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Etiology of Acute Infectious Meningitis and Meningoencephalitis in Karachi, Pakistan: Retrospective Observational Study from a Tertiary Care Center

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Abstract. Meningoencephalitis (ME) is potentially fatal and is caused by a wide array of pathogens. Diagnostic and health-care access gaps prevent accurate estimation of the pathogen-specific burden in low-resource settings. We present pathogen-specific etiologies among patients hospitalized with ME in Karachi, Pakistan. We performed a retrospective hospital database evaluation of pathogen etiology and outcomes of community-acquired infectious ME at a single tertiary care center in Karachi, Pakistan. Annual rates of hospitalization (ARH) were calculated by adjusting for missed cases and are reported per 100,000 population. From May 2017 to April 2020, 522 episodes of infectious ME were identified in 514 patients. The overall ARH from ME was 5.7/100,000 population (95% CI, 5.1–6.1). Among children younger than 5 years, the ARH was 9.8/100,000 population (95% CI, 8.1–11.8). Unknown causes of ME resulted in the greatest burden, with an ARH of 1.9/100,000 population (95% CI, 1.7–2.2). Among known causes, the greatest burden of hospitalizations resulted from tuberculous ME (0.8/100,000; 95% CI, 0.6–0.97), followed by pneumococcal and enteroviral ME (both 0.6/100,000 population; 95% CI, 0.5–0.8). The burden of ME caused by pathogens preventable through vaccination or public health measures outweighed that of ME from other causes ($P = 0.0092$, Fisher's exact test). We report a broad range of pathogens causing ME in southern Pakistan and show a high burden of preventable illness. Synergistic actions to improve diagnostic strategies, increase vaccinations, and introduce measures to reduce water-borne and vector-borne diseases are required to reduce the ME burden in Pakistan and prevent future outbreaks.

INTRODUCTION

Acute meningitis, encephalitis, and meningoencephalitis (ME) can be caused by a variety of pathogens, can be difficult to differentiate clinically, and all can result in severe disease.[1–3] Risk factors vary depending on the causative pathogen, but immunocompromised individuals in the community, especially at the extremes of age and including those without predisposing factors, can be affected.

Despite advances in the diagnosis and management of these conditions, mortality remains high, especially in low-income countries (LICs) and lower middle-income countries (LMICs), particularly among neonates and children.[1] Pathogen-specific incidence and mortality estimates have wide uncertainty intervals resulting from a lack of access to diagnostics in LICs and LMICs,[4] which also results in delayed or inappropriate patient management. Moreover, low vaccination rates affect incidence of and mortality from vaccine-preventable causes. Reduction in disability-adjusted life years (DALYs) attributable to ME requires comprehensive etiology-specific surveillance that can inform management and control strategies. These data underscore the need for enhanced detection of acute meningitis and ME, especially in children, and improved diagnostic capacity and access to diagnostics, with a view to improving etiological diagnosis and surveillance.[1] Knowledge of pathogen-specific etiology is therefore essential to informing diagnostic and preventive strategies at various levels of health care.

Pakistan is among the top 10 countries with the greatest number of deaths from meningitis; however, it is important to note the limitations of global burden estimates for low- and middle-income settings such as Pakistan, resulting from the paucity of pathogen-specific surveillance data.[1] Estimates do not include pathogens associated more commonly with chronic meningitis and encephalitis (notably, tuberculous ME).[1,5] The burden of ME in Pakistan therefore remains unknown. Prior to the introduction of childhood vaccinations against *Haemophilus influenzae* type b (HiB) (introduced circa 2009) and invasive pneumococcal serotypes (10-valent vaccine introduced in 2013),[6] HiB and pneumococci were estimated to cause a significant proportion of childhood bacterial meningitis.[7] The effect of these vaccinations reduced the burden of childhood HiB meningitis significantly and is expected to have reduced the meningitis burden resulting from pneumococcal vaccine serotypes.[8] The Meningitis or ME burden among adults has not been estimated pre- or postvaccination in Pakistan. Globally, the postvaccination etiology of meningitis and ME has changed significantly and shows greater diversity of pathogens.[1] No data are available from Pakistan that reflect the diversity of pathogens causing the clinically overlapping and often indistinguishable syndromes of meningitis, encephalitis, and ME. Although recent reports of primary amoebic ME[9,10] and arboviral ME outbreaks[11,12] have informed the facility-based management of community-acquired ME in Pakistan, systematic data on hospitalized ME informing etiology-specific estimates are unavailable.

We present the relative proportion of pathogens among acute infectious ME in a hospitalized cohort admitted to a center with accredited laboratory and inpatient services in southern Pakistan.

MATERIALS AND METHODS

Study objectives. The main objective of this analysis was to determine the etiologies of community-acquired ME among patients hospitalized at a tertiary care center in the city of Karachi in southern Pakistan to determine the pathogen-specific proportions, hospitalization rates, in-hospital mortality rates, and proportion of infectious ME of undetermined etiology.

Study design and setting. We conducted a retrospective evaluation of patients hospitalized from May 2017 to April 2020

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with discharge diagnoses of meningitis, ME, or encephalitis. The study site is a Joint Commission International–accredited emergency and tertiary care hospital in Karachi, Pakistan, with 700 hospital beds and active neurology and infectious disease services. The hospital admits ~61,000 patients of all ages per year and has a catchment population of ~15 million.[13]

Data sources. Patients were identified through a database search of hospital records with a primary or associated discharge diagnosis of meningitis, encephalitis, or ME (International Classification of Diseases, Ninth Revision, coding), and with cerebrospinal fluid (CSF) samples sent to the laboratory for evaluation. Patients without CSF sampling and analysis were excluded to ensure data completeness (determination of etiology) and to increase specificity of the data set. To ensure the search was complete and discharge records did not miss any of the diagnoses, we complemented this search strategy with a search of laboratory records of CSF microbiological tests performed on hospital inpatients during the study period. Records were then matched, and duplicates removed. Medical records of all patients identified through the search were reviewed for inclusion and exclusion criteria to improve the specificity of community-acquired infectious ME diagnosis.

Study definitions and inclusion criteria. Meningoencephalitis was defined as acute brain dysfunction and/or evidence of either meningeal or cerebral inflammation. Thus, the syndrome encompasses both meningitic and encephalitic presentations, and includes patients who have meningeal inflammation with an encephalitic component.[14]

Patients of all ages meeting the following diagnostic criteria were considered to have ME[14,15]: fever ($\geq$ 38°C or 100.4°F within 72 hours before presentation) and/or altered mental status (altered level of consciousness, lethargy or irritability, personality changes in adults or poor feeding in infants) for $>$ 24 hours with no alternative cause and any two of the following: manifest or subclinical new-onset seizure activity (per an abnormal electroencephalogram), signs of meningeal inflammation/irritation or bulging fontanelle in infants, new onset of focal neurological findings, or CSF pleocytosis ($\geq$ 5 cells/mm^3) or neuroimaging abnormalities consistent with an acute infectious process. Those with infections acquired in the community who were evaluated at and admitted to the study facility and who received at least one CSF microbiological test (culture, antigen detection, or nucleic acid amplification) for determination of infectious etiology were included.

Patients with health-care and ventricular device-associated ME, posttraumatic ME (e.g., skull fractures), autoimmune ME, drug-induced or chemical ME, and a metabolic or extracranial infectious cause for signs and symptoms were excluded.

Any episode of ME occurring in a patient 3 weeks or more after recovery from a previous episode, regardless of pathogenic etiology, was considered a recurrent episode.[16]

Preventable causes of ME were categorized as vaccine preventable and preventable through other public health strategies. Pneumococcal, meningococcal, *Haemophilus*, tuberculous, measles, and subacute sclerosing panencephalitis, mumps, influenza, and rabies were considered vaccine preventable. Meningoencephalitic etiologies for which public health strategies can be implemented to prevent the incidence of illness included, in addition to vaccine-preventable causes, *Naegleria fowleri*, flaviviruses, alphaviruses, malaria, neurolisteriosis, neurobrucellosis, and neurosyphilis.

Etiological classification and diagnostic criteria. Etiology was determined based on laboratory confirmation by CSF pathogen detection, laboratory confirmation by ancillary tests, or clinical diagnosis. Table 1 shows the etiological classification categories used in our study. When multiple pathogens were detected, or multiple criteria fulfilled, the pathogen more likely to be associated with clinical symptoms and for which physicians prescribed antimicrobial treatment was considered to be the cause of an episode. For calculation of hospitalization rates, episodes with multiple etiologies were included in rates for both pathogens.

Diagnostic laboratory methods. All diagnostic tests were performed at the facility's affiliated clinical laboratory, which has been accredited by the College of American Pathologists since 2016. Microbiological diagnostic procedures for ME included a CSF Gram stain and bacterial culture at a minimum. Other tests for etiological diagnosis were requested variably per the attending physician's clinical judgment: whole blood malaria immunochromatographic test (BinaxNOW™; Abbott, Scarborough, ME) or a malarial parasite film; CSF latex agglutination test for *Cryptococcus* (IMMY, Norman, OK); mycobacterial testing by culture and/or the Xpert Ultra MTB/RIF nucleic acid amplification test for *Mycobacterium tuberculosis* (MTB) (Cepheid, Sunnyvale, CA); CSF wet mount and polymerase chain reaction (PCR) for *Naegleria fowleri* (laboratory-developed test); nucleic acid amplification panel test for 14 pathogens viz. enterovirus (EV), Herpes simplex virus 1 and 2 (HSV-1 and -2), cytomegalovirus (CMV), Varicella zoster virus, human herpesvirus 6, human parechovirus, *Escherichia coli* K1, *Listeria monocytogenes*, *Haemophilus influenzae*, *Streptococcus pneumoniae*, *Neisseria meningitidis*, *Streptococcus agalactiae* or group B *Streptococcus*, and *Cryptococcus neoformans*, (Filmarray ME, bioMereiux, France); and the CSF Venereal Disease Research Laboratory (BD, Sparks, MD) test for neurosyphilis. Blood cultures, when performed, were processed using automated BACT/ALERT Standard Aerobic/Anaerobic and Peds Plus (bioMerieux, Durham, NC) bottles.

The following alternative specimen tests (serum/plasma or nasopharyngeal swab) were used as ancillary etiological diagnostic tests for ME when consistent with clinicoradiological presentation: Dengue or West Nile viruses IgM tests (InBios International, Seattle, WA), the Dengue NS-1 antigen test (PanBio, Abbott Diagnostics Korea Inc., Yongin, South Korea), Chikungunya virus IgM test (InBios International), a *Toxoplasma* IgM or IgG test (Immulite DPC-Siemens, France), a measles IgM test (NovaTek, Launch Diagnostics, UK), a rapid plasma reagin test (Immutrep Omega Diagnostics, UK), and a nasopharyngeal PCR test for influenza A or B (Cepheid). Supplemental Table 1 presents the various laboratory tests used to confirm pathogen etiologies as well as additional criteria used for diagnosis when applicable.

Medical record review and data extraction. Medical records were reviewed and the following variables were recorded in an MS Excel database: age, gender, recognized predisposing factors viz. CSF leak, diabetes mellitus, otitis media, sinusitis, pneumonia, immunocompromised state (HIV, steroid use, primary or secondary immune deficiency syndromes),[16] presenting symptoms, duration of acute illness before presentation to the emergency/hospital system, neuroimaging results, laboratory test results (CSF and alternative specimens), duration of hospitalization (from day of admission until day of discharge or death), discharge dispositions, and duration of follow-up.

TABLE 1
Etiological classification categories used for infectious ME diagnosis among patients fulfilling study inclusion criteria

Category	Definition	Pathogens captured with diagnostic tests available	Additional clinical or radiological features required to support diagnostic test results
Laboratory-confirmed diagnosis	Primary etiological pathogen detected through culture or NAAT in CSF sample obtained from the patient at the time of presentation *or* Pathogen detected in CSF by NAAT and treatment initiated/continued *or* Antibody response to pathogen detected in CSF with low inherent rate of biological false positives for diagnostic test	*Streptococcus pneumoniae*, *Neisseria meningitidis*, *Haemophilus influenzae*, *Streptococcus agalactiae*, *Escherichia coli* K1, *Listeria monocytogenes*, *Mycobacterium tuberculosis*, *Salmonella* spp. *Naegleria fowleri*, *Cryptococcus neoformans*, Herpes simplex virus-1 and -2, Varicella zoster virus, Enterovirus, human herpesvirus-6, cytomegalovirus, *Treponema pallidum*	–
Laboratory-confirmed ancillary diagnosis	Etiological pathogen detected in CSF not a primary ME etiological agent *or* Pathogen or its immune response detected in serum/blood or nasopharyngeal swab of patient at the time of presentation	*Staphylococcus aureus,* CONS, *Streptococcus viridans*, Dengue virus/other flaviviruses, Measles virus, Chikungunya virus, *Toxoplasma gondii, Plasmodium* spp., *Brucella* spp., Influenza viruses	Exclusion of alternative etiologies (CSF tests negative for recognized pathogens of ME); clinical history, EEG, and radiological imaging consistent with characteristics described in literature
Clinical diagnosis	CSF analysis and etiology negative or inconclusive and diagnosis made based on CSF pleocytosis (lymphocytic or polymorphonuclear predominance), clinical and epidemiological risk factors, and/or clinical/radiological electroencephalographic signs	Measles, mumps, Rabies virus, *M. tuberculosis*	Presence of pathognomonic clinical signs (typical measles rash/nonsuppurative parotitis for mumps), consistent clinical history (e.g., dog bite for rabies), clinical course consistent with natural history of illness, absence of alternative cause and evidence of infection in other samples (e.g., respiratory samples positive for *M. tuberculosis*), other sustained improvement after appropriate treatment (> 2-month follow-up for tuberculosis)

CONS = coagulase-negative staphylococci; CSF = cerebrospinal fluid; EEG = electroencephalogram; ME = meningoencephalitis; NAAT = nucleic acid amplification test.

Data analysis. Data were exported to GraphPad Prism 9.3.1 (GraphPad Software, LLC, Boston, MA) for further analysis. Quantitative variables were analyzed for median and interquartile ranges, and compared between pathogen groups using the Kruskal-Wallis test with multiple comparisons. Categorical comparisons were performed using the χ^2 or Fischer's exact tests. A P value of < 0.05 was considered significant.

Annual rates of hospitalization were calculated for all episodes, episodes in children 5 years and younger, and for all pathogens. Annual rates and 95% CIs were calculated per 100,000 population, for a catchment population of 15 million[13] using a WHO-recommended method[17] and the open epi calculator (OpenEpi, Version 3.01, Dean AG, Sullivan KM, Soe MM. OpenEpi: Open Source Epidemiologic Statistics for Public Health). Calculated rates were adjusted for both the health-care use at the tertiary care hospital and uptake of lumbar puncture (LP) and CSF sampling. Based on previous data, we assumed that 70% of the catchment population sought care at the private hospitals in the city,[6,18] of which we assumed 50% received care at the study hospital, which is the largest tertiary care private center in the city. Based on LP deferral rate in the study hospital and previously reported LP deferral rates among children in Pakistan,[19,20] we estimated that 40% of ME cases do not undergo diagnostic CSF sampling. Supplemental Note 1 shows an example calculation for the hospitalization rate and adjustment for care use and CSF sampling.

RESULTS

Hospital discharge database search results. A total of 1,382 records were identified with a discharge diagnosis of meningitis, encephalitis, or ME. Of these, only 896 (64.8%) underwent CSF sampling, and 522 fulfilled diagnostic criteria of community-acquired infectious ME. Study profile and patient selection stages are shown in Figure 1. No additional episodes or patients were identified through review of the institutional laboratory database of 1,305 CSF examinations.

Study cohort and diagnostic classification. We identified 522 episodes of infectious ME in 514 patients during the 36-month study period. The median age of patients at first episode was 28 years (interquartile range, 49–9 years). The male-to-female ratio was 1.7. Of 522 episodes, etiology was determined for 339 (64.9%) episodes; of these, 74.4% (n = 252) were laboratory-confirmed (LC), 15.3% (n = 52) were

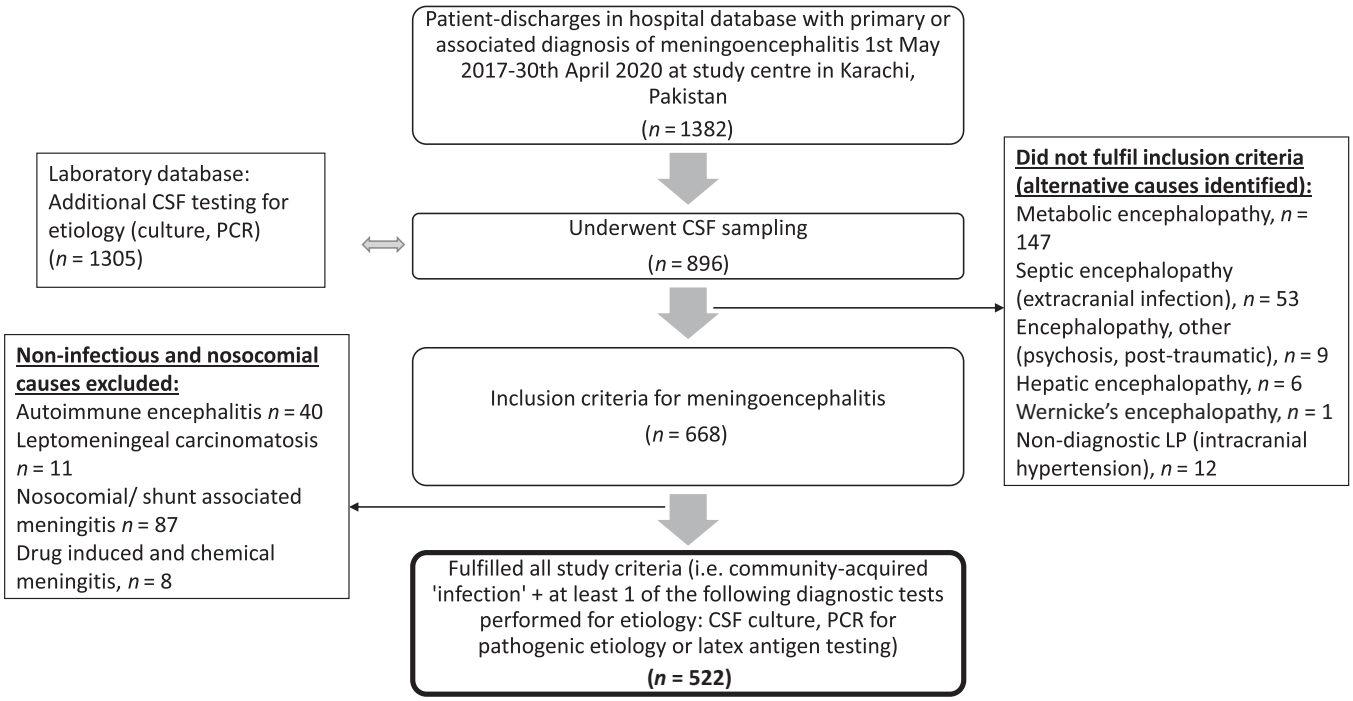

FIGURE 1. Study profile, and refinement and derivation of study database of 522 ME episodes with etiology evaluation. CSF = cerebrospinal fluid; LP = lumbar puncture; ME = meningoencephalitis; PCR = polymerase chain reaction.

laboratory-confirmed ancillary, and 10.3% (n = 35) were clinical diagnoses. Of the 252 LC diagnoses, two episodes with CMV had additional clinical and ancillary test-based diagnoses of tuberculous meningoencephalitis (TBME) and influenza encephalitis, respectively. Of the 183 episodes with undetermined etiology, only one episode had a positive CSF laboratory result for CMV, which was considered clinically insignificant and not treated.

Risk factors and characteristics on admission. Table 2 describes the characteristics of patients at presentation, risk factors, signs and symptoms of ME, duration of illness before presentation, and CSF biochemical and cellular analysis. Cerebrospinal fluid to serum glucose ratio was not included, because results for glucose tested at the point of care at the time of CSF analysis were not available from medical records. Table 2 also shows an analysis of clinical features by bacterial, viral, or unknown etiology. Patients presenting with viral etiologies were significantly younger than those with fungal ME (post hoc Dunn test, P = 0.016). Among risk factors, sinusitis and CSF leak were significantly more common among patients with bacterial etiologies, whereas immunocompromised status was common for fungal etiologies (cryptococcal ME). Diabetes was observed among all etiologies except amebic ME (*N. fowleri*). Altered mental status was significantly more common in amebic and fungal ME; the meningitis triad was observed significantly more frequently in TBME. Symptom duration was significantly longer in TBME (post hoc Dunn test, $P < 0.05$ for all except mycobacterial versus fungal). Symptom duration of fungal ME was significantly longer than that of bacterial and amebic ME (post hoc Dunn test, P = 0.048 and 0.027, respectively). A significant difference between Glasgow Coma Scale (GCS) scores of various etiologies was driven by a reduced GCS for amebic ME, whereas differences in CSF leukocyte counts, lymphocytosis, and protein were also driven by high counts, low lymphocytes (neutrophil pleocytosis), and high protein in bacterial and amebic ME. Notable features of ME of unknown etiology were younger median age, median CSF pleocytosis less than that of other etiologies, and lymphocytic predominance (Table 2).

Laboratory tests and diagnostic yield. Cerebrospinal fluid cytological and biochemical analysis results were available for 99.6% of episodes. In two episodes, the CSF sample obtained was insufficient for analysis and the multiplex PCR panel test and CSF culture were prioritized. Cerebrospinal fluid culture results were available for all episodes, whereas the CSF multiplex panel PCR test was performed in 97.7% of all episodes (n = 510). Mycobacterial culture and/or PCR tests were performed in 53% of episodes (n = 279). Supplemental Figure 1 shows diagnostic test use in the cohort.

Of the 339 episodes with etiological diagnoses, 252 (74.3%) were LC, of which two episodes had dual pathogen etiologies based on clinical parameters (MTB and CMV; n = 1) and ancillary tests (influenza and CMV; n = 1). Overall, 15.4% (n = 52) were considered LCA diagnoses. In 10.3% of episodes (n = 35), a diagnosis was made on clinical grounds, of which 85.7% (n = 30) were TBME and 14.3% (n = 5) were viral/postviral.

When etiology was determined, CSF analysis (culture, PCR, or antibody tests) yielded an etiological diagnosis in 78.4% of episodes (265 of 338), with the CSF multiplex panel PCR test providing the diagnosis in 70.9% (188 of 265), a CSF MTB PCR test (Xpert MTB/RIF Ultra) in 10.9% (29 of 265), a CSF culture (bacterial or mycobacterial) in 14% (37 of 265), and a CSF *Naegleria* PCR test in the remaining 4.2% (11 of 265). In 10.9% episodes (39 of 339), serum antibody tests for viral agents of encephalitis yielded a diagnosis.

Among patients with undetermined etiology, laboratory test use for CSF bacterial culture with a multiplex panel PCR

TABLE 2

Demographics, risk factors, symptoms, signs, and CSF parameters at presentation in 522 hospitalized ME episodes, Karachi, Pakistan, 2017–20

Feature	All episodes	Bacterial	Mycobacterial*	Viral*	Fungal*	Amoebic	Unknown etiology	*P* value†
n	522	90	70	149	10	11	183	–
Demographic								
Age, years; median (IQR)	28 (9–49)	32 (4–56.3)	26.5 (16–45.8)	20 (5–45)	48 (40.8–64.3)	32 (27–38)	27 (11–53)	**0.0209**
Male gender, % (*n*)	63.4 (331)	67.8 (61)	57.1 (40)	60.4 (90)	60 (6)	90.9 (10)	63.9 (117)	0.2937
Risk factors, % (*n*)								
AOM/COM	2.5 (13)	7.8 (7)	1.4 (1)	–	10 (1)	–	2.2 (4)	0.0566
Sinusitis	4.4 (23)	16.7 (15)	–	0.7 (1)	10 (1)	–	3.3 (6)	**< 0.0001**
Pneumonia	0.6 (3)	2.2 (2)	1.4 (1)	–	–	–	–	> 0.99
CSF leak	4 (21)	17.8 (16)	–	–	–	–	2.7 (5)	**< 0.0001**
Diabetes mellitus	15.1 (79)	25.6 (23)	17.1 (12)	11.4 (17)	20 (2)	–	12 (22)	**0.0266**
Immunocompromised‡	4.9 (26)	3.3 (3)	7.1 (5)	2 (3)	60 (6)	–	1.6 (3)	**< 0.0001**
Presentation, % (*n*)								
Fever	98.7 (515)	98.9 (89)	98.6 (69)	99.3 (148)	90 (9)	100	98.9 (181)	0.2028
Headache	49.4 (258)	52.2 (47)	55.7 (39)	49.7 (74)	80 (8)	63.6 (7)	43.2 (79)	0.1138
Neck rigidity	11.5 (60)	8.9 (8)	14.3 (10)	16.8 (25)	–	–	9.3 (17)	0.1345
Seizures	35.1 (183)	36.7 (33)	30 (21)	28.2 (42)	20 (2)	54.5 (6)	41.5 (76)	0.0705
Altered mental status	50 (261)	52.2 (47)	61.4 (43)	41.6 (62)	80 (8)	90.9 (10)	47 (86)	**0.0015**
Meningitis triad	2.1 (11)	2.2 (2)	8.6 (6)	0.7 (1)	–	–	1.1 (2)	**0.0014**
Lethargy	27 (141)	23.3 (21)	24.3 (17)	30.9 (46)	20 (2)	9.1 (1)	27.9 (51)	0.5316
Personality changes	6.7 (35)	6.7 (6)	8.6 (6)	3.4 (5)	20 (2)	18.2 (2)	7.7 (14)	0.1395
Acute flaccid paralysis	0.2 (1)	–	–	0.7 (1)	–	–	–	–
Other focal neurological signs	11.7 (61)	10 (9)	18.6 (13)	13.4 (20)	20 (2)	–	8.7 (16)	0.2027
Rash	0.9 (5)	–	–	2.7 (4)	–	–	0.6 (1)	–
Diarrhea	0.2 (1)	1.1 (1)	–	–	–	–	–	–
Symptom duration, days; median (IQR)	4 (2–7)	3 (2–4)	10 (6–14)	3 (2–6.5)	6.5 (3–15)	2 (2–3)	3 (2–5)	**< 0.0001**
GCS/pediatric score, points; median (IQR)	14 (13–15)	14 (13–14)	13 (12–14)	15 (13–15)	13 (11.5–14)	6 (6–8)	14 (13–15)	**< 0.0001**
Investigations								
CSF leukocyte count, $\times 10^3$ cells/mm^3; median (IQR)	0.2 (0.04–0.5)	1.9 (0.5–6.2)	0.15 (0.06–0.37)	0.12 (0.03–0.3)	0.12 (0.04–0.26)	4.9 (2.4–10)	0.08 (0.03–0.2)	**< 0.0001**
Lymphocytes, %; median (IQR)	80 (35–90)	20 (11–49)	80 (60–90)	90 (80–95)	72.5 (40–95)	10 (10–30)	85 (54.8–90)	**< 0.0001**
CSF protein, mg/dL; median (IQR)	72 (39–141)	180 (108–238.8)	92 (55–168.8)	47.5 (30–74.3)	99 (62.8–234)	330 (305–358)	63.5 (37.8–106.8)	**< 0.0001**
Abnormal EEG, % (*n*/*N*)	91.9 (227/247)	95.6 (43/45)	95 (38/40)	93.2 (55/59)	100 (5/5)	100 (7/7)	86 (74/86)	0.2734
CT/MRI abnormalities, % (*n*/*N*)	74.6 (344/461)	88.4 (76/86)	97.1 (68/70)	70.5 (86/122)	100	100	55.8 (86/154)	**< 0.0001**

AOM/COM = acute otitis media/chronic otitis media; CSF = cerebrospinal fluid; CT = computed tomography; EEG = electroencephalogram; GCS = Glasgow Coma Scale; IQR = interquartile range; ME = meningoencephalitis; MRI = magnetic resonance imaging.

*The following were excluded from analysis: subacute sclerosing panencephalitis (postviral), multiple pathogen etiologies (tuberculous + cryptococcal, and tuberculous + cytomegalovirus), toxoplasmosis, and malaria.

†*P* values for quantitative variables represent results from the Kruskal-Wallis test for difference in medians of independent variables. For qualitative variables, *P* values were drawn from Pearson's χ^2 test. *P* values in bold type are significant.

‡Includes HIV, steroid use, and primary or secondary immune deficiency syndromes. HIV was detected in 7 patients and included no bacterial, two mycobacterial (including mixed mycobacterial infections not included in the column), two viral (including mixed viral infections not included in the column), two fungal, one amebic (including toxoplasmosis not included in the column), and no unknown etiologies.

TABLE 3
Age-specific etiology (bacterial/viral, parasitic/fungal) among 522 episodes of ME hospitalized in Karachi, Pakistan, from 2017–20

Variable	Age group						Total
	< 1 month	1–12 months	13–60 months	6–17 years	18–64 years	≥ 65 years	
No. of patients captured	14	36	63	69	276	57	515
Patients with > 1 episode	–	1	1	–	5	–	7
Episodes with known etiology, % (*n*)	57 (8)	64.9 (24)	71.9 (46)	66.7 (46)	62.3 (175)	70.2 (40)	64.9 (339)
Etiology, % (*n*)							
Bacterial	62.5 (5)	54.2 (13)	37 (17)	32.6 (15)	51.4 (90)	50 (20)	30.7 (160)
Viral	37.5 (3)	41.6 (10)	63 (29)	67.4 (31)	34.3 (60)	42.5 (17)	28.7 (150)
Fungal	0	0	0	0	4.6 (8)	5 (2)	1.9 (10)
Parasitic	0	4.2 (1)	0	0	8.6 (15)	2.5 (1)	3.3 (17)
Mixed	0	0	0	0	1.1 (2)	0	0.4 (2)
Disposition at hospital discharge, % (*n*)							
Died	0	0	1.6 (1)	4.3 (3)	14.9 (41)	19.3 (11)	10.7 (56)
PVS	0	0	1.6 (1)	0	0.4 (1)	1.8 (1)	0.6 (3)
TRO/LTFU	0	8.3 (3)	3.2 (2)	0	4 (11)	1.8 (1)	3.3 (17)

ME = meningoencephalitis; PVS = persistent vegetative state; TRO/LTFU = transferred out of hospital and lost to follow-up.

test, mycobacterial PCR test, and viral antibody test were 100%, 28.4%, and 69.4%, respectively.

Age-specific etiology. Patients were grouped into five age categories (neonates, infants, children age 2–5 years, children age 6–17 years, adults age 18–64 years, and elderly adults ≥ 65 years). Table 3 describes the number of patients and episodes within each age group, percentage of known etiologies, pathogen etiology by group, and outcomes at hospital discharge.

Among neonates and infants, bacterial etiologies predominated; viral causes were most frequent in children up to 17 years of age. Among young adults and older adults, bacterial causes predominated, with most episodes attributable to TBME (Supplemental Figure 2). No TBME cases were observed in neonates, whereas among infants 2 to 12 months of age, 5.4% of the total episodes were a result of TBME. Mycobacterial etiology (among all age groups) was established by CSF testing in 56.9% of TBME episodes (*n* = 41), whereas 43.1% of episodes were determined based on clinical criteria. In all clinically diagnosed cases, either a PCR test or a mycobacterial culture test was performed. Supplemental Figure 2 shows the breakdown of age-specific etiologies by pathogens.

Age-specific death rates are also shown in Table 3. Among in-hospital deaths from ME, older age groups incurred the greatest rate.

Recurrence. Recurrence within the study period was observed in seven patients, all of whom experienced at least one additional episode each within the study period. One patient experienced three episodes within the study period. In three patients, two episodes of recurrent ME were pneumococcal; in one patient, two episodes were enteroviral; and in one patient, the first episode was pneumococcal and the second was caused by *S. agalactiae*. In two patients, the etiology of both recurrent episodes remained undetermined. Predisposing risk factors, etiology, and patient demographics for recurrent episodes are presented in Supplemental Table 2.

Annual rates of hospitalization. The overall annual rate of hospitalization from ME, adjusted for health care and LP use, was 5.7/100,000 population (95% CI, 5.1–6.1) . Among children younger than 5 years of age, the annual rate of hospitalization was 9.8/100,000 population (95% CI, 8.1–11.8). Of these, vaccine-preventable causes resulted in an annual hospitalization rate of 2.5/100,000 children 0 to 5 years old (95% CI, 1.7–3.6).

Figure 2 shows etiology-specific annual rates of hospitalization in Karachi. The greatest burden of hospitalization was the result of unknown infectious agents, with annual rates of 1.9/100,000 population (95% CI, 1.7–2.2). Among known causes, the greatest burden of hospitalizations resulted from TBME, followed by pneumococcal and enteroviral ME.

Analysis of annual hospitalization rates by preventable versus nonpreventable causes is shown in Figure 3. Annual rates of hospitalization resulting from vaccine-preventable

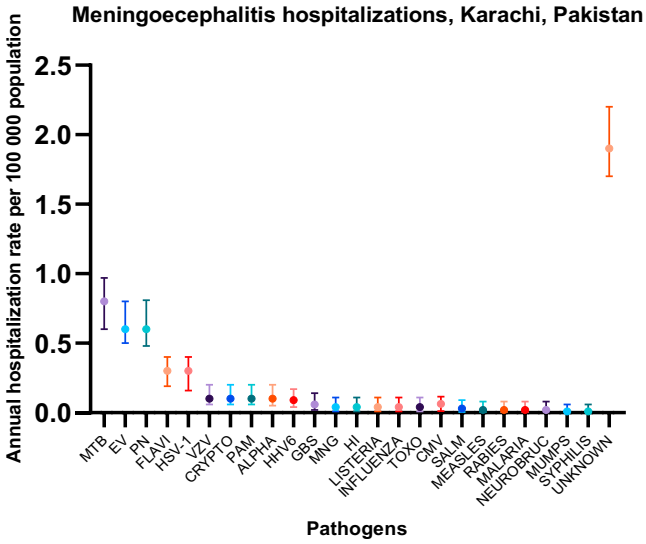

FIGURE 2. Annual hospitalization rates per 100,000 population for ME by etiology; Karachi, Pakistan, 2017–20. Rates adjusted for health-care use (at the study hospital) and for deferral rate of lumbar puncture. ALPHA = alphavirus (Chikungunya); CMV = cytomegalovirus; CRYPTO = *Cryptococcus neoformans*; EV = enterovirus; FLAVI = flaviviral (Dengue and West Nile viruses); GBS = *Streptococcus agalactiae* (group B *Streptococcus*); HHV-6 = human herpesvirus 6; HI = *Haemophilus influenzae* (all capsule types); HSV-1 = Herpes simplex virus 1; INFLUENZA = Influenza virus; LISTERIA = *Listeria monocytogenes*; Malaria = Malaria with ME presentation; ME = meningoencephalitis; MEASLES = Measles virus; MNG = *Neisseria meningitidis* (meningococcal); MTB = *Mycobacterium tuberculosis*; MUMPS = Mumps virus; NEUROBRUC = neurobrucellosis (i.e., *Brucella* spp.); PAM = primary amebic meningoencephalitis (*Naegleria fowleri*); PN = *Streptococcus pneumoniae* (pneumococcal); Rabies = Rabies virus; SALM = *Salmonella* spp.; SYPHILIS = neurosyphilis (*Treponema pallidum*); TOXO = *Toxoplasma gondii*; Unknown = no pathogen identified; VZV = Varicella zoster virus.

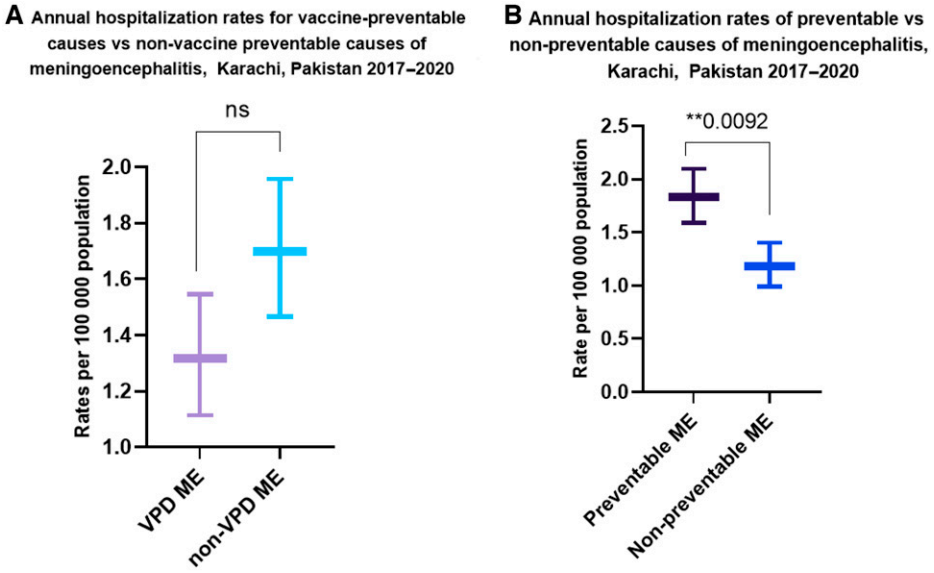

FIGURE 3. (**A**) Comparison of vaccine-preventable disease (VPD) hospitalization rates vs. non-VPD. (**B**) Comparison of all preventable pathogen causes vs. nonpreventable causes. Statistical test used was Fisher's exact test. VPD includes pneumococcal, meningococcal, *Haemophilus influenzae* (all types), tuberculous, measles, mumps, rabies, and influenzae meningoencephalitis (ME). Preventable ME pathogens or diseases include, in addition to VPD, *Naegleria fowleri*, flavivirus, alphavirus, malaria ME, and neurolisteriosis, neurobrucellosis, and neurosyphilis. ns = not significant.

causes were not significantly different from hospitalization rates for nonvaccine-preventable causes (Figure 3A). When all preventable strategies are considered, preventable causes of ME resulted in significantly greater rates of hospitalization than nonpreventable ME (Figure 3B).

Outcomes. The median duration of hospitalization in the entire cohort was 4 days (95% CI, 4–5). Median duration of hospitalization differed significantly by pathogen etiology of the ME episode ($P < 0.0001$). Overall, the median duration of hospitalization was significantly shorter for *N. fowleri* (fulminant course and early death) and enteroviral ME (shorter length of stay) than for other etiologies. Median duration was significantly longer for alphaviral (Chikungunya), flaviviral, HSV-1, tuberculous, and cryptococcal ME than other etiologies (Supplemental Figure 3). Of note, 97.8% of those (179 of 183) with undetermined etiology were treated with antibiotics.

Survival outcomes were not available for 17 patients who were transferred out during an ongoing ME episode. Of 505 patients for whom the hospital discharge dispositions could be determined, 11% ($n = 56$) died in the hospital, whereas 0.6% ($n = 3$) were discharged in a persistent vegetative state. Pathogen-specific analysis showed death rates to be significantly different among etiologies ($P < 0.0001$). Figure 4 shows pathogen-specific proportions of in-hospital deaths and population in-hospital mortality rates adjusted for transfers. The pathogen-specific proportion of deaths was greater for rabies, *Listeria* ME, and neurobrucellosis; however, the related mortality rates were not high as a result of overall low incidence or capture rate of ME from these pathogens. The greatest mortality rates were attributed to unknown causes, followed by *N. fowleri* ME, cryptococcal ME, and TBME.

DISCUSSION

We report the first-ever etiology-specific annual hospitalization rates for acute infectious ME from Pakistan, identified through a rigorous review of data from patients hospitalized at a tertiary care center in southern Pakistan during a 3-year period. Despite extensive testing, the pathogen etiology in the majority of cases remained unknown, suggesting deficiencies of currently available clinical and diagnostic algorithms to determine the true etiology of ME episodes.

Among known etiologies, the greatest burden was a result of TBME. This reflects the high disease burden in Pakistan.[21] No previously published studies estimate the burden of TBME in the study area of Karachi, Pakistan. Moreover, TBME burden is not known, because data reported by the national control program are not disaggregated by type and site of extrapulmonary disease. Our study is therefore the first to estimate the burden of hospitalization from TBME. Of note, rates we report are based on comprehensive clinical as well as microbiological criteria to determine the tuberculous etiology of ME. This approach to the determination of extrapulmonary tuberculosis rates is preferred to avoid underestimating the burden of disease, which may result from a low uptake of sensitive diagnostic tests (Xpert MTB Ultra) and low sensitivity of available diagnostics. We did not, however, adjust crude rates for the low uptake of Xpert diagnosis in ME cases, as diagnostic use is affected by CSF analysis criteria and evaluation/exclusion of other risk factors. The rates we report are therefore a cautious compromise to avoid large deviations from the true incidence of TBME. We also considered TBME a preventable cause of ME. The illness is vaccine preventable, especially among infants and children,[22] and preventable through treatment directed toward latent tuberculous infection (LTBI) in adults.[23] However, low uptake of the Bacille Calmette-Guérin (BCG) vaccine in Pakistan,[24] along with very low uptake of LTBI treatment in children and adults[21] in a highly endemic setting, have likely impacted greater rates of TBME. Tuberculous ME has been shown to result in significant economic losses, and we also show longer hospital stays resulting from TBME. It

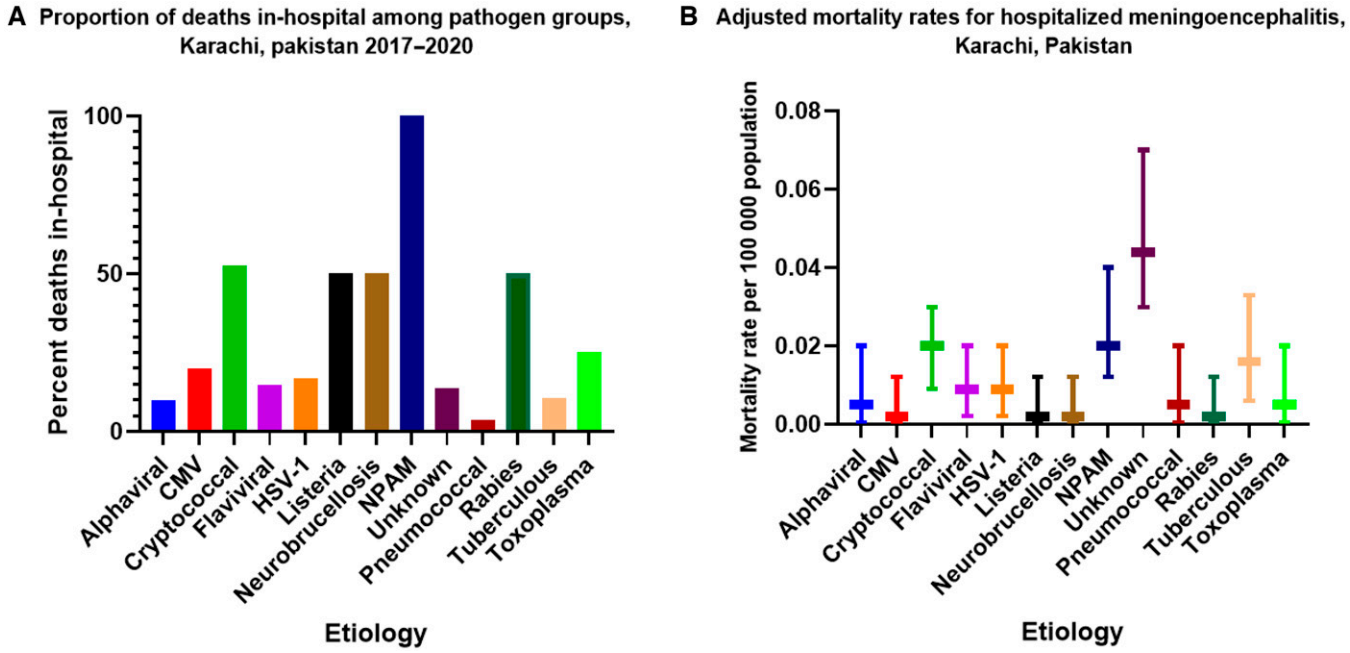

FIGURE 4. Death rates in hospital and population mortality rates (per 100,000) adjusted for transfers and losses to follow-up within the hospitalized meningoencephalitis cohort, Karachi, Pakistan, 2017–20. (**A**) Proportion of deaths in the hospital for specific pathogenic etiologies. (**B**) Adjusted mortality rates per 100,000. CMV = cytomegalovirus; HSV-1 = Herpes simplex virus 1; NPAM = *Naegleria* primary amebic meningoencephalitis.

follows that renewed BCG vaccination campaigns and an investment in strategies to improve the uptake and use of LTBI treatment, especially among high-risk groups, can reduce the current burden of TBME. Our results call for urgent public health action toward improving access to and upscaling strategies for the prevention of extrapulmonary tuberculosis in Pakistan.

Enteroviral and pneumococcal ME followed TBME as the next most frequent etiologies of hospitalized ME among all age groups. Although enteroviral ME predominated in pediatric age groups, pneumococcal ME was common across all age groups. Of note, one patient presented with enteroviral ME with acute flaccid paralysis, which may be associated with poliovirus and other enteroviruses (such as enterovirus D-68).[25] Because Pakistan remains a polio-endemic setting,[26] and the Filmarray ME test detects all species of EV (A–D, with Poliovirus 1–3 as part of EV serotype C),[27] it is unknown whether the infecting EV was a poliovirus or EV D-68. Serotyping and/or genomic analysis are not performed routinely; such information, however, remains critical and has public health implications.

Serotyping was also not performed for pneumococcal meningitis. The 10-valent pneumococcal conjugate vaccine (PCV-10) has been a part of the expanded program of immunization in Pakistan since 2013.[28] Recent analyses of colonizing serotypes among children from Pakistan have revealed serotype replacement[29]; however, serotype replacement among invasive pneumococcal disease has not been documented. Nevertheless, incomplete vaccination and suboptimal coverage of three-dose PCV-10 among children in Pakistan[30] indicate that much of the burden of the current ME and other invasive pneumococcal illnesses among children is preventable, especially as some nonvaccine serotype illnesses may be prevented through cross-protective immunity against vaccine serotypes.[31] The pneumococcal conjugate vaccine also exerts indirect effects in nonimmunized adults against invasive disease through reduced colonization among companion children.[32] Vaccination of at-risk adults is not a public-funded program in Pakistan, and there are also no data on the uptake of the polysaccharide or the conjugate vaccine among adults in Pakistan. With inadequate vaccination rates among both children and adults, the burden of pneumococcal ME among both adults and children therefore is likely to be reduced through improved rates of vaccination. Low rates of *Haemophilus* ME in this study reveal that the HiB vaccination program has had an impact on bacterial ME. We also report low rates of meningococcal meningitis. Previous reports of epidemics from Pakistan are rare, with the last epidemic reported from 1988,[33] and sporadic infection accounts for most cases. Recent data suggest that rates of sporadic disease have decreased during the past two decades.[34,35]

Another major preventable cause of ME is mosquito-borne flaviviral, alphaviral, and malarial ME. Currently, vector control interventions are triggered by outbreak reports, and are implemented through federal and provincial control programs in coordination with the malaria control program.[36] However, during the past 3 years, high caseloads and several reports of dengue outbreaks from Pakistan[37] highlight the inadequacy of current control measures. Moreover, most of the flaviviral ME cases were detected using dengue serology (NS1 antigen, IgM, or both). However, because of extensive cross-reactivity among flaviviruses, it is possible that other encephalitic flaviviruses such as West Nile virus or Japanese encephalitis are cocirculating. Additional PCR diagnosis of flaviviral ME is essential to inform epidemiology of flaviviral illness in Pakistan, especially as the availability of a dengue vaccine becomes imminent.

Public health measures to control amebic ME seem similarly inadequate. Several reports highlight the inadequacy of chlorination of the water supply,[9,38] and—coupled with rising temperatures—this creates ideal conditions for *N. fowleri* to proliferate in Karachi's water supply. Taken together, the low vaccination rates and poor public health measures together result in a large proportion of preventable ME.

The large proportion of infectious ME of unknown etiology suggests that the testing algorithms and/or the currently available tests for ME can be improved. As a result of study design limitations, we could not evaluate various testing algorithms and pathways, because testing was subject to physicians' discretion. It is clear, however, that uptake for certain tests such as Xpert Ultra for TBME and dengue serology could be improved through the validation of clinical algorithms triggering various testing pathways. Although a wider array of clinical and laboratory services and data linkages allowed us to determine infectious etiology from laboratory samples other than CSF (i.e., serum and nasopharyngeal samples for viral etiologies), improving the performance metrics of available tests and expanding the pathogen array range of panel tests to include contextually prioritized pathogens will not only improve surveillance, but also will provide opportunities for early diagnosis and improved management of most frequent ME etiologies. The minimization of patients with no pathogens identified can avert inappropriate empiric antibiotic and antiviral use in ME.

Data we present should be interpreted while keeping in mind the study design and other limitations. Risk factors presented provide insight into the characteristics of the study cohort; however, we urge caution in overinterpreting these data to assess the burden of risk factor-associated ME. Milder episodes of community-acquired ME may not have been captured in our hospital database study. However, hospital database studies are appropriate to determine ME burden, because facility-specific health-care use estimates for ME are likely to be greater than those for other febrile illnesses, as individuals and families are more likely to seek care for a serious illness such as ME.[39] Nevertheless, we caution readers about estimating population incidence from hospitalization rates, because there may be a significant proportion of ME cases occurring out-of-hospital, given that health-care access to populations remains limited.[40] Results we present for the burden of hospitalizations should be interpreted with limitations of the assumptions we have made in mind. Lacking differential health-care use data, our assumptions of the total hospitalization burden of 35% in the study hospital may be greater or less than the true burden. We also adjusted study data for a high LP deferral rate; however, published literature from Pakistan concurs with a high LP deferral rate. Because of inconsistencies in documentation of vaccination (status and antigens) medical records, and the unavailability of records of vaccinations performed elsewhere than the study hospital, we were unable to determine the proportion of vaccinated subjects within our cohort. We were also unable to report on post-ME disability and sequelae of ME. Disability scales or morbidity assessment scales were not reported consistently in the hospital notes. We were also unable to comment on the impact of prehospital antibiotic use on diagnostic yield, annual rates of hospitalization, or outcomes of ME.

Our study provides valuable insights to the prevention and control of ME in Pakistan. We expect these results to affect the development and validation of diagnostic and management algorithms for ME in hospitals, as well as public health measures to reduce the burden of preventable ME. Specifically, differential pathogen proportions and annual hospitalization rates should inform vaccination strategies for the prevention of pneumococcal disease in all ages, renewed vaccination efforts for tuberculosis, and public health measures to prevent mosquito-borne and waterborne illnesses. Timely development and implementation of such strategies can avert loss of DALYs resulting from ME, and can prevent major outbreaks.

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