Clinical Impact of Human Coronaviruses 229E and OC43 Infection in Diverse Adult Populations

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Background. The incidence and clinical impact of coronavirus (CoV) infection in elderly persons and those with underlying cardiopulmonary disease over a long duration is not well described. We determined the incidence and clinical impact of 229E and OC43 CoV in this population during 4 consecutive winters, and compared illnesses to influenza A, respiratory syncytial virus, and human metapneumovirus.

Methods. CoV 229E and OC43 were detected by reverse transcription polymerase chain reaction and serology in 4 adult populations under surveillance for acute respiratory illness during the winters of 1999–2003. Cohorts included healthy young adults, healthy elderly adults, high-risk adults with underlying cardiopulmonary disease, and a hospitalized group.

Results. Three hundred ninety-eight CoV infections were identified, with annual infection rates ranging from 2.8% to 26% in prospective cohorts, and prevalence ranging from 3.3% to 11.1% in the hospitalized cohort. The incidence of infections with each strain was similar, although asymptomatic infection and viral coinfection was significantly more common with 229E than OC43 infection. Although the incidence and clinical manifestations were similar for each strain, OC43-infected subjects tended to seek more medical care, as OC43 was twice as common as 229E among the hospitalized cohort.

Conclusions. CoV infections in the elderly are frequent, likely causing substantial medical disease burden.

Keywords. coronavirus; adults; elderly.

Coronaviruses (CoVs) are enveloped, nonsegmented positive strand RNA viruses of the family Coronaviridae and cause a wide spectrum of illness including respiratory and gastrointestinal disease in a variety of mammalian species including humans [1]. CoVs are divided into 4 genera and are capable on occasion of interspecies transmission, as postulated for the severe acute respiratory syndrome (SARS) coronavirus that emerged briefly in 2003 and a very recently identified lethal novel coronavirus (MERS-CoV) in the Middle East [2, 3]. Since the 1960s, CoV strains 229E and OC43, classified as alpha- and betacoronaviruses, respectively, have been recognized as frequent causes of the "common cold" [4]. In the past decade, CoV NL63 (an alphacoronavirus) and HKU1 (a betacoronavirus) were discovered as causes of respiratory illnesses and together with 229E and OC43 continue to circulate widely in humans during the winter months in temperate climates [5–7]. In addition to upper respiratory tract illness, these latter 4 strains are also associated with bronchitis, acute exacerbation of chronic obstructive pulmonary disease (AECOPD), and pneumonia in all age groups [8–11]. Infection rates range widely from 2% to 20% with marked variation in the predominant circulating strain. Coronaviruses are rarely isolated in routine tissue culture and without molecular diagnostic tests the majority of infections go unrecognized. Most descriptions of the epidemiology and clinical impact of CoV are in infants and young children, with only a few

small long-term studies in adults, and none that evaluated illness in outpatient and hospitalized populations simultaneously. With the exception of SARS and CoV-EMC, there is little information regarding the relative virulence among coronavirus strains.

In this report we describe and compare the incidence, clinical characteristics, and outcome of infection with CoV strains 229E and OC43 in 4 large adult cohorts with a variety of underlying medical conditions over 4 consecutive winter seasons. In addition, we contrast the relative clinical impact of these CoV strains to influenza A, respiratory syncytial virus (RSV), and human metapneumovirus (hMPV).

METHODS

Subjects

The 4 adult populations included in this study have previously been described in detail [12, 13]. Coronavirus infections were identified using archived serum and nasal respiratory secretions previously collected during respiratory illness surveillance encompassing 4 consecutive winters (15 November—15 April) of 1999–2003 in Rochester, New York. Three prospective cohorts (healthy persons ≥65 years of age, young adults 19–40 years of age, and high-risk adults with underlying chronic obstructive pulmonary disease [COPD] or congestive heart failure) were enrolled in the late summer/early fall of each year and participated for a maximum of 2 winters. Approximately half of the subjects in the prospective cohorts were replaced each year using a rolling enrollment strategy. Upon enrollment, demographics, medical history, and directed respiratory examination were recorded and a serum specimen was obtained. Prospective subjects were asked to notify study staff of acute respiratory symptoms (cough, sore throat, increased or new sputum, dyspnea, wheezing with or without fever), at which time the signs and symptoms of illness were recorded and nose and throat swabs and acute serum specimen were collected. Four to 6 weeks later, a follow-up assessment was performed and convalescent serum samples were collected. At the conclusion of each season, subjects were queried about any other respiratory illnesses during the season, and a serum sample was obtained.

Simultaneously, a hospital cohort was recruited from among persons admitted to Rochester General Hospital with acute respiratory symptoms. Patients with acute coronary syndromes, documented pulmonary embolism, or aspiration pneumonia were excluded [12]. Acute illness and follow-up evaluations were similar to the prospective cohorts. The University of Rochester and Rochester General Hospital institutional review boards approved the study, and all participants or guardians provided written informed consent.

Laboratory Testing

Serum samples and nose and throat swab specimens were stored at −80°C for 3–6 years before testing for CoV. Nose and throat swabs were analyzed for human coronavirus 229E and OC43 RNA in single runs using real-time multiplex reverse transcription polymerase chain reaction (RT-PCR) with primers and probes targeting the polymerase gene. RNA was extracted into 12 µL of water from 250 µL of stored respiratory specimens. For reverse transcription, 5.25 µL of RNA was incubated at 42°C for 30 minutes with 10 U of avian myeloblastosis virus reverse transcriptase, 10 U of RNasin, 200 µM dNTPs, and primers specific for strain OC43 (5′AATACCTTTCTTGG CTCGAGTAAT) and strain 229E (5′AATACCTTTCTTTGCT CTAGTAAT) at a concentration of 200 nM each. The amplification mixture contained 5 µL of complementary DNA, 5 mM $MgCl_2$, 400 µM dNTP (dUTP replacing dTTP), 5 U of Taq polymerase, and 1 U of uracil-DNA glycosylase, 200 nM of OC43 forward primer, 229E forward primer, a common reverse primer (5′GTGGATTCTGCTCAAG), and strain-specific probes (Sigma-Genosys); OC43 (5′FAM-TACAGAAT GCGCTGTTTCTGCAGTCTGTGAAT-BHQ1); 229E (5′-TR-ACAGGCATGAGCAGTATCTGATGTCTGTGCGA-BHQ2). Amplification was performed on a Bio-Rad iCycler for 42 cycles of 95°C for 5 seconds, 42°C for 40 seconds, and 68°C for 10 seconds. The cycle threshold was defined when the relative fluorescent units (RFUs) exceeded 15% of the highest RFU value for the positive control. Strain-specific specificity of the assay was confirmed using CoV 229E and OC43 obtained from the American Type Culture Collection (ATCC; VR 740 and VR 759, respectively).

Serologic testing for CoV 229E and OC43 was performed by enzyme immunoassay using serum samples spanning each winter season (fall through spring) separately. CoV 229E from ATCC was propagated in MRC-5 cells (Viromed Laboratories) until cytopathic effect was extensive. Cells were scraped into the medium and sonicated followed by low-speed clarification. The supernatant fluid was pelleted in a Beckman SW27 rotor at 20 000 rpm for 90 minutes, resuspended in TNE buffer (50 mM Tris/140 mM NaCl/10 mM EDTA, pH 7.5), and sonicated, and the virus was purified on a 20%/60% discontinuous sucrose gradient (32 000 rpm in a SW41 rotor for 100 minutes). The virus band was diluted to 3.5 mL in TNE buffer and stored at −80°C until use. OC43 (ATCC VR 759) was grown in HRT-18G cells (obtained from ATCC, CRL-11663) until cytopathic effect was extensive, at which time cells were scraped into the supernatant and clarified [14]. The pellet was resuspended into phosphate-buffered saline/0.5% NP40, placed on ice, sonicated 4 times for 15 seconds, and then clarified. The supernatant was stored at −80°C until use. CoV antigens coated onto enzyme immunoassay plates and control plates were coated with uninfected cell preparations. Serial 2-fold dilutions of sera were incubated in antigen-coated plates and developed by addition of alkaline phosphatase–conjugated goat antihuman immunoglobulin G (IgG) followed by substrate. The usual convention of a ≥4-fold rise in serum IgG specific for the CoV antigens was considered evidence of infection.

Infection with other viruses were also identified by culture, RT-PCR, or serology (influenza A and B, RSV, hMPV) or culture alone (parainfluenza virus) in the same samples as previously described [12, 13].

Definition of Infection

Symptomatic CoV infection was defined as an illness with upper or lower respiratory symptoms associated with a positive RT-PCR at the time of symptoms and/or a ≥4-fold rise in CoV-specific IgG between acute and convalescent serum samples. Incompletely evaluated CoV illnesses were those for which participants failed to report symptoms during the winter surveillance, or were out of town at the time of the illness, but were reported to study staff at the final spring interview and demonstrated a significant increase in CoV-specific serum IgG. Asymptomatic infection was defined as a ≥4-fold increase in CoV-specific serum IgG bracketing a period when no respiratory symptoms were reported.

Statistical Analysis

Differences between groups were analyzed using the χ^2 test of independence for dichotomous variables and unpaired, 2-tailed t tests for continuous variables. P values were corrected for multiple comparisons using the Bonferroni correction method.

RESULTS

Populations Studied

The prospective cohorts included 611 healthy elderly adults, 537 high-risk adults with underlying cardiopulmonary disease, and 291 healthy young adults, and the hospital cohort numbered 1388 (Table 1). These cohorts have previously been described in detail [12, 13]. The young adult cohort was approximately 40 years younger on average than the other cohorts, and had a substantially greater frequency of living with or exposure to children. Compared to the healthy elderly cohort, the high-risk and hospital cohorts had a relatively high prevalence of cardiac and pulmonary disease and greater impairment in the Instruments of Activities of Daily Living (IADL) scores. During the 4 winters, 398 coronavirus infections in 394 subjects were identified (4 subjects had a dual OC43/229E infection). In all years, OC43 was more common than 229E early in the surveillance period, and in years 2 and 4, 229E peaked later (Figure 1).

Incidence of Coronavirus Infections in the Prospective Cohorts

A total of 302 CoV infections occurred in the prospective cohorts, with slightly more OC43 than 229E infections (Table 2). Overall, there were 113 CoV infections in the healthy elderly cohort (54 229E, 59 OC43), 112 in the high-risk group (57 229E, 55 OC43), and 77 in the young cohort (36 229E, 41 OC43). The percentage of subjects infected with coronaviruses varied considerably from year to year, with infection rates for

Table 1. Demographic and Clinical Characteristics of Study Groups

Characteristic	Healthy Elderly (n = 611)	High-Risk (n = 537)	Young Adults (n = 291)	Hospital (n = 1388)
Age, mean ± SD	75 ± 6	70 ± 11	33 ± 5	75 ± 12
Male, %	42.4	54.4	16.2	44.7
Race, %				
White	97.7	93.7	82.1	84.9
Black	2.1	5.4	11.0	9.6
Hispanic	0.2	0.9	5.2	5.5
Live with children, %	2.2	6.0	88	7.4
Exposed to children[a], %	58	65	100	52
Chronic illnesses, %				
Lung disease	2.1	64.6	10.0	58.6
Cardiac disease	16.5	47.9	1.4	54.6
Smoking (ever), %	55.3	81.9	38.1	78.1
IADL[b] score, mean ± SD	0.31 ± 1.1	1.2 ± 2.2	0.03 ± 0.4	3.8 ± 4.1
Use of home oxygen, %	0	18	0	24
No. of illnesses	525	519	314	1471

[a] Defined as exposure at least once per month.

[b] Instruments of Activities of Daily Living (6 items scored from 0 to 2, with a maximum of 12 indicating severe disability).

229E ranging from 0.5% to 15.0% and OC43 from 2.3% to 11.4%. Overall, infection rates were greatest during the winter of year 2 (2000–2001), when 23%–26% of subjects in each of the prospective cohorts were infected with 1 of the 2 strains.

Of note, asymptomatic infection in the prospective cohorts (defined as a serologic response without a reported illness) was relatively common, and was significantly greater for 229E than for OC43 infections (36.7% vs 18.1%; $P = .0003$) when the 3 cohorts are considered together. Mixed viral infections were also significantly more frequent with symptomatic 229E (16.1%) than OC43 infection (6.3%, $P = .03$). For 229E-infected persons, coinfecting viruses were hMPV (6), RSV (5), influenza A and B (1 each), and OC43 (1). For OC43-infected persons, coinfecting viruses were RSV (3), hMPV (2), influenza A and B (1 each), and 229E (1).

Clinical Characteristics and Outcome in Prospective Cohorts

To compare clinical characteristics and outcomes associated with 229E and OC43 infections in the prospective cohorts, we analyzed fully evaluated symptomatic illnesses not associated with other viruses (Table 3). Upper respiratory symptoms were relatively common in all 3 cohorts, and nasal congestion and rhinorrhea were nearly universal regardless of infecting strain.
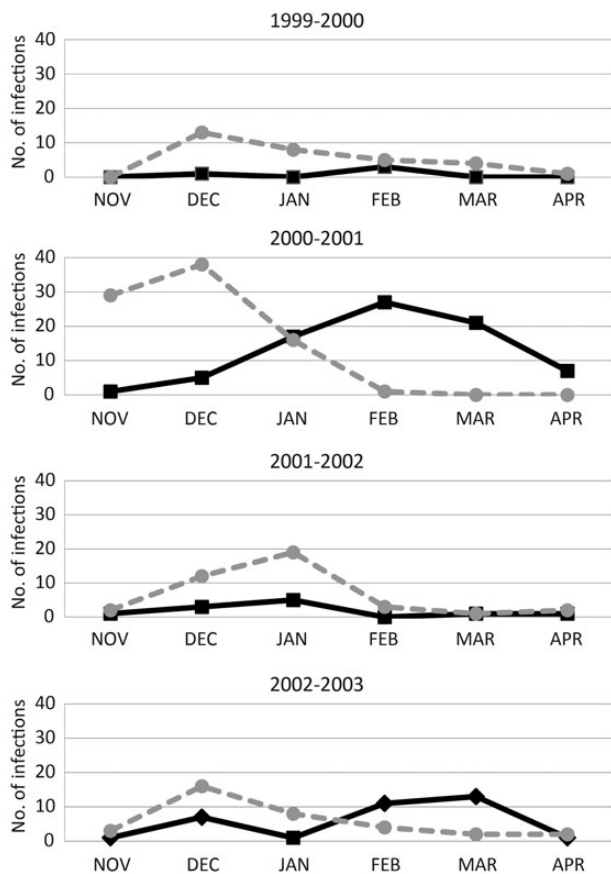
Figure 1. Incidence of symptomatic 229E (solid black line) and OC43 (dashed gray line) coronavirus infections in the combined prospective cohorts and the hospital cohort for each of the 4 winters.

Other upper respiratory symptoms were less common, with 30%–63% of subjects complaining of sore throat and 25%–44% complaining of hoarseness. Cough was more common in OC43 infections when all 3 cohorts were considered together, although lower respiratory signs and symptoms were similar for both strains.

The outcome of coronavirus illness in these same subjects is shown in Table 4. For each cohort, there was a trend toward longer duration of illness with OC43 infection. In aggregate, OC43-infected subjects used significantly more cough suppressants and bronchodilators. In addition, there was a trend toward more health service use, such as physician or emergency room visits in the healthy elderly and high-risk groups during OC43 infection.

Prevalence and Clinical Impact of Coronavirus Infection in the Hospital Cohort

The hospital cohort included 1388 subjects admitted for 1471 illnesses (Table 1). Subjects were similar in age to the healthy elderly group and slightly older than the high-risk group. Rates of underlying cardiopulmonary disease and tobacco use were

also similar to the high-risk group, whereas home oxygen use was significantly greater in the hospital cohort (24% vs 18%, $P = .003$). During the 4 winters, 96 coronavirus infections were identified in the 1388 subjects in the hospital cohort. Of these, 34 (2.4%) were 229E and 62 (4.5%) OC43, with 2 subjects having both 229E and OC43 identified (Table 2). Similar to the prospective cohorts, the highest incidence of coronavirus infections occurred in year 2 (11.8%) and ranged from 3.3% to 7.5% in other years.

Because all subjects had respiratory symptoms, none were classified as asymptomatic. However, overall, 25% of CoV infections in the hospital cohort were associated with a second virus. As in the prospective cohorts, a significantly greater percentage of dual virus infection was noted in 229E-infected subjects than in OC43-infected subjects (16/34 [47%] vs 7/62 [11%]; $P = .0002$). For the 229E-infected persons, the coinfecting viruses were RSV (7), hMPV (9), OC43 (2), and influenza A and B (1 each). For the OC43-infected persons, the viruses were RSV (2), 229E (2), hMPV (1), parainfluenza virus (1), and influenza A (1). Admission diagnoses were similar for 229E and OC43, with pneumonia (32% and 35%, respectively) and AECOPD (36% and 41%, respectively) most common. The clinical presentation of the 2 strains in the hospital cohort was similar, although there was a trend toward longer hospital stay, and greater need for intensive care unit care and ventilatory support in OC43-infected subjects (Table 5). The only significant difference between strains was that OC43-infected subjects were more likely to have pulmonary infiltrates noted on chest radiographs.

Laboratory Diagnosis of 229E and OC43 Infections

For both strains, the combination of serology and RT-PCR greatly enhanced the diagnosis of infection. Results from symptomatic subjects in whom both tests were available indicate that a significantly greater proportion of OC43-infected subjects were RT-PCR positive (109/158 [69%]) than 229E-infected subjects (52/108 [48%], $P = .001$). For both strains, approximately 30% were positive by both RT-PCR and serology.

Relative Impact of Coronavirus Infection Compared to Other Respiratory Viruses

We had previously reported the incidence and clinical outcomes of several other viruses in these same cohorts, thus providing an opportunity to compare 229E and OC43 to influenza A, RSV, and hMPV [12, 13]. Figure 2A and 2B shows the virus-specific medical care utilization for the 2 older prospective cohorts in subjects with symptomatic illness not associated with dual virus infection. Influenza A infection, followed by hMPV and RSV, had the greatest impact and 229E the least. It should be noted that the majority of influenza A illnesses occurred when H3N2 strains were the dominant circulating viruses.

Table 2. Incidence of 229E and OC43 Coronavirus Infections During 4 Winters

Year	Healthy Elderly	High-Risk	Young Adults	Hospital
1999–2000				
No. in cohort	212	206	103	274
No. of illnesses	123	146	78	289
No. of 229E infections (% of cohort)	1 (0.5%)	2 (0.9%)	2 (1.9%)	3 (1.1%)
No. asymptomatic 229E infections	1	2	1	0
No. of OC43 infections (% of cohort)	5 (2.3%)	11 (5.3%)	10 (9.7%)	6 (2.2%)
No. asymptomatic OC43 infections	1	0	0	0
2000–2001				
No. in cohort	280	271	180	296
No. of illnesses	159	160	113	307
No. of 229E infections (% of cohort)	37 (13.2%)	36 (13.3%)	27 (15.0%)	11 (3.7%)
No. asymptomatic 229E infections	9	10	14	0
No. of OC43 infections (% of cohort)	28 (10.0%)	31 (11.4%)	20 (11.1%)	24 (8.1%)
No. of asymptomatic OC43 infections	6	10	3	0
2001–2002				
No. in cohort	180	195	164	434
No. of illnesses	102	115	101	165
No. of 229E infections (% of cohort)	1 (0.6%)	6 (3.1%)	1 (0.6%)	5 (1.2%)
No. asymptomatic 229E infections	0	2	0	0
No. of OC43 infections (% of cohort)	9 (5.0%)	7 (3.6%)	7 (4.3%)	18 (4.1%)
No. asymptomatic OC43 infections	0	1	1	0
2002–2003				
No. in cohort	295	210	90	384
No. of illnesses	135	103	37	410
No. of 229E infections (% of cohort)	15 (5.1%)	13 (6.2%)	6 (6.7%)	15 (3.9%)
No. asymptomatic 229E infections	9	5	1	0
No. of OC43 infections (% of cohort)	17 (5.8%)	6 (2.9%)	4 (4.4%)	14 (3.6%)
No. asymptomatic OC43 infections	1	2	3	0
All years combined				
No. of 229E infections	54	57	36	34
% asymptomatic	35.1%	33.3%	44.4%	NA
No. of OC43 infections	59	55	41	62
% asymptomatic	13.6%	23.6%	17.1%	NA

We next compared the incidence of each virus in the prospective cohorts to the incidence in the hospital cohort. This should provide a reasonable gauge of the relative virulence of each virus, with a low ratio indicating that fewer outpatient infections are required to produce similar numbers of hospitalizations compared to a virus with a high ratio. For this analysis, asymptomatic infections and dual virus infections are included in the prospective cohorts. Figure 2C shows that influenza A has the lowest ratio (0.30), followed in ascending order by RSV (0.85), hMPV (0.95), OC43 (2.1), and 229E (6.2).

DISCUSSION

Noninfluenza respiratory viruses are increasingly recognized as significant pathogens in adult populations, especially in elderly adults. However, unlike influenza, the impact and importance of other respiratory viruses are less well documented in this age group. Although coronaviruses have been identified in elderly adults and high-risk populations such as those with COPD, cystic fibrosis, or immune deficiencies, only a few studies have included extended periods or directly compared results to other viruses [10, 11, 15–19]. Our data indicate that depending upon the year, 3%–26% of prospectively enrolled outpatients were infected with OC43 and 229E CoV strains. More importantly, among the hospital cohort the incidence of coronavirus infection varied from 3.3% to 11.8%, with an average of 6.8% per year. These findings are consistent with reports from other investigators. In a 1-year analysis of 2215 patients with COPD enrolled in an influenza vaccine efficacy study, Gorse et al identified coronaviruses in 14% of acute influenza-like illnesses (defined as ≥3 respiratory symptoms or fever plus 2 respiratory symptoms), a rate similar to that of influenza [10]. In that

Table 3. Clinical Characteristics of 229E and OC43 Coronavirus Infections in the Prospective Cohorts[a]

Characteristic	Healthy Elderly		High-Risk		Young Adults	
	229E (n = 27)	OC43 (n = 42)	229E (n = 20)	OC43 (n = 34)	229E (n = 15)	OC43 (n = 32)
Symptoms, No. (%)						
Congestion	27 (100)	41 (98)	20 (100)	34 (100)	15 (100)	32 (100)
Sore throat	9 (33)	17 (40)	6 (30)	15 (44)	7 (47)	20 (63)
Hoarseness	9 (33)	17 (40)	5 (25)	17 (50)	5 (33)	14 (44)
Cough[b]	14 (52)	30 (71)	10 (50)	27 (79)	6 (40)	20 (63)
Sputum	6 (22)	14 (33)	7 (35)	20 (59)	3 (20)	6 (19)
Dyspnea	1 (4)	6 (14)	9 (44)	18 (53)	5 (33)	7 (19)
Wheezing	2 (7)	6 (14)	5 (25)	10 (29)	2 (13)	5 (16)
Constitutional	20 (74)	25 (60)	14 (70)	21 (62)	8 (53)	19 (59)
Feverishness	7 (26)	8 (19)	4 (20)	11 (32)	6 (40)	7 (22)
Signs						
Rhinorrhea	27 (100)	37 (88)	19 (95)	32 (94)	13 (87)	29 (91)
Wheezing	0	3 (7)	2 (10)	5 (15)	0	2 (6)
Rales	0	1 (2)	1 (5)	5 (15)	0	0
Rhonchi	0	0	0	0	0	0
Temperature, °C, mean ± SD	36.4 ± 0.4	36.5 ± 0.6	36.8 ± 0.4	36.7 ± 0.9	36.8 ± 0.4	36.6 ± 0.6
SaO2 on room air	96.6 ± 2.1	95.6 ± 4.0	95.8 ± 1.9	95.2 ± 3.4	98.3 ± 1.4	98.3 ± 1.2

Abbreviation: SaO2, saturation of arterial oxygen.

[a] Dual virus infections excluded; only fully evaluated illnesses are included in this analysis.

[b] $P = .065$ comparing OC43 to 229E when all groups are combined.

study, OC43 was approximately 3 times more common than 229E; however, asymptomatic infection was not sought and thus the true number of infections was likely greater. In our study, we used less stringent criteria for evaluation and thus we likely detected a broader spectrum of illness. In contrast to our study, Gorse et al found that coronavirus and influenza infections were equally associated with the need for hospitalization, although only influenza was temporally linked to impairment of pulmonary function. In a community-based study from the Netherlands, Graat et al detected coronaviruses by RT-PCR in

Table 4. Outcome of Coronavirus Infections in the Prospective Cohorts[a]

Outcome	Healthy Elderly		High-Risk		Young Adults	
	229E (n = 27)	OC43 (n = 42)	229E (n = 20)	OC43 (n = 34)	229E (n = 15)	OC43 (n = 32)
Days of illness, mean (range)	10.0 ± 5.2	12.7 ± 8.1	11.1 ± 6.6	12.4 ± 10.8	6.7 ± 4.2	12.0 ± 9.5
Days house bound, mean ± SD	0.9 ± 2.1	0.8 ± 1.5	1.2 ± 1.8	2.1 ± 5.2	0.1 ± 0.5	0.3 ± 0.8
Called MD, No. (%)	0	6 (14)	3 (15)	8 (26)	0	1 (3)
Visited MD, No. (%)	1 (4)	7 (17)	4 (20)	11 (36)	0	0
Visited ED, No. (%)	0	0	0	0	0	0
Hospitalized, No. (%)	0	0	0	1 (3)	0	0
Medications						
Antipyretics	14 (52)	18 (43)	10 (50)	13 (41)	12 (80)	21 (68)
Cough suppressants[b]	5 (19)	19 (45)	1 (5)	10 (31)	4 (27)	11 (35)
Bronchodilators	0	2 (5)	1 (5)	6 (18)	0	3 (10)
Systemic steroids	0	3 (7)	0	6 (18)	0	1 (3)
Antibiotics	1 (4)	4 (10)	2 (10)	10 (29)	1 (7)	0

Abbreviations: ED, emergency department; MD, physician.

[a] Dual virus infections were excluded, and only fully evaluated illnesses are included in this analysis.

[b] $P = .02$ for differences between 229E and OC43 when all 3 prospective groups combined.

Table 5. Clinical Characteristics of Coronavirus Infections in the Hospital Cohort

Characteristic	229E (n = 18)	OC43 (n = 53)
Symptoms, No. (%)		
Congestion	6 (33)	29 (55)
Sore throat	4 (18)	15 (28)
Hoarseness	3 (19)	17 (32)
Cough	15 (83)	51 (96)
Sputum	11 (65)	36 (68)
Dyspnea	18 (100)	51 (96)
Wheezing	10 (63)	35 (66)
Constitutional	6 (35)	23 (43)
Feverishness	8 (44)	23 (43)
Signs		
Rhinorrhea	3 (17)	5 (9)
Wheezing	12 (67)	40 (76)
Rales	12 (67)	31 (60)
Rhonchi	7 (41)	20 (38)
Temperature, °C, mean (SD)	37.6 ± 1.0	37. 5 ± 1.0
SaO2 on room air	87.9 ± 5.8	89.3 ± 6.9
Outcome		
Days in hospital, mean ± SD	5.9 ± 4.7	8.8 ± 9.6
ICU care, No. (%)	1 (3)	9 (15)
Ventilatory support	2 (6)	8 (15)
Infiltrate on chest radiography	4 (12)	19 (37)[a]
Death	1 (3)	2 (4)

Dual virus infections not included.

Abbreviations: ICU, intensive care unit; SaO2, saturation of arterial oxygen.

[a] P = .05 compared to 229E.

19% of 97 elderly subjects during a 1-year period, none of whom required hospitalization [20]. In studies from Houston, Texas, Greenberg identified OC43 or 229E in 16 of 62 COPD patients during 30 months of surveillance, with the majority of infections caused by OC43, whereas Glezen et al diagnosed serologically confirmed 229E or OC43 infection in 3.4% of 417 hospitalized adults during a 4-year period [11, 15]. In an analysis of a large number of outpatient respiratory illnesses in the United Kingdom, Gaunt identified CoV in approximately 1.1% of older adults over 3 years [8]. In that study, lower respiratory tract illness was noted in 43% of OC43 infections, but in no 229E infections.

Our data also suggest that OC43 has greater clinical impact than 229E in adults. OC43 infection was significantly less likely to be asymptomatic or associated with mixed viral infection, the latter a common finding also noted by other investigators. We defined asymptomatic infection by a positive serologic rise during an asymptomatic interval rather than by a positive PCR in asymptomatic persons. The latter may represent "colonization" whereas the former likely represents true infection. The true frequency of coinfections is likely to be considerably
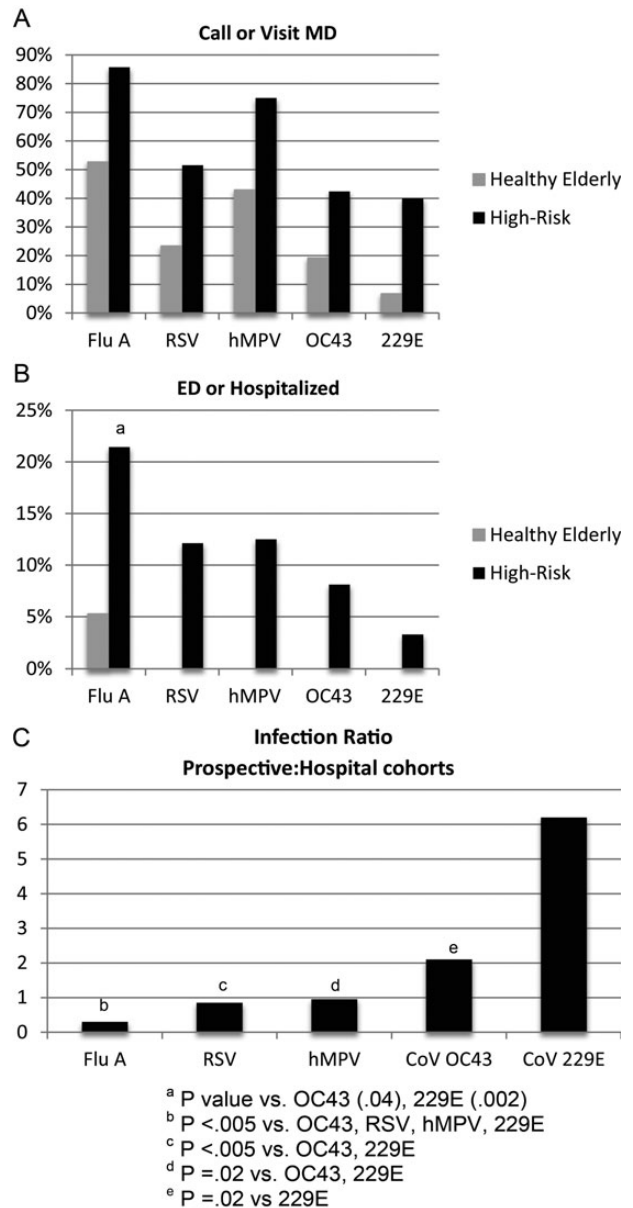

Figure 2. Comparative clinical impact of coronavirus (CoV) 229E and OC43 to influenza A (Flu A), respiratory syncytial virus (RSV), and human metapneumovirus (hMPV) in the healthy elderly and high-risk cohorts. *A* and *B*, Percentage of subjects infected with each virus who called or visited their physician (MD; *A*) or sought care in an emergency department (ED) or were admitted to the hospital (*B*). Data used include symptomatic fully evaluated infections, excluding viral coinfections. Healthy elderly cohort (gray bars), high-risk cohort (black bars). *C*, Ratio of infections in combined healthy elderly and high-risk cohorts to the number of infections in the hospital cohort. Data for this analysis include asymptomatic and viral coinfections in the prospective cohorts.

greater, as we did not assess subjects for rhinovirus and we only performed culture for parainfluenza viruses. The most frequent coinfecting viruses, RSV and hMPV, have previously been shown by us and others to account for significant morbidity in

elderly adults [12, 13, 21–23]. Thus, the observed illness in coinfected subjects may primarily be attributable to the coinfecting virus, a suggestion made by Prill et al in a study of CoV infection in infants [24]. Perhaps most significant is the lower ratio of outpatient infections to inpatient infections for OC43 compared to 229E, suggesting a virulence difference between strains. Our results imply that in elderly adults with a high incidence of underlying cardiopulmonary disease, it would require approximately 3-fold more outpatient 229E infections than OC43 infections to result in an equivalent number of hospitalizations. Although another group of investigators also simultaneously measured the incidence of infection in outpatient and hospitalized adults in the same community with a similar trend, they did not differentiate the coronavirus or influenza strains in the outpatient groups [11, 15].

In a similar fashion, we were also able to compare the relative impact of both coronaviruses to that of other previously characterized respiratory viruses in these same cohorts. Not surprisingly, the results indicate that the clinical impact is greatest for influenza A followed by RSV, hMPV, OC43, and 229E in order of decreasing importance. Although the relative impact of coronavirus infection appears to be less than the other viruses, the overall burden, particularly for OC43, is not insignificant. Less virulent organisms, such as rhinoviruses, have also been shown to have significant overall disease burden in the elderly due to their relatively high prevalence [25]. Using our coronavirus data in relation to influenza A during the 4-year period of surveillance and eliminating viral coinfections, the hospital burden of these 2 coronaviruses combined was approximately 47% of the influenza A.

Our study has several important limitations. The first is that we did not test for NL63 and HKU1 infections, and thus are unable to provide a complete picture of the clinical impact of coronavirus infection in adults. In some studies, NL63 or HKU1 has been more frequently identified than OC43 and 229E, although the majority reported OC43 to be the most common coronavirus strain identified [8, 9, 17–19, 24, 26]. It is possible that some of our subjects diagnosed with OC43 or 229E by serology alone could include HKU1 or NL63 infections due to antigenic cross-reactivity within coronavirus groups [27]. In addition, we also did not include an asymptomatic control group for the hospital cohort to assess illness causality, as done in one pediatric study [24]. Futhermore, viral coinfections were likely underestimated, and thus the attributable impact of CoV infection may be overestimated. Finally, our study was limited to winter seasons, and although coronaviruses tend to be most active in the winter, we likely missed some CoV infections, especially in year 2 when OC43 was highly active at the start of our surveillance period. Because we performed serology for each winter season separately, we would not have detected summer illnesses.

In conclusion, the overall incidence and impact of combined coronavirus 229E and OC43 infections in diverse adult populations over 4 consecutive winters is relatively high and the overall medical burden of disease is substantial.

Notes

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