RESEARCH PAPER

Hospital-based Surveillance of Invasive Pneumococcal Disease and Pneumonia in South Bangalore, India

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Objective: To estimate the incidence of invasive pneumococcal disease and pneumonia, distribution of pneumococcal serotypes, and antibiotic susceptibility in children aged 28 days to <60 months.

Design: Hospital-based surveillance.

Setting:; South Bangalore, India.

Participants: 9950 children aged 28 days to <60 months with clinical suspicion of invasive pneumococcal disease or pneumonia.

Results: The estimated at-risk population included 224,966 children <5 years of age. Forty cases of invasive pneumococcal disease were identified. Estimated invasive pneumococcal disease incidence was 17.8/100,000 with incidence being highest among children aged 6 months to <12 months (49.9/100,000). Clinical pneumonia syndrome was the most frequent diagnosis (12.5/100,000). Pneumococcal serotypes included: 6A (*n*=6, 16.7%); 14 (*n*=5, 13.9%); 5 (*n*=4, 11.1%); 6B (*n*=4, 11.1%); 1, 18C, and 19A (*n*=3 each, 8.3%); 9V (*n*=2, 5.6%); and 3, 4, 10C, 18A, 18F, and 19F (*n*=1 each, 2.8%). Serotypes 6A, 14, 6B, 1, 18C, 19A, 9V, 4, 10C, and 18A showed antibiotic resistance. Clinical pneumonia incidence was 2109/100,000, with incidence being highest among children aged 28 days to <6 months (5033/100,000). Chest radiograph-confirmed pneumonia incidence was 1114/100,000, with incidence being highest among children aged 28 days to <6 months (2413/100,000).

Conclusion: Invasive pneumococcal disease and pneumonia were found to be common causes of morbidity in young children living in South Bangalore, India.

Keywords: *Epidemiology, Streptococcus pneumoniae, Surveillance*.

Globally, approximately 541,000 children aged less than 5 years die from pneumococcal disease annually with a disproportionate number of deaths occurring in developing countries [1]. In India, recent data examining the overall incidence of invasive pneumococcal disease and pneumococcal serotype distribution are scarce.

Pneumococcal conjugate vaccines are not currently included in the national immunization program in India. To evaluate the health impact of a national immunization program with pneumococcal conjugate vaccine, it is crucial to assess disease burden and serotype distribution before vaccine introduction [2]. Pneumonet (Pan Asia Epidemiologic Surveillance Network) is a 2-year hospital-based surveillance study that assessed the burden of invasive pneumococcal disease in India, Thailand, Indonesia, and the Philippines. The primary objective was to estimate incidence rates of invasive pneumococcal disease and pneumococcal serotype distribution in children aged 28 days to less than 60 months. For each area of surveillance, the secondary objectives were to estimate incidence rates of clinical pneumonia and chest radiograph-confirmed pneumonia; to estimate case fatality rates for invasive pneumococcal disease and clinical and chest radiograph-confirmed pneumonia; describe antibiotic resistance rates of invasive pneumococcal isolates and serotype distribution of these resistant isolates; assess neurological sequelae of pneumococcal meningitis; and describe the risk factor distribution for invasive pneumococcal disease.

Accompanying Editorial: Pages 199-200.

METHODS

This was a hospital-based study with 3 participating hospitals in South Bangalore, India: Kempegowda Institute of Medical Sciences Hospital, Vanivilas Hospital, and Indira Gandhi Institute of Child Health. These are the main hospitals catering to children from South Bangalore, and were chosen based on their ability

to conduct the study assessed by a pre-determined set of criteria. The study was approved by the institutional review board/independent ethics committee. The parent(s) or legal guardian(s) of participants completed the informed consent process.

Final case diagnoses were recorded either on Day 10 or the day of hospital discharge, whichever occurred first. Case definitions were as follows:

- Sepsis: Presence of danger signs (unable to drink, lethargy, hypothermia, severe malnutrition, convulsions) with or without pneumonia clinical syndrome (meningitis excluded).
- Meningitis clinical syndrome: At least one of the following usually with fever: stiff neck, altered or decreased consciousness, bulging fontanelle, toxic appearance, lethargy, poor sucking and irritability, petechial or purpural rash, convulsions [3,4]
- Invasive pneumococcal disease (IPD) Isolation of *S. pneumoniae* from a sterile body site
- Definite pneumococcal meningitis: Isolation of *S. pneumoniae* by culture from the cerebrospinal fluid (CSF) or a blood culture of a subject with >10 white blood cells per 1 μL of CSF, and CSF protein >100 mg/dL or CSF glucose <40 mg/dL; or glucose CSF-to-serum ratio <0.6; or a positive result from a nonculture method for identification of *S. pneumoniae* in the CSF [3,4].
- Probable pneumococcal meningitis: CSF with >10 white blood cells per 1 μl and other evidence suggestive of pneumococcal infection; or isolation of *S. pneumoniae* in the blood by culture or nonculture method in a subject admitted for meningitis clinical syndrome, who lacked the CSF characteristics for definite pneumococcal meningitis; or CSF not obtained [3,4].
- Clinical pneumonia syndrome: Tachypnea (>50 breaths/minute if subject was aged <12 months or >40 breaths/minute if subject was aged 12 months to <60 months) and/or cough and/or difficulty breathing.
- Chest radiograph (CXR)-confirmed pneumonia: Lobar consolidation and/or pleural effusion [3].
- C-reactive protein (CRP) + CXR-confirmed pneumonia: CXR-confirmed pneumonia, or clinical pneumonia with an abnormal CXR result plus a CRP value ≥40 mg/L [4].
- Bacteremia: Isolation of bacteria from the blood; *S. pneumoniae* was isolated from the blood for pneumococcal bacteremia.

The study enrolled a sample of convenience consisting of all children aged 28 days to 36 months residing in the surveillance area from February 27, 2009 to February 26, 2011, who presented to any of the study hospital with a measured temperature or history of measured temperature ≥39.0°C within 24 h prior to screening; or with clinical suspicion of pneumonia, meningitis, sepsis, or other invasive pneumococcal disease (*e.g.,* arthritis, osteomyelitis, peritonitis), regardless of temperature; and children aged >36 months to <60 months with clinical suspicion of pneumonia, meningitis, sepsis, or other invasive pneumococcal disease, regardless of temperature. Signs and symptoms used to identify suspected meningitis included fever, headache, stiff neck, nausea, vomiting, seizures, and lethargy. Signs and symptoms of suspected pneumonia included fever, cough, shortness of breath, rapid breathing, sputum production, lack of appetite, and lethargy. Signs and symptoms of sepsis included fever, lethargy, and lack of appetite. Children with suspected or diagnosed dengue fever, malaria, or chronic or relapsing/recurring febrile illness, with or without laboratory confirmation, were excluded.

For each subject, up to 3 study visits were planned. The first visit was for screening and enrolment. The second visit was for collection of data related to final diagnosis and outcome. This visit was performed either on Day 10 after enrollment or on the day of hospital discharge (whichever came first) in hospitalized subjects, all subjects whose cultures yielded *S. pneumoniae*, and those in whom there was clinical suspicion of meningitis. The third visit was performed 3-4 months after enrollment in subjects with a final diagnosis of probable or definite pneumococcal meningitis. At this follow-up visit, a neurologic examination was conducted for evaluation of neurologic sequelae.

Sample collection and laboratory testing: In all subjects who met the inclusion criteria, a medical history was taken by the treating physician, risk factors for invasive pneumococcal disease were recorded by interviewing the parents, and a blood specimen was collected. Risk factors recorded included residence (urban/not urban), antibiotic use in the previous 7 days, use of herbal or traditional medicine in the previous 7 days, attendance at daycare or kindergarten, smokers in the household, exposure to cooking smoke, malnutrition, number of individuals in the household, number of children <60 months, number of sleeping rooms in the house, and presence of underlying medical illnesses or chronic conditions known to increase the risk for pneumococcal disease. CSF was collected for culture when meningitis was suspected. Specimens from other sterile sites (*e.g.,* pleural fluid) were collected as per

routine medical practice. Blood, CSF, and pleural fluid cultures were performed at the Central Research Laboratory, Kempegowda Institue of Medical Sciences by using BACTEC 9050 (Becton Dickinson, Gurgaon, Haryana, India). *S. pneumoniae* isolates were subcultured and sent to a central laboratory for confirmation of identification, and serotyping using type-specific sera by Quellung reaction. Antibiotic susceptibility testing was performed at a central laboratory for penicillin, amoxicillin, ampicillin, erythromycin, trimethoprim-sulfamethoxazole, ceftriaxone, levofloxacin and vancomycin was carried out using standard methodology, including microbroth dilution and/or E-test. A chest radiograph was obtained in children with clinically-suspected pneumonia. In addition, blood specimens from children with clinically-suspected pneumonia were tested for CRP.

All blood, CSF, and pleural fluid specimens were stored and tested using polymerase chain reaction (PCR) for nonculture identification of *S. pneumoniae*. Samples were stored at the local laboratory at –80°C and were transferred to the testing laboratory by packing on dry ice. Only those blood samples not exceeding 3 months of storage since sample collection were shipped and tested. Whole blood and CSF were tested from children with meningitis, and whole blood and pleural fluid were tested from children with pneumonia. For PCR analysis, purified DNA from each specimen was first tested with duplex PCR to detect *lytA* and *ply* genes. Specimens with high concentrations of these genes were tested using 13 serogroup/serotypes real-time PCR assays. Samples were considered PCR positive for each serotype based on qualitative results. If a specimen tested positive in the *lytA* assay but negative in the 13 serogroup/serotype specific assays, the specimen was assigned a non-13-valent pneumococcal conjugate vaccine serotype. Each PCR assay contained PCR positive and negative controls, extraction negative controls, and extraction/detection positive controls (*S. pneumoniae* type 1).

Quality control and assurance checks included periodic monitoring of surveillance activities. During these site visits, information recorded in case report forms for individual subjects was verified against source documents. In addition, trained research staff conducted a daily review of screening logs to ensure complete and accurate documentation of eligible subjects. Investigators also reviewed computerized patient lists from outpatient clinics and emergency department lists to identify patients who may be eligible for study enrolment. Regular meetings were held with practitioners in the study area to encourage referral of patients for evaluation and possible study enrollment. An external surveillance system monitor visited the study site each month to conduct a weeklong assessment of the quality of patient capture among those patients with suspected meningitis, pneumonia, and sepsis who were treated at each study hospital.

Statistical analysis: Incidence rates with 95% confidence intervals (CIs) were determined based on numbers of cases identified by the current study and the at-risk population for the three hospitals in the surveillance area. Cases were counted only once in the numerator, unless there were separate episodes of occurence. At-risk populations (denominator for incidence rates calculations) consisted of children aged 28 days to less than 60 months in the catchment areas of the three study hospitals. The age group specific, at-risk populations were calculated based on data from the National Polio Surveillance Project, with age and gender breakdown based on the Demographic and Health Survey (National Family Health Survey) for the state of Karnataka, where the surveillance area was located [5]. We assumed that some children in the target study population would seek care at medium-sized hospitals (i.e., >100 beds) with the capacity to care for severely ill children and therefore would not be identified in our study hospitals. By counting these hospitals and the number of patients they serve, we could better estimate the total number of children <5 years of age who were at risk for hospitalization due to severe diseases, such as meningitis, pneumonia and septicemia. Data on key demographic, clinical, and laboratory characteristics were collected at study enrollment. Additional data were collected as they became available during hospitali-zation. Univariate analyses were the only analyses performed on these data and determined percentages of subjects within groups having a specific characteristic or risk factor.

Frequency distributions (numbers and percentages) were generated for each risk factor. Descriptive statistics were calculated for the following continuous variables: number of individuals in the household, number of children aged <60 months, and number of sleeping rooms. Descriptions of risk factor distributions were generated for the full study population and for subjects with clinical pneumonia, chest radiograph-confirmed pneumonia, or CRP+CXR-confirmed pneumonia.

Standard statistical methods for categorical comparisons of distributed data were employed, including the chi square test and the Fisher exact test, where applicable.

RESULTS

A total of 9950 children (56% males), 5249 in year 1 and 4701 in year 2, were enrolled from the estimated at-risk

population of 224,966 children. The mean (SD) age of the children was 19.7 (13.8) months and the majority (66.2%) were aged <24 months. The majority of enrolled subjects (55.9%) were male. The gender and age distributions among the enrolled patient population mirrored the distribution of residents in communities of Karnataka as reported in the National Family Health Survey [5]. Premature infants (gestation <37 weeks) represented 2.6% of enrolled subjects.

At the time of patient discharge, 1300 of 1802 patients had a final diagnosis consistent with one of the targeted conditions, including pneumonia clinical syndrome (*n*=883, 49%), bacteremia (*n*=216, 12.0%), sepsis (*n*=114, 6.3%), and meningitis clinical syndrome (*n*=78, 4.3%). Four of the children with a final diagnosis of meningitis clinical syndrome had definite pneumococcal meningitis, three of whom had a follow-up neurological assessment. At follow-up, two children had a normal neurological assessment and one had an abnormal neurological assessment. Hospitalization was required for 18.1% of the enrolled children; 75.6% of those hospitalized were aged <24 months. Almost half (49%) of hospitalized subjects had a final diagnosis of pneumonia clinical syndrome.

Forty children met the case definition for invasive pneumococcal disease; 22 in Year 1 and 18 in Year 2. The mean (SD) age of those with invasive pneumococcal disease was 19.7 (15.5) months; 65% were aged <24 months. Prior pneumococcal conjugate vaccination occurred in 0.3% of all enrolled subjects, and in none of the invasive pneumococcal disease cases. Thirty of the 40 children with invasive pneumococcal disease were hospitalized.

TABLE I ESTIMATED INVASIVE PNEUMOCOCCAL DISEASE INCIDENCE RATES

	No. of cases	*At-risk population*	*Incidence rate (95% CI) per 100,000 children*
Overall	40	224,966	17.8 (12.7, 24.2)
By gender			
Male	26	117,388	22.2 (14.5, 32.5)
Female	14	107,578	13.0 (7.1, 21.8)
By age group			
28 d to <6 mo	6	16,372	36.7 (13.5, 79.8)
6 to <12 mo	13	26,080	49.9 (26.5, 85.2)
12 to <24 mo	7	45,554	15.4 (6.2, 31.7)
24 to <36 mo	7	44,940	15.6 (6.3, 32.1)
36 to <60 mo	7	92,020	7.6 (3.1, 15.7)

The overall estimated incidence rate for invasive pneumococcal disease was 17.8 per 100,000 children (95% CI 12.7, 24.2). The highest estimated incidence rate was observed in children aged 6 months to <12 months, 49.9 per 100,000 children (95% CI 26.5, 85.2) (***Table I***). The overall estimated incidence rates for pneumonia clinical syndrome, bacteremia, and meningitis were 12.5, 3.6, and 1.8 per 100,000 children, respectively.

There were no significant differences between the total study population and those with invasive pneumococcal disease for smokers in the household (28.6% *vs* 27.5%), breastfeeding <2 months (13.1% vs 15.0%). Exposure to smoke from cooking occurred in 27.5% of the total population in comparison to 37.5% of those with invasive pneumococcal disease. Malnutrition diagnosed by a healthcare provider was reported in 5.8% of the study population and 12.8% of those with invasive pneumococcal disease. Antibiotics were used within 7 days prior to study enrolment in 19.8% of the total population and in 23.1% of those with invasive pneumococcal disease.

Laboratory Results

PCR results: Due to concerns about sample storage, PCR analysis was only performed on samples that had been stored for <3 months (358 out of 9950). Of all cultures analyzed using PCR, 62 were positive for *S. pneumoniae*: 20 blood samples (13.8%, 20 of 145), 29 CSF samples (15.1%, 29 of 192), and 13 pleural fluid samples (56.5%, 13 of 23). *S. pneumoniae* serotypes identified by PCR were: 1 (*n*=2), 3 (*n*=1), 5 (*n*=6), 6A (*n*=2), 6B (*n*=1), 7 (*n*=1), and 19F (*n*=3). Four non–13-valent pneumococcal conjugate vaccine serotypes were identified. Of the 45 samples that were positive for *S. pneumoniae* from routine culture, 11 were tested by PCR, and samples from 6 subjects were positive by both methods. Of the samples from 9910 subjects that were negative from routine culture, 347 were tested by PCR, and 56 were positive for *S. pneumonia.*

Bacteriology and antibiotic resistance: A total of 9894 subjects (99.4% of 9950 total enrolled subjects) had 10,149 samples cultured. Overall, positive bacterial cultures were obtained in 788 (7.8%) samples. The rate of positive growth in blood, CSF, and pleural fluid was 7.3%, 20.1%, and 46.7%, respectively. The most commonly isolated pathogens were *Salmonella* species (*n*=80, 10.2%), *Staphylococcus aureus* (*n*=48, 6.1%), and *S. pneumoniae* (*n*=45, 5.7%). Serotype information of *S. pneumonia* was obtained from 36 samples. Serotyping information was not performed at the central laboratory for four cases. These four cases were culture-positive for *S. pneumoniae* at the local laboratory, and were therefore

included in the IPD population. Serotypes 6A (16.7%, 6 of 36) and 14 (13.9%, 5 of 36) were seen most frequently (***Table* II**). Maximum resistance was seen to erythromycin (***Table* II**).

Children aged 28 days to <6 months had the highest estimated incidence rates for clinical pneumonia, CXR-confirmed pneumonia, and CRP+CXR-confirmed pneumonia (***Table* III**). Over the 2-year study period, ≥1 positive blood culture was reported in 354 children with clinical pneumonia, 202 with CXR-confirmed pneumonia, and 212 with CRP+CXR-confirmed pneumonia.

TABLE II SEROTYPE DISTRIBUTION AND ANTIBIOTIC RESISTANCE OF INVASIVE PNEUMOCOCCAL DISEASE CASES IN CHILDREN AGED 28 DAYS TO <60 MONTHS (*N*=36)*

Serotype	*n(%)*	*Antibiotic resistance (No. of isolates)*
6A	6 (16.7)	Erythromycin (4), Trimethoprim/ sulfamethoxazole (1)
14	5 (13.9)	Trimethoprim-sulfamethoxazole (4) Erythromycin (2), Penicillin (1)
5	4 (11.1)	–
6B	4 (11.1)	Erythromycin (2) Trimethoprim-sulfamethoxazole (2)
1	3 (8.3)	Trimethoprim-sulfamethoxazole (1)
18C	3 (8.3)	Trimethoprim-sulfamethoxazole (2)
19A	3 (8.3)	Erythromycin (2) Trimethoprim-sulfamethoxazole (2)
9V	2 (5.6)	Levofloxacin (1) Trimethoprim-sulfamethoxazole (1)
3	1 (2.8)	–
4	1 (2.8)	Trimethoprim-sulfamethoxazole
10C	1 (2.8)	Trimethoprim-sulfamethoxazole
18A	1 (2.8)	Trimethoprim-sulfamethoxazole
18F	1 (2.8)	–
19F	1 (2.8)	–

**4 invasive pneumococcal disease cases were confirmed by a local laboratory only; thus serotyping was not performed. –:No resistance to antibiotics tested.*

A total of 59 deaths were reported among study participants. Death was reported in 28 of the sepsis cases and 6 of the meningitis cases, with corresponding fatality rates of 24.6% and 7.7%, respectively. There were 39 deaths among subjects with clinical pneumonia, 14 in subjects with CXR-confirmed pneumonia, and 14 in those with CRP+CXR-confirmed pneumonia (some children had >1 diagnosis); the corresponding fatality rates were 0.82%, 0.56%, and 0.55%, respectively. These deaths include 5 (12.5%) deaths among the 40 children with invasive pneumococcal disease. The final diagnoses among those with invasive pneumococcal disease who died were bacteremia and pneumonia clinical syndrome; right-sided empyema and pneumothorax with necrotic middle lobe and pneumonia clinical syndrome; bacteremia, definite pneumococcal meningitis, and sepsis; bacteremia and pneumonia clinical syndrome; and bacteremia, definite pneumococcal meningitis, and sepsis.

DISCUSSION

This prospective study examined the burden of invasive pneumococcal disease and *S. pneumoniae* serotype distribution among young children in an urban area in Southern India. Notably, this is the first prospective study to document the presence of serotypes 3, 6A, and 19A in children with invasive pneumococcal disease in India.

A lower proportion of invasive pneumococcal disease was identified by routine culture techniques compared with PCR. However, the PCR results were obtained on a small fraction of the total number of subjects (those with samples stored for <3 months), and thus cannot be

TABLE III ESTIMATED INCIDENCE RATES (PER 100,000 CHILCREN) OF CLINICAL PNEUMONIA, CHEST RADIOGRAPH (CXR)-CONFIRMED PNEUMONIA, AND C-REACTIVE PROTEIN (CRP)+CXR-CONFIRMED PNEUMONIA IN THE STUDY AREA

Age (at risk population)	*Clinical pneumonia**		*CXR-confirmed pneumonia**		*CRP+CXR-confirmed pneumonia**	
	Cases (n)	*Incidence rate (95% CI)*	*Cases (n)*	*Incidence rate (95% CI)*	*Cases (n)*	*Incidence rate (95% CI)*
28 d to <6 mo (16372)	824	5033 (4695, 5389)	395	2413 (2181, 2663)	406	2480 (2244, 2733)
6 to <12 mo (26080)	986	3781 (3548, 4024)	492	1887 (1723, 2061)	501	1921 (1756, 2097)
12 to <24 mo (45554)	1230	2700 (2551, 2855)	700	1537 (1425, 1655)	721	1583 (1469, 1703)
24 to <36 mo (44940)	765	1703 (1584,1827)	388	863 (780.59, 954)	397	883 (799, 975)
36 to <60 mo (92020)	939	1020 (956, 1088)	530	576 (528, 627)	542	589 (540, 641)
Overall (224966)	4744	2109 (2049, 2170)	2505	1114 (1070, 1158)	2567	1141 (1097, 1186)

**Some patients had more than one diagnosis.*

WHAT IS ALREADY KNOWN?

- Invasive pneumococcal disease burden in children aged <5 years is significant in India and data regarding overall invasive pneumococcal disease incidence are scarce.

WHAT THIS STUDY ADDS?

- The estimated incidence of pneumococcal disease among children aged 28 days to <60 months in South Bangalore was 17.8/100,000. Frequently occurring serotypes are 6A, 14, 5, and 6B.

considered representative of the total samples. In addition, because samples were considered PCR- positive based on qualitative results, there is an increased risk of false positives. However, it appears that using traditional culture techniques to identify cases of invasive pneumococcal disease may result in a considerable underestimation of the true burden of disease. Furthermore, important limiting factors for the blood culture technique include previous antibiotic use and inability to identify serotypes.

There were several limitations of this study. First, this was a hospital-based study which may underestimate the true number of cases in the region. Hospitals other than those selected for this study as well as private clinics in the catchment area may also treat some inpatients. In addition, the data may not be representative of the entire country. Furthermore, calculation of the denominator and age- and gender-adjustment based on Karnataka state data might have led to an over- or under-estimate of the incidence rates because the surveillance area may have a different age- and gender-distribution. Finally, although almost all enrolled subjects had a culture performed, positive culture was observed in only 8% of subjects. Obtaining positive blood cultures in the pediatric population is a challenge [6]. In addition, many different factors, including prior antimicrobial use, may influence the ability to isolate pathogens. Although the sample size was limited, PCR results support the need for exploring the inclusion of non-culture methods in clinical and epidemiologic surveillance.

In the Invasive Bacterial Infection Surveillance study conducted almost 15 years ago, no serotype 3 isolates were detected in children aged <5 years and serotyping was not provided for the serogroup 6 and 19 isolates obtained [7]. In a retrospective examination of serotypes and susceptibility in patients with invasive and clinically significant *S. pneumoniae* infections in Puducherry [8], one serotype 3 isolate was identified in a child aged <5 years and serotyping was not performed for serogroup 6 and 19 isolates. Several recent publications suggest that the incidence of antibiotic-resistant strains of *S. pneumoniae* is increasing in India [9-11]. Southeast Asian estimates of *S. pneumoniae* burden of disease in children aged <5 years are 2991 per 100,000 children (95% CI 2329, 3717) for invasive pneumococcal disease and 2911 per 100,000 children (95% CI 2265, 3622) for pneumonia [12]. Other studies, including the Million Death Study [4] and the Child Health Epidemiology Reference Group (CHERG) analysis [13], similarly confirmed a higher incidence of pneumonia in younger children.

In summary, continuous larger-scale surveillance throughout India is needed to better understand the burden of pneumococcal disease. The variability of serotype epidemiology and antibiotic resistance are crucial data for determining country-specific pneumococcal vaccination needs.

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Competing interests: IB and SJ were employees of Pfizer, Global Research and Development, Paris, France during conduct of study. PEK was an employee and SAK is still an employee of The International Vaccine Institute, Seoul, Republic of Korea, contracted to Pfizer Inc, Collegeville, PA, USA at the time of this study and during development of this manuscript. JY is a former employee of On Assignment Inc, and was a paid contractor to Pfizer to assist with study design, statistical planning, data analysis and manuscript development. MM is a current employee of Pfizer Inc, Collegeville, PA, USA.
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