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COMPARING INFLUENZA POSITIVITY RATES BY REAL-TIME RT-PCR, ELISA AND VIRAL CULTURE METHODS IN CÔTE D'IVOIRE, WEST AFRICA, IN 2009

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Abstract

Detection of circulating influenza strains is a key public health concern especially in limited-resource settings where diagnosis capabilities remain a challenge. As part of multi-site surveillance in Côte d'Ivoire during the 2009 influenza A(H1N1) pandemic, we had the opportunity to test respiratory specimens collected from patients with acute respiratory illness (ARI). We analyzed and compared the percentage of specimens testing positive using three laboratory methods (rtRT-PCR, ELISA, viral culture). From January to October 2009, 1,356 respiratory specimens were collected from patients with acute respiratory illness and shipped at the WHO NIC (Institut Pasteur) Cote d'Ivoire, and 453 (33%) tested positive for influenza by one or more laboratory methods. The proportion of positive influenza tests did not differ by the sex or age of the patient or presenting symptoms, but did differ depending on the timing and site of specimen collection. Of the 453 positive specimens, 424 (93.6%) were detected by PCR, 199 (43.9%) by ELISA and 40 (8.8%) by viral culture. While seasonal influenza A(H1N1) virus strains were prominent, only four 2009 pandemic influenza A(H1N1) cases were detected. Use of molecular biology method (rtRT-PCR) increased sensitivity and diagnosis capabilities. Among all three methods used, rRT-PCR was the most sensitive and rapid method. More capacity building is still required for viral culture. Need to collect denominator data in order to have an accurate estimate of the burden of influenza. There was delayed introduction of pandemic influenza A(H1N1)2009 in Cote d'Ivoire

Key words: influenza, patients, rRT-PCR, ELISA, viral culture, Africa

Introduction

The relatively recent introduction of influenza surveillance and laboratory-based confirmation of influenza in Africa has expanded understanding of the burden of influenza in previously overlooked regions of the world (Gressner et al., 2011). As part of multi-site surveillance in Côte d'Ivoire during the 2009 influenza A(H1N1) pandemic, we had the opportunity to test respiratory specimens collected from patients with acute respiratory illness (ARI) using three laboratory methods. This allowed us to replicate analyses conducted in previous studies in the United States (Grody et al., 2009) and Europe (Quinlivan et al., 2004; Steininger et al., 2002), compare the percentage of specimens testing positive using three laboratory methods. The timing of the surveillance from January 1 to October 31, 2009 also allowed us to identify the types and subtypes of locally circulating influenza during a period when 2009 pandemic A(H1N1) was widespread throughout other parts of the world.

Methods

Setting and Population: As part of local monitoring of the 2009 influenza A(H1N1) pandemic, respiratory specimens were collected from January 1 to October 31, 2009 from 18 surveillance sites, which included 10 existing surveillance sites and 8 health facilities serving urban areas in Côte d'Ivoire. Most sites served outpatients only, though three in Abidjan (Treichville, Cocody, and Yopougon) were hospital settings. Patients that presented at the surveillance sites with symptoms of acute respiratory infection (ARI) were screened. In the outpatient settings, if the patient met WHO case definition for influenza-like-illness (ILI) (temperature ≥ 38.0°C and either cough or sore throat) they were enrolled. Hospitalized patients were enrolled if they met criteria for severe acute respiratory infection (SARI) (which for the age range 2 months to 4 years includes cough or shortness of breath or difficulty breathing and any sign of severe illness; for those age 5 years and older symptoms include temperature ≥ 38.0°C and cough with shortness of breath or difficulty breathing). Patient information, symptom reports, and days since onset were recorded using a standardized surveillance questionnaire.

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Human Use Statement: The protocol for this study did not require approval neither from the Cote d'Ivoire Ministry of Health nor the U. S. Naval Medical Research Unit No. 3 Institutional Review Board; simply because this activity was considered as a routine surveillance activity and not research. However, it was conducted in compliance with all applicable Federal regulations governing the protection of human subjects.

Respiratory Specimens: Whole respiratory specimens collected with nasal pharyngeal (NP) swabs at the outpatient department for ILI and at the hospitalization ward for SARI patients were stored in Viral Transportation Medium (VTM) at + 4°C in a refrigerator at the laboratory of the sentinel site. Twice a week (Wednesdays and Fridays) collected specimens were shipped in cool boxes containing cold-packs to the WHO National Influenza Center located at the Institut Pasteur of Côte d'Ivoire along with the completed questionnaires. Dates of illness onset and specimen collection were recorded. Specimens were accepted up to 14 days after illness onset.

Influenza Testing: Three methods of influenza detection were used to confirm the diagnosis: (a) real time reverse transcription polymerase chain reaction (rRT-PCR) assay, (b) enzyme-linked immunosorbent assay (ELISA) and (c) viral culture. Influenza typing and subtyping were performed with rRT-PCR using the automated ABI 7300 for the detection of M genes and hemagglutinin proteins (H1, H3 and pandemic H1) using CDC developed primers and probes. ELISA antigen detection of influenza A and B was performed using monoclonal and polyclonal antibodies of immunized rabbits. Viral culture was performed using Madin-Darby Canine Kidney (MDCK) cells.

For each specimen that was collected, 3 aliquots were made; the first (500 microliters) was used for both rRT-PCR (140 microliters) & ELISA (200 microliters), the second (500 microliters) was used for viral culture and the last was stored at -80 °C. Testing was done in parallel for each specimen using ELISA and rRT-PCR techniques and, only influenza positive specimens with any of the 2 methods were cultured.

Analysis: Proportions positive by any of the three methods and by each method separately were compared by gender, age, illness symptoms, clinical diagnosis, and days elapsed since illness onset. Proportions were also compared between seven large surveillance sites (with ≥ 40 specimens collected) and between the capital district (Abidjan) and the interior country districts (Adzope, Agnibilekro, Akoupe, and Bouake). Chi-square tests were conducted to test the significance of differences in the distribution of positive results between patient groups and the distribution of types and subtypes identified by rRT-PCR. Kappa tests of association were used to quantify the agreement between testing methods using STATA version 11.0 (STATA Corporation Texas, USA).

Results

From January 1 to October 31, 2009, respiratory specimens were collected from 1,356 patients with ARI in Côte d'Ivoire. As listed in Table 1, about half of the ARI patients were male (55%) and under age 19 (56%).

Of 1,356 patients, 453 (33%) ARI patients had laboratory-confirmed influenza by one or more of the three detection assays. Influenza positivity proportions did not differ significantly across age groups, but ranged from 30% among patients age 40 to 54 years to 38% among patients age 5 to 19 years. Positivity proportions were similar for those clinically diagnosed with ILI (39%) and SARI (42%) and similar for those presenting with fever (32%), cough (33%), or sore throat (34%). However, patients who had one or more ARI symptoms, but did not have a clinical diagnosis in their charts, had significantly fewer positive specimens (14%).

The most substantial variation in influenza positivity was associated with the time elapsed between illness onset and specimen collection. The proportion of specimens testing positive for influenza by any method was highest among specimens collected within 5 days of illness onset (74%) and lowest among specimens collected 6 or more days after illness onset (7%). The mean number of days from specimen collection to delivery at the reference laboratory was 1.3 days (standard deviation (SD) = 1.8) within the capital district and approximately 3.9 days (SD = 2.8) in the interior districts.

Statistically significant differences in the proportion of respiratory specimens testing influenza positive by any method were noted between the seven large surveillance sites, which varied from 20% among specimens collected at the United Nations Mission in Côte d'Ivoire (ONUCI) to 42% among specimens from Adzope General Hospital. Laboratory-confirmed influenza was also higher among specimens collected from interior districts of the country (40%) compared to the large capital district, Abidjan (32%).

As expected, there was strong and statistically significant agreement across methods. Agreement between rRT-PCR and ELISA results was especially high (Kappa = 0.43; $z = 17.4$, $p < .001$). Nevertheless, rRT-PCR was the most sensitive method. rRT-PCR detected 225 cases (or 17% of the study population) that were not detected by ELISA and 384 cases (28% of the study population) not detected by viral culture.

The majority of influenza viruses were influenza type A, with H3N2 the most commonly detected sub-type. During this pandemic period, we detected seasonal influenza A(H1N1) virus strains, but less than 1% of specimens were 2009 pandemic influenza A(H1N1). Types and subtypes of influenza virus strains detected by rRT-PCR did not differ significantly by age group, district or site, or by clinical diagnosis (ILI, SARI).

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Table 1: Descriptive characteristics and proportions of influenza positive specimens by method of assay among 1356 patients in Côte d'Ivoire from January 1 to October 31, 2009.

	Total N (%)	**N (% of all tested specimens) Positivity by any method**	**N (% of all positive tests) RT-PCR Confirmed**	**N (% of all positive tests) ELISA Confirmed**	**N (% of all positive tests) Viral Culture Confirmed**
All	**1356 (100)**	**453 (33.4)**	**424 (93.6)**	**199 (43.9)**	***40 (8.8)***
Influenza type and subtype					
A(H1N1) seasonal	-	-	28 (6.6)	-	-
A(H1N1) pandemic	-	-	4 (0.9)	-	-
A(H3N2)	-	-	169 (39.9)	-	-
A (unsubtypable)	-	-	115 (27.1)	-	-
B	-	-	108 (25.5)	-	-
Gender					
Male	740 (54.6)	235 (31.8)	223 (94.9)	107 (45.5)	*24 (10.2)*
Female	580 (42.8)	203 (35.0)	189 (93.1)	83 (40.9)	*13 (6.4)*
Not classified	36 (2.6)	-	-	-	-
Age					
0-4 years	592 (43.7)	204 (34.5)	192 (94.1)	87 (42.7)	*15 (7.4)*
5-19 years	166 (12.2)	63 (38.0)	61 (96.8)	26 (41.3)	*9 (14.3)*
20-39 years	405 (29.9)	126 (31.1)	113 (89.7)	65 (51.6)	*12 (9.5)*
40-54 years	111 (8.2)	33 (29.7)	32 (97.0)	9 (27.3)	*1 (3.0)*
55+	36 (2.7)	13 (36.1)	13 (100.0)	6 (46.2)	*1 (7.7)*
Unknown	46 (3.4)	-	-	-	-
Illness/Symptoms					
Fever	1060(78.2)	342 (32.3)	319 (93.3)	151 (44.2)	*28 (8.2)*
Cough	963 (76.4)	322 (33.4)	304 (94.4)	131 (40.7)	*28 (8.7)*
Sore throat	181 (13.4)	62 (34.3)	59 (95.2)	31 (50.0)	*8 (12.9)*
Clinical recorded diagnoses					
ILI among outpatients	1017(75.0)	398 (39.1)	380 (95.5)	175 (44.0)	*34 (8.5)*
SARI among inpatients	24 (1.8)	10 (41.7)	8 (80.0)	5 (50.0)	*3 (30.0)*
Not classified	315 (23.2)	45 (14.3)	36 (80.0)	19 (42.2)	*3 (6.7)*
Days since onset when specimen collected					
0-3 days	832 (61.4)	288 (34.6)	274 (95.1)	129 (44.8)	*31 (10.8)*
4-5 days	128 (9.4)	46 (35.9)	43 (93.5)	18 (39.1)	*3 (6.5)*
6-7 days	89 (6.6)	20 (22.5)	16 (80.0)	7 (35.5)	*2 (10.0)*
8+ days	52 (3.8)	13 (25)	12 (92.3)	6 (46.1)	*0 (0.0)*
Unknown	255 (18.8)	-	-	-	-
Large surveillance sites					
AGEFOSYN	205 (15.1)	47 (22.9)	41 (87.2)	18 (38.3)	*1 (2.1)*
ATTECOUBE	521 (38.5)	186 (35.7)	178 (95.7)	80 (43.0)	*17 (9.1)*
M.A.C.A	198 (14.6)	55 (27.8)	49 (89.1)	30 (54.6)	*2 (3.6)*
ADZOPE	45 (3.3)	19 (42.2)	18 (94.7)	11 (57.9)	*2 (10.5)*
AGNIBILEKRO	103 (7.6)	41 (39.8)	39 (95.1)	17 (41.5)	*4 (9.8)*
INHP	43 (3.2)	18 (41.9)	18 (100.0)	5 (27.8)	*1 (5.6)*
U.N. Mission CI	55 (4.1)	11 (20.0)	11 (100.0)	5 (45.5)	*2 (18.2)*
Large districts					
ABIDJAN district	1097(80.9)	345 (31.5)	322 (93.3)	148 (42.9)	*29 (8.4)*
INTERIOR districts	*212 (15.6)*	*85 (40.1)*	*80 (94.1)*	*40 (47.1)*	*8 (9.4)*

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Discussion

About 1 in 3 patients with ARI had laboratory-confirmed influenza. This prevalence, which is similar to or higher than, estimates of influenza infection incidence in the United States (Brammer et al., 2002), other western countries (Terletskaia-Ladwig et al., 2009), and Asia (Simmerman et al., 2008) reaffirms influenza virus infection is a prominent cause of ARI in Côte d'Ivoire. Surprisingly, seasonal influenza strains predominated in this region during a time when 2009 pandemic A(H1N1) was widespread in other parts of the world.

Positivity proportions using any of the three diagnostic methods pointed to similar rates of influenza across age and gender groups. Contrary to expectations, positivity rates were not higher among young children in Côte d'Ivoire, neither for hospitalized nor for non-hospitalized children. Findings in hospitalized children in the U.S. (Dawood et al., 2010) also showed low positivity rates.

The influenza positivity rates were higher among specimens collected within 5 days of illness onset, which fits with other surveillance findings (Belongia et al., 2009).

We also noted significant differences in influenza positivity rates across surveillance sites. This may reflect differences in local screening practices. Lacking a denominator for all ARI patients at each surveillance site limits our ability to interpret these differences.

A higher incidence of influenza in the interior region was noted despite transportation delays and other specimen management issues that may have reduced detection among these specimens.

Although there was high agreement between the laboratory methods, rRT-PCR was the most sensitive influenza diagnostic method. ELISA missed 1 in 5 influenza cases, and viral culture missed 1 in 4 influenza cases, compared with rRT-PCR. Given that rRT-PCR takes hours to complete versus days for viral culture, it also has advantages in feasibility and timeliness. Although viral culture will continue to play a key laboratory role (e.g., for isolate characterization), expanding rRT-PCR capacity in Africa could increase the number of appropriately diagnosed patients and expand our understanding of the epidemiology of influenza.

Conclusion

Seasonal influenza strains were a prominent cause of ARI in western Africa during the 2009 pandemic and were the predominant circulating strain in Côte d'Ivoire through October, 2009 while pandemic influenza A(H1N1) was the predominant circulating strain in most parts of the world. rRT-PCR proved to be the most sensitive method of detection, which reinforces the importance of expanding rRT-PCR capacity in Africa where laboratory diagnosis of diseases with epidemic and pandemic potential is especially important.

Authors' Disclaimer Statement: The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the U. S. Department of the Navy, U.S. Department of Defense, Institut Pasteur Côte d'Ivoire (IPCI), Institut National d'Hygiene Publique, Côte d'Ivoire (INHP); National Institute for Communicable Diseases, South Africa (NICD), or the Centers for Disease Control and Prevention (CDC).

Conflict of Interest Statement: I, Talla Nzussouo Ndahwouh, declare that I have no proprietary, financial, professional or other personal interest of any nature or kind in any product, service and/or company that could be construed as influencing the position presented in, or the review of this manuscript.

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