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High performance of rapid influenza diagnostic test and variable effectiveness of influenza vaccines in Mexico

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ABSTRACT

Objectives: To evaluate the performance of rapid influenza diagnostic tests (RIDT) and influenza vaccines' effectiveness (VE) during an outbreak setting.
Methods: We compared the performance of a RIDT with RT-PCR for influenza virus detection in influenza-like illness (ILI) patients enrolled during the 2016/17 season in Mexico City. Using the test-negative design, we estimated influenza VE in all participants and stratified by age, virus subtype, and vaccine type (trivalent vs quadrivalent inactivated vaccines). The protective value of some clinical variables was evaluated by regression analyses.
Results: We enrolled 592 patients. RT-PCR detected 93 cases of influenza A(H1N1)pdm09, 55 of AH3N2, 141 of B, and 13 A/B virus infections. RIDT showed 90.7% sensitivity and 95.7% specificity for influenza A virus detection, and 91.5% sensitivity and 95.3% specificity for influenza B virus detection. Overall VE was 33.2% (95% CI: 3.0–54.0; $p = 0.02$) against any laboratory-confirmed influenza infection. VE estimates against influenza B were higher for the quadrivalent vaccine. Immunization and occupational exposure were protective factors against influenza.
Conclusions: The RIDT was useful to detect influenza cases during an outbreak setting. Effectiveness of 2016/17 influenza vaccines administered in Mexico was low but significant. Our data should be considered for future local epidemiological policies.
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Background

Influenza is a leading infectious cause of morbimortality worldwide. Annually, about 5 to 10% of adults and 20 to 30% of children have symptoms of influenza-like illness (ILI), and approximately 650,000 deaths secondary to influenza occur each epidemic season (World Health Organization 2018). Influenza vaccination is a major strategy to stop this global threat. However, mutational rates and local dynamics of circulating influenza virus strains affect the effectiveness of vaccines (Boni 2008). Furthermore, in 2009, a new pandemic influenza A(H1N1)pdm09 virus emerged in Mexico (Dawood et al. 2009; Perez-Padilla et al. 2009) for which there was no specific vaccine available when it appeared. Since then, global circulation of this virus has been reported (Bedford et al. 2015).

During the 2016/17 season, the Mexican Ministry of Health acquired a trivalent influenza vaccine containing an A/California/7/2009 (H1N1)pdm09–like virus, A/Hong Kong/4801/2014 (H3N2)–like virus, and a B/Brisbane/60/2008-like virus, as well as a quadrivalent influenza vaccine adding a B/Phuket/3073/2013-like virus, prioritizing their application to high risk groups in accordance with the World Health Organization (WHO) recommendations for influenza vaccination in the Northern Hemisphere (World Health Organization 2016). Despite this effort, 2727 cases of influenza A(H1N1)pdm09, 1306 of influenza AH3N2, and 1304 of influenza B occurred during the same period in Mexico, mainly affecting children (Secretaria de Salud and Dirección General de Epidemiología, 2017), either reflecting an insufficient immunization coverage or a low vaccine effectiveness (VE). Recently, we identified novel genetic substitutions affecting the virulence of influenza A(H1N1)pdm09 virus isolated from Mexicans that may potentially impact the influenza VE (Arellano-Llamas et al. 2017). Taken together, these data remark on the necessity for studies addressing influenza VE in Mexico.

On the other hand, timely diagnosis and early antiviral therapy are crucial to counteract influenza spread. However, current diagnostic tools such as the real-time polymerase chain reaction (RT-PCR) are expensive and time-consuming. Some rapid influenza diagnostic tests (RIDTs) are also used to rapidly support treatment decision during influenza outbreaks (Petrozzino et al. 2010). Nonetheless, RIDTs' performance varies according to the prevalence of different influenza virus strains and the method used to determine their results (Makkoch et al. 2012; Peci et al. 2014; Ryu et al. 2018). Therefore, the diagnostic accuracy of RIDTs must be validated in each population. Here, we evaluated the diagnostic performance of an RIDT and the influenza VE during the 2016/17 season in Mexico City.

Subjects, materials, and methods

Participants

The Ethics Committee of the National Institute of Respiratory Diseases (INER) in Mexico City approved the study. We enrolled all ILI patients attending to the INER from October 2016 to March 2017. ILI was defined as an acute respiratory illness with a temperature $\geq$38 °C and cough, with onset within the past 10 days (Fitzner et al. 2018). Clinical and demographic data were retrieved from all participants. In addition, we reviewed their immunization records looking for influenza vaccination for the current season. Patients who received vaccination within the last 14 days were ineligible. Most of the immunized participants received the 2016/17 influenza inactivated trivalent vaccine (IIV3; Vaxigrip® 2016/17, Sanofi Pasteur Inc., Canada). A subgroup of INER workers received the 2016/17 influenza inactivated quadrivalent vaccine (IIV4; Fluzone® Quadrivalent 2016–2017 formula, Sanofi Pasteur Inc., Canada). We collected respiratory swab samples to perform viral detection studies as described below. All patients provided written consent to participate in the study.

Rapid influenza diagnostic test

We used the Fuji dri-chem immuno AG cartridge FluAB kit (Fujifilm Corp., Tokyo, Japan) for detection of influenza viruses in fresh respiratory specimens. This test utilizes the immunochromatographic

Table 1
Clinical and demographic characteristics of patients with ILI.

Variable	n	%
Total	592	100
Age, median (Q_{25}-Q_{75})	14	6–40
Gender		
Male	270	45.6
Female	322	54.3
Influenza RT-PCR test positive results	302	51
Influenza A (H1N1)pdm09 virus	93	15.7
Influenza A H3N2 virus	55	9.3
Influenza B virus	141	23.8
Influenza A and B coinfection	13	2.2
Influenza RIDT positive results	298	50.3
Influenza A virus	145	24.5
Influenza B virus	140	23.6
Influenza A and B coinfection	13	2.2
Influenza vaccination during current season	171	28.9
Contact with ILI patients	311	52.5
Travel within the last 15 days	75	12.7
Median of days since onset of symptoms (Q25-Q75)	3	1–4
Vaccinated	3	1–4
Unvaccinated	3	1–4
Signs and symptoms		
Cough	526	88.9
Fever	497	83.9
General malaise	487	82.3
Headache	476	80.4
Rhinorrhea	465	78.5
Sudden onset of symptoms	458	77.4
Nasal congestion	456	77.0
Myalgia	421	71.1
Chills	411	69.4
Odynophagia	397	67.1
Arthralgia	371	62.7
Prostration	301	50.8
Conjunctivitis	284	47.9
Dysphonia	275	46.4
Abdominal pain	245	41.4
Chest pain	235	39.7
Dyspnea	231	39.0
Diarrhea	112	18.9
Cyanosis	45	7.6
Disturbance of consciousness	24	4.1
Comorbidities		
Asthma	71	12.0
Tobacco use	27	4.6
Diabetes	23	3.9
Immunosuppression	5	0.8
COPD	4	0.7
Use of antiviral drugs		
Amantadine	61	10.3
Oseltamivir	15	2.5
Rimantadine	7	1.1
Antibiotics prescription		
Amoxicillin	22	3.7
Clarithromycin	20	3.4
Ceftriaxone	19	3.2
Azithromycin	15	2.5
Amoxicillin/Clavulanate	14	2.4
Cefalexin	13	2.2
Penicillin	12	2.0
Levofloxacin	6	2.4
Erythromycin	5	2.0

COPD, chronic obstructive pulmonary disease; ILI, Influenza-like illness; RIDT, rapid Influenza diagnostic test; RT-PCR, real time polymerase chain reaction.

principle of virus detection as other conventional RIDTs, adding the silver amplification principle of photographic development to improve its sensitivity (Mori et al. 2012). Briefly, after dropping the sample into the test cartridge, the influenza A and B viruses react with two sets of specific antibodies conjugated either with colloid gold or colored latex, forming immune complexes on a labeling particle coated area. Such complexes flow through a porous carrier towards a membrane filter coated with capture monoclonal antibodies (detection line), forming sandwich complexes. Thus, two lines for colored latex and colloidal gold conjugates are formed in the detection line. The silver amplification occurs after the system supplies silver ions and a reducing agent (Fe2+), whereas 50 nm gold particles placed on the porous carrier function as catalysts. In this manner, large silver particles are produced during the reaction amplifying the colloidal gold particles conjugated to detection antibodies and making the two detection lines strongly visible. The test's sensitivity is further improved by washing off non-attached antibodies prior to the development reaction to avoid false positive results. We used the densitometry analyzer Fuji dri-chem immune AG1 ((Fujifilm Corp., Tokyo, Japan) which executes automatic results determination in up to 15 min, preventing visual interpretation errors (Mori et al. 2012).

We also determined the limit of viral detection of the RIDT using aliquots containing 10^6 to 10^2 viral particles of an influenza A/California/07/2009 (H1N1) pdm09-like virus (ATCC® VR-1894) propagated in MDCK cells and titrated by plate assay. The detection limit of the RIDT was compared with RT-PCR.

Influenza virus detection by RT-PCR

Detection of influenza viruses was assessed by RT-PCR according to WHO/CDC guidelines (WHO 2009; WHO 2017; CDC 2014). Briefly, nucleic acid extraction from clinical samples was performed with the PureLink Viral RNA/DNA mini kit (Thermo Fisher Scientific, Inc., USA). For the RT-PCR reactions we used the following specific primers: influenza A (forward 5′-GACCRATCCTGTCACCTCTGAC-3′, reverse 5′-AGGGCATTYTGGACAAAKCGTCTA-3′, probe 5′-(FAM)-TGCAGTCCTCGCTCACTGGGCACG-(BHQ1)-3′); influenza A(H1N1) pdm09 virus (forward 5′- GTGCTATAAACACCAGCCTYCCA -3′, reverse 5′- CGGGATATTCCTTAATCCTGTRGC-3′, probe 5′-(FAM)-CAGAATATACA"T"CCRGTCACAATTGGARAA-(BHQ1)-3′); influenza AH3N2 (forward 5′-ACCCTCAGTGTGATGGCTTTCAAA-3′, reverse 5′-TAAGGGAGGCATAATCCGGCACAT-3′, probe 5′-(FAM)- ACGAAG-CAAAGCCTACAGCAACTGTT-(BHQ1)-3′); influenza B (forward 5′-TCCTCAACTCACTCTTCGAGCG-3′, reverse 5′-CGGTGCTCTTGACCAAA TTGG-3′, probe 5′-(JOE)-CCAATTCGAGCAGCTGAAACTGCGGTG-(BHQ1)-3′). The reaction mixture was prepared with the Invitrogen Superscript III Platinum® One Step Quantitative Kit (Thermo Fisher Scientific, Inc., USA) using the following reagent volumes per reaction: nuclease-free water 5.5 μL, forward primer 0.5 μL, reverse primer 0.5 μL, SuperScript™ III RT/Platinum® Taq Mix0.5 μL, 2X PCR Master Mix12.5 μL, and isolated viral RNA 5 μL. The RT-PCR was conducted as follows: 50 °C for 30 min for cDNA synthesis, 95 °C for 2 min for Taq inhibitor activation, 45 PCR amplification cycles of 95 °C for 15 seconds and 55 °C for 30 s, using the CFX96 Real Time System Bio-Rad Platform (Bio-Rad Laboratories Inc.). We interpreted amplification curves according to WHO/CDC guidelines (WHO 2009, WHO 2017).

Statistical analysis

Descriptive statistics were used to characterize the study population. Considering the RT-PCR as the gold standard for the detection of influenza viruses in respiratory specimens, we determined the diagnostic value of the RIDT by calculating its sensitivity, specificity, positive predictive value (PPV), negative predictive value (NPV), diagnostic accuracy, and their corresponding 95% confidence intervals (CI). We estimated influenza VE in all participants and stratified by age, causative virus subtype, and vaccine type using the equation $(1–OR) \times 100$, where OR is the odds ratio for influenza among vaccinated compared with unvaccinated participants, determined from logistic regression models (Foppa et al. 2013). The 95% CI for VE was calculated using the equation 1–CIOR $\times 100$, where CIOR is the CI of the OR estimates. Values of $p < 0.05$ were considered significant. To determine the clinical characteristics associated with higher probability of influenza, we compared descriptive statistics between influenza positive and negative ILI cases by Xi^2 test, obtaining the corresponding ORs by bivariate and multivariate non-conditional logistic regression tests. OR values that did not include the null value in the 95% CI were considered significant.

Results

Participant characteristics

We enrolled 592 participants, 45.6% males and 54.3% females; their median age was 14 years. From these, 171 subjects (28.9%) received influenza vaccination for the evaluated season: 127 were immunized with the IIV3 and 44 received the IIV4. The median time of disease duration since symptom onset was 3 days with not significant differences between vaccinated and unvaccinated participants. The most common symptoms were cough, fever, general malaise, headache and rhinorrhea. Asthma was the most prevalent comorbidity (12.0%) and approximately 14 % of participants referred usage of antiviral drugs (Table 1).

RIDT's performance

The detection limit for the identification of influenza A(H1N1) pdm09 virus was 10^2 viral particles for RT-PCR and 10^3 viral particles for the analyzer-based RIDT. RT-PCR detected 302 influenza cases: 93 (15.7%) positive for influenza A(H1N1)

Table 2
Performance of the rapid Influenza diagnostic test compared with RT-PCR.

		RT-PCR			Sensitivity % (95% CI)	Specificity % (95% CI)	PPV % (95% CI)	NPV % (95% CI)	Accuracy % (95% CI)
		Positive	Negative	Total					
Influenza A									
RIDT	Positive	146	12	158	90.7 (85.2–94.3)	95.7 (92.6–97.5)	92.4 (87.2–95.6)	94.7 (91.4–96.8)	93.9 (91.2–95.7)
	Negative	15	267	282					
	Total	161	279	440					
Influenza B									
RIDT	Positive	140	13	153	91.5 (86.0–95.0)	95.3 (92.2–97.2)	91.5 (86.0–95.0)	95.3 (92.2–97.2)	94.0 (91.3–95.9)
	Negative	13	265	278					
	Total	153	278	431					

95% CI, 95% confidence interval; RIDT, rapid Influenza diagnostic test; RT-PCR, real time polymerase chain reaction.

Table 3
Estimates of Influenza vaccines effectiveness during 2016-2017 season.

Group	n	All influenza subtypes			A(H1N1) pdm09			A H3N2			B			A/B coinfection		
		VE (%)	95% CI	p	VE (%)	95% CI	p	VE (%)	95% CI	p	VE (%)	95% CI	p	VE (%)	95% CI	p
All	592	33.2	3.0–54.0	0.02	44.6	1.6–69.8	0.03	43.6	−15.2 to 74.1	0.09	17.1	−31.0 to 48.0	0.39	63.2	−73.8 to 96.1	0.18
<5 years*	112	43.7	−39.4 to 77.1	0.16	57.2	−52.6 to 89.5	0.14	78.3	−15.9 to 97.8	0.04	−37.5	−326.5 to 56.1	0.53	0	−36.4 to 0	0.08
5-17 years*	197	28.4	−62.3 to 68.1	0.37	37.0	−123.0 to 86.0	0.44	47.5	−166.3 to 94.6	0.41	22.6	−95.0 to 70.2	0.55	−31.5	−1658.2 to 97.6	0.81
≥18 years	283	16.6	−40.8 to 50.8	0.47	38.3	−38.1 to 74.0	0.20	0	−177.0 to 66.0	1.00	0.4	−99.5 to 51.2	0.99	45.8	−593.3 to 99.0	0.59
IIV3	518	21.0	−20.3 to 48.2	0.24	28.2	−31.8 to 62.2	0.26	40.3	−33.9 to 75.8	0.18	3.5	−61.4 to 42.9	0.88	50.5	−136.3 to 94.8	0.35
≥18 years	209	−7.4	−105.3 to 44.1	0.81	6.2	−127.4 to 63.6	0.87	-19.3	−308.9 to 70.0	0.75	−20.3	−190.9 to 52.5	0.65	12.5	−1037.2 to 98.4	0.90
IIV4 (≥18 years)	74	25.0	−134.3 to 75.5	0.57	37.5	−5048.6 to 99.2	0.74	6.25	−1115.5 to 90.1	0.94	28.5	−171.9 to 80.73	0.56	–	–	–

* No comparison between both types of vaccines were done in these age-groups, as quadrivalent vaccine was administered only to participants older than 18 years-old. IIV3, influenza inactivated trivalent vaccine; IIV4, influenza inactivated quadrivalent vaccine; VE: vaccine effectiveness; 95% CI: 95% Confidence interval.

pdm09 virus, 55 (9.3%) for influenza AH3N2 virus and 141 (23.8%) for influenza B virus. Influenza A/B virus co-infection occurred in 13 cases (2.2 %). Meanwhile, RIDT detected 298 (50.3%) influenza cases: 145 (24.5%) positive for influenza A virus, and 140 (23.6%) for influenza B virus. The 13 influenza A/B virus coinfections were also identified by RIDT (Table 1). For the diagnosis of influenza A, both tests overlapped in 146 positive cases, but 15 infections detected by RT-PCR were negative in the RIDT, whereas 12 RT-PCR-negative cases resulted positive in the RIDT. Similarly, for the detection of influenza B virus, RT-PCR and RIDT results overlapped in 140 positive cases, but 13 cases detected by RT-PCR were not identified by RIDT, and another 13 patients behaved in the opposite way (Table 2). Despite these discrepancies, we found a high correlation of the diagnostic performance of RIDT and RT-PCR. Indeed, for influenza A virus detection the RIDT had a 90.7% sensitivity, 95.7% specificity, 92.4% PPV, 94.7% NPV, and 93.9% accuracy. Similarly, for influenza B virus detection the RIDT showed a 91.5% sensitivity, 95.3% specificity, 91.5% PPV, 95.3% NPV, and 94.0% accuracy (Table 2).

Vaccine effectiveness

Overall VE against any laboratory-confirmed influenza virus infection was 33.2% (95% CI: 3.0–54.0; p = 0.02; Table 3). We observed significant influenza VE only against influenza A(H1N1)pdm09 virus (p = 0.03) among participants of all ages independently of the type of vaccine. In children younger than 5 years old, the VE was significant against influenza AH3N2 (78.3%, 95% CI: −15.9 to 97.8; p = 0.04), but not for influenza A(H1N1)pdm09, influenza B nor influenza A/B virus coinfection. No significant VE was observed against infection with any influenza virus subtype among the other age groups (Table 3). Nevertheless, our VE estimates tended to be higher against influenza A viruses as compared with influenza B virus in all participants.

We were unable to compare the effectiveness of both vaccine types against influenza A/B virus coinfection, since all the cases occurred among the trivalent vaccine group. Direct comparison between vaccine types was not performed in all age groups, as all the participants that received IIV4 were ≥18 years-old. Nonetheless, we did not find significant VE for any type of vaccine in participants ≥18 years-old, although VE estimates tended to be higher for the IIV4 compared with IIV3 against influenza overall, as well as for disease caused by influenza A(H1N1)pdm09 and influenza B viruses (Table 3). The number of cases and non-cases among vaccinated and unvaccinated groups used for VE estimations are shown in Table 4.

Clinical predictors of influenza

Bivariate logistic regression analysis showed that gender, age, fever, rhinorrhea, myalgias, arthralgias, headache, cough, asthma, antibiotic use, and recent travel history were potential predictors of influenza A diagnosed by RT-PCR, whereas occupational exposure (INER worker) and influenza vaccination were negatively associated with infection by type A viruses (Figure 1A). Similarly, gender, age, fever, rhinorrhea, myalgias, cough, asthma, and antibiotic use were associated with influenza B virus infection, whereas arthralgias, headache, tobacco consumption, occupational exposure, history or recent traveling, and influenza vaccination were inversely associated with influenza B (Figure 1B).

Multivariate analysis showed that age, fever, rhinorrhea and arthralgias were predictors of influenza A (Figure 2A), whereas fever, arthralgias and antibiotic use were predictors of influenza B (Figure 2B). Interestingly, occupational exposure (Figure 2A) and headache (Figure 2B) were inversely associated with influenza A and influenza B, respectively.

Table 4
Absolute numbers of influenza cases and non-cases among vaccinated and unvaccinated ILI patients.

Group	n	All influenza subtypes		A(H1N1) pdm09		A H3N2		B		A/B coinfection	
		Cases	Non-cases	Cases	Non-cases	Cases	Non-cases	Cases	Non-cases	Cases	Non-cases
All	59										
Vaccinated	2	75	96	20	96	12	96	41	96	2	96
Unvaccinated		227	194	73	194	43	194	100	194	11	194
<5 years*	11										
Vaccinated	2	18	16	5	16	2	16	11	16	0	16
Unvaccinated		52	26	19	26	15	26	13	26	5	26
5–17 years*	19										
Vaccinated	7	18	16	4	16	2	16	11	16	1	16
Unvaccinated		99	64	25	64	15	64	56	64	3	64
>18 years	28										
Vaccinated	3	39	64	11	64	8	64	19	64	1	64
Unvaccinated		76	104	29	104	13	104	31	104	3	104
IIV3	51										
Vaccinated	8										
Unvaccinated		63	64	19	64	9	64	33	64	2	64
		217	174	72	174	41	174	93	174	11	174
IIV3 (>18 years)	209										
Vaccinated		27	32	10	32	5	32	11	32	1	32
Unvaccinated		66	84	28	84	11	84	24	84	3	83
IIV4 (>18 years)	74										
Vaccinated											
Unvaccinated		12	32	1	32	3	32	8	32	–	–
		10	20	1	20	2	20	7	20	–	–

* These groups only received the trivalent influenza vaccine. IIV3, influenza inactivated trivalent vaccine; IIV4, influenza inactivated quadrivalent vaccine.

Discussion

Here we analyzed the utility of an RIDT to detect influenza cases among ILI patients seeking medical attention during the 2016/17 season. We observed an excellent RIDT performance, contrary to previous reports showing a suboptimal sensitivity of other RIDTs, particularly in outbreak settings (Agoritsas et al. 2006; Chartrand et al. 2012; Makkoch et al. 2012; Peci et al. 2014; Trombetta et al. 2018). These investigations demonstrated that the RIDTs' performance was affected by the anatomical site and time of respiratory samples collection, the patients' age, and the local influenza virus strain dominance. However, most previous studies assessed conventional immunochromatographic RIDTs, whose results may be inaccurate as they are interpreted by the human eye. Recently, novel RIDTs that include digital automatic analyzers have shown a higher sensitivity (Ryu et al. 2016, 2018; Merckx et al. 2017). Indeed, the analyzer-based RIDT evaluated here showed a limit of detection lower than other conventional observer-based RIDTs (Mori et al. 2012). However, its sensitivity was lower than RT-PCR which relies on the detection of nucleic acids, whereas RIDTs detect viral proteins. Despite this, we found a high diagnostic performance of the evaluated analyzer-based RIDT to detect influenza A and B viruses in respiratory samples comparable to RT-PCR, the gold standard diagnostic test (Table 2).

We choose this analyzer-based RIDTs as it was one of the first tests commercially available in Mexico offering an easy operation system that allows the user to easily execute automatic result determination after dropping respiratory samples onto the test cartridge, getting rapid results with lower risk of determination errors (Mori et al. 2012). Moreover, in an initial performance evaluation made by the manufacturer, this test was more sensitive than other conventional RIDTs to detect influenza A and B viruses in respiratory samples from patients with influenza confirmed by RT-PCR (Mori et al. 2012). In fact, the sensitivity of this analyzer-based RIDT was at maximum after 48 h of sample collection, suggesting that the test has a lower likelihood to overlook the infection even at recovery phases of influenza when few viral particles are detectable (Mori et al. 2012). Similarly, other researchers showed that this test was one of the most sensitive among several observer-based and analyzer-based RIDTs to detect influenza A(H1N1)pdm09 virus, influenza AH3N2 virus, influenza B virus, and even other zoonotic strains (Sakai-Tagawa et al. 2017). However, these investigators did not probe the RIDT in a real clinical setting. Thus, our report is among the first studies evaluating an analyzer-based RIDT during an outbreak, showing an excellent performance comparable to RT-PCR and other recent fluorescent immunoassay-based tests (Lewandrowski et al. 2013; Ryu et al. 2016, 2018; Selove and Rao 2016).

Nevertheless, we could not stratify participants by age-group and time of sample collection to address differences in the RIDT utility, but the age range of our population encompassed children and older adults, and the median time of disease duration at attendance (when all the samples were taken) was 3 days. Hence, our results support a high performance of the analyzer-based RIDT to detect influenza from early stages of disease in different age groups. Despite this, it is relevant to mention that a limitation of RIDTs in general is that they do not differentiate between seasonal/pandemic influenza A viruses nor influenza B virus lineages, which restricts their utility for epidemiological studies addressing seasonal influenza virus strains prevalence, but they are helpful to provide a rapid confirmation of influenza in hospitals without access to RT-PCR.

We also evaluated the VE of 2016/17 influenza vaccines. Our overall VE estimates against any type of influenza were lower compared to the previous season (Jackson et al. 2017) and almost similar to estimates from other Northern Hemisphere countries that also showed a low VE of the 2016/17 vaccines (Pebody et al. 2017; Mira-Iglesias et al. 2018; Wu et al. 2018; Baselga-Moreno et al., 2019; Flannery et al., 2019). Our results also showed that VE against influenza AH3N2 virus was higher in children and young adults compared to older patients during the evaluated season (Kissling et al. 2017; Pebody et al. 2017; Wu et al. 2018; Flannery et al., 2019). Of note, VE against influenza A(H1N1)pdm09 virus was not significant in other countries that reported low circulation of this virus (Baselga-Moreno et al., 2019). Conversely, we found a significant 44.6% VE against this virus, which remains similar to previous seasons (Belongia et al. 2016; Darvishian et al. 2017;

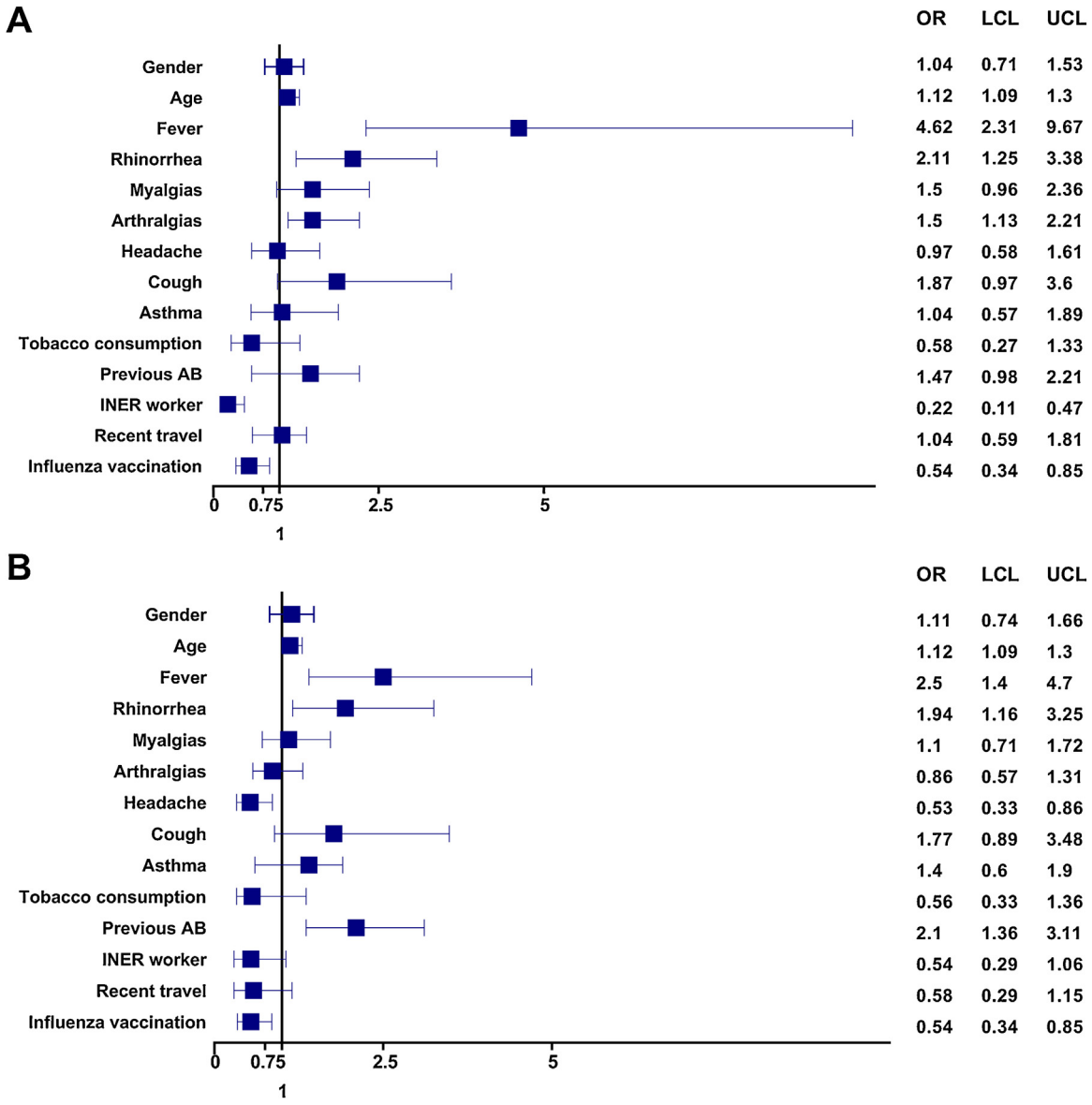

Figure 1. Potential factors associated with influenza in Mexican ILI patients during the 2016-2017 winter season. **(A)** Bivariate analysis of potential factors associated with influenza A virus infection. **(B)** Bivariate analysis of potential factors associated with Influenza B virus infection. AB, antibiotic usage; INER, National Institute of Respiratory Diseases; OR, Odds ratio; LCL, lower confidence limit; UCL, upper confidence limit.

Jackson et al. 2017; Kuliese et al. 2017). Thus, due to the high circulation of the influenza A(H1N1)pdm09 virus in Mexico, our results may provide more accurate VE estimates that may serve as a global reference about protective effectiveness of the 2016/17 influenza vaccines against this virus. Regarding influenza B virus, some studies have suggested that quadrivalent formulations are unnecessary to improve influenza VE as there is a certain degree of cross-protection between B lineages included in seasonal vaccines (Jang and Seong 2013; Rudenko et al. 2018) and trivalent formulations have not shown lower effectiveness (Milne et al. 2016). Conversely, although our VE estimates were not significant for influenza B, we found better VE of the quadrivalent vaccine like other studies (Jackson et al. 2017; Pebody et al. 2017). These data strongly suggest that it would be wise to switch to the use of quadrivalent vaccines to prevent influenza B in Mexico.

Strikingly, despite our low VE estimates, we found that immunization was protective for laboratory-confirmed influenza. Occupational exposure was also protective, which may be due to the continuous exposure of healthcare workers to respiratory viruses, which provides them a higher pre-existing immunity that reduces viral shedding and therefore detection of infection. This observation contrasts with previous data about healthcare workers having a higher risk for influenza A(H1N1)pdm09 virus infection, but not for other seasonal influenza viruses (Williams et al. 2010; Pujol et al. 2016). Nonetheless, our findings may also reflect the effect of the IIV4 administered only to INER workers. We also observed that fever, rhinorrhea, cough, and myalgia were associated with influenza. Previous reports showed that fever, cough and other constitutional symptoms are predictors of influenza (Kuo et al. 2011). Indeed, the fever-cough dyad has shown a high NPV (Yang et al. 2015), and here we found that fever and cough have the highest OR values for influenza. Furthermore, although it is believed that differentiation of the causative virus subtype is impossible by clinical presentation (Caini et al. 2018), we showed that headache is a predictor of influenza A, but it inversely associates with influenza B.

Finally, our RT-PCR results showed that influenza A(H1N1) pdm09 virus was the predominant strain in our population,

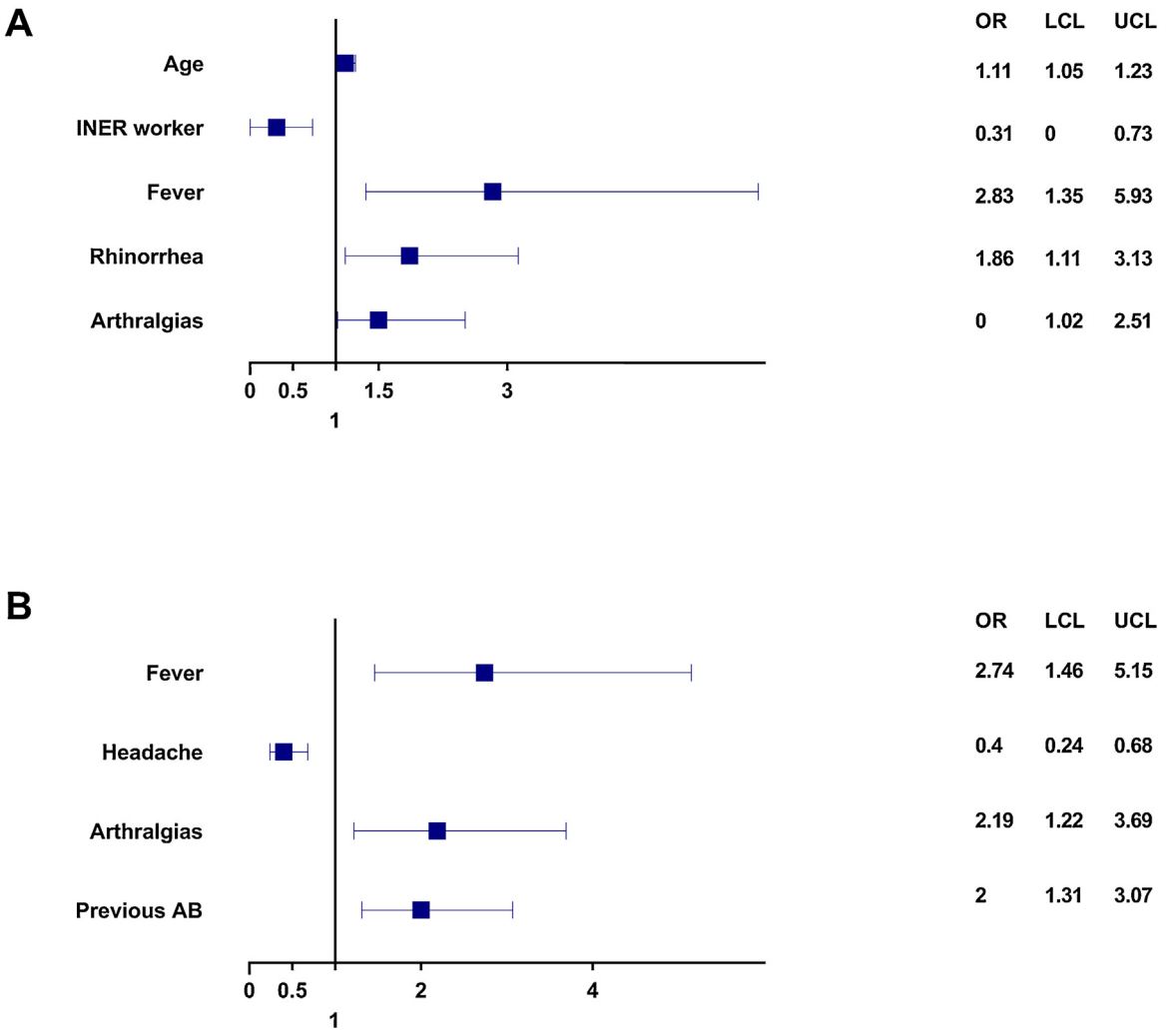

Figure 2. Independent factors associated with influenza in Mexican ILI patients during the 2016-2017 winter season. **(A)** Multivariate analysis of independent factors associated with influenza A virus infection. **(B)** Multivariate analysis of independent factors associated with influenza B virus infection. AB, antibiotic usage; OR, Odds ratio; LCL, lower confidence limit; UCL, upper confidence limit.

coinciding with other studies reporting a high circulation of this strain in Mexico during the same period (Baselga-Moreno et al., 2019), while most other Northern Hemisphere countries reported a predominance of influenza AH3N2 virus (Pebody et al. 2017; Mira-Iglesias et al. 2018; Wu et al. 2018; Baselga-Moreno et al., 2019; Flannery et al., 2019). Interestingly, the incidence of influenza B in our study was higher than previous local seasonal averages (Cortes-Alcala et al. 2018; Fernandes-Matano et al 2019), but contrasts with official national statistics showing a lower incidence of influenza B during the same period (Secretaria de Salud and Dirección General de Epidemiología, 2017). Perhaps our national reference center receives more cases not detected at other hospitals around the country due to differences in the availability of diagnostic tests, although these discrepancies may also result from variations in influenza virus strains dominance across different regions of Mexico.

Some study limitations include that we did not characterize the prevalence of B/Victoria/2/87-like and B/Yamagata/16/88-like lineages in our population to estimate influenza VE against these lineages. However, except for some Asian countries, epidemiological studies performed in different regions around the same period showed that the dominant influenza B virus lineage was the B/Yamagata/16/88-like lineage (Adlhoch et al. 2018; Baselga-Moreno et al. 2019; Flannery et al. 2019; Korsun et al. 2019). These data may explain our higher VE of the 2016-2017 IIV4 against influenza B, as the predominant lineage was absent in the IIV3. Since we lacked data about the time between immunization and disease onset, as well as history of immunization in previous seasons, we were unable to determine the effect of time on influenza VE, and the impact of consecutive immunizations on the protective effectiveness of seasonal vaccines (Bartoszko et al. 2018). Moreover, although no participant had influenza before getting vaccinated during the current season, we could not rule out possible bias in our VE estimates as some participants could have had influenza in previous seasons. Despite this, our study accomplishes most of the assumptions required for the validity of the VE estimations using the test-negative design (Foppa et al. 2013). Finally, further follow up of influenza cases would have informed about the impact of vaccination on their clinical outcome. Also, further exploration of RT-PCR negative ILI-patients by sampling bronchoalveolar lavage would have detected more influenza cases (Bogoch et al. 2013; Chen et al., 2010). This fact would have constituted a caveat for the validity of our VE estimates only if there had been a higher probability to detect extra cases among one but not the two vaccinated and unvaccinated groups.

In conclusion, we showed a high performance of an analyzer-based RIDT to detect positive influenza cases in Mexicans, supporting its usage a cost-effective test that along with the clinical judgment of

physicians can improve the diagnostic approach to influenza. Finally, we showed low VE of 2016/17 influenza vaccines in Mexico. Therefore, our data must be considered for local epidemiological policies during future seasons.

Conflict of interest

The authors declare no conflict of interest to disclose.

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Ethical approval

The current study was approved by the Ethics Committee of the National Institute of Respiratory Diseases (INER), a referral center for respiratory diseases in Mexico City. All participants or their legal guardian provided written consent to be enrolled in the study. All the samples were processed and stored according to the Mexican Constitution law NOM-012-SSA3-2012, that establishes criteria for the execution of clinical research projects in humans.

Author contribution

Collected clinical data and biological samples for the study: MC, CCG, JCP, JR, LJA, CHC, EBV, JMO, JSG, CG, EOM, ACL, GR, EM, LP, NRZ, CM, AG, RPP, RB, AS, DM, AH, LAR, EHM, GDC. Performed the RT-PCR test in respiratory samples: CCG, VH, FHS. Performed RIDT: MC, JZ. Analyzed the data: MC, CCG, JCP, JZ. Contributed to the writing of the paper: JCP, JZ, MC, JSH, RPP. Conception and design of the study: JZ, JSH, PSD, JR, RPP, MC, CCG, RB.

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