on independent, manual reviews of medical records by three physicians, with inter-reviewer agreement evaluated using Cohen's $\boldsymbol{\kappa}$ statistic. The performance of 16S-rRNA sequencing (index test) was compared to bacterial cultures for EVDI diagnosis. Additionally, a 16S-guided treatment protocol was developed and its potential impact on antibiotic stewardship was assessed, then validated in an external cohort of 135 patients from Norrlands University Hospital. **Results:** Of the 265 patients treated with EVDs during the study period, 191 had paired results from 16S-rRNA sequencing and bacterial cultures. Among these, 8 cases of verified infection (VI) and 61 cases of suspected infection (SI) were identified. Both methods identified 7 of 8 VI cases, yielding a per-patient sensitivity of 87.5%. On a per-sample basis, 16S-rRNA sequencing and culture demonstrated sensitivities of 92.9% vs. 91.7%, specificities of 92.7% vs. 97.8%, a negative predictive values (NPV) of of 99.7%, and a positive predictive values (PPV) of 35.1% vs. 61.1%, respectively. Implementing a 16S-rRNA sequencing-guided treatment protocol could reduce antibiotic exposure by 43–71%, and a 40-55% reduction in the number of treated patients was estimated in an external cohort. **Conclusion:** Our findings suggest that 16S-rRNA sequencing is a valuable adjunct to traditional EVDI surveillance, primarily functioning as a rapid rule-out test to support withholding or de-escalation of broad-spectrum antibiotic treatment if results are readily available. Furthermore, exploratory findings suggest that semi-quantitative reads ratios may help differentiate clinically relevant findings from contamination.

Keywords: External ventricular drain-related infections, Ventriculostomy associated infections, External ventricular drain-related infection surveillance, Molecular diagnostics, Antibiotic Stewardship

1 Introduction

External ventricular drain (EVD)–associated infections (EVDIs) are serious infections associated with prolonged hospital stay, long-term neurological deficits, and increased morbidity and mortality [1–5]. EVDIs are challenging complications to accurately identify in a timely manner. The reported incidence rate in the literature ranges from 1-35%, highlighting one of the major challenges in EVDI research: the lack of an internationally established EVDI definition [6–8]. Current EVDI surveillance relies heavily on clinical, cytological and biochemical indicators, which are often non-specific and overlap with aseptic inflammation, especially in critically ill neurological patients.

Cerebrospinal fluid (CSF) bacterial cultures are regarded as the diagnostic gold standard but are often limited in their utility for guiding treatment decisions, in part because prior antibiotic exposure reduces culture sensitivity, and prolonged incubation

times hinder timely results [9–14]. Additionally, the microbiological profiles seen in EVDIs are dominated by indolent pathogens which are difficult to distinguish from contamination [14–17]. Consequently, many patients receive empiric broad-spectrum antibiotics for suspected EVDI, contributing to antimicrobial overuse and resistance development, with few later having their diagnosis microbiologically confirmed [6, 14, 18–20].

The high prevalence of antimicrobial resistance in intensive care units (ICUs) is a growing global health concern. ICUs account for a substantial proportion of in-hospital antibiotic consumption, and broad-spectrum antibiotic use is a key driver of antimicrobial resistance [21–25]. Antimicrobial stewardship programs are vital in mitigating this problem and have demonstrated improved patient outcomes and reduced healthcare costs in ICU populations [23, 24]. However, the urgent need to initiate empirical therapy must often be balanced against the risk of overtreatment, complicating efforts to optimize antibiotic use in neurocritical care especially. EVDI treatment accounted for roughly 15% of total antibiotic use over the last 17 years at our institution, primarily attributed to a high rate of empirical EVDI treatments due to suspected infections. This relationship is likely generalizable to other institutions, suggesting that the opportunity for improved antimicrobial stewardship if more rapid and accurate diagnostics were available is significant [6, 14, 26].

Given these challenges, alternative diagnostic approaches are needed to improve EVDI surveillance. Sequencing techniques targeting the 16S ribosomal RNA (rRNA) gene have emerged as promising tools for rapid and sensitive pathogen detection, potentially overcoming many limitations associated with culture-based diagnostics. These methods are faster and capable of amplifying genetic material from non-viable bacteria, providing results in a clinically relevant time frame and potentially reducing false negatives caused by prior antibiotic use [27–29]. While increased sensitivity may increase the false-positive rate, previous studies have demonstrated that 16S-rRNA sequencing can improve diagnostic accuracy and enable earlier diagnosis and treatment adjustments [30–32]. However, data on the use of next-generation 16S-rRNA sequencing for EVDI surveillance in a critical care setting are limited [26, 28, 33].

In this study we retrospectively evaluate the performance of next-generation 16S-rRNA sequencing compared to conventional CSF bacterial cultures for EVDI diagnostics, and evaluate our approach through validation in an external cohort. Our primary objective was to determine whether 16S-rRNA sequencing can reliably guide the initiation and de-escalation of EVDI treatment in a critical care setting.

2 Methods

Study design, participants and data collection

This retrospective cohort included all ICU patients treated with EVDs who had paired CSF bacterial cultures and 16S-rRNA sequencing data (index test). Electronic health records covering the ICU stay were reviewed for demographics, EVD timelines, Glasgow Coma Scale (GCS) scores, temperature, administered antimicrobials, routine blood/CSF laboratory analyses, and microbiological results, including culture incubation times and 16S-rRNA sequencing read counts. During the study period blood was

sampled daily and CSF twice weekly or on clinical indication while CSF microbiology was analyzed upon suspected infection, defined by clinical indicators or abnormal CSF granulocyte counts ($\geq 100 \times 10^6$ cells/L).

At our institution, empiric treatment for EVDI typically consists of meropenem combined with linezolid, or cefotaxime monotherapy, with intrathecal vancomycin added in complex cases. Full duration EVDI treatment was routinely 14 days, with extensions determined by identified pathogen, clinical trajectory, and potential complicating factors. EVDI treatment was defined as the administration of appropriate antibiotics (empirical or targeted EVDI treatment in cases with known pathogens) for a minimum of 24 hours at dosages consistent with bacterial central nervous system (CNS) infections, which was used to identify patients for manual review.

The study was approved by the Swedish Ethical Review Authority (DNR 2017/1544-31/1, DNR 2018/1701-32, DNR 2024-07464-02).

Infection classification and microbiology

Infection classification was based on a manual review of medical records. The results were used as the reference against which the diagnostic performance of 16S-rRNA sequencing and cultures were evaluated.

First, all patients with a positive microbiological CSF finding (n=35) were reviewed by a resident in anaesthesia and intensive care (MS), a senior consultant in infectious diseases (JKV), and a senior consultant in anaesthesia and intensive care (DWN) to determine whether the findings represented a clinically relevant infection or contamination. EVDI classification was reached by consensus, accounting for factors such as bacterial species, growth pattern, clinical suspicion, and length of treatment.

Second, all suspected EVDI cases, defined as patients receiving at least 24 hours of EVDI treatment (n=49), but with no positive microbiological findings, were reviewed by MS and classified as a true infection or not. In addition, patients with positive microbiological findings but no EVDI treatment were reviewed to verify that treatment was not missed during data extraction or initiated immediately after ICU discharge (and therefore not captured in our dataset). To assess classification reliability, 20 randomly selected cases were reviewed by JKV, blinded to MS's assessment. There was perfect agreement between the reviewers (Cohen's $\kappa = 1.00$).

Infection group characteristics:

These are retrospective characteristics observed across groups, and do not reflect predefined classification criteria.

Verified infection (VI):

1. Full duration EVDI treatment (and as such persistent clinical assessment as EVDI by treating clinicians and infectious disease consults).
2. Microbiological findings assessed as clinically relevant based on manual review.
3. Clinical trajectory consistent with CNS infection, characterized by progressive clinical symptoms or CSF abnormalities that progressively resolve following EVDI treatment.

Suspected infection (SI):

1. Empirical EVDI treatment discontinued prematurely due to decreased clinical suspicion, as assessed by treating physicians and infectious diseases consults.
2. Negative microbiological results or consistent with sample contamination based on primary review.
3. Clinical trajectory unaffected by EVDI treatment, with suspicion primarily raised due to:

   - A non-progressive, isolated increase in CSF cell counts.
   - A non-progressive, persistent increase in CSF lactate.
   - Non-specific clinical signs of infection.
   - A temporary (< 24 hours) clinical deterioration of unclear etiology.
   - Insufficiently documented, but no clear signs suggestive of EVDI on review.

No infection (NI):

1. No EVDI treatment.
2. Negative microbiological results or consistent with contamination based on primary review.

16S-rRNA sequencing and cultures

16S-rRNA sequencing was performed using the IonTorrent next-generation sequencing (NGS) platform (Thermo Fisher Scientific) targeting the hypervariable V1/V2 and V3/V4 regions of the 16S rDNA gene. CSF samples were collected under sterile conditions without any additives and sent to the Department of Clinical Microbiology at Karolinska University Hospital for sample processing and storage. All 16S-rRNA sequencing analyses underwent routine evaluation by a physician in microbiology, whose assessment of sequencing data alone determined the classification of results as negative, or positive in which case relevant pathogens were provided. The reviewers performing manual infection classification were blinded to 16S-rRNA sequencing reads counts and reads ratios to allow independent evaluation of their potential to differentiate clinically relevant findings from contamination. The reads ratio, the number of sequencing reads in the V1/V2 or V3/V4 regions (whichever was highest) relative to the reads of the negative control, was evaluated in a sensitivity analysis to assess its discriminatory capacity to differentiate clinically relevant findings from contamination.

Cultures were obtained under sterile conditions and incubated for up to 12 days before being finalized as negative. Results included detailed information on the cultured pathogen, including antimicrobial resistance patterns, growth characteristics, and comments pertaining to the need for enrichment.

Validation cohort

A total of 376 paired culture and 16S-rRNA polymerase chain-reaction () samples from 135 patients who received EVDs at Norrland University Hospital, Umeå, Sweden, between January 2016 and December 2018, were included as an external validation cohort in this study. Norrland University Hospital employed similar EVDI surveillance

protocols and conformed to similar treatment routines as ours, with the difference of employing a different method for sequencing the 16S gene, Sanger sequencing. CSF samples were routinely collected twice weekly or upon clinical suspicion, and all samples were submitted for microbiological diagnostics using culture and 16S-rRNA PCR. The number of days with EVDI treatment was not available in the external cohort, thus only calculations on a patient basis were possible. EVDI treatment decisions adhered to the same clinical routines and national guidelines as our institution, with routine involvement of an infectious disease specialist.

Statistical analyses

Categorical and continuous variables are presented as counts and percentages and as means or medians, respectively. Errors are reported as standard deviation (SD) or interquartile ranges (IQR) All analyses were conducted on non-imputed data. The Chi-square test or Fisher's exact test was used to compare categorical variables, while the Kruskal-Wallis test was employed for continuous variables.

Diagnostic performance metrics, including positive predictive value (PPV), negative predictive value (NPV), sensitivity, and specificity, were calculated on a per-sample basis. For this analysis, follow-up microbiological results that turned negative following the initiation of EVDI treatment in VI patients (i.e., after initial confirmed pathogens in CSF) were excluded. This approach was based on the expected clearance of viable pathogens following appropriate antimicrobial therapy and the limited available data on the persistence of detectable microbial DNA or RNA by molecular diagnostics in CSF during or after treatment.
All statistical analyses were performed using R version 4.6.1 (2026-06-24).

3 Results

Study cohort

Patients (n=265) were treated with EVDs between November 2021 and October 2024. In total, 34 patients admitted with primary CNS infections, and 40 patients who completely lacked results from 16S-rRNA sequencing *or* paired 16S-rRNA sequencing *and* cultures were excluded. Patients excluded due to lack of 16S-rRNA sequencing results were predominantly from the beginning of the study period, when the new surveillance protocol was under implementation. Of the remaining 191 patients, 69 were initiated on EVDI treatment due to clinical suspicion of EVDI. Of these, 61 patients were classified as SI and 8 patients as VI. Thus, 32% of the cohort was initiated on EVDI treatment due to suspected infection, while the confirmed EVDI rate was 4.2%. The 191 patients yielded a total of 344 CSF samples, of which all were analyzed with 16S-rRNA sequencing and 332 by culture. The study cohort is visualized in Figure 1.

Patient characteristics are presented in Table 1. ICU length of stay (LoS) and EVD duration were not significantly different across groups. Treatment was generally initiated later in the VI group compared to the SI group, and the duration was longer.

Subarachnoid hemorrhage (SAH) was more common among SI patients, and was otherwise the most common admission diagnosis, followed by intracerebral hemorrhage (ICH). The 30- and 90-day mortality rates for VI patients were both 0.0%, whereas for SI patients, the rates were 16.4% at both time points, and in NI patients mortality rates of 18.0% and 25.0% at 30 days and 90 days were observed.

Microbiology

Microbiological findings are summarized in Table 2. Bacteria were identified in 37/344 (10.8%) samples by 16S-rRNA sequencing and 18/332 (5.4%) samples by cultures. A total of 44 CSF samples tested positive by either 16S-rRNA sequencing or culture, which are presented in detail in Table 3. Among 16S-rRNA sequencing-positive samples, thirteen (35.1%) were assessed as clinically relevant, compared to 11/18 culture-positive samples (61.1%). Polymicrobial findings were identified in 7 CSF samples by 16S-rRNA sequencing, compared to none by cultures, and were in all cases considered contamination except for one case with *coagulase-negative staphylococci* (CoNS) and *Cutibacterium acnes (C. acnes)*, where the former was considered clinically relevant (Table 3). Thus, while 16S-rRNA sequencing demonstrated a higher overall detection rate, it was also associated with a higher frequency of contaminations and polymicrobial findings compared to conventional cultures.

Identified bacteria were primarily skin colonizers, predominantly *C. acnes* and CoNS (Table 2). CoNS was also the most common clinically relevant pathogen, followed by *Enterococcus faecalis (E. faecalis)* and *Klebsiella oxytoca (K. oxytoca)*. *C. acnes* was the most commonly identified contaminant by 16S-rRNA sequencing, but equally common as CoNS in culture results. Of note, no pathogens exhibiting multi-resistance antibiotic patterns were identified in our cohort.

16S-rRNA sequencing and cultures were generally in agreement in cases of true infection. Among 8 VI patients, 6 were positive by both 16S-rRNA sequencing and culture, and 2 were positive by either culture or 16S-rRNA sequencing (Table 3). Two instances of contamination with *C. acnes* were identified in two VI patients: one occurred concurrently with a clinically relevant finding of CoNS, and the other was identified one week prior to EVDI treatment in a patient with *E. faecalis*-related EVDI. In the SI and NI groups, 12 and 15 patients respectively had microbiological findings consistent with contamination by either 16S-rRNA sequencing or culture. Contamination-associated results were generally discordant between 16S-rRNA sequencing and cultures, except for one case of *C. acnes* (Table 3). Overall, 16S-rRNA sequencing and cultures demonstrated high concordance in detecting clinically relevant pathogens, whereas contaminations were characterized by discordant findings.

Incubation times may help distinguish true infection from contamination. The mean incubation time for all positive cultures in the VI group was just under 3 days, whereas contaminations had an average incubation time of approximately 9.5 days, possibly representing lower bacterial loads. The longest culture incubation time for a clinically relevant pathogen was 7 days, observed with *E. faecalis*. In two VI cases, 16S-rRNA sequencing remained positive for an additional day after cultures had turned negative following the initiation of EVDI treatment. 16S-rRNA sequencing exhibited

one false negative, a culture positive *E. faecalis* infection, in an unsedated, clinically symptomatic patient with progressive pleocytosis. Likewise, culture exhibited one false negative in relation to 16S-rRNA sequencing. Notably, in the one VI case that was 16S-rRNA sequencing-positive but culture-negative, no antibiotics had been administered in the 24 hours prior to sampling, thus representing a possible false negative of the current gold standard.

The reads ratio, defined as the number of sequencing reads attributed to the identified pathogen relative to the negative controls, was significantly higher in 16S-rRNA sequencing samples classified as clinically relevant compared to those classified as contamination (median 15.9 [IQR 6.7–177.2] vs. 0.4 [IQR 0.2–1.0], Wilcoxon rank-sum, $p < 0.01$).

Diagnostic performance

The higher rate of contamination observed with 16S-rRNA sequencing relative to cultures contributed to a lower PPV for 16S-rRNA sequencing (Table 4). Both methods demonstrated high NPVs and specificity, consistent with the high prevalence of true negatives in the cohort. The sensitivity for 16S-rRNA sequencing and culture was 92.9% and 91.7%, respectively. On a patient level, both 16S-rRNA sequencing and culture correctly identified 7 out of 8 VI cases, resulting in a sensitivity of 87.5% for each method. Contingency tables are presented in Supplementary Table 2 and 3.

Potential impact of 16S-rRNA sequencing-guided EVDI treatment on antibiotic exposure

The total cumulative duration of EVDI treatment in the ICU was 406 days in our cohort. Of these, 73 days (18%) were appropriately administered for confirmed infections in 8 patients.

Here, three clinical scenarios are modeled, comparing projected antibiotic use with both our ICU baseline (406 days) and the ideal benchmark (73 days). The scenarios assume varying diagnostic turnaround times and management strategies (see supplement), and diagnostic performance of the sequencing method in parity to what was observed in this study. The potential reductions compared to baseline were 71%, 58%, and 43% for scenario 1 through 3. Potential treatment reductions under different clinical assumptions of molecular diagnostics availability are visualized in Figure 2.

External validation

In the external cohort, 1 patient was classified as having a confirmed EVDI (VI), 19 as suspected EVDIs (SI), and 115 as no infection (NI). Patient demographic data are detailed in supplementary Table 1. Applying the same criteria, clinical suspicion or abnormal CSF, if EVDI treatment was strictly initiated based on positive 16S-rRNA sequencing findings the number of treated patients would decrease to 12. When excluding patients without clinical suspicion (i.e., true negatives), the number further reduces to 9 patients receiving EVDI-targeted therapy. Importantly, the single confirmed EVDI case is retained in both scenarios.

Within our cohort, a 16S-rRNA sequencing-guided treatment approach could potentially reduce antibiotic exposure by approximately 43-71%. In the external cohort, employing similar surveillance routines but utilizing a different sequencing method for the 16S gene, the potential reduction in number of treated patients ranged from 40-55%.

4 Discussion

In this retrospective cohort study, we evaluated the performance of next-generation 16S-rRNA sequencing alongside conventional CSF bacterial cultures for diagnosing EVDIs. Our findings suggest that when 16S-rRNA sequencing is readily available, it can effectively complement cultures to guide antimicrobial therapy in the neurocritical care setting. Specifically, a 16S-guided strategy has the potential to reduce empiric broad-spectrum antibiotic exposure by 43% to 71%. Furthermore, we explored this stewardship estimate in an independent cohort employing an alternative 16S sequencing platform.

Importantly, 16S-rRNA sequencing exhibited diagnostic sensitivity on par with conventional CSF cultures, the current clinical gold standard. Both modalities accurately identified 7 of 8 confirmed EVDIs, with a per-sample sensitivity of 92.9% for 16S-rRNA sequencing compared to 91.7% for cultures. Although specificity remained high for both methods (92.7% vs. 97.8%), the PPV was considerably lower for 16S-rRNA sequencing (35.1% vs. 61.1%), driven by a higher rate of contaminations. Conversely, NPV was 99.7% for both methods. While this high NPV is partly expected given the low disease prevalence, it nonetheless underscores the primary utility of these assays in ruling out active infection. However, because evaluators were not blinded to microbiological results during manual review, microbiological findings may have inherently influenced infection classification despite neither positive nor negative results serving as predefined criteria for infection status. As such, potential incorporation bias cannot be excluded, and these performance metrics must be interpreted as descriptive.

The validity of the manual infection classification applied in this study warrants discussion. The approach has previously been used in sepsis research [34, 35], which faces similar difficulties as EVDI research in defining and identifying true infections. The complete agreement between our evaluators ($\kappa = 1$) in a random subset suggests that the classification is robust and reproducible. Moreover, the observed infection rate aligned with our prior studies utilizing strict culture-based definitions, as well as benchmarks from other low-incidence centers [8, 14]. This concordance, coupled with the fact that only a single probable false-negative culture was identified, demonstrates that manual evaluation was consistent with a strict "culture-based" definition while providing a pragmatic baseline to assess molecular diagnostic performance.

Incorporating two diagnostic approaches appears to mitigate the risk of misclassification. In our cohort, verified pathogens were characterized by high inter-test concordance, whereas contaminants were overwhelmingly discordant (Table 3). Although the overlap between true pathogens and common skin flora often obscures diagnostic interpretation [11, 15], simultaneous assessment allows one modality to cross-validate

the other. Given this high concordance in true infections, we believe that the likelihood of missing a clinically significant EVDI when employing dual diagnostics over time is minimal.

A high contamination rate nonetheless remains the principal hurdle of broad-range 16S-rRNA sequencing. In our exploratory analysis, the reads ratio (amplification relative to negative controls) was significantly higher in clinically relevant findings compared to contaminants and sequentially declined following initiation of EVDI treatment, except in one case where a rebound was observed, potentially indicating treatment failure or catheter colonization (Table 3). These dynamics suggest that semi-quantitative read metrics may potentially reflect bacterial burden in the CSF. However, because NGS quantification is constrained by variable ribosomal gene copy numbers across species, PCR amplification biases, and the low event rate in our cohort, these metrics must currently be considered hypothesis-generating and require prospective validation before being formally incorporated into clinical decision-making [36].

Translating these retrospective insights into a prospective clinical approach requires practical strategies to mitigate false-positive sequencing results, particularly because conventional culture results arrive later. To prevent inappropriate or excessive molecular testing, our institution applies an objective pre-test screening threshold of CSF granulocytes $\geq 100 \times 10^6$ cells/L before submitting samples for 16S-rRNA sequencing when clinical suspicion is otherwise low. Derived from institutional data spanning 17 years and over 1,800 patients, this screening threshold captured every verified EVDI case [14], potentially filtering out low-yield sampling. When sequencing is performed and returns positive, management could follow either a weighted approach integrating clinical probability with read ratios, or a pragmatic strategy treating positive results as presumptive infections until culture incubation rules out growth. Reassuringly, the microbiological profile and proportion of clinically relevant findings in our cohort align with previous findings from our institution and the broader literature [11, 14]. In aggregate, while 16S-rRNA sequencing exhibits a higher contamination rate than traditional cultures, combining pre-test cellular thresholds with structured post-test decision algorithms may effectively mitigate this limitation.

Beyond the challenge of positive contaminants, the effectiveness of 16S-rRNA sequencing as a rule-out assay critically depends on the validity of negative results, as false negatives risk untreated infection which may be deleterious. In our series, 16S sequencing failed to detect a single culture-confirmed *E. faecalis*. Unfortunately, rapid transfer to intermediate care precluded repeat testing. Notably, Widén et al. [28] reported an identical missed *E. faecalis* case using real-time 16S PCR. While such episodes highlight that molecular tests currently cannot supersede bedside clinical judgment, the overall high sensitivity of 16S-rRNA sequencing suggests that serial testing may recover missed organisms in persistently symptomatic patients. 16S-rRNA sequencing should therefore be deployed as an adjunct to de-escalate or refrain from EVDI treatment in a critical care setting, but does not replace cultures.

The clinical safety of relying on such a rule-out strategy is further reinforced by the low-virulence microbiology typical of modern neurocritical care units. When rigorous hygienic protocols are maintained, initial bacterial inocula are likely small, producing

an indolent course that routine CSF surveillance parameters cannot reliably differentiate from blood-induced aseptic inflammation [14]. Importantly, no excess mortality was observed historically at our institution among patients with confirmed infections compared to those with suspected cases [14]. Because these indolent skin commensals rarely precipitate fulminant deterioration, a brief diagnostic deferral while awaiting molecular confirmation is unlikely to impact patient survival. A negative 16S result may therefore support withholding or de-escalating EVDI treatment, provided cultures are followed and testing is repeated if suspicion persists.

Aside from the initial treatment decision, post-treatment bacterial clearance kinetics also dictate how molecular results should be interpreted longitudinally. In community-acquired meningitis, Brink et al. documented persistent PCR positivity up to day 7 after initiation of antimicrobial therapy, whereas conventional cultures rapidly turned negative [37]. However, it is important to recognize that bacterial loads are likely higher in community-acquired meningitis, which may limit the direct translation of these findings to EVDI diagnostics. Additionally, heterogeneous drug penetration across variable blood-brain barrier disruption may differ between patients with community-acquired meningitis and ICU patients with EVDIs, potentially further complicating direct comparisons [38]. In our cohort, clearance patterns varied substantially. One patient with *E. faecalis* remained positive by both culture and sequencing for 10 days, while another cleared 16S signals within 4 days of EVDI treatment. Beyond drug penetration, these variations may reflect host factors such as differences in bacterial burden, or catheter colonization rather than parenchymal infection. Consequently, persistent molecular signals during active treatment must be evaluated alongside clinical trajectory rather than assumed to indicate treatment failure.

Together, these clinical and diagnostic characteristics underpin the primary potential benefit suggested by our stewardship modeling: the potential of 16S-rRNA sequencing to drastically reduce unnecessary antibiotic exposure. Similar observations have been reported in other critical care cohorts [30]. In this study, we modeled three clinical scenarios with varying diagnostic turnaround times and management strategies, comparing projected antibiotic use against both our observed ICU baseline and an ideal benchmark treating verified infections alone (see supplement). Notably, even under our most conservative scenario, where every molecular false positive received five days of treatment corresponding to the average for patients with suspected infection at our institution, a 16S-guided strategy achieved a 43% reduction in total antibiotic days, extending up to 71% under optimized turnaround scenarios. While NGS pipeline turnarounds currently vary across centers, rapid molecular platforms, such as real-time 16S PCR, loop-mediated isothermal amplification, and targeted nanopore sequencing (recently demonstrated by Widén et al. [28] to achieve high diagnostic concordance in neuro-ICU patients), confirm that same-day reporting is clinically realistic. Consequently, integrating rapid molecular rule-out testing into neurocritical care workflows can potentially eliminate up to two-thirds of EVDI treatment days.

The stewardship estimate in this study was explored in in an independent cohort of 135 EVD patients at Norrland University Hospital, where a similar EVDI surveillance protocol was employed. Notably, that center routinely performed Sanger sequencing rather than NGS. Their cohort exhibited a lower incidence of confirmed infections

based on a strict "culture-based" classification approach, with only one verified case, and a reduced proportion of patients initiated on EVDI-targeted therapy (approximately 15% compared to 36% at our institution). The lower treatment rate at Norrland University Hospital potentially reflects a smaller institution, where management decisions are primarily influenced by a few infectious disease consultants with a strong CNS focus. Despite this lower baseline treatment rate, applying a 16S-rRNA sequencing-guided strategy based on clinical suspicion or CSF abnormalities still suggested a substantial reduction in antibiotic exposure, estimated between 40% and 55%, while successfully capturing the confirmed infection. These findings suggest that a 16S rule-out strategy offers significant promise for antimicrobial stewardship, suggesting that the approach may be transferable across different sequencing technologies and institutional prescribing patterns.

5 Limitations

We recognize several limitations associated with this study. As discussed, the authors were not blinded to the results of microbiological findings during manual classification, which may introduce incorporation bias, and the diagnostic performance of both 16S-rRNA sequencing and traditional cultures will have to be regarded as descriptive. Our institution employs a strict EVD management routine, likely resulting in a low-incidence and low-virulence EVDI setting. A threshold of granulocytes $\geq 100 \times 10^6 cells/L$ to guide the selective use of molecular diagnostics may therefore not be generalizable to other units, and individual adjustments based on local conditions are likely appropriate to reduce the proportion of false positive results. All data in this study were limited to the duration of each patient's ICU stay and results cannot be generalized to the non-ICU setting.

6 Conclusions

This study suggests that 16S-rRNA sequencing can serve as a valuable adjunct to traditional EVDI surveillance, primarily functioning as a rapid rule-out test to guide the withholding or de-escalation of unnecessary empiric broad-spectrum antibiotics if results are readily available. Furthermore, exploratory findings suggest that semi-quantitative reads ratios may represent a promising tool to differentiate clinically relevant findings from contaminations. By helping to excluding infection, 16S-rRNA sequencing has substantial potential to advance antimicrobial stewardship and reduce broad-spectrum antibiotic exposure in the neurocritical care setting.

List of abbreviations

EVD = External ventricular drain
EVDI = External ventricular drain associated infection
CSF = Cerebrospinal fluid
ICU = Intensive care unit
rRNA = ribosomal RNA
GCS = Glascow Coma Scale

CNS = Central nervous system
MS = Marcus Ståhlberg
JKV = John Karlsson Valik
DWN = David W Nelson
VI = Verified infection
SI = Suspected infection
NI = No infection
NGS = Next-generation sequencing
SD = Standard deviation
IQR = Interquartile range
PCR = Polymerase chain-reaction
PPV = Positive predictive value
NPV = Negative predictive value
LoS = Length of stay
SAH = Subarachnoid hemorrhage
ICH = Intracerebral hemorrhage
CoNS = Coagulase-negative staphylococci
C. acnes = Cutibacterium acnes
E. faecalis = Enterococcus faecalis
K. Oxytoca = Klebsiella Oxytoca

7 Tables

Characteristics	**NI**	**SI**	**VI**
Total (*n*)	122	61	8
Age (*mean (SD)*)	56.8 (16.6)	52.6 (17.9)	47.4 (18.3)
Female (%)	47.0	61.0	63.0
ICU LoS (*median (IQR)*)	17.0 (11.0-23.0)	15.0 (12.0-21.0)	22.5 (13.8-28.8)
EVD duration (*median (IQR)*)	12 (7.0-18.8)	13 (8.0-17.0)	17 (11.5-26.5)
EVDI treatment duration (*mean (SD)*)	NA	5.3 (2.7)	9.1 (4.9)
Admission to treatment (*mean (SD)*)	NA	6.9 (5.0)	13.4 (7.5)
EVD to treatment (*mean (SD)*)	NA	6.6 (5.1)	13.0 (7.5)
30-day mortality (%)	18.0	16.4	0
90-day mortality (%)	25.0	16.4	0
Admission diagnosis			
Subarachnoid hemorrhage (%)	43.0	74.0	50.0
Intracerebral hemorrhage (%)	24.0	16.0	38.0
Other cerebral diagnoses (%)	22.0	8.2	0.0
Traumatic brain injury (%)	7.4	1.6	13.0
Other non-cerebral diagnoses (%)	3.3	0.0	0.0

Table 1: Patient characteristics. EVD and admission to treatment refers to number of days from insertion of EVD and admission to the ICU to initiation of EVD-targeted treatment. ICU LoS refers to total length of ICU admission. All data, except for mortality rates, were limited to the ICU period and catheterization or treatment after discharge was not recorded. Length of stay (LoS), external ventricular drain (EVD), not applicable (NA), no infection (NI), suspected infection (SI), verified infection (VI), inter-quartile range (IQR), standard deviation (SD).

	Clinically relevant	Contamination
Culture	11	7
Coagulase-negative staphylococci		
S. epidermidis	1	1
CoNS [a]	3	3
Enterococcus faecalis	4	0
Klebsiella oxytoca	2	0
Staphylococcus aureus	1	0
Cutibacterium acnes	0	3
16S-rRNA sequencing	13	29
Coagulase-negative staphylococci	5	1
Enterococcus spp.	3	0
Klebsiella spp.	3	0
Streptococcus spp.		
S. agalactiae	1	0
S. mitis	0	1
S. anginosus	0	1
Staphylococcus aureus	1	0
Cutibacterium acnes	0	17
Prevotella spp.	0	2
Corynebacterium spp.	0	1
Polymicrobial growth [b]	0	1
Escherichia coli	0	1
Paracoccus spp.	0	1
Anaerobic mixed species [b]	0	1
Peptoniphilus spp.	0	1
Porphyromonas spp.	0	1

Table 2: Summary of all microbiological findings from samples analyzed with 16S. CoNS is unspecified species of coagulase-negative staphylococci, but not *S. epidermidis*.
a: Species unidentified.
b: Agents unspecified.

Patient	ICU day	Culture	16S-rRNA sequencing	Reads ratio	Assessment	
1	5	CoNS	CoNS	988.0	VI	✓
	9	CoNS	CoNS	7.8		✓
2	7	neg	S. agalactiae	0.2	VI	×
3	9	CoNS	CoNS / C. acnes[a]	0.1 / 0.4	VI	✓
4	26	S. epidermidis	CoNS	999.0	VI	✓
	27	neg	CoNS	1.6		∘
5	16	K. oxytoca	Klebsiella spp.	999.0		✓
	19	K. oxytoca	Klebsiella spp.	15.7	VI	✓
	20	neg	Klebsiella spp.	6.7		∘
6	17	neg	C. acnes[a]	0.4		
	26	E. faecalis	Enterococcus spp.	29.2	VI	✓
	30	E. faecalis	Enterococcus spp.	15.9		✓
	33	E. faecalis	Enterococcus spp.	29.8		✓
7	10	S. aureus	S. aureus	177.2	VI	✓
8	6	E. faecalis	neg	0.0	VI	×
9	10	NA	C. acnes	0.2	SI	
10	23	neg	C. acnes	1.0	SI	
11	13	neg	Prevotella spp. / Porphyromonas spp.	0.2 / 0.2	SI	
12	25	CoNS	neg	0.0	SI	
13	4	neg	C. acnes	0.3	SI	
	19	neg	C. acnes	0.5		
14	5	neg	Mix flora[b]	0.4[1]	SI	
15	4	neg	C. acnes	0.3	SI	
16	4	neg	Corynebacterium spp.	0.5	SI	
17	14	neg	C. acnes / Prevotella spp.	1.1 / 0.1	SI	
18	14	S. epidermidis	neg	0.0	SI	
19	27	CoNS	neg	10.2[2]	SI	
20	12	neg	C. acnes	0.1	SI	
21	10	neg	Anaerobic mix flora[b]	0.2[1]	NI	
22	5	neg	C. acnes	0.3	NI	
23	23	neg	C. acnes	0.0	NI	
24	11	C. acnes	C. acnes	0.3	NI	✓
25	6	neg	C. acnes	NA	NI	
26	6	neg	S. anginosus	9.4	NI	
27	1	neg	C. acnes	52.0	NI	
	6	neg	C. acnes	0.0		
28	11	neg	CoNS	0.2	NI	
29	8	neg	C. acnes / S. mitis	0.0/1.5	NI	
30	7	neg	Paracoccus spp.	NA	NI	
31	2	CoNS	neg	0.0	NI	
32	2	neg	E. coli / Peptoniphilus spp.	NA	NI	
33	5	C. acnes	neg	0.0	NI	
34	19	C. acnes	neg	0.0	NI	
35	5	neg	C. acnes	2.4	NI	

Table 3: All positive 16S-rRNA sequencing or culture results. Reads ratio refers to the ratio of reads relative to negative control (spike). ✓refers to concordant results. ∘ refers to expected discordance with negative cultures due to administered antibiotics. × refers to discordant results where the identified pathogen by 16S-rRNA sequencing or culture is considered clinically relevant. Contaminations are unmarked. Assessment includes external ventricular drain-related infection (EVDI) classifications, no infection (NI), suspected infection (SI) where positive findings are consistent with contamination, and verified infection (VI). Coagulase-negative staphylococci (CoNS). Rows are colored per patient.

1: Three most prevalent identified species combined.

2: Negative 16S-rRNA sequencing result, CoNS corresponded to only 6.2% of total reads but negative control relatively reduced due to high (92%) amplification of Moraxella spp. which was assessed as a non-relevant contamination.

a: Contamination.

b: Agents unspecified.

Diagnostic test performance		
	16S-rRNA sequencing (%)	Culture (%)
Positive predictive value	35.1	61.1
Negative predictive value	99.7	99.7
Sensitivity	92.9	91.7
Specificity	92.7	97.8

Table 4: Descriptive diagnostic performance of 16S-rRNA sequencing and cultures measured as positive predictive value, negative predictive value, sensitivity, and specificity, on a per sample basis.

8 Figures

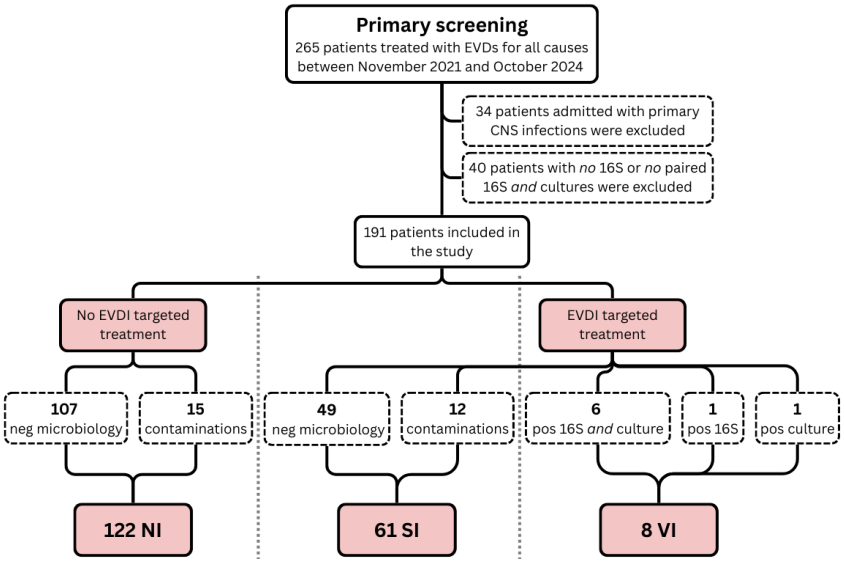


Figure 1: Flowchart of study cohort. Numbers in the flow chart refer to number of patients, not samples. Contaminations indicate microbiological findings with 16S-rRNA sequencing *or* cultures consistent with contamination. Neg microbiology refers to patients with negative 16S-rRNA sequencing *and* cultures. CNS is central nervous system. 16S is 16S-rRNA sequencing. EVDI is external ventricular drain-related infection. NI is no infection. SI is suspected infection. VI is verified infection.

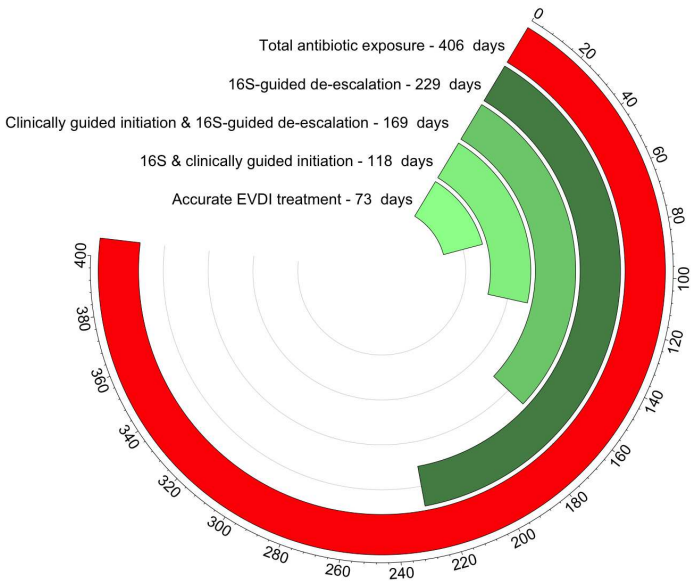


Figure 2: Potential impact of 16S-rRNA sequencing-guided EVDI treatment on antibiotic exposure, measured as treatment duration in days. The calculations are based on different clinical assumptions of molecular diagnostics availability and performance on par with 16S-rRNA sequencing in this study. Total days of EVDI treatment with current EVDI surveillance and treatment algorithms compared to total days of accurately administered treatment are illustrated, highlighting potential reductions achieved by applying 16S-rRNA sequencing & clinically guided initiation, clinically guided initiation & 16S-rRNA sequencing-guided de-escalation, and 16S-rRNA sequencing-guided de-escalation of EVDI treatment. Accurate EVDI treatment refers to days of EVDI treatment administered to patients with true EVDI. EVDI treatment data were confined to each patient's ICU stay.

Declarations

- Ethics approval and consent to participate: This study was conducted in accordance with the Declaration of Helsinki and was and approved by the Swedish Ethical Review Authority (DNR 2017/1544-31/1, DNR 2018/1701-32, DNR 2024-07464-02). The need for written informed consent was waived by the Swedish Ethical Review Authority due to the retrospective nature of the study.
- Consent for publication: Not applicable.
- Availability of data and materials: Data and code will be made available upon reasonable request to the corresponding author at marcus.stahlberg.2@ki.se.
- Competing interests: The authors declare that they have no competing interests.
- Funding: This study was in part funded by the regional Agreement on Medical Training and Clinical Research (ALF).
- Author contribution: D.W.N. conceived of the study and participated in study design, data analysis, data interpretation, manual infection classification, and contributed to drafting and critical revision of the manuscript. M.S. participated in study design, data collection, data analysis, data interpretation, manual infection classification, and drafted the manuscript. J.K.V. assisted with study design and methodology, participated in data interpretation and manual infection classification, and contributed to drafting and critical revision of the manuscript. J.R. participated in data interpretation and contributed to drafting and critical revision of the manuscript. C.M., J.B., E.W., and C.G.G. participated in data interpretation and contributed to manuscript drafting and critical revision. All authors read and approved the final manuscript.
- Acknowledgements: We would like to thank the nursing staff and assistant nurses at Karolinska University Hospital whose daily sampling routines, catheter bundles, and clinical documentation enabled the retrospective data collection.

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