

Frequency and Effects of Bacterial Infection in Children with Influenza Under Oseltamivir Treatment

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Abstract

Background: New antiviral drugs can rapidly improve the symptoms of influenza, but some patients still have prolonged fever and complications. The cause of the prolonged symptoms despite antiviral therapy remains unclear. Recent studies have shown a synergistic effect between influenza viruses and bacteria. This study investigated the frequency of bacterial infection in such patients and its effects on the clinical course to determine the need for antibiotics.

Methods: In two seasons (December 2001 through March 2002, and December 2002 through March 2003), throat cultures were obtained from 387 patients with influenza being treated with oseltamivir, and clinical courses were observed. Control throat cultures were obtained from 109 healthy children.

Results: The detection rate of pathogens was higher in patients with influenza (54.3%) than in control (23.9%, $p < 0.001$). The most common pathogen was *Streptococcus pneumoniae* (49.7%) in patients with influenza and was *Haemophilus influenzae* (69.2%) in controls. Of the patients with normal flora, 4.1% had fever for 4 or more days and showed pathogens in throat cultures on day 4. Of the patients with pathogen-positive cultures who did not receive antibiotics, 40.3% had fever for 4 or more days.

Conclusion: Throat cultures obtained on the first or fourth day of treatment with oseltamivir were positive for pathogenic bacteria in all patients with fevers for 4 or more days. Our observations suggest that patients with influenza and prolonged fever despite receiving oseltamivir should be given antibiotics.

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Key words: influenza, bacteria, oseltamivir, superinfection, antibiotics

Introduction

Influenza viral infection increases the risk of hospitalization in infants and young children¹ and

sometimes leads to bacterial superinfections with associated complications and accounts for morbidity and mortality². Animal models and molecular microbiologic studies provide evidence for understanding the synergistic effects between

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influenza viruses and bacteria³⁻¹⁰. These studies suggest that some strains of *Staphylococcus* and *Streptococcus*³ and some protease-producing bacteria may increase viral replication and pathogenicity⁴. However, influenza viruses can directly damage the respiratory epithelium^{5,6} and cause bacterial invasion. Recent studies have shown that influenza A viral infection also induces immunosuppression, which may cause bacterial superinfection^{5,7,8,10}.

Influenza viral infection can now be confirmed with rapid diagnostic kits¹¹, and early administration of antiviral agents rapidly relieves symptoms¹². There have been no reports on the risk and frequency of bacterial superinfection with such therapy. The purpose of this study was to examine the frequency and effects of bacterial infection in children with influenza being treated with oseltamivir and to determine the necessity of antibiotics.

Materials and Methods

Study Design

To detect bacteria in the throats of patients, cultures were obtained by swabbing the posterior pharynx with a rayon-tipped swab at the same time that the influenza viral infection was diagnosed with nasal swabbing. Microorganisms were identified by standard throat culture with 5% sheep's blood containing trypticase-soy agar and polyvitex containing chocolate agar (BBL Becton Dickinson Microbiology Systems, USA) incubated at 37°C in a 5% CO₂ incubator. Susceptibility to antibiotics was determined with the use of Kirby-Bauer disk diffusion test (BBL Becton Dickinson Microbiology Systems).

Patients received oseltamivir (4 mg/kg/day) for 5 days in two divided daily doses. The parents recorded the children's body temperature four times a day until the symptoms were resolved. Patients followed up every 2 to 4 days and compliance was confirmed. The patients with symptoms and throat cultures positive for pathogenic bacteria were given antibiotics, according to the sensitivity test of the isolates at the second visit, for 3 or 4 days.

The second cultures were obtained from the

patients with normal flora if the fever lasted for 4 or more days after the start of treatment with oseltamivir.

Study Population

Infection with influenza A or influenza B viruses was diagnosed with rapid diagnostic kits (Flu AB Quick, Denka Co., Japan) in 625 patients at the pediatric department of Nissan Tamagawa Hospital during 2001/02 and 2002/03 influenza seasons. Patients were eligible for the study if, at presentation to the hospital, the duration of their fever ($\geq 37.5^{\circ}\text{C}$) was less than 48 hours and they had not received any medication for 1 week. Patients were not eligible if they were younger than 1 year or had received influenza vaccines for the current season. Patients with any congenital or chronic diseases were also excluded. The parents were given a thorough explanation of the purpose of the study, and informed consent was obtained.

Control Subjects

Throat swabs for culture were obtained from 109 healthy children at the Minobusan Nursery School near the hospital in November 2001. Children who had chronic illnesses or had been treated with antibiotics were excluded. Written informed consent was obtained from the parents of the children.

Statistical Analysis

Data are expressed as means \pm SD. We performed a one-way factorial analysis of variance for repeated measurements of variance followed by Fisher's protected least-significant difference for comparison among means of several groups and Student's *t*-test for comparison between two groups. For comparison of category data we performed the χ^2 test. We considered differences significant at $p < 0.05$. All analyses were performed with SPSS 11.0J software (SPSS Inc. USA).

Results

Of 625 patients with influenza, 387 were eligible for the study. Patients with influenza A were younger than patients with influenza B, but the

Table 1 Characteristics of study patients and throat cultures at presentation

		Number of children	Sex (male/female)	Age (years)	Body temperature (°C)
Patients	Influenza A	189	98/91	6.9 ± 4.1	38.9 ± 0.9
	Influenza B	198	96/102	7.9 ± 3.4	38.7 ± 0.6
	p value		0.575	0.008	0.829
Throat cultures	Normal flora	177	86/91	7.5 ± 3.8	38.5 ± 1.0
	Pathogenic bacteria	210	108/102	7.3 ± 3.9	38.8 ± 0.6
	p value		0.92	0.61	0.76

Data are number of children or mean ± SD. p values were determined by paired t tests.

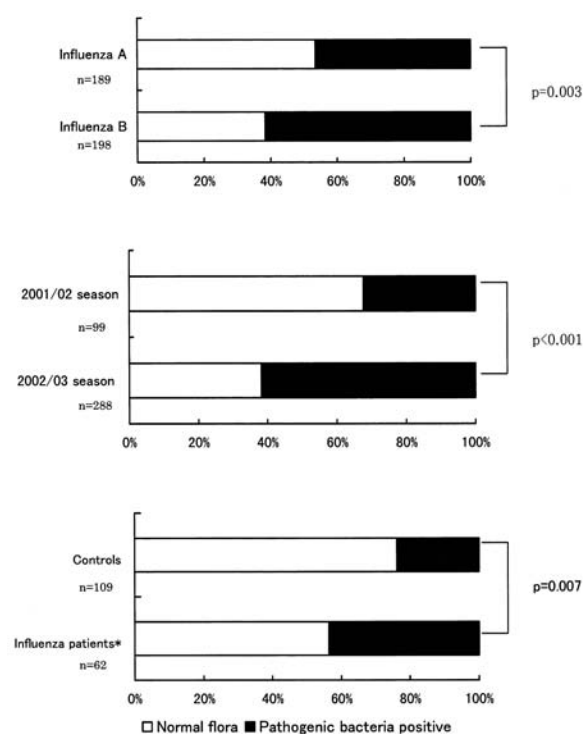


Fig. 1 Throat cultures at the first presentation to the hospital. Controls were healthy children aged 1 to 6 years in November 2001. Influenza patients* were aged 1 to 6 years in the 2001/02 season. P values were determined with the χ^2 tests for categorical variables.

male/female ratio and body temperature at the first hospital presentation were not different (Table 1).

Throat Culture

Of 387 patients, 210 (54.3%) had pathogens on throat cultures and 177 (45.7%) had normal flora. Age and body temperature did not differ between these groups (Table 1). The detection rate of pathogens was higher in patients with influenza B (61.6%) than in patients with influenza A (46.6%, $p=$

0.003). Pathogens were more frequently detected in the 2002/03 season (61.8%) than in the 2001/02 season (32.3%, $p<0.001$; Fig. 1).

A total of 286 pathogenic bacteria were isolated from 210 patients. Half of the isolates were *Streptococcus pneumoniae* (49.7%, Fig. 2). The isolates were different between patients with influenza A and those with influenza B and also between the 2001/02 season and the 2002/03 season (Fig. 2).

The pathogenic bacteria groups did not differ significantly in age, except for the penicillin-resistant *S. pneumoniae* (PRSP) group, which was younger than the other groups (Table 2).

Comparison with Controls

A total of 109 throat cultures were obtained from healthy children (controls) in November 2001. For comparison with controls, 62 patients aged 1 to 6 years with influenza in the 2001/02 season were chosen to minimize the confounding effects of age and season. The characteristics of these two groups did not differ significantly: the age of controls and influenza patients were 4.1 ± 1.5 and 3.8 ± 1.8 years, respectively, and the male/female ratios were 56/53 and 35/27, respectively. Of the 109 controls, 26 (23.9%) had pathogens and 83 (76.1%) had normal flora. The detection rate of pathogens was lower than that in selected patients with influenza (43.5% (27 of 62), $p=0.007$) (Fig. 1). The most common isolate was *Haemophilus influenzae* (69.2%) in controls and *S. pneumoniae* (33.3%) in selected patients with influenza. The isolates of these two groups were significantly different ($p<0.001$, Fig. 2).

Bacterial Infection in Children with Influenza

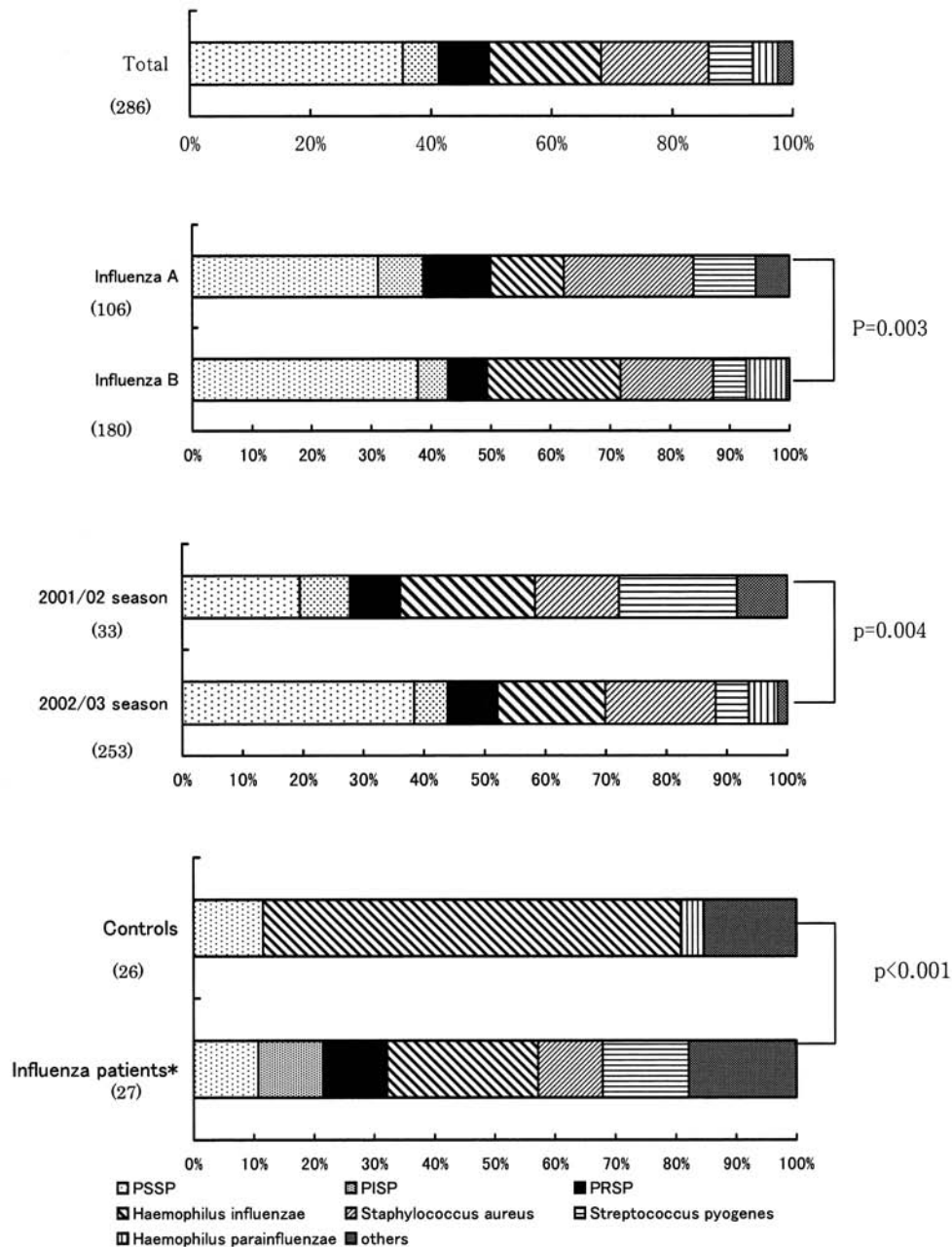


Fig. 2 Isolates from throat cultures. (), number of isolates. Controls were healthy children aged 1 to 6 years in November 2001. Influenza patients* were aged 1 to 6 years in the 2001/02 season. P values for differences between groups were determined with the χ^2 test for categorical variables.

Clinical Course

The clinical courses of 339 patients were observed, and the results are summarized in **Figure 3**. The remaining 48 patients (36 with normal flora and 12 with pathogens) did not return to the hospital after the first visit. A total of 147 cultures (77 in patients with influenza A and 70 in patients with influenza B) showed normal flora. The duration of fever after treatment with oseltamivir was 3 or fewer days (1.6

± 0.7 days) in 141 of these patients (95.9%) and was 4 or more days in 6 patients (4.1%). All 6 patients (1 patient with influenza A and 5 patients with influenza B) had second throat cultures positive for pathogenic bacteria on the fourth day.

Of 198 patients with pathogens, 67 had not received antibiotics for 4 days after treatment with oseltamivir. Of these 67 patients, 27 (40.3%) had fevers for 4 or more days and 40 (59.7%) had fevers

Table 2 Age of patients with bacteria-positive throat cultures

Age (years)	<i>S. pneumoniae</i>			<i>H. influenzae</i> n=53	<i>Staphylococcus aureus</i> n=51	<i>Streptococcus pyogenes</i> n=21	<i>Haemophilus Parainfluenzae</i> n=14
	PSSP n=101	PISP n=17	PRSP n=24				
	8.1 ± 3.7	6.0 ± 3.8	3.9 ± 3.1	6.8 ± 3.2	8.3 ± 3.7	8.5 ± 3.8	9.2 ± 4.1

Data are mean ± SD. PSSP, Penicillin-sensitive *S. pneumoniae*; PISP, Penicillin-intermittent *S. pneumoniae*; PRSP, Penicillin-resistant *S. pneumoniae*.

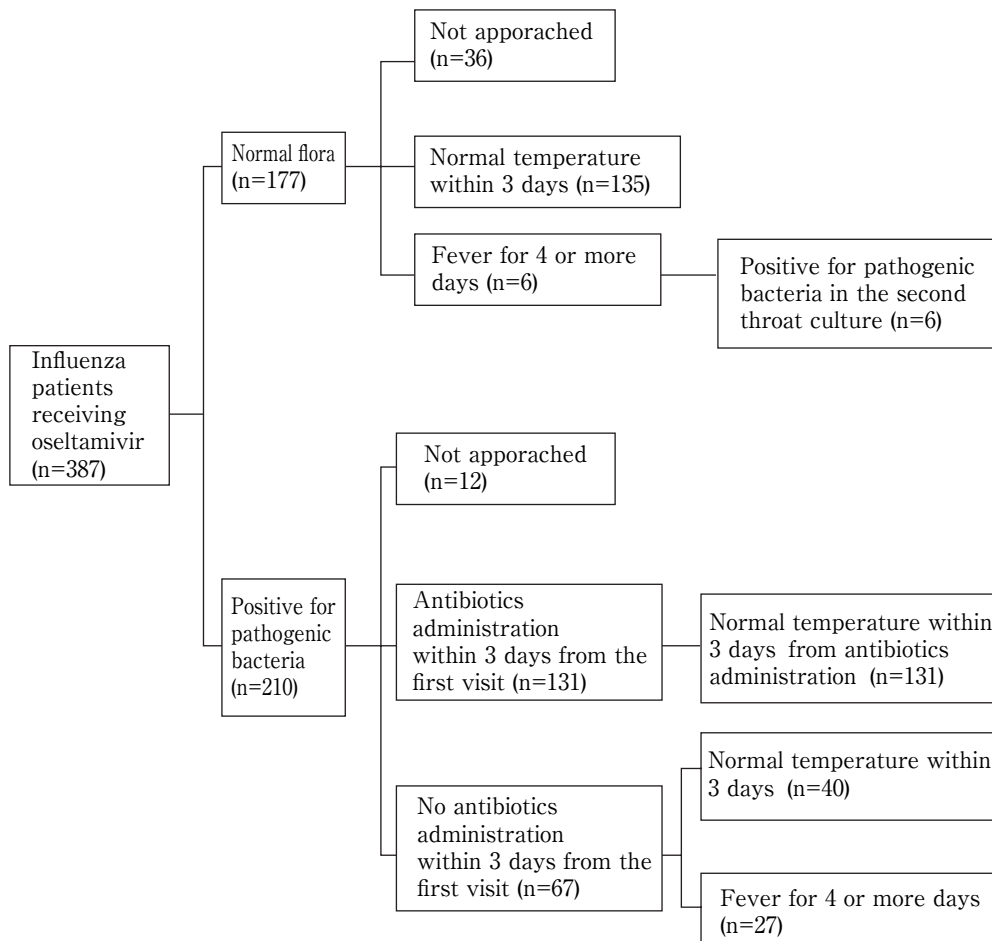


Fig. 3 Clinical course. n, number of patients.

for 3 or fewer days. The remaining 131 patients received antibiotics within 3 days, on the basis of the sensitivity of isolates, and had fevers for 3 or fewer days (2.0 ± 1.1 days). The percentage of the patients with fevers for 4 or more days was significantly higher in patients with pathogenic bacteria who did not receive antibiotics than in patients with normal flora (40.3% vs 4.2%, $p < 0.001$).

None of the patients were hospitalized, although pneumonia developed in one patient with influenza B whose second throat culture revealed PRSP and

H. influenzae infection in spite of normal flora in the first culture.

Discussion

Our examinations revealed that 54.3% of patients with influenza viral infection had throat cultures positive for pathogenic bacteria. This percentage was higher than in healthy children. Of the patients with normal flora, 95.9% had fevers for 3 or fewer days after receiving oseltamivir. The remaining 4.1%

of patients still had fever on the fourth day and had second cultures that were positive for pathogenic bacteria. Of the patients who had cultures positive for pathogenic bacteria and did not receive antibiotics, 59.7% had fevers for 3 or fewer days, but 40.3% had fevers for 4 or more days. These differences in the detection rate of pathogens suggest that pathogenic bacteria in the pharynx may affect the clinical course of patients with influenza.

Official reports of influenza virus showed no marked changes in epidemic strains in these two seasons^{13,14}. AH3N2 were A/Panama/2007/99 and its antigenic variations. AH1N1 were A/New Caledonia. Of the influenza B viruses, 97% were B/Shandong/7/97. The rapid diagnostic kit (Flu AB Quick, Denka Co.) that were used in this study are highly specific (100%) and sensitive (influenza A; 95%, influenza B; 88%) for these viruses¹¹.

There was no difference in the body temperature at the first visit to the hospital between patients with normal flora and those with pathogens. Therefore, it was difficult to predict the bacterial infection and clinical course from early clinical manifestations.

The detection rate of pathogens was higher in patients with influenza B than in patients with influenza A and differed with the season. These differences may result from the prevalent bacteria strains and different immune responses to influenza A and influenza B viruses¹⁵.

In the patients with normal flora, the duration of fever after treatment with oseltamivir was 1.6 ± 0.7 days, which was in accord with other reports¹². Recent studies have found that strains resistant to oseltamivir did not appear easily¹⁶. Therefore, resistant strains may not cause prolonged fevers. The higher detection rate of pathogens suggests an interaction between influenza viruses and bacteria.

We did not obtain blood samples, so it is difficult to know whether bacterial infection was limited to the pharynx or was systemic. However, several studies have reported the synergistic effects of viral and bacterial pathogens³⁻¹⁰.

Potential mechanisms of the effects of influenza viruses include: suppressing the excretory function

of the respiratory tract, destruction and apoptosis of respiratory epithelial cells, which may increase bacterial adhesion^{6,7,9}; virus-induced immunosuppression, such as acceleration of neutrophil apoptosis^{5,10}, which may cause bacterial superinfections; and inflammatory response to viral infection which may up-regulate expression of molecules that bacteria utilize as receptors, especially viral neuraminidase, which exposes pneumococcal receptors on host cells^{5,11}. Indeed *S. pneumoniae* was the bacteria most frequently detected in our study.

Other reports suggest that bacteria promote viral invasion and replication in epithelial cells: proteases from some bacterial strains, streptokinase and staphylokinase, may activate influenza virus hemagglutinin, which facilitates viral invasion and replication^{3,4}.

These studies were performed in vitro, and more studies may be needed to clarify the synergistic effects of viral and bacterial pathogens in vivo.

In conclusion, bacterial superinfection may cause prolonged fevers in patients with influenza treated with oseltamivir, and antibiotics may be required in these patients.

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