Unbiased metagenomic sequencing for pediatric meningitis in Bangladesh reveals neuroinvasive Chikungunya virus outbreak and other unrealized pathogens

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Abstract

The disease burden due to meningitis in low and middle-income countries remains significant and failure to determine an etiology impedes appropriate treatment for patients and evidence-based policy decisions for populations. Broad-range pathogen surveillance using metagenomic next-generation sequencing (mNGS) of RNA isolated from cerebral spinal fluid (CSF) provides an unbiased assessment for possible infectious etiologies. In this study, our objective was to use mNGS to identify etiologies of pediatric meningitis in Bangladesh.

We conducted a retrospective case-control mNGS study on CSF from patients with known neurologic infections (n=36), idiopathic meningitis (n=25), without infection (n=30) and six environmental samples collected between 2012-2018. Using an open-access, cloud-based bioinformatics pipeline (IDseq) and machine learning, we identified potential pathogens which were confirmed through qPCR and Sanger sequencing. These cases were followed-up through phone/home-visits. The CSF samples were collected from children with WHO-defined meningeal signs during prospective meningitis surveillance at the largest pediatric referral hospital in Bangladesh.

The 91 participants (42% female) ranged in age from 0-160 months (median: 9 months). In samples with known infectious causes of meningitis and without infections (n=66), there was 83% concordance between mNGS and conventional testing. In idiopathic cases (n=25), mNGS identified a potential etiology in 40% (n=10), including bacterial and viral pathogens. There were three instances of neuroinvasive Chikungunya virus (CHIKV). The CHIKV genomes were >99% identical to each other and to a Bangladeshi strain only previously recognized to cause systemic

illness in 2017. CHIKV qPCR of all remaining stored CSF samples from children who presented with idiopathic meningitis in 2017 at the same hospital (n=472) revealed 17 additional CHIKV meningitis cases. Orthogonal molecular confirmation of each mNGS-identified infection, case-based clinical data, and follow-up of patients substantiated the key findings.

Using mNGS, we obtained a microbiological diagnosis for 40% of idiopathic meningitis cases and identified a previous unappreciated pediatric CHIKV meningitis outbreak. Case-control CSF mNGS surveys can complement conventional diagnostic methods to identify etiologies of meningitis and facilitate informed policy decisions.

Introduction:

Globally there are 10.6 million cases of meningitis and 288,000 deaths every year.[1,2] In addition, at least a quarter of survivors suffer from long-term neurological sequelae.[3] The vast majority of meningitis cases occur in low- and middle-income countries (LMICs) .[4] In a World Health Organization (WHO)-supported meningitis surveillance study in Dhaka, Bangladesh,[5] we collected 23,140 cerebrospinal fluid (CSF) samples from patients with suspected meningitis between 2004 and 2016, 8,125 of which contained ≥10 WBC/µl. We were able to detect a bacterial etiology in only 1,585 (20%) of these cases despite the use of multiple diagnostic tools including culture, serologic and antigen assays and pathogen-specific qPCR. Such low rates of microbiological diagnosis are common in many settings globally, hampering implementation of evidence-based policy decisions for optimizing local empiric treatment protocols and disease prevention strategies.[6,7]

The challenges of obtaining a microbiological diagnosis may be due to a combination of multiple factors including (i) meningitis is caused by a wide variety of microbes, some of which are uncommon and lack diagnostic assays, (ii) prior antibiotic exposure and delay in care-seeking can lower the yield of culture and PCR-based methods, and/or (iii) non-infectious causes of inflammation can mimic infectious meningitis. Drawing on recent studies demonstrating the promise of unbiased metagenomic next-generation sequencing (mNGS) approaches to identify pathogens in diverse biological specimens,[8–11] we sought to conduct a retrospective case-control study to investigate CSF of children with idiopathic meningitis in Bangladesh.

Methods:

Study site and population:

All CSF samples used in this study were collected as part of the meningitis surveillance study supported by the WHO conducted in Dhaka Shishu Hospital (DSH). Children admitted at DSH were enrolled if they met WHO-defined inclusion criteria of meningitis and if a CSF specimen was collected (Table S1).[12]

Protocols were approved by the ethical review board of the Bangladesh Institute of Child Health. Samples were collected for routine clinical care, at the discretion of the attending physician. Informed consent was obtained from parents/caregivers.

Laboratory methods and data collection:

CSF specimens were cultured (Figure 1A) using the standard procedure and pneumococcal antigen was detected by immunochromatographic test (BinaxNow).[13–15] White blood cells (WBC) in the specimens were counted and differentiated into lymphocytes and polymorphonuclear neutrophils (PMNs). Culture-negative and pneumococcus-antigen-negative CSF specimens underwent latex agglutination and PCR testing for *Haemophilus influenzae*, pneumococcus and meningococcus, the predominant bacterial etiologies of meningitis in the region. Surplus CSF was stored at -80ºC. Detection of chikungunya virus (CHIKV) was conducted using qPCR with published primers on all CSF specimens stored and collected in 2017 (n=472) (Table S2).[16]

Sample selection:

Samples collected between 2012 and 2018 were selected and clinical details of all selected samples are provided in Table S3. For positive controls, CSF specimens where an etiology could be successfully established through culture, serology, antigen testing, and/or qPCR were chosen. For idiopathic samples, specimens were randomly chosen from a set that contained ≥20 WBC/µl (≥40% PMNs) (median: 314 WBC/µl), and ≥40 mg/dl protein (median: 220 mg/dL) (normal range is 15-45 mg/dL).

Negative controls consisted of randomly chosen CSF specimens from patients in whom an alternate diagnosis was ultimately made, the child was discharged within 6 days of hospitalization, and CSF samples contained ≤6 WBC/µl (median: 0 WBC/µl) and ≤30 mg/dl protein (median: 20 mg/dL). This set also included environmental samples, which were nuclease-free water (Invitrogen, 10977-015) samples (n=5) transferred into CSF collection tubes in the patient wards and treated and stored in the laboratory like CSF specimens in a blinded fashion. A "no template" water control sample was included during RNA extraction.

mNGS and confirmatory testing: Total RNA was extracted from 100 µl of unspun CSF, and mNGS libraries were prepared following published methods.[17] External RNA Controls 103 Consortium collection (ERCC) (ThermoFisher, 4456740) spike-in controls were used in every sample as markers of potential library preparation errors and to permit calculation of input RNA mass. Libraries were sequenced on a NovaSeq 6000 to generate 150 bp, paired-end sequences. All non-human sequence reads were deposited in Sequence Read Archive, National Center for Biotechnology Information (NCBI) BioProject PRJNA516582.

Pathogens were identified from the raw fastq files using the IDseq portal, a cloud-based, open-source bioinformatics platform designed for detection of microbes from metagenomic data (Figure S1, Methods S1). Similar to published methods, potentially pathogenic microbes were distinguished from both ubiquitous environmental contaminants and commensal flora using a Z-score metric for each genus relative to a background distribution derived from the set of CSF specimens from non-meningitis cases and water controls.[8] Taxa with a Z-score less than one were removed from analysis. To further aid analysis, we employed a published logistic regression method to classify and assign potential etiological candidates in each sample.[18] We retrained the model using the following features: RNA-seq reads per million (rpM), rank amongst all detected microbes within the sample, and a binary variable indicating whether the microbe has established pathogenicity.[18,19] Microbes with probability scores >0.2 were reported as potential pathogens. In cases where more than one potential pathogen was identified, only the top scoring pathogen was considered. Based on the water controls, a minimum calculated RNA input threshold of 3.0 pg was required for pathogen prediction. The average RNA input of the set of non-infectious CSF samples was 1.6 pg (range: 0.9–2.8).

Potential pathogens identified by mNGS were confirmed through PCR and Sanger sequencing (Table S2).

Genome assembly, microbial typing, phylogenetics:

For *de novo* assembly and annotation of draft genomes, we used the St. Petersburg genome assembler (SPAdes, v3.11.1)[20] and Geneious (v10.3.2).[21] Genotype assignments for viruses were identified using BLASTn, with the assembled genome sequence of the virus as query. We

specifically compared the assembled CHIKV genomes with selected CHIKV genomes available in NCBI data for time-resolved phylogenetics (Method S2).

Clinical Data collection and patient follow-up: Clinical and demographic data were collected from electronically stored surveillance forms. The number and distribution of suspected and confirmed CHIKV febrile cases were collected from the microbiology laboratory records of Shishu Shasthya Foundation Hospital and DSH, the two largest pediatric hospitals of Bangladesh, serving the same catchment area. Clinical follow-up was conducted through telephone and/or home visits using structured questionnaires.

Results

CSF specimens were tested from 91 patients (42% female), ranging in age from 0-160 months (median: 9 months). Comparative clinical characteristics of the three case types: infectious, idiopathic and non-infectious, are provided in Figure 1B and Table S3. The majority (71%, n=65) of patients originated in Dhaka division, while the remaining cases sought care from 11 outlying districts (Figure S2A, B).

mNGS libraries (n=97) were prepared and sequenced (Methods), resulting in an average depth of 72 million reads/sample (IQR: 53-86 million). The resulting fastq files were processed using the open-source IDseq (www.IDseq.net, v1.8) platform (Figure S1, Methods S1). Using ERCC spike-in control RNAs (Methods), the calculated RNA input masses were highly correlated with WBC count (log scaled Pearson r=0.69).[17] RNA input masses for the non-infectious CSF

samples and template-free controls were significantly less than both the samples of known and unknown etiology (2 pg vs 273 pg, p <0.0001 by Wilcoxon rank sums) (Fig 2, Table S4).

Specimens of known etiology (n = 36)

Positive control samples were drawn from cases with previously identified pathogens, via a combination of standard lab diagnostics, including culture (n=8), qPCR and antigen/serology (n=26), and antigen-only (n=2). Using logistic regression (Methods), we correctly identified pathogens in 7 of 8 (88%) samples that were culture positive (Figure 2B). For specimens that were previously PCR and antigen or serology positive, mNGS identified 24 of 27 (89%) samples whose confirmatory qPCR had a cycle threshold (Ct) of <32. Taking into account all specimens that were culture, PCR and/or antigen or serology positive (n=36) regardless of Ct value, 25 (69%) specimens were classified as containing potential pathogens by mNGS (Figure 2B).

Negative specimens (n = 36)

Among the non-infectious specimens, only 4 (11%) had an input RNA mass greater than 3.0 pg. No potential pathogens were identified in these samples.

Idiopathic specimens (n = 25)

Potential pathogens were identified in 10 of 25 (40%) idiopathic cases: four cases with bacterial pathogens (*Salmonella enterica, Stenotrophomonas maltophilia, Bacillus cereus, Mycobacterium tuberculosis)* and six with viral pathogens (CHIKV (n=3), mumps virus (n=2), enterovirus B) (Figure 2). All these were further confirmed with orthogonal PCR testing and Sanger sequencing investigation of case histories and patient follow-up.

Salmonella enterica

CHRF0082: A 4-month old boy was admitted with suspected meningitis, with fever and bulging fontanel. The CSF specimen tested in this study was collected 8 days after admission and contained 400 WBC/µl (80% PMNs) and 450 µg/dl protein. The child was treated with ceftriaxone, netilmicin and amoxicillin, and discharged after 23 days with residual Bell's palsy. mNGS identified *S. enterica* (Table S4), and retrospective investigation revealed that a CSF specimen was also collected the day of admission and that first specimen was culture positive for *S. enterica.* The child was healthy upon follow-up at 15 months of age.

Mycobacterium tuberculosis

CHRF0058: A 13-year old girl was admitted after 30 days of fever, vomiting, and headache with neuroimaging observations of intracranial space occupying lesions. Her CSF had 360 WBC/µl (80% PMNs) and 200 µg/dl protein. After 15 days of treatment with empiric ceftriaxone, meropenem, azithromycin, and acyclovir, the family left against medical advice. mNGS identified *Mycobacterium tuberculosis* (TB). Follow-up revealed that the child went to several health care facilities, where she was ultimately diagnosed with TB meningitis based on clinical suspicion and initiated on anti-tubercular chemotherapy. The grandfather of the child lived in the same household and died from pulmonary TB 2-3 months before the onset of her symptoms. The child, after almost a year, remains bedridden with persistent neurocognitive impairment.

Stenotrophomonas maltophilia

CHRF0059: A 4-month old girl was admitted for 10-days of fever, convulsion and cough. She was treated at home with empiric cefixime and azithromycin before seeking care at DSH. Her CSF sample contained 120 WBC/µl (80% PMNs) and 300 µg/dl protein. She was treated with ceftriaxone, meropenem, vancomycin, and amikacin and discharged after 28 days. *S. maltophilia* was detected by mNGS (Table S4). This child was lost to follow-up.

Bacillus cereus

CHRF0070: A 6-day old boy was admitted with fever, convulsion, lethargy and yellow coloration of skin; the treating physicians provisionally diagnosed him with sepsis and neonatal jaundice. His CSF contained 12,000 WBC/µl (95% PMNs) and 500 µg/dl protein. He was discharged after 15 days following empiric treatment with ceftazidime and amikacin. mNGS identified *B. cereus* as the potential etiology. Follow-up at the age of 1 year revealed that the child required ventriculoperitoneal shunt placement for hydrocephalus. The child currently does not have any significant health problems.

Mumps virus

CHRF0036: A 13-year old boy was admitted after 7 days of fever with irritability and headache. His CSF had 1,500 WBC/µl (60% PMNs) and 160 mg/dl protein. The patient was treated empirically with ceftriaxone for 10 days, after which the family left against medical advice. The logistic regression classifier failed to identify a potential pathogenic microbe. However, manual inspection of the data identified two reads that mapped mumps virus. This was further confirmed with validated qPCR of the original CSF sample to detect Mumps and Sanger sequencing of the resultant amplicon. Mumps virus was not detected in any other samples, except for CHRF0011

(see below). During a follow-up conversation with the father, he reported that the child had had parotitis and fever preceding the headaches and that he had made a full recovery.

CHRF0011: An 18-month old boy was admitted with fever and convulsion. His CSF revealed 100 WBC/ µl (60% PMNs) and 60 µg/dl protein. He was treated with ceftriaxone and discharged after 9 days. mNGS identified mumps virus with sufficient read coverage to determine the genotype as G. At the age of 2.5 years, the child was healthy.

Enterovirus B

CHRF0010: A 7-month old boy was admitted due to fever, convulsion and lethargy. His CSF analysis unveil 314 WBC/µl (80% PMNs) and 40 µg/dl protein. He was discharged after 6 days of empiric treatment with ceftriaxone. mNGS identified enterovirus B. Almost a year after the episode, the father reported his child falls frequently.

Chikungunya virus:

CHRF0071: A 1-month old girl was admitted with fever, rash, convulsion, diarrhea and lethargy. Her CSF contained 180 WBC/µl (80% PMNs) and 250 µg/dl protein. She was treated with ceftazidime and amikacin and discharged after 6 days. mNGS detected CHIKV with complete genome coverage. The child is currently healthy.

CHRF0094: A 5-day old girl was admitted with fever, convulsion and lethargy. CSF contained 1,000 WBC/µl (90% PMNs) and 220 µg/dl protein. She was treated with ceftazidime and

amikacin, followed by meropenem and was discharged after 10 days. mNGS detected CHIKV with complete genome coverage. The child was healthy during our follow up.

CHIRF0012: An 86-month old girl was admitted with acute glomerulonephritis, fever, convulsion, abdominal distension, edema, lethargy and generalized weakness. A LP was performed after 36 days, presumably due to development of meningitis-like symptoms. The CSF contained 180 WBC/µl (60% PMNs) and 55 µg/dl protein. She was treated empirically with cefuroxime, metronidazole, ciprofloxacin, ceftazidime, and acyclovir. The child finally died after 45 days. mNGS identified CHIKV, and the complete genome was assembled.

Neuroinvasive CHIKV in Bangladesh

All belonging to the East/Central/South African (ECSA) CHIKV genotype, the three CHIKV genomes from the above cases were >99% identical to each other, and to the genome of the strain that caused a febrile outbreak in Dhaka in the summer of 2017. Two of the three children with CHIKV were admitted in June and July of 2017, the peak of the febrile outbreak. To determine if there were additional cases of meningitis admitted in DSH during that period, we performed CHIKV-specific qPCR on 472 idiopathic CSF specimens collected and stored in 2017 and identified 17 additional CHIKV cases. We detected significant overlap between dates of collection of the 20 CHIKV-positive CSF samples with the dates when suspected and confirmed febrile CHIKV cases appeared in our hospitals (Figure 3). Most of these cases originated in Dhaka city, where the febrile outbreak occurred (Figure S2). The median age of these 20 CHIKV-positive patients was 8 months (range: 8 days – 96 months), 35% were female. The mean CSF WBC count was 188/µl (range: 12–1200 WBC/ µl), and the mean PMNs was 48%

(range: 10-90%). The average hospital length of stay was 11 days (range: 2-45 days), and the 30 day mortality rate was 0.05% (1/20).

Subsequent mNGS of CSF from these 17 additional cases identified CHIKV RNA in all (Table S3). Comparison with other CHIKV genomes available in NCBI showed close relationship of the Bangladeshi strain with other strains that caused outbreaks in Asia in recent years, specifically to the one that caused an outbreak in Pakistan in 2016 (99.8% identity (Figure 4).

Discussion

Prevention and effective and timely treatment of pediatric meningitis in LMICs is essential for achieving the United Nation's Sustainable Development Goal 3 of ensuring healthy lives and promoting well-being for all at all ages. Obtaining a microbiological diagnosis can improve outcomes by informing targeted antimicrobial therapy for individual patients. On a population level, improved surveillance better informs region-specific treatment and prevention policy decisions and monitor outbreaks. Here, we coupled unbiased mNGS and machine learning with traditional diagnostics to bridge knowledge gaps and identify the etiology of meningitis in Bangladeshi children.

For samples with known etiology previously determined by qPCR and/or by antigen or serology testing, unbiased mNGS correctly identified the pathogen in 25 of 36 (69.4%) samples. After excluding cases with low pathogen abundance as defined by a confirmatory qPCR Ct of >32, mNGS identified 24 of 27 (89%) known infections. The decreased sensitivity of CSF mNGS for very low abundance pathogens has been previously reported.[22] A potential pathogen was

identified in 40% (10/25) of previously idiopathic meningitis cases. The microbes detected in these cases included bacteria, mycobacteria and viruses with established CNS pathogenicity that were likely not detected due to a variety of reasons including prior antibiotic consumption, the lack of available clinical laboratory assays, and in the case of the CHIKV cases, lack of clinical suspicion for a newly emerging virus with underrecognized neuroinvasive potential.

Among potential bacterial pathogens, we identified *S. enterica* in one sample. Even though that CSF specimen was culture-negative, a culture obtained one week earlier grew *S. enterica*, suggesting that mNGS identified an infection even after antibiotic treatment cleared live pathogens. *B. cereus,* detected in a 6-day old neonate, is commonly recognized as a CNS pathogen in immunocompromised patients including neonates, and at least 7 cases of neonatal *B. cereus* meningitis have been reported.[23,24] *S. maltophila* was identified in an 4-month old patient; this is a global emerging multidrug resistant gram-negative species of bacteria frequently associated with infections in young or immunocompromised patients[25]. *Mycobacterium tuberculosis*, a well-known cause of severe and chronic meningitis in TB-endemic countries,[26] was detected from one patient. This finding was strengthened by the patient's potential exposure in the household and a serendipitous subsequent clinical diagnosis unrelated to this study. The time-consuming nature of culture, the low yield of smear-based detection, makes detection of TB meningitis notoriously challenging and suggests the potential value of mNGS for broad range screening of diverse pathogens including TB.

Among the viral pathogens detected in this study, mumps virus was detected in two cases, an organism well-associated with meningitis.[27,28] Enterovirus B was detected in one case, also a known cause of pediatric meningitis in the South Asia region.[29]

One of the most interesting findings in this study was the detection of CHIKV in three children, two of which occurred amidst a 2017 CHIKV outbreak in Bangladesh. In recent years, there have been increasing reports of neurological complications associated with CHIKV infection,[30,31] but no neuroinvasive cases have been identified previously in Bangladesh, and no clinical testing for CHIKV in CSF was being performed in Bangladesh at the time. Our mNGS findings led us to retrospectively screen 472 CSF samples from DSH collected through 2017 with a CHIKV qPCR assay. This identified an additional 17 CHIKV cases, bringing the total to 20. The rate of detection of CHIKV meningitis was consistent with the rate of detection of CHIKV fever outbreak cases reported in Dhaka during the same time period. This study has enhanced the viral detection capabilities at the DSH laboratory, consistent with outbreak preparedness goals. Future studies will include a health-care utilization survey in the catchment area surrounding the DSH to ascertain disease incidence,[32] in addition to follow-up surveillance for neurodevelopmental assessment of the affected children.

The findings of this study should be considered within the context of several limitations. Importantly, CSF specimens were not collected and stored specifically for metagenomic analysis; this study used samples stored following no stringent or consistent guidelines, with no specific reagents, over a period of six years, which may have affected the quality of the RNA in the samples. Furthermore, this was a retrospective study, and thus, the timing of sample

collection with respect to days of disease evolution and prior antibiotic exposure was uncontrolled and unknown in most cases. The quantity of pathogen material may be limiting or undetectable with time, which may contribute to the proportion of unresolved cases in this study. Future studies will include prospective surveillance following improved guidelines for sample collection, storage, processing, and sequencing. The availability of open-source cloud-based pipelines, such as IDseq, for bioinformatic interrogation of metagenomic samples, obviates the need for expensive on-premise compute, storage, and IT personnel support, and future studies can be conducted on site, in real time.

Conclusions

Hypothesis-free pathogen identification methods like mNGS can facilitate the identification of infectious causes of meningitis that had eluded standard laboratory testing, including previously unrecognized outbreaks of neuroinvasive viral infections like CHIKV. These improved patient and population-level data can inform better health policy decisions, including but not limited to vaccine deployment, antibiotic stewardship, vector control and pandemic preparedness.

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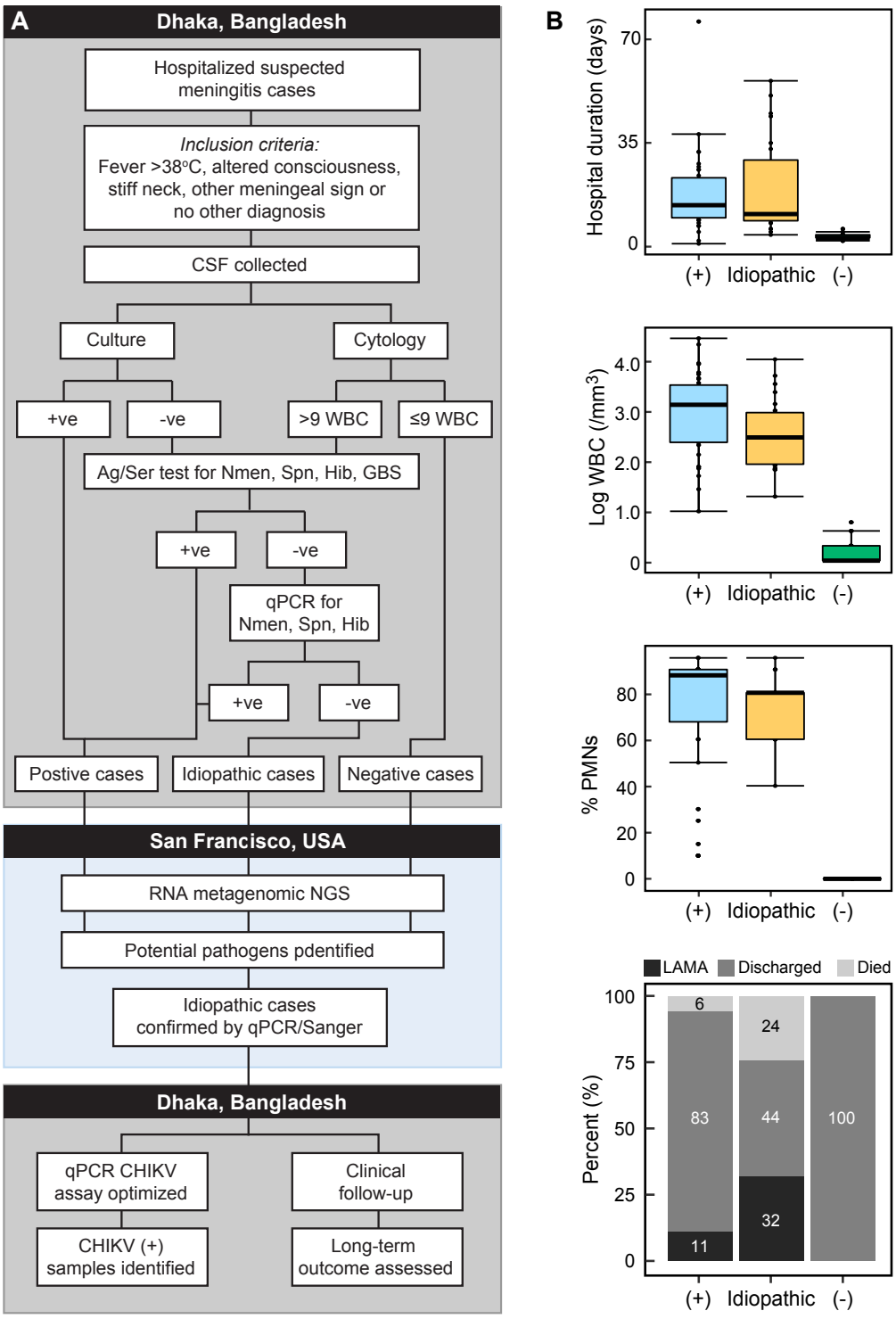

Figure 1. **Selection and characteristics of samp les used in this study.** A. Study flow diagram. B. Comparison of clinical characteristics between the three types of samples, positive (+), idiopathic, and negative (-) chosen for this study. Ag: antigen; Ser: serology; Spn: *Streptococcus pneumoniae*; Nmen: *Neisseria meningitidis*; Hib: *Haemophilus influenzae* tybe b; GBS: Group B Streptococcus; CHIKV: Chikungunya virus; PMN: Polymorphonuclear neutrophils

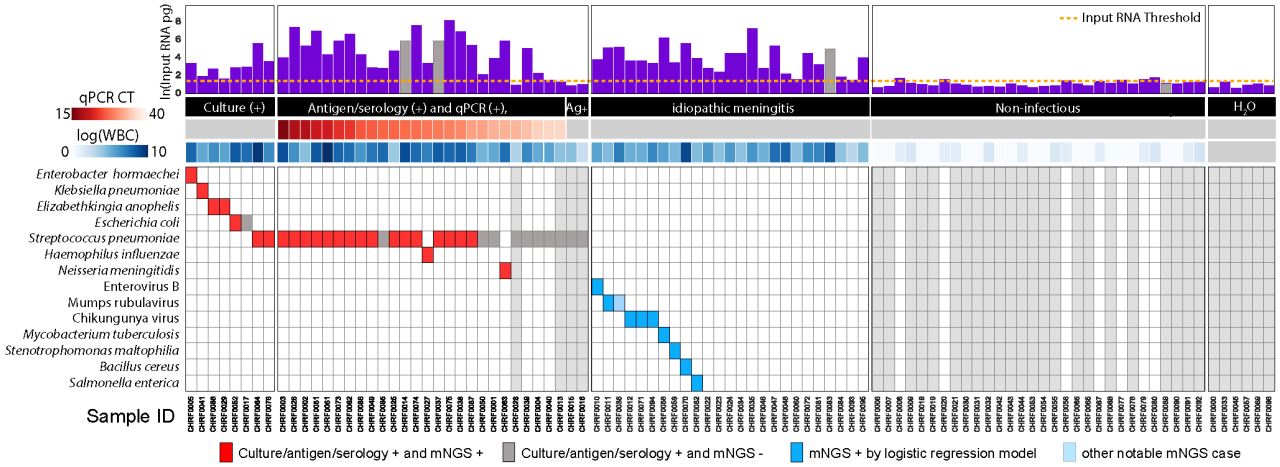

Figure 2. **Pathogen Identification through mNGS and logistic regression in all sample types.** Total input RNA (log pg) is shown for all samples. Samples for which the input RNA values could not be reliably calculated (outliers), are shown as grey bars with imputed input RNA values based on the mean value in their respective groups (known infection, no infection, idiopathic samples). Samples in the known infection group are ordered by increasing cycle threshold, depicted as a heat map below the x-axis. Next, the WBC counts obtained by the clinical lab are also plotted as a heatmap. The predicted pathogens for all samples are shown as filled-in squares. Grey squares indicate samples which were considered positive by clinical diagnostic, but for whom no pathogen was detected by the pathogen-calling algorithm using mNGS data. Red boxes indicate concordant findings and blue boxes indicate new putative pathogens identified by mNGS data that were not identified by standard clinical methods. The light blue squares indicate pathogens that were not picked up by the logistic regression method but were flagged as potentially interesting by manual review and followed-up as if detected. Ag+: Antigen positive

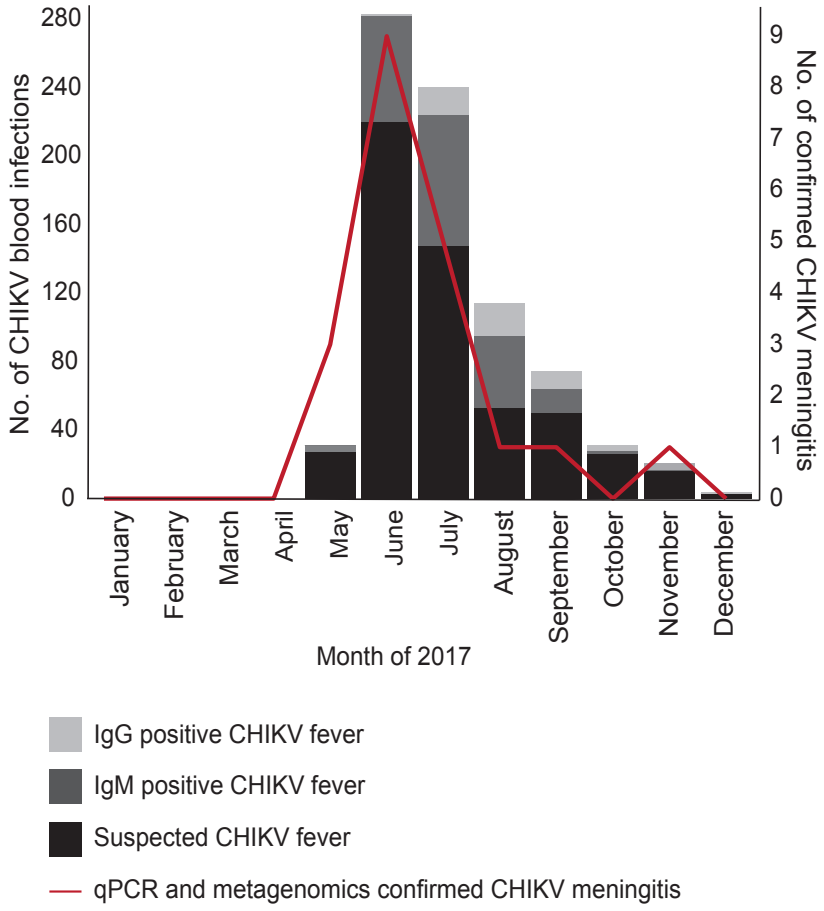

Figure 3. **Chikungunya meningitis outbreak in Bangladesh. CHIKV meningitis outbreak overlapped with CHIKV febrile outbreak.** The months in 2017 when the CHIKV-positive meningitis CSF samples were collected and when suspected febrile CHIKV cases sought care in the two largest pediatric hospitals of Bangladesh, Dhaka Shishu Hospital and Shishu Shasthya Foundation Hospital. The blood samples of suspected febrile CHIKV cases were detected by a specific diagnostic test for CHIKV-IgG and IgM (SD Biosensor, South Korea) as part of clinical care and results were collected retrospectively from laboratory records.

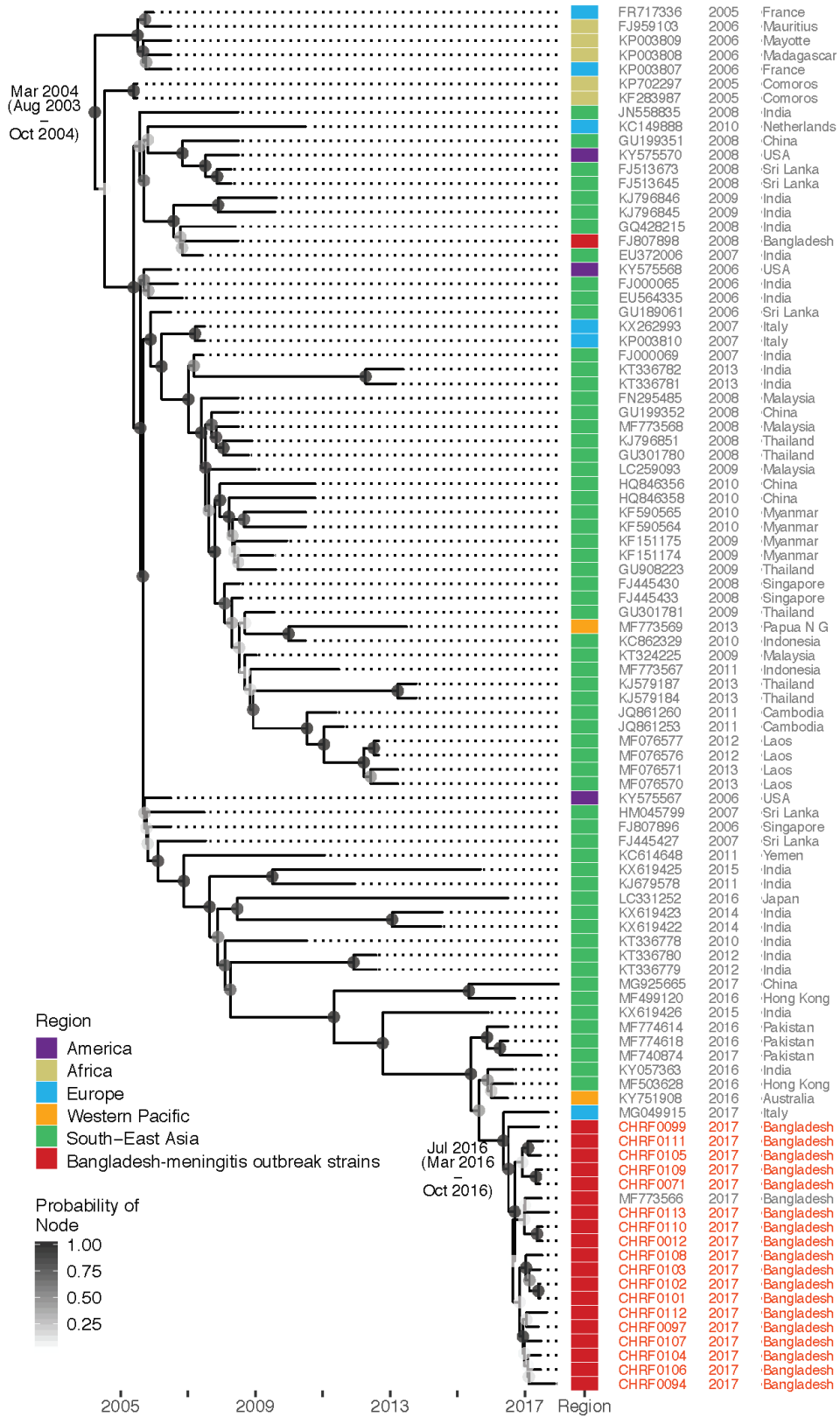

Figure 4. **Genetic relationship of Bangladeshi CHIKV-meningitis strain with strains that caused recent outbreak/s in Bangladesh and elsewhere.** All CHIV genomes identified and assembled in this study were compared with selected CHIKV genomes available in NCBI data for time-resolved phylogenetics.

Table 1. Summary of clinical characteristics of idiopathic cases (n=25).

Sample ID	Age (months)	WBC/ul (PMN%) / Protein (mg/dl)	Pathogen detected	Provisional/ Final diagnosis	Hospital duration (days)/Outcome	Long-term outcome
Resolved						
CHRF0010	7/m	314 (80) / 40	Enterovirus B	Meningitis/Meningitis	6/Dis	Heathy
CHRF0058	160/f	360 (80) / 200	*M. tuberculosis*	ICSOL/ ICSOL	15/LAMA	Sequalae
CHRF0070	0/m	12000 (95) / 500	*B. cereus*	Sepsis	15/Dis	Heathy
CHRF0082	4/m	90 (70) / 600	*S. enterica*	Meningitis/Meningitis	23/Dis	Healthy
CHRF0094	0/f	1000 (90) / 220	CHIKV	Meningitis/Meningitis	10/Dis	Healthy
CHRF0011	18/m	100 (60) / 60	Mumps	Meningitis/Meningitis	9/Dis	Healthy
CHRF0035	21/f	600 (90) / 700	Human herpes virus 6	Acute stroke syndrome/ Acute stroke syndrome	56/Died	Died
CHRF0059	4/f	120 (80) / 300	*S. maltophilia*	Meningitis/Meningitis	28/Dis	NA
CHRF0071	1/f	180 (80) / 250	CHIKV	Meningitis/Meningitis	6/Dis	Heathy
CHRF0012	86/f	180 (60) / 55	CHIKV	Acute Glomerulonephritis/Meningoencephalitis	45/Died	Died
CHRF0036	156/m	1500 (60) / 160	Mumps	Meningitis/Meningitis	10/LAMA	Healthy
Unresolved						
CHRF0081	72/m	1100 (40) / 350	NA	Meningitis/Meningitis	37/LAMA	Died
CHRF0093	13/m	20 (80) / 80	NA	Heart disease/ Dextocardia	11/Died	Died
CHRF0022	3/m	460 (80) / 300	NA	Meningitis/Meningitis	9/LAMA	Died
CHRF0034	23/f	70 (70) / 150	NA	Meningitis/Meningitis	11/Dis	Sequelae
CHRF0046	1/f	160 (80) / 70	NA	Pneumonia/ Pneumonia	4/LAMA	Healthy
CHRF0023	4/m	74 (70) / 100	NA	Meningitis/ Meningitis	8/Dis	Sequelae
CHRF0047	16/m	2600 (90) / 400	NA	ARI/Meningoencephalitis	5/Died	Died
CHRF0083	7/m	5600 (80) / 1000	NA	Hydrocephalus/Pneumonia	10/Died	Died
CHRF0095	98/m	90 (70) / 60	NA	Meningoencephalitis/Tubercular meningitis	33/LAMA	Died
CHRF0024	96/f	460 (60) / 200	NA	Meningitis/Meningitis	8/LAMA	NA
CHRF0048	2/m	3800 (80) / 400	NA	Meningitis/Meningitis	35/Dis	NA
CHRF0060	4/m	84 (70) / 150	NA	Meningitis/Meningitis	11/LAMA	Died
CHRF0072	3/f	900 (60) / 300	NA	Meningitis/Meningitis	44/Dis	Sequelae
CHRF0084	4/f	70 (60) / 250	NA	Meningitis/Meningitis	51/Died	Died

NA: not available; LAMA: Left against medical advice; Dis: Discharged; ICSOL: intracranial space-occupying lesion. NA: not available

Supplemental Material

Method S1. **Bioinformatic analysis and pathogen identification.**

Method S2. **Phylogenetic analysis of Chikungunya virus strains responsible for the meningitis outbreak in Bangladesh, 2017.**

Figure S1. **Schematic representation of the bioinformatic approach in IDseq for pathogen identification.**

Figure S2. **Residential locations of all cases and CHIKV-positive meningitis cases.**

Table S1. **WHO-defined clinical criteria used to enroll children in the meningitis surveillance in Dhaka Shishu Hospital, Bangladesh.**

Table S2. **Detection of Chikungunya virus and orthogonal confirmation of probable pathogens through qPCR.**

Table S3. **Case-based clinical and laboratory metadata of all cases included in this study (n=115).**

Table S4. **Case-based metagenomic data derived from all sequenced samples (n=115)**

Table S5. **List of microbes included in the logistic regression model as potential pathogens.**

Method S1. Bioinformatic analysis and pathogen identification

Microbial pathogens were identified from raw sequencing reads using the IDseq Portal (https://idseq.net), a cloud-based, open-source bioinformatics platform designed for detection of microbes from metagenomic data (eFigure 1). IDseq scripts and user instructions are available at https://github.com/chanzuckerberg/idseq-dag and the graphical user interface web application for sample upload is available at https://github.com/chanzuckerberg/idseq-web. IDseq is conceptually based on previously implemented platforms,[1–3] but is optimized for scalable Amazon Web Services (AWS) cloud deployment. Bioinformatics data processing jobs are carried out on demand as Docker containers using AWS Batch. Alignments to the National Center for Biotechnology Information (NCBI) database are executed on dedicated auto scaling groups (ASG) of Amazon Elastic Compute Cloud (EC2) instances, with the number of server instances varied with job load. Fast downloads of the NCBI database from the Amazon Simple Storage Service to each new server instance are enabled by the open-source tool s3mi (https://github.com/chanzuckerberg/s3mi). Initial alignment and removal of reads derived from the human genome is performed using the Spliced Transcripts Alignment to a Reference (STAR) algorithm.[4] Low-quality reads, duplicates, and low-complexity reads are then removed using the Paired-Read Iterative Contig Extension (PRICE) computational package,[5] the CD-HIT-DUP tool[6] and a filter based on the Lempel-Ziv-Welch (LZW) compression score, respectively. A second round of human read filtering is carried out using bowtie2[7] Remaining reads are queried against the most recent version of the NCBI nucleotide (NT) and non-redundant (NR) protein databases (updated monthly) using GSNAPL and RAPSearch2 respectively.[8,9] Reads matching GenBank records in the superphylum Deuterostomia are removed, given the high likelihood that such residual reads are of human origin. The relative abundance of microbial taxa is calculated based on reads per million (rpM) mapped at the genus level. An overview of this pipeline is represented in eFigure 1.

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Method S2. Phylogenetic analysis of Chikungunya virus strains responsible for the meningitis outbreak in Bangladesh, 2017.

To study the phylogeny of the Chikungunya virus (CHIKV), BLASTn was used to extract all complete CHIKV genomes that had greater then 85% identity to the draft genomes assembled using SPAdes v3.11.1.[1] These genomes were then aligned using the default settings in MUSCLEv3.8.1551.[2] Annotation from one of the NCBI genomes (accession number: HM045823) was used to divide the genome into coding, and non-coding regions, and ModelTest-NGv0.1.5 was used to identify the best-fitting evolutionary models for each genomic region. Using the best-fitting models for evolution for each genomic region, we reconstructed a maximum-likelihood phylogeny using RAxML-ng v0.6.0 using default settings.[3] The maximum likelihood phylogeny was used to select a lineage of most closely related NCBI sequences to the assembled chikungunya virus genomes. We then created a time-resolved phylogeny for this lineage in BEAST v1.10.1 using substitution models selected using ModelTest-NG a strict molecular clock for all genomic sites, and selected the Bayesian skyline model as the tree prior.[4,5] Two separate BEAST runs of 300 million Markov chain Monte Carlo iterations sampling every 50,000 iterations were used to explore the posterior distribution of phylogenies and evolutionary parameters. Convergence was visually checked after removing the first 50% of samples, and samples from the two chains were merged. A maximum clade credibility phylogeny was created using median node heights. Using the above described method, another time-resolved phylogeny of the Bangladesh CHIKV was generated using the genomes assembled earlier and E1 viral structural glycoprotein sequence from 2016-2017 South Asian cluster [Pakistan 2016 CHIKV outbreak (accession number: MF774613—MF774619, MF74074—MF74081), Bangladesh 2017 outbreak (accession number: MG697262—MG697282), Australia (accession number: KY751908), Italy (accession number: MG049915) Hong-Kong (accession number: MF503628) and India (accession number: KY057363)].

Accession number of Enterovirus B, CHRF0010, is MK468615 of the CHIKV genomes are CHRF_0071: MK468608; CHRF_0103: MK468609; CHRF_0094: MK468610; CHRF_0099: MK468611; CHRF_0101: MK468612; CHRF_0106: MK468613; CHRF_0108: MK468614; CHRF_0110: MK468615; CHRF_0104: MK468616; CHRF_0105: MK468617; CHRF_0107: MK468618; CHRF_0109: MK468619; CHRF_0012: MK468620; CHRF_0111: MK468621; CHRF_0112: MK468622; CHRF_0098: MK468623; CHRF_0100: MK468624; CHRF_0097: MK468625; CHRF_0102: MK468626; CHRF_0113: MK468627; CHRF_0012: MK468628; CHRF_0012: MK468629

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Figure S1. **Schematic representation of the bioinformatic approach in IDSEQ for pathogen identification.**

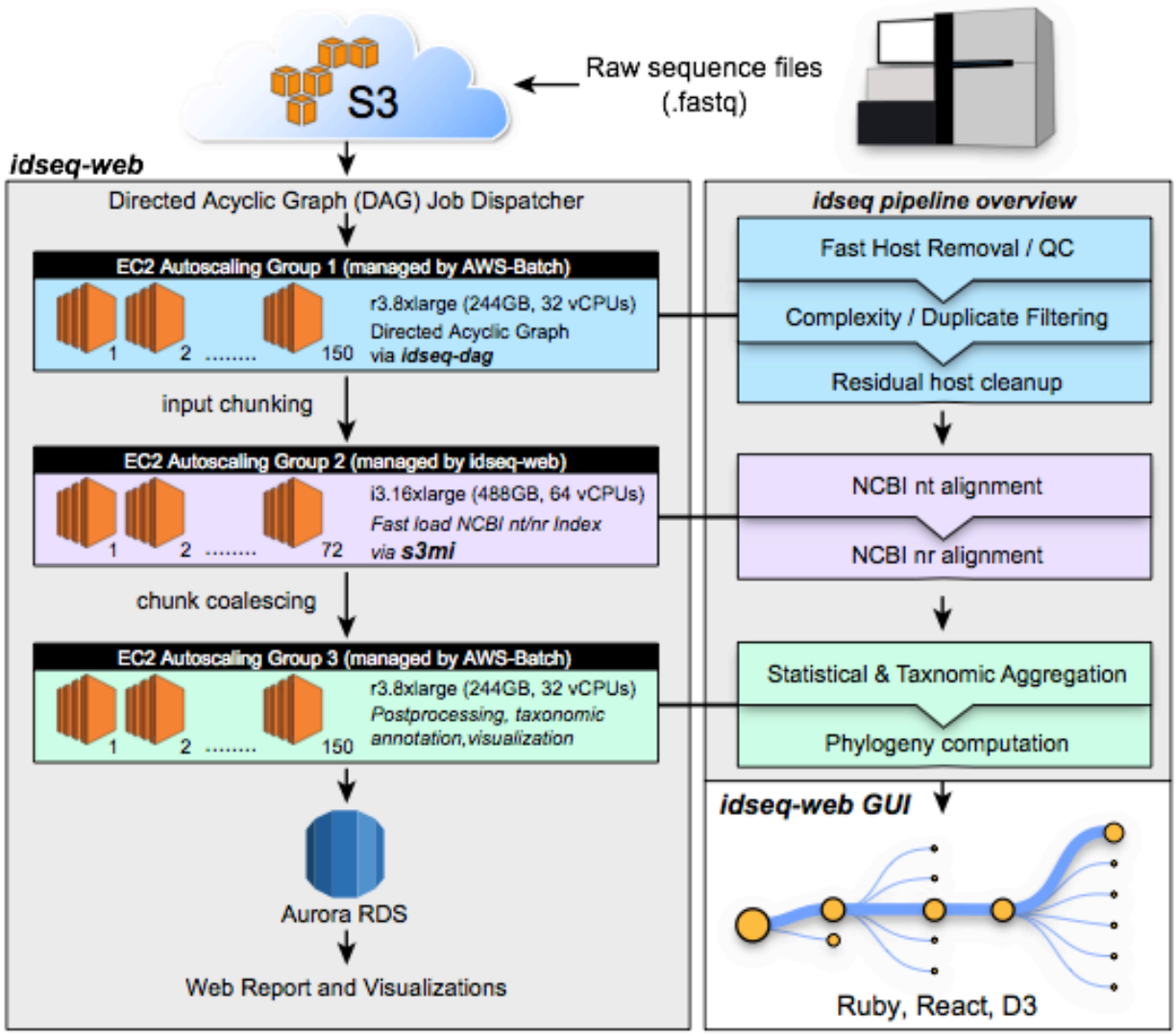

Figure S2. **Residential locations of all selected cases and CHIKV-positive meningitis cases.** A. The map of Bangladesh depicting national distribution of all cases. B. Magnified map of Dhaka division from where majority of cases arrived. C The map of Bangladesh depicting national distribution of all CHIKV-positive meningitis cases during the outbreak of 2017. D. Magnified map of Dhaka division from where almost of CHIKV-meningitis cases arrived from during the outbreak in 2017.

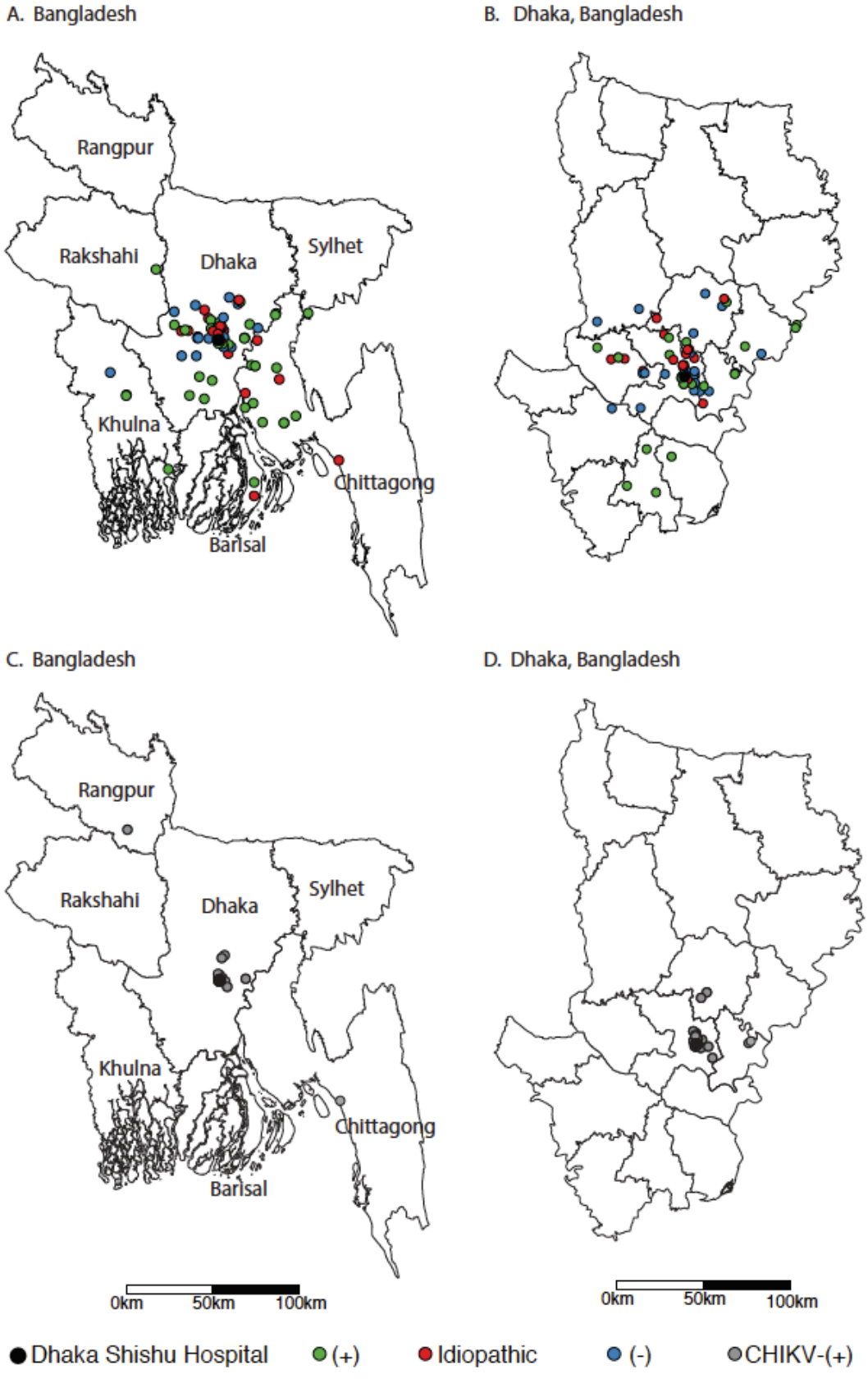

Table S1. **WHO-defined clinical criteria used to enroll children in the meningitis surveillance in Dhaka Shishu Hospital, Bangladesh.**

WHO case definition	Inclusion criteria
Meningitis	Any child aged 0-59 months hospitalized with sudden onset of fever >100.4 °F and one of the following signs: • Stiff neck • Altered or reduced level of consciousness • Bulging fontanel (if < 12 months of age) • Prostration/lethargy • Convulsions • Toxic appearance • Petechial or purpuric rash • Poor Sucking • Irritability (>2 months) Or any child aged 0-59 months hospitalized with a clinical diagnosis of meningitis.

Table S2. **Detection of Chikungunya virus and orthogonal confirmation of probable pathogens through qPCR.** For CHIKV confirmation, we used qScript XLT 1-Step RT-qPCR ToughMix Low-ROX (Cat. No. 95134-100). For the remaining confirmations, we used Quantabio PerfeCTa qPCR ToughMix, Low Rox (Cat. No. 95114-012). For reverse transcription, we used Quantabio qScript cDNA Super Mix (Cat. No. 95048-100): 25°C for 5 min, 42°C for 30 min, 85°C for 5 min.

Sample ID	Pathogen	Template	Primers	Probes	qPCR conditions
CHRF_0012, CHRF_0071, CHRF_0094	CHIKV	Whole CSF*	F:5'AAAGGGCAAACTCAGCTTCAC; R:5'GCCTGGGCTCATCGTTATTC	FAM-CACCGACGGCGAGACCGACTTT-BHQ1	50°C for 10 min, 95°C for 1 min, 45 cycles of 95°C for 15 s, 60°C for 1 min.
CHRF_0010	Enterovirus B	CSF-RNA	F:5'CCTGAATGCGGCTAATCC; R:5'TTGTCACCATWAGCAGYCA	FAM-CCGACTACTTTGGGWGTCCGTGT-BHQ1	95°C for 30 s, 40 cycles: 95°C for 15 s, 60°C for 30 s
CHRF_0011, CHRF_0036	Mumps	CSF-RNA	F:5'GTATGACAGCGTACGACCAACCT; R:5'GCGACCTTGCTGCTGGTATT 3'	FAM-CCGGGTCTGCTGATCGGCGAT-BHQ	95°C for 30 s, 40 cycles: 95°C for 5 s, 60°C for 15 s, 70°C for 10 s
CHRF_0059	*S. maltophilia*	CSF-RNA	F:5'GCCGAAAGCCCAAGGTTT; R:5'CGACTTTCGTCCTCGCCTTA 3'	FAM-CGCAACGTTAATCGGCGCAGG-TAMRA	95°C for 30 s, 40 cycles: 95°C for 5 s, 60°C for 15 s, 70°C for 10 s
CHRF_0082	*S. enterica*	Whole CSF	F:5'CTCACCAGGAGATTACAACATGG; R:5'AGCTCAGACCAAAAGTGACCATC	FAM-CACCGACGGCGAGACCGACTTT-BHQ1	95°C for 30 s, 40 cycles: 95°C for 15 s, 60°C for 30 s
CHRF_0058	*M. tuberculosis*	Whole CSF	F:5'GTCGAACGGCTGATGACCAAACT; R:5'TCCGAAGCGGCGCTGGACGA; F1:5'AACGGCTGATGACCAAACTC 3'; R1:5'AAGCGGCGCTGGACGAGATC	Probe1: FAM-ACCACGATCGCTGATCCGGCCACA-BHQ1; Probe2: FAM-ACCCGCGGCAAAGCCCGCAGG-BHQ1	95°C for 10 min, 95°C 10 min; 10 cycles: 95°C 15 s, 65°C 30 s; 40 cycles: 95°C 15s, 60°C 30 s
CHRF_0070	*B. cereus*	Whole CSF	F:5'ACTGTATTAGAAGGGCGCGG; R:5'TAAAGCCACCGCCAAGTACA 3'	N/A	95°C for 10 min, 95°C for 30 s , 40 cycles: 95°C for 5 s, 57°C for 15 s, 70°C for 10 s

Table S3. Case-based clinical and laboratory metadata of all cases included in this study (n=115).

Sample ID	DSH result	Age (m)	CSF collection date	WBC/ul (PMN%)/ Protein mg/dl	Hospital duration/ outcome	Organism detected in DSH	qPCR Ct	Provisional/ Final Diagnosis
CHRF0052	Culture (+)	f/3	14-Nov-17	6000 (90)/600	21/Dis	*E. coli*	-	Sepsis/Hydrocephalus
CHRF0064	Culture (+)	m/81	6-Jun-16	24000 (95)/1000	12/Dis	*S. pneumoniae*	-	Meningoencephalitis/Meningitis
CHRF0076	Culture (+)	m/6	20-Feb-15	860 (90)/300	1/LAMA	*S. pneumoniae*	-	Meningitis/Meningitis
CHRF0088	Culture (+)	f/0	25-Sep-16	750 (90)/500	32/Dis	*Flavobacterium sp.*	-	Meningitis/Meningitis
CHRF0005	Culture (+)	m/0	5-Jun-16	3500 (90)/700	23/Dis	*Enterobacter sp.*	-	perinatal asphyxia/Meningitis
CHRF0017	Culture (+)	m/132	13-Mar-16	2600 (90)/150	24/Dis	*E. coli*	-	Meningitis/Meningitis
CHRF0029	Culture (+)	f/0	10-Jan-16	280 (85)/300	38/LAMA	*Flavobacterium sp.*	-	perinatal asphyxia /perinatal asphyxia
CHRF0041	Culture (+)	m/6	03-Nov-12	250 (50)/300	76/Dis	*K. pneumoniae*	-	Meningitis/Hydrocephalus
CHRF0001	Antigen, PCR (+)	m/0	16-May-17	250 (80)/300	15/Dis	*S. pneumoniae*	32.9	Meningitis/Meningitis
CHRF0013	Antigen, PCR (+)	m/4	7-Mar-17	74 (25)/60	13/Dis	*S. pneumoniae*	36	Meningitis/Meningitis
CHRF0025	Antigen, PCR (+)	f/3	19-Mar-17	52 (10)/200	15/Dis	*S. pneumoniae*	27.2	Meningitis/Meningitis
CHRF0037	Antigen, PCR (+)	m/60	18-Feb-17	5000 (90)/250	15/Dis	*S. pneumoniae*	28.7	Meningoencephalitis/Meningitis
CHRF0049	Antigen, PCR (+)	100	5-Sep-17	2000 (80)/800	14/Dis	*S. pneumoniae*	26.5	Meningitis/Meningitis
CHRF0061	Antigen, PCR (+)	f/4	10-Apr-17	32000 (90)/300	26/Dis	*S. pneumoniae*	21.5	Meningitis/Pneumonia
CHRF0073	Antigen, PCR (+)	m/1	10-Apr-17	4000 (95)/700	7/Died	*S. pneumoniae*	23.4	Meningitis/Meningitis
CHRF0085	Antigen, PCR (+)	f/4	28-Mar-17	3200 (85)/500	26/Dis	*S. pneumoniae*	27.1	Meningitis/Meningitis
CHRF0002	Antigen, PCR (+)	m/3	27-Feb-17	80 (60)/300	7/LAMA	*S. pneumoniae*	19.2	Meningitis/Meningitis
CHRF0014	Antigen, PCR (+)	m/7	26-Feb-17	10000 (90)/400	5/LAMA	*S. pneumoniae*	27.6	Meningitis/Meningitis
CHRF0026	Antigen, PCR (+)	m/108	30-Aug-17	700 (90)/300	9/Died	*S. pneumoniae*	19	NS/NS
CHRF0038	Antigen, PCR (+)	m/79	19-Mar-16	4800 (80)/300	10/Dis	*S. pneumoniae*	29.7	Meningitis/Meningitis
CHRF0050	Antigen, PCR (+)	f/6	16-Mar-16	140 (70)/150	2/Dis	*S. pneumoniae*	31.8	Meningitis/Meningitis
CHRF0062	Antigen, PCR (+)	f/9	7-May-16	2500 (90)/250	12/Dis	*S. pneumoniae*	23.8	Meningitis/Meningitis
CHRF0074	Antigen, PCR (+)	f/85	27-Jun-16	3000 (85)/350	18/Dis	*S. pneumoniae*	27.7	Meningitis/Meningitis
CHRF0086	Antigen, PCR (+)	m/6	14-Dec-15	550 (90)/300	14/Dis	*S. pneumoniae*	26.2	Meningitis/Meningitis
CHRF0003	Antigen, PCR (+)	m/71	18-Jan-15	9600 (90)/220	14/Dis	*S. pneumoniae*	16.3	ICSOL/Meningitis
CHRF0015	Antigen (+)	m/78	22-Mar-15	80 (10)/15	18/Dis	*S. pneumoniae*	NA	NS/NS
CHRF0027	Antigen, PCR (+)	m/1	27-Sep-17	1300 (80)/250	15/Dis	*H. influenzae*	28.4	Meningitis/Meningitis

Sample ID	DSH result	Age (m)	CSF collection date	WBC/ul (PMN%)/ Protein mg/dl	Hospital duration/ outcome	Organism detected in DSH	qPCR Ct	Provisional/ Final Diagnosis
CHRF0039	Antigen, PCR (+)	f/4	4-Sep-16	950 (85)/70	9/Dis	*S. pneumoniae*	34.1	Meningitis/Meningitis
CHRF0051	Antigen, PCR (+)	m/3	14-May-16	6500 (90)/200	12/Dis	*S. pneumoniae*	21.3	Meningitis/Meningitis
CHRF0063	Antigen, PCR (+)	f/40	22-Jul-16	2400 (90)/200	9/Dis	*N. meningitidis*	33	Meningitis/Meningitis
CHRF0075	Antigen, PCR (+)	m/72	16-Feb-15	3400 (90)/200	27/Dis	*S. pneumoniae*	29.3	Meningoencephalitis/Meningoencephalitis
CHRF0087	Antigen, PCR (+)	f/82	27-Nov-17	1600 (90)/80	28/Dis	*S. pneumoniae*	31.7	Meningoencephalitis/Meningitis
CHRF0004	Antigen, PCR (+)	m/61	9-Sep-17	220 (10)/100	14/Dis	*S. pneumoniae*	35.5	Meningitis/Meningitis
CHRF0016	Antigen (+)	f/4	5-Oct-17	10 (60)/150	32/Dis	*S. pneumoniae*	NA	Encephalitis/Meningitis
CHRF0028	Antigen, PCR (+)	m/55	30-Oct-17	28 (30)/100	8/Dis	*S. pneumoniae*	33.2	Meningitis/Meningitis
CHRF0040	Antigen, PCR (+)	m/9	18-Dec-17	280 (15)/160	12/Dis	*S. pneumoniae*	36	Pneumonia
CHRF0053	(-)	f/12	23-Jul-14	0 (0)/15	3/Dis	-	-	UTI/AGE
CHRF0065	(-)	m/8	24-Jul-14	0 (0)/20	2/Dis	-	-	AFC/AFC
CHRF0077	(-)	m/5	19-Sep-14	0 (0)/25	4/Dis	-	-	AFC/AFC
CHRF0089	(-)	f/12	21-Sep-14	0 (0)/30	3/Dis	-	-	AFC/AFC
CHRF0006	(-)	f/5	21-Sep-14	0 (0)/15	4/Dis	-	-	Meningitis/ARI
CHRF0018	(-)	f/24	24-Sep-14	0 (0)/15	2/Dis	-	-	Meningitis/AFC
CHRF0030	(-)	m/14	29-Sep-14	0 (0)/15	3/Dis	-	-	AFC/AFC
CHRF0042	(-)	m/0	12-Oct-14	0 (0)/20	4/Dis	-	-	Perinatal asphyxia/ Perinatal asphyxia
CHRF0054	(-)	m/12	30-Oct-14	0 (0)/25	3/Dis	-	-	Meningitis/AFC
CHRF0066	(-)	m/16	21-Nov-14	0 (0)/25	3/Dis	-	-	AFC/AFC
CHRF0078	(-)	m/14	16-Jan-15	0 (0)/20	4/Dis	-	-	AFC/AFC
CHRF0090	(-)	m/12	22-Jan-15	0 (0)/15	4/Dis	-	-	AFC/AFC
CHRF0007	(-)	f/8	29-Jan-15	0 (0)/20	4/Dis	-	-	Meningitis/AFC
CHRF0019	(-)	f/12	1-Feb-15	0 (0)/20	4/Dis	-	-	Meningitis/AFC
CHRF0031	(-)	m/9	2-Feb-15	0 (0)/15	3/Dis	-	-	Down's syndrome/AFC
CHRF0043	(-)	f/5	5-Feb-15	0 (0)/20	2/Dis	-	-	AFC/AFC
CHRF0055	(-)	m/43	3-Feb-18	4 (0)/20	3/Dis	-	-	AFC/AFC
CHRF0067	(-)	f/28	3-Feb-18	2 (0)/20	3/Dis	-	-	AFC/AFC
CHRF0079	(-)	f/8	5-Feb-18	2 (0)/20	5/Dis	-	-	AFC/AFC

Sample ID	DSH result	Age (m)	CSF collection date	WBC/ul (PMN%)/ Protein mg/dl	Hospital duration/ outcome	Organism detected in DSH	qPCR Ct	Provisional/ Final Diagnosis
CHRF0091	(-)	m/17	10-Feb-18	2 (0)/20	3/Dis	-	-	AFC/AFC
CHRF0008	(-)	m/15	11-Feb-18	2 (0)/15	3/Dis	-	-	AFC/AFC
CHRF0020	(-)	m/6	11-Feb-18	2 (0)/20	3/Dis	-	-	Meningitis/AFC
CHRF0032	(-)	m/6	15-Feb-18	4 (0)/20	3/Dis	-	-	Pseudotumor cerebri/Pseudotumor cerebri
CHRF0044	(-)	f/27	15-Feb-18	2 (0)/20	3/Dis	-	-	Pseudotumor cerebri
CHRF0056	(-)	f/18	20-Feb-18	2 (0)/20	6/Dis	-	-	Seizure disorder/AFC
CHRF0068	(-)	m/8	17-Feb-18	0 (0)/20	2/Dis	-	-	Meningitis/AFC
CHRF0080	(-)	f/14	18-Feb-18	4 (0)/20	4/Dis	-	-	AFC/AFC
CHRF0092	(-)	m/11	19-Feb-18	6 (0)/25	3/Dis	-	-	AFC/AFC
CHRF0009	(-)	m/14	19-Feb-18	4 (0)/25	5/Dis	-	-	AFC/AFC
CHRF0021	water	f/8	28-Feb-18	2 (0)/25	4/Dis	-	-	AFC/AFC
CHRF0033	water	-	4-Mar-18	-	-	-	-	-
CHRF0045	water	-	4-Mar-18	-	-	-	-	-
CHRF0057	water	-	4-Mar-18	-	-	-	-	-
CHRF0069	water	-	4-Mar-18	-	-	-	-	-
CHRF0096	water	-	4-Mar-18	-	-	-	-	-
CHRF0000	water	-	-	-	-	-	-	-
CHRF0081	Idiopathic	m/72	5-Feb-18	1100 (40)/350	/LAMA	-	-	Meningitis/Tubercular meningitis
CHRF0093	Idiopathic	m/13	22-Feb-18	20 (80)/80	11/Died	-	-	Congenital heart disease/Dextrocardia
CHRF0010	Idiopathic	m/7	4-Jan-18	314 (80)/40	6/Dis	-	-	Meningitis/Meningitis
CHRF0022	Idiopathic	m/3	8-Jan-18	460 (80)/300	9/LAMA	-	-	Meningitis/Meningitis
CHRF0034	Idiopathic	f/23	22-Jan-18	70 (70)/150	11/Dis	-	-	Meningitis/Meningitis
CHRF0046	Idiopathic	f/1	28-Jan-18	160 (80)/70	4/LAMA	-	-	Pneumonia/Pneumonia
CHRF0058	Idiopathic	f/160	27-Dec-04	360 (80)/200	15/LAMA	-	-	ICSOL/ICSOL
CHRF0070	Idiopathic	m/0	14-Dec-17	12000 (95)/500	15/Dis	-	-	Sepsis/Sepsis
CHRF0082	Idiopathic	m/4	9-Dec-17	90 (70)/600	23/Dis	-	-	Meningitis/Meningitis
CHRF0094	Idiopathic	f/0	22-Nov-17	1000 (90)/220	10/Dis	-	-	Meningitis/Meningitis
CHRF0011	Idiopathic	m/18	5-Nov-17	100 (60)/60	9/Dis	-	-	Meningitis/Meningitis

Sample ID	DSH result	Age (m)	CSF collection date	WBC/ul (PMN%)/ Protein mg/dl	Hospital duration/ outcome	Organism detected in DSH	qPCR Ct	Provisional/ Final Diagnosis
CHRF0023	Idiopathic	m/4	18-Sep-17	74 (70)/100	8/Dis	-	-	Meningitis/Meningitis
CHRF0035	Idiopathic	f/21	13-Sep-17	600 (90)/700	56/Died	-	-	ASS/ASS
CHRF0047	Idiopathic	m/16	5-Sep-17	2600 (90)/400	5/Died	-	-	ARI/Meningoencephalitis
CHRF0059	Idiopathic	f/4	20-Aug-17	120 (80)/300	28/Dis	-	-	Meningitis/Meningitis
CHRF0071	Idiopathic	f/1	17-Jun-17	180 (80)/250	6/Dis	-	-	Meningitis/Meningitis
CHRF0083	Idiopathic	m/7	12-Aug-17	5600 (80)/1000	10/Died	-	-	Hydrocephalus/Pneumonia
CHRF0095	Idiopathic	m/98	16-Jul-17	90 (70)/60	33/LAMA	-	-	Meningoencephalitis/TB meningitis
CHRF0012	Idiopathic	f/86	11-Jul-17	180 (60)/55	45/Died	-	-	AGN/Meningoencephalitis
CHRF0024	Idiopathic	f/96	25-Apr-17	460 (60)/200	8/LAMA	-	-	Meningitis/Meningitis
CHRF0036	Idiopathic	m/156	23-Apr-17	1500 (60)/160	10/LAMA	-	-	Meningitis/Meningitis
CHRF0048	Idiopathic	m/2	6-Jun-17	3800 (80)/400	35/Dis	-	-	Meningitis/Meningitis
CHRF0060	Idiopathic	m/4	4-Jun-17	84 (70)/150	11/LAMA	-	-	Meningitis/Meningitis
CHRF0072	Idiopathic	f/3	25-May-17	900 (60)/300	44/Dis	-	-	Meningitis/Meningitis
CHRF0084	Idiopathic	f/4	20-May-17	70 (60)/250	51/Died	-	-	Meningitis/Hydrocephalus
CHRF0097	PCR (+)	f/1	31-May-17	1200 (90)/250	12/Dis	CHIKV	36.0	Septicaemia/Meningitis
CHRF0098	PCR (+)	m/96	31-May-17	26 (10)/80	11/Dis	CHIKV	38.5	Encephalitis/Meningitis
CHRF0099	PCR (+)	f/11	31-May-17	20 (20)/30	4/Dis	CHIKV	35.0	AFC/Meningitis
CHRF0100	PCR (+)	m/22	14-Jun-17	60 (25)/70	2/Dis	CHIKV	37.7	AFC/AFC
CHRF0101	PCR (+)	m/4	17-Jun-17	160 (70)/110	8/Dis	CHIKV	31.3	AFC/Meningitis
CHRF0102	PCR (+)	m/3	17-Jun-17	220 (80)/250	15/Dis	CHIKV	35.4	AFC/Meningitis
CHRF0103	PCR (+)	m/93	19-Jun-17	20 (10)/40	4/Dis	CHIKV	33.7	Meningitis/AFC
CHRF0104	PCR (+)	m/11	19-Jun-17	20 (10)/30	4/Dis	CHIKV	37.9	Meningitis/AFC
CHRF0105	PCR (+)	f/14	21-Jun-17	32 (20)/35	2/LAMA	CHIKV	31.5	AFC
CHRF0106	PCR (+)	m/20	24-Jun-17	120 (90)/70	7/Dis	CHIKV	35.1	Meningitis/AFC
CHRF0107	PCR (+)	m/17	29-Jun-17	12 (10)/30	3/Dis	CHIKV	34.7	Meningitis/AFC
CHRF0108	PCR (+)	f/8	10-Jul-17	120 (30)/35	9/Dis	CHIKV	35.8	AFC/Meningitis
CHRF0109	PCR (+)	m/2	8-Jul-17	24 (70)/50	28/Dis	CHIKV	33.8	Pneumonia/Pneumonia
CHRF0110	PCR (+)	m/0	10-Jul-17	60 (90)/150	16/Dis	CHIKV	30.3	Sepsis/Meningitis

Sample ID	DSH result	Age (m)	CSF collection date	WBC/ul (PMN%)/ Protein mg/dl	Hospital duration/ outcome	Organism detected in DSH	qPCR Ct	Provisional/ Final Diagnosis
CHRF0111	PCR (+)	m/8	18-Jul-17	42 (20)/35	7/Dis	CHIKV	34.3	AFC/Meningitis
CHRF0112	PCR (+)	m/7	31-Aug-17	180 (10)/50	12/Dis	CHIKV	29.3	Meningitis/Meningitis
CHRF0113	PCR (+)	m/1	18-Sep-17	90 (70)/150	17/Dis	CHIKV	32.7	Meningitis/Meningitis
CHRF0114	Negative - Water	-	-	-	-	-	-	-

ICSOL: Intracranial; UTI: Urinary tract infection; ARI: Acute respiratory tract infection; AGN: acute glomerulonephritis; AGE: acute gastroenteritis; AFC: Acute febrile convulsion; ASS: Acute stroke syndrome; NS: Nephrotic syndrome; LAMA: Left against medical advice; NA: the data are not available or applicable

Table S4. Case-based metagenomic data derived from all sequenced samples (n=115).

Sample ID	mNGS output	Total reads	Nonhost reads	Non-host reads %	No of reads/million of the called pathogen	ERCC reads	RNA input
CHRF0052	*E. coli*	70250230	212176	0.3	465.9	41572416	17.2
CHRF0064	*S. pneumoniae*	71227742	308236	0.4	3008.2	6063522	268.7
CHRF0076	*S. pneumoniae*	63375670	98018	0.2	356.2	25658770	36.7
CHRF0088	*E. anophelis*	85955422	162734	0.2	116.5	53048838	15.5
CHRF0005	*E. hormaechei*	49785764	100862	0.2	465.9	23464986	28.0
CHRF0017	0	44873536	71250	0.2	-	25545366	18.9
CHRF0029	*E. anophelis*	54869610	373468	0.7	5758.7	46651102	4.4
CHRF0041	*K. pneumoniae*	125053116	104932	0.1	21.0	101372810	5.8
CHRF0001	0	102302082	216946	0.2	-	33395452	51.6
CHRF0013	0	12772456	93332	0.7	-	11518604	2.7
CHRF0025	*S. pneumoniae*	87017766	110194	0.1	36.0	15448776	115.8
CHRF0037	*S. pneumoniae*	95449142	9212	0.0	7.5	516	n/r
CHRF0049	*S. pneumoniae*	63460278	72664	0.1	18.8	37940022	16.8
CHRF0061	*S. pneumoniae*	102545870	74388	0.1	74.2	25325942	76.2
CHRF0073	*S. pneumoniae*	109138694	105100	0.1	554.8	6998288	364.9
CHRF0085	0	80961046	158908	0.2	-	49273376	16.1
CHRF0002	*S. pneumoniae*	141979356	2837444	2.0	19144.3	14875054	213.6
CHRF0014	*S. pneumoniae*	5138438	1520	0.0	29.7	47076	n/r
CHRF0026	0	150000000	216484	0.1	659.0	2209684	1670.2
CHRF0038	*S. pneumoniae*	131599198	57492	0.0	15.4	3102744	1035.3
CHRF0050	0	71994986	231934	0.3	-	55364152	7.5
CHRF0062	*S. pneumoniae*	150000000	47112	0.0	40.9	4619612	786.7
CHRF0074	*S. pneumoniae*	117315916	38716	0.0	5.1	1435378	2018.3
CHRF0086	*S. pneumoniae*	66783636	69142	0.1	82.0	15896928	80.0
CHRF0003	*S. pneumoniae*	56010100	88082	0.2	17.4	17328160	55.8
CHRF0015	0	45328346	208836	0.5	-	42675752	1.6
CHRF0027	*H. influenzae*	93564596	93914	0.1	56.3	43933980	28.2

Sample ID	mNGS output	Total reads	Nonhost reads	Non-host reads %	No of reads/million of the called pathogen	ERCC reads	RNA input
CHRF0039	0	88964960	47550	0.1	-	12434208	153.9
CHRF0051	*S. pneumoniae*	150000000	42228	0.0	64.5	3295142	1113.2
CHRF0063	*N. meningitidis*	83295482	338442	0.4	3.8, 3424.4	5436750	358.0
CHRF0075	*S. pneumoniae*	111766028	12132	0.0	9.3	774910	3580.8
CHRF0087	*S. pneumoniae*	69829522	30968	0.0	3.1	7217626	216.9
CHRF0004	0	60813922	237838	0.4	-	45038786	8.8
CHRF0016	0	56699154	127496	0.2	-	52922396	1.8
CHRF0028	0	39764866	166378	0.4	-	37251668	1.7
CHRF0040	0	85263302	282996	0.3	-	74509638	3.6
CHRF0053	0	46124002	83482	0.2	-	44273832	1.0
CHRF0065	0	44707958	182912	0.4	-	41350484	2.0
CHRF0077	0	75937524	102300	0.1	-	66630914	3.5
CHRF0089	0	6302354	184446	2.9	-	56770	n/r
CHRF0006	0	80267448	145860	0.2	-	77153088	1.0
CHRF0018	0	47931332	84642	0.2	-	44741152	1.8
CHRF0030	0	128845460	113374	0.1	-	120459392	1.7
CHRF0042	0	56269302	88386	0.2	-	53806038	1.1
CHRF0054	0	109272300	127002	0.1	-	103557262	1.4
CHRF0066	0	57896582	95220	0.2	-	54678742	1.5
CHRF0078	0	42345140	63466	0.1	-	39235528	2.0
CHRF0090	0	67481750	106452	0.2	-	62447376	2.0
CHRF0007	0	52481998	126918	0.2	-	49759666	1.4
CHRF0019	0	9003804	46672	0.5	-	8476152	1.6
CHRF0031	0	65032886	190336	0.3	-	62205370	1.1
CHRF0043	0	58224438	311660	0.5	-	53771664	2.1
CHRF0055	0	65419488	115050	0.2	-	61576086	1.6
CHRF0067	0	66072270	158258	0.2	-	58759328	3.1
CHRF0079	0	60221934	86664	0.1	-	52120452	3.9
CHRF0091	0	68421536	123050	0.2	-	62355960	2.4

Sample ID	mNGS output	Total reads	Nonhost reads	Non-host reads %	No of reads/million of the called pathogen	ERCC reads	RNA input
CHRF0008	0	56664264	127858	0.2	-	47826772	4.6
CHRF0020	0	75031264	545870	0.7	-	64841106	3.9
CHRF0032	0	52942812	94000	0.2	-	50417636	1.3
CHRF0044	0	74679170	126652	0.2	-	70394724	1.5
CHRF0056	0	58958602	100272	0.2	-	52111098	3.3
CHRF0068	0	58263836	81770	0.1	-	53534916	2.2
CHRF0080	0	58480150	77640	0.1	-	48353780	5.2
CHRF0092	0	51999596	83734	0.2	-	46960542	2.7
CHRF0009	0	42981288	122776	0.3	-	39365830	2.3
CHRF0021	0	38396862	145968	0.4	-	35594906	2.0
CHRF0033	0	50925620	68302	0.1	-	45749064	2.8
CHRF0045	0	50455298	80706	0.2	-	48657850	0.9
CHRF0057	0	50024148	95218	0.2	-	46905102	1.7
CHRF0069	0	45846480	59750	0.1	-	42479872	2.0
CHRF0096	0	49342992	69600	0.1	-	46646414	1.4
CHRF0000	0	135087088	156310	0.1	-	130150782	0.9
CHRF0081	0	64453194	38766	0.1	-	31709728	25.8
CHRF0093	0	49670048	99216	0.2	-	43264818	3.7
CHRF0010	*Enterovirus B*	61327650	70042	0.1	9.3	22230004	44.0
CHRF0022	0	118348946	181994	0.2	-	70802894	16.8
CHRF0034	0	60169132	112136	0.2	-	13310622	88.0
CHRF0046	0	42195836	158576	0.4	-	25483100	16.4
CHRF0058	*M. tuberculosis*	65072802	34314	0.1	9.5	3104784	499.0
CHRF0070	*B. cereus*	69060574	98978	0.1	247.4	5797930	272.8
CHRF0082	*S. enterica*	60637346	98414	0.2	97.8	19935498	51.0
CHRF0094	CHIKV	61336096	412890	0.7	5495.9	28094424	29.6
CHRF0011	Mumps	60553510	25850	0.0	0.7	7677666	172.2
CHRF0023	0	53928028	285306	0.5	-	38094584	10.4

Sample ID	mNGS output	Total reads	Nonhost reads	Non-host reads %	No of reads/million of the called pathogen	ERCC reads	RNA input
CHRF0035	Human herpes virus 6 (manual)	96343858	16208	0.0	-	1643200	1440.8
CHRF0047	0	57593626	54800	0.1	-	6349378	201.8
CHRF0059	*S. maltophilia*	85986872	1362544	1.6	3240.9	38318362	31.1
CHRF0071	CHIKV	58234020	85112	0.1	242.8	23316766	37.4
CHRF0083	0	139233556	48796	0.0	-	286176	n/r
CHRF0095	0	61324940	84592	0.1	-	19572240	53.3
CHRF0012	CHIKV	8760674	56784	0.6	1830.1	3462238	38.3
CHRF0024	0	98585738	86354	0.1		20945142	92.7
CHRF0036	Mumps (manual)	72872708	62864	0.1	0 rpm, 2 total reads	8675260	185.0
CHRF0048	0	77389688	62590	0.1	-	58257176	8.2
CHRF0060	0	81192516	193074	0.2	-	70471964	3.8
CHRF0072	0	150000000	89218	0.1	-	31927140	92.1
CHRF0084	0	59849024	57728	0.1	-	49233396	5.4
CHRF0097	CHIKV	150000000	120342	0.1	24.9	130431532	3.8
CHRF0098	CHIKV	150000000	187842	0.1	20.9	141728870	1.5
CHRF0099	CHIKV	150000000	414592	0.3	6423.7	136277954	2.5
CHRF0100	CHIKV	150000000	311012	0.2	8.0	95075520	14.4
CHRF0101	CHIKV	150000000	1632694	1.1	20200.8	96521140	13.9
CHRF0102	CHIKV	150000000	436436	0.3	111.5	113727008	8.0
CHRF0103	CHIKV	150000000	381190	0.3	3254.2	121280236	5.9
CHRF0104	CHIKV	150000000	371278	0.2	106.0	116330080	7.2
CHRF0105	CHIKV	150000000	228930	0.2	881.3	105689416	10.5
CHRF0106	CHIKV	150000000	359446	0.2	4169.4	117445236	6.9
CHRF0107	CHIKV	150000000	171530	0.1	966.1	138854562	2.0
CHRF0108	CHIKV	150000000	241328	0.2	248.9	131571546	3. 5
CHRF0109	CHIKV	150000000	157336	0.1	565.6	138399578	2.09
CHRF0110	CHIKV	150000000	6795359	4.5	37386.1	16650300	200.3
CHRF0111	CHIKV	150000000	223136	0.1	1065.6	136500544	2.5

Sample ID	mNGS output	Total reads	Nonhost reads	Non-host reads %	No of reads/million of the called pathogen	ERCC reads	RNA input
CHRF0112	CHIKV	150000000	25772	0.0	3.0	319182	n/r
CHRF0113	CHIKV	150000000	1357528	0.9	34.8	72993550	26.7
CHRF0114	0	150000000	231476	0.2	-	139254112	1.9

n/r: the RNA input could not be back-calculated from ERCC counts.

Table S5. **List of microbes included in the logistic regression model as potential pathogens.**

Bacteria	**Viruses**
Escherichia coli	Enterovirus
Shigella spp	Adenovirus
Ureaplasma spp	Influenza A
Streptococcus pyogenes	Influenza B
Salmonella spp	Parainfluenza-1
Pseudmonas aeruginosa	Parainfluenza-2
Staphylococcus aureus	Parainfluenza-3
Streptococcus pneumoniae	Respiratory syncytial virus
Streptococcus agalactiae	Parechovirus
Klebsiella spp	Enterovirus
Haemophilus influenzae	Human metapneumovirus
Neisseria meningitidis	Rubulavirus
Mycoplasma pneumoniae	Rhinovirus
Chlamydia trachomatis	Cytomegalovirus
Chlamydophila pneumoniae	Mumps virus
Bordetella pertussis	Chikungunya virus
Stenotrophomonas spp	Enterobacter
Acinetobacter spp	Alphavirus
Mycobacterium tuberculosis	Human herpesvirus
Bacillus cereus	
Elizabethkingia spp	