A Cluster of Patients Infected With I221V Influenza B Virus Variants With Reduced Oseltamivir Susceptibility—North Carolina and South Carolina, 2010–2011

Shikha Garg,1,2 Zack Moore,3 Nicole Lee,3 John McKenna,2 Amber Bishop,2 Aaron Fleischauer,3 Chasisis B. Springs,4 Ha T. Nguyen,2 Tiffany G. Sheu,2 Katrina Sleeman,2 Lyn Finelli,2 Larisa Gubareva,2 and Alicia M. Fry2

1Epidemic Intelligence Service and 2Influenza Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, Georgia; 3North Carolina Department of Health and Human Services, Raleigh; and 4South Carolina Department of Health and Environmental Control, Columbia

Background. During 2010–2011, influenza B viruses with a novel neuraminidase substitution, denoted I221V (B/I221V), associated with reduced in vitro oseltamivir susceptibility were detected in North Carolina.

Methods. We determined the prevalence of I221V among B viruses submitted to the Centers for Disease Control and Prevention for antiviral resistance surveillance, including all B viruses submitted to North Carolina and South Carolina state laboratories, during October 2010–September 2011. We conducted chart reviews and telephone interviews to characterize North Carolina and South Carolina patients with B/I221V vs wild-type B virus infection (B/WT).

Results. We detected I221V in 45 (22%) of 209 B viruses from North Carolina and 8 (10%) of 82 B viruses from South Carolina. We detected I221V in 3 (0.3%) of 881 B viruses tested from 45 other states. B/I221V infection was not associated with differences in underlying conditions or illness severity, compared with B/WT infection. No patients with B/I221V infection received oseltamivir prior to specimen collection. Among patients who completed oseltamivir, those with B/I221V infection reported a longer duration until illness resolution (5 vs 3 days; P = .02).

Conclusions. B/I221V cocirculated with B/WT in North Carolina and South Carolina during 2010–2011. I221V did not alter illness severity but may have reduced oseltamivir effectiveness. Thus, global surveillance for I221V is important.

Keywords. influenza B virus; neuraminidase substitution; oseltamivir; antiviral resistance.

Influenza B viruses cause annual epidemics and can be associated with severe disease resulting in hospitalizations and deaths [1–3]. Neuraminidase (NA) inhibitors (NAIs), including oseltamivir and zanamivir, are the only class of antiviral agents currently licensed to treat influenza B virus infections. The Centers for Disease Control and Prevention (CDC) and the World Health Organization Global Influenza Surveillance program conduct year-round surveillance to monitor for susceptibility of circulating influenza viruses to antiviral agents in the United States and globally.

During the 2010–2011 influenza season, through routine surveillance, the CDC influenza laboratory identified a cluster of influenza B viruses from North Carolina with an elevated median inhibitory concentration (IC50) to oseltamivir on a functional NA inhibition (NI) assay, interpreted as reduced susceptibility to oseltamivir, when compared with reference influenza B viruses [4]. All influenza B viruses in this cluster were found by sequencing to have a novel NA substitution, denoted I221V [4].
Prior to this cluster, influenza B viruses with NA substitutions associated with reduced oseltamivir susceptibility had been rarely described [5–11]. We sought to characterize the epidemiologic and clinical characteristics of patients infected with influenza B viruses with the I221V substitution (B/I221V) as compared to patients infected with influenza B viruses without the I221V substitution (B/wild-type). We further sought to evaluate the clinical effectiveness of oseltamivir among patients infected with B/I221V viruses.

METHODS

Laboratory Testing
During the 2010–2011 influenza season, states were requested to send a sample of 5 influenza viruses, representing a mixture of circulating types and subtypes, to the CDC every 2 weeks for routine antiviral resistance surveillance. During routine drug susceptibility testing, several influenza B viruses from North Carolina were found in vitro assays to have reduced oseltamivir susceptibility. When sequenced, a novel mutation, I221V (ATC → GTA), consisting of an isoleucine (I) to valine (V) substitution at position 221 (B NA numbering corresponds to 222 in the N2 NA amino acid numbering) was detected in a conserved residue of the NA active site of these influenza B viruses [4]. After the cluster of B/I221V viruses was identified [4], we requested that all influenza B viruses submitted to North Carolina and South Carolina state public health laboratories be sent to the CDC for expanded B/I221V testing. In addition, all virus isolates submitted to the CDC for antiviral resistance surveillance from 44 other state public health laboratories were tested for this marker by conventional sequencing and/or pyrosequencing [4, 9].

Epidemiologic Investigation
An epidemiologic investigation was initiated in North Carolina and South Carolina, the states where B/I221V viruses were identified. Information on age, sex, specimen collection date, and county of residence was obtained from laboratory requisition forms of all patients with influenza B virus infections who had clinical specimens submitted to state public health laboratories in North Carolina and South Carolina during October 2010–March 2011. Healthcare providers who submitted clinical specimens that were subsequently tested for B/I221V were asked to complete standardized medical record abstraction forms that included information on past medical history, details of the influenza illness at the time of the clinic visit, prescribed treatment, and any clinical outcomes that were known. Standardized telephone interviews were also attempted for all patients or for their parent or guardian, for whom contact information was available. Patients were asked details about their influenza illness, potential exposures related to their influenza illness, and whether they completed oseltamivir treatment. Patient surveys were translated into Spanish, and all interviews were conducted in Spanish for patients who spoke Spanish as their primary language. All telephone interviews were conducting in March 2011. Calendars and medical office visit dates were used as memory aids during the interview.

The CDC determined that this investigation represented a public health response and did not require institutional review board authorization. All patients who were contacted as part of the investigation were given the opportunity to decline the interview.

Data Analysis
We used t tests and Wilcoxon rank sum tests, for continuous variables, and χ² tests and Fisher exact tests, for categorical variables, to compare clinical and epidemiologic characteristics between patients infected with B/I221V and B/wild-type viruses. Among a subset of patients who reported completing a course of oseltamivir treatment, we also compared treatment effectiveness between patients with B/I221V infection and those with B/wild-type infection. Many factors potentially biased receipt and completion of antiviral treatment, including severity of illness, age, time since illness onset, socioeconomic factors, and underlying conditions [12]. Since we did not collect all of these data systematically and had a small sample, we did not attempt to compare antiviral treatment effectiveness between treated and untreated patients. We assumed these biases did not affect comparison of clinical outcomes between treated patients with B/I221V and those with B/wild-type infection, because clinicians were unaware of I221V status. A 2-tailed P value of ≤ .05 was considered statistically significant. Analyses were performed in SAS, version 9.2 (Cary, NC).

RESULTS

Prevalence of B/I221V Viruses
During October 2010–September 2011, the prevalence of I221V among influenza B viruses from patients who had clinical specimens submitted for surveillance was 22% (45 of 209) in North Carolina and 10% (8 of 82) in South Carolina. The I221V substitution was also detected in 3 (0.3%) of 881 B viruses tested from 45 other states; this includes 1 virus (6%) among 17 tested from Florida and 2 viruses (2.2%) among 91 tested from New York. The individual from Florida had no epidemiologic links or travel to North Carolina or South Carolina, and epidemiologic links were not identified for the 2 individuals from New York. Within North Carolina and South Carolina, most B/I221V viruses were detected from an area surrounding Charlotte, North Carolina, near the North Carolina/South Carolina border (Figure 1). B/I221V was detected in clinical specimens from residents of 15 counties and
accounted for >40% of influenza B virus infections in 7 of these counties.

All B/I221V viruses were of the Victoria lineage and were antigenically similar to the influenza B virus strain contained within the 2010–2011 influenza vaccine [4]. In a previous publication, we reported that phylogenetic analysis showed that the B/I221V viruses from North Carolina formed a distinct cluster within the NA gene of Victoria lineage B viruses; no such cluster was observed in the HA gene [4]. Furthermore, no additional mutations were exclusively or consistently observed in the NA gene of the B/I221V viruses. Use of the fluorescent NA inhibition assay revealed that the mean oseltamivir IC50 of the B/I221V viruses (19.86 nM) was 6-fold greater than that of the CDC reference wild-type oseltamivir-susceptible influenza B viruses (3.25 nM) and 2-fold higher than that of the B/wild-type influenza viruses circulating in North Carolina and South Carolina during 2010–2011 (8.33 nM) [4]. Virologic details are described fully elsewhere [4].
B/I221V was first detected in an influenza B virus from a North Carolina patient on 10 November 2010 and was last detected in an influenza B virus from a South Carolina patient on 28 February 2011 (Figure 2). There was some clustering of B/I221V-infected patients among students at a college in North Carolina (4 persons infected during 24 January 2011–27 January 2011; 2 persons infected during 7 February 2011–9 February 2011) and a college in South Carolina (3 persons infected during 21 February 2011–28 February 2011).

Nationally, during the 2010–2011 influenza season, 74% of circulating influenza viruses were influenza A viruses, and 26% were influenza B viruses. While the proportion of circulating viruses in South Carolina that were influenza B (32%) was similar to the national estimate, the proportion of influenza viruses in North Carolina that were influenza B (42%) was higher than seen nationally [13]. Among 742 influenza B viruses tested nationally, 699 (94%) belonged to the B/Victoria lineage, and 43 (6%) belonged to the B/Yamagata lineage. The distribution of B/Victoria vs B/Yamagata viruses in North Carolina and South Carolina was similar to that seen nationally [13]. Peak influenza-like activity was higher than the national peak (4.5%) for both South Carolina (7%) and North Carolina (6%) during the 2010–2011 season.

Epidemiologic and Clinical Characteristics of Patients With Influenza B Virus Infections

The North Carolina and South Carolina state public health laboratories identified influenza B virus in clinical specimens from 302 patients during the 2010–2011 influenza season. Specimens from 291 (96%) of these patients were tested by conventional sequencing and/or pyrosequencing for I221V. Of these 291 patients, we sought to conduct medical record reviews and telephone interviews for 233 (80%) patients. In South Carolina, of 82 patients tested for I221V, we sought to interview 24 patients from counties in South Carolina where B/I221V was known to be circulating, because of limited resources. Medical record reviews and telephone surveys were completed for 168 patients (72%; 149 from North Carolina and 19 from South Carolina). Medical records were used in lieu of telephone interviews for patients who had cognitive dysfunction (n = 2) or were deceased (n = 7).

There were no significant differences in age, sex, or underlying medical conditions of patients infected with influenza B viruses with and those infected with influenza B virus without I221V (Table 1). The majority of patients with influenza B virus infections were younger than 30 years and enrolled in prekindergarten–fourth grade (38%) or college (53%). A significantly higher proportion of patients with B/I221V infection spoke only Spanish, compared with patients infected with B/wild-type viruses (33% vs 20%; P ≤ .05).

No patients infected with influenza B viruses with or without I221V had received oseltamivir for prophylaxis or treatment prior to collection of their specimen for influenza testing (Table 1). A small number of patients in both groups reported exposure prior to specimen collection to a household contact who was receiving oseltamivir. About half of the patients in both groups reported exposure to a sick contact at school or work. Nine patients with B/I221V infection were college...
students at a single university in North Carolina, and 5 were college students at a single university in South Carolina. These patients had no other epidemiologic exposures in common. Two patients from South Carolina had traveled prior to illness onset to a North Carolina county in which B/I221V was circulating; one was found to be infected with a B/I221V virus.

The median number of days from illness onset to influenza specimen collection was 2 days for patients infected with influenza B virus with and those infected with influenza B virus without I221V (Table 2). Among all patients, 94% took over-the-counter antipyretic medications to treat their symptoms. There were no significant differences in the proportion of patients infected with B/I221V virus as compared to B/wild-type virus who had fever, cough, sore throat, or other symptoms associated with their infection. There were also no significant differences in the proportion of patients who missed school or work as a result of illness, required additional office visits, were hospitalized as a result of their illness, or died.

### Effectiveness of Oseltamivir

On the basis of data obtained from patient interviews, 14 of 38 patients (37%) with B/I221V infection and 75 of 128 patients (59%) with B/wild-type infection confirmed starting
oseltamivir treatment for their influenza episode. Among patients who started oseltamivir treatment and with available data on oseltamivir completion, 12 of 14 (86%) with B/I221V infection and 61 of 75 (81%) with B/wild-type infection reported completing the entire course of oseltamivir treatment (P = .70).

Among the 12 patients with B/I221V infections and 61 patients with B/wild-type infections who completed oseltamivir treatment, a similar proportion took antipyretic medications for their illness (Table 3). Among patients who completed oseltamivir treatment, those with B/I221V infection reported significantly longer intervals between the date of their initial physician visit and the time they felt better and the time they felt like themselves, compared with patients with B/wild-type infection. The duration of several clinical symptoms was longer and more days of work or school were missed among patients with B/I221V infection; however, no values were significantly different from those for patients with B/wild-type infection. In contrast, among patients who did not receive oseltamivir treatment, the duration of most symptoms and illness did not differ between patients with B/I221V and B/wild-type infections, except for days of school or work missed. Among the 73 patients who completed oseltamivir treatment, 64 (88%) had information available on timing of oseltamivir treatment, and all reported starting oseltamivir on the same day as their initial physician visit. Patients who reported completing only partial treatment with oseltamivir (duration, 1–4 days) were excluded.

**DISCUSSION**

We describe the epidemiologic and clinical characteristics of a large cluster of individuals infected with influenza B viruses with reduced oseltamivir susceptibility and a novel I221V NA substitution. B/I221V viruses cocirculated with wild-type influenza B viruses in 1 geographic region in the United States throughout the 2010–2011 influenza season. These B/I221V viruses circulated in the absence of drug pressure. We found no evidence to suggest that B/I221V viruses behaved differently than B/wild-type viruses with respect to populations affected or illness severity Although the number of oseltamivir-treated patients in our analysis was small, our data suggest that oseltamivir may have had less effect on reducing illness among those infected with B viruses with the I221V substitution.

This is the first cluster of influenza B viruses with an NA substitution and reduced oseltamivir susceptibility to be reported. Prior reports have described the prevalence of influenza B viruses with substitutions at the 221 residue to be rare [6, 10]. Amino acid 221 is a highly conserved residue of the NA enzyme active site. I221T has been reported sporadically from 2011 belonged to the Victoria lineage, while 88 (58%) belong to the Yamagata lineage [13].

### Update for the 2011–2012 Influenza Season

During 1 October 2011–31 March 2012, none of the 132 influenza B viruses tested from 41 states for antiviral resistance were found to have the I221V mutation, including 3 influenza B viruses tested from North Carolina. Only 64 (42%) of the 152 influenza B viruses tested during 2010–2011 belonged to the Victoria lineage, while 88 (58%) belong to the Yamagata lineage [13].

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Completed Oseltamivir Treatment</th>
<th>Did Not Receive Oseltamivir Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patients With B/I221V (n = 12)</td>
<td>Patients With B/Wild-Type (n = 61)</td>
</tr>
<tr>
<td>Antipyretic use, no.</td>
<td>10 (91)</td>
<td>49 (92)</td>
</tr>
<tr>
<td>Fever duration, d</td>
<td>5 (2–13)</td>
<td>3 (1–10)</td>
</tr>
<tr>
<td>Cough duration, d</td>
<td>7 (2–21)</td>
<td>6 (2–30)</td>
</tr>
<tr>
<td>Sore throat duration, d</td>
<td>4 (2–7)</td>
<td>3 (1–14)</td>
</tr>
<tr>
<td>Chills duration, d</td>
<td>4 (2–7)</td>
<td>3 (1–14)</td>
</tr>
<tr>
<td>Myalgias duration, d</td>
<td>5 (3–10)</td>
<td>3 (1–14)</td>
</tr>
<tr>
<td>Extra medical visits, no.</td>
<td>4 (33)</td>
<td>13 (21)</td>
</tr>
<tr>
<td>Hospitalization, no.</td>
<td>2 (17)</td>
<td>9 (15)</td>
</tr>
<tr>
<td>Death, no.</td>
<td>0</td>
<td>3 (6)</td>
</tr>
<tr>
<td>School/work time missed, d</td>
<td>5 (3–7)</td>
<td>4 (1–14)</td>
</tr>
<tr>
<td>Time until feeling better, d</td>
<td>5 (3–12)</td>
<td>3 (1–14)</td>
</tr>
<tr>
<td>Time until feeling like self, d</td>
<td>9 (7–20)</td>
<td>7 (2–21)</td>
</tr>
</tbody>
</table>

Data are no. (%) of subjects or median value (range).
through global antiviral resistance surveillance [7, 9, 11, 14, 15] and have been reported rarely among influenza B virus specimens from patients after receipt of NAi therapy [5, 6, 8]. Although it is not possible to know whether B/I221V viruses initially emerged spontaneously or in relation to drug pressure, the lack of exposure to oseltamivir prior to specimen collection among patients infected with B/I221V viruses suggests that the substitution may not have developed in response to oseltamivir use. In vitro studies and animal models have found that NA substitutions may affect NA activity and compromise the ability of the virus to infect individuals and cause disease. [16, 17]. However, our investigation suggests that the presence of the I221V substitution did not compromise the ability of influenza B viruses to cause illness or to be transmitted efficiently within populations. Furthermore, B/I221V viruses infected people with similar demographic characteristics and caused similar illness as wild-type influenza B viruses.

In vitro functional NA assays revealed that all B/I221V viruses had an elevated IC50 to oseltamivir, compared with reference viruses [4]. Notably, wild-type, oseltamivir-susceptible influenza B viruses have a higher baseline IC50 for oseltamivir than do influenza A viruses [14, 18, 19]. Several studies have questioned whether the higher baseline IC50 values translate into altered drug effectiveness [20–22]. A few observational studies have suggested that oseltamivir may reduce fever quicker in patients with influenza A virus infections, compared with patients with influenza B virus infections [20–22]. Thus, a NA substitution, such as I221V, that increased the IC50 in influenza B viruses even higher might be clinically important. Our findings suggest that patients with uncomplicated illness due to B/I221V infection may not have responded to oseltamivir treatment as effectively as patients with B/wild-type virus infection, as evidenced by a longer duration from initiation of antiviral therapy to illness resolution. A trend toward a longer duration of several clinical outcomes suggests these findings may not be spurious. However, we cannot exclude the possibility of random effects from multiple comparisons in a small sample, and we were not able to compare treated and untreated patients, since we did not collect information on biases related to antiviral receipt. Although we were not able to assess the effectiveness of oseltamivir treatment for severe B/I221V infection, our findings suggest that oseltamivir may not be optimal treatment for severe infections due to B/I221V. Because of the high prevalence of B/I221V at the time of the investigation and the uncertain clinical significance of the in vitro finding of reduced oseltamivir susceptibility, the North Carolina Department of Health and Human Services alerted clinicians about the cluster and advised them to consider this information when treating patients with severe influenza B virus infections who did not appear to be responding to oseltamivir. Intravenous zanamivir, an investigational agent, was recommended as an alternative treatment.

Influenza B viruses can be associated with severe disease [1–3], and in this investigation, a total of 26 patients with influenza B virus infection were hospitalized as a result of their influenza episode, 10 patients required intensive care unit admission, and 7 patients died. Although numbers were small, there were no significant differences in the proportion of patients with B/I221V vs B/wild-type who were hospitalized, required intensive care unit admission, or died. In accordance with guidelines, all individuals >6 months of age should receive influenza vaccination annually to prevent complications associated with influenza virus infection [23, 24].

This investigation had several limitations. The number of influenza specimens submitted for surveillance varied by state and county, and several counties submitted no specimens; thus, the prevalence of B/I221V viruses could have been higher and the circulation more widespread than we describe. A higher proportion of patients infected with B/I221V spoke only Spanish. Thus, it is possible that there were epidemiological links that we were unable to identify during the investigation and that there may have been more localized circulation of B/I221V. Clinical illness information was collected retrospectively after resolution of illness, and patient memory regarding certain clinical details may have declined over time; however, we used calendars and medical office visit dates as memory aids and medical record data to corroborate patient information. Finally, given the small sample size, there was limited power to detect clinically significant differences in illness course and outcomes among patients infected with influenza B viruses with and without I221V.

Influenza B viruses with reduced oseltamivir susceptibility and a novel NA substitution cocirculated with wild-type influenza B viruses in a limited geographic area within the United States during the 2010–2011 influenza season. As of 31 March 2012, no B/I221V viruses were detected during the 2011–2012 influenza season, although circulation of Victoria-like B viruses was low. Thus, it appears unlikely that influenza B viruses with the I221V substitution will circulate more widely in the near future. However, the ability of B/I221V viruses to be transmitted within the community, their unaltered ability to cause human illness, and the possible effect of the NA substitution on the clinical effectiveness of oseltamivir, make any future widespread circulation of B/I221V viruses concerning. Thus, surveillance for NA substitutions at the 221 residue should be included in global surveillance for antiviral resistance among influenza B viruses.

**Supplementary Data**

Supplementary materials are available at The Journal of Infectious Diseases online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary
data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

**Notes**

**Acknowledgments.** We thank the residents of North Carolina and South Carolina, as well as the county health departments in North Carolina and South Carolina, for their assistance with this investigation. We also thank the following individuals for their contributions: N. Janine Dailey Garnes, Susan Kilpatrick, and Peggy Brantley (North Carolina Department of Health and Human Services, Raleigh, NC); Jennifer Meredith (South Carolina Department of Health and Environmental Control (Columbia, SC); Matthew Biggerstaff and Krista Kniss (Infection Division, National Center for Immunization and Respiratory Diseases, Centers for Disease Control and Prevention, Atlanta, GA); Rob Garmin (Tennessee Department of Health, Nashville, TN); and Kirstin St George and Jennifer La Plante (New York State Department of Health, Albany, NY).

**Disclaimer.** The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

**Financial support.** This work was supported by the Centers for Disease Control and Prevention.

**Potential conflicts of interest.** All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

**References**