

Original Article

Pertussis in north-central and northwestern regions of Algeria

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Abstract

Introduction: Pertussis outbreaks continue to occur in many countries despite high vaccination coverage. Under-diagnosed cases in adolescents and adults may result in increased transmission to infants, who are at risk of severe pertussis. Additional measures to protect both groups should be considered.

Methodology: Nasopharyngeal samples and sera were collected from patients and household contacts with clinically suspected pertussis. Diagnoses were confirmed by culture, real-time polymerase chain reaction (PCR), and serology. *Bordetella pertussis* isolates were characterized by antimicrobial sensitivity and fimbrial serotyping.

Results: Of 392 participants, 134/248 patients (54%) and 66/144 contacts (45.8%) had confirmed pertussis infections. *B. parapertussis* was not detected. All *B. pertussis* isolates were sensitive to the antibiotics tested, and all expressed the Fim3, not the Fim2, fimbrial serotype. Most patients (81.2%) were <6 months (51.8% of whom were <3 months) of age; 77.6% were unvaccinated, and most positive contacts were mothers 20–40 years of age.

Conclusions: Despite high vaccination coverage, pertussis is circulating in Algeria. Most infections occur in unvaccinated infants <6 months of age, with mothers as the main source of infection. An adolescent/adult booster should be considered. Adoption of sensitive and specific laboratory tests would improve pertussis diagnosis and surveillance.

Key words: Algeria; pertussis; infants; contacts; real-time PCR; serology.

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Introduction

Whooping cough or pertussis is a highly communicable human respiratory disease with epidemic peaks occurring every two to five years [1]. The primary etiologic agent is *Bordetella pertussis*. Other less-common *Bordetella* species (*e.g.*, *B. parapertussis*, *B. holmesii*, and *B. bronchiseptica*) have been implicated in similar illnesses [1]. Adolescents and adults are the most likely source of infections that occur in infants and newborns [1,2]. After the introduction of the whole-cell pertussis vaccine in 1950s, the incidence of pertussis in children decreased dramatically [1]. However, despite decades of ongoing high vaccination coverage, pertussis outbreaks have been reported in many countries in the past 10 years [3,4]. Waning vaccine-induced immunity in adolescents and adults and genetic changes in circulating *B. pertussis* strains have been implicated in the resurgence of pertussis. Furthermore, the

availability and use of increasingly more sensitive diagnostic tests may have contributed to an increase in the number of reported pertussis outbreaks [4,5].

In Algeria, mandatory pertussis vaccination began in 1969 with use of whole-cell pertussis vaccine and a primary vaccination schedule of three doses at 3, 4, and 5 months of age followed by one booster at 18 months of age. Currently, the whole-cell vaccine is the only vaccine in use in our country; the acellular pertussis vaccine has not yet been introduced. The National Institute of Public Health reported a subsequent, significant decrease of childhood pertussis morbidity and mortality that reached the lowest level in 1994 [6]. Since the 2000s, a recrudescence of the disease has been observed with peaks reported in 2001, 2004, and 2009. However, annual epidemiological reports may underestimate the incidence of pertussis because of underreporting of cases, reporting only clinically diagnosed cases [7], under-diagnosis of pertussis in

adolescents and adults, and the unavailability of sensitive and/or specific laboratory diagnostics tests [8]. This is the first report of pertussis surveillance data in different regions for Algeria.

Methodology

Clinical samples

Nasopharyngeal aspirates (NPAs) and nasopharyngeal swabs (NPSs) were obtained from patients with clinically suspected pertussis and from household contacts between February 2012 and September 2013. The samples were collected at several hospitals in north-central and northwestern regions of Algeria. NPSs were inoculated in Amies transport medium, and when possible, household contacts provided both NPSs and sera. All sera were obtained from individuals not recently vaccinated (less than one year). All NPAs and NPSs were analyzed by culture and real-time polymerase chain reaction (PCR). Clinical data associated with each sample, including age, sex, presence of household contacts, presence and duration of cough, administration of antibiotics prior to sampling, and immunization status were collected using a questionnaire. The work described below was carried out in the Medical Bacteriology Laboratory of the Institut Pasteur of Algeria.

Culture

When received, NPSs were inoculated onto Bordet-Gengou medium (Becton Dickinson, Franklin Lakes, USA) containing 10% defibrinated horse blood and incubated at 35°C for 7 days. Plates were observed after 24 hours of culture and then monitored every 48 hours for 1 week for the presence of suspicious colonies. The *B. pertussis* ATCC 9797 strain was cultured as a positive, media quality control.

Identification, serotyping, and susceptibility testing of isolates

Bordetella isolates were identified by colony morphology, oxidase and catalase reactions, and by using the API 20NE system (bioMérieux, Marcy L'Etoile, France). Serotyping was carried out by agglutination as previously described [9]. *B. pertussis* strain FDA 460 (Fim2+/Fim3+) and sera containing C10C2D5 monoclonal anti-Fim3 and F2β2G8 anti-Fim2 antibodies were used as references. Susceptibility to ampicillin (10 µg), erythromycin (15 µg), spiramycin (15 µg), azithromycin (15 µg), clarithromycin (15 µg), gentamicin (10 µg), ofloxacin (5 µg), cotrimoxazole (1.25/23.75 µg), doxycycline (30 µg), and rifampicin (5 µg) was determined on Mueller-

Hinton medium supplemented with 10% defibrinated horse blood using interpretive criteria. *B. pertussis* ATCC 9797 was used as an internal control.

Real-time PCR assay

Nucleic acid extraction was performed using High Pure PCR Template Kits (Roche Diagnostics, Mannheim, Germany) following the manufacturer's instructions. Genomic DNA was extracted from the *B. pertussis* ATCC 9797 reference strain using DNeasy Tissue Kits (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. *Bordetella* strains harboring *IS481* specific to *B. pertussis*, *B. bronchiseptica*, and *B. holmesii* or *IS1001* specific to *B. parapertussis*, were identified by real-time PCR.

Detection of *IS481* was carried out using an in-house reference real-time PCR method as previously described [10] until August 2012. After that time, validated commercial kits were used for the detection of both *IS481* and *IS1001*, *i.e.*, Simplexa *Bordetella pertussis/parapertussis* (FOCUS Diagnostics Cypress, California, USA) until March 2013 and *Bordetella pertussis/parapertussis* triplex (Bio-Evolution, Bry-sur-Marne, France) until September 2013, following the manufacturer's protocols [11]. *B. pertussis* ATCC 9797 DNA was used as positive control for the in-house real-time PCR.

Serology

Anti-pertussis toxin (PT) IgG antibodies were detected and quantified using a commercial enzyme-linked immunosorbent assay (ELISA) kit (EUROIMMUN, Lübeck, Germany) according to the manufacturer's instructions and as previously described [12]. Titers ≥ 100 UI/mL indicated an acute infection if the patient had not been immunized during the previous year. Titers ≥ 40 and < 100 UI/mL were consistent with infection if the patient had not received a dose of vaccine in the previous year. The results were verified by analysis of a second serum sample obtained 10 days after the first. A negative serological result was defined as a single anti-PT IgG antibody titer < 40 UI/mL.

In this study, pertussis cases were laboratory confirmed by culture and/or real-time PCR and/or serology, or were epidemiologically linked to a laboratory-confirmed case (*i.e.*, a known contact with a case confirmed by culture, real-time PCR, or serology).

Statistical analysis

Data were reported as numbers and percentages. Chi-square tests were used to compare percentages. All reported *p* values <0.05 were considered statistically significant.

Results

Patients and samples

A total of 458 samples were collected from 392 study participants between February 2012 and September 2013. There were 248 samples from patients with clinically suspected pertussis and 144 from household contacts. The participants resided in northwestern, north-central and southern regions of Algeria. Most were from Oran (*n*=265), Blida (*n*=62), and Algiers (*n*=59). Only a few enrolled patients lived in Tizi-Ouzou (*n*=2), Médéa (*n*=1), and Adrar (*n*=3). The majority of clinical specimens were NPAs (*n*=245); 115 were NPSs. Sera were obtained from 98 participants; single sera from 55 and dual sera from 43. Both NPSs and sera were obtained from 66 participants. The majority of NPAs (*n*=196) were collected from 1–2 weeks after the onset of clinical symptoms, and the remaining specimens were obtained at 3–5 weeks (*n*=34) and 6–16 weeks (*n*=15). Sera were obtained between 1 and 8 weeks after the onset of cough. Real-time PCR targeted *IS481* in 362 of the NPAs and NPSs; *IS1001* was targeted in 264 samples.

Laboratory findings

Among the 392 study participants, a total of 200 (51%) pertussis cases were confirmed. Of these, 192 (49%) were laboratory confirmed by culture, real-time PCR, and/or testing for anti-PT antibodies, and 8 (2%) were epidemiologically linked to a laboratory-confirmed case (Table 1). Culture and real-time PCR were both positive for *B. pertussis* in 4 patients (1%). In 137 patients (35%), *B. pertussis* was detected by real-

time PCR only. Anti-PT IgG antibodies were detected in 51 cases (13%), 11.5% in single sera, and 1.5% in dual sera, with titers ranging from 100 to over 200 UI/mL. Both real-time PCR and serology were positive in 17 patients (4%), and 34 cases (9%) were confirmed by serology only. Titers between 40 and 70 UI/mL were observed in four cases. Second sera were not available for those cases; however, *IS481* was positive in all of them. Positive results for *IS481* with negative serology, *i.e.*, titers under 40 UI/mL, were reported in 5 cases. *B. parapertussis* was negative in all samples evaluated.

Real-time PCR was positive in 118 of the 126 patients (93.5%) with confirmed pertussis; culture and serology with or without real-time PCR were positive in 3% and 2.7% of cases, respectively. Pertussis was confirmed in 31 of the 66 household contacts (47%) by serology alone and in 35 household contacts (53%) by serology with or without real-time PCR (Figure 1). Real-time PCR detected *B. pertussis* in patients and household contacts much more frequently (82%) than did culture (2%; *p* <0.001; Table 1).

All 4 of the isolated strains were from infants between 47 days and 7 months of age who had not been vaccinated. All isolates were found to be *B. pertussis*, all were susceptible to the antibiotics tested, and all expressed Fim3 but not Fim2.

Incidence of pertussis infection

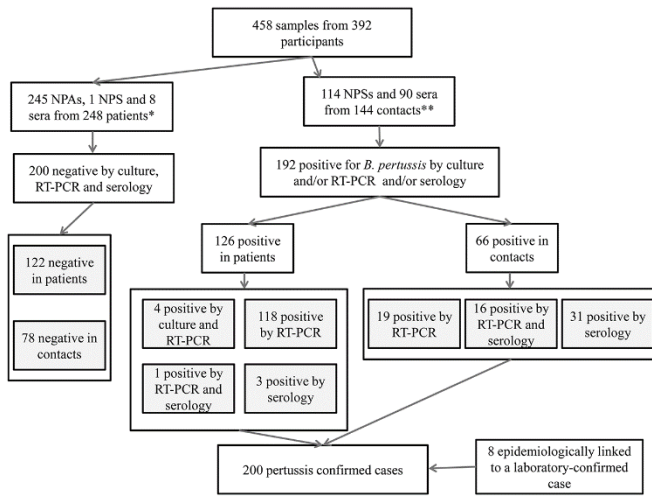
Pertussis was confirmed in 200 of the 392 study participants (51%). In 2012, the incidence was 1.73/100,000 in the study population and 7.33 in Oran city. Pertussis was confirmed in 134 of the 248 patients (54%), an incidence rate of 1.04/100,000, and in 66 of 144 household contacts (46%), an incidence rate of 0.69/100,000.

Table 1. Epidemiologic characteristics of 134 patients with, and 114 without, confirmed pertussis, Algeria, 2012–2013.

		Confirmed n (%)	Not confirmed n (%)	P value
Age	< 3 months	57 (42.5)	60 (52.6)	NS
	≥ 3 to < 6 months	53 (39.5)	30 (26.3)	< 0.05
	≥ 6 to < 18 months	9 (6.7)	12 (10.5)	
	≥ 18 to < 6 years	8 (6)	7 (6.1)	
	≥ 6 to < 16 years	5 (3.7)	2 (1.7)	
	≥ 16 years	2 (1.5)	3 (2.6)	
Sex	Female	63 (47)	53 (46.5)	NS
	Male	71 (53)	61 (53.5)	NS
Immunizationstatus	Unvaccinated	104 (77.6)	92 (80.7)	NS
	Incomplete	5 (3.7)	5 (4.4)	
	Up-to-date	9 (6.7)	8 (7.9)	
	Unknown	16 (11.9)	9 (7.9)	

P < 0.05: significant difference between percentages; NS: not significant.

Figure 1. Study flowchart.



*Six patients submitted both NPS and serum; **60 contacts submitted both NPS and serum; RT-PCR: real-time polymerase chain reaction; NPA: nasopharyngeal aspirate; NPS: nasopharyngeal swab

Epidemiologic characteristics and clinical findings in patients with confirmed pertussis

Of the 134 pertussis confirmed cases, 94% (126 cases) were laboratory confirmed (Figure 1); 6% (8 cases) were epidemiologically linked to a laboratory-confirmed case. None of the laboratory-confirmed cases were positive for *B. parapertussis*.

The median age of the patients with confirmed pertussis was 3 months (range: 26 days to 47 years); 110 (82%) were < 6 months of age and of those, 52% were < 3 months of age. No significant differences were observed in participants with confirmed versus unconfirmed pertussis who were < 3 months of age, but pertussis was confirmed in significantly more patients between 3 and 6 months of age ($p < 0.05$). The overall male-to-female ratio was 1.14, with no significant difference between the confirmed and unconfirmed pertussis patient groups (Table 1).

The seasonal distribution of confirmed pertussis cases reported throughout the study period showed a clustering in the three-month period from July to September ($n=89$ cases) compared with the number of cases ($n=31$) reported during the same period that were not confirmed ($p=0.05$, (Figure 2). The regional distribution of confirmed cases included 79 in Oran, 28 in Algiers, 23 in Blida, 3 in Adrar, and 1 in Tizi-Ouzou (Figure 3).

Available data from 85 of the pertussis-positive patients (63.4%) revealed that 78.8% presented with >14 days of cough, and 21.2% had coughs persisting for ≤14 days. Corresponding data from 63 unconfirmed

Figure 2. Seasonal distribution of case patients during the 2012–2013 study period.

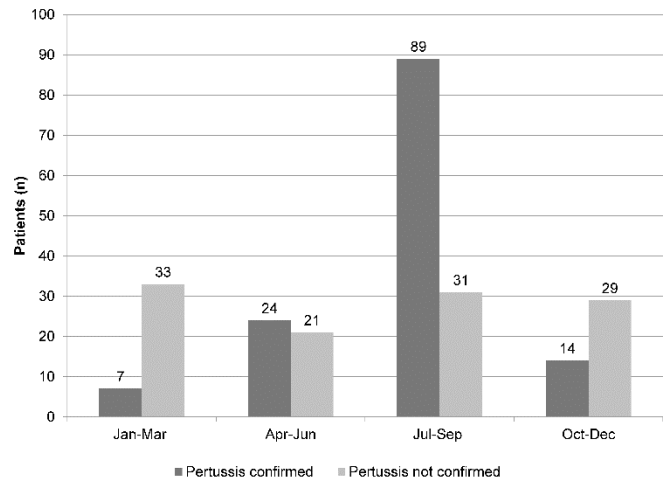
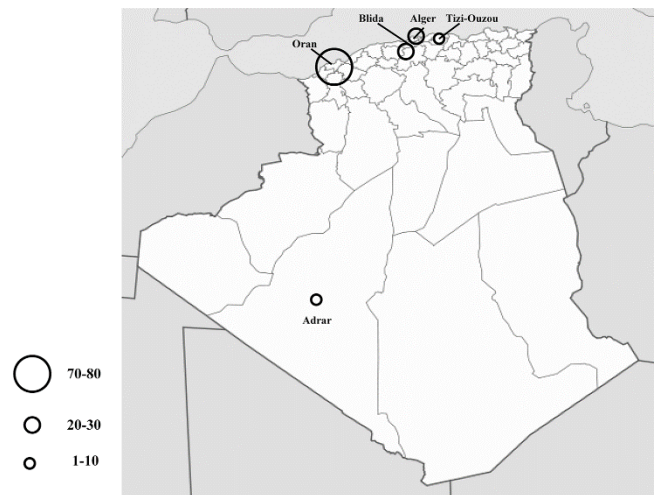


Figure 3. Geographic distribution of pertussis confirmed case patients during the 2012–2013 study period.



Source: SVG maps of Algeria at https://commons.wikimedia.org/wiki/Category:SVG_maps_of_Algeria.

cases (55%) showed that 49% of them presented with >14 days of cough and 51% had cough persisting for ≤14 days. The differences in cough duration of >14 days ($p < 0.001$) and ≤14 days ($p < 0.05$) were both significant.

Of the pertussis-confirmed patients, 100 (75%) had received prior antimicrobial treatment. Most of them (85%) were given macrolides, and 11% were given other antimicrobials; 4% of patients with confirmed pertussis did not receive antimicrobials. Seventy-nine of the patients who did not have confirmed pertussis (90%) received macrolides, 5% were given other

antimicrobials, and 5% were not given antimicrobials. The differences in antimicrobial treatment received by the two groups before study enrollment were not significant.

Immunization status is shown in Table 1. Most patients with confirmed pertussis (78%) had not been vaccinated. Of those, 89% were < 6 months of age, 61% were < 3 months of age, 4% were incompletely vaccinated, and 7% had an up-to-date vaccination status. The vaccination histories of 12% of the pertussis patients were not available. The differences in vaccination status observed in the two study groups were not significant (Table 1).

Epidemiologic characteristics of case contacts with confirmed pertussis

Pertussis was confirmed in 66 cases. *B. parapertussis* was not detected in any of the household contacts. The median age of household contacts was 30 years (range: 6 to 72 years); most (80%) were between 20 and 40 years of age. The ratio of males to females was 0.14. There was no significant difference in the percentages of female household contacts observed in the two groups, but the percentage of male household contacts was significantly higher in the group with confirmed pertussis than in the unconfirmed group ($p < 0.05$, Table 2).

The primary source of exposure and transmission of infection was the mother, comprising 82% of confirmed cases and 90% of unconfirmed cases ($p > 0.05$). Other close contacts made up only 18% of confirmed cases (Table 2). Of the 66 cases of confirmed pertussis in household contacts, 63 occurred in Oran and 3 in Blida.

Discussion

Laboratory findings

Pertussis cases were confirmed by culture and/or real-time PCR and/or presence of anti-PT antibodies. The finding that real-time PCR detected *B. pertussis* much more frequently than did culture (82% versus 2%; $p < 0.001$) highlights the importance of that assay method. Culture is the reference method because of its high specificity, but it has poor sensitivity, ranging from 12% to 80% [13]. In this study, most cultures were negative, which might be partially explained by the time between the onset of cough and specimen collection and the time between specimen collection and culture, which was more than 24 hours for many specimens received from Oran. Additional factors include possible bacterial and fungal contamination of specimens, and the antimicrobial treatment received by 95.5% of the patients, most of whom (87.5%) had received a macrolide before enrollment.

The real-time PCR results were positive for pertussis in 93.5% of patients. Real-time PCR and/or serology, and real-time PCR alone, confirmed pertussis in 71% and 29% of household contacts, respectively. These results are consistent with those of previous studies [14]; real-time PCR is the most sensitive tool in infants because pertussis is diagnosed early in the course of the disease. Serologic assays are more sensitive in adolescents and adults because they often seek medical attention for a prolonged cough, resulting in a delayed diagnosis of pertussis [14].

Real-time PCR for *B. pertussis* that targets *IS481* has increased sensitivity and rapidity, but false-positive results have been noted [15]. Indeed, other *Bordetella* species including *B. holmesii* and *B. bronchiseptica*, can cause respiratory illness similar to that caused by *B.*

Table 2. Epidemiologic characteristics of 66 household contacts with, and 78 without, confirmed pertussis, Algeria, 2012–2013.

		Confirmed n (%)	Not confirmed n (%)	P value
Age (years)	0 to < 10	1 (1.5)	1 (1.3)	
	≥ 10 to < 20	2 (3.0)	2 (2.6)	
	≥ 20 to < 40	53 (80.3)	70 (89.7)	< 0.05
	≥ 40 to < 60	9 (13.6)	4 (5.1)	
	≥ 60	1 (1.5)	1 (1.3)	
Sex	Female	58 (87.9)	71 (91.0)	NS
	Male	8 (12.1)	7 (9.0)	< 0.05
Contact	Mother	54 (81.8)	70 (89.7)	NS
	Father	5 (7.6)	5 (6.4)	
	Brother	0 (0)	1 (1.3)	
	Sister	1 (1.5)	1 (1.3)	
	Grandfather	1 (1.5)	0 (0)	
	Uncle	4 (6.1)	0 (0)	
	Aunt	1 (1.5)	1 (1.3)	

P < 0.05: significant difference between percentages; NS: not significant.

pertussis [16-19]. *IS481* is present in higher copy numbers in *B. pertussis* (80 to 193 copies per genome) than in *B. holmesii* (28 to 36 copies per genome) and *B. bronchiseptica* (≤ 1 copy per genome) [20]. However, a retrospective study of 11,319 PCR assays obtained in 1992–2003 using an *IS481* target in Finnish and Dutch patients with suspected pertussis did not detect *B. holmesii* despite finding 1,856 PCR-positive isolates [21]. The results of a similar four-year prospective study using an *IS481* target and conducted from 2007 to 2011 in 599 Tunisian infants did not detect *B. holmesii* in a total of 126 confirmed cases [22].

Five patients with negative serology were found positive for *IS481* by real-time PCR. This result can be explained by the presence of another species of *Bordetella* or by the serum samples having been obtained too soon after the onset of illness. In this study, we were unable to implicate or exclude *B. holmesii* or *B. bronchiseptica* in the pertussis case patients. However, 55 of the *B. pertussis* cases were confirmed by culture or serology.

Incidence of pertussis infection

A total of 200 of the 392 patients and household contacts (51%) evaluated during this 20-month period in 2012–2013 had a confirmed diagnosis of pertussis; 134 were patients and 66 were household contacts. The incidence rate in our study population was 1.73/100,000 in 2012, whereas in 2010 and 2011, the pertussis incidence rates reported by the National Public Health Institute were 0.09 and 0.07/100,000, respectively [8]. In 2012, the incidence rate in Oran city was 7.33/100,000 (seven times higher than the national incidence rate), while in 2011, the incidence rate was 0.06/100,000 [8].

Our findings are in line with a previous study conducted between 2003 and 2004 on patients living in Algiers [23] and with reports from other countries of a resurgence of pertussis despite high vaccination coverage [22-30]. Several explanations have been suggested, including the occurrence of pertussis outbreaks, or the two- to five-year cycles of increasing and decreasing incidence. This periodicity is transient, differs from one country to another, and may result from an increase in the size of susceptible populations or changes in vaccination coverage [31]. Other possible causes are low vaccination coverage that hinders optimal vaccine effectiveness, waning vaccine-induced immunity in adolescent and adult populations who had received whole-cell or acellular vaccines without subsequent boosters, *B. pertussis* adaptation (antigenic divergence of circulating and vaccine strains), and the

presence of asymptomatic transmitters, which potentially biases the estimation of vaccine effectiveness [2,4,5,28-34]. The resurgence of pertussis has also been attributed, at least in part, to increased routine use of real-time PCR and more sensitive serologic assays (real-time PCR was introduced in Algeria in 2012), and increased awareness of clinicians [2,4,5,28-32].

Pertussis confirmed case patients

A total of 134 of the 248 patients (54%) were confirmed pertussis cases, and only *B. pertussis* was found in those patients. This is in agreement with several studies reporting that *B. pertussis* was the main etiological agent of pertussis [35]. A negative result for *B. parapertussis* is consistent with the low rate of isolation of this bacterium previously reported in infants [36]. The limited number of patients evaluated in this study probably contributed to the negative result.

As previously described by other investigators, the occurrence of pertussis had a seasonal distribution with a peak occurring between July and September [1,37]. The majority of confirmed pertussis cases (59%) occurred in Oran, which suggests that an outbreak or a cycle of increased incidence as described by Mattoo and Cherry [1] had occurred. Alternatively, the relatively large number of specimens received from Oran may have resulted from an increased awareness of pertussis among clinicians. Similar to previous reports [23,38], the highest rate of infection (82%) was in infants < 6 months of age, with more than half of those cases diagnosed in infants < 3 months of age. In addition, most patients with confirmed pertussis (79%) presented with a cough of >14 days' duration [1].

Prior antimicrobial treatment, predominantly with macrolides, was reported by 96% of patients. Because pertussis is a severe disease in very young infants, and sensitive diagnostic tests may not be readily available, physicians frequently administer empirical antimicrobial treatment, including macrolides.

As reported by others, most confirmed patients (78%) had not been vaccinated. A large majority (89%) were < 6 months of age; 61% of those patients were < 3 months of age and were thus too young to be fully vaccinated [22,23,29,36]. These findings lead us to question the homogeneity of vaccine coverage rate in our country. Confirmed cases in infants < 3 months of age, which comprised more than half of the confirmed cases, were expected, as these patients were not eligible for vaccination. However, the confirmed cases among unvaccinated infants > 3 months of age reflect deficiencies in immunization coverage. Although the

national diphtheria-tetanus-pertussis (DTP3) vaccination coverage rate in Algeria is high, and ranged from 86% to 95% between 2000 and 2013 [39], low coverage (*i.e.*, <80%) has been reported in some regions, including Oran (71%), Algiers (78%), and Adrar (76%) (unpublished data). On the other hand, coverage in Médéa, Blida, and Tizi-Ouzou ranged from 89% to 92% (unpublished data), indicating that vaccination coverage may be heterogeneous.

Pertussis-confirmed case contacts

Of the 144 household contacts, 66 (45.8%) were confirmed cases, and most of them (80%) were between 20 and 40 years of age. These results confirm that, as in other countries, adults are the primary source of infection [2,40]. Increased incidence of pertussis in adolescents and adults has been observed in recent years, and those populations have been identified as sources of *B. pertussis* infection in unvaccinated or incompletely vaccinated infants [2,41-45]. However, these results may have been biased by increased surveillance and the lack of extended follow-up of all asymptomatic contacts. In this study, mothers were most frequent pertussis-confirmed contact of confirmed pertussis patients. They were thus the most likely source for transmitting the infection [22,46,47]. However, it should be noted that investigation of close contacts was systematic in mothers even if they were not symptomatic. Other contacts who were symptomatic or had a history of cough were evaluated. Furthermore, the high number of diagnosed case-contacts in Oran resulted from systematic case investigations conducted by clinicians. The introduction of a booster vaccination to this target population with attention to immunization of pregnant women must be evaluated.

Conclusions

Our study confirms that *B. pertussis* is still circulating in Algeria, especially in unvaccinated infants, indicating that the incidence of pertussis is probably underestimated in our country. Bacterial culture is required to monitor both antimicrobial resistance and spatio-temporal evolution of circulating *Bordetella* stains. Real-time PCR is the most sensitive method of detection, but PCR results should be confirmed by a reference laboratory. Serology is useful, particularly in adult populations. Establishment of national pertussis surveillance in both infants and adults using sensitive and specific biological methods is needed in Algeria. In order to control and prevent pertussis, vaccination programs should increase

population immunity by early immunization of infants at two months of age (currently being implemented in Algeria), achieving a primary vaccination coverage rate of over 90% in infants throughout the country, and considering the introduction of booster vaccinations for adolescents and adults.

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