RESEARCH PAPER

Multicentric Hospital-Based Surveillance of Pertussis Amongst Infants Admitted in Tertiary Care Facilities in India

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Objective: To estimate the disease and economic burden of pertussis amongst hospitalised infants in India.

Design: Multicentric hospital-based surveillance study.

Participants: Hospitalised infants with clinical suspicion of pertussis based on predefined criteria.

Outcome measures: Proportion of infants with laboratory-confirmed pertussis, economic burden of pertussis amongst hospitalised infants.

Results: 693 clinically suspected infants were recruited of which 32 (4.62%) infants had laboratory-confirmed pertussis. Progressive cough with post-tussive emesis (50%) and pneumonia (34%) were the common clinical presentations; apnea in young infants was significantly associated with pertussis. Infants with pertussis were more likely to be younger (median age 102.5 days vs.157 days) and born preterm (42.9% vs 24.5%). Almost 30% infants with pertussis had not received vaccine for pertussis with 50% of these infants aged less than 2 months. Pertussis was associated with higher costs of hospitalisation, pharmacy and loss of working days by caregivers as compared to non-pertussis cases.

Conclusion: Younger infants, those born preterm and those inadequately immunised against pertussis are at higher risk of pertussis infection. Timely childhood immunisation and introduction of maternal immunisation for pertussis can help in reducing the disease burden.

Keywords: *Bordetella pertussis, Burden, Whooping cough*.

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Despite the introduction of Diphtheria pertussis tetanus (DPT) vaccine in the expanded program on immunization, pertussis (whooping cough) caused by *Bordetella pertussis* continues to be an important public health problem with about 151000 cases reported globally in 2018 [1]. According to a recent modelling study on the global burden of pertussis, there are 5.1 million estimated pertussis cases and 85900 estimated pertussis deaths amongst infants [2] and India contributes to 26.7% (11,875 cases) of the global burden of pertussis [3,4].

In the recent years, resurgence in pertussis has been reported amongst infants and adolescents from many countries around the world including United States, England [5,6], Brazil, Argentina [7] and China [8]. The possible reasons for resurgence include waning immunity, inadequate vaccine coverage, failure to administer booster doses after the initial vaccination, differential herd immunity between whole cell (wP) and acellular (aP) vaccines, diagnostic and epidemiologic surveillance systems, and genetic changes in the pathogen [6,9,10]. Several hospital-based and community-based surveillance studies from developed countries have reported a high rate of hospital-admissions due to pertussis amongst infants, especially the youngest [11-13] and a significant economic burden in infants hospitalised with complications due to pertussis [14]. Maternal vaccination with the tetanus, diphtheria reduced dose and acellular pertussis (Tdap) vaccine in third trimester of pregnancy, neonatal vaccination, cocooning, adult and adolescent immunization, addition of new antigens to the existing vaccine are some of the strategies recommended towards reducing the resurgence of pertussis [5,15].

A recent systematic review from Asia has highlighted the burden of pertussis in neonates and the paucity of systematic data in this regard [16]. In India, although the reported incidence of pertussis has reduced significantly since 1987, due to lack of routine laboratory diagnosis and uniformity in the clinical definition of pertussis, large number of cases may go undetected and many non-pertussis cases may be getting misdiagnosed as whooping cough [4,17]. Thus, although India contributes significantly to global burden of pertussis, country-specific estimates on the burden of pertussis in infants at

community or hospital level are not available, which are important to inform the national immunization policy [17].

The national average for full immunization is only 62%, and nation-wide coverage for the 3rd primary dose of DPT/pentavalent vaccine (containing DPT with H. influenzae B and hepatitis B) is 78.4% as per National Family Health Survey-4 (NFHS-4) [18]. In line with WHO recommendations, the public health programs in India continue to use wP vaccines rather than aP vaccines [19]; although, the Indian Academy of Pediatrics recommends both wP and aP vaccines for primary immunization [4,20]. The present study was designed to estimate the disease and economic burden of pertussis amongst hospitalized infants in a network of four tertiary care hospitals in India.

METHODS

This cross-sectional, observational, multicentric hospital-based active surveillance of pertussis was conducted in four tertiary care hospitals in India – KEM Hospital, (KEMH) Pune, Maharashtra; Sri Ramachandra Medical College, (SRMC) Chennai, Tamil Nadu; Christian Medical College (CMC), Ludhiana, Punjab and Institute of Child Health (ICH), Kolkata, West Bengal. The sites were chosen from four different zones across the country to account for geographical, seasonal and socioeconomic variations. The study was conducted from October, 2018 to April, 2020 in the given four hospital sites.

The overall conduct of the multicentric study was coordinated by a team of investigators and project managers at KEM Hospital Research Centre, Pune (KEMHRC). This team was responsible development of study protocol and study tools, training of all site teams, site monitoring, data management and analysis. The sites teams were trained for the study protocol, case record forms and nasopharyngeal swab collection during investigators meetings arranged before initiation of the study.

The study protocol was approved by the institutional ethics committees of all the sites. The study was registered with Clinical Trial Registry of India. The study participants were recruited after obtaining written informed consent from their parents or guardians.

The study was conducted in hospitalized infants with clinical suspicion of pertussis. Three of the sites (KEMH, SRMC and ICH) pre-screened potential study participants from hospital registers before screening them using the study criteria whereas at CMC, all infants admitted were screened using study criteria. The clinical case definition for pertussis was adapted from the criteria published by Cherry, et al. [21], which were generally consistent with the case definitions from the United States Centers for Disease Control and Prevention (US CDC) [22], European Centre for Disease Prevention (ECDC) [23] and World Health Organisation (WHO) [24].

A laboratory confirmed case (LCP) of pertussis was defined as one with clinical criteria with at least one of the following laboratory criteria: *i)* Detection of *Bordetella pertussis, Bordetella parapertussis* or *Bordetella holmesii* nucleic acid in a clinical specimen using real-time polymerase chain reaction (RT-PCR); *ii)* Detection of *B. pertussis* or *B. parapertussis* in a clinical specimen using culture.

For the enrolled infants, information on demography, history of the present illness, vaccination records and socioeconomic status was collected from the caregivers by trained clinical coordinators on the case record forms at each site. The demographic variables collected included gender, date of birth, birthweight, gestational age and mode of delivery. Information on birthweight, gestational age and anthropometric parameters was collected from the hospitalisation records. The variables collected for vaccination status included number of doses of DPT or pentavalent vaccine received, type of vaccine (aP or wP) and the dates of vaccination. Details about the onset, duration and clinical course of the current disease were collected during the course of hospitalization till the child was discharged/transferred from the inpatient facility.

Data about economic burden of the present illness at household level were collected by interview method using a questionnaire which included costs on use of health care resources (cost of out-patient consultation, hospitalization, laboratory tests, medications, physician/ emergency room visits), use of non-health care resources (travel, food and miscellaneous expenditure) and productivity costs (loss of wages) for all clinically suspected cases for the given episode of illness. Cost data towards management of pertussis-related complications were collected till the end of present hospitalisation. In addition, the socioeconomic status of the household was determined using modified Kuppuswamy scoring [25]. The income of non-earning members of the family e.g., housewives was assumed to be equivalent to minimum daily wages of unskilled labour as per Government of India depending upon their geographical area [26].

Two posterior nasopharyngeal swabs were collected by trained clinical coordinators, nurse or laboratory technicians for all children with clinical suspicion of pertussis, not later than 72 hours following hospital admission and preferably before administration of systemic antibiotics. The swabs were transported dipped in Amies medium with charcoal/viral transport medium on dry ice in vaccine carrier to the local microbiology laboratory.

In the local laboratory, the swabs for cultures were immediately streaked on Bordet Gengou (BG) medium supplemented with 15% defibrinated horse blood and containing cephalexin to inhibit normal flora (40µg/mL). These culture plates were incubated for 7 days at 35-36°C and were inspected daily. Presence of any *Bordetella* colonies were identified based on colony morphology, colony smear showing Gram-negative coccobacilli and biochemical tests [27].

The swabs collected for RT-PCR were stored immediately after collection for refrigeration at –20ºC to -80ºC till further processing. The swabs were periodically (once in 2 months) shipped on dry ice to microbiology laboratory, KEM Hospital, Pune for analysis of RT-PCR (central laboratory). In the central laboratory, DNA was extracted from the submitted specimens using a QIAamp DNA mini kit (Qiagen) according to the manufacturer's recommendations. The assays for *B. pertussis* and *B. parapertussis* were done by RT-PCR using Taqman technology for the amplification of the insertion elements IS481 and IS1001 of *Bordetella spp*. Threshold cycle of ≥35 was considered positive for IS481. PCR assay for PtxA-S1 was carried out on all specimens that tested positive for IS481 to confirm the diagnosis of *B. pertussis*. Interpretation of results and identification of species was done using WHO algorithm for diagnosis of pertussis [28] (**Web Table I)**.

During the study period, the central laboratory completed a clinical proficiency testing program for *B. pertussis* with Wisconsin State Laboratory Hygiene (WSLH), USA as a part of external quality assurance.

Data management and analysis: At individual sites, the data including clinical and laboratory data from the case record forms were entered into a centrally managed electronic case record forms generated using Open Clinica (community version). Source data verification and quality control was managed by the central team at KEMHRC. The anonymized dataset for the entire study was extracted for analysis following source data verification. The dataset was archived at local servers at KEMHRC.

The demographic factors were compared between pertussis and non-pertussis cases. Young infants were defined as infants with age <60 days [29]. Age-appropriate pertussis vaccination was defined as vaccination within 4 weeks of the exact age of eligibility (i.e. for first dose of pertussis vaccine, vaccination within 10 weeks of age is considered age appropriate). The proportion of laboratory-confirmed pertussis was calculated and compared amongst different age sub-populations (i.e. <2 months, 2-6 months, and ≥6-12 months). Occurrence of total cases and pertussis positive cases per month was charted for total numbers as well as for site-specific cases.

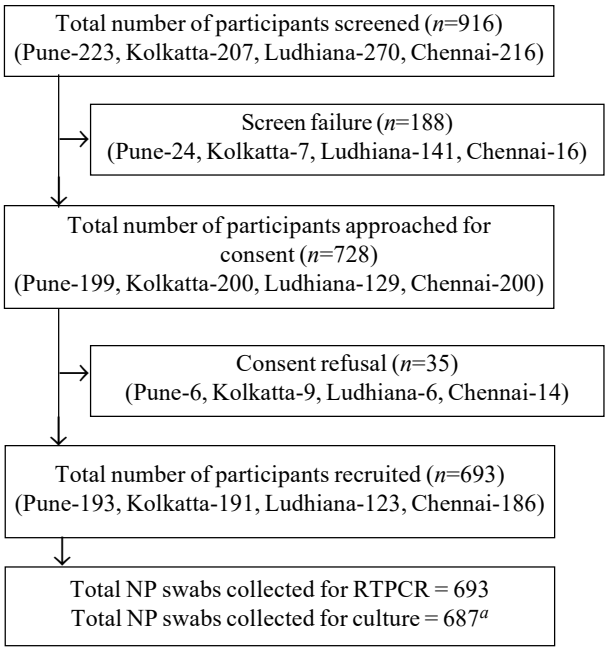
Pearson Chi-square test was used for comparing the proportions and Kolmogrov-Smirnoff test was used for comparison of numerical data (non parametric data). All the analysis was done using STATA 15.0.

RESULTS

A total of 916 infants were screened using clinical case definition criteria, and 693 study participants were recruited (**Fig. 1)**. Thirty two infants were detected with LCP; 8 from Pune, 17 from Kolkata, 2 from Ludhiana and 5 from Chennai (**Table I**). The median age of infants with LCP was about 3.6 months, and boys contributed 62.5% cases of LCP (**Table II**).

Web Table II shows characteristics of study participants recruited overall, and at each site. Approximately, two third of the study participants were boys with a median age of 5 months. About 85% of the participants were older than 2 months. Approximately, one-fourth of the recruited children had low birthweight and/or were born preterm.

About 75% of the study participants belonged to lower or lower middle socioeconomic class as per modified Kuppuswamy classification. About 50% (*n*=355) of



[a]Culture data not available for 6 swabs due to non-availability of culture plates. NP-nasopharyngeal.

Fig. 1 Study flow chart.

Table I Number of Infants With Laboratory Confirmed Bordetella Infection

Number of clinically suspected cases	*Total (n=693)*	*KEMH (n=193)*	*ICH (n=191)*	*CMC (n=123)*	*SRMC (n=186)*
Total Bordetella, n (%)	32 (4.6)	8 (4.1)	17 (8.9)	2 (1.6)	5 (2.7)
B. pertussis, n (%)	25 (3.6)	7 (3.6)	12 (6.3)	2 (1.6)	4 (2.1)
B. parapertussis, n (%)	7 (1.0)	1 (0.5)	5 (2.6)	0	1 (0.5)

the study participants had received age-appropriate vaccination for pertussis and 30% (n=214) of them had received less than adequate vaccination. A total of 124 study participants had not received any pertussis vaccination of whom 81 were aged less than 2 months. Amongst these, 39 were aged less than 6 weeks and thus were not eligible to receive first dose of pertussis vaccine.

Of the 687 cultures done, bacterial growth was detected in 164 cultures. None of the 164 cultures grew *Bordetella* species. Of 693 nasopharyngeal swabs collected for RT-PCR, *Bordetella* species were detected in 32 (4.62%) swabs, of which 25 were *B. pertussis* and 7 were *B. parapertussis* **(Table I).**

Presence of classical whoop was reported in only one child. Apnea was significantly more associated with pertussis especially in younger infants (aged <2 months). In addition to cough and fever, the presenting symptoms for LCP included worsening of symptoms at night in 59%, post-tussive emesis in 50% and pneumonia in 34% children. Although leukocytosis was reported in slightly higher proportion of children with LCP, this difference was not statistically significant (**Table II**).

Infants with LCP were significantly younger than those without LCP. Infants with LCP were more likely to have been born preterm and were smaller in size. About 68% of infants with LCP were not age-appropriately vaccinated for pertussis as compared to 48% of infants without LCP. Amongst children aged less than 2 months, all the 5 cases of LCP occurred in children who did not receive single dose of pertussis vaccine and only one of these was aged less than 6 weeks and was thus not eligible to receive first dose of pertussis vaccine (**Table II**). There were no significant differences in the gender, birth weight or socioeconomic status or receipt of antibiotic treatment in the two groups.

Fig. 2 shows age-wise proportion of laboratory-confirmed pertussis cases. In Pune and Chennai, the proportion of LCP was higher in infants aged less than 2 months whereas in Kolkata the highest number of cases was in the 2-6 months age group. As a result, overall, there were more cases of LCP in the 2-6 months age category as compared to less than 2 months and more than 6 months.

Table II Characteristics of Infants With and Without Laboratory-Confirmed Pertussis

Characteristics	*Pertussis (n=32)*	*Non-Pertussis (n=661)*
Age (d)[a,b]	102.5 (61-168)	157 (87-242)
Age <60 d	5 (15.63)	96 (14.52)
Male gender	20 (62.5)	439 (66.41)
Signs and symptoms		
Progressive cough	32 (100)	661 (100)
Presence of whoop	1 (3.13)	14 (2.12)
Apnea[d]	2 (6.25)	3 (0.45)
Post-tussive emesis	16 (50)	266 (40.2)
Cyanosis	0	7 (1.06)
Seizure	1 (3.1)	36 (5.4)
Pneumonia	11 (34.4)	320 (48.4)
Worsening of symptoms at night	19 (59.4)	322 (48.7)
Increased WBC counts	3 (9.4)	49 (7.4)
Weight (kg)[a,b]	5.25 (4.25-6.55)	6.2 (4.8-7.5)
Length (cm)[a,c]	59.5 (56-62.5)	62 (57-68)
Head circumference (cm)[a,b]	39 (36.5-4)	41 (39-43)
Low birth weight[e]	11 (34.4)	154 (23.44)[e]
Preterm[b,f]	12 (42.9)	150 (24.51)
Age-appropriate vaccination		
Full	10 (31.2)	345 (52.2)
Partial	12 (37.5)	202 (30.6)
None	10 (31.2)	114 (17.2)
Antibiotics in last 72 h	15 (46.9)	341 (51.6)
Socioeconomic status		
Upper	0	16 (2.4)
Upper middle	5 (15.6)	147 (22.3)
Lower middle	20 (62.5)	294 (44.5)
Lower	7 (21.9)	203 (32.8)

Data presented as no. (%) or [a]median (IQR). [b]P<0.05, [c]P=0.01, [d]P=0.001. [e]out of 657, [f]out of 28 for pertussis and out of 612 for non-pertussis. WBC: white blood cells.

Amongst the infants with LCP, 18% (n=6) required ICU admission as compared to 23.6% (n=156) amongst infants without LCP. Of these, only one infant with

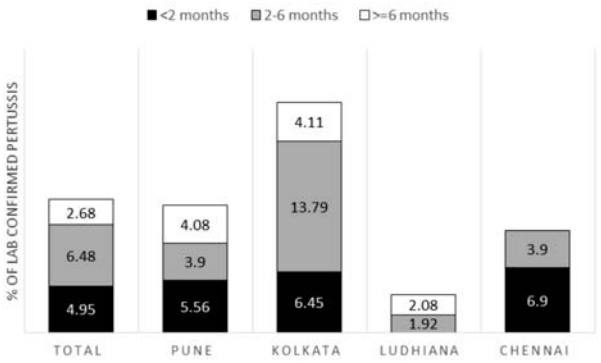


Fig. 2 Age-wise proportion of laboratory confirmed pertussis cases.

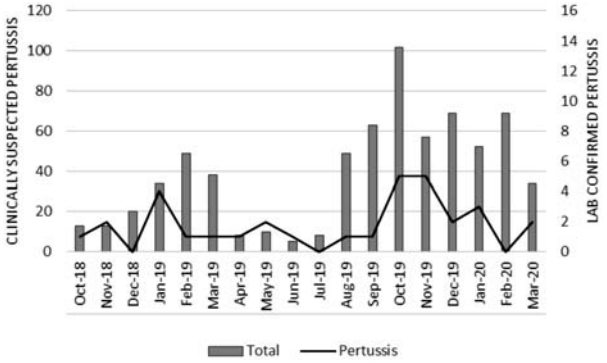


Fig. 3 Seasonality of occurrence of pertussis.

pertussis required mechanical ventilation. The remaining 5 infants were treated with oxygen therapy. Antibiotics were used in 23 infants, which mainly included macrolides and cephalosporins.

There was complete recovery at the time of discharge in 28 (87.5%) cases of pertussis. Two study participants with pertussis had partially recovered at the time of hospital discharge without any permanent debility and two children were discharged against medical advice. There were no death during hospitalization amongst infants with LCP as compared 8 deaths amongst infants without LCP.

Fig. 3 shows seasonal trends in occurrence of the total cases and pertussis. Clinically suspected pertussis as well as LCP cases were most frequent from October to January, which coincides with winter season in India.

Table III shows economic burden of pertussis at household level. Hospitalization of an infant with LCP resulted in a median hospitalization cost of approx. Rs.15000, median hospitalization duration of 5.5 days and a median loss of worktime of 2 weeks by the caregivers for taking care of the infant during the illness. This led to median loss of income of Rs 6921 to caregivers of infants suffering from LCP. The total cost for hospitalization including pharmacy cost was more in infants with LCP. The days spent away from work by the caregivers during illness were also significantly higher for LCP. For all the infants with LCP, the families used their savings to meet the expenses incurred. In addition, 15% families accepted donations from others and 28% families borrowed money to meet the expenses. It is noteworthy that 3% of the families had to sell their assets or use donations by non-government organizations or hospitals to meet the hospitalisation expenses.

Table III Economic Burden of Pertussis

Cost	*Laboratory confirmed pertussis(n=32)*	*Non-pertussis (n=661)*	*P value*
Direct costs (*in INR*), *median (IQR)*			
Pre-hospitalization expenses	590 (250-1050)	530 (250-1150)	0.92
Hospitalization cost[a]	10520 (8030-14685)	8950 (3499-19815)	0.06
Pharmacy cost	2270 (1150-3750)	1080 (0-3000)	0.01
Other costs (food, travel, accommodation)	1750 (700-2500)	1000 (500-2400)	0.18
Total cost	15035 (11959-21713)	12626 (5665-24976)	0.04
Indirect costs, median (IQR)			
Person time spent in outpatient consultation (h)	2 (1.5-4.5)	3 (1.9-5)	0.76
Days of hospitalization	5.5 (3.5-8)	4 (3-7)	0.53
Days of ICU hospitalization	1.5 (1-2)	2 (1-6)	0.6
No of person-days lost by caregivers	14 (2-21)	12 (8-22)	0.02
Income lost by caregivers (in INR)	6921 (5050-10446.67)	6065 (3441.67-10613.33)	0.08

[a]*Hospitalization cost includes cost of hospital stay, nursing and consultancy charges.*

DISCUSSION

This is the first hospital-based prospective surveillance study for LCP amongst infants in India. The earlier reported literature from India was an outbreak of suspected pertussis in Arunachal Pradesh in 2007, with 71% of the suspected cases of pertussis being under one year of age [30]. However, none of these children underwent laboratory confirmation for pertussis. Although two retrospective studies from tertiary care hospitals in India have been reported recently with 30 and 36 cases of LCP in infants and children, respectively; these studies present retrospective data from single centers [31,32].

In our study, 4.62% of Indian infants hospitalized with clinical suspicion of pertussis were found to have LCP. This is much less than the numbers reported from hospital-based studies conducted in Peru (39.5%) [12], Thailand (19%) [33], and a seven-country multinational study including Brazil, Germany, Spain, Costa Rica, Taiwan, Singapore and Uruguay (12%) [34]. A possible reason could be the difference in the clinical definitions used for diagnosis of pertussis. In the multinational study conducted by Kowalzik, et al. [34], infants admitted in pediatric wards with any one of the clinical symptoms i.e. respiratory failure, apnea, bradycardia, or cough accompanied by paroxysms, vomiting, whoop or cyanosis were included. Both the Thailand [33] and the Peruvian [12] study used a clinical definition similar to that of CDC [22]. However, the Thailand study recruited children presenting to the outpatient clinic, whereas Peruvian study recruited hospitalized children. In both these studies, children with chronic respiratory or cardiac diseases were excluded. Two community-based surveillance studies from other parts of South Asia have reported relatively low incidence of pertussis amongst infants (13.3 and 3.96 cases per 1000 infant-years from Nepal [35] and Pakistan [36]).

Amongst the clinical features of LCP, progressive cough with post-tussive emesis, pneumonia and worsening of symptoms at night were common presenting features whereas classical whoop was found in only one child with LCP. This highlights that inspiratory whoop, which is mainstay of clinical diagnosis for pertussis in older children and adults, may not present in infants [21]. Apnea and seizure were presenting features in young infants with LCP but leukocytosis with absolute lymphocytosis was present only in one child aged less than 2 months. This is not consistent with Cherry, et al. [21] and other hospitalized studies of pertussis [31,32,37,38] where leukocytosis with absolute lymphocytosis was largely reported in young infants with pertussis. Few studies have reported severe leukocytosis in critically ill patients with pertussis [39,40]. Pneumonia was found in over 30% of infants with LCP in our study; however, this is one of the many causes of pneumonia. Overall, per-tussis contributes to only a fraction of pneumonia hospitalizations amongst infants from low- and middle-income countries [38,41]. These observations point towards equivocality of clinical criteria and need for more frequent laboratory diagnosis of pertussis amongst children.

Almost 75% of the infants with LCP were aged less than 6 months and 15% were aged less than 2 months in our study. Retrospective studies from Indonesia [42], Philippines [38,43] and Singapore [37] have also reported pertussis cases with higher occurrence and mortality in infants aged less than 6 months. Bhattacharya, et al. [31] reported about 60% of cases in infants aged less than 16 weeks and 30% cases in infants aged less than 8 weeks. Our findings emphasize the earlier observation that pertussis can present with severe morbidity in younger infants requiring hospitalisation [40]. However, only 18-20% of our study participants with LCP required admissions in the intensive care and only one child required mechanical ventilation as against substantial morbidity and mortality reported from studies done elsewhere [37,38,40,42]. Children born as preterm presented as an additional risk factor for pertussis which has also been reported earlier [44]. This could partly be due to delay in the vaccination for preterm children (46.9% full vaccination in preterm as compared 54% amongst others).

In our study, inadequate vaccination or delayed vaccination for pertussis was found to be an important risk factor. Almost 30% infants with LCP had not received vaccine for pertussis, 50% of these infants aged less than 2 months. Another 30% had received less than adequate pertussis vaccination. Similar results were reported in earlier retrospective Indian study conducted by Kavita, et al. [32] and a recently conducted Chinese study by Wang, et al. [40]. Lack of timely vaccination has been reported to be an important preventable risk factor for pertussis amongst young infants globally [16,43,45] not only as a direct risk from lack of protection but also indirectly as infected young infants and children can contribute to increase circulation and cause infection of infants who are too young to get vaccinated and but at high risk of developing complications due to pertussis. This Indian scenario is different from the Western world where resurgence of pertussis has been documented despite high coverage of childhood pertussis immunisation and where the main postulated cause of pertussis is reported to be waning immunity from childhood vaccine in mothers [46].

WHAT IS ALREADY KNOWN?

- Pertussis can lead to severe manifestations in infants requiring hospitalization.

WHAT THIS STUDY ADDS?

- Laboratory confirmed pertussis was seen in 4.6% of children hospitalized with a clinically diagnosed pertussis.
- Younger age, prematurity and inadequate immunization against pertussis were the major risk factors for pertussis.

Introduction of maternal immunization with TdaP has been shown to protect young infants from pertussis and can be useful strategy in our setup as well [15].

Majority of the infants in our study had received wP vaccine and only 4-6% of them received aP vaccine for their primary immunization. National immunization program in India continues to use wP based on the WHO recommendations to continue wP vaccine in countries where it is part of the program in order to minimise the risk of pertussis resurgence associated with aP vaccines [45].

The costs associated with LCP were higher than that of non-LCP due to increased hospitalization and pharmacy costs. As almost 75-80% of the families belonged to lower or lower-middle socioeconomic status, the hospitalization posed significant economic burden on the households leading to stretching of the existing resources. Thus, 3% of the families had to resort to selling their assets or borrowing to meet the expenditure.

Our study has few limitations. Since it only focused on the hospitalized cases of pertussis, children admitted in the day care centres or visiting outpatient departments of the tertiary care centres with similar symptoms were not recruited. The clinical outcome after hospital discharge was not monitored. Although the nasopharyngeal swabs were collected within 72 hrs of hospitalization and preferably before administration of antibiotics, large proportion of the infants had received antibiotics before hospitalization. Although uniform clinical criteria were used for identification of clinically suspected pertussis cases, one of the study sites did not pre-screen potential study participants giving rise to higher screen failures as compared to the other three sites. The study did not collect information about household contacts for pertussis and does not provide population-based incidence of pertussis. None-the-less, the study emphasizes increased risk of pertussis amongst young Indian infants, especially those not fully vaccinated.

Our study provides the first systematic evidence for burden of pertussis amongst hospitalized infants in India. Younger infants, those born preterm and inadequately immunized against pertussis are at higher risk of infection. Efforts to reduce delay in primary immunization and introduction of maternal immunization for pertussis can clearly help in reducing the disease burden in young infants.

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Contributors: AA, SS: concept or design, supervision, data analysis and interpretation, drafting publication, critical revision, final approval, accountable for accuracy and data integrity; RS: data acquisition, project management, drafting publication, critical revision, final approval, accountable for accuracy and data integrity; MM,PVR,SJ: data acquisition, data interpretation, critical revision, final approval, accountable for accuracy and data integrity; JC: data acquisition, supervision, data interpretation, critical revision, final approval, accountable for accuracy and data integrity; JC,SP,AP: data acquisition, critical revision, final approval, accountable for accuracy and data integrity; RK, GK: data acquisition, supervision, critical revision, final approval, accountable for accuracy and data integrity; NJ: data acquisition, data analysis and interpretation, critical revision, final approval, accountable for accuracy and data integrity; PK: design or concept, critical revision, final approval, accountable for accuracy and data integrity; DM: design or concept, funding acquisition, critical revision, final approval, accountable for accuracy and data integrity; AB: design or concept, supervision, funding acquisition, critical revision, final approval, accountable for accuracy and data integrity. All authors approved the final version of manuscript, and are accountable for all aspects related to the study.

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Competing interest: Denis Macina is currently employed by Sanofi Pasteur SA and also reports holding of shares in the Sanofi group of companies as part of his employee remuneration. All other authors declare no competing interests.

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