9 Supplementary material

Characteristics	**NI** (n=115)	**SI** (n=19)	**VI** (n=1)
Age (mean)	57	61	78
Female (%)	57	68	0
ICU LoS (days, median)	15.0	18.0	16
EVD duration (days, median)	11	15	10
EVD to treatment (days, median)	NA	8	5
ICU diagnosis			
Subarachnoid hemorrhage (%)	40.0	73.6	0
Intracerebral hemorrhage (%)	29.5	10.5	0
Other cerebral diagnoses (%)	15.6	0	0
Traumatic brain injury (%)	14.7	15.7	100
Other non-cerebral diagnoses (%)	3.3	0.0	0.0

Supplementary table 1: External validation cohort characteristics. EVD to treatment is number of days from insertion of EVD to initiation of EVD-targeted treatment. ICU LoS refers to total length of admission. Length of stay (LoS), external ventricular drain (EVD), intensive care unit (ICU), not applicable (NA), no infection (NI), suspected infection (SI), verified infection (VI).

Per Sample

A			**B**		
Group	No Inf	Inf	**Group**	No Inf	Inf
16S			**Cult**		
Negative	306	1	Negative	313	1
Positive	24	13	Positive	7	11

Per Patient

C			**D**		
Group	No Inf	Inf	**Group**	No Inf	Inf
16S			**Cult**		
Negative	162	1	Negative	176	1
Positive	21	7	Positive	7	7

Supplementary Table 2: **A**: 16S-rRNA sequencing results per sample (n=344), stratified by infection group. **B**: CSF bacterial culture results per sample (n=332), stratified by infection group. **C**: 16S-rRNA sequencing results per patient, stratified by infection group. **D**: CSF bacterial culture results per patient, stratified by infection group.

Culture	NA	Negative	Positive
16S			
NA	0	0	0
Negative	14	286	7
Positive	1	25	11

Supplementary Table 3: Concordance between 16S-rRNA sequencing and traditional CSF cultures (per sample analysis).

Clinical antibiotic stewardship scenarios

(1) 16S-rRNA sequencing & clinically guided initiation: a perfect scenario, where only patients with positive molecular diagnostics and strong clinical suspicion are initiated on EVDI treatment. In this scenario, NI patients are not initiated on treatment due to no clinical suspicion, and SI patients with positive 16S-rRNA sequencing were standardized to 5 days of treatment, reflecting the median treatment duration in the SI group. This scenario assumes that the results from molecular diagnostics are available within the same time-frame as regular cytological and biochemical CSF parameters (hours).

(2) Clinically guided initiation & 16S-guided de-escalation: an almost perfect scenario, where EVDI treatment is initiated based on clinical suspicion and discontinued if the results from molecular diagnostics are negative after 1 day of treatment. In this scenario, NI patients are not initiated on treatment due to no clinical suspicion, and SI patients with positive 16S-rRNA sequencing were standardized to 5 days of treatment, reflecting the median treatment duration in the SI group. SI patients with negative 16S-rRNA sequencing were standardized to 1 day of treatment, reflecting

realistic termination. This scenario assumes that the results from molecular diagnostics are available within 1 day of sampling.

(3) 16S-guided de-escalation: a scenario, where EVDI treatment is initiated based on clinical suspicion and discontinued if the results from molecular diagnostics are negative after 1 day of treatment. In this scenario, both NI and SI patients with positive results from 16S-rRNA sequencing were standardized to 5 days of treatment, reflecting the median treatment duration in the SI group. SI patients with negative 16S-rRNA sequencing were standardized to 1 day of treatment, reflecting realistic termination. This scenario assumes that the results from molecular diagnostics are available within 1 day of sampling.