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Human Coronavirus-HKU1 Infection Among Adults in Cleveland, Ohio

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Background. Human coronaviruses (CoV) have been long recognized as a common cause of respiratory tract disease including severe respiratory tract illness. Coronavirus-HKU1 has been described predominantly among children less than 5 years of age in the United States with few studies characterizing the disease spectrum among adults.

Methods. Nasopharyngeal specimens of patients with respiratory symptoms were analyzed for CoV-HKU1 by NxTAG Respiratory Pathogen Panel multiplex assay from February 7, 2016 to April 30, 2016. Epidemiologic, clinical, and laboratory data were collected on adults (patients >18 years) whose samples screened positive.

Results. Of 832 adult respiratory specimens screened, 13 (1.6%) cases of CoV-HKU1 were identified. Adults age ranged between 23 and 75 years and 6 (46%) were males. All of whom had 1 or more respiratory symptoms, and 5 (38%) also reported 1 or more gastrointestinal symptoms. Eleven (85%) reported history of smoking and 5 (38%) used inhaled steroids. Seven (54%) required hospitalization, 5 (71%) of these needed supplemental oxygen, and 2 (29%) were admitted to intensive care. Median length of hospitalization was 5 days. Eight (62%) received antibiotics despite identification of CoV-HKU1. Infectious work-up in 1 patient who died did not reveal any other pathogen. In 2 (15%) CoV-HKU1-positive adults, the only viral coinfection detected was influenza A.

Conclusions. Coronavirus-HKU1 accounted for 1.6% of adult respiratory infections and should be considered in differential diagnosis of severe respiratory illnesses among adults.

Keywords. adults; clinical features; coronavirus; coronavirus-HKU1; respiratory infections.

Human coronaviruses (CoVs) have been long recognized as a common cause of respiratory tract disease with first reports being of CoV 229E and CoV OC43 as a cause of common cold in adults during the mid-1960s [1]. In 2003, the severe acute respiratory syndrome (SARS)-CoV pandemic sparked renewed interest in study of CoVs, and since that time, 3 additional CoV species have been identified: CoV-NL63, CoV-HKU1, and Middle East respiratory syndrome (MERS)-CoV [2–4]. Although MERS-CoV circulation has remain restricted to specific regions of the Middle East, Europe, and South Asia, both CoV-HKU1 and NL63 have been reported worldwide. Disease manifestations associated with CoV-HKU1 and CoV-NL63 include common cold, bronchitis, asthma, chronic obstructive pulmonary disease (COPD) exacerbations, and pneumonia [1].

Coronavirus-HKU1 has been predominantly reported in children in United States [5, 6] but less commonly among adults [7–9]. Silva et al [9] found 1 case (0.5%) among 200 adults presenting with flu-like symptoms over a 5-month period, and Gorse et al [7] reported 1 patient with CoV-HKU1 among 585 adults (0.17%) with history of COPD. In this study, we report one of the largest case series of CoV-HKU1 infections among adults presenting with respiratory tract illness and describe their clinical characteristics.

METHODS

Population Characteristics

Cleveland is a city on Cuyahoga county in the state of Ohio, with a population of 396 815 per 2010 US Census [10]. The MetroHealth System serves the residents of the city of Cleveland and Cuyahoga County.

Sample Collection

Between February 7 and April 30, 2016, a total of 1208 adult and pediatric respiratory samples were submitted to MetroHealth Medical Center (Cleveland, OH) for respiratory pathogen screening. Of these, 832 samples were from adults (age >18 years). All samples were collected by nasopharyngeal swabs (Puritan 6" Sterile Standard Polyester Swab, Guilford, ME). Samples were collected at the outpatient clinics, inpatient

wards, and emergency department (ED) at the discretion of the on-service physicians. Samples were placed in M4-RT viral transport media (Remel, Lenexa, KS) and transported to the laboratory where they were frozen until testing.

Sample Preparation and Viral Screening

Testing was performed using the NxTAG Respiratory Pathogen Panel CE-IVD assay kits (Luminex, Austin, TX) per the manufacturer's guidelines (Package Insert, NxTAG Respiratory Pathogen Panel Assay; Luminex). In brief, 35 μL of extracted nucleic acid were added directly to NxTAG Respiratory Pathogen Panel pre-plated, lyophilized reagents. Multiplexed reverse-transcription polymerase chain reaction and bead hybridizations were performed in each plate well under a single cycling program per manufacturer's instructions. The sealed plates were placed directly on the MAGPIX instrument (Luminex) for data acquisition. Raw signals generated by the MAGPIX instrument were subsequently analyzed by the assay-specific Software Accessory Package using SYNCT software to establish the presence or absence of each pathogen in each sample.

For detection of CoVs, the nucleocapsid protein gene (N) was used to identify CoVs 229E, OC43, and NL63. The open reading frame 1laboratory region of the ribonucleic acid polymerase was used to identify CoV-HKU1. The limit of detection titer for CoV-HKU1 was 1.57E+04 genome copies/mL (Package Insert, NxTAG Respiratory Pathogen Panel Assay).

Clinical Data Collection

Sample collection and data analysis were approved by the MetroHealth Medical Center Institutional Review Board (IRB16-00162). Clinical data analysis for adults (patients >18 years of age) included the following: age, gender, hospitalization status, length of hospitalization, clinical features, discharge diagnosis, outcome (death or survived), comorbidities (smoking, lung disease), laboratory and radiology results, intensive care unit (ICU) stay, oxygen use and duration, treatment provided, and exposure history. Clinical data were collected using Redcap Software (REDCap 6.17.0, Vanderbilt University).

RESULTS

Coronaviruses detected from all respiratory specimens during the study period is summarized in Table 1. Of these, 832 originated from adult patients (>18 years) with 35 (4.2%) screening positive for CoVs. Thirteen (1.6%) samples tested positive for CoV-HKU1 and represented 37% of all CoV species detected among adults. Median age of adult patients was 52 years (range, 23 to 79 years). Eleven CoV-HKU1-positive adults (85%) had an underlying respiratory comorbidity, predominantly history of smoking, with 6 (46%) being current smokers (Table 2). Five patients (38%) reported use of inhaled corticosteroids. One patient was on systemic immunosuppression (prednisone, azathioprine). In addition, 2 patients had underlying gastrointestinal comorbid conditions.

All adults with CoV-HKU1 infection had respiratory symptoms (Table 3). Shortness of breath was the most common respiratory complaint (77%) followed by cough (62%) and rhinorrhea (54%). However, fever, wheezing, and chest pain were less common (38%, 23%, and 15%, respectively). Upper or lower gastrointestinal symptoms were reported in 5 (38%) adults. Other symptoms included myalgias (31%) and sore throat (23%). Seven patients (54%) required hospital admission, 5 (71%) of whom were diagnosed with pneumonia by the on-service team. Among hospitalized adults, 2 (29%) were admitted to the ICU. Both patients were male. Three patients (23%) were diagnosed with sepsis, 1 of whom developed septic shock and died. This individual had a history of smoking (64-pack years) and COPD. Another individual admitted to the ICU developed pancreatitis, pericarditis, peritonitis, and altered mental status during hospitalization and recovered. All blood, urine, and sputum cultures from these individuals were negative for other bacterial, fungal, and viral infections.

Laboratory findings (Table 3) were obtained in 9 (69%) of the CoV-HKU1 adults. Of these, leukocytosis (white blood cell count [WBC] >11 000 cells/μL) was observed in 5 (56%), with an average of 29 600 cells/μL (range, 13 600–49 700 cells/μL). Of these, neutrophilic predominance (neutrophil count >8000 cells/μL) was seen in 4 (80%) and bandemia (>10% bands) was seen in 2 (40%). One (8%) adult had leukopenia (WBC of 3700 cells/μL). Twelve (92%) adults had chest radiography performed with 6 (50%) showing infiltrative disease. The chest imaging findings included prominence of interstitial markings with a reticulonodular pattern, ground-glass opacities, patchy consolidation, interlobular and intralobular septal thickening, basilar atelectasis, small pleural effusions, and hyperinflated lungs (Figure 2). Eight (62%) received antibiotics, and 2 (15%) received oseltamivir, despite identification of CoV-HKU1 as the

Table 1. Distribution of Human Corona Viruses (CoVs) From Patients Presenting With Respiratory Tract Symptoms Between February and April 2016

Human CoV Isolated	Adults, N (%)	Children, N (%)	Viral Coinfections, N (%)
HKU1 (N = 18)	13 (72.2)	5 (27.7)	4 (22.2)
OC43 (N = 18)	15 (83.3)	3 (16.6)	4 (22.2)
NL63 (N = 12)	6 (50.0)	6 (50.0)	8 (66.7)
229E (N = 1)	1 (100)	0	0
Total (N = 49)	35 (71.4)	14 (28.6)	16 (32.6)

Table 2. Demographics of 13 Adult Patients With HCoV-HKU1 Infection

Variables	N = 13, n (%)	Age (18–30 yrs), N = 2 [n (%)]	Age (31–50 yrs), N = 4 [n (%)]	Age >50 yrs, N = 7 [n (%)]
Males	6 (46)	1 (50)	2 (50)	3 (43)
History of smoking	11 (85)	1 (50)	4 (100)	6 (86)
Current smokers	6 (46)	1 (50)	3 (75)	2 (29)
Inhaled corticosteroids	5 (38)	0	1 (25)	4 (57)
COPD	3 (23)	0	0	3 (43)
Asthma	1 (8)	0	1 (25)	0
Systemic immunosuppression	1 (8)	0	0	1 (14)

Abbreviations: COPD, chronic obstructive pulmonary disease; HCoV, human coronavirus.

sole pathogen on work-up. Half of those receiving antibiotics were outpatients. Outpatient antibiotics prescribed included clindamycin, azithromycin, ciprofloxacin, moxifloxacin, and doxycycline. The antibiotics were continued for a median duration of 7 days.

Coinfections were detected in 13 (27%) of all CoV-positive samples (Table 1). These included influenza A (5), rhinovirus (4), respiratory syncytial virus ([RSV] 2), human bocavirus (2), adenovirus (1), metapneumovirus (1), and enterovirus (1). No CoV sample had coinfection with a second CoV species. Two

Table 3. Clinical Manifestations and Laboratory Findings of 13 Adults With HCoV-HKU1 Infection

Clinical Manifestations, N (%)	
Constitutional Symptoms	
Fever	5 (38)
Myalgias	4 (31)
Sore throat	3 (23)
Respiratory symptoms	13 (100)
Shortness of breath	10 (77)
Cough	8 (62)
Rhinorrhea	7 (54)
Wheezing	3 (23)
Chest pain	2 (15)
Gastrointestinal symptoms	5 (38)
Diarrhea	4 (31)
Nausea or vomiting	3 (23)
Abdominal pain	2 (15)
Hospital Course, N (%)	
Required hospitalization	7 (54)
Median length of stay (days)	5 (range: 3–11)
Required supplemental oxygen	5 (38)
Median length of oxygen use (days)	5 (range, 4–6)
ICU admission	2 (15)
Median length of ICU stays (days)	7.5 (range, 4–11)
Required intubation	1 (8)
Use of antimicrobials	8 (62)
Median length of antibiotic use (days)	7 (range, 4–7 days)
Laboratory Findings, N (%)	
Chest x-ray findings	
Abnormal	6 (46)
Unilateral infiltrates	4 (31)
Bilateral infiltrates	2 (15)
Leukocytosis (>11 000/µL)	5 (38)

Abbreviations: HCoV, human coronavirus; ICU, intensive care unit.

respiratory samples had ≥3 pathogens. Of the 18 CoV-HKU1-positive samples, 4 (22%) had coinfections occurring in 2 children (adenovirus, bocavirus) and 2 adults (influenza A).

Eighty-three percent of all CoV-HKU1-positive samples occurred within a 4-week period between February 7 and March 5 (Figure 1). This period included 10 (67%) of the adult cases. Coronaviruses OC43 and NL63 had a more prolonged circulation but also predominated during these same 4 weeks.

DISCUSSION

Our report describes the largest number of CoV-HKU1 infections among the general adult population in the United States to date. We find that 1.6% of adults screened for respiratory viruses have evidence of CoV-HKU1. Previous studies in North America have reported lower rates during a typical viral respiratory season [7–9]. On the other hand, CoV-HKU1 infection among adults outside the United States have been reported with rates ranging between 0.04% and 2.1% [11–15]. Our rate of 1.6% is similar to these findings. We hypothesize that the overall rate might change if surveillance occurred throughout the year. It is likely that the study period represents peak CoV-HKU1 circulation in our community.

We identified smoking as the predominant comorbid condition (85% of all CoV-HKU1-positive adults). Smoking has been found to be an independent predictor of MERS-CoV acquisition in Saudi Arabia [16] and among 50% of smokers with CoV-HKU1 pneumonia in Hong Kong, China [11]. Increased respiratory infections are hypothesized to be secondary to continuing parenchymal trauma and suppression of host resistance over time [17]. In our study, smoking was identified commonly among all age groups of adults with CoV-HKU1 disease irrespective of the age of onset of smoking.

We found a high rate of CoV-HKU1 infections (38%) in patients using inhaled corticosteroids. Although inhaled steroids have been accepted as safe agents with no or minimal systemic absorption, the effects on local cell-mediated immunity is unclear. They have been shown to be a risk factor for bacterial pneumonia in patients with asthma and COPD, as well as influenza in COPD patients [18, 19]. In a recent meta-analysis by Dong et al [20], there was an increased but nonsignificant trend towards influenza infection among patients taking inhaled
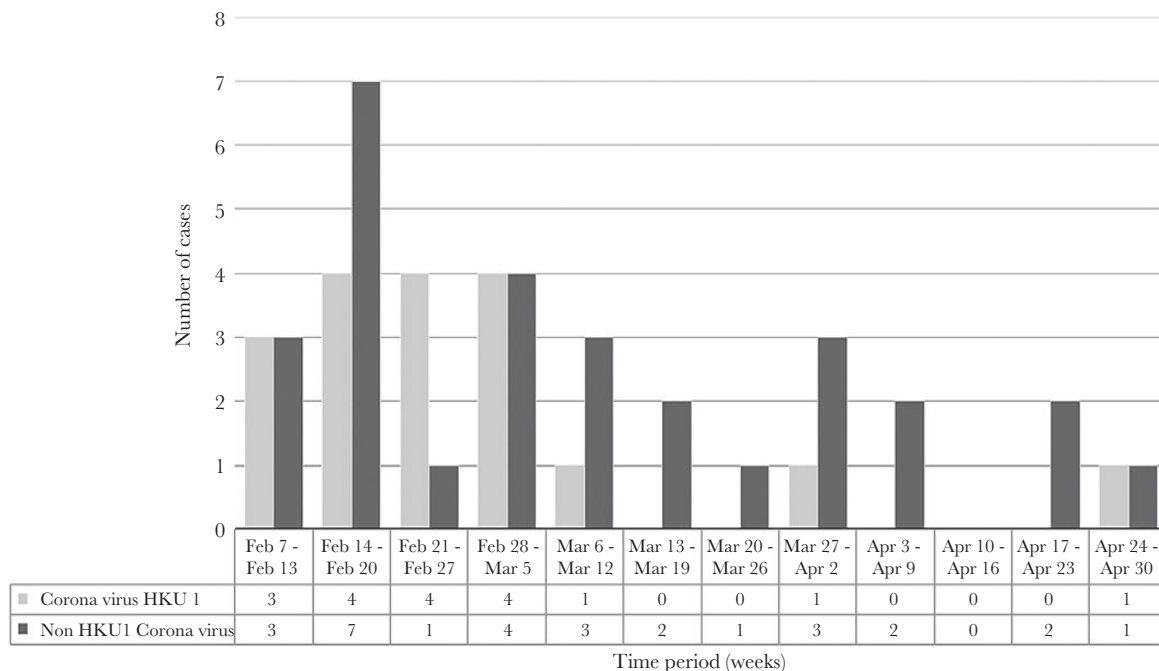
Figure 1. Weekly distribution of human coronavirus (HCoV) detection in Cleveland from February to April 2016. The number of CoV-HKU1-positive samples (light grey) and non-CoV-HKU1 CoV species (black) are displayed. Peak circulation of all CoV species was seen in February and early March.

	Feb 7 - Feb 13	Feb 14 - Feb 20	Feb 21 - Feb 27	Feb 28 - Mar 5	Mar 6 - Mar 12	Mar 13 - Mar 19	Mar 20 - Mar 26	Mar 27 - Apr 2	Apr 3 - Apr 9	Apr 10 - Apr 16	Apr 17 - Apr 23	Apr 24 - Apr 30
Corona virus HKU 1	3	4	4	4	1	0	0	1	0	0	0	1
Non HKU1 Corona virus	3	7	1	4	3	2	1	3	2	0	2	1

Time period (weeks)

fluticasone. Further studies are needed to confirm any relationship between respiratory illness due to CoV-HKU1 and other CoV with inhaled corticosteroids use.

All patients we identified with CoV-HKU1 infection had respiratory symptoms with dyspnea, cough, and rhinorrhea being the most common. This is not unexpected due to our study design. Similar studies describing HKU1 in pediatric and adult patients have also found an association with upper and lower respiratory tract illness [7, 9, 12, 15, 21]. However, CoV-HKU1 has also been reported in patients presenting with nonrespiratory symptoms [8]. We identified gastrointestinal symptoms in 38% of CoV-HKU1-positive adults, consisting of diarrhea and nausea and/or vomiting. The mechanism for gastrointestinal

symptoms seen in CoV-HKU1 disease remains unclear. Vabret et al [22] and Esper et al [23] have isolated CoV-HKU1 from stool samples in patients with diarrhea and nausea and/or vomiting. Non-CoV, such as influenza [24], and other CoVs, such as SARS-CoV [25] and MERS-CoV [26], have also been detected in stool specimens among patients with and without gastrointestinal symptoms. Further studies are needed to determine whether CoV-HKU1 detection in stools is part of gastrointestinal disease versus asymptomatic viral shedding and whether it plays any role in disease transmission.

It is interesting to note that adults identified with Cov-HKU1 had higher than expected severity of disease in our study, with increased occurrence of hospital admissions, oxygen use, and
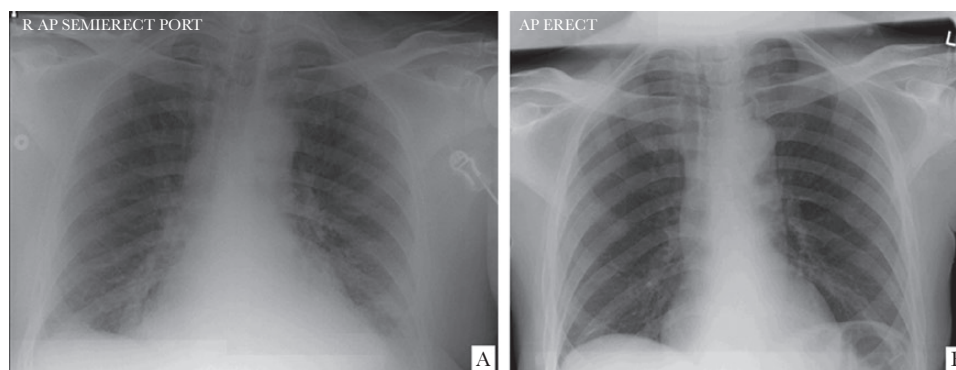

Figure 2. Representative anteroposterior (AP) chest x-ray of adult hospitalized with respiratory tract illness associated with coronavirus (CoV)-HKU1. Chest x-ray obtained from a patient at time of CoV-HKU1 diagnosis (A) is compared with chest x-ray obtained 1-year prior (B). Radiologic findings during this respiratory illness include prominence of interstitial markings with reticulonodular pattern, consolidative changes in right lower lobe with small right pleural effusion. No other respiratory pathogens were identified in this patient.

ICU admission. This is in contrast to other studies in which at-risk patient populations had lower morbidity and hospitalization with CoV-HKU1 infections [7, 8, 27]. Looking at other CoVs, the rates of hospitalization and morbidity were relatively similar in combined pediatric and adult studies [12]. Our finding of 54% hospitalization and 15% ICU admission could be accounted for by selection bias of the sample population (screening those presenting to the ED or hospitalized with respiratory tract illness). Studies focusing on outpatient and asymptomatic adults will provide a better understanding of disease severity. However, this study does demonstrate CoV-HKU1's potential for severe illness. A 75-year-old with no history of immunosuppression died due to shock while meeting clinical criteria for sepsis and negative infectious work-up. It remains unclear what, if any, role Cov-HKU1 played in this patient's demise. Most studies of Cov-HKU1 have demonstrated lower mortality [11]. In the 2 deaths reported by Woo et al [11], both patients were immunocompromised with history of cancer, diabetes mellitus, advanced age, and lymphopenia. Another death linked to CoV-HKU1 was reported in a severely immunocompromised patient lymphodepleted by conditioning for autologous hematopoietic stem cell transplantation [28]. In this case, CoV-HKU1 was isolated from his lung tissue and bronchoalveolar lavage with autopsy revealing bronchopneumonia, organizing pneumonitis, and diffuse alveolar damage.

In our study, HKU1-positive adults who did not require hospitalization had a normal chest x-ray, whereas 86% among inpatients with CoV-HKU1 had chest infiltrates. Garbino et al [29] reported interstitial and alveolar infiltrates in 52% of hospitalized patients with respiratory samples positive for CoV-OC43, CoV-NL63, CoV-229E, and CoV-HKU1. Given paucity of chest imaging data, complete characterization of radiographic findings in CoV-HKU1 infections is incomplete.

Approximately 62% of all patients with CoV-HKU1 infection in our study received antibiotics. This is similar to other studies in which up to 76% of patients with CoV-positive bronchoalveolar lavage received antibiotics [29]. Antibiotic use after a positive respiratory pathogen panel for viral pathogen was observed for median duration of 7 days in our study. This is consistent with other studies suggesting that mere disease diagnosis with respiratory pathogen panel might not alter antibiotic prescribing practices among healthcare providers for viral respiratory illnesses [30]. Such practices are concerning for unnecessary antibiotic exposure and the potential to aid the development of multidrug-resistant organisms [31]. This highlights the need for education of providers to consider CoV-HKU1 as a pathogen associated with upper and lower respiratory tract infections and de-escalate antibiotics with negative bacterial cultures and antigen [32].

Among all CoV-positive adults, 17% had coinfections, the most common being influenza A and rhinoviruses. These findings are comparable to other studies [21]. Higher coinfections

with CoV and other viruses could suggest common reservoirs, overlap in seasonality, and persistence of virus or viral genome in respiratory secretions [33]. Among our 2 adults with CoV-HKU1 and influenza A coinfection, it was unclear whether symptoms were due to CoV-HKU1 or influenza or both. The clinical relevance of respiratory viral coinfections is not fully understood. Higher severity of respiratory disease has been reported in certain pathogen coinfection combinations, including influenza A with CoVs [34]. In other studies, there was no increased severity of illness in CoV-associated coinfections [15, 27, 35]. Gaunt et al [27] showed that a quantitative viral load for CoV was unchanged in cases of coinfection with RSV and monoinfection. Further investigation on the role viral coinfections play in alteration of respiratory disease severity should be undertaken.

Our study has several limitations. By evaluating only symptomatic patients, we could have missed asymptomatic or subclinical infections. This could lead to an underestimation of the disease prevalence in the adult population and an overestimation of disease severity. The retrospective aspect of our study is insufficient to establish causality. Larger study periods will also be required to establish a complete understanding regarding seasonal distribution patterns of this virus in our community. However, the majority of CoV-HKU studies in the literature are single-center experiences occurring over 1 season [12, 21].

CONCLUSIONS

Our study provides needed insight into clinical characteristics and severity associated with CoV-HKU1 infection in adults in the Northeast region of the United States. We opine that CoV-HKU1 infection should be considered in differential diagnosis of community-acquired pneumonia in adults, including those that require hospitalization. More studies are needed to fully understand the seasonality, risk factors, incidence, and epidemiology of CoV-HKU1.

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