Etiological and epidemiological features of acute meningitis or encephalitis in China: a nationwide active surveillance study

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Summary

Background Acute meningitis or encephalitis (AME) results from a neurological infection causing high case fatality and severe sequelae. AME lacked comprehensive surveillance in China.

Methods Nation-wide surveillance of all-age patients with AME syndromes was conducted in 144 sentinel hospitals of 29 provinces in China. Eleven AME-causative viral and bacterial pathogens were tested with multiple diagnostic methods.

Findings Between 2009 and 2018, 20,454 AME patients were recruited for tests. Based on 9,079 patients with all-four-virus tested, 28.43% (95% CI: 27.50%–29.36%) of them had at least one virus-positive detection. Enterovirus was the most frequently determined virus in children <18 years, herpes simplex virus and Japanese encephalitis virus were the most frequently determined in 18−59 and ≥60 years age groups, respectively. Based on 6,802 patients with all-seven-bacteria tested, 4.43% (95% CI: 3.94%–4.91%) had at least one bacteria-positive detection, *Streptococcus pneumoniae* and *Neisseria meningitidis* were the leading bacterium in children aged <5 years and 5−17 years, respectively. *Staphylococcus aureus* was the most frequently detected in adults aged 18−59 and ≥60 years. The pathogen spectrum also differed statistically significantly between northern and southern China. Joinpoint analysis revealed age-specific positive rates, with enterovirus, herpes simplex virus and mumps virus peaking at 3−6 years old, while Japanese encephalitis virus peaked in the ≥60 years old. As age increased, the positive rate for

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Streptococcus pneumoniae and *Escherichia coli* statistically significantly decreased, while for *Staphylococcus aureus* and *Streptococcus suis* it increased.

Interpretation The current findings allow enhanced identification of the predominant AME-related pathogen candidates for diagnosis in clinical practice and more targeted application of prevention and control measures in China, and a possible reassessment of vaccination strategy.

Research in context

Evidence before this study

In Aug 2021, we did a search of PubMed for papers reporting acute meningitis or encephalitis (AME) from Jan 1, 2009 to Aug 5, 2021, using search terms "viral/virus/viruses" or "bacterial/bacteria/bacterium" and "meningitis" or "encephalitis" and "China", with no data or language restrictions. Among the 463 papers from the preliminary screening, 27 papers have reported the etiological detection with or without the clinical and epidemiological investigation of AME in China during 2009–2021. Thirteen papers reported single pathogen detection including five papers on Japanese encephalitis virus (JEV), four on enterovirus (EV), one on tick-borne encephalitis virus, one on cytomegalovirus, one on *Neisseria meningitidis* (*N. meningitidis*), and one on *Klebsiella pneumoniae*. Fourteen papers reported multiple AME pathogens detection, including three papers focusing on viral infections, six papers only investigating bacterial infections and five papers investigating both viral and bacterial pathogens. No studies were performed on a nationwide scale and over a long time.

Added value of this study

To the best of our knowledge, this study provided an unprecedented and comprehensive analysis of the etiological and epidemiological features of patients with AME in the mainland of China. Based on the detection of 11 commonly seen pathogens on 20,454 all-age patients that were recruited from 144 sentinel hospitals in 29 provinces of the Chinese mainland from 2009 to 2018, the prevalence and pathogen spectrum of AME patients were disclosed for the first time. The important findings included:

1. The viral detection rates of EV>JEV>HSV>MuV and bacterial detection rates of *N. meningitidis*>*S. pneumoniae*>*S. aureus*>*E. coli*>*S. suis*>Hib>*M. tuberculosis* were revealed respectively.

2. The pathogen spectrum differed among age groups, with children aged 5–17 years old having the highest viral detection rate with EV as the leading pathogen. The children aged <5 years old had the highest bacterial detection rate, with *S. pneumoniae* as the leading pathogen. The joinpoint regressions demonstrated different turnaround points at specific age or age range that marked increased or decreased positive rate for each of the detected pathogens.

Implications of all the available evidence

Our findings fill crucial gaps of how the distributions of AME-related pathogens change across China in AME patients. This allows enhanced identification of the predominant AME-related pathogen candidates for diagnosis in clinical practice and more targeted application of prevention and control measures in China, as well as, a possible reassessment of vaccination strategy.

Background

Infection of the central nervous system, often presenting as acute meningitis or encephalitis (AME), is associated with substantial mortality and long-term neurological sequelae worldwide.[1−3] A wide variety of infectious agents, including viruses, bacteria, fungi and parasites are causative of AME.[1] The incidence of infectious encephalitis is estimated at 1.5−7 cases/100,000 inhabitants/year, with the highest incidence reported in children.[2,4] Over one hundred viral and bacterial species have been shown to cause AME,[5] and with viral pathogens composing the most part of them.[6,7] For example, in the USA (1993–2008), 31% of visits in emergency department admissions for all-cause meningitis were viral meningitis.[8] Furthermore, in England and Wales, the reporting number of viral meningitis or encephalitis increased seven times from 2004 to 2013.[9] The studies usually focused on the viral infections in AME patients, while few studies reported both viral and bacterial etiologies of them in China.[10,11]

In China, high case fatality and severe sequelae are associated with AME.[12−14] A nationwide vaccination

program that included vaccines against serogroup A and serogroup A&C meningococcal, conjugate vaccines against *Haemophilus influenzae type b* (Hib) and Japanese encephalitis virus (JEV), resulted in pronounced declines in the invasive diseases caused by these pathogens across all age groups.[15−17] It is not mandatory to notify the public health authorities about infectious encephalitis. *Neisseria meningitidis* (*N. meningitidis*) and JEV are the only two causative agents of AME included in the National Infectious Diseases Surveillance System. In recent years, an increasing body of work has shown a wider range of other common pathogens in encephalitis patients in China, resulting in a much larger list of potential causative pathogens. For example, in Guangxi, enterovirus (EV) was the most frequently detected pathogen, accounting for more than 31.5% of all confirmed viral meningitis cases.[18] Moreover, in a study by Li et al., *Streptococcus pneumoniae* (*S. pneumoniae*) accounted for 46.9% of bacterial meningitis cases in children in China during 2014–2016, substantially more than the rate derived from previous studies in other countries.[19−21] Although interesting and suggestive, these studies were usually limited by small specimen sizes, limited coverage of geographic range and population types, short surveillance duration and restricted testing of pathogens, making it difficult to generalize to the overall burden of meningitis/encephalitis. Here we present the results of a nationwide surveillance for patients with AME that overcomes many of these limitations and identify the etiological and epidemiological features of AME in all-age population for a decade in China.

Methods

Hospital based surveillance network
Between 2009 and 2018, an active surveillance was conducted on patients with AME at national level in China. The surveillance was administered under the tertiary network system, managed by Chinese Center for Disease Control and Prevention (China CDC). The number of sentinel hospitals was determined in proportion to the total population size at the scale of ecological region, and the surveillance hospitals were chosen after full consideration of the surveillance and laboratory testing capacities and representativeness of their geographical locations (Supplementary Figure 1). In all participating hospitals, a standard operating procedure (SOP) was applied to recruit and test AME patients, that regulated and standardized patient enrollment, specimen collection and storage, laboratory testing and data collection.[22] Pre-study training, whole-procedure supervision, monthly enrollment reports, data audits, and annual study-site visits were also conducted to ensure uniform procedures were employed as guided among the study sites and across the years of the study. The SOP was approved by the institutional review board at each institution and at the CDC (2015-025) and remained unchanged across the reported surveillance period.

Patients' enrollment, specimen collection and laboratory procedures
Case definitions of AME were created prior to study initiation and clinically based: having acute onset of fever, headache or vomiting, accompanied with at least one of neural paralysis, disturbance of consciousness, meningeal irritation sign or changes in mental status.[22] In order to exclude nosocomial infection, patients who developed AME-like symptoms after hospitalization were not enrolled in this study. For patients who met the inclusion criteria and who were willing to participate in the study, samples were collected on admission and before therapy was administered. The samples were required to be tested within 24 hours after collection, and if this was not possible, were stored at -70°C until tested. The sample size was determined by the time window and presenting population.

Cerebrospinal fluid (CSF) and blood samples were collected for detection of four viruses, including EV, JEV, mumps virus (MuV), herpes simplex virus (HSV). Throat swabs were additionally collected from patients who were suspected with viral infection for the test of EV, MuV and HSV, while stool samples were additionally collected for the test of EV from patients who were suspected to have hand, foot, and mouth disease (Supplementary Figure 2). For the laboratory test for viruses, polymerase chain reaction (PCR) or reverse transcription-polymerase chain reaction (RT-PCR) or enzyme-linked immunosorbent assay (ELISA) was performed (Supplementary Table 1). Bacterial culture mainly included culture of samples collected from sterile sites, such as CSF and whole blood, for the isolation of bacteria, including *N. meningitidis, S. pneumoniae, Staphylococcus aureus* (*S. aureus*), Hib, *Streptococcus suis* (*S. suis*), *Mycobacterium tuberculosis* (*M. tuberculosis*) and *Escherichia coli* (*E. coli*). For isolated bacteria, morphological identification according to the colony morphology on different media and gram stain microscopic examination were conducted. After morphological identification, biochemical assays specific to different bacteria were conducted. Latex agglutination, PCR and real-time PCR were used to detect negative bacterial culture specimens (Supplementary Tables 2 and 3). All steps of the nucleic acid extraction and PCR/RT-PCR/real-time PCR test were conducted in parallel with positive and negative controls. If any targeted pathogen was detected in any of the specimens, the patient was considered as pathogen-positive (detailed in supplemental material and methods).

Data management and statistical analysis
Data on individual demographic (age, sex), case type (inpatient or outpatient), location, clinical data and

medication use were collected by reviewing medical records of the patients. All data were entered into a standardized database by trained clinicians and uploaded to the online management system generated by the China CDC. Data collection was considered as public health surveillance by the National Health Commission of the People's Republic of China and verbal informed consent was obtained from patients or their legal guardians. The project and the above procedure for obtaining consent were approved by the ethical review committee of China CDC. The location of southern or northern China was defined according to latitude (Supplementary Figure 1).

Data were sorted to remove the redundant and the incomplete before analysis. Descriptive statistics were performed for categorical variables to estimate frequency, proportion and rate, for continuous variables to estimate medians and interquartile ranges (IQR). The nonparametric test with Mann-Whitney U test method was used to perform two-group comparison for continuous variables. Pearson chi-square test or Fisher exact test was applied to perform comparison for categorical variables. No allowance for multiplicity was made in the analyses. The Cochran-Armitage trend test was used for the trend analysis.[23] Joinpoint regression analysis was used to identify the best-fitting points where a statistically significant change in the log-linear slope of trend occurred.[24] The associations between positive detection rate and meteorological and socioeconomic factors were explored by using a negative binomial regression at the city level (detailed in the supplementary appendix). Statistical analysis was performed using R version 3.6.3 (R Foundation for Statistical Computing, Vienna, Austria), ArcGIS version 10.5 (ESRI, ArcGIS, Redlands, CA, USA) and Join-Point Trend Analysis Software version 4.7.0.0 (Statistical Research and Applications Branch, National Cancer Institute, USA). A two-sided P value of <0.05 was statistically significant.

Role of the funding source
The funders for this study had no role in study design, data collection, data analysis, data interpretation, writing of this report, or decision to submit this paper for publication. The corresponding author had full access to all the data in the study and had final responsibility for the decision to submit for publication.

Results
From January 2009 to December 2018, a total of 24,679 patients with clinical diagnosis of AME were enrolled from 144 sentinel hospitals in 29 provinces of the mainland of China (Supplementary Figure 1). Among those patients, 4,225 were excluded due to incomplete data (Supplementary Table 4), and the remaining 20,454 patients were included for further analysis (Figure 1). Among those recruited, 12,956 (63.34%) were male,

7,666 (37.48%) were children <5 years old, 6,506 (31.81%) cases were 5–17 years old, 5,123 (25.05%) were 18–59 years old and 1,159 (5.67%) were ≥60 years old. The median interval from disease onset to clinic visit was 3 (IQR: 1–6) days. The majority of the recruits were hospitalized patients (81.79%) (Table 1). A higher proportion of patients (60.23%) were recruited from sentinel hospitals in southern China. There were 15,101 patients tested for at least one virus, among them 9,079 patients were tested for all four viruses. There were 9,115 patients who were tested for at least one bacterium, and 6,802 patients were tested for all seven bacteria. There were 1,951 patients who were tested for all 11 pathogens (Figure 1).

The virus spectrum based on patients with all-four-virus test
Overall, 28.43% (95% CI: 27.50%–29.36%) of the patients had at least one virus-positive detection. EV was the most common virus, accounting for 48.89% of all positive test (1,504/3,076), followed by HSV (27.34%), MuV (12.97%) and JEV (10.79%) (Supplementary Figure 3a, Table 2). EV was the most commonly identified virus in the CSF samples as well (Supplementary Figure 4a). The positive rate and pathogen spectrum differed by age. The patients aged 5–17 years old and children <5 years old had comparable positive rates (36.96% [95% CI: 35.25%–38.67%] and 30.57% [95% CI: 29.04%–32.11%]) and the same viral ranking EV>HSV>MuV>JEV. Lower positive rates were obtained from both adult groups aged 18–59 years old (15.20% [95% CI: 13.66%–16.73%]) and ≥60 years old (15.56% [95% CI: 12.21%–18.90%]). These groups also had a different viral ranking, i.e., HSV>EV>JEV>MuV for patients aged 18–59 years old, while JEV>HSV>EV>MuV for patients aged ≥60 years old. Geographical differences were observed, with positive rate from northern China higher than southern China (37.75% vs. 20.51%, rate difference 17.23%, 95% CI: 15.38%–19.09%). In both regions, EV was the top-ranking virus, although JEV attained a higher overall ranking in southern China. The overall positive rates were comparable between female and male (28.65% vs. 28.30%, rate difference 0.36%, 95% CI: -1.57%–2.28%) and case types (28.53% in inpatients and 28.01% in outpatients, rate difference 0.52%, 95% CI: -1.84%–2.87%).

The bacterial spectrum based on patients with all-seven-bacteria test
Overall, 4.43% (95% CI: 3.94%–4.91%) of the patients had at least one bacteria-positive detection. *S. pneumoniae* was the most common bacterium, accounting for 27.33% of all the bacterial-positively patients (85/311), followed by *S. aureus* (24.76%), *N. meningitidis* (21.19%), and *E. coli* (16.72%) (Supplementary Figure 3b, Table 2). The same ranking was also observed when the test results from CSF samples were used
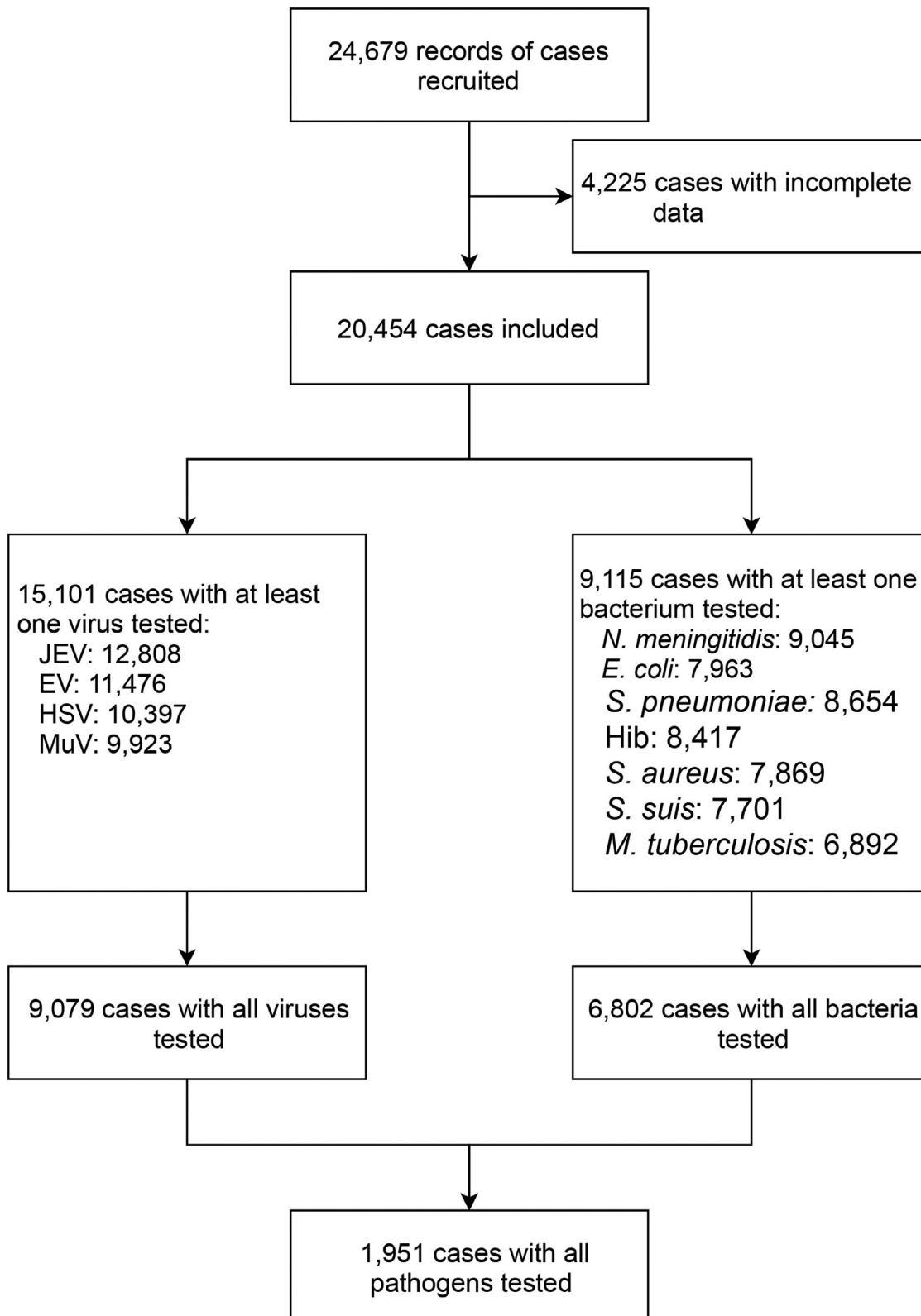
Figure 1. The flowchart of patient recruit and sorting procedures. This flow diagram summarizes the number of AME patients subject to each analysis in this study.

Characteristics	All patients (N=20,454)	At least one virus tested (n=15,101)	At least one bacterium tested (n=9,115)	All viruses tested (n=9,079)	All bacteria tested (n=6,802)	All pathogens tested (n=1,951)
Sex, male, n (%)	12,956 (63.34)	9,493 (62.86)	5,793 (63.55)	5,704 (62.83)	4,369 (64.23)	1,229 (62.99)
Age, year, median (IQR)	7 (3-26)	6 (3-18)	15 (4-42)	6 (3-23)	17 (3-43)	25 (6-48)
Age group, n (%)						
Children (<5 years)	7,666 (37.48)	5,820 (38.54)	2,593 (28.45)	3,467 (38.19)	2,003 (29.45)	373 (19.12)
<6 months	1,122 (5.49)	559 (3.70)	713 (7.82)	363 (4.00)	567 (8.34)	79 (4.05)
6–12 months	736 (3.60)	507 (3.36)	316 (3.47)	333 (3.67)	251 (3.69)	43 (2.20)
1– years	1,855 (9.07)	1,460 (9.67)	544 (5.97)	853 (9.40)	426 (6.26)	75 (3.84)
2– years	1,395 (6.82)	1,122 (7.43)	387 (4.25)	624 (6.87)	295 (4.34)	51 (2.61)
3– years	1,307 (6.39)	1,103 (7.30)	310 (3.40)	648 (7.14)	235 (3.45)	62 (3.18)
4– years	1,251 (6.12)	1,069 (7.08)	323 (3.54)	646 (7.12)	229 (3.37)	63 (3.23)
5–17 years old	6,506 (31.81)	5,445 (36.06)	2,158 (23.68)	3,063 (33.74)	1,459 (21.45)	478 (24.50)
18–59 years old	5,123 (25.05)	3,077 (20.38)	3,598 (39.47)	2,099 (23.12)	2,751 (40.44)	870 (44.59)
≥60 years old	1,159 (5.67)	759 (5.03)	766 (8.40)	450 (4.96)	589 (8.66)	230 (11.79)
Days from disease onset to clinic visit, median (IQR)	3 (1-6)	3 (1-6)	2 (0-6)	3 (1-6)	2 (0-6)	3 (1-6)
Case type, n (%)						
Inpatient	16,729 (81.79)	12,286 (81.36)	7,376 (80.92)	7,344 (80.89)	5,399 (79.37)	1,406 (72.07)
Outpatient	3,725 (18.21)	2,815 (18.64)	1,739 (19.08)	1,735 (19.11)	1,403 (20.63)	545 (27.93)
Surveillance location, n (%)						
Northern China	8,134 (39.77)	7,178 (47.53)	2,554 (28.02)	4,170 (45.93)	1,524 (22.41)	563 (28.86)
Southern China	12,320 (60.23)	7,923 (52.47)	6,561 (71.98)	4,909 (54.07)	5,278 (77.59)	1,388 (71.14)

Table 1: **Demographic and epidemiological characteristics of patients with acute meningitis and encephalitis in China during 2009–2018.**
Data are n (percentage %) unless otherwise indicated. Percentages may not total 100 because of rounding. IQR, interquartile range. Provinces located in southern and northern China were defined according to latitude.

	Virus					Bacterium							
	Positive rate	Proportion (Total positive=3,076)				Positive rate	Proportion (Total positive=311)						
		EV	HSV	MuV	JEV		S. pneumoniae	S. aureus	N. meningitidis	E. coli	S. suis	Hib	M. tuberculosis
Overall cases	2,581 (28.43)	1,504 (48.89)	841 (27.34)	399 (12.97)	332 (10.79)	301 (4.43)	85 (27.33)	77 (24.76)	69 (22.19)	52 (16.72)	18 (5.79)	7 (2.25)	3 (0.96)
Sex													
Female	967 (28.65)	537 (46.41)	350 (30.25)	141 (12.19)	129 (11.15)	85 (3.49)	26 (29.89)	21 (24.14)	20 (22.99)	16 (18.39)	2 (2.30)	1 (1.15)	1 (1.15)
Male	1,614 (28.30)	967 (50.39)	491 (25.59)	258 (13.44)	203 (10.58)	216 (4.94)	59 (26.34)	56 (25.00)	49 (21.88)	36 (16.07)	16 (7.14)	6 (2.68)	2 (0.89)
P value	0.072	0.027				0.005	0.697						
Age													
<5 years old	1,060 (30.57)	719 (56.48)	321 (25.22)	136 (10.68)	97 (7.62)	99 (4.94)	49 (48.51)	8 (7.92)	14 (13.86)	23 (22.77)	0 (0)	6 (5.94)	1 (0.99)
5-17 years old	1,132 (36.96)	687 (50.78)	351 (25.94)	189 (13.97)	126 (9.31)	64 (4.39)	12 (17.39)	13 (18.84)	30 (43.48)	14 (20.29)	0 (0)	0 (0)	0 (0)
18-59 years old	319 (15.20)	86 (23.06)	147 (39.41)	62 (16.62)	78 (20.91)	110 (4.00)	21 (18.58)	47 (41.59)	17 (15.04)	13 (11.50)	12 (10.62)	1 (0.88)	2 (1.77)
≥60 years old	70 (15.56)	12 (15.58)	22 (28.57)	12 (15.58)	31 (40.26)	28 (4.75)	3 (10.71)	9 (32.14)	8 (28.57)	2 (7.14)	6 (21.43)	0 (0)	0 (0)
P value	<0.001	<0.001				0.456	<0.001						
Case type													
Inpatient	2,095 (28.53)	1,272 (50.70)	708 (28.22)	295 (11.76)	234 (9.33)	244 (4.52)	79 (31.10)	75 (29.53)	22 (8.66)	50 (19.69)	18 (7.09)	7 (2.76)	3 (1.18)
Outpatient	486 (28.01)	232 (40.92)	133 (23.46)	104 (18.34)	98 (17.28)	57 (4.06)	6 (10.53)	2 (3.51)	47 (82.46)	2 (3.51)	0 (0)	0 (0)	0 (0)
P value	0.669	<0.001				0.459	<0.001						
Geographic location													
Northern	1,574 (37.75)	875 (43.77)	651 (32.57)	346 (17.31)	127 (6.35)	124 (8.14)	52 (39.69)	6 (4.58)	36 (27.48)	29 (22.14)	0 (0)	6 (4.58)	2 (1.53)
Southern	1,007 (20.51)	629 (58.40)	190 (17.64)	53 (4.92)	205 (19.03)	177 (3.35)	33 (18.33)	71 (39.44)	33 (18.33)	23 (12.78)	18 (10.00)	1 (0.56)	1 (0.56)
P value	<0.001	<0.001				<0.001	<0.001						

Table 2: **Pathogen spectrum of patients with acute meningitis or encephalitis in China during 2009–2018.**

The viral positive rate was estimated based on 9,079 cases with all four viruses tested and the bacterial positive rate was estimated based on 6,802 cases with all seven bacteria tested. Pathogen spectrum was presented as Number of positive test (proportion of positive %) unless otherwise indicated. The total positive counts for viruses and bacteria were 3,076 and 311, which were larger than the number of positive patients because of the coinfection. Proportions may not total 100 because of rounding. Chi-square test or Fisher's exact test were used for comparisons among different groups. EV, enterovirus; JEV, Japanese encephalitis virus; HSV, herpes simplex virus; MuV, mumps virus; *N. meningitidis, Neisseria meningitidis; S. pneumoniae, Streptococcus pneumoniae; S. aureus, Staphylococcus aureus;* Hib, *Haemophilus influenzae type b; S. suis, Streptococcus suis; M. tuberculosis, Mycobacterium tuberculosis; E. coli, Escherichia coli.*

Characteristics	EV	JEV	HSV	MuV
Overall cases	18.44 (2116/11,476)	11.02 (1,412/12,808)	9.79 (1,018/1,0397)	6.25 (620/9,923)
Sex				
Male	18.72 (1,353/7,228)	10.40 (831/7,991)	9.14 (596/6,519)	6.84 (428/6,259)
Female	17.96 (763/4,248)	12.06 (581/4,817)	10.88 (422/3,878)	5.24 (192/3,664)
Rate difference (95% CI)	0.76 (-0.71, 2.22)	-1.66 (-2.80, -0.52)	-1.74 (-2.94, -0.54)	1.60 (0.64, 2.55)
Age				
Children (0–5 years)	23.66 (1,110/4,692)	8.97 (411/4,580)	9.86 (384/3,894)	5.17 (193/3,734)
<6 months	10.49 (49/467)	5.58 (26/466)	7.21 (30/416)	3.10 (12/387)
6–12 months	13.95 (60/430)	6.19 (26/420)	5.66 (21/371)	1.42 (5/353)
1– years	21.65 (265/1,224)	6.76 (75/1,109)	6.86 (65/947)	3.08 (28/909)
2– years	27.79 (259/932)	9.37 (77/822)	10.26 (72/702)	4.28 (29/677)
3– years	31.26 (267/854)	13.20 (116/879)	14.43 (103/714)	8.25 (58/703)
4– years	26.75 (210/785)	10.29 (91/884)	12.50 (93/744)	8.65 (61/705)
P value*	<0.001	<0.001	<0.001	<0.001
5–17 years old	23.29 (885/3,800)	12.21 (555/4,547)	11.49 (418/3,637)	10.16 (348/3,425)
18–59 years old	4.16 (102/2,454)	9.69 (286/2,950)	7.66 (181/2,363)	2.94 (67/2,278)
≥60 years old	3.58 (19/530)	21.89 (160/731)	6.96 (35/503)	2.47 (12/486)
P value#	<0.001	<0.001	0.002	<0.001
Case type				
Inpatient	18.86 (1,736/9,206)	10.63 (1,115/10,492)	10.25 (877/8,558)	5.90 (477/8,085)
Outpatient	16.74 (380/2,270)	12.82 (297/2,316)	7.67 (141/1,839)	7.78 (143/1,838)
Rate difference (95% CI)	2.12 (0.39, 3.85)	-2.20 (-3.68, -0.71)	2.58 (1.21, 3.96)	-1.88 (-3.21, -0.55)
Geographic location				
Northern	24.10 (1,328/5,511)	11.25 (654/5,812)	13.48 (698/5,179)	8.79 (381/4,334)
Southern	13.21 (788/5,965)	10.83 (758/6,996)	6.13 (320/5,218)	4.28 (239/5,589)
Rate difference (95% CI)	10.89 (9.47, 12.31)	0.42 (-0.67, 1.51)	7.34 (6.21, 8.48)	4.51 (3.52, 5.51)

Table 3: **Positive detection rate of viral or bacterial pathogen for patients with acute meningitis or encephalitis in China during 2009–2018.** The data were presented as positive rate (positive number/tested number) unless otherwise indicated. * indicates comparison among six age groups of children by performing Cochran-Armitage trend test. # indicates comparison among children <5 years old, patients aged 5–17 years old, 18–59 years old and ≥60 years old by performing Cochran-Armitage trend test. JEV, Japanese encephalitis virus; EV, Enterovirus; MuV, Mumps virus; HSV, Herpes simplex virus.

(Supplementary Figure 4b). The positive rate was comparable across four age groups, while the bacteria ranking differed. For children <5 years old, the top three bacteria were *S. pneumonia*>*E. coli*>*N. meningitidis*, which were switched to *N. meningitidis*>*E. coli*>*S. aureus* in patients aged 5–17 years old. In a more divergent pattern still, both adult groups had *S. aureus* up ranked to the first place, followed by *S. pneumonia* and *N. meningitidis* in 18–59 years old, *N. meningitidis* and *S. suis* in ≥60 years old, respectively. Geographical differences were observed, showing higher positive rate from northern than from southern China (8.14% vs. 3.35%, rate difference 4.78%, 95% CI: 3.33%–6.24%). *S. pneumoniae* was the top listing bacteria in northern China, while *S. aureus* was the highest ranking in southern China. *S. pneumonia* and *S. aureus* were more frequently seen among inpatients, while *N. meningitidis* was more frequently seen among outpatients (P<0.001) (Supplementary Figure 3b).

Age and sex specific pattern of viral pathogen

The age pattern of positive detection for the four viruses differed. EV, HSV and MuV were more frequently identified from children <5 years old and 5–17 years old, while JEV had the highest positive rate in the ≥60 years old (Supplementary Figure 5a, Table 3). By applying joinpoint model, EV had shown three descending turnpoints at three, 11 and 16 years old, with the greatest drop of positive rate seen at 11 years old (APC=-18.74 [95% CI: -29.2–-6.8]). Both HSV and MuV had shown one descending turnpoint at four and six years old, respectively (Figure 2). For JEV, a descending turnpoint at seven years old (APC=-4.55 [95% CI: -6.7–-2.4]) and an ascending turnpoint at 25 years (APC=3.17 [95% CI: 2.5–3.8]) were observed, leading to a different age pattern from those of the other three viruses. The positive rates also differed between sex in an age-dependent manner. For example, JEV was detected more often in females (Supplementary Figure 5a), but only among the ≥60 years old. HSV was more often detected in females, but only among the children <5 years and adults aged 18–59 years old (Table 4). While MuV was statistically significantly more frequent in males, which was only among patients aged 5–17 years old. Moreover, higher positive rates for EV and HSV were observed among inpatients, and with a reversed manner for JEV
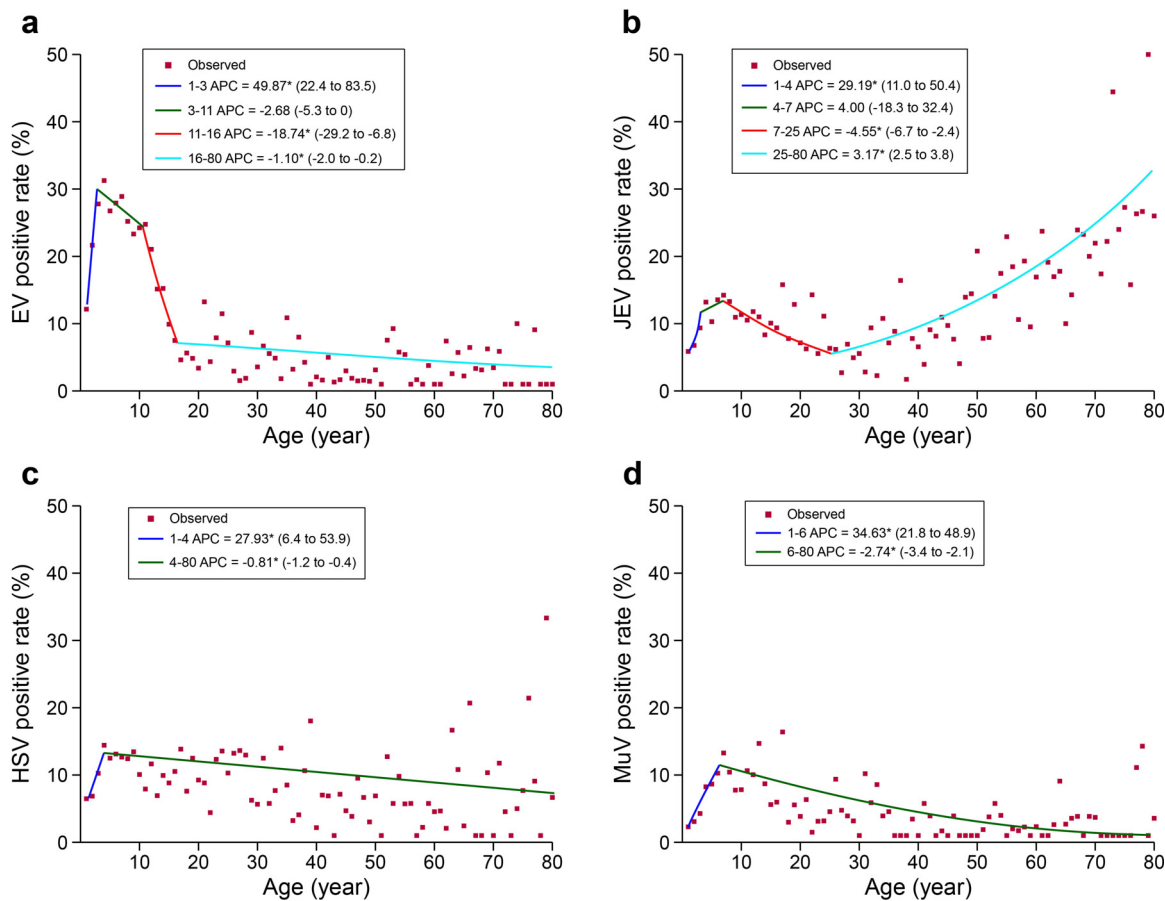
Figure 2. Joinpoint regression analysis of the positive rates by age of the patient. (a) EV; (b) JEV; (c) HSV; (d) MuV. EV, Enterovirus; JEV, Japanese encephalitis virus; HSV, Herpes simplex virus; MuV, Mumps virus. Red points indicate the mean positive rate of patients in terms of age and the colored curves indicate fitted patterns by age groups. The annual percent change (APC) value of each fitted curves is given for each virus. *indicates that the APC is statistically significantly different from zero at P<0.05.

and MuV. Higher rates of EV (24.10% vs. 13.21%, rate difference 10.89%, 95% CI: 9.47%–12.31%), HSV (13.48% vs. 6.13%, rate difference 7.34%, 95% CI: 6.21%–8.48%) and MuV (8.79% vs. 4.28%, rate difference 4.51%, 95% CI: 3.52%–5.51%) were observed in patients from northern China than patients from southern China (Table 3). EV had the highest positive rate in the samples of CSF and throat swabs, and HSV was more frequently identified from blood samples (Supplementary Table 5).

Age and sex specific pattern of bacterial pathogen
The age pattern of positive detection for the seven bacteria differed. The positive rates for *S. pneumoniae* and *E. coli* statistically significantly decreased with age (Chi-square for trend, P<0.001 and P=0.003; Supplementary Figure 5b, Table 5), while increased positive rates were observed for *S. aureus* and *S. suis* (Chi-square for trend, both P<0.001). Notably, the *N. meningitidis* was

detected with a remarkably higher rate in patients aged 5–17 years old. The positive rates for *S. pneumonia, S. aureus, E. coli* and *S. suis* were higher in inpatients than in outpatients, while for *N. meningitidis* and Hib, the positive rates were higher in outpatients. Geographic differences were observed for *S. pneumoniae, N. meningitidis, E. coli* and Hib, which were more frequently identified among patients in northern China than in southern China (Table 5). *S. pneumoniae* had the highest positive rate in the CSF specimens, and *N. meningitidis* was more frequently identified from blood specimens (Supplementary Table 5).

Temporal and spatial pattern
Joinpoint analysis along the study years revealed decreased positive rate of JEV during 2009–2016 (APC=-19.40 [95% CI: -29.5–-7.9]), but a statistically significant increase in positive rate of HSV was found during 2009–2014 (APC=22.87 [95% CI: 2.5–47.3],

	<5 years old			5–17 years old			18–59 years old			≥60 years old		
	Female	Male	Rate difference (95% CI)	Female	Male	Rate difference (95% CI)	Female	Male	Rate difference (95% CI)	Female	Male	Rate difference (95% CI)
EV	23.86	23.53	0.33	22.52	23.69	-1.17	4.51	3.92	0.59	2.40	4.35	-1.94
	(424/1,777)	(686/2,915)	(-2.18, 2.84)	(290/1,288)	(595/2,512)	(-3.99, 1.65)	(44/975)	(58/1,479)	(-1.04, 2.23)	(5/208)	(14/322)	(-4.99, 1.10)
JEV	9.28	8.79	0.49	12.37	12.12	0.25	10.50	9.14	1.36	31.37	14.43	16.94
	(161/1,735)	(250/2,845)	(-1.22, 2.21)	(193/1,560)	(362/2,987)	(-1.76, 2.26)	(126/1,200)	(160/1,750)	(-0.84, 3.56)	(101/322)	(59/409)	(10.84, 23.05)
HSV	11.27	8.98	2.29	12.35	11.05	1.30	9.42	6.51	2.91	5.58	7.84	-2.26
	(169/1,500)	(215/2,394)	(0.32, 4.25)	(154/1,247)	(264/2,390)	(-0.91, 3.52)	(88/934)	(93/1,429)	(0.65, 5.18)	(11/197)	(24/306)	(-6.66, 2.14)
MuV	4.41	5.64	-1.23	8.36	11.07	-2.72	3.44	2.61	0.83	1.07	3.34	-2.27
	(63/1,428)	(130/2,306)	(-2.65, 0.20)	(96/1,149)	(252/2,276)	(-4.77, -0.66)	(31/900)	(36/1,378)	(-0.63, 2.29)	(2/187)	(10/299)	(-4.79, 0.24)
N. meningitidis	1.33	1.87	-0.53	3.59	3.15	0.44	1.28	1.75	-0.47	1.47	1.84	-0.38
	(13/974)	(30/1,606)	(-1.51, 0.45)	(26/724)	(45/1,427)	(-1.19, 2.07)	(17/1,327)	(39/2,225)	(-1.29, 0.34)	(4/273)	(9/489)	(-2.23, 1.48)
S. pneumoniae	2.26	2.36	-0.10	0.59	1.66	-1.06	1.01	1.43	-0.42	1.53	0.63	0.90
	(21/929)	(36/1,524)	(-1.32, 1.12)	(4/674)	(22/1,328)	(-1.96, -0.16)	(13/1,291)	(31/2,170)	(-1.16, 0.32)	(4/262)	(3/476)	(-0.75, 2.54)
S. aureus	0.23	0.49	-0.26	1.48	0.51	0.98	0.88	2.14	-1.27	1.74	1.40	0.34
	(2/879)	(7/1,436)	(-0.74, 0.22)	(9/607)	(6/1,184)	(-0.07, 2.02)	(10/1,142)	(42/1,962)	(-2.10, -0.43)	(4/230)	(6/429)	(-1.68, 2.36)
E. coli	0.93	1.19	-0.26	0.97	0.67	0.30	0.17	0.64	-0.47	0	0.46	-0.46
	(8/863)	(17/1,427)	(-1.12, 0.59)	(6/620)	(8/1,203)	(-0.59, 1.20)	(2/1,163)	(13/2,019)	(-0.89, -0.05)	(0/235)	(2/433)	(-1.10, 0.18)
S. suis	0.12	0	0.12	0	0	0	0.09	0.82	-0.73	0.88	1.41	-0.53
	(1/855)	(0/1,402)	(-0.11, 0.35)	(0/583)	(0/1,143)	(0, 0)	(1/1,114)	(16/1,950)	(-1.17, -0.29)	(2/228)	(6/426)	(-2.18, 1.12)
Hib	0.22	0.47	-0.25	0	0.08	-0.08	0.16	0.19	-0.03	0	0	0
	(2/913)	(7/1,498)	(-0.71, 0.21)	(0/659)	(1/1,302)	(-0.23, 0.07)	(2/1,239)	(4/2,101)	(-0.32, 0.26)	(0/248)	(0/457)	(0, 0)
M. tuberculosis	0.13	0.08	0.05	0	0	0	0.10	0.17	-0.07	0.49	0	0.49
	(1/776)	(1/1,264)	(-0.25, 0.35)	(0/493)	(0/986)	(0, 0)	(1/1,002)	(3/1,778)	(-0.34, 0.20)	(1/204)	(0/389)	(-0.47, 1.45)

Table 4: **Positive detection rate for tested viruses and bacteria by age and sex in patients with acute meningitis or encephalitis in China during 2009–2018.**
The data were presented as positive rate (positive number/tested number) unless otherwise indicated. Chi square test or Fisher's exact test were used for comparisons between male and female in different age groups. EV, enterovirus; JEV, Japanese encephalitis virus; HSV, herpes simplex virus; MuV, mumps virus; *N. meningitidis, Neisseria meningitidis; S. pneumoniae, Streptococcus pneumoniae; S. aureus, Staphylococcus aureus;* Hib, *Haemophilus influenzae type b; S. suis, Streptococcus suis; M. tuberculosis, Mycobacterium tuberculosis; E. coli, Escherichia coli.* .

Characteristics	N. meningitidis	S. pneumoniae	S. aureus	E. coli	S. suis	Hib	M. tuberculosis
Overall cases	2.02 (183/9,045)	1.55 (134/8,654)	1.09 (86/7,869)	0.70 (56/7,963)	0.34 (26/7,701)	0.19 (16/8,417)	0.10 (7/6,892)
Sex							
Male	2.14 (123/5,747)	1.67 (92/5,498)	1.22 (61/5,011)	0.79 (40/5,082)	0.45 (22/4,921)	0.22 (12/5,358)	0.09 (4/4,417)
Female	1.82 (60/3,298)	1.33 (42/3,156)	0.87 (25/2,858)	0.56 (16/2,881)	0.14 (4/2,780)	0.13 (4/3,059)	0.12 (3/2,475)
Rate difference (95% CI)	0.32 (-0.27, 0.91)	0.34 (-0.18, 0.87)	0.34 (-0.11, 0.80)	0.23 (-0.13, 0.60)	0.30 (0.07, 0.54)	0.09 (-0.09, 0.27)	-0.03 (-0.19, 0.13)
Age							
Children (0–5 years)	1.67 (43/2,580)	2.32 (57/2,453)	0.39 (9/2,315)	1.09 (25/2,290)	0.04 (1/2,257)	0.37 (9/2,411)	0.10 (2/2,040)
<6 months	0.99 (7/709)	1.71 (12/700)	0.74 (5/672)	2.32 (15/646)	0 (0/650)	0.15 (1/689)	0.17 (1/578)
6–12 months	1.59 (5/315)	4.04 (12/297)	0.34 (1/290)	0 (0/285)	0 (0/279)	0.68 (2/294)	0 (0/257)
1– years	1.29 (7/542)	2.88 (15/520)	0.20 (1/491)	0.21 (1/481)	0 (0/477)	0.59 (3/511)	0 (0/434)
2– years	1.82 (7/385)	2.15 (8/372)	0.30 (1/333)	0.59 (2/340)	0.31 (1/325)	0.55 (2/366)	0 (0/301)
3– years	3.55 (11/310)	1.44 (4/278)	0.38 (1/262)	1.88 (5/266)	0 (0/258)	0 (0/272)	0.42 (1/239)
4– years	1.88 (6/319)	2.10 (6/286)	0 (0/267)	0.74 (2/272)	0 (0/268)	0.36 (1/279)	0 (0/231)
P value*	0.031	0.749	0.114	0.121	0.572	0.888	0.981
5–17 years old	3.30 (71/2,151)	1.30 (26/2,002)	0.84 (15/1,791)	0.77 (14/1,823)	0 (0/1,726)	0.05 (1/1,961)	0 (0/1,479)
18–59 years old	1.58 (56/3,552)	1.27 (44/3,461)	1.68 (52/3,104)	0.47 (15/3,182)	0.55 (17/3,064)	0.18 (6/3,340)	0.14 (4/2,780)
≥60 years old	1.71 (13/762)	0.95 (7/738)	1.52 (10/659)	0.30 (2/668)	1.22 (8/654)	0 (0/705)	0.17 (1/593)
P value#	0.375	<0.001	<0.001	0.003	<0.001	0.055	0.432
Case type							
Inpatient	1.47 (108/7,332)	1.71 (120/7,033)	1.31 (84/6,398)	0.84 (54/6,450)	0.42 (26/6,245)	0.13 (9/6,800)	0.09 (5/5,478)
Outpatient	4.38 (75/1,713)	0.86 (14/1,621)	0.14 (2/1,471)	0.13 (2/1,513)	0 (0/1,456)	0.43 (7/1,617)	0.14 (2/1,414)
Rate difference (95% CI)	-2.91 (-3.91, -1.90)	0.84 (0.30, 1.39)	1.18 (0.84, 1.51)	0.71 (0.42, 0.99)	0.42 (0.26, 0.58)	-0.30 (-0.63, 0.03)	-0.05 (-0.26, 0.16)
Geographic location							
Northern	3.24 (81/2,498)	3.75 (88/2,346)	0.53 (10/1,880)	1.54 (31/2,008)	0 (0/1,742)	0.52 (12/2,292)	0.25 (4/1,571)
Southern	1.56 (102/6,547)	0.73 (46/6,308)	1.27 (76/5,989)	0.42 (25/5,955)	0.44 (26/5,959)	0.07 (4/6,125)	0.06 (3/5,321)
Rate difference (95% CI)	1.68 (0.93, 2.44)	3.02 (2.22, 3.82)	-0.74 (-1.17, -0.30)	1.12 (0.56, 1.69)	-0.44 (-0.60, -0.27)	0.46 (0.16, 0.76)	0.20 (-0.06, 0.46)

***Table 5*: Positive detection rate of viral or bacterial pathogen for patients with acute meningitis or encephalitis in China during 2009–2018.**
The data were presented as positive rate (positive number/tested number) unless otherwise indicated. * indicates comparison among six age groups of children by performing Cochran-Armitage trend test. # indicates comparison among children <5 years old, patients aged 5–17 years old, 18–59 years old and ≥60 years old by performing Cochran-Armitage trend test. *N. meningitidis, Neisseria meningitidis; S. pneumoniae, Streptococcus pneumoniae; S. aureus, Staphylococcus aureus;* Hib, *Haemophilus influenzae type b; S. suis, Streptococcus suis; M. tuberculosis, Mycobacterium tuberculosis; E. coli, Escherichia coli.*
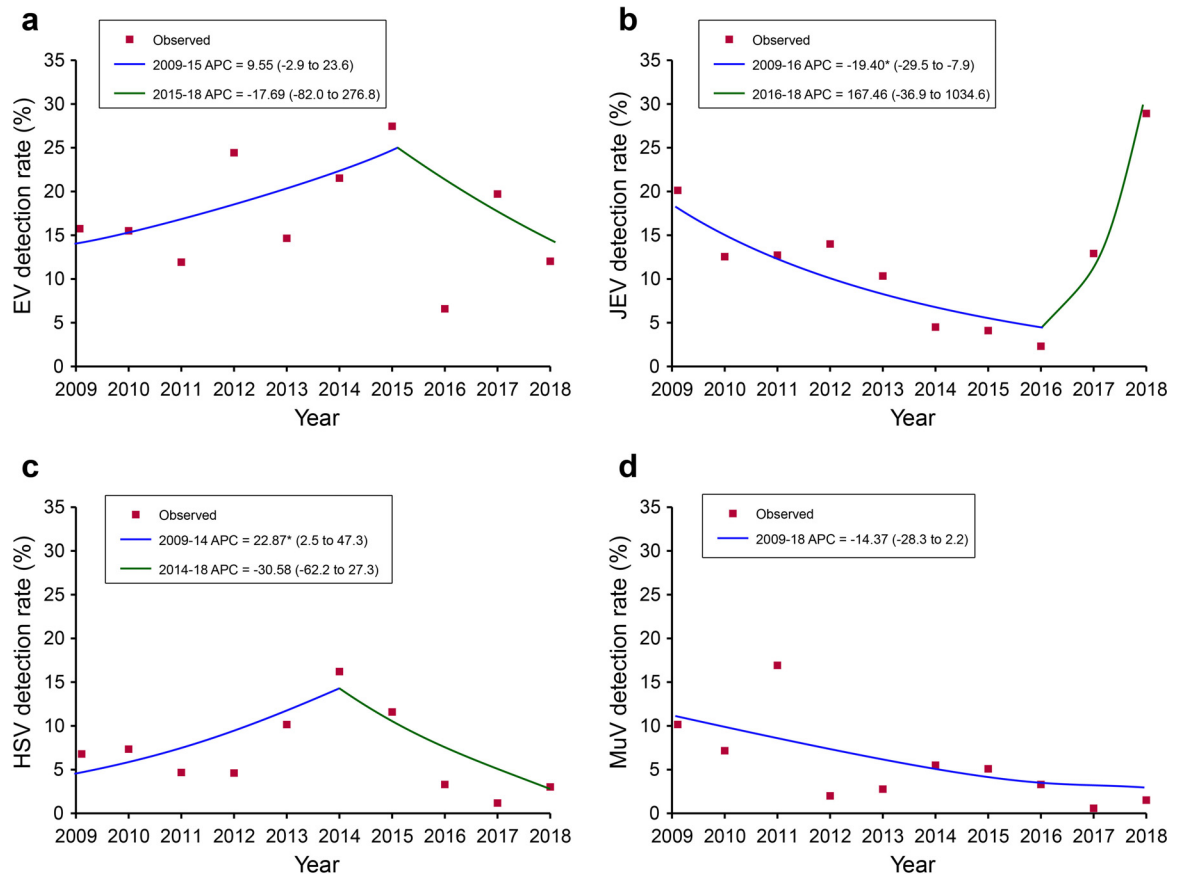
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Figure 3. Joinpoint regression analysis of the annual positive rates for four viruses. (a) EV; (b) JEV; (c) HSV; (d) MuV. EV, Enterovirus; JEV, Japanese encephalitis virus; HSV, Herpes simplex virus; MuV, Mumps virus. Red points indicate the mean positive rate of tested viruses in terms of year. The colored curves indicate fitted patterns by the red points. Legends give the annual percent change (APC) value of each fitted curve for each virus. *indicates that the APC is statistically significantly different from zero at P<0.05.

Figure 3). No autocorrelations were observed (Supplementary Figure 6). Seasonal pattern for tested viruses was observed based on the monthly positive rate for each pathogen. Both EV and HSV peaked during April–September, while JEV and MuV peaked during July–September and April-June, respectively (Supplementary Figure 7). No obvious seasonal trend was shown for any of the tested bacteria.

The tested pathogens with positive rates greater than 1% were further investigates for their relationship with meteorological and socioeconomic factors at the city level. The weekly average temperature had statistically significantly affected most of the tested agents, however with different directions of effects between viruses and bacteria. The weekly incidence rate ratios (IRRs) for viruses ranged from 1.08 (95% CI: 1.00–1.17) to 1.74 (95% CI: 1.59–1.92), indicating a positive association between temperature and viral activity, while IRRs for *S. pneumoniae* was 0.86 (95% CI: 0.76–0.98), indicating a negative association between temperature and bacterial activity (Supplementary Table 6). Relative humidity also displayed a bidirectional effect, with the

negative association shown for MuV, HSV and *N. meningitidis*, while the positive association for *S. aureus*. The weekly precipitation affected JEV and *S. aureus* statistically significantly, but with different directions of effects. Longer sunshine hours were additionally related to the increased circulation of JEV and *S. pneumoniae*. GDP per capita was statistically significant for viruses and bacteria, with the IRRs ranging from 0.89 (95% CI: 0.79–0.99) to 1.21 (95% CI: 1.04–1.41). The population density was statistically significant for only few tested viruses.

Discussion

The longitudinal surveillance described herein spanned a decade and has provided the first comprehensive understanding of etiological and epidemiological features of AME across China. Age was the most important factor that can impact on the pathogen spectrum. Viral pathogens were the main cause among pediatric patients with AME (usually EV or HSV). This was consistent with previous findings in Chinese children with

AME from 2009–2012.[25] Vaccines against mumps and JEV had been included in the expanded program on immunization (EPI) in China since the year of 2007, with the first vaccination given at eight months (Supplementary Table 7). The vaccines were related to the obvious decline of risk of infection among young children. For JEV, a further obvious increase of positive detection was observed after 25 years, indicating increased burden of disease in older adults with potential greater exposure, and also likely related to the decay of vaccine elicited immunity as children age because of the first introduction of JEV vaccine in China in the 1960s. The peak in activity of EV, MuV and HSV was observed among young children, commensurate with likely increases in transmission due to close contacts among school aged populations.[26,27]

We found the main pathogens of bacterial meningitis or encephalitis in China were *S. pneumoniae, S. aureus* and *N. meningitidis*, with each of them responsible for the bacterial meningitis or encephalitis in different age groups. In the studies from other countries, such as Spain and France, the reduction of pneumococcal meningitis was likely to be achieved through immunization with 13-valent pneumococcal conjugated vaccine (PCV 13) in children less than 18 years.[28,29] In China, PCV 13 in children were not included in the EPI, leaving *S. pneumoniae* as the leading cause of pediatric bacterial meningitis or encephalitis. It would clearly be advantageous to supplement PCV 13 vaccines into the EPI schedule to reduce the burden of *S. pneumoniae* related meningitis or encephalitis in China. Similarly, decrease in *N. meningitidis* infections has been observed after introduction of the meningitis b vaccine.[30]

By analyzing aggregated nationwide data over a decade, in combination with meteorological and socio-economic factors, we identified a consistent temperature effect that positively affected viral and negatively affected bacterial activity. Longer sunshine hours and lower relative humidity also enhanced viral activity. These findings were also reflected and corroborated by the seasonal pattern of the tested pathogen in the current study, showing the peak of viral detection in summer and bacterial detection in winter. Further research to narrow the geographic scope or pathogen transmission,[31−35] may assist the specific diagnosis and prevention of the most frequently identified infectious pathogens, and targeting of vaccination.

Annually, the activity of most tested viruses decreased throughout the study period, with exception of JEV, which showed elevated activity in 2017–2018. The year to year increase reflected the increased trend of the disease that might be attributed to the expansion of the competent vectors as a result of warming climate in recent years.[36] According to the current study, other factors involving population density and GDP also affected the viral and bacterial circulation in a significant manner, supporting the hypothesis that crowded conditions and lower living standards also favored infection.[37]

The joinpoint analysis showed the age threshold that clearly defined the descending turnpoint at 6–7 years old for JEV and MuV, and after 25 years old for JEV increasing. Therefore, immunization strategies should be accordingly adjusted for JEV and MuV. A booster immunization is therefore advocated according to the age turnpoint that mark these highly vulnerable populations. For example, booster immunity JEV vaccines at around 25 years, might be recommended, in addition to the first shot at 6 years (Supplementary Table 7). PCR-based results were of value in providing pathogen-specific and serotype/group data for the timely prevention and treatment of AME disease. These data also help to warn against the antimicrobial resistance for some bacteria causing AME, which might be a major source of nosocomial infection.

Our study was subject to several limitations. First, we failed to identify any of the candidate etiology in more than 70% of cases. This limitation is common to surveillance studies with similar study design.[18,38] Only pre-specified pathogens were tested, thus those negative patients might be infected with organisms not included in our diagnostic algorithms. Previous studies indicated that about 11.8%, 7.2%, 18.9%, and 0.1% of AEM patients were caused by *Listeria monocytogenes, group B streptococcus, Cryptococcus neoformans,* varicella zoster virus respectively in China, which might account for the undetermined organisms in the current AME patients.[39−42] In addition, autoimmune meningitis or encephalitis are increasingly recognized as accounting for large amounts of AME illness of non-infectious cause,[43,44] which was not taken into account in our study. Second, only part of the recruited patients has been tested for all four viruses and seven bacteria tested, which may lead to the selection bias in the analysis of clinical differential among viral and bacterial infections. Third, some of the current positive detection was obtained from a non-sterile site, which confers less diagnostic weight than detection in CSF. This is especially important as the pathogenesis role of the organism causing AME, cannot be determined merely from positive detection, especially for those from samples of unsterile sites (i.e., stool samples), which are less likely to indicate an invasive infection. Identification of organisms outside CNS indeed occurs with high frequency, however, particularly for viruses that cause acute encephalitis through an immune-mediated pathogenesis.[45] Finally, the clinical definition of meningitis and encephalitis were used in combination for the recruit of patients, which made the separate analysis for their pathogen spectrum unlikely to be performed.

Despite of these concerns, the determination of the pathogen spectrum, as well as their specific pattern in terms of age and gender might assist in identifying predominant pathogen candidates for applying clinical diagnosis, prevention control, and even vaccination strategy.

Long-lasting surveillance is advocated not only for the purpose of establishing a baseline for AME related pathogens in China, but also for the sake of evaluation of public health measures, for example, the nonpharmaceutical interventions (NPI) used to combat the COVID-19 in the mainland of China may have broad impact on the occurrence of meningitis and encephalitis, which warrant to be investigated in the future.

Declaration of Competing Interest
We declare no competing interests.

Contributors
GFG, WZY, JGW, SIH, LQF, WL and ZJL conceived, designed and supervised the study. WZY, JL, MFL, SWZ, ZGY, ZHY, XC, YC, ZSY, LM, XHW, YLL, JGW, LYH, HQJ, XW, ALC, SJL and WBX formulated the protocols, guideline and SOP of the active sentinel pathogenic surveillance. YY, QBL, SXZ, HYZ, XAZ, MYL, LSS, XR, YFW, SHL, CHZ, MJG and YLZ collected, cleaned, and analyzed the data. LQF, WL, LPW, QBL and YY wrote the drafts of the manuscript. LQF, WL, ZJL, LPW, YY, QBL and YLL interpreted the findings. GFG, WZY, JGW, SIH, LQF, WL, ZJL, MFL, ZHY and YC commented on and revised drafts of the manuscript. All authors read and approved the final report.

Availability of data and materials
The raw data analyzed during the current study is available from the corresponding author on reasonable request.

Supplementary materials
Supplementary material associated with this article can be found in the online version at doi:10.1016/j.lanwpc.2021.100361.

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