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Influenza A(H1N1)pdm09 outbreak detected in inter-seasonal months during the surveillance of influenza-like illness in Pune, India, 2012–2015

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SUMMARY

An outbreak of influenza A(H1N1)pdm09 was detected during the ongoing community-based surveillance of influenza-like illness (ILI). Among reported 119 influenza A(H1N1)pdm09 cases (59 cases in the year 2012 and 60 cases in 2015) in summer months, common clinical features were fever (100%), cough (90·7%), sore throat (85·7%), nasal discharge (48·7%), headache (55·5%), fatigue (18·5%), breathlessness (3·4%), and ear discharge (1·7%). Rise in ILI cases were negatively correlated with the seasonal factors such as relative humidity (Karl Pearson's correlation coefficient, i.e. $r = -0{\cdot}71$ in the year 2012 and $r = -0{\cdot}44$ in the year 2015), while rise in ILI cases were positively correlated with the temperature difference ($r = 0{\cdot}44$ in the year 2012 and $r = 0{\cdot}77$ in the year 2015). The effective reproduction number R, was estimated to be 1·30 in 2012 and 1·64 in 2015. The study highlights the rise in unusual influenza activity in summer month with high attack rate of ILI among children aged $\leqslant 9$ years. Children in this age group may need special attention for influenza vaccination. Influenza A(H1N1)pdm09 outbreak was confirmed in inter-seasonal months during the surveillance of ILI in Pune, India, 2012–2015.

Key words: Community surveillance, disease outbreak, influenza A(H1N1)pdm09, seroepidemiology, transmission dynamics, urban slum.

INTRODUCTION

The first outbreak of the pandemic influenza A(H1N1) virus was reported in Mexico in April 2009 [1]. Subsequently, the first case of pandemic influenza A (H1N1) from India was reported in June 2009 in Hyderabad [2] and the first pandemic influenza A (H1N1) outbreak was reported from residential school at Panchgani, Maharashtra [3]. Transmission of influenza was started in local populations in various part of India in mid-June 2009 and pandemic influenza A(H1N1) cases were also reported from Pune, Maharashtra in large numbers [4]. Influenza viruses are known to cause periodic epidemics and rare pandemics as experienced recently. Community-based surveillance studies are valuable tools to assess the burden and epidemiology of influenza-like illness (ILI) in a given area. Population-based epidemiologic studies with laboratory data on influenza and information on confirmed pandemic (H1N1) 2009 cases, through household survey provides a complete picture of the incidence and epidemiology of ILI in the community [5].

To understand the pattern of transmission of influenza in Pune city, community-based surveillance

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of ILI was undertaken since 2011 in Janata Vasahat slum. In February 2012, an unusual rise in ILI cases was detected during the surveillance and similar rise in ILI cases was detected in February 2015. The present study reports confirmation of influenza A (H1N1)pdm09 outbreak in summer season (inter-seasonal period) in the year 2012 and 2015, genetic characterization and susceptibility of virus isolates of influenza virus to oseltamivir. The study also describes characteristics of the influenza outbreak, correlation of outbreak with climatic factors and immunity status of the influenza cases, family members of confirmed influenza cases. The epidemiological parameters required for disease propagation during the outbreak were also estimated in the study.

METHODS

Setting, study period and procedure

A prospective community-based incidence study was undertaken from the year 2011 in a population of 29 797 in Janata Vasahat slum in Pune city, India (Fig. 1a). In the study area, houses are in close proximity. The study area is subdivided into 25 small clusters (number of houses between 350 and 500) separated by small roads, population density in each cluster does not differ and all study population has easy access to private health clinics in study area. Field activities and weekly survey for each cluster was monitored by independent community health volunteer (CHV) and community health worker (CHW). Active surveillance of ILI case was carried by CHV by giving weekly visits to the each house and recording the health status of the individuals. These CHVs were females, living in the same study area, had formal education and were adequately trained for survey. Their work has been supervised by the CHWs and project staff. All the CHWs were females, living in the same study area, had formal education and were adequately trained for survey activity and supervision of the work done by CHV. In the study population, male to female ratio was 1:0·9 (Fig. 1b). The majority of the population is young working adults. The entire population in the field was surveyed on weekly basis. The population has access to a health clinic run by Pune Municipal Corporation along with private clinics run by private practitioner's. Those cases suspected for ILI were referred to medical general practitioners clinics by CHWs and specimens were collected by project staff from eligible ILI case (onset of symptoms within 72 h). Influenza A(H1N1)pdm09 activity in the year 2012 (weeks 7–15) and the year 2015 (weeks 4–13) was analyzed.

Case definitions

A case of ILI was defined as a person of any age living in the study area and presented or reported with an acute onset of fever (>38 °C) with a cough and/or sore throat within 7 days in the absence of any other diagnosis. A person with ILI with laboratory confirmation for influenza A(H1N1)pdm09 virus on a throat swab and nasal swab by real-time reverse transcriptase polymerase chain reaction (RT–PCR) was considered as confirmed case of influenza A(H1N1) pdm09. The rise in a number of ILI and laboratory confirmation of influenza cases was considered as start of influenza season and decline in a number of ILI and laboratory confirmation of influenza cases was considered as an end point of influenza activity during the influenza outbreak period in the year 2012 and 2015.

For serosurvey purpose, among 25 clusters in the study area, a case area was defined as those clusters where one or more confirmed influenza A(H1N1) pdm09 cases were reported; while control area was defined as those clusters where no confirmed case of influenza A(H1N1)pdm09 was reported during the outbreak. Influenza vaccination history was inquired from study participants. Haemagglutination inhibition (HI) assay was performed for detecting antibodies against influenza A(H1N1)pdm09 virus and a titer of $\geqslant$1:40 was considered seropositive [4]. All the persons living in the study area and meeting the above case definitions were included in the study.

Data collection

Clinical details from ILI cases were recorded in standardized clinical proforma by trained project staff. Information collected from all the cases of ILI included the demographic details, residence, date of onset of illness, clinical details, results of laboratory investigations, history of travel, and history of contact with a positive case of influenza A(H1N1)pdm09 virus.

Specimen collection and laboratory investigations

Throat swabs and nasal swabs were collected from ILI cases having post onset date of illness within 3 days

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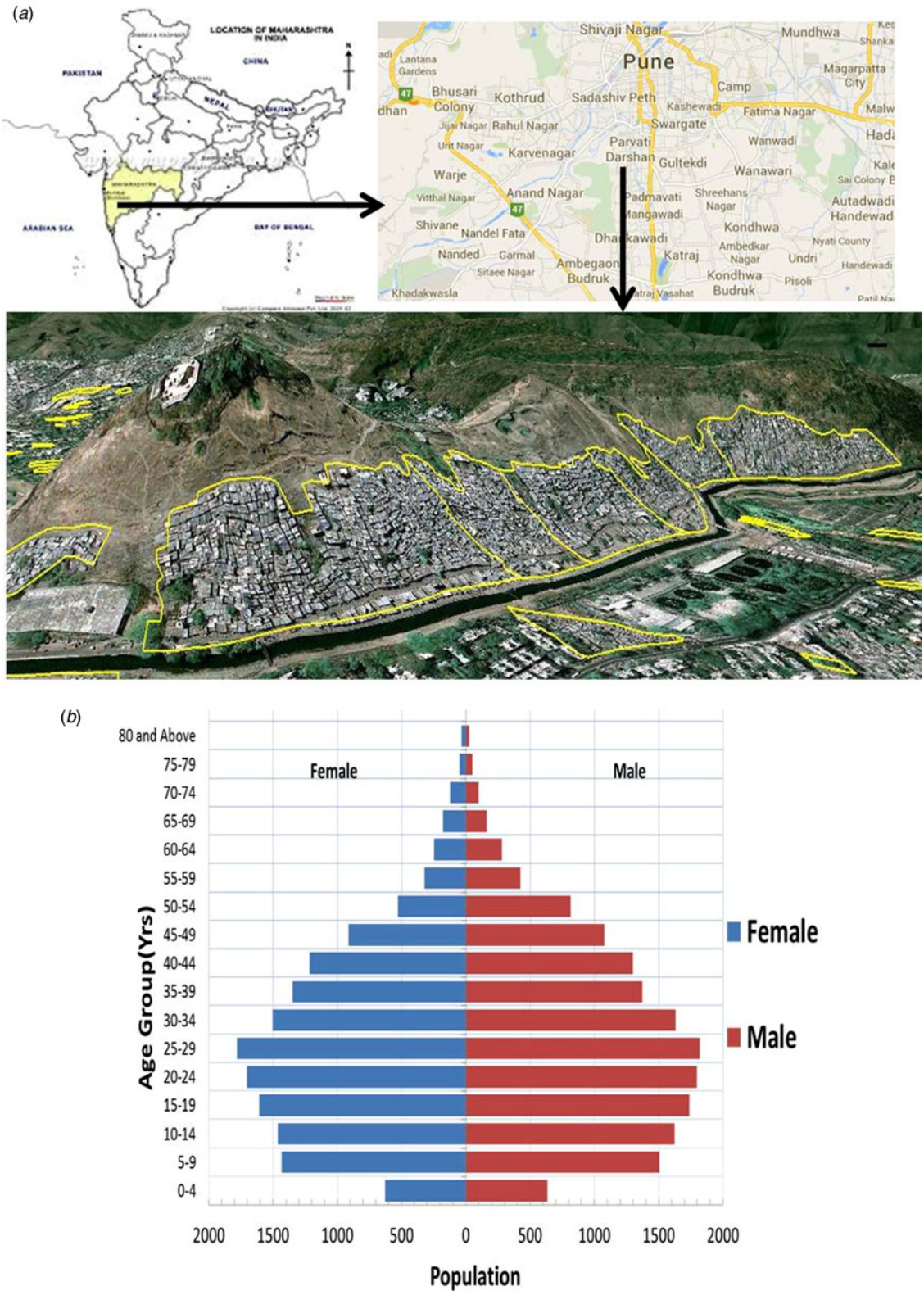

Fig. 1. Study area and study population. (a) Location of community site in Pune city, Maharashtra, India. (b) Population pyramid showing age and sex distribution of study population.

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and came at general practitioner's clinics for taking treatment. Throat swabs and nasal swabs were collected in sterile viral transport medium were transported at 4 °C to the National Institute of Virology, Pune. All the specimens were tested by real-time RT–PCR for influenza type (A and B), and influenza A positives further subtyped for H3, H1 (CDC Primer probe) and novel H1N1 [6]. Attempts were also made to isolate the influenza virus using Madin–Darby canine kidney (MDCK) cells as per the World Health Organization (WHO) manual (WHO 2002) [7]. Influenza results were informed to Municipal Corporation health authorities in Pune. Blood samples were also collected from influenza A(H1N1) pdm09 cases, family members of the influenza A (H1N1)pdm09 cases and from control area during the outbreak in the year 2012. The serum samples were tested by hemagglutination inhibition assay for detection of antibodies against pandemic influenza A (H1N1)pdm09 virus, using 0·5 percent Turkey red blood cells. Briefly all serum samples were treated with receptor-destroying enzyme (Denka Seiken, Japan) for removal of non-specific inhibitors. Sera with non-specific agglutinins were treated with Turkey red blood cells. The final dilution of the serum was 1:10. Pandemic (H1N1) 2009 virus isolated at National Institute of Virology, Pune, was grown in 10-day-old specific pathogen-free embryonated chicken eggs, inactivated by β-propiolactone and was used in the assay. Antigens of seasonal influenza viruses were obtained from the WHO Collaborating Centre for Influenza, Centres for Disease Control and Prevention, Atlanta, USA. Titers were reported as the reciprocal of the highest dilution for complete inhibition. [7].

Pandemic influenza-positive samples and type B positives were analyzed genetically for known resistance markers and 1146 bp type B NA gene was sequenced to cover all known mutation sites 119, 152, 198, 222, 274, responsible for drug resistance. Pandemic H1N1 viruses were assessed for H274Y mutation in NA gene by allelic discrimination real-time PCR [8].

Sequencing of HA gene and phylogenetic analysis

Full HA gene (1700 bp) was amplified in four overlapping fragments using WHO-recommended sequencing primer. The PCR products were purified using PCR purification kits (Qiagen). DNA sequencing was carried out using Big Dye terminator V 3.1 cycle sequencing ready reaction kit (ABI, Foster City, CA) and unincorporated labeled ddNTP's were removed using Dye-X removal column purification kit (Qiagen). The sequencing was done on ABI 3730 DNA analyzer and pairwise sequence alignment and protein translation, was performed with MEGA 6 program. Phylogenetic analysis of pandemic influenza A(H1N1)pdm09 viruses from the study was done and compared with the viruses isolated from severe cases (hospitalized cases in intensive care unit in tertiary care hospitals in Pune) of the same period and WHO reference viruses. These severe cases were referred to NIV (National Institute of Virology, Pune) for pandemic influenza detection. MEGA version 6 was used for constructing NJ (neighbor-joining) trees using the Tamura–Nei model.

Data analysis

We calculated the cumulative and weekly incidence, overall and age-specific attack rates, a proportion of suspected and laboratory-confirmed cases. The χ^2 test was used to compare the seropositivity for influenza A(H1N1)pdm09 among different population groups. Karl Pearson's correlation coefficient was calculated to assess the correlation between ILI cases and different climatic factors.

Calculation of growth rate, basic reproduction number and transmission rate

During the initial phase of the outbreak, the numbers of secondary cases are assumed to increase at an exponential rate. The growth rate of the epidemic (r) was calculated from the estimates of cumulative number of confirmed infections (y) and the estimated start date and size of the outbreak (t_0 and y_0), respectively, and using the following equation [9, 10].

$$y = y_0\, e^{r(t-t_0)} \tag{1}$$

The effective reproduction number R is defined as the average number of secondary cases generated by the introduction of one infective into a population made up of both susceptible and non-susceptibles during the course of infection of the infective. In the present case, R was computed using the formula

$$R = n\left(1 + \frac{r}{\alpha}\right)\left(1 + \frac{r}{k}\right) \tag{2}$$

with r being the growth rate of the epidemics obtained from eqn (1), the mean infective period, $1/\alpha$ and mean incubation period, $1/k$ and n is the proportion of the

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population susceptible to the virus. Since all types of influenza involve a definite incubation period in the host (exposed or latent state) and a definite infectious period for the symptomatic host (infectious state), effective modeling of such an epidemic should account for both these periods.

The transmission rate (β) was computed as: $\beta = R\,\alpha/N$, where N is the population size [11]. The doubling time (the time period in which the size of the outbreak doubles) is given by $t_d = \ln(2/r)$, where r is the exponential growth rate of the epidemic [12].

Graph plotting and mathematical calculations were performed using the MATLAB® software package. The epidemic growth rate has been estimated from the growth in the number of infected persons during the initial phase of the outbreak, as available from the clinico-epidemiological data. The R has been estimated from eqn (2) using the estimated growth rate and assuming: (i) mean duration of symptoms (infectious period) as 4 days ($=1/\alpha$; $\alpha = 0{\cdot}25$) and (ii) mean latent period as 1·5 days ($1/k$; $k = 0{\cdot}66$), both standard values for human infections of influenza [9, 11–15].

Ethical clearance

The study was approved by Institutional Ethics Committee of National Institute of Virology, Pune. Written consent was collected from the participants in the study before collection of clinical samples.

RESULTS

Observations during surveillance period (2012–2015)

During surveillance of ILI between the year 2012 and April 2015, among 29 797 population in a study area, influenza A(H1N1)pdm09 outbreak was reported in the year 2012 (February–April) and the year 2015 (January–April) (Fig. 2).

In the year 2012, the rise in influenza A(H1N1)pdm09 cases were reported in February and March 2012 (59 cases in late winter and early summer months) (Fig. 2a, c). In the same year (2012), both influenza A(H1N1)pdm09 and influenza A(H3N2) activity were also noted between July and October months (Fig. 2a). Influenza A(H3N2) activity was predominantly observed from February to September in the year 2013, sporadic influenza A(H1N1)pdm09 cases were reported in February–April in 2013 and a case in August. In the year 2014, influenza type B cases were reported between May and September (Fig. 2a) and in the year 2015, again rise in influenza A(H1N1)pdm09 cases (60) were reported between February and March and an influenza type B case in March (Fig. 2a). Proportions of sampled ILI cases varied during the surveillance period (2012–2015) (Fig. 2b). The detailed observations in the year 2012 and 2015 are being reported separately.

Observations in the year 2012

In year 2012, a total of 499 ILI cases were reported between 6 February and 29 April 2012 (i.e., between 6th and 17th week). The rise in ILI cases started in 8th week and peaked on 11th week (Fig. 2c). In 2012, ILI cases reported in adults (20–59 years) were 39·3% (198/499) followed by children ($\leqslant$9 years) 39·3% (196/499). Age-specific attack rate of ILI cases among children (aged $\leqslant$9 years), adolescents (10–19 years), adults (20–59 years) and elderly ($\geqslant$60 years) was 39·6, 14·3, 11·1, and 9·2 per 1000 persons in that specific age group (Fig. 3a).

Percentage positivity for influenza A(H1N1)pdm09 among children (aged $\leqslant$9 years) was 15·1% (16/106) and among adults (aged 20–59 years) was 29·4% (30/102) and the difference was found to be statistically significant (χ^2 statistics: 6·1862; $P = 0{\cdot}0131$). Percentage positivity for influenza A(H1N1)pdm09 among adolescent (aged 11–19 years) was 25·9% (14/54). The first influenza A(H1N1)pdm09 case was detected in the 8th week (Fig. 2c). A total of 59 influenza A(H1N1)pdm09 cases were reported from 55 families and 15 influenza B cases were reported from 15 families. Clustering of influenza A(H1N1)pdm09 cases was observed in the family where two influenza A(H1N1)pdm09 cases were reported in three families and four influenza A(H1N1)pdm09 case in a family within 1–4-day duration. The overall attack rate of ILI was 1·67% and attack rate for influenza A(H1N1)pdm09 was 0·20%.

Common comorbid conditions reported among influenza A(H1N1)pdm09 cases were hypertension (7), diabetes (5) and obesity (6). Among these eight influenza A(H1N1)pdm09 cases (13·5%) required hospitalization. Other influenza A(H1N1)pdm09 cases had mild illness.

Average relative humidity decreased from weeks 7 to 11 (Fig. 2c). Karl Pearson's correlation coefficient (week 7–16) showed that ILI cases negatively correlated with relative humidity (Karl Pearson's correlation coefficient, $r = -0{\cdot}71$; $P = 0{\cdot}02$), while ILI cases were positively correlated with the temperature

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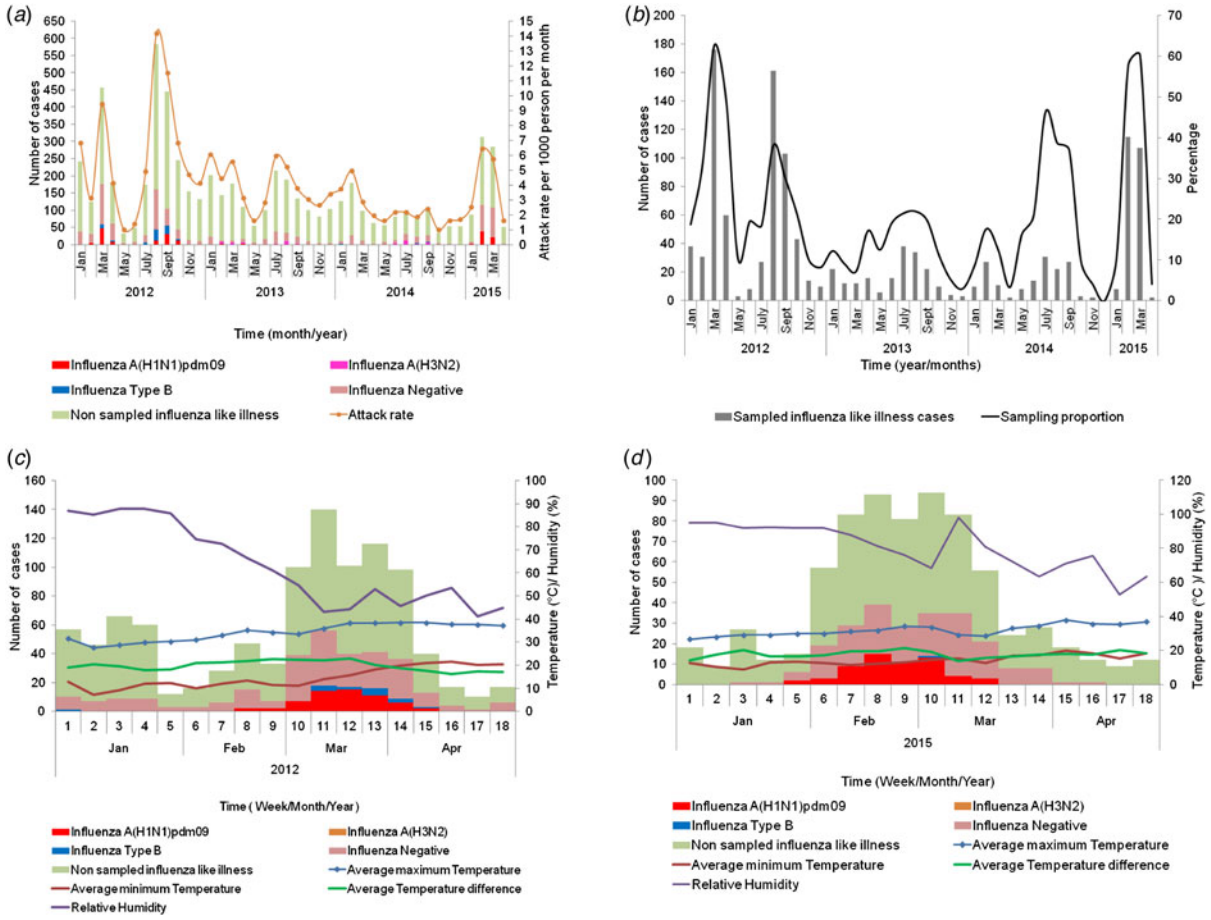

Fig. 2. (a) Time distribution of incidence density of influenza-like illness cases showing influenza etiology (b) sampling proportion from year January 2012 to April 2015. (c) Time distribution of influenza like illness cases showing influenza etiology and its correlation with seasonal factors during influenza A(H1N1)pdm09 outbreak in year 2012 and (d) in year 2015.

difference ($r = 0{\cdot}44$; $P = 0{\cdot}21$), i.e. difference between maximum and minimum temperature.

HI assays were performed for detecting antibodies against influenza A(H1N1)pdm09 and a titer of $\geqslant$1:40 were considered seropositive. In case area, blood samples could be collected from 31 influenza A(H1N1)pdm09 cases and seropositivity among influenza A(H1N1)pdm09 cases was 83·9% (95% CI 70·9–96·8). However, seropositivity among contacts of influenza A(H1N1)pdm09 cases (41/82, 50%) was significantly higher (χ^2 test = 10·12, df 1, $P = 0{\cdot}0017$) as compared with control area (30/109, 27·5%) (Table 1). No influenza vaccination was received by any study participants.

Based on the growth of the cumulative confirmed cases for the first 30 days (Fig. 5a), the intrinsic exponential growth rate (r) was calculated and the value was found to be 0·1121 per day. Assuming the mean incubation period to be 1·5 days and the mean infectious period (duration of symptomatic and infectious state) as 4 days, R was estimated to be 1·30. This indicated a moderate transmission of influenza A (H1N1)pdm09 in the population. Also, considering the total residing population 29 797, the transmission rate (β) was estimated as $1{\cdot}09 \times 10^{-5}$ per day. The doubling time of the epidemic was estimated to be 2·88 days.

Observations in the year 2015

A total of 377 ILI cases were reported between weeks 4 and 13 (Fig. 2d). ILI cases reported in adults (20–59 years) were 48·3% (182/377) followed by children ($\leqslant$9 years) 35·1% (132/377). Age-specific attack rate of ILI cases among children (aged $\leqslant$9 years), adolescents (10–19 years), adults (20–59 years) and elderly ($\geqslant$60 years) was 24·9, 9·3, 9·7, and 7·8 per 1000 persons in that specific age group (Fig. 3b). Average relative

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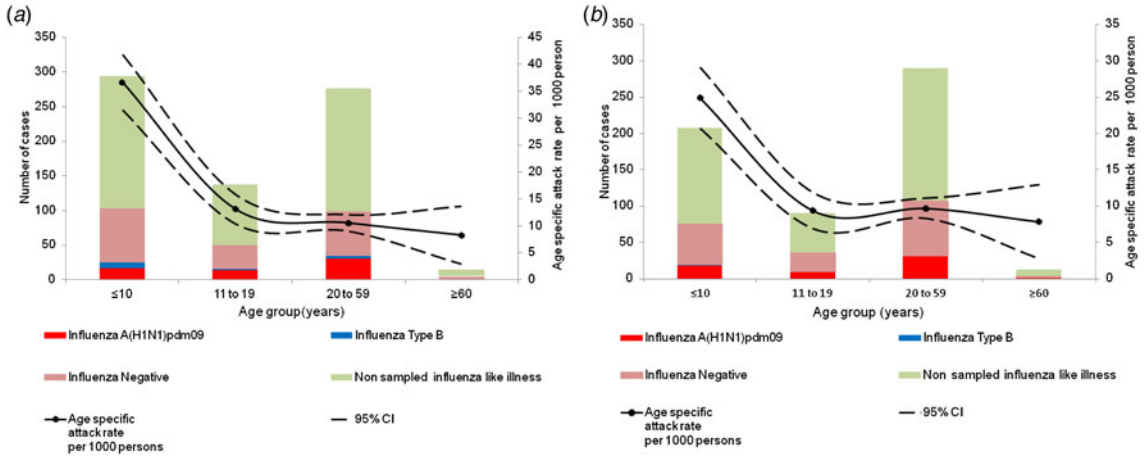

Fig. 3. Age-specific attack rate of influenza-like illness cases per 1000 persons showing influenza etiology in (a) January–April 2012 and (b) January–April 2015.

Table 1. *Seropositivity for influenza A(H1N1)pdm09 among various subgroup population detected by haemagglutination inhibition (HI) assay*

Subgroup population	Seropositive (total subjects)	Seropositive percentage With HI titer ⩾40 (95% CI)
Influenza A(H1N1) pdm09 cases	26 (31)	83·9 (70·9–96·8)
Family member of influenza A(H1N1) pdm09 cases	41 (82)	50 (39·18–60·82)
Population in control area*	30 (109)	27·5 (19·14–35·91)

* Representative study population in study area where no influenza A(H1N1)pdm09 case was reported during the outbreak.

humidity decreased from weeks 6 to 10 (Fig. 2d). Karl Pearson's correlation coefficient (week 4–13) showed that ILI cases were negatively correlated with relative humidity (Karl Pearson's correlation coefficient, $r = -0{\cdot}44$; $P = 0{\cdot}20$), while ILI cases were positively correlated with the temperature difference ($r = 0{\cdot}77$; $P = 0{\cdot}01$) (Fig. 2d).

Considering the growth of cumulative confirmed cases for the first 16 days (Fig. 5b) the intrinsic exponential growth rate (r) was 0·1743 per day. Assuming all other parameters for influenza A(H1N1)pdm09 to remain the same (as in 2012), the R was estimated to be 1·64. This indicated a slightly higher transmission in the population compared with 2012. Also, considering the total residing population of the area under surveillance (Janata Vasahat) to be $N = 29\,797$, the transmission rate (β) was estimated as $1{\cdot}37 \times 10^{-5}$ per day. The doubling time of the epidemic was estimated to be 2·44 days.

Clinical findings, sequencing of HA gene and phylogenetic analysis observations

Among reported 119 influenza A(H1N1)pdm09 cases reported in the year 2012 and 2015 in summer months (59 cases in 2012 and 60 cases in 2015), common clinical features were fever (100%), cough (90·7%), sore throat (85·7%), nasal discharge (48·7%), headache (55·5%), fatigue (18·5%), breathlessness (3·4%), and ear discharge (1·7%). All influenza A(H1N1)pdm09 cases received oseltamivir treatment. No case fatality was reported among influenza A(H1N1)09pdmcases in the year 2012 and 2015.

Seven viruses of 2015 and six viruses of 2012 were isolated using MDCK and HA gene were sequenced and submitted to Gene bank (Accession nos KX792276, KX792277, KX792280, KX792281,KX792286, KX792287 & KX 792333, KX 792349, KX 792356, KX 792361, KX 792364, KX 792373, KX 79 2393).

In these outbreaks, influenza A(H1N1)09pdm strains circulating were similar to A/California/07/2009 2009–2016 vaccine component. The phylogenetic analysis showed that 2015 viruses were grouped in clade 6B with signature mutations K163Q & A256T and 2012 viruses were grouped in clade 7 with signature mutation S143G & A197T (Fig. 4). HA-based analysis of 89 type b viruses showed that both the lineages were in circulation however Yamagata

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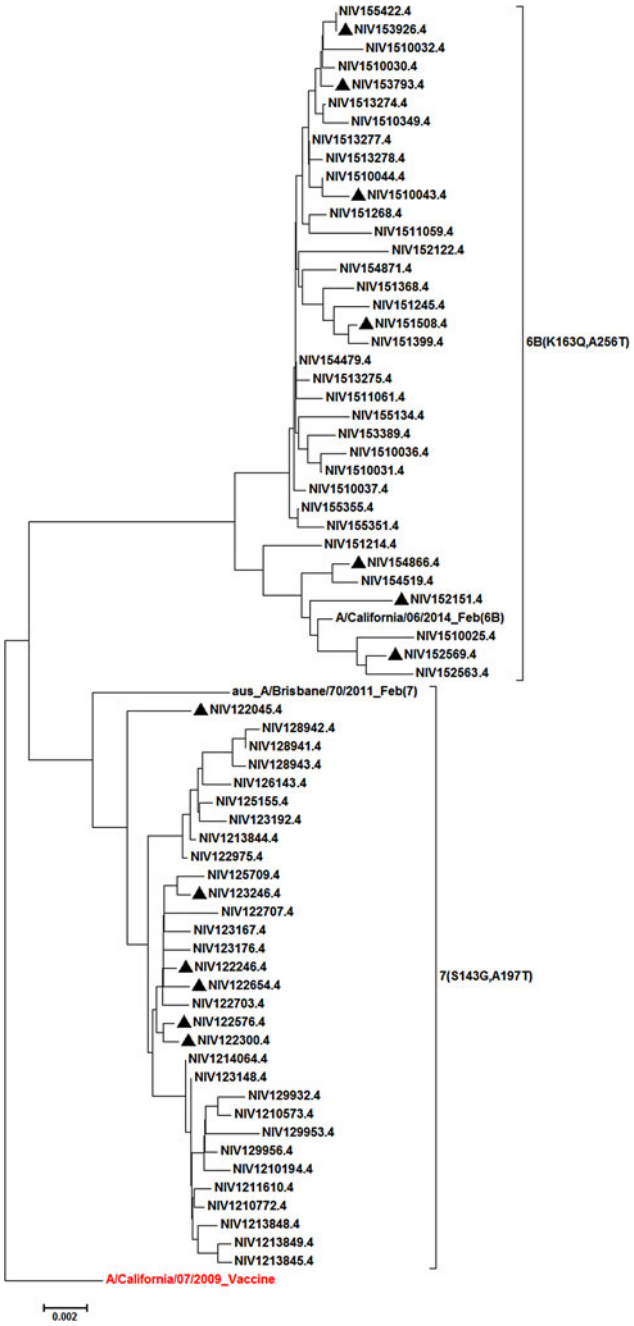

Fig. 4. HA gene phylogenetic analysis of 2012 and 2015 influenza A(H1N1)pdm09 strains from the study denoted with solid black triangle and compare with viruses from severe cases of same period. The vaccine component is shown in red color.

lineage was predominantly circulated (Yamagata (n = 75) and Vitoria (n = 14)).

Allelic discrimination real-time PCR to detect H275Y mutation in pandemic H1N1 showed that both the 2012 and 2015 viruses were sensitive to oseltamivir. NA gene sequencing of type B isolates confirmed that the viruses were sensitive to oseltamivir.

DISCUSSION

An outbreak of influenza A(H1N1)pdm09 virus was confirmed during the routine surveillance of ILI cases in the slum community in Janata Vasahat, Pune, India. The study highlights the rise in influenza activity in the inter-seasonal period of the year as evident in the year 2012 and 2015 (Fig. 2). The present

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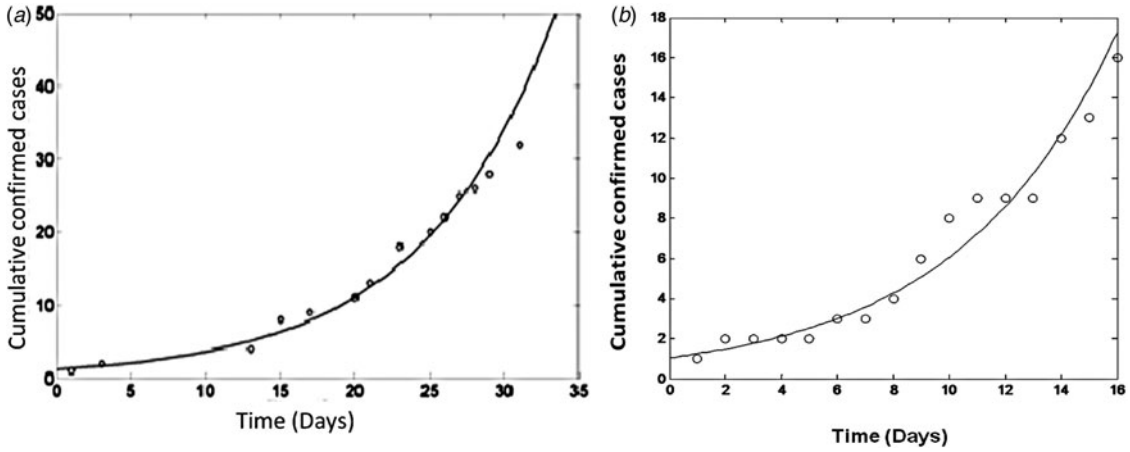

Fig. 5. Cumulative growth of confirmed cases of influenza A(H1N1)pdm09 with time. Data plot and curve fitting in MATLAB for (a) year 2012 (data set: 21 February–22 March 2012) and (b) year 2015 (data set: 1 February–21 March 2015).

study showed that most of the influenza A(H1N1) pdm09 cases reported mild symptoms with self-limiting illness. High influenza transmission was noted in close contacts of influenza A(H1N1)pdm09 cases and moderate transmission of influenza was observed in a study area which did not report influenza A(H1N1)pdm09 cases.

Outbreaks of influenza A(H1N1)pdm09 have been reported in close institutional settings [3, 16] as well as in community settings in India [17]. Earlier, an outbreak of influenza reported in Pune among children presented with severe respiratory illness [18] and the majority of influenza cases were only reported in children age group 5–15 years [19]. Influenza surveillance multi-centric study in India reported majority (45%) of ILI among children [20]. An outbreak of influenza A(H1N1)pdm09 in Kolkata, West Bengal, India reported cases predominantly among young and middle-aged persons. However, in the present influenza outbreak, ILI cases were predominantly among children aged $\leqslant$9 years of age (Fig. 3).

During the influenza A(H1N1)09pdm outbreak in the year 2012, percentage positivity for influenza A (H1N1)pdm09 among children aged $\leqslant$9 years was lower (15·1%) as compared with adults (29·4%) in age group 20–59 years of age and difference was found to be statistically significant ($P = 0{\cdot}01$). A study in Delhi reported 18·6% positivity of influenza A(H1N1)pdm09 among adults [21]. As seen elsewhere, attack rate of influenza A(H1N1)pdm09 have varied among different age group, however, in most of the studies have reported children as a high-risk group [22, 23]. In the present study age-specific attack rate was high in children (aged $\leqslant$9 years) as compared with adolescents, adults and this could be a result of overcrowded conditions in the home, schools, and social environment in a slum.

In an influenza outbreak in Kolkata, most of the suspected (61·1%) and laboratory-confirmed (59·7%) cases were in the age group of 15–49 years, and most (74%) laboratory-confirmed patients need hospitalization and had comorbid conditions (11%) [17]. In the present outbreak, only eight influenza cases with comorbid conditions among adults needed hospitalization which may be due to most of the mild influenza cases and timely administration of oseltamivir therapy. No complications and case fatality was reported in the reported outbreaks as compared with case fatality ratio (CFR) of 0·90 percent reported in Kolkata [17] and other CFR estimates of 0·004–1·5 percent from other countries [24]. In the present study, none of the cases with influenza B positivity had complications. Similar findings were reported in a Nomadic community in Jammu and Kashmir [25]. However, severe complications such as encephalitis/encephalopathy, influenza-associated myositis and ARDS (acute respiratory distress syndrome) have been reported from fatal cases with influenza B in Taiwan [26].

As evident by the presence of antibodies against influenza A(H1N1)pdm09 virus (Table 1), transmission of influenza in the families reported by seropositivity of influenza A(H1N1)pdm09 case in families was significantly higher (50%) as compared with the area not reporting with influenza A(H1N1)pdm09 cases (27·5%). This shows active transmission of the

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influenza A(H1N1)pdm09 virus in close family contacts only. Earlier, serosurvey conducted in Pune during the influenza pandemic in 2009, seropositivity of influenza A(H1N1)pdm09 in slum-dwellers was 3·5% and 6% in general population.[4]. In this study, large number of subjects in the study population use to visit different places in Pune city and outside the city for their job. Hence, possible links of contacting the infection of influenza A(H1N1)pdm09 virus to the subjects in the study population from surrounding areas cannot be ruled out. Influenza vaccination was not reported by any study participants, which suggests need of health education to slum population regarding various approaches for prevention of influenza.

During influenza outbreaks in 2012, the moderate transmission of influenza was noted ($R = 1{\cdot}30$) (Fig. 5a) as compared with the intense transmission of the influenza virus observed in clustered population in earlier studies [9, 27, 28]. Hence, the doubling time is longer in this outbreak compared with earlier observations [9, 28]. During 2015 outbreak, the R was 1·64, which is slightly higher than that in 2012 and it indicates a higher rate of transmission. This reflected in a slightly shorter doubling time, 2·44 days in 2015 as compared with 2·88 days in 2012 (Fig. 5).

Tropical monsoon climate is observed in Pune, India with March–May months represent hot season (summer), June–September a cooler wet season (rainy season) with October as a transitional hot month, and November–February being cold season [18, 20, 29]. Surveillance studies of influenza in Pune showed that influenza outbreak occurred predominantly during the rainy months [20, 29, 30]. A multi-centric study done in India in which analysis of monthly data over a 5-year period (2009–2013) from Pune showed influenza circulation primarily from June to October for most years, with discrete peaks in July–September. The predominant subtype was influenza A(H1N1)pdm09 in 2009, 2010, and 2012; influenza A(H3) in 2011 and 2013 [29]. Outbreaks of influenza A and influenza B were also reported in Pune city during summer season (March and April) in 1979 [19, 31]. However, in the year 2005, 2006, and 2007, during July and August (rainy season) the predominant influenza activity was observed with minor activity in the months of January–March [20]. Various influenza A(H1N1) pdm09 outbreaks in India reported peak influenza A (H1N1)pdm09 activity in rainy season [3,17]. In the present study in the year 2012, influenza cases started increasing gradually in the month of February and peaked in March month (summer season).

Various studies showed that weather factors do influence the manifestation of influenza-like symptoms [32]. In the present study correlation of ILI cases with climatic factors was analyzed for outbreak period only. The analyses of correlation of ILI cases with climatic factors in non-outbreak period could not be undertaken. The rise in ILI cases were negatively correlated with the seasonal factors such as relative humidity (Karl Pearson's correlation coefficient, i.e $r = -0{\cdot}71$ in the year 2012 and $r = -0{\cdot}44$ in the year 2015), while rise in ILI cases were positively correlated with the temperature difference ($r = 0{\cdot}44$ in the year 2012 and $r = 0{\cdot}77$ in the year 2015) (Fig. 2). A study in India showed that rainfall, relative humidity, and small differences between minimum and maximum temperatures has influenced the occurrence of influenza outbreak in the rainy season. The study also suggested the possibility of the involvement of other unidentified factors. [30]. A significant correlation between the temperature and absolute humidity at the time of infection has been shown in a study conducted in European countries [32].

The present outbreak could establish influenza etiology among 27·8% cases. Similar findings were noted in an outbreak of influenza in Kolkata with 29% influenza positivity in cases [17]. In the present outbreak, influenza A(H1N1)09pdm strains circulating were similar to vaccine component A/California/07 and sensitive to oseltamivir. Yamagata lineage type B strains were in circulation and were also sensitive to oseltamivir drug. The influenza A(H1N1) pdm09 activity was predominantly observed in summer month in the year 2012 in Pune (Fig. 2). A surveillance study of influenza viruses in Delhi, influenza B circulation was peaked in January and April 2010 in the urban area, but not until May 2010 in peri-urban area [21].

The early detection of the virus, and implementation of control measures are essential to limit the transmission of influenza virus during outbreaks. In the present influenza A(H1N1)pdm09 outbreak, Pune Municipal Corporation implemented several preventive and control measures to limit the spread of the infection in the community. Oseltamivir was administered to all influenza A(H1N1)pdm09 cases and their close contacts. Extensive information, education and communication (IEC) activities were also carried out in the communities to educate the people about prevention of influenza. The present study has the following limitations, the etiology of the other respiratory viruses and bacterial infections could not

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be established. The reasons for different transmission levels of influenza among influenza-negative ILI cases in case and control area (present in the same slum) could not be studied. A subset of ILI could be sampled due to sampling strategy (specimen collection for ILI cases having onset of illness within 3 days) and consent requirements during the surveillance period. This may have affected in calculation of reproduction number and other statistical analysis.

Influenza A(H1N1)pdm09 outbreak was confirmed during the surveillance of ILI cases in the slum community in Janata Vasahat, Pune, India. The study highlights the rise in unusual influenza activity in summer month with high attack rate of ILI among children aged $\leqslant 9$ years. Children in this age group may need special attention for influenza vaccination. The present study also highlights the fact that most of the influenza A(H1N1)pdm09 cases presented mild symptoms and were self-limiting. The study reports the high influenza virus transmission in close contacts of influenza A(H1N1)pdm09 cases and moderate transmission of influenza in an area not-reporting the influenza A(H1N1)pdm09 cases. This suggests the need of early detection of influenza cases, which could help in prevention and control of further influenza virus transmission.

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DECLARATION OF INTEREST

None.

ETHICAL STANDARDS

The authors assert that all procedures contributing to this work comply with the ethical standards of the relevant national and institutional committees on human experimentation and with the Helsinki Declaration of 1975, as revised in 2008.

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