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Detection of influenza C viruses among outpatients and patients hospitalized for severe acute respiratory infection, Minnesota, 2013–2016

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Abstract

Background—Existing literature suggests that influenza C typically causes mild respiratory tract disease. However, clinical and epidemiological data are limited.

Methods—Four outpatient clinics and three hospitals submitted clinical data and respiratory specimens through a surveillance network for acute respiratory infection (ARI) during May 2013 through December 2016. Specimens were tested using multi-target nucleic acid amplification tests (NAAT) for 19–22 respiratory pathogens, including influenza C.

Results—Influenza C virus was detected among 59 of 10,202 (0.58%) hospitalized SARI cases and 11 of 2,282 (0.48%) outpatients. Most detections occurred from December to March, with 73% during the 2014–2015 season. Influenza C detections occurred among patients of all ages, with similar rates between inpatients and outpatients. The highest rate of detection occurred among children aged 6 to 24 months (1.2%). Among hospitalized cases, seven required intensive care. Medical co-morbidities were reported in 58% of hospitalized cases and all who required intensive care. At least one other respiratory pathogen was detected in 40 (66%) cases, most commonly rhinovirus/enterovirus (25%) and respiratory syncytial virus (RSV) (20%). The hemagglutinin-esterase-fusion (HEF) gene was sequenced in 37 specimens, and both C/Kanagawa and C/Sao Paulo lineages were detected in inpatients and outpatients.

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Conclusions—We found seasonal circulation of influenza C with year-to-year variability. Detection was most frequent among young children, but occurred in all ages. Some cases positive for influenza C, particularly those with co-morbid conditions, had severe disease, suggesting a need for further study of the role of influenza C virus in the pathogenesis of respiratory disease.

Keywords

influenza virus; hospitalization; influenza-like illness; biosurveillance

Acute respiratory infections (ARI) are a major cause of morbidity and mortality worldwide [1], and viral pathogens, including influenza viruses, cause many of these infections [2]. Two genera of human influenza—influenza A and B—are well studied and are thought to cause most influenza-associated human disease. In contrast, less is known about a third genus, influenza C. Influenza C was first described in 1947 as antigenically distinct from influenza A and B [3]. Like other influenza types, influenza C is a negative-sense, segmented RNA virus that circulates worldwide [4–6] and can cause disease in both the upper [7, 8] and lower [9–13] respiratory tract in humans as well as pigs [14] and dogs [15]. In experimentally infected individuals, the virus caused a febrile illness with mild upper respiratory symptoms [7], similar to most described cases of naturally acquired infections [8], and is thought to be less severe than other influenza types. However, some studies have reported episodic occurrences of more serious disease and hospitalizations [9, 11, 16]. The underlying reasons for these differences in disease severity remain unclear.

Although less well studied than other influenza viruses, influenza C infection appears to be common. Cross-sectional, population-based serological studies show peak prevalence of influenza C-specific antibody responses reaching 78-100% [6, 17–19]. The primary target of influenza C-specific antibody responses is the surface glycoprotein hemagglutinin-esterase-fusion protein (HEF), which is analogous to the two separate hemagglutinin and neuraminidase proteins of influenza A and B [20]. Unlike influenza A viruses, which undergo regular antigenic drift, influenza C viruses appears to be more antigenically stable with the same antigenic types circulating over a period of multiple years [21]. Interestingly, the presence of influenza C-specific antibodies does not confer complete protection, as adults with serological evidence of past exposure can develop symptoms and shed viruses [8]. A potential explanation for this may be the circulation of at least six distinct lineages of influenza C viruses [21], but the ways in which these lineages evolve and co-circulate in a population are not well understood.

In addition to the perceived low pathogenicity, limitations of diagnostics testing have hindered surveillance for influenza C virus. While there are commercially available tests for influenza A and B viruses, no such tests exist for influenza C virus. Furthermore, the virus is difficult to culture, even in confirmed outbreaks [22]. The expansion of molecular diagnostic techniques into research and surveillance settings could significantly increase knowledge of the spectrum of influenza C disease and patterns of circulation. Thus, beginning in May 2013, the Minnesota Department of Health (MDH) with the support of CDC incorporated molecular testing for influenza C into existing sentinel surveillance systems for outpatient and inpatient ARI, allowing us to study the epidemiology of influenza C virus infection.

METHODS

Inpatient Surveillance

The Minnesota Severe Acute Respiratory Illness (SARI) sentinel surveillance program was established at three hospitals in Minneapolis and St. Paul in May 2013, including two general hospitals and a large pediatric hospital system. Patients qualified as a possible SARI case if they were admitted to an inpatient unit for ARI or asthma exacerbation with ≥1ARI symptom. Testing of possible cases was encouraged but not required. After completion of clinician-ordered testing at the submitting facility, any residual upper or lower respiratory specimens were placed in viral transport media and submitted to the MDH public health laboratory for testing. Medical records were reviewed for all patients with submitted specimens using a standardized case report form (CRF) [23].

Outpatient Surveillance

The Influenza Incidence Surveillance Project (IISP) was established in 2009 and conducts surveillance for influenza-like-illness (ILI) and ARI through four primary care clinics that serve patients of all ages with sites currently in Hennepin, Kandiyohi, Kittson and Rock counties. ILI is defined as fever with cough or sore throat and ARI as any two of the following symptoms: fever (temperature ≥38 ° C or after 2015, patient/family-reported fevers), cough, sore throat, rhinorrhea, or congestion. Clinical staff collected an upper respiratory specimen for testing in the MDH public health laboratory and a limited CRF with from the first ten patients presenting each week with ILI and with ARI, as described previously [23].

Laboratory Testing

Specimens submitted from SARI between May 2013 and December 2016 and from IISP between September 2014 and December 2016 were tested at the MDH public health laboratory. Total nucleic acids (DNA and RNA) were extracted from specimens and nucleic acid testing for viral and bacterial pathogens was performed (Supplementary Figure 1). usingTaqMan ® real-time-PCR (rPCR) and real-time RT-PCR (rRT-PCR) assays with oligonucleoide primers/probes obtained from CDC [24, 25] or a Luminex Respiratory Pathogen Panel. Pathogens detected in referring hospital laboratories were also included in the analysis when available.

Sequencing of HEF genes

HEF genetic sequence analysis was performed by the Diagnostic Development Team, Influenza Division, CDC on influenza C-positive specimens with ample residual volume and rRT-PCR Ct values ≤32. Invitrogen SuperScript™III One-Step RT-PCR System with Platinum® Taq High-Fidelity kits were used for PCR amplifications. Primers are available upon request. PCR products were purified by ExoSAP-IT® for PCR Product Clean-Up kit (USB Corporation, USA). Sequencing reactions were performed using an Applied Biosystems BigDye® Terminator v3.1 Cycle Sequencing Kit and an Applied Biosystems Sequencer 3730 DNA Analyzer. Sequences analyzed were either obtained from this study or from the NCBI Influenza Resource (http://www.ncbi.nlm.nih.gov/genomes/FLU/ and

Supplementary figure 2). and were aligned using the CLUSTALW program. Phylogenetic analyses were performed using Molecular Evolutionary Genetics Analysis software (MEGA, version 5.1) [26]. The evolutionary history was inferred using the Neighbor-Joining method [27].

Data Analysis

Patients were included as influenza C cases if they tested positive for influenza C virus. Data were analyzed using Epi Info 7.2.0.1. The data presented are public health surveillance data and not subject to institutional review board approval for human research protections.

RESULTS

Study Population

During the study period, we completed influenza C testing on 12,484 specimens including from 10,202 hospitalized SARI patients and from 2,282 outpatients with ARI or ILI. The SARI population was younger than IISP with a median age of 2.72 years (IQR 0.5-31.7 years; range 0 days – 101.2 years) compared to 20.29 years (IQR 10.1-33.9 years; range 27 days-95 years) for IISP. However, older patients are represented as well, with 10.9% of SARI patients and 5.9% of IISP patients aged >65 years.

A total of 70 individuals tested positive for influenza C virus; 59 were identified among SARI patients and 11 from IISP, giving detection rates of 0.58% and 0.48%, respectively (Table 1). Four hospitalized cases had multiple specimens that tested positive for influenza C: two with positive detections within one day of each other, one with two positive detections 21 days apart, and one case with a total of five positive specimens detected over a period of four months. In cases with multiple detections, only the data corresponding to the date of the initial positive specimen was included in the analysis.

The median age of all influenza C cases was 20 months (range 3 weeks - 84 years; interquartile range [IQR] 8 months – 9 years) and 59% were <2 years old. Among hospitalized patients, influenza C was detected among 0.80% of children aged <5 years and 0.41% of children ≥5 years old. Among outpatients, influenza C was detected among 0.39% of children <5 years old and 0.49% of cases aged ≥5 years. Influenza C virus was detected in one of 1,114 individuals aged >65 years, equal to detection rate of 0.09% (data not shown). Influenza C virus detections were distributed similarly by sex, and 39% were non-white. Of cases with a known county of residence, 57% resided within either Hennepin (Minneapolis) or Ramsey (St. Paul) counties and the remainder were distributed among 10 surrounding counties (data not shown).

Clinical Characteristics

Among all influenza C cases, cough (60%), fever (47%), and congestion (41%) were most commonly reported. Among the 59 hospitalized cases for whom a more extensive symptom inventory was obtained, 54% reported respiratory distress or shortness of breath. At least one comorbid condition was reported for 58% of hospitalized cases (55% of cases <18 years; 83% of cases ≥18 years). The most common co-morbidities included asthma orchronic

obstructive pulmonary disease (COPD) and prematurity. For hospitalized cases, the median length of stay (LOS) was 2 days (IQR 1-4). Seven cases were admitted to the intensive care unit (ICU), four received mechanical ventilation, and none died. Among the cases admitted to the ICU, all were < 3 years old and had at least one underlying condition, the most common of which were prematurity and congenital heart disease. Influenza C virus was the sole pathogen detected for two of the ICU-admitted cases, both of which were premature, one of which had ventilator-dependent chronic respiratory failure and the other had cyanotic congenital heart disease. Of the two cases with widely spaced influenza C virus detections suggestive of prolonged shedding, both had underlying medical co-morbidities; the case with five detections had acute lymphoblastic leukemia and was undergoing maintenance chemotherapy and the other had multiple chronic medical co-morbidities including a history of prematurity and neurological and upper airway abnormalities.

Co-pathogen Detections

More than one pathogen was detected in 46 (66%) influenza C cases (Table 2). Although co-detections of multiple pathogens were more frequent in younger children, occurring in 34 (71%) cases <5 years old, we also found more than one pathogen in 12 (55%) cases ≥5 years old. Rhinovirus/enterovirus (RV/EV) and respiratory syncytial virus (RSV) were the most frequently co-detected, similar to their overall prevalence among all SARI cases (data not shown). Among influenza C cases aged ≥5 years, influenza A virus, human metapneumovirus, coronavirus NL63, and rhinovirus were most commonly co-detected with influenza C virus. Of the five cases admitted to the ICU, two had only influenza C virus detected and three had co-detections including adenovirus, parainfluenza 2, influenza B virus, RSV or *Moraxella catarrhalis*.

Seasonality

Overall, influenza C exhibited a peak of infections in 2014–15 that overlapped with the seasonal pattern of other influenza viruses. The peak quarter of detection was January–March (Figure 1). Interestingly, there was variability from year to year with 51 cases detected during the October–June season in 2014–2015 but only two cases detected during the same period in 2013-2014, eight cases in 2015-2016, and seven in October–December 2016.

Lineages

To determine which lineages were circulating, we sequenced the HEF genes of the influenza C isolates from 39 cases who tested positive from December 2014 through February 2016. We detected only the C/Kanagawa and C/Sao Paulo lineages in our isolates (Supplementary Figure 2). The C/Kanagawa linages originated from eight counties and the C/Sao Paulo lineages from five counties without apparent geographic clustering or differences in age (data not shown). Interestingly, the C/Kanagawa lineage was over-represented among hospitalized cases whereas the C/Sao Paulo lineage more evenly split between inpatients and outpatients.

DISCUSSION

Although the first published case of influenza C was described in New York in 1949 [3], few subsequent studies have characterized the burden of disease in the United States. Other respiratory viruses show seasonal and geographic variability in circulation [28] so we reasoned that influenza C might show similar variability and thus merit direct study of the epidemiology. Furthermore, because most routine laboratory tests do not detect influenza C virus, we hypothesized that influenza C may be an underappreciated cause of respiratory illness. To address this deficit, we incorporated molecular testing into two existing large-scale surveillance programs drawn from a largely urban and suburban population.

We detected influenza C virus infections in two of the three years of the surveillance program. The average frequency of influenza C detection over the 44-month study period was similar among outpatients and hospitalized SARI patients (0.48% and 0.58%, respectively), and was consistent with previous reports ranging from 0.2% [29] to 2.6% [30]. However, we noted substantial year-to-year variability in the number of cases with 73% of cases occurring during a single season, similar to previous observations [19], and other seasons with few or no detections. The annual variability seen in our study illustrates the risk of missing outbreaks of this disease with intermittent testing, highlighting the value of including influenza C in ongoing surveillance programs. Furthermore, while some previous reports suggested no seasonal pattern [31], studies from Japan [19] and Canada [30] have found peaks in the winter and spring seasons consistent with our findings. We observed a peak from December 2014 to May 2015, overlapping with the seasonal peak for influenza A and B. Further surveillance is needed to further clarify whether true seasonality exists.

In addition to characterizing the burden and temporal patterns of influenza C virus circulation, our data provide insights into the characteristics of individuals with possible influenza C virus-associated disease. We showed a statistically higher percentage of influenza C virus detection in the 6 month-2 year age group (1.22%) with lower rates of infection through the remainder of childhood and adulthood. There was also a lower rate of detection in those <6 months, corresponding to a time when children have residual maternal antibodies which could provide some immunological protection [32]. The lowest rates of detection occurred among teenagers, with slightly higher rates in adults, though this was not statistically significant. While we cannot rule out the possibility that rates of influenza C detection in adults were artificially low due to a bias against testing for respiratory pathogens in older age groups, another recent study found almost identical rates (0.3%) of influenza C infection in adults systematically tested for viral infection [33].

Although we do not have corresponding serological data from our population, multiple studies from varied locations have consistently demonstrated high levels of seropositivity against influenza C virus in older children and adults [6, 17–19]. Our observation that rates of influenza C virus detection decline with age fits with previous studies demonstrating increased prevalence of influenza C antibodies with increasing age. Interestingly, 20% of our influenza C virus detections were among symptomatic adults. These data may suggest that while many adults have had prior exposure to influenza C virus, the immunological protection from past exposure may wane over time. Alternatively, it may be possible to have

serial infections with antigenically distinct circulating lineages. The presence of at least six influenza C lineages raises the question of whether exposure to new strains might allow infection of previously exposed individuals. Consistent with reports from Japan (19), we identified two lineages—C/Kanagawa and C/Sao Paulo—co-circulating in our study population. The sample numbers were small and we were unable to find any differences between strains with respect to age or geographic distribution (data not shown). Nevertheless, we did find the C/Kanagawa lineage more consistently among hospitalized cases. Ongoing testing through established surveillance systems will make it possible to identify the emergence of new antigenic lineages.

Our data may also challenge the perception that influenza C virus is rarely associated with severe disease [31]. We detected influenza C virus at similar rates in hospitalized cases and outpatients with ARI, raising the possibility that it may be associated with a spectrum of disease. The reasons for the variability of disease severity are likely multi-factorial and may include both host and viral factors. From a host perspective, all cases admitted to the ICU had at least one underlying medical condition, suggesting that underlying illness is a risk factor for severe disease and consistent with literature on other respiratory infections such as RSV [34]. Specifically, prematurity was noted in 17% of all cases and 80% of cases admitted to the ICU. Interestingly, we observed prolonged shedding of virus in two cases with underlying medical illness. From a viral standpoint, many of our cases had co-infections, and we detected two different circulating influenza C lineages, which may differ in pathogenicity. At this time, no specific anti-viral therapy is available [35] so treatment is primarily supportive. Nevertheless, awareness of respiratory viral infections such as influenza C that are not currently detected on routine respiratory pathogen panels may inform hospital infection control policy and would support presumptive respiratory isolation based on symptoms, even in the setting of negative test result.

Although our study provides valuable information about naturally occurring influenza C virus detections, it has several limitations. First, clinical testing was done at the discretion of the treating clinician. Thus, some patients may not have had testing ordered or had residual specimen for testing at MDH, leading to an underestimation of cases. A second caveat is that children, particularly infants <1 year of age, are more likely to be hospitalized for respiratory infections and therefore have respiratory specimens obtained for testing [36]. This is reflected in the fact that 57% of patients from the SARI surveillance program were aged <5 years. Third, no information was obtained about underlying medical conditions or follow up data for outpatient cases, and thus, a comparative analysis of comorbidities and outcomes with SARI cases was not possible. Finally, influenza C virus was commonly co-detected with other viral pathogens. As compared to culture-based methods, molecular detection methods such as ours frequently detect multiple infections that pose a challenge to interpretation [37]. Viral nucleic acid may be detectable for prolonged periods of time, including in asymptomatic individuals, and it can be difficult to distinguish among symptomatic infection, asymptomatic shedding or non-infectious viral debris [37]. Therefore, it will also be important to measure influenza C virus prevalence in asymptomatic controls to address the potential for asymptomatic shedding. Nevertheless, by conducting surveillance in a large population and incorporating multiplexed testing for other known pathogens, we identified 24 cases with influenza C virus but no co-pathogens detected. This

included two cases who became ill enough to warrant ICU admission. While we cannot rule out the possibility that another agent is the primary cause of symptoms, these data raise the possibility that influenza C virus has the potential to cause more disease than previously appreciated.

In conclusion, we employed a large-scale surveillance system to identify cases of influenza C in the Upper Midwest. Our data suggest that it is detected in a minority of patients with symptomatic respiratory illness but may be a cause of severe disease and periodic outbreaks.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Short summary of main findings

We detected influenza C viruses mostly in children in both outpatients and inpatient surveillance populations. Our findings suggest that influenza C may be an under-recognized cause of outpatient and severe hospitalized illness in the United States.

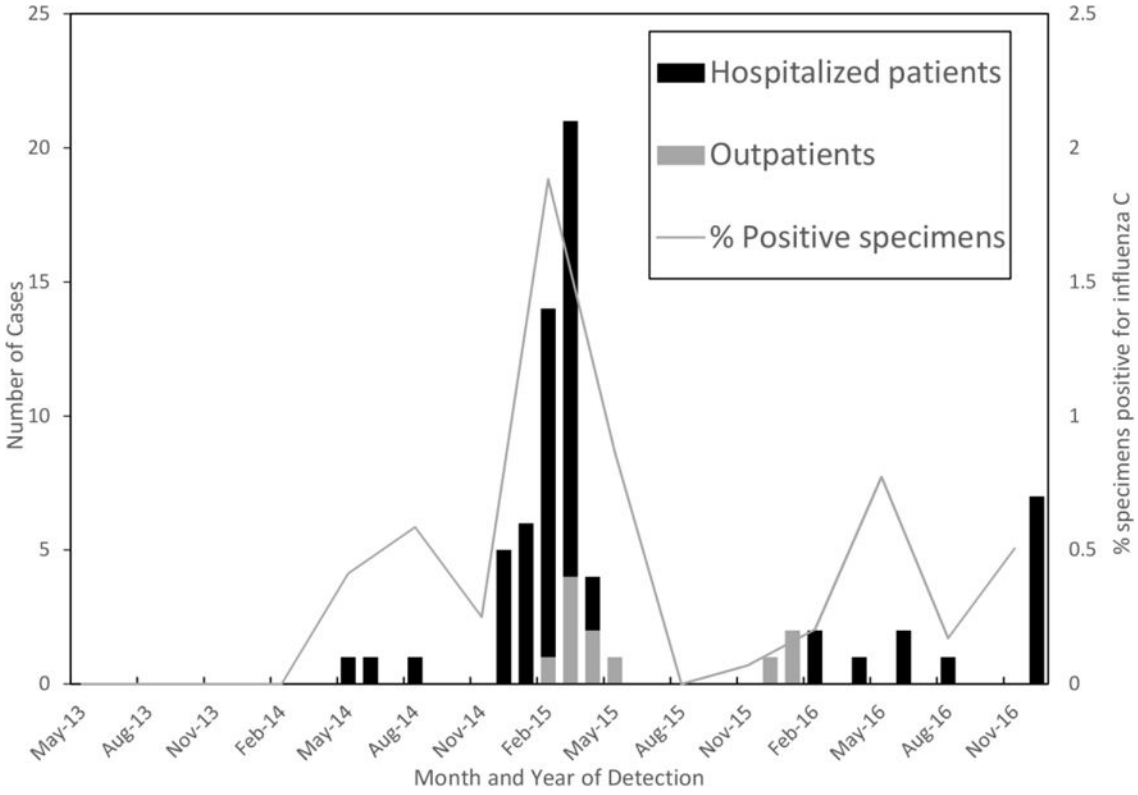

Figure 1.

Number of influenza C virus detections among hospitalized patients (black bar) and outpatients (gray bar) with acute respiratory infection in Minnesota by month and year of detection, May 2013-December 2016 and percentage of submitted respiratory specimens testing positive for influenza C by quarter (line).

Table 1

Rates of influenza C virus detected among surveillance population by demographic and clinical characteristics.

	Total	Hospitalized	Outpatients	Influenza C only
Demographics	**No./Total (%)**	**No./Total (%)**	**No./Total (%)**	**No./Total (%)**
Age				
<6 months	14/2560 (0.55)	14/2539 (0.54)	0/21 (0.00)	5/2560 (0.20)
6 months–2 years	27/2216 (1.22)***	26/2139 (1.20)**	1/77 (1.30)	7/2216 (0.32)
2–4 years	7/1347 (0.52)	7/1187 (0.58)	0/160(0.00)	2/1347 (0.15)
5–11 years	7/1358 (0.52)	5/923 (0.54)	2/435 (0.46)	2/1358 (0.15)
12–17 years	1/778 (0.13)	1/535(0.20)	0/243 (0.00)	1/778 (0.13)
18+ years	14/4225 (0.33)	6/2879(0.57)	8/1346 (0.59)	7/4225 (0.17)
Sex				
Male	37/6447 (0.57)	29/5129 (0.57)	8/1318 (0.61)	15/6447 (0.23)
Female	33/5413 (0.61)	30/4451 (0.67)	3/962 (0.31)	9/5413(0.17)
	0/614 (0)	0/612 (0)	0/2 (0)	0/614 (0)
Race				
White	31/6022 (0.51)	26/4636 (0.56)	5/1386 (0.36)	9/6022 (0.15)
Black	19/2319 (0.82)	18/2223 (0.81)	1/96 (1.04)	8/2319 (0.34)
Asian/Pacific Islander	5/869 (0.58)	3/837 (0.36)	2/32 (6.25)***	1/869 (0.12)
American Indian/Alaska Native	1/211 (0.47)	1/208 (0.48)	0/3 (0)	1/211 (0.47)
Mixed	2/231 (0.87)	2/229 (0.87)	0/2 (2)	2/231 (0.87)*
Other	0/11 (0)	–	0/11 (0)	0/11 (0)
Unknown	12/2811 (0.43)	9/2059 (15)	3/752 (0.40)	3/2811 (0.11)
Total	70/12484 (0.56)	59/10202 (0.58)	11/2282 (0.48)	24/12484 (0.19)
Clinical characteristics	No. (%)	No. (%)	No. (%)	No. (%)
Symptom on presentation				
Cough	42 (60)	35 (59)	7 (64)	12 (50)
Shortness of breath/Respiratory distress[a]	38 (54)	38 (64)		9 (38)
Fever	33 (47)	28 (47)	5 (45)	11 (46)
Congestion	29 (41)	24 (41)	5 (45)	11 (46)
Vomiting[a]	20 (29)	20 (34)		7 (29)
Wheezing	16 (23)	16 (27)	0 (0)	3 (13)
Sore throat	10 (14)	2 (3)	8 (73)	6 (25)
Diarrhea[a]	8 (11)	8 (14)		3 (13)
Myalgias	8 (11)	4 (7)	4 (36)	4 (17)
Headache	6 (9)	2 (3)	4 (36)	4 (17)
Rash	5 (7)	5 (8)	3 (27)	2 (8)
Seizure[a]	5 (7)	5 (8)		2 (8)

	Total	Hospitalized	Outpatients	Influenza C only
Demographics	**No./Total (%)**	**No./Total (%)**	**No./Total (%)**	**No./Total (%)**
Conjunctivitis	4 (6)	4 (7)	0 (0)	2 (8)
Co-morbidities				
Any underlying medical condition		34 (58)		10 (59)
Asthma/chronic obstructive pulmonary disease		10 (17)		2 (12)
Prematurity		10 (17)		1 (6)
Neurological/neuromuscular		5 (8)		2 (12)
Genetic disorder		5 (8)		2 (12)
Abnormality of upper airway		4 (7)		2 (12)
Cardiovascular disease		4 (7)		0 (0)
Diabetes		1 (2)		1 (6)
Unknown Co-morbidities		11		7
Hospital Length of Stay				
<1 day	12 (17)	1 (2)	11 (100)	7 (29)
1–2 days	31 (44)	31 (53)		9 (38)
3–4 days	11 (16)	11 (19)		3 (13)
5–6 days	9 (13)	9 (15)		4 (17)
7–20 days	6 (9)	6 (10)		0 (0)
>20 days	1 (1)	1 (2)		1 (4)
Known Treatment/Outcome				
Received influeza anti-viral		3 (5)		1 (5)
Intensive care unit admission		7 (12)		2 (11)
Received mechanical ventilation		4 (7)		1 (5)
Received extracorporeal membrane oxygenation		0 (0)		0 (0)
Died		0 (0)		0 (0)

[a] Patients hospitalized compared to outpatients, with or without co-detection of pathogens. The last column represents patients with influenza C virus infections without any other pathogen detected in respiratory sample.

[b] Signs/symptom data collected only for hospitalized patients

Table 2

Number and type of additional pathogens detected in patients testing positive for influenza C virus stratified by location (hospitalized vs. outpatients).

	Total	Hospitalized	Outpatients
Number of Co-pathogens	**No. (%)**	**No. (%)**	**No. (%)**
0	24 (34)	17 (29)	7 (64)
1	34 (49)	30 (51)	4 (36)
2	7 (10)	7 (12)	0 (0)
2	4 (6)	4 (7)	0 (0)
4	0 (0)	0 (0)	0 (0)
5	1 (1)	1 (2)	0 (0)
Co-pathogens			
Rhinovirus/enterovirus	15 (25)	14 (24)	1 (9)
Respiratory syncytial virus	12 (20)	12 (20)	0 (0)
Adenovirus	6 (10)	6 (10)	0 (0)
Parainfluenza 3	6 (10)	6 (10)	0 (0)
Human Metapneumovirus	6 (10)	6 (10)	0 (0)
Influenza A	4 (7)	3 (5)	1 (9)
Coronavirus NL63	2 (3)	1 (2)	1 (9)
Influenza B	2 (3)	1 (2)	1 (9)
Parainfluenza 1	1 (2)	1 (2)	0 (0)
Parainfluenza 2	1 (2)	1 (2)	0 (0)
Parainfluenza 4	1 (2)	1 (2)	0 (0)
Bordatella parapertussis	1 (2)	1 (2)	0 (0)
Chlamydia pneumoniae	1 (2)	1 (2)	0 (0)
Coronavirus 229E	1 (2)	1 (2)	0 (0)
Moraxella catarrhalis	1 (2)	1 (2)	0 (0)
Total	70	59	11