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Multi-year study of human metapneumovirus infection at a large US Midwestern Medical Referral Center

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Abstract

Background—Because of its recent identification, few multi-year epidemiologic studies of hMPV infection have been reported.

Objective—We sought to retrospectively describe hMPV infections among patients evaluated by a large US Midwestern referral laboratory.

Study design—Clinical specimens were submitted to a large US Midwest referral hospital from 1 October 2001 to 18 May 2004. RT-PCR was used to retrospectively screen the clinical specimens for human metapneumovirus. Demographic and clinical data were retrieved.

Results—34 (2.6%) of 1294 specimens were hMPV positive. Among these, 21 (62%) were culture positive and available for genetic typing. A previously considered rare genotype of hMPV, B1, was the most common single genotype identified, comprising 9 (43%) of the 21 isolates. Multivariate logistic regression modeling identified patients aged 0.4–9 years (OR = 8.9; 95% CI = 2.0–38.5) and those under intensive care (OR = 3.2; 95% CI = 1.1–8.7) as more likely to have hMPV infection than their peers.

Conclusion—In this large referral hospital viral assays more often had evidence of hMPV when they were collected from children receiving intensive care.

Keywords

Human metapneumovirus; Respiratory viruses; Epidemiology; Genotyping

1. Introduction

Since hMPV was first reported (van den Hoogen et al., 2001) in 2001, much progress has been made in its detection (Cote et al., 2003; Ebihara et al., 2003, 2004; Hamelin and Boivin, 2005; Leung et al., 2005; Mackay et al., 2003; Maertzdorf et al., 2004), in understanding its

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genetic diversity (Boivin et al., 2002; Falsey et al., 2003; Ludewick et al., 2005; Mackay et al., 2003) and in documenting its varied clinical presentation (Falsey et al., 2003; Principi et al., 2006). By linking demographic and clinical data to a large, 3-year, viral culture specimen repository, we sought to describe hMPV infections among patients evaluated by a large US Midwestern referral laboratory.

2. Methods

2.1. Specimen collection and clinical data

We retrospectively studied a convenient sample of 1500 available, archived respiratory specimens, collected from 1 October 2001 to 18 May 2004, at the at the University of Iowa Hospitals and Clinics' Clinical Microbiology Laboratory. Respiratory specimen data (75% collected by nasal wash) were linked to patient demographic and clinical data: gender, age, race/ethnicity, clinic/ward source, date and site of culture, clinical diagnoses, patient disposition, admission/discharge date, and co-infections with other respiratory pathogens such as adenovirus, influenza A, influenza B, parainfluenza (1, 2, 3) and respiratory syncytial virus (RSV).

2.2. Reverse-transcription polymerase chain reaction (RT-PCR)

Viral specimens were evaluated for molecular evidence of hMPV as per our previous report (Gray et al., 2006). Briefly, specimens were screened with a one-step RT-PCR procedure using primer set F2 (Table 1). Screened specimens that yielded bands within 50 bp of the expected 347 bp product, were further tested with a two-step RT-PCR, using the F1, F2 and N-gene primer sets (Table 1). Specimens were designated as "RT-PCR+" if the N-gene primer set and at least one of the confirmatory F-gene primer sets yielded appropriately sized bands.

2.3. hMPV isolation

RT-PCR+ specimens were further studied with shell-vial LLC-MK2 cell culture for the presence of viable hMPV. Shell vials were incubated for 3–4 weeks at or until cell disruption occurred. Infected cell supernatant media was harvested each week upon cell media replacement. From an aliquot of the infected media, RNA was extracted and a subsequent RT-PCR was performed via the hMPV F2 gene one-step protocol.

2.4. Sequencing

Amplification products obtained using G_{univ} primers (Table 1) adapted for amplification of a 800–1000 bp region of the hMPV G protein gene were electrophoresed on 1.0% agarose gels and purified. The purified DNA was then sequenced in both directions.

2.5. Phylogenetic analysis

Alignments of the partial nucleotide sequences of the hMPV G protein gene were generated with the program ClustalW (National Center for Biotechnology Information, Bethesda, MD).

2.6. Statistical analysis

The Cochran–Armitage test was used to verify prevalence trends among infants. Binomial confidence intervals were computed about crude prevalence statistics. Unadjusted and adjusted odds ratios were computed for each risk factor using logistic regression. The final multivariable model was identified by using a saturated model and manual backwards elimination.

3. Results

3.1. Diagnostics and genetic characterization

Considering only the first specimen per medical event and good linkage data, 1294 specimens were available for study. Thirty-four (2.6%) of these specimens were positive by the RT-PCR screening assay and all were confirmed by the additional RT-PCR assays. Of the RT-PCR positive specimens, 21 (62%) grew hMPV virus in LLC-MK2 cell cultures. CPE was detectable at 14 days post-inoculation.

3.2. Clinical features

Among the 34 hMPV+ patients, seven presented with respiratory failure (Table 2). One of these, a 3-year-old Caucasian boy was respirator-dependent. Twenty-seven of the 34 hMPV+ patients were hospitalized at the time of culture (median stay 8 days). Frequent diagnoses among the hMPV+ patients included pneumonia, bronchiolitis, and nonspecific acute upper respiratory infection. Among the patients from whom hMPV genotype B was detected, 42% (5 isolates) had leukemia, in contrast to 11% (1 isolate) of patients from whom hMPV genotype A was grown. Among these five hMPV genotype B isolates, four (80%) were of subtype B1 (Table 2).

3.3. Risk factor modeling

Data from the 1294 patients were analyzed. The median patient age was 5.3 years: 52% were male and 66.8% were Caucasian (Table 3). Most specimens, 83.5%, were collected during the months of December–April.

Most (61.8%) of the hMPV+ specimens were from patients with ages of 0.4–9 years (Table 3). In fact, a significant increasing trend ($p < .001$) of hMPV+ prevalence by age was noticed in infants up to 6 years old (Fig. 1). Specimens that were positive for parainfluenza viruses were similarly more likely to be statistically associated with this age group, in contrast to the age distributions and influenza A virus, which were not statistically associated (Table 3).

All 34 hMPV+ specimens were also screened for RSV, and one was positive. Similarly, 33 of the hMPV+ specimens were screened for influenza A and B infection, and only one yielded evidence of co-infection with influenza B (Table 2). Ninety-three percent of the RSV+ specimens were collected during the same “high-risk” period (December–April) as were many of the hMPV+ specimens (Table 3).

3.4. Multivariable modeling

Logistic regression modeling revealed that only age group and patient group were associated with hMPV+ specimens (Table 4). Gender was not an important predictor of hMPV infection. The final multivariate logistic model indicated that children between 0.4 and 9 years of age had 8.9 times the odds of having hMPV infection compared with infants 0.4 years old or younger (Table 4). In addition, when adjusting for age group, intensive care unit patients had a higher odds (OR = 3.2) of being hMPV+ as comparing to outpatients.

3.5. Phylogenetic analyses of hMPV strains

The 21 specimens that were culture positive for hMPV were successfully sequenced. Phylogenetic studies (Fig. 2) revealed a high prevalence of subtypes B1 (nine isolates) and A2 (six isolates).

4. Discussion

Previous studies have associated hMPV infection with rhinorrhea, pharyngitis, otitis media, bronchiolitis, bronchitis, pneumonia, respiratory failure, and death (Pelletier et al., 2002; Williams et al., 2004). hMPV infections may exacerbate reactive airway disease among both the young and the old (Boivin et al., 2002; Greensill et al., 2003). There is evidence that some patients suffer repeat hMPV infections (Boivin et al., 2003), that mixed hMPV infections may be common (Schildgen et al., 2005), that many hMPV infections occur in winter months, and that the immunocompromised may be at greater risk of hMPV disease (van den Hoogen et al., 2001). Our data are consistent with many of these observations but offer some new possible risk factor associations. Our 34 hMPV+ specimens were obtained from patients with a wide array of clinical conditions, many of whom were immunocompromised. Among the 34 hMPV + specimens, two (Table 2, patients 20 and 25) were taken from patients >60 years of age who died; however, their deaths occurred 2 months after the specimens were collected and had underlying cancers that could explain their deaths. A third severely ill patient who survived, a 3-year-old boy (Table 2, patient 24) diagnosed with acute encephalomyelitis and coma, also had hMPV detected (genotype B1). Again while his respiratory culture yielded hMPV, it is difficult to clearly implicate hMPV as the etiology for his encephalitis. However, encephalitis has been previously found to be associated with hMPV (Boivin et al., 2002).

Although it is difficult to determine the primary etiology of many of the patients' diagnoses through this retrospective review of medical records, it seems safe to assume that clinicians were at least concerned about a viral cofactor, as they took the initiative to order viral cultures. Thus far asymptomatic carriage of hMPV has been only rarely reported (Boivin et al., 2002; Williams et al., 2004). Among the 34 hMPV+ patients, it is interesting to note that four (Table 2, patients 14, 19, 25, 30) had bacterial infections, four had other viral infections (Table 2, patients 4, 16, 26, 27), and one had a fungal infection (Table 2, patient 20).

Consistent with previous reports, our hMPV+ patients ranged in age from less than a year to 79 years (Boivin et al., 2003) and most infections occurred during the winter months and early spring (van den Hoogen et al., 2004). Also consistent with a previous report, we found that patients from the 0.4 to 9 years had a markedly increased risk (OR = 8.9, Table 4) of infection compared to those less than 0.4 years of age (Ludewick et al., 2005). This observation is likely due to waning maternal immunity. The increased risk and the increasing trend in hMPV prevalence (Fig. 1) up to age six suggests hMPV surveillance and preventive measures may be most efficiently focused upon patients less than 6 years of age.

Recent studies suggest that hMPV infection is not without clinical morbidity. Many of our hMPV+ patients and those previously reported (Boivin et al., 2004; Ludewick et al., 2005; Peret et al., 2004) have been hospitalized with severe disease. While severe disease association observations in clinical case series studies may represent a bias in sample selection (e.g. only the most severely ill are cultured), in our study we were able to compare the odds of a specimen being hMPV+ through comparisons of patient groups' clinical status (e.g. outpatient, observation, hospitalization ward, intensive care unit). This comparison revealed that after adjusting for age differences, intensive care unit patients had increased odds of being hMPV + compared to similarly tested outpatients, suggesting that hMPV infections may be more common among intensive care unit patients. This is likely due to the patients' underlying conditions, but it might also be explained by better clinical diagnosis or nosocomial transmission.

Our data were also unique in that we had a relative high proportion of isolates (43% of 21 sequenced) that were classified in the B1 genome subgroup (Fig. 2) (Ludewick et al., 2005; Mackay et al., 2004). This subgroup has previously been found to be rather uncommon

in Europe, Canada, and South Africa (Fox et al., 1977,1982;Hall et al., 1971). Some have speculated that the prevalence of circulating genome groups may change over time reflecting community immunity rather than viral emergence (Peret et al., 2002).

Like most previously published studies of hMPV, our analysis was limited to archived specimens collected for clinical purposes. This of course results in potential sampling biases and does not provide accurate community acquired incidence or risk factor data. However, because hMPV infections are rather rare, collecting such data would be difficult and very expensive.

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Abbreviations

hMPV, human metapneumovirus; RT-PCR, reverse-transcription polymerase chain reaction; E-MEM, Eagle's Minimum Essential Media; CPE, cytopathic effect.

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Fig. 1. Distribution of human metapneumovirus RT-PCR+ specimens from patients with ages 0–9 years old.

Fig. 2.

Neighbor joining tree computed from alignment of partial nucleotide sequences of the hMPV G protein gene using the MEGA 2 program (University of Pittsburgh, Pittsburgh, PA). Sequences obtained from hMPV isolates in this study (GenBank accession numbers are listed in Table 2) are compared to prototypic Canadian viruses: HMPV-13-00 (genotype A1), Can97-83 (genotype A2), HMPV-33-01 (genotype B1), and CAN98-75 (genotype B2), GenBank accession numbers AY485232, AY485253, AY485242, and AY485245, respectively.

Table 1
Oligonucleotide primers used for amplification and sequencing of hMPV

Primer set name	Target	Amplicon size (bp)	Sequence
F1 (Peret et al., 2002)	F gene	450	Forward: 5'-CTTTGGAAGTAAATGACAGATG-3'; Reverse: 5'-GTCTTCCTGTGCTAACTTTG-3'
F2 (Falsey et al., 2003)	F gene	347	Forward: 5'- GAGCAAATTGAAAATCCCAGACA-3'; Reverse: 5'- GAAACTGCCGCACAACATTAG-3'
N1 (Mackay et al., 2003)	N gene	210	Forward: 5'- AACCGTGTACTAAGTGATGCACTC-3'; Reverse: 5'-CATTGTTTGACCGGCCCCATAA-3'
G _{univ} (Ludewick et al., 2005)	G gene	800–1000	Forward: 5'- GAGAACATTCGRRCRATAGAYATG-3'; Reverse: 5'- AGATAGACATTRACAGTGGATTCA-3'

Table 2

Demographics and clinical features of patients RT-PCR positive for human metapneumovirus (hMPV)

Case	Date (month/year)	hMPV isolate, genotype (Peret et al., 2004), GenBank accession number	Race	Gender	Age (year)	Days in hospital	Co-infection ^a	Reason for assessment (diagnosis)	Other diagnoses of interest
1	March 2002	—	Caucasian	F	54.7	14	—	Obstructive bronchitis without exacerbation	Heart replaced by transplant
2	March 2002	IA3-2002, A1, DQ312444	Caucasian	M	0.6	8	—	Congestive heart failure, unspecified	Congenital mitral insufficiency
3	March 2002	—	Asian	F	4.6	3	—	Bronchitis, not specified as acute or chronic	
4	March 2002	—	Caucasian	F	8.9	2	—	Pneumonia, organism unspecified	Congenital cytomegalovirus infection
5	December 2002	IA4-2002, B1, DQ312445	Caucasian	F	6.3	3	—	Fever	Acute lymphoid leukemia in remission; anemia, unspecified
6	December 2002	IA5-2002, B1, DQ312446	Caucasian	F	5.8	—	—	Osteopetrosis	Bone marrow transplant
7	January 2003	—	Caucasian	F	78.5	10	—	Obstructive bronchitis with (acute) exacerbation	Respiratory failure
8	January 2003	IA6-2003, B1, DQ312447	Caucasian	F	9.5	—	—	Chronic myeloid leukemia without mention of remission	
9	January 2003	—	Caucasian	M	2.7	2	—	Upper respiratory infection (acute), unspecified site	Chronic respiratory disease arising in the perinatal period
10	January 2003	IA7-2003, B1, DQ312448	Caucasian	M	17.8	9	—	Respiratory failure	Congenital quadriplegic
11	January 2003	—	Caucasian	M	3.5	6	—	Respiratory failure	Chronic respiratory disease arising in the perinatal period; dependence on respirator; pulmonary collapse, other chronic pulmonary heart disease
12	January 2003	—	African American	M	0.8	1	—	Acute bronchiolitis due to other infectious organisms	Pulmonary collapse, congenital anomaly of lung unspecified
13	January 2003	IA8-2003, A2, DQ312449	African American	F	2.7	3	—	Pneumonia, organism unspecified	Prader-Willi syndrome
14	January 2003	IA9-2003, B1, DQ312450	Caucasian	F	5.4	5	—	Bacteremia (micrococcus species)	Acute lymphoid leukemia without remission, agranulocytosis
15	January 2003	IA10-2003, A1, DQ312451	Caucasian	F	22.8	8	—	Respiratory failure	Acute renal failure; systemic lupus erythematosus; nephritis and nephropathy, not specified as acute or chronic, in diseases classified elsewhere
16	January 2003	IA11-2003, B1, DQ312452	Caucasian	F	18.6	—	—	Influenza with other respiratory manifestations	Pneumonia, organism unspecified; agenesis of lung
17	January 2003	—	Caucasian	F	0.8	11	—	Respiratory failure	
18	February 2003	IA13-2003, B1, DQ312454	Unknown	F	0.9	—	—	Cough	
19	February 2003	IA14-2003, A2, DQ312455	Caucasian	M	57.7	18	—	Pneumonia due to streptococcus group A	
20 ^b	February 2003	—	Caucasian	M	61.4	—	—	Myeloid leukemia	Pneumonia; aspergillosis
21	February 2003	IA15-2003, A1, DQ312456	Caucasian	F	0.3	20	—	Acute bronchiolitis due to other infectious organisms	Tetralogy of fallot; infundibular pulmonic stenosis; ostium secundum type atrial septal defect
22	February 2003	IA16-2003, B2, DQ312457	Caucasian	F	6.3	4	Influenza B	Other specified aplastic anemias	Acute lymphoid leukemia in remission
23	February 2003	IA17-2003, A2, DQ312458	Unknown	F	45.0	—	—	Screening for unspecified condition	
24	February 2003	IA-18-2003, B1, DQ312459	Caucasian	M	3.9	8	—	Unspecified infectious and parasitic diseases	Postinfectious encephalitis; coma
25 ^b	February 2003	—	Caucasian	M	68.7	14	—	Malignant neoplasm lower lobe, bronchus or lung	Pneumococcal pneumonia; abscess of lung; respiratory failure
26	February 2003	—	Caucasian	M	0.1	6	—	Acute bronchiolitis due to respiratory syncytial virus ^c	Pulmonary collapse; respiratory failure

Case	Date (month/ year)	hMPV isolate, genotype (Peret et al., 2004), GenBank accession number	Race	Gender	Age (year)	Days in hospital	Co- infection ^a	Reason for assessment (diagnosis)	Other diagnoses of interest
27	March 2003	—	Unknown	M	1.4	—	RSV	Acute bronchiolitis due to respiratory syncytial virus	Chronic maxillary sinusitis
28	March 2003	IA19-2003, B2, DQ312460	Caucasian	M	3.9	13	—	Respiratory failure	Hypertrophic obstructive cardiomyopathy; complications of transplanted liver
29	March 2003	—	Caucasian	F	30.6	17	—	Other pulmonary insufficiency, not elsewhere classified	Other shock without mention of trauma; pneumonia, organism unspecified; anoxic brain damage
30	April 2003	IA20-2003, B1, DQ312461	Caucasian	M	4.3	45	—	Acute lymphoid leukemia without remission	Other specified aplastic anemias; bacteremia; pseudomonas;
31	January 2004	IA21-2003, A2, DQ312462	Unknown	F	4.4	3	—	Fever	hyperpotassemia; other pulmonary insufficiency, not elsewhere classified; hypopotassemia; abnormal liver scan Acute lymphoid leukemia in remission; other diseases of nasal cavity and sinuses; agranulocytosis; Other nonspecific abnormal serum enzyme levels
32	February 2004	IA22-2003, A2, DQ312463	Unknown	M	10.2	6	—	Other specified aplastic anemias	Hypopotassemia; Hodgkin's disease, unspecified
33	March 2004	IA23-2004, A2, DQ312464	Hispanic	M	1.2	1	—	Foreign body in esophagus	Food/vomit pneumonia; pulmonary insufficiency following trauma and surgery
34	March 2004	IA24-2004, B2, DQ312465	Unknown	F	2.1	8	—	Epilepsy, grand mal status	Pneumonitis due to inhalation of food or vomitus; periventricular leukomalacia; unspecified otitis media; esophageal reflux

Diagnoses were recorded using the International Classification of Diseases, Ninth Revision.

^a Another virus detected in the same culture specimen where hMPV was detected.

^b Patient died.

^c Not confirmed by laboratory microbiological tests.

Table 3

Characteristics of clinical specimens tested for human metapneumovirus in comparison to other respiratory viruses, without duplicates^a

Risk factor	Number of specimens (%)					
	Total sample (n = 1294)	Human metapneumovirus- virus-positive (n = 34)	Respiratory syncytial virus-positive (n = 174)	Parainfluenza virus- positive (n = 22)	Influenza virus- ^b positive (n = 120)	Adenovirus-positive (n = 11)
Age group (year)						
<0.4	288 (22.3)	2 (5.9)	59 (33.9)	3 (13.6)	5 (4.2)	0 (0.0)
0.4–9	415 (32.1)	21 (61.8)	97 (55.8)	17 (77.3)	50 (41.7)	6 (54.6)
10–40	261 (20.2)	5 (14.7)	7 (4)	0 (0.0)	41 (34.2)	2 (18.2)
>40	329 (25.4)	6 (17.7)	10 (5.8)	2 (9.1)	24 (20)	3 (27.3)
Unknown	1 (0.1)	0 (0.0)	1 (0.6)	0 (0.0)	0 (0.0)	0 (0.0)
Gender						
Female	621 (48)	19 (55.9)	78 (44.8)	11 (50)	61 (50.8)	5 (45.5)
Male	673 (52)	15 (44.1)	96 (55.2)	11 (50)	59 (49.2)	6 (54.6)
Race/ethnicity						
Caucasian	864 (66.8)	24 (70.6)	108 (62.1)	13 (59.1)	69 (57.5)	7 (63.6)
African American	68 (5.3)	2 (5.9)	9 (5.2)		3 (2.5)	1 (9.1)
Hispanic	45 (3.5)	1 (2.9)	5 (2.9)	1 (4.6)	8 (6.7)	0 (0.0)
Asian	27 (2.1)	1 (2.9)	3 (1.7)	1 (4.6)	4 (3.3)	0 (0.0)
Others ^c	43 (3.4)	0 (0.0)	6 (3.5)	0 (0.0)	0 (0.0)	0 (0.0)
Unknown	247 (19.1)	6 (17.7)	43 (24.7)	7 (31.8)	36 (30)	3 (27.3)
Patient group						
Intensive care unit	223 (17.2)	9 (26.5)	16 (9.2)	5 (22.7)	4 (3.3)	0 (0.0)
Observation ^d	70 (5.4)	2 (5.9)	8 (4.6)	1 (4.6)	2 (1.7)	0 (0.0)
Hospital ward	607 (46.9)	16 (47.1)	73 (42)	12 (54.6)	32 (26.7)	5 (45.5)
Outpatient	394 (30.5)	7 (20.6)	77 (44.3)	4 (18.2)	82 (68.3)	6 (54.6)
Month						
January–March	759 (58.7)	31 (91.2)	144 (82.8)	5 (22.7)	69 (57.5)	9 (81.8)
April–June	116 (9.0)	1 (2.9)	13 (7.5)	4 (18.2)	0 (0.0)	0 (0.0)
July–September	54 (4.2)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
October–December	365 (28.2)	2 (5.9)	17 (9.8)	9 (40.9)	51 (42.5)	2 (18.2)
Culture sites						
Nasopharyngeal wash	11 (0.9)	1 (2.9)	1 (0.6)	0 (0.0)	1 (0.8)	0 (0.0)
Nasal wash	972 (75.1)	24 (70.6)	164 (94.3)	16 (72.7)	111 (92.5)	10 (90.9)
Bronch wash	30 (2.3)	1 (2.9)	0 (0.0)	1 (4.6)	0 (0.0)	0 (0.0)
Bronchoalveolar lavage	135 (10.4)	2 (5.9)	2 (1.2)	0 (0.0)	1 (0.8)	1 (9.1)
Tracheal aspirate	101 (7.8)	6 (17.7)	7 (4)	5 (22.7)	2 (1.7)	0 (0.0)
Others	45 (3.5)	0 (0.0)	0 (0.0)	0 (0.0)	5 (4.2)	0 (0.0)

^aDuplicates were defined as multiple specimens with the same laboratory results taken from the same patient during a 2-week period.

^bInfluenza A, influenza B or both.

^cThe other race/ethnicity category includes Native Americans.

^dPatients held for observation but not hospitalized.

Table 4

Prevalence and odds ratio of RT-PCR positivity for human metapneumovirus by risk factor, without duplicates^a

Risk factor	Crude prevalence %hMPV PCR+ (95% CI)	Bivariate analyses OR (95% CI)	Multivariable logistic regression OR (95% CI)
Age group (year)			
<0.4	0.7 (0.1–2.5)	Reference	Reference
0.4–9	5.1 (3.2–7.6)	7.6 (1.8–32.8)	8.9 (2.0–38.5)
10–40	1.9 (0.6–4.4)	2.8 (0.5–14.5)	3.2 (0.6–16.9)
>40	1.8 (0.7–3.9)	2.7 (0.5–13.3)	2.7 (0.5–13.4)
Gender			
Female	3.1 (1.9–4.7)	1.4 (2.7–0)	—
Male	2.2 (1.3–3.7)	Reference	—
Patient group			
Intensive care unit	96 (1.9–7.5)	2.3 (0.9–6.3)	3.2 (1.1–8.7)
Observation ^b	2.9 (0–6.8)	1.6 (0.3–8)	1.9 (0.4–9.3)
Hospital ward	2.6 (1.5–4.3)	1.5 (0.6–3.7)	1.9 (0.8–4.6)
Outpatient	1.8 (0.7–3.6)	Reference	Reference

Due to missing data the total counts per risk factor are not identical. OR: odds ratio; CI: confidence interval.

^aDuplicates were defined as multiple specimens with the same laboratory results taken from the same patient during a 2-week period.

^bPatients held for observation but not hospitalized.