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Comparison of Rapid Detection Methods for Influenza A Virus and Their Value in Health-Care Management of Institutionalized Geriatric Patients

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Respiratory specimens from 160 geriatric patients with suspected influenza illness were used to evaluate the abilities of two enzyme immunoassays (EIAs; Directigen FLU-A [Becton Dickinson Microbiology Systems, Cockeysville, Md.] and Prima EIA [Baxter/Bartels Diagnostics, Inc., Issaquah, Wash.]) and direct immunofluorescence testing (immunofluorescence assay [IFA]) to identify influenza A virus. In comparison with culture isolation, the sensitivities and specificities of the IFA, Directigen FLU-A, and Prima EIA were 92.5 and 97.2%, 86.8 and 99.1%, and 92.5 and 98.1%, respectively. In contrast to EIA, IFA was labor intensive and required a high degree of technical expertise, and the results of IFA were difficult to interpret. These factors may preclude the use of IFA for testing large numbers of specimens. A retrospective epidemiologic survey of influenza infection was done in six geriatric institutions which had used either rapid and culture testing or culture alone. Preventable cases of influenza A virus infection ranged from 9 to 38% of all cases in facilities which used culture testing only and which had not instituted amantadine prophylaxis. The use of direct specimen testing is recommended as an adjunct to culture isolation for the identification of influenza A virus. Use of a combination of these methods permits the timely administration of appropriate antiviral therapy and infection control measures, while it also permits the antigenic surveillance of circulating influenza strains, which is necessary for present vaccine efficacy evaluations and the creation of future effective vaccine formulations.

Age-associated declines in both humoral and cell-mediated immunity contribute to the increased susceptibility of elderly people to infection (5). Residents of long-term-care facilities are particularly burdened because infection may exacerbate existing chronic medical problems, and the potential for nosocomial transmission increases the risk of infection. Acute infection may also facilitate the recrudescence of formerly acquired pathogens (herpes simplex virus, cytomegalovirus, varicella-zoster virus) or permit a secondary bacterial infection to occur.

Viruses are important causes of acute respiratory infections in nursing facilities during the winter months (5). Estimates of the total number of excess cases of influenza-associated mortality from 1972 and 1973 through 1980 and 1981 were reported to be 200,000, with an 80 to 90% occurrence rate found among people older than 64 years (10). Respiratory syncytial virus has also been associated with high rates of morbidity and mortality in nursing home outbreaks (5, 14).

The rapid identification of respiratory viruses is invaluable to the health care management of the institutionalized elderly by indicating the appropriateness of prophylactic and symptomatic antiviral therapies, eliminating the unnecessary use of antibiotics, and determining the proper regimen of infection control (11–13).

Cell culture is a highly sensitive and specific method that has traditionally been used to identify influenza virus. Unfortunately, the time period required for culture isolation and identification can range between 2 and 10 days, thereby limiting its impact on patient care. Alternatively, shell vial culture techniques allow results within 2 to 3 days (4, 11).

In contrast to culture techniques, rapid direct specimen testing of influenza A virus by immunofluorescence assay (IFA) and enzyme immunoassay (EIA) methods can produce same-day results (4, 6, 7, 15, 17). A number of studies have compared these methods with culture isolation by using a variety of specimen sources in pediatric (15, 17) and mixed-age (4, 6, 7) populations.

In the investigation described here, we examined the performances of a monoclonal IFA (Baxter Diagnostics) and two EIAs (Directigen FLU-A [Becton Dickinson Microbiology Systems, Cockeysville, Md.] and Prima Influenza A EIA [Baxter/Bartels Diagnostics, Inc., Issaquah, Wash.]) to identify influenza A virus in a population of symptomatic institutionalized geriatric patients. The efficacies of rapid methods on patient-care management were also evaluated.

MATERIALS AND METHODS

Study population. Specimens were obtained from residents of 24 geriatric-care centers in south eastern New York State during the 1992 and 1993 winter season. These patients demonstrated one or more signs or symptoms of flu-like illness, including abrupt onset of fever, sore throat, nonproductive cough, headache, myalgia, and malaise (3). Nursing staff members were advised of the proper means of specimen collection and transport (9), including the completion of a

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viral history form containing demographic and clinical information for each patient.

Specimen collection and handling. Nasopharyngeal and throat specimens were obtained by vigorously swabbing these sites with dacron-tipped swabs. Both swabs were placed into a single vial of viral transport medium (Hank's balanced salt solution, 10 μg of gentamicin per ml, 4 μg of amphotericin B per ml, 0.5% gelatin, sodium bicarbonate [pH 7.2]). Generally, patient materials were received and processed within 4 h of collection. Vials were vortexed, and a 0.6-ml aliquot for rapid testing was obtained prior to additional treatment of specimens with antibiotics.

Culture techniques. Aliquots of each specimen (0.2 ml) were adsorbed onto monolayers of two Rhesus monkey kidney and human lung carcinoma (A549) cell cultures (Whittaker Bioproducts, Walkersville, Md.) for 30 min. Following replacement of maintenance medium (minimal essential medium with Earle's salts, L-glutamine, 10 μg of gentamicin per ml, 4 μg of amphotericin B per ml, 15 mM HEPES [*N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid], 9.5 mM sodium bicarbonate), cultures were incubated at 33 to 34°C for 14 days. Cultures were examined for a cytopathic effect every other day. Hemadsorption testing was performed at 7 and 14 days postinoculation by using guinea pig erythrocytes (0.5%; Whittaker Bioproducts) suspended in phosphate-buffered saline (PBS) as described previously (15). Hemadsorption and cytopathic effect-positive specimens were confirmed by fluorescent-antibody techniques (8). Hemagglutination inhibition techniques (16) were used to identify subtypes of influenza A virus, and selected positive isolates were forwarded to the World Health Organization Influenza Control Branch (Centers for Disease Control and Prevention, Atlanta, Ga.) for further antigenic determination.

Direct specimen procedures. Specimen material was centrifuged at 1,000 × *g* for 10 min, and the resulting pellet was washed in PBS. After repeating this procedure, the pellet was resuspended in 0.4 ml of PBS and equally apportioned (125 μl) for each direct specimen test.

One aliquot was placed onto Teflon-coated microscope slide wells and air dried. Following fixation in cold acetone, the specimens in the slide wells were stained with a purified mouse monoclonal antibody directed against influenza A and an anti-mouse immunoglobulin G $F(ab')_2$ fluorescein isothiocyanate conjugate (Baxter/Bartels Diagnostics, Inc.) according to the procedures described by the manufacturer. Slides were examined at a ×400 magnification by using a Nikon episcopic microscope equipped with a 460- to 490-nm excitation filter (Nikon Inc., Garden City, N.Y.). The presence of at least two cells with apple green fluorescent nuclei and irregularly sized cytoplasmic inclusions was considered a positive result. Specimens containing fewer than 30 cells were excluded from the study.

Two EIAs were evaluated. The Directigen FLU-A (Becton Dickinson Microbiology Systems) is an enzyme immunomembrane filter assay which detects influenza A nucleoprotein extracted from nasopharyngeal washes, aspirates, or swabs. The appearance of a purple triangle (of any intensity) was considered a positive result, whereas the appearance of a purple control dot was considered a negative result.

The Prima influenza A EIA (Baxter/Bartels Diagnostics, Inc.) uses microwells coated with anti-influenza A virus monoclonal antibodies to selectively capture influenza A virus. Positive, negative, and indeterminate results were determined by comparative analysis of colorimetric absorbance values (by using a plate reader at an A_{450}) for patient specimens and negative controls. For the purpose of the present study, samples with indeterminate results were repeat tested if possible or were considered negative. Each assay was performed according to the manufacturer's instructions.

Sensitivity, specificity, and positive and negative predictive values were calculated for the direct specimen tests by using culture results as the "gold standard." In addition, seven culture-negative specimens which were positive by all three rapid tests were considered true positives.

Epidemiologic survey of geriatric institutions. Epidemiologic data were gathered from six skilled-nursing-care facilities in New York State (three sites used a combination of rapid and culture testing, three sites used culture alone) which had self-reported, laboratory-confirmed influenza outbreaks. Data from the first three facilities that agreed to participate in rapid and culture testing were chosen for analysis. Facilities that used culture testing alone were self-selected.

Our analysis compared initial attack rates (the point that a facility identified an increased incidence of influenza-like illness above endemic rates) and final attack rates (the point of culmination of influenza-like illness) by the following formula: number of cases of influenza-like illness/patient census) × 100. The projected attack rate (initial attack rate + rate of new cases within the next 48 h) and the percentage of preventable cases (final attack rate − projected attack rate) were then hypothetically calculated for the facilities which used culture testing only. These hypothetical values are based on the assumption that rapid testing would have permitted timely reporting of results (within 24 h), would have allowed the prophylactic administration of amantadine, and subsequently, would have prevented any further cases in the outbreak after 48 h (1).

RESULTS

Specimens from 160 institutionalized elderly patients (mean age, 84.1 ± 8.5 years; median age, 86 years; male: female ratio, 1:2.9) with symptoms of influenza-like respiratory illness were evaluated. Reported symptoms for 144 of these patients included a documented fever of greater than 100°F (37.8°C) (92.4%), cough (60.4%), rhinorrhea (25.0%) headache (13.9%), and myalgia (7.6%). Pneumonia was reported in five patients.

A comparison of direct specimen methods with culture isolation methods is summarized in Table 1. The sensitivities and specificities of the IFA, Directigen FLU-A, and Prima EIA tests were 92.5 and 97.2%, 86.8 and 99.1%, and 92.5 and 98.1%, respectively. The IFA technique generated the highest number of false-positive results (three specimens) among the products that tested specimens directly. Instances of multiple false-positive results by different rapid tests on the same specimen were not observed. The Directigen test generated the highest number of false-negative results (seven specimens); however, for four of these specimens, IFA and Prima EIA also produced false-negative results.

Cell culture identified influenza A/H3N2 virus in 46 specimens. Selected viral isolates forwarded to the World Health Organization Collaborating Center for Influenza were further characterized as A/Beijing/32/92-like (eight isolates) and A/Washington/15/91-like (one isolate) agents. The A/Beijing/32/92-like isolates demonstrated significant antigenic variation from the A/Beijing/353 virus contained in the 1992 and 1993 trivalent influenza vaccine. Other isolated viruses included influenza type B (four patients), herpes simplex virus

TABLE 1. Comparison of direct specimen testing with tube culture isolation for the identification of influenza type A in symptomatic, institutionalized geriatric patients

Assay[a]	No. of specimens[b]				Percent[c]			
	TP	TN	FP	FN	Sensitivity	Specificity	PPV	NPV
IFA	49	104	3	4	92.5	97.2	94.2	96.3
Directigen	46	106	1	7	86.8	99.1	97.8	93.8
Prima EIA	49	105	2	4	92.5	98.1	96.1	96.3
Culture	46	107	0	7[d]	86.8	100.0	100.0	93.9

[a] Directigen (Becton Dickinson) and Prima (Baxter/Bartels Diagnostics) are membrane disk and 96 well-plate EIAs, respectively.

[b] A total of 160 specimens (nasopharyngeal and throat) were examined. TP, true positive; TN, true negative; FP, false positive; FN, false negative.

[c] Sensitivity = number of true-positive-specimens/(number of true-positive specimens + number of false-negative specimens) × 100; specificity = number of true-negative specimens/(number of true-negative specimens + number of false-positive specimens) × 100; PPV, positive predictive value; NPV, negative predictive value.

[d] Seven specimens which were culture negative but positive by all three rapid tests were considered to be true positives. Patients who provided three of these specimens began amantadine treatment prior to specimen collection.

type 1 (three patients), and respiratory syncytial virus (two patients). Both influenza type A and herpes simplex virus type 1 were isolated from two patients.

In seven specimens, influenza A virus was identified by all direct tests but failed to be isolated in culture (the gold standard). For three specimens, the results may be attributable to the fact that amantadine hydrochloride was administered prior to specimen collection, thereby inhibiting viral growth in culture. Findings for the other four specimens remain unclear. For the purposes of rapid testing evaluation, the culture results were "corrected" and all seven specimens were considered positive.

Among the tests that directly tested the specimen, IFA was labor intensive and required a high degree of technical expertise, and the results of IFA were the most difficult to interpret. Identification was also impeded by the presence of nonspecific fluorescent cellular staining, fluorescent debris, and overlapping cells. In contrast, these difficulties were not encountered in the EIA.

Epidemiologic data for nursing facilities obtained by different diagnostic regimens and the hypothetic calculation of the projected attack rate and preventable morbidity in facilities that used culture testing alone are summarized in Fig. 1 and Table 2. Influenza outbreaks varied by the point at which the facility identified the outbreak, with initial attack rates ranging from 7 to 26%. In one facility (site 1), deviation from the standard infection curves suggested that the initial cases of influenza were probably not identified. Although the initial attack rates were higher in facilities that used rapid and culture testing (ranges of 10 to 26% versus 7 to 17%), the final attack rates were greater in facilities that used culture testing alone (ranges of 26 to 58% versus 10 to 36%); this is probably because these facilities chose not to institute amantadine prophylaxis. Nursing facilities that used a rapid detection and culture regimen obtained results in less than 24 h after specimen collection. Positive cultures were reported for facilities that used culture alone (sites 1 to 3) at 5, 8, and 6 days postcollection, respectively.

The value of timely infection control measures was suggested from the experience of one facility (Fig. 1, site 4) because patient isolation (prior to reporting of diagnostic results) combined with amantadine prophylaxis readily controlled the spread of disease.

The use of projected attack rates allowed us to obtain estimates of preventable morbidity. These hypothetical values ranged from 9 to 38% for facilities which used culture diagnostic testing only.

DISCUSSION

The seasonal circulation of influenza A virus has historically been responsible for excess cases of pneumonia and mortality among elderly people (2). Prevention of influenza in nursing facilities is primarily accomplished by the yearly vaccination of both residents and staff. Although vaccination is recommended by state and federal agencies, a high rate of vaccine nonacceptance among staff has been demonstrated (13, 18).

The effective formulation of these vaccines requires the continued surveillance of the circulating influenza strains, thereby mandating the use of isolation methods. In the 1992 and 1993 winter influenza season, surveillance efforts allowed the identification of a variant strain [A/Beijing/32/92 (H3N2)] and its subsequent incorporation into the 1993 and 1994 influenza vaccine (2).

In situations of influenza A outbreaks, the use of amantadine and the institution of appropriate infection control measures can limit nosocomial disease spread (1). The effectiveness of these strategies depends on their timely implementation, thereby necessitating rapid and accurate viral diagnosis. The ability of IFA and EIA methods to perform this function was investigated. While we did not conduct a controlled study (in that nursing homes self-selected the use of culture or rapid testing protocols, made their own decisions about the use of amantadine, and may have had different rates of vaccination of staff and patients against influenza), our data suggest that a significant number of influenza cases could be prevented if a rapid diagnosis of influenza was available and acted on by the medical staff.

The sensitivity and specificity of direct specimen testing by IFA and EIA were comparable to those of the culture isolation method. The process of centrifugation (which was done in the present study because the gelatin found in the collection medium was not acceptable for the Prima EIA) may have slightly reduced the sensitivity of the EIA products, because these assays can also detect cell-free antigen in respiratory secretions.

IFA was labor intensive and required the greatest technical expertise, and the results of IFA were often difficult to interpret. These findings, previously reported in other reports of studies that compared rapid diagnostic methods (7, 11), may preclude the use of IFA when examining large numbers of specimens.

Little difficulty was experienced in the operation of the EIAs, and both EIAs allowed batch testing of low numbers of specimens (breakaway well strip format in the Prima EIA). Use of the Directigen FLU-A product required no instrumentation and the product provided results in less than 15 min. Although the Prima EIA product required the use of instrumentation and a longer period of time was required to obtain results (approximately 2 h), it offered an objective, interpretable result (numerical values versus a color change) and demonstrated a slightly greater sensitivity than that of the Directigen FLU-A EIA (92.5 versus 86.8%). Other studies have reported sensitivity values of 62 and 100% for Directigen FLU-A EIA when this product was compared with culture isolation (7, 17).

For diagnostic testing, nasopharyngeal specimens obtained via aspiration and washing are preferred over swab

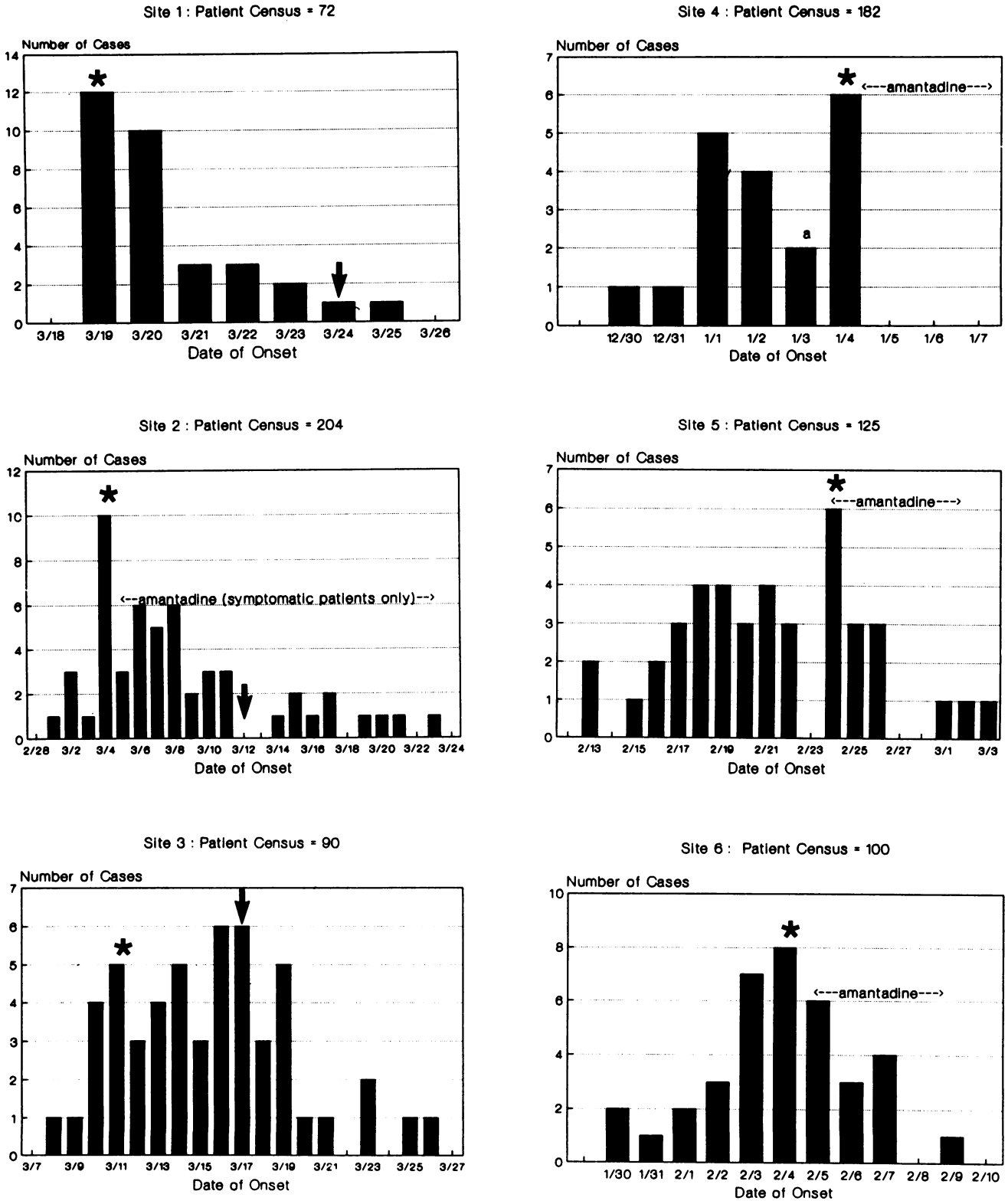

FIG. 1. Epidemiologic comparison of influenza A virus outbreaks in nursing facilities by culture (sites 1 to 3) and by culture and direct testing of specimens (sites 4 to 6). In both diagnostic regimens, the date of testing is indicated by an asterisk. For facilities that used culture alone, laboratory result reporting occurred at times indicated by the arrows. Results of rapid testing were reported within 24 h of specimen collection. Patient isolation prior to testing occurred in one facility (a).

TABLE 2. Comparison of illness in patients with symptoms of influenza in geriatric institutions that used culture alone versus those that used culture and rapid testing methods

Test and site no.	No. of patients	Percent			
		Initial attack rate	Final attack rate	Projected attack rate	Preventable cases of illness
Culture alone					
Site 1	72	17	44	35	9
Site 2	204	7	26	12	14
Site 3	90	12	58	20	38
Culture and rapid tested					
Site 4	182	10	10		
Site 5	125	26	33		
Site 6	100	23	37		

specimens. With respect to our specialized patient population, the latter method was chosen for several reasons. Concern over the potential for aspiration pneumonia was expressed because both bacterial colonization and a reduced "gag reflex" are often demonstrated in this patient group. The staff at the nursing facilities were unfamiliar with aspiration and were unequipped to aspirate specimens. Swabs were considered easier to obtain, because collection of specimens from elderly patients is often difficult. Throat and nasopharyngeal specimens were combined in one vial in order to maximize the potential number of respiratory viruses recoverable in culture while minimizing the monetary costs associated with testing of multiple specimens.

While not endorsing any particular product, the value of combined rapid and culture testing for influenza A virus is strongly suggested by the lower final attack rates observed among the nursing facilities that used that regimen. Although many factors contribute to infection control (i.e., level of staffing, physical environment, patient isolation), the value of the appropriate use of amantadine therapy in patients infected with influenza A virus is well documented (1). Direct specimen testing allows for rapid reporting of results, thereby permitting timely, appropriate amantadine prophylaxis. Subsequently, morbidity and mortality from influenza A virus infection among institutionalized elderly individuals may be reduced. Importantly, this combined regimen also permits the continued antigenic surveillance of circulating influenza strains, which is necessary for testing vaccine efficacy and formulating effective vaccines in the future.

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