The epidemiology of meningococcal meningitis: multicenter, hospital-based surveillance of meningococcal meningitis in Iraq

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ABSTRACT

Objectives: Outbreaks of Neisseria meningitidis have reached alarming levels due to the pathogen's ability to cause severe complications, presenting as meningitis or septicemia. Our study reports the results of the first wide-scale surveillance of meningococcal meningitis in Iraq.

Methods: The study included all consecutive cases of clinically suspected meningitis between June 2018 and May 2020 at 18 major hospitals around Iraq ($n = 2314$). Laboratory analysis of biological samples and real-time polymerase chain reaction tests were conducted to confirm bacterial etiology. Demographical and medical data were collected for statistical analysis.

Results: In total, 370 patients were confirmed to have bacterial meningitis (215 had N. meningitidis, 154 had Streptococcus pneumoniae, and one case had Haemophilus influenzae type b). The most common N. meningitidis serogroup was B (77.7%), followed by W (18.1%) and X (4.2%). The annual incidence rate of N. meningitidis per 100 000 population was 0.86, with the highest being in Karbala (1.52 per 100 000 population). Cases of meningococcal meningitis were more likely to occur in children younger than 15 (OR = 3.526), and in the winter (OR = 1.474).

Conclusions: Continuous surveillance of N. meningitidis is necessary in Iraq, and can only be achieved through improved detection methods. The incidence of meningococcal meningitis in Iraq warrants improved vaccination programs.

Introduction

Several bacterial organisms have been associated with causing meningitis in humans. Streptococcus pneumoniae, Haemophilus influenzae type b (Hib), and Neisseria meningitidis constitute the three major causes of vaccine-preventable bacterial meningitis. S. pneumoniae, a Gram-positive diplococcus, has been described as a major contributor to community-acquired meningitis outbreaks in Europe and the United States (Mook-Kanamori et al., 2011). Hib, a Gram-negative coccobacillus, also causes meningitis, particularly in children under the age of 5 (Khattak and Anjum, 2021). N. meningitidis, an encapsulated Gram-negative diplococcus, is known to cause severe clinical manifestations in the form of invasive meningococcal disease (IMD) (Cohn and MacNeil, 2015). IMD is caused by the ability of N. meningitidis to colonize the mucosal surface of the nasopharynx and invade sterile tissues, in-

cluding the meninges, bloodstream, and vital organs. Clinically, IMD often presents as meningococcal meningitis or septicemia that progresses rapidly, leading to death in 10–15% of cases, or to permanent disabilities in 20% of survivors (Parikh et al., 2020).

The effects of outbreaks of bacterial meningitis and IMD on lower-income communities can be particularly devastating due to the lack of appropriate capacities for quick diagnosis and therapy, as well as low vaccination rates. Epidemiological reports on N. meningitidis infections have been relatively lacking in the Eastern Mediterranean region, with only one publication from Iraq reporting two cases of N. meningitidis in Baghdad in 2013 (Razak et al., 2013). Due to the major demographic and socioeconomic changes that have occurred in the country in recent years, an investigation of the epidemiological patterns of meningococcal meningitis in Iraq was warranted, especially considering the intensifi-

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cation of religious mass gatherings, which are associated with increased risk of IMD outbreaks (Muttalif et al., 2019). While the government does require travelers arriving from endemic countries to provide proof of a quadrivalent vaccination prior to entry (The Ministry of Health of Iraq, 2019), these requirements do not account for certain disease serotypes; nor are they required for locals or arrivals from neighboring countries, many of whom participate in annual mass gathering events in Iraq.

For these reasons, our aim was to conduct a prospective surveillance study of meningococcal meningitis in Iraq. Reported herein are the results of our investigation, which took place between June 2018 and May 2020, highlighting the causative agents linked with bacterial meningitis in Iraq, the prevalent serogroups of *N. meningitidis*, and associated clinical features.

Methods

Study design and sites

This was a prospective hospital-based surveillance study aimed at estimating the etiology and incidence of vaccine-preventable bacterial meningitis, and identifying the most commonly circulating serogroups of *N. meningitidis* among the Iraqi population. The study team, in collaboration with the Iraqi Ministry of Health (MOH), identified and selected sites from multiple geographical regions in the country, including mid, northern, and southern provinces. The criteria for site inclusion considered respective catchment areas at each study site (having a minimum population of 12 million in all selected governorates), their accessibility, and their capacity to perform biological specimen collection via lumbar puncture.

The selected sites included 18 major hospitals: 12 in Al-Rusafa and Karkh districts in the governorate of Baghdad, and two hospitals each in the Karbala, Kirkuk, and Maysan governorates. Three investigators were assigned to each hospital and served as focal points to follow-up on data and specimen collection, and on specimen transportation.

Geographical information

Al-Rusafa is an Eastern district of the city of Baghdad, the capital of Iraq. It comprises a population of 4 830 885 according to latest census data provided by the Iraqi MOH. Karkh is the western district of Baghdad, and has a population of 3 550 260. Karbala is a central city in Iraq with a population of 1 283 484. Kirkuk governorate is in northern Iraq, and has a population of 1 682 809. Maysan is a southeastern governorate located on the border with Iran, with a population of around 1 171 802 (Kirmanc, 2013).

Patients

The investigators followed the guidelines and case definitions described by the World Health Organization (WHO) to identify patients with suspected bacterial meningitis (World Health Organization & Centers for Disease Control and Prevention (US), 2011). Patients were considered as suspected meningitis cases if they fulfilled the criteria of presenting with acute fever (> 38.5°C, rectal or 38.0°C, axillary), headache, and one of the following signs: neck stiffness, altered consciousness, or other meningeal signs. Consecutive cases, comprising patients of all ages, who were admitted at the selected study sites during the study period of June 1, 2018 to May 30, 2020, and who satisfied the case definition of suspected meningitis, were considered eligible for the study.

Data collection

An investigation form was developed by the Iraqi MOH using Epi Info^{TM} software (Atlanta, Georgia, USA). For each case, data collected included demographic characteristics (gender, age, area of residency),

clinical data (signs and symptoms, date of onset), cerebrospinal fluid (CSF) description, and patient status (alive or dead) at the end of the study. In addition, the laboratory test results, causative agent (for confirmed cases), subtype (if the causative agent was *N. meningitidis*), and antimicrobial treatment data were collected.

Specimen collection and confirmed diagnosis

CSF specimens were collected from the study participants upon hospital arrival. In rare cases when participants refused to provide a CSF sample, serum samples were obtained. Due to the limited PCR testing capacities in Iraqi hospitals at the time of the study, and to avoid delays in patient treatment, two samples were collected from each patient. One sample was processed on-site following routine laboratory examination protocols, including: culturing the bacterial pathogen from CSF or blood samples; Gram-stain identification; WBC, protein, and sugar assays; and CSF examination. Turbidity, leukocytosis (> 100 cells/mm^3), elevated protein levels (> 100 mg/dl), or decreased glucose (< 40 mg/dl) were considered to be indicative of probable bacterial meningitis and sufficient evidence to initiate antimicrobial treatment. The other CSF sample was sent to the Central Public Health Laboratory (CPHL) in Baghdad for detection of *N. meningitidis, S. pneumoniae*, and Hib by real-time polymerase chain reaction (RT-PCR), and for subtyping the *N. meningitidis*-positive specimens. The CPHL-Baghdad personnel followed the WHO and US Centers for Disease Control and Prevention's (CDC) RT-PCR testing protocol for confirming diagnosis of *N. meningitidis*. Results produced by the CPHL of Baghdad were relayed to primary healthcare teams for all participants. Biosafety, storage, and shipping for all specimens collected for this study also followed the WHO and CDC guidelines (World Health Organization & Centers for Disease Control and Prevention (US), 2011).

Ethical considerations

Ethical approval for this study was obtained from the Iraqi MOH. Given the anonymous nature of specimen collection, written consent was waived. In line with to the ministry's routine practices, informed consent for CSF and serum collection was obtained verbally from all adult participants and from the parents or guardians of all child participants; serum was collected if participants or guardians refused lumbar puncture. Collected data were coded and stored in a secure database. The participants were informed that no personal identifiers would be disseminated.

Statistical analysis

Statistical analyses were performed using IBM SPSS version 24. Quantitative data were described using medians, means, and standard deviations. Categorical variables were described using frequencies and percentages. Annual incidence rates were calculated with a denominator representing the population of the catchment area, based on the most recent census data. Chi-square and Fischer's exact tests were used to compare the associations between different categorical variables and cases of *N. meningitidis* with those of *S. pneumoniae*. Additionally, univariate logistic regression was used to identify risk factors associated with *N. meningitidis* cases. Significant factors were then entered into a multivariate regression model. A *p*-value < 0.05 was considered as statistically significant for all statistical tests.

Results

Patients with suspected meningitis

Patient demographics and characteristics

In total, 2314 patients with clinically suspected meningitis were admitted to the 18 designated hospitals from the four Iraqi governorates
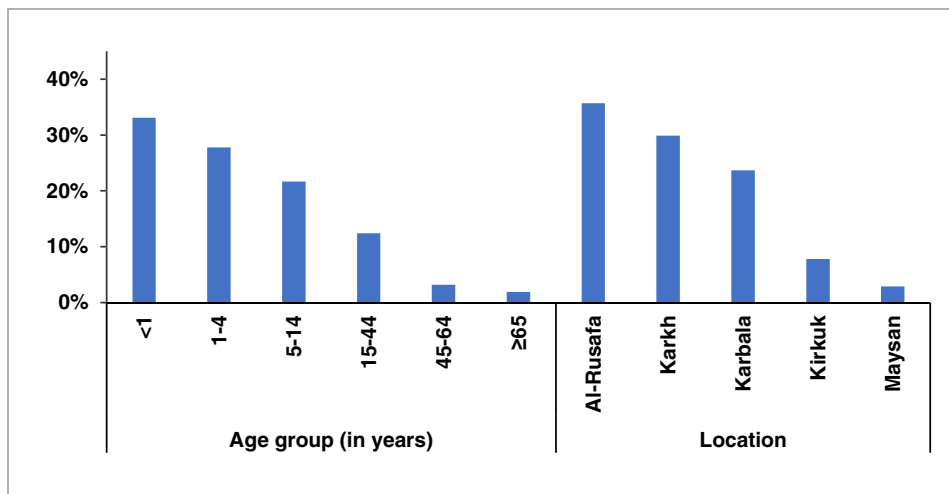
Figure 1. Distribution of patients with suspected meningitis in Iraq, according to age and location, June 2018 to May 2020.
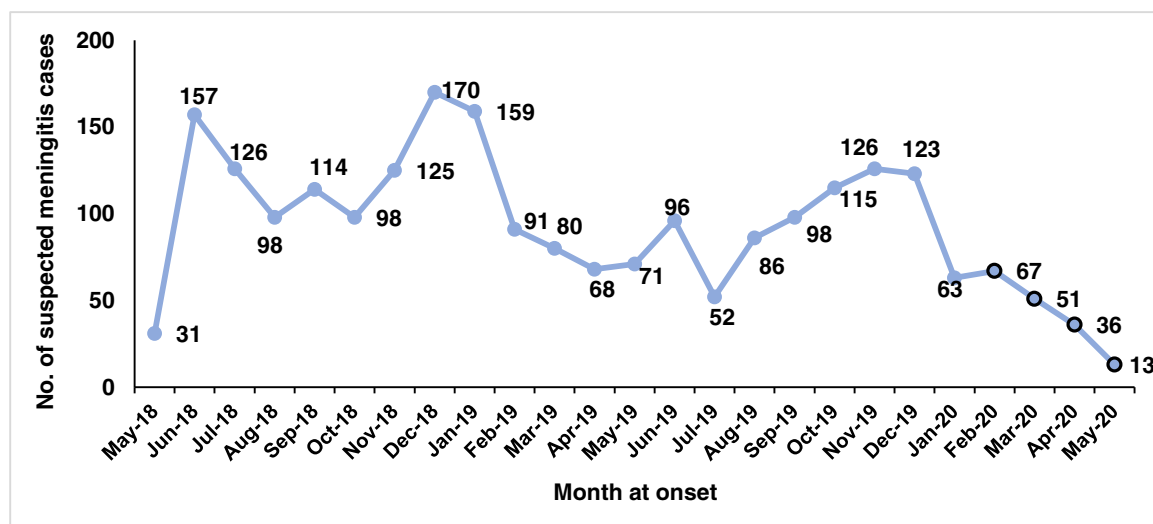

Figure 2. Frequency of suspected meningitis cases by month of onset, June 2018 to May 2020. Data points outlined in black signify cases that occurred after the implementation of COVID-19 regulations.

during the study period. Their ages ranged from 3 days to 91 years, with a median age of 2 years (mean ± SD = 8.8 ± 15.0 years). Of the total suspected cases, 33.1% were less than 1 year old on hospital arrival, while 27.8% were between 1 and 4 years, and 21.7% were aged between 5 and 14 years. Of the suspected cases, 826 (35.7%) were reported in Al-Rusafa, 693 (29.9%) in Karkh, 548 (23.7%) in Karbala, 180 (7.8%) in Kirkuk, and 67 (2.9%) in Maysan (Figure 1).

Distribution of suspected meningitis cases over time

The highest number of cases (N = 180) was recorded in December 2018; this declined to 13 cases in May 2019. The number of suspected cases declined from February 2020 through the rest of the surveillance period. This decrease in case admissions coincided with logistical issues that occurred during the COVID-19 pandemic, and affected the delivery of samples between the governorates and the CPHL of Baghdad, reducing the overall number of participants (Figure 2).

Bacterial meningitis

Causative agents

Of all the cases of suspected meningitis, 370 (16.0%) were confirmed by the CPHL of Baghdad to have bacterial meningitis. Of these, 215 cases

(58.1%) were *N. meningitidis*, 154 (41.6%) were *S. pneumoniae*, and only one case (0.03%) was caused by Hib, as confirmed by RT-PCR. Among the *N. meningitidis* cases, the most common serogroup was B (n = 167, 77.7%), followed by W (n = 39, 18.1%), and X (n = 9, 4.2%). None of the *N. meningitidis* cases were of the C or Y serogroups.

Patient characteristics and risk factors of meningococcal disease

Almost two thirds of the patients diagnosed with *N. meningitidis* (63.2%) or *S. pneumoniae* (61.0%) were males. Patients with *N. meningitidis* were aged between 2 months and 36 years, with a median of 5 years (mean ± SD = 6.5 ± 6.6 years), and the majority (90.7%) were younger than 15. The age range of patients with *S. pneumoniae* was between 1 week and 40 years, with a median of 0.8 years (mean ± SD = 2.5 ± 5.1 years). The majority (83.7%) of patients diagnosed with *S. pneumoniae* were less than 5 years old, with 58.4% being less than 1 year old. While the percentage of infants diagnosed with *S. pneumoniae* was higher than that of those with *N. meningitidis* (7.9%), children aged between 1 and 14 years were more likely to be diagnosed with *N. meningitidis* than with *S. pneumoniae* (82.8% vs 38.3%, p < 0.001) (Table 1).

Children younger than 15 had a high association with meningococcal meningitis compared with those aged 15 years or older (odds ratio (OR) = 3.526, 95% CI = 4.706–12.643, p < 0.001), and patients aged ≥ 1 year were more associated with *N. meningitidis* diagnosis compared

Table 1

The distribution of *N. meningitidis, S. pneumoniae* and *H. influenzae* cases according to patient characteristics

Characteristics	*N. meningitidis* n=215	*S. pneumoniae* n=154	Hib n= 1	Total n=370	*p* value
Age (years)					
Mean ± SD	6.6 ± 7	2.4 ± 5.2	6 months	9.7 ± 15.0	
Range	2 months- 36 years	17 days – 40 years		17 days – 40 years	
Age group-n (%)	<.001*				
<1 year	17 (7.9%)	90 (58.4%)	1	108 (29.2%)	
1- 4	89 (41.4%)	39 (25.3%)		128 (34.6%)	
5-14	89 (41.4%)	20 (13.0%)		25 (6.8%)	
15-44	20 (9.3%)	5 (3.2%)		109 (29.5%)	
Sex-n (%)	.665				
Males	136 (63.3%)	94 (61%)	1	231 (62.4%)	
Females	79 (36.7%)	60 (39%)		139 (37.6%)	
Health Directorates-n (%)	.604				
Al-Rusafa	93 (18.1%)	56 (36.4%)		149 (40.3%)	
Karkh	63 (29.3%)	46 (29.9%)		109 (29.5%)	
Karbala	39 (18.1%)	40 (26.0%)	1	80 (21.6%)	
Kirkuk	19 (8.8%)	10 (6.5%)		29 (7.8%)	
Maysan	1 (0.5%)	2 (1.3%)		3 (0.8%)	
Antibiotic prior to admission-n (%) [a]	.910				
Yes	81 (37.7%)	61 (39.6%)		142 (38.4%)	
No	105 (48.8%)	77 (50%)		182 (49.2%)	
Unknown	29 (13.5%)	16 (10.4%)	1	46 (12.4%)	
Reported signs and symptoms-n (%)					
Fever	203 (94.4%)	146 (94.3%)	1	350 (94.6%)	1.00
Vomiting	142 (66.0%)	86 (55.8%)	1	229 (61.9%)	.0510
Seizures	93 (43.3%)	77 (50%)	1	171 (46.2%)	.2058
Irritability	86 (40%)	71 (46.1%)	1	158 (42.7%)	.2857
Headache	102 (47.4%)	39 (25.3%)		141 (38.1%)	<.001*
Neck rigidity	98 (45.6%)	49 (31.8%)		147 (39.7%)	.0096*
Altered consciousness	46 (21.4%)	41 (26.6%)		87 (23.5%)	.2642
Bulging fontanelle	25 (11.6%)	30 (19.5%)		55 (14.9%)	.0392*
Kernig's sign	39 (18.1%)	11 (7.1%)		50 (13.5%)	.0020*
Purpura	14 (6.5%)	4 (2.6%)		18 (14.9%)	.0929

[a]Unknown cases were excluded from analysis. * Denotes statistical significance level corresponding to a *p* value <.05. Statistical comparisons were made of *N. meningitidis* and *S. pneumoniae* cases only.

Table 2

Univariate and multivariate logistic regression models showing the risk factors for confirmed diagnosis of *N. meningitidis*.

Variable	Univariate analysis		Multivariate analysis	
	OR (95% CI)	***p*-value**	**OR (95% CI)**	***p*-value**
Age group	1.990 (1.298–3.051)	0.001*	3.526 (4.706–12.643)	< 0.001*
Age <15 vs ≥ 15				
Infancy	4.330 (2.771–6.764)	< 0.001*	7.714 (2.188–5.684)	< 0.001*
Age ≥ 1 vs < 1				
Season at onset	1.150 (1.034–1.279)	0.004*	1.474 (1.094–1.985)	0.011*
Winter vs non-winter seasons				
Spring seasons	0.849 (0.565–1.279)	0.434		
Fall seasons	0.935 (0.685–1.276)	0.670		
Summer seasons	0.720 (0.513–1.011)	0.058		
Gender	1.116 (0.932–1.598)	0.218		
Male vs female				

*Denotes the statistical significance level corresponding to a *p*-value < 0.05. OR = odds ratio; 95% CI = 95% confidence interval.

with infants aged < 1 year (OR = 7.714, 95% CI = 2.188–5.684, *p* < 0.001) (Table 2). Gender did not differ significantly between cases of *N. meningitidis* and *S. pneumoniae* (*p* = 0.665) (Table 1) and was not significantly associated with meningococcal meningitis (OR = 1.116, 95% CI = 0.932–1.598, *p* = 0.218) (Table 2).

Most of the patients who were diagnosed with bacterial meningitis presented with fever and vomiting (94.6% and 61.9%, respectively). Less than half of the patients suffered from seizures (46.2%) or irritability (42.7%) at presentation. Headaches, neck rigidity, and Kernig's sign were significantly more likely to be reported by patients with *N. meningitidis* than by patients with *S. pneumoniae* (*p* < 0.001, *p* = 0.0096, and *p* = 0.0020, respectively). Only bulging fontanelle was significantly associated with *S. pneumoniae* cases (19.5% vs 11.6%, *p* = 0.0392) (Table 1).

The distribution of cases per month of onset revealed seasonal variations in the numbers of cases per causative agent (Figure 3). Of note, cases of *N. meningitidis* peaked significantly during the winter months (December, January, and February). Of a total of 215 *N. meningitidis* cases, 82 (38.1%) occurred during the winter, followed by 60 (27.9%) in the fall, 46 (21.4%) in the summer, and 27 (12.7%) in the spring (Figure 3). Indeed, logistic regression analysis revealed that the winter months corresponded to increased odds of 1.474 for meningococcal meningitis (95% CI = 1.094–1.985, *p* = 0.011), while the other seasons were not significantly associated (Table 2). On the other hand, the highest number of *S. pneumoniae* cases occurred during the fall season, with a total of 76 cases (49.4%), followed by 43 in the winter (27.9%), 24 (15.6%) in the summer, and 11 cases (7.1%) in the spring
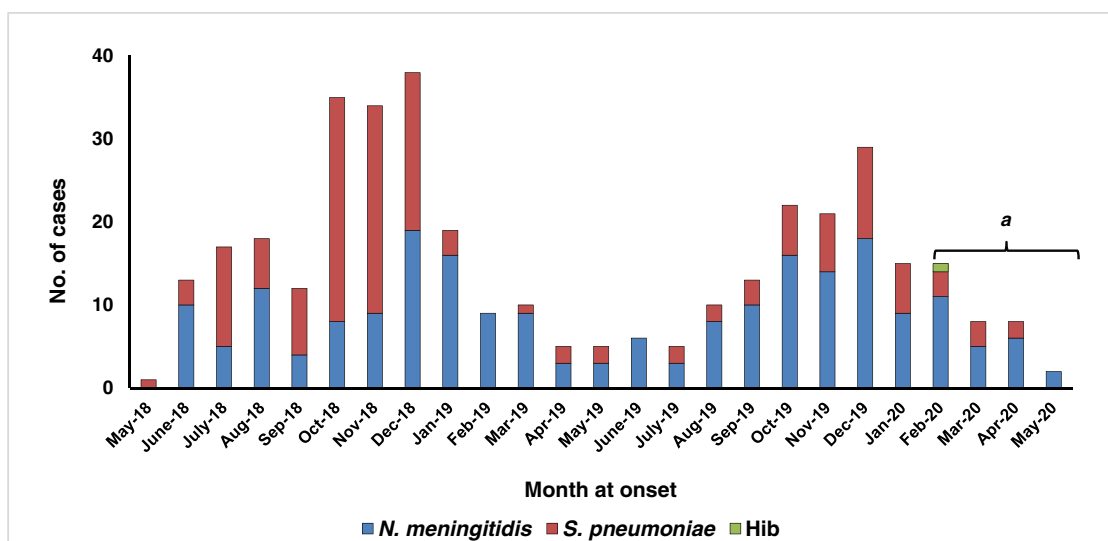
Figure 3. Distribution of confirmed cases of bacterial meningitis according to causative agent, June 2018 to May 2020. [a]Indicates the period of time when COVID-19 regulations were implemented in Iraq.

Table 3

The annual incidence rate of laboratory-confirmed bacterial meningitis per 100 000 populations according to governorate

Governorate	Population	N. meningitidis		S. pneumoniae		Laboratory-confirmed bacterial meningitis	
		Number of cases	Annual IR[a]	Number of cases	Annual IR	Total	Annual IR
Karkh	3 550 260	63	0.89	46	0.65	109	1.54
Al-Rusafa	4 830 885	93	0.96	56	0.58	149	1.54
Karbala	1 283 484	39	1.52	40	1.56	79	3.08
Kirkuk	1 682 809	19	0.56	10	0.30	29	0.86
Maysan	1 171 802	1	0.04	2	0.09	3	0.13
Total	12 519 240	215	0.86	154	0.62	369	1.47

[a] Annual incidence rate (IR) is per 100 000 population.

of our study period. The sole case of Hib was recorded in February 2020 (Figure 3).

Incidence rates

Overall, the annual incidence rate (IR) of laboratory-confirmed bacterial meningitis in Iraq was 1.47 per 100 000 population. The annual IR was 0.86 per 100 000 population for meningococcal meningitis and 0.62 per 100 000 population for pneumococcal meningitis. The annual IR per 100 000 population for bacterial meningitis ranged between a 0.13 in Maysan to 3.08 in Karbala. The highest annual IRs of *N. meningitidis* and *S. pneumoniae* were detected in Karbala (1.52 and 1.56, respectively) and the lowest rates were found in Maysan, with *N. meningitidis* and *S. pneumoniae* IRs of 0.04 and 0.09, respectively (Table 3).

Characteristics of patients with different serogroups of N. meningitidis

All patients diagnosed with *N. meningitidis* were under 36 years of age, with the youngest being only 2 months old at diagnosis; the median age at the time of diagnosis was 4 years. For *N. meningitidis* serogroup B (MenB), the median age at diagnosis was 2 years (range: 6 months to 36 years), while the median age for MenW was also 2 years (range 2 months to 24 years). The median age for MenX was 3 years (range 4 months to 12 years). Most patients with MenB (83.2%), MenW (82.0%), and MenX (77.8%) were between 1 and 15 years old. Patients with MenB and MenW were predominantly males (62.9% and 71.8%, respectively), while patients with MenX were predominately females (66.7%) (Table 4).

Mortality

Of the 2314 patients with suspected meningitis, 40 (1.7%) died post diagnosis. Of the total deaths, five were patients diagnosed with bacterial meningitis (two with *S. pneumoniae,* two with MenB, and one patient with MenW). The case-fatality rate was 2.3% for patients with *N. meningitidis* and 1.3% for patients with *S. pneumoniae.*

Discussion

Our study was the first extensive investigation of vaccine-preventable meningitis in Iraq. It showed that bacterial meningitis is endemic and is impacting a significant number of individuals in the country. Our results also showed that the etiological distribution differed by age, with meningococcal meningitis cases more common in older children compared with streptococcal meningitis, which was more common in infants. The distribution of IMD cases was also higher during the winter months compared with other months of the year, and were mostly caused by serogroup B, with lower occurrences of serogroups W and X.

Increased odds of meningococcal meningitis were observed among children younger than 15 when compared with patients who were 15 or older ($p < 0.001$), which, combined with the reduced risk of the disease among infants, presents an urgent call for increased vaccination of Iraqi youths. This need for childhood meningitis vaccination is supported by CDC recommendations that meningococcal vaccines be administered to preteens aged 11–12 (Robinson et al., 2019).

When comparing the clinical presentations of patients with *N. meningitidis* with those associated with *S. pneumoniae,* it was found that that while fever, being a criterion for case suspicion, was present in almost

Table 4
Characteristics of patients with *N. meningitidis* according to serogroup.

N. meningitidis serogroup	MenB *N* = 167		MenW *N* = 39		MenX *N* = 9	
Variable	*N*	%	*n*	%	*n*	%
Age (years)						
< 1	12	7.2%	3	7.7%	2	22.2%
1–4	69	41.3%	19	48.7%	1	11.1%
5–14	70	41.9%	13	33.3%	6	66.7%
15–44	16	9.6%	4	10.3%	0	0.0%
Sex						
Female	62	37.1%	11	28.2%	6	66.7%
Male	105	62.9%	28	71.8%	3	33.3%
Health directorate						
Karbala	31	18.6%	7	17.9%	1	11.1%
Karkh	56	33.5%	6	15.4%	1	11.1%
Kirkuk	16	9.6%	2	5.1%	1	11.1%
Maysan	1	0.6%	0	0.0%	0	0.0%
Al-Rusafa	63	37.7%	24	61.5%	6	66.7%
Reported signs and symptoms						
Fever	160	95.8%	34	87.2%	9	100.0%
Vomiting	110	65.9%	24	61.5%	8	88.9%
Seizures	72	43.1%	16	41.0%	5	55.6%
Irritability	69	41.3%	15	38.5%	2	22.2%
Headache	83	49.7%	16	41.0%	3	33.3%
Neck rigidity	78	46.7%	17	43.6%	3	33.3%
Altered consciousness	34	20.4%	11	28.8%	1	11.1%
Bulging fontanelle	22	13.2%	2	5.1%	1	11.1%
Kernig's sign	32	19.2%	7	17.9%	0	0.0%
Purpura	12	7.2%	2	5.1%	0	0.0%

all cases of both infections, neck stiffness and headaches were more highly associated with *N. meningitidis.* This suggests that patients with *N. meningitidis* infections are more likely to present with the conventional signs of meningitis: the triad of fever, headache, and neck rigidity (Hausdorff et al., 2007). In addition, purpura, bulging fontanelle, and Kernig's sign are all regarded as signs of IMD in the literature (Bénard et al., 2016). In our cohort, bulging fontanelle was associated more with cases of *S. pneumoniae,* while Kernig's sign and purpura were more frequently observed with cases of *N. meningitidis* ($p = 0.002$ and $p = 0.0929,$ respectively). It is also worth noting that patients with MenB presented with purpura, bulging fontanelle and Kernig's signs more frequently than did the other serogroups, and that none of the MenX patients presented with either purpura or Kernig's. Taken together, these observations suggest MenB as a predictor for IMD manifestation in our patients, in addition to their inherent value in improving the diagnosis of suspected cases by serving as visual signs of possible IMD for clinicians and civilians of at-risk communities.

The significance of these observations is enhanced further by most of the IMD during the study period being MenB cases (77.7%) and by the lack of access to vaccinations against the B serogroup in Iraq. In fact, the current Iraqi national immunization program only provides the MenACWY vaccine to citizens traveling to Mecca for Hajj, and only requires proof of vaccination from travelers arriving from countries of the 'African belt' (The Ministry of Health of Iraq, 2019), which could also explain the lack of MenC cases and the low incidence of MenW.

In investigating the burden of meningococcal meningitis in Iraq, our results also revealed the incidence of *S. pneumoniae*-induced meningitis, corresponding to 41.6% of cases. Pneumococcal meningitis poses particular public health challenges due to its increasing resistance to many classes of antibiotic (Peyrani et al., 2019). There are vaccines targeting *S. pneumoniae,* namely the PCV13 and PPSV23 pneumococcal vaccines (Shapiro et al., 1991), but they are not yet included in Iraqi vaccination programs, due to cost considerations and the lack of updated data on incidence rates prior to conducting this study. On the other hand, the apparent scarcity of Hib infections may be a result of national vaccination efforts by the Iraqi MOH, starting in 2012 (Hikmat and Abdulmunem, 2012), which has been essential due to the high mortality rate of Hib among children (Hausdorff et al., 2007).

Our results showed both temporal and geographical trends in the incidence of bacterial meningitis in Iraq. Cold temperatures coincided with a rise in *N. meningitidis* infections, as the largest percentages of cases were observed in the winter months of 2019 and 2020 ($p = 0.011$), a trend that has been observed in previous reports (Palmgren, 2009; Vescio et al., 2015). Moreover, our study found that the annual IRs per 100 000 population for both *N. meningitidis* and *S. pneumoniae* were highest in Karbala. In fact, at 3.08, the IR of bacterial meningitis in Karbala was twice the IR rate of 1.54 per 100 000 population recorded both in Karkh and Al-Rusafa. Karbala also recorded an annual IR rate that was almost 24 times the IR of the city with the lowest portion of cases — Maysan — and twice the overall IR per 100 000 population of Iraq as a whole. The relatively high number of cases in the city of Karbala may be explained by the 'Arba'een' pilgrimage, which takes place annually in Karbala. Each year it is estimated that 17–21 million Shi'ite Muslims from Iraq and close to 3 million people from neighboring countries flock to the city on foot to visit the holy shrine of Imam Hossein (Al-Ansari et al., 2021), creating dense mass gatherings that are conducive to *N. meningitidis* and *S. pneumoniae* transmission. The Arba'een pilgrimage took place on October 30, 2018 and on October 19, 2019 during our study period. However, our investigators could not make conclusive claims as to whether the spikes of cases in Karbala were due to the pilgrimage, since the study participants were not asked if they attended the event. It would nonetheless be interesting to investigate whether the ceremony is indeed linked with IMD in future studies. In the same regard, a study of IMD and mass gatherings revealed that in 2001 and 2002, 249 Hajj and Umrah pilgrims contracted IMD in Mecca, and this coincided with increased IMD cases overall in the Gulf region. The numbers later decreased to 17 Hajj and Umrah pilgrims between 2002 and 2011 after increased vaccination campaigns were implemented in Saudi Arabia and other Gulf countries (Muttalif et al., 2019).

Most importantly, the results of our study reflect the urgent need for improvements in testing and surveillance capacities in Iraq. At the time of study initiation, the designated hospitals in each of the study sites relied on routine testing to identify and treat cases they suspected of having bacterial meningitis. The RT-PCR protocol was newly introduced to the MOH by the study team, who arranged training workshops for the staff of the CPHL in Baghdad prior to study initiation. When comparing

RT-PCR with the methods used by the hospital laboratories included in this study, it was apparent that the routine analyses were insufficient, due to their lower sensitivity, an observation that has been corroborated by previous reports (Sacchi et al., 2011; Wu et al., 2013). From the 370 patients who had PCR-confirmed bacterial meningitis, 172 samples were not cultured successfully, 59 did not show any bacterial organisms by Gram-stain, and 204 were classed as 'clear'. Unfortunately, due to limited resources, it is improbable that all hospital laboratories in the country will introduce PCR testing capabilities to improve diagnosis and case management. For this reason, the study team recommended to the Iraqi MOH the implementation of latex agglutination testing in public hospitals of the country, in addition to their routine analyses, to improve the diagnosis and etiological identification of meningitis. Establishing a system of referral, similar to that implemented for this study, to the CPHL of Baghdad, could also prove useful for the future surveillance of IMD cases in Iraq.

In conducting this study, our investigators faced several limitations. First, with the advent of the COVID-19 pandemic, governmental regulations reducing inter-district travel began on January 2020 (the first case of COVID-19 in Iraq was reported on February 24, 2020). This affected subject enrollment because the shipment of CSF samples to Baghdad was disrupted, and thus the resultant drop in cases of suspected meningitis during the last 3 months of our study period may have unintentionally affected the analyses. Additionally, 844 (36.5%) of the 2314 patients with suspected meningitis reported obtaining antimicrobial treatments, which are available as over-the-counter medication in the country, prior to hospital admission. This may have led to an underestimation of the true incidence of bacterial meningitis in Iraq. Moreover, future studies could benefit from collecting data on patients' long-term morbidity and mortality, which would provide helpful insights into the prognosis of meningococcal meningitis in the Iraqi population.

Our study was yet another demonstration of the need for improved surveillance of infectious diseases in lower- to middle-income countries. Ultimately, the results of our study provided various new insights into the epidemiological landscape of bacterial meningitis in Iraq. Having a better understanding of the frequency and etiology of meningococcal meningitis in Iraq will be of benefit to public health officials in prioritizing programs aimed at managing the spread of the disease. In particular, the discovery of its seasonal variations, its high incidence rate in Karbala, and the prevalence of MenB provide incentives for intervention, and highlight the urgency for increased vaccination and improved laboratory detection methods.

Contributions

TS, AB, AM, AA, RA, and SM contributed to the study design and implementation. IA and SI conducted data cleaning and analysis. RA, AD, and MQ contributed to lab work and data collection. TS, SI, and AA wrote the final manuscript.

Potential competing interests

AA and AD are employees of Sanofi Pasteur. All other co-authors report no competing interests.

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Declaration of interests

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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