ance enables Japanese citizens to consult a physician or to be hospitalized easily with a low financial burden.

Underlying neurological conditions are thought to be an independent risk factor for admission to an ICU. One previous report has suggested that asthma is a risk factor for admission to an ICU.[3] However, asthma was not significantly associated with admission to an ICU in the present study. One possible explanation is that children with asthma are likely to be hospitalized with milder illness, especially in Japan. Our results suggest that health care providers should pay significant attention to hospitalized children with underlying neurological diseases.

The large proportion of subjects with atypical behavior was also a unique characteristic of our study. Atypical behavior, which may have been caused by influenza virus infection or oseltamivir, attracted great public attention in several previous influenza seasons in Japan. This concern may have lowered the clinical thresholds for diagnosing atypical behavior and ensuing hospital admission in Japan below those in other countries. All of the 83 patients with atypical behavior received neuraminidase inhibitors (61 of them received oseltamivir), while 398 of 432 patients (92.1%) without that diagnosis received these medications ($P = 0.008$). However, we cannot discuss a causal relation between the administration of neuraminidase inhibitors and the appearance of atypical behavior, primarily because we could not obtain data on the time elapsed between them.

In addition to that, our regression analyses failed to demonstrate a preventive effect of neuraminidase inhibitors on admission of hospitalized children to the ICU. The timing of administration of these medications was similar in ICU and non-ICU patients ($P = 0.764$). However, we have to take into account the following three important factors in the discussion: (i) a considerably large proportion of subjects received early treatment with these medications; (ii) patients with severe illnesses were more likely to receive these medications; and (iii) we analyzed data only of hospitalized patients. A number of studies have reported the clinical effectiveness of neuraminidase inhibitors in reducing risks with seasonal and pandemic influenza infections in both children and adults.[8,18–26] The results of our regression analyses do not directly indicate the ineffectiveness of neuraminidase inhibitors. Substantially widespread and early administration of neuraminidase inhibitors and early hospitalization should be considered as rather important findings. Such proactive treatments and good access to medical services may have contributed to the lower incidence of severe illness and the lower mortality in Japan.

Our study has several limitations. It is not a population-based study, and the results may have been affected by the selection of participating hospitals. In addition, we could not estimate hospitalization rates from our data, and the effects of differences in judgment on admission to hospital or an ICU between Japan and other countries could not be completely excluded.

In summary, our results reflect the lower mortality rate and lower incidence of severe illness among the 2009 pandemic influenza-virus-infected Japanese children. In addition, our study suggests the possible contribution of good access to medical services and prompt treatment in Japan. However, we could not specify the main factors that contributed to the lower disease burden in Japan. Several possible factors could be considered, including the difference in ethnicity, in addition to the factors we have suggested. Further investigations are necessary to identify the main factors that have contributed to the relatively lower disease burden caused by the 2009 influenza pandemic in Japan. Such studies could provide efficient options for reducing the worldwide burden of influenza.

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Immunogenicity and Reactogenicity of a Monovalent Inactivated 2009 Influenza A Vaccine in Adolescents: With Special Reference to Pre-Existing Antibody

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Objective To evaluate the immunogenicity and reactogenicity of a monovalent 2009 pandemic influenza vaccine in Japanese adolescents.

Study design A total of 111 junior high school and high school students aged 13 to 18 years participated. Subjects received two doses of a monovalent inactivated unadjuvanted 2009 influenza A vaccine. Immunogenicity of the vaccine was evaluated according to the international criteria. We also asked subjects to report adverse reactions.

Results After the first dose of vaccine, the seroprotection rate was 91% (95% CI, 85%-96%), the seroconversion rate was 78% (70%-86%), and the geometric mean titer ratio was 11.9 in all subjects. Antibody titers achieved did not differ significantly after the first and the second doses. With multivariate analysis, an independent negative effect of a prevaccination titer of ≥1:40 on ≥4 fold antibody increase was indicated. No serious adverse reaction was reported.

Conclusion The monovalent pandemic vaccine generally was safe, and a single dose of the vaccine given to adolescents induced sufficient immunity. Pre-existing antibody showed substantial effect on antibody response. The effect of pre-existing titer should be considered when evaluating the immunogenicity of influenza vaccines, especially in studies conducted during pandemic waves. *(J Pediatr 2012;160:632-7).*

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A novel swine-origin influenza A (H1N1), first identified in the United States in April 2009, rapidly spread to many countries around the world, and the World Health Organization declared a pandemic on June 11, 2009.[1] In Japan, the peak of pandemic influenza activity was observed during October and November 2009. However, it was not until October 2009 that monovalent pandemic H1N1 vaccine was developed and distributed for tiered use. Vaccination was scheduled first for health care workers, and then provided to junior high school and high school students from January 2010, according to the order of priority of the groups.

A number of studies have suggested that a single dose of inactivated pandemic vaccine was enough to induce a protective antibody response in adults.[2-5] In contrast, only few studies have evaluated the immunogenicity in adolescents, despite 45% of 2009 H1N1 infection-associated hospitalizations having occurred in patients <18 years of age.[3,5,6] In this study, we evaluated the immunogenicity and reactogenicity of a monovalent H1N1 pandemic vaccine in Japanese adolescents aged 13 to 18 years.

In assessing the immunogenicity, pre-existing antibody can affect the antibody response.[7,8] However, there is no reference to consideration of pre-existing antibody in the conventionally used licensing criteria. We therefore attempted to evaluate the immunogenicity in adolescents, while considering the effect of pre-existing titer, by using the epidemiologic methodologies of stratification and multivariate analysis.

Methods

This study, including recruitment of subjects, vaccination, and serum collection, was conducted during October and November 2009, at 9 pediatric clinics and 3 hospitals in Osaka, Hyogo, and Fukui prefectures, Japan, involving 111 subjects aged 13 to 18 years (junior high school students and high school students). Participating institutions were enlisted through the network of Osaka Pediatric Association. Exclusion criteria included previously confirmed or suspected infection with 2009 H1N1 virus,

GMT	Geometric mean titer
GMTR	Geometric mean titer ratio
H1N1	Influenza A
HAI	Hemagglutination-inhibition
ORS	Oculo-respiratory syndrome

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acute febrile illness, or signs of severe acute illness at the time of vaccination, history of anaphylaxis caused by vaccine components, known or suspected immunosuppressive disease, recent history of immunosuppressive treatment, and other conditions that made vaccination inappropriate. We were required to enroll study subjects as soon as possible to provide data on immunogenicity in adolescents to the Ministry of Health, Labor and Welfare, Japan, mainly because of the urgency to evaluate the necessity for the second dose of the vaccine.

Vaccine

The vaccine administered was a monovalent inactivated unadjuvanted split-virus 2009 H1N1 vaccine (HP01A in 2009, Biken, Osaka, Japan). The seed virus was prepared from reassortant vaccine virus A/California/7/2009 NYMC X-179A (New York Medical College, New York, New York), distributed by the Centers for Disease Control and Prevention in the United States. The vaccine was prepared in embryonated chicken eggs with standard methods used for the production of seasonal trivalent inactivated vaccine. The vaccine was supplied as multidose vials containing 0.0008% thimerosal. Vaccine dose was 0.5 mL, containing 15 μg of hemagglutinin antigen, and two doses were administered subcutaneously 3 weeks apart. We instructed participating institutions not to co-administer seasonal trivalent influenza vaccines. The Ministry of Health, Labor and Welfare, Japan, reserved the vaccine for this study on priority.

Serum Collection and Antibody Titer Measurement

Serum samples were collected before the first dose (S0), 3 weeks after the first dose (S1), and 4 weeks after the second dose (S2). Antibody titer against the vaccine strain was measured with the hemagglutination-inhibition (HAI) assay according to standard methods with chicken erythrocytes.[9,10] Serum samples were treated with receptor destroying enzyme (RDE, Vibrio cholera filtrate, Denka Seiken, Tokyo, Japan) to inactivate nonspecific inhibitors. All samples were assayed at the same time at the laboratory of the Research Foundation for Microbial Diseases of Osaka University.

Survey of Reactogenicity

Subjects and their guardians were asked to record on a questionnaire, after each dose, symptoms of the oculo-respiratory syndrome (ORS) noted within 24 hours, and systemic and local reactions noted within 48 hours. The specified symptoms of the ORS included red eye, facial edema, and respiratory symptoms (cough, wheezing, chest tightness, difficulty breathing, difficulty swallowing, hoarseness, and throat tightness), as in an earlier report.[11] Systemic reactions included fever, fatigue, myalgia or arthralgia, headache, and rash, whereas local reactions included erythema, swelling, induration, itching, and pain. We also asked about medical visits caused by these symptoms.

Statistical Analysis

On the basis of conventional international criteria, these 3 immunogenic end points were selected: seroprotection rate, seroconversion rate, and geometric mean titer ratio (GMTR).[12,13] Seroprotection was defined as an HAI titer of 1:40 or more. Seroconversion was defined as a prevaccination HAI titer <1:10 and a postvaccination HAI titer ≥1:40, or a prevaccination HAI titer ≥1:10 and ≥4-fold antibody increase after vaccination. In addition, we calculated the seroresponse rate, the proportion of subjects who achieved ≥4-fold increase. In this calculation, an HAI titer <1:10 was treated as 1:5, and reciprocal antibody titers were analyzed after logarithmic transformation. The results were presented in the original form by obtaining the antilogarithms.

The data was stratified for analysis by sex, school age (ie, junior high school or high school), and prevaccination titer (ie, <1:10, 1:10-1:20, or ≥1:40). The significance of titer increase by vaccination within a category was assessed with the Wilcoxon signed-rank test, and inter-category comparisons of the geometric mean titer (GMT) or GMTR were made with the Wilcoxon rank sum test or the Kruskal-Wallis test. The χ^2 test, Mantel-extension trend test, and McNemar test were also used when appropriate.

The independent effects of potential predictors, such as prevaccination titer and school age, on the antibody response were evaluated with a logistic regression model. The model was constructed with seroresponse as the dependent variable.

Reactogenicity data were expressed as the number and proportion of subjects who had each symptom. Differences in frequencies after the first and the second doses were evaluated with the McNemar test.

All reported P values are two-sided, and a P value <.05 was considered to be statistically significant. All statistical analyses were performed with SAS software version 9.1 (SAS Institute Inc, Cary, North Carolina).

The study was approved by the ethics committee of the Osaka City University Graduate School of Medicine and conducted in accordance with the principles of the Declaration of Helsinki and Japanese regulatory requirements. Written informed consent was obtained from each subject's legal representative.

Results

Immunogenicity

A total of 111 subjects, 63 junior high school students and 48 high school students, consented to participate in the study. Fourteen of these subjects had underlying illness (10 had asthma, 2 were being treated with growth hormone, 1 had diabetes mellitus, and 1 had epilepsy), but all were in a stable medical condition. Of all the subjects, 5 (3 junior high school and 2 high school students) were confirmed to have H1N1 virus infection with the rapid test between the first and the second doses. Additionally, 4 junior high school students were confirmed with H1N1 virus infection between the second dose and the last serum sampling. After excluding these cases, 106 and 102 subjects provided immunogenicity data for analysis after the first and the second dose, respectively.

GMTs are shown in Table I. Before vaccination, 22 of the subjects (21%) had a seroprotective antibody titer (≥1:40). The first dose of vaccine induced significant titer rise in all

Table I. Antibody response to each dose of vaccine

Category	N	GMT* S0	GMT* S1	GMT* S2‡	GMTR† S1/S0		GMTR† S2/S0‡	
All subjects	106	12	145	147	11.9	$P < .001$	12.1	$P < .001$
Sex								
Male	57	12	154	156	13.3	$P < .001$	13.8	$P < .001$
Female	49	13	135	138	10.5	$P < .001$	10.4	$P < .001$
		$P = .963$	$P = .619$	$P = .400$	$P = .481$		$P = .342$	
School age								
Junior high school	60	10	162	158	15.6	$P < .001$	15.4	$P < .001$
High school	46	15	126	136	8.3	$P < .001$	9.0	$P < .001$
		$P = .132$	$P = .343$	$P = .616$	$P = .031$		$P = .034$	
Prevaccination titer								
<1:10	48	5	102	107	20.5	$P < .001$	21.3	$P < .001$
1:10-1:20	36	12	170	166	13.7	$P < .001$	13.4	$P < .001$
≥1:40	22	83	241	246	2.9	$P < .001$	3.0	$P < .001$
		$P < .001$	$P = .090$	$P = .001$	$P < .001$		$P < .001$	

*Wilcoxon rank sum test or Kruskal-Wallis test.
†Wilcoxon signed-rank test for intra-category, Wilcoxon rank sum test or Kruskal-Wallis test for inter-category comparisons.
‡The analysis population comprised 102 subjects (male, 55; female, 47; junior high school, 56; high school, 46; pre-titer <1:10, 46; pre-titer 1:10-1:20, 35; pre-titer ≥1:40, 21).

subjects (GMTR, 11.9; $P < .001$), but the second dose induced little additional antibody response (post-dose 2 versus pre-dose 1 GMTR, 12.1). GMTRs (S1/S0 and S2/S0) were significantly lower in high school students than junior high school students ($P = .031$ versus $P = .034$). Subjects with higher prevaccination titer also had lower GMTRs (S1/S0 and S2/S0, $P < .001$ for each). There was no significant difference by sex.

Seroprotection rates and seroconversion rates are summarized in Table II. After the first dose, the seroprotection rate was 91% (95% CI, 85%-96%) and the seroconversion rate was 78% (70%-86%) for all subjects. The second dose led to only a small increase in seroprotection and seroconversion rates. Subjects with higher prevaccination titer showed lower seroconversion rate (trend $P < .001$).

These findings suggested that: (1) a single dose of vaccine induced significant antibody response, as reflected in the GMTR, seroprotection rate, and seroconversion rate; and (2) school age and prevaccination titer may have substantially affected antibody response.

In the stratified analysis in which the effects of school age and prevaccination titer were examined (Table III), high school students had a higher proportion of subjects with a prevaccination titer ≥1:40 (13/46, 28%) compared with junior high school students (9/60, 15%). In addition, higher prevaccination titer was significantly associated with lower GMTR in both junior high school and high school students ($P = .004$, $P < .001$). Similarly, prevaccination titer had an inverse dose-response relationship with seroconversion in both the student groups (trend $P = .033$, $P = .031$). The mutual effect of school age and prevaccination titer on seroresponse (S1/S0 ≥4) was examined by using a logistic regression model (Table IV). Compared with junior high school students, high school

Table II. Seroprotection rate and seroconversion rate after each dose of vaccine

Category	Seroprotection: After the first dose n/N (%, 95% CI)	Seroprotection: After the second dose n/N (%, 95% CI)	Seroprotection: P value	Seroconversion: After the first dose* n/N (%, 95% CI)	Seroconversion: After the second dose† n/N (%, 95% CI)	Seroconversion: P value
All subjects	96/106 (91, 85-96)	96/102 (94, 90-99)	.180	83/106 (78, 70-86)	84/102 (82, 75-90)	.103
Sex						
Male	51/57 (89, 82-97)	50/55 (91, 83-99)	.564	46/57 (81, 70-91)	45/55 (82, 72-92)	.564
Female	45/49 (92, 84-100)	46/47 (98, 94-102)	.157	37/49 (76, 63-88)	39/47 (83, 72-94)	.083
	$P = .678$	$P = .136$		$P = .518$	$P = .879$	
School age						
Junior high school	55/60 (92, 84-106)	53/56 (95, 89-101)	.564	50/60 (83, 74-93)	48/56 (86, 77-95)	.564
High school	41/46 (89, 80-98)	43/46 (93, 86-101)	.157	33/46 (72, 58-85)	36/46 (78, 66-90)	.083
	$P = .658$	$P = .804$		$P = .151$	$P = .326$	
Prevaccination titer						
<1:10	40/48 (83, 79-93)	42/46 (91, 83-99)	.180	40/48 (83, 73-93)	42/46 (91, 83-99)	.180
1:10-1:20	34/36 (94, 87-102)	33/35 (94, 87-102)	NA	34/36 (94, 87-102)	33/35 (94, 86-102)	NA
≥1:40	22/22 (100, 100)	21/21 (100, 100)	NA	9/22 (41, 30-51)	9/21 (43, 22-64)	.317
	Trend $P = .018$	Trend $P = .170$		Trend $P = .001$	Trend $P < .001$	

NA, not applicable.
χ^2 test for two categories, Mantel-extension method for the trend test among 3 categories comparisons.
McNemar test for the comparisons between the first and the second dose.
*Seroresponse rates were 83%, 84%, 81%, 87%, 78%, 94%, 94%, and 41% for the 8 listed categories, from top to bottom.
†Seroresponse rates were 84%, 85%, 83%, 88%, 80%, 96%, 94%, and 43% for the 8 listed categories, from top to bottom.

Table III. Stratified immunogenicity analysis by school age and prevaccination titer (after the first dose)

Prevaccination titer	N	Seroprotection* n (%)	Seroconversion* n (%)	GMTR†
Junior high school	60			
<1:10	30	26 (87)	26 (87)	25.9
1:10-1:20	21	20 (95)	20 (95)	13.6
≥1:40	9	9 (100)	4 (44)	4.0
		Trend $P = .151$	Trend $P = .033$	$P = .004$
High school	46			
<1:10	18	14 (78)	14 (78)	13.7
1:10-1:20	15	14 (93)	14 (93)	13.9
≥1:40	13	13 (100)	5 (38)	2.4
		Trend $P = .047$	Trend $P = .031$	$P < .001$

*Mantel-extension method.
†Kruskal-Wallis test.

students had a decreased OR in univariate analysis (0.55), but the OR shifted toward null (0.86) when the effect of prevaccination titer was simultaneously considered. However, a prevaccination titer ≥1:40 indicated decreased OR (0.05) in univariate analysis, and even after adjusting for school age in multivariate analysis.

Additional calculations were performed to elucidate the effect of earlier vaccination history, with 53 study subjects whose data on vaccination status in 2008/2009 season was available (46 vaccinated and 7 unvaccinated). No significant association was observed between an earlier season's vaccination and prevaccination serostatus or any measurement of antibody response against the 2009 pandemic virus or its vaccine (eg, prevaccination GMT was 13 in vaccinated versus 12 in unvaccinated; seroprotection rate and seroconversion rate after the first dose were 91% versus 100% and 78% versus 100%, respectively).

Reactogenicity

Of 111 subjects who received the first dose of vaccine, 109 (98%) completed the questionnaire. One of these 109 subjects was confirmed to have H1N1 infection within 48 hours after vaccination and therefore excluded from the reactogenicity analysis. Of the 106 subjects who received the second dose, 103 (97%) completed the questionnaire.

Six percent and 3% of the subjects reported symptoms of ORS after the first dose and the second dose, respectively. Respiratory symptoms were reported most frequently (Table V; available at www.jpeds.com).

Systemic reactions were reported in 17% (for the first dose) and 7% (for the second dose) of subjects ($P = .008$). Fatigue was the most frequent synptom after the first dose, but it was less frequent after the second dose ($P = .003$). Local reactions were reported by 36% and 34% of the subjects after the first and second doses, respectively. The most frequent local reaction was pain, both after the first dose and the second dose. No medical visit because of adverse reactions was reported.

Discussion

This study showed that the monovalent inactivated pandemic vaccine evaluated was safe and immunogenic in adolescents. A single dose of the vaccine induced sufficient antibody to meet both the international licensing criteria of European Agency for the Evaluation of Medical Products and the US Food and Drug Administration. A second dose did not elicit significant additional increase in antibody titer. GMTs after the second dose were somewhat lower than those after the first dose in some of the stratified groups (junior high school students, $P = .827$; subjects with prevaccination titer of 1:20-1:40, $P = 1.000$), as observed in some earlier studies.[3,5] We also found that subjects with high antibody titer (≥1:320) after the first dose were more likely to experience titer decrease after the second dose (ie, exceeding the range of random variation [$P = .028$], although the decrease observed in no case exceeded the minimum-fold change in the HAI assay). Reduced GMTs seen after the second dose in some groups could be explained by these findings. Both seroprotection and seroconversion tended to have the same or slightly higher rates after the second dose even in these two groups (Table II).

Table IV. Effect of each variable on seroresponse (≥4 fold antibody increase) after the first dose of vaccine

Category	n/N (%)	Univariate OR (95% CI)	Univariate *P* value	Multivariate* OR (95% CI)	Multivariate* *P* value
Sex					
Male	48/57 (84)	1.00			
Female	40/49 (81)	0.83 (0.30-2.30)	.725		
School age					
Junior high school	52/60 (87)	1.00		1.00	
High school	36/46 (78)	0.55 (0.20-1.54)	.257	0.86 (0.25-3.03)	.817
Prevaccination titer					
<1:10	45/48 (93)	1.00		1.00	
1:10-1:20	34/36 (94)	1.13 (0.18-7.16)	.894	1.14 (0.18-7.22)	.889
≥1:40	9/22 (41)	0.05 (0.01-0.20)	<.001	0.05 (0.01-0.21)	<.001
		Trend $P < .001$		Trend $P < .001$	

Logistic regression model.
*Adjusted for school age and prevaccination titer.

Detailed analyses clearly indicated that higher prevaccination titer was associated with lower seroresponse, a phenomenon known as "negative feedback" or "law of initial value."[7,8] In the primary analysis, GMTR was significantly lower in high school students than junior high school students. In stratified analysis (**Table III**) and multivariate analysis (**Table IV**), the independent effect of pre-existing titer on antibody response, but not of school age per se, was revealed. This report specifically documents the effect of pre-existing titer on the parameters of antibody response to a 2009 pandemic influenza vaccine; a similar "negative feedback" association has been reported for seasonal vaccines.[7,8] The effect of pre-existing titer has an important impact on evaluation of influenza vaccines, especially against pandemic strains. Because novel vaccine's potency usually would be examined during a pandemic wave, substantial numbers of study subjects may have had earlier infection. Immunogenicity of the 2009 H1N1 vaccine was high enough, that the results sufficiently met international licensing criteria. This, however, may not be the case with future vaccines for newly identified pandemic strains. It is quite possible that such a study could infer incorrectly the necessity for the second dose of vaccine, despite a single dose eliciting sufficient antibody. We have thus shown the considerable usefulness of epidemiologic methods of assessing immunogenicity while taking the pre-existing antibodies in account.

The effect of pre-existing antibody, however, is not a simple comprehensive suppression of antibody increase, because post-vaccination GMT still was higher in subjects with higher pre-existing titer. The existence of a potential upper limit for the antibody response to the vaccine (ie, a ceiling effect) may be a more appropriate interpretation. Hence, the aforementioned "negative feedback" can be attributed to such a ceiling effect and does not necessarily contradict an "anamnestic response."

The high proportion of subjects with pre-existing titer in this study also is noteworthy. At baseline (October-November 2009), 21% of subjects had a protective level of antibody ($\geq$1:40). This proportion was lower than that in adults in Australia in July 2009 (26.8% during the pandemic period),[2] but higher than that in adults reported in China in July 2009 (4.3%, during the prepandemic period).[3] The differences in those studies might be explained in part by the spread of influenza, because the Australian study and ours were conducted during the peak of the epidemic. Considering our study protocol in which subjects with a history of confirmed or suspected infection were excluded, one possible explanation for this high proportion is subclinical infection. A few studies have shown frequent asymptomatic infection during the pandemic.[14-17] In a Finnish garrison, 50% of subjects with 2009 H1N1 virus-specific antibodies reported no recent history of upper respiratory tract infection.[15] However, the characteristics of that study population were so unusual that they precluded simple comparison with our findings. A household transmission study in China reported that 9.4% of laboratory-confirmed household secondary cases were asymptomatic, and a seroprevalence study in elementary school students in Japan demonstrated that 23.5% to 25.0% of students who had pandemic virus-specific neutralizing antibodies had no symptoms.[16,17] In these studies, the frequencies of asymptomatic infections were expressed as a proportion of those with no earlier influenza episode in laboratory-confirmed infections. However, the frequency of asymptomatic infection defined in our study is the proportion of subjects with high antibody titer in subjects with no earlier episode. Thus, it is difficult to compare frequency data in these studies. Regardless, it is clear that subjects in this study included individuals who had influenza infection that was not detected in the screening and were not a representative sample of the general population.

Another possible explanation for this high prevaccination seroprevalence might be the presence of cross-reactive antibodies. A recent study reported that administration of seasonal vaccine in the earlier 5 seasons was associated with higher prevaccination antibody against 2009 H1N1 virus.[4] However, our additive analysis showed no significant association between influenza vaccination history in the earlier season and prevaccination serostatus.

Possible inaccuracy of the HAI assay should be considered. However, the HAI assay generally is thought to be well established and standardized and does not pose large necessity for considering inter-laboratory variability. Even when low specificity of the HAI assay or other mechanisms, such as earlier infection, unrepresentativeness of the study population, and cross-reactive immunity contributed substantially to finding a high proportion of subjects with pre-existing titer $\geq$1:40, these results showing negative association between pre-existing titer and antibody increase could not be explained fully.

A limitation of our study is the possibility of intercurrent unnoticed infection, because our study was conducted during the peak of a pandemic wave. Using unvaccinated or placebo controls would have overcome this weakness. However, we could not adopt such study designs or rigorous randomization, primarily because of the urgent need to generate data for formulating a national immunization program. The small sample size is another limitation. Although the reported adverse reactions generally were mild, and no medical intervention was needed, we cannot infer from this data the probability of rare but severe adverse events.

The effect of pre-existing titer should be carefully considered when evaluating the immunogenicity of influenza vaccines, especially in studies conducted during pandemic waves. ■

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Table V. Numbers and proportions of subjects with adverse reactions

Symptom	First dose (n = 108) n (%)	Second dose (n = 103) n (%)
ORS		
Any	7 (6)	3 (3)
Red eye	1 (1)	0 (0)
Facial edema	1 (1)	0 (0)
Respiratory symptom*	5 (5)	3 (3)
Systemic reaction		
Any†	18 (17)	7 (7)
Fever‡	0 (0)	0 (0)
Fatigue†	11 (10)	2 (2)
Myalgia/arthralgia	1 (1)	4 (4)
Headache	9 (8)	3 (3)
Rash	1 (1)	0 (0)
Local reaction		
Any	39 (36)	35 (34)
Erythema	15 (14)	11 (11)
Swelling	19 (18)	10 (10)
Induration	9 (8)	8 (8)
Itching	14 (13)	13 (13)
Pain	20 (19)	15 (15)
Medical visits	0 (0)	0 (0)

*Cough, wheezing, chest tightness, difficulty breathing, sore throat, hoarseness, and throat tightness were included.
†$P < .05$ (McNemar test).
‡Defined as body temperature ≥37.5°C.

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Japanese Guidelines for the Management of Respiratory Infectious Diseases in Children 2007 with focus on pneumonia

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Abstract Members of the Japanese Society of Pediatric Pulmonology and the Japanese Society for Pediatric Infectious Diseases developed the *Guidelines for the Management of Respiratory Infectious Diseases in Children* with the objective of facilitating the appropriate diagnosis and treatment of childhood respiratory infections. To date, a first edition (2004) and a revised edition (2007) have been issued. Many problems complicate the diagnosis of the pathogens responsible for bronchopulmonary infections in children. The *Guidelines* were the first pediatric guidelines in the world to recommend treatment with antimicrobials suited to causative pathogens as identified from cultures of sputum and other clinical specimens collected from infection sites and satisfying assessment criteria. The major causative microorganisms for pneumonia in infants and children were revealed to be *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Mycoplasma pneumoniae*. This manuscript describes the *Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007*, with a focus on pneumonia.

Key words appropriate use of antimicrobials, causative microorganism, children, guidelines, respiratory infections.

The *Guidelines for the Management of Respiratory Infectious Diseases in Children* were developed by members of the Japanese Society of Pediatric Pulmonology and the Japanese Society for Pediatric Infectious Diseases to facilitate proper management primarily for pneumonia and other childhood respiratory infections. The first edition[1] was issued in 2004, and a revised edition[2] was released in 2007.

The causative microorganisms of bronchopulmonary infections in children have not been sufficiently examined and assessed either in Japan or in other countries. The *Guidelines* were developed to recommend the appropriate use of antimicrobials for treating respiratory infections based on identification of the causative microorganisms. The *Guidelines* were the first pediatric guidelines in the world to utilize sputum cultures and other clinical specimens from infection sites to identify causative microorganisms. Clinical research has scrutinized the appropriateness of the recommendations in the *Guidelines*, and it is hoped that such scrutiny can improve the appropriateness of the recommended use of antimicrobials in childhood respiratory infections. This manuscript focuses on pneumonia, which is addressed in the 150-page *Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007* that is used in clinical practice in Japan.

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Principles for the development of *Guidelines for the Management of Respiratory Infections in Children*

The *Guidelines* were created with the objectives of: (i) improving the quality of the management and treatment of childhood respiratory infections; and (ii) considering antimicrobial treatment that minimizes the advent of drug-resistant pathogens. The *Guidelines*, which cover childhood respiratory infections, were developed in consideration of age-specific and other characteristics of children.[1] The *Guidelines* are subject to revision when necessitated by trends associated with causative microorganisms, the emergence of resistant pathogens, the occurrence of adverse events, and the development of new drugs. The revised 2007 edition makes more information available about viral infections,

Table 1 Table of contents from *Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007*

Full color photographs and schematics of key findings	
Dosage recommendations for antimicrobials approved for the treatment of pediatric respiratory infections	
Chapter 1	Principles for the development of guidelines for the management of respiratory infections in children
Chapter 2	Concept and classification of childhood respiratory infections
Chapter 3	Characteristics of childhood respiratory infections
Chapter 4	Pathogens of childhood respiratory infections and their detection: Bacteria, mycoplasma, chlamydia, viruses
Chapter 5	Upper respiratory infections: common cold (nasopharyngitis), pharyngitis/tonsillitis, croup syndrome (epiglottitis)
Chapter 6	Bronchitis: Acute bronchitis, protracted bronchitis
Chapter 7	Bronchiolitis: RSV and other viruses
Chapter 8	Pneumonia: Severity criteria, first-line antimicrobial treatment
Chapter 9	Pleurisy and pyothorax: Diagnosis and treatment
Chapter 10	Pneumonia with underlying disease: Blood diseases, immunodeficiency, neonates, heart diseases
Chapter 11	Nosocomial pneumonia: Fundamentals of infection control, infections from hospital environment/medical practice
Chapter 12	Diseases mainly controlled by vaccination: Influenza, measles, pertussis, diphtheria, tuberculosis
Chapter 13	Pathogenic resistance in community-acquired childhood respiratory infections: Pneumococcus; *Haemophilus influenzae, Moraxella catarrhalis*, Group A Streptococcus, *Staphylococcus aureus*, *Mycoplasma pneumoniae*
Appendix	Table 1: List of reagents for rapid diagnosis of pathogenic microorganisms Table 2: Contact details for organizations supporting the national stockpile of vaccines and antitoxins
Appendix	Chest X-rays of pneumonia

addresses pneumonia in children with underlying diseases and nosocomial pneumonia, and includes tuberculosis and measles in the scope of the *Guidelines*.

Classification of childhood respiratory infections and content of the *Guidelines* (Table 1)

Causative microorganisms of childhood respiratory infections and their detection

Bacteria[3]

1 The problem of identifying the causative pathogens of respiratory infections:[1,2] Identifying the causative bacteria of respiratory infections is more difficult than for other infectious diseases. Deep respiratory infections do not allow non-invasive collection of specimens from the affected site; and bronchopulmonary secretions are unavoidably contaminated by upper respiratory tract and oral flora on expectoration. Thus, isolating bacteria from these clinical specimens is not a reliable method for identifying the causative microorganism(s).

2 Upper respiratory tract flora: The detection of pharyngeal flora and percentage of bacterial colonies in healthy, symptom-free children differ in neonates, infants, preschool children, and school children. *Streptococcus pneumoniae* and *Haemophilus influenzae* are more frequently isolated and accounts for a greater percentage of colonies in infants and preschool children than in other age groups (Fig. 1).

3 Causative bacteria of childhood respiratory infections by disease location: Table 2 lists causative pathogens based primarily on data from the Department of Pediatrics of Chiba University and associated medical institutions.

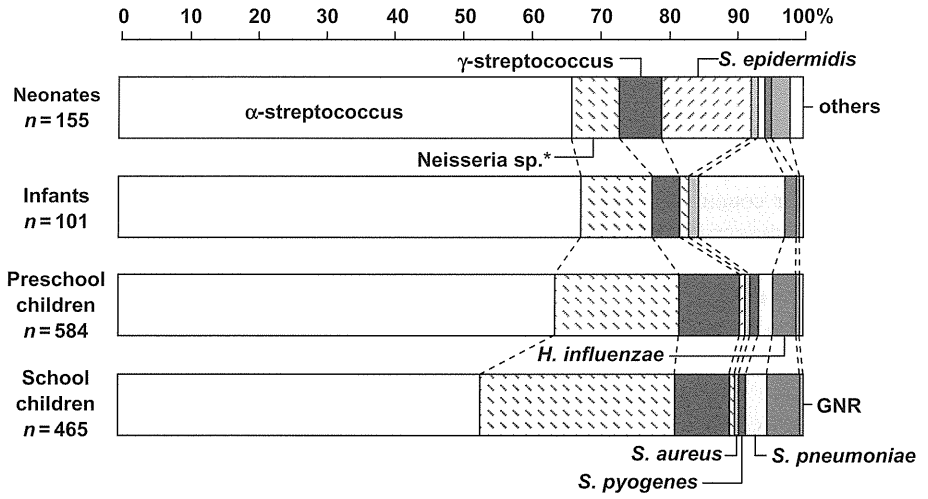

Fig. 1 Distribution of bacteria by age group in throat cultures from healthy children (average % of colonies). **M. catarrhalis* not classified. GNR, gram-negative rods. (Reproduced from *The Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007*, Uehara and Sunakawa [eds.][2] with permission.)

Table 2 Causative bacteria of childhood respiratory infections by disease location (Adapted from *The Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007*, Uehara and Sunakawa [eds.][2] with permission)

	Group A Streptococcus	Group B Streptococcus	*Streptococcus viridans*	*Streptococcus pneumoniae*	*Staphylococcus aureus*	*Corynebacterium diphtheriae*	*Moraxella catarrhalis*	*Haemophilus influenzae*	*Bordetella pertussis*	*Pseudomonas aeruginosa*	*Klebsiella*	Anaerobic bacteria	*Mycobacterium tuberculosis*	*Nocardia*	*Actinomycetes*	*Legionella*
Acute nasopharyngitis (common cold)	◎								△							
Acute pharyngotonsillitis	●			○		○		○				○				
Acute laryngitis (croup)						○		○								
Acute epiglottitis	○			○	○			◎								
Acute tracheitis				○	◎			○								
Acute bronchitis	△			○	○		○	○				*Bacteroides*				
Protracted bronchitis				◎			○	◎	○							
Acute bronchiolitis				○				○								
Pneumonia	○	○	○	●	◎		○	●	△	○	○	○	○	△	△	△
Lung abscess			○		○						○	○		○	○	
Pleurisy												○	○			
Pyothorax	○			○	◎			○								

●◎○△: frequency of occurrence from high to low.

4 Causative bacteria of upper respiratory infections and their detection: The *Guidelines* describe detection methods for Group A Streptococcus (GAS), including rapid diagnostics, and for *Corynebacterium diphtheriae*.

5 Causative bacteria of bronchopulmonary infections and their detection: The major bacteria responsible for childhood bronchopulmonary infections are *S. pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus*. These organisms are isolated with blood agar medium and chocolate agar medium. The clinical laboratory should be contacted in advance about suspected cases of pertussis and *Legionella* infections, which require specialized media for isolation.

6 Selection and determination of causative bacteria of bronchopulmonary infections: As previously stated, contamination with bacteria from the upper respiratory tract is a problem when diagnosing the causative bacteria for bronchitis, pneumonia, and other bronchopulmonary infections. Clinical specimens for culturing the causative bacteria for pneumonia as proposed by Moffet[4] are presented in Table 3. Sputum and nasopharyngeal and throat secretions are categorized as being of dubious value for diagnosing the causative bacteria of pneumonia. Moffet states that bacteria cultured from blood, pleural fluid, and lung puncture are definitive. Blood culture is less sensitive than culture from lung puncture.[5] Surveys conducted by Uehara of the causative bacteria determined from blood, pleural fluid, and lung puncture at pediatric training hospitals throughout Japan showed that the number of cases caused by *S. aureus* became fewer and those caused by *S. pneumoniae* and *H. influenzae* increased, beginning in the 1990s (Fig. 2).[6] It must be noted that only a small number of the total cases were confirmed by these conclusive culture sources. Pneumonia is transmitted via the airways as well as the bloodstream. We were able to raise the significance of sputum from "3. Cultures of dubious significance", which included sputum and nasopharyngeal and throat secretions to "2. Occasionally significant culture sources".

7 Assessment of causative bacteria identified in sputum culture: As sputum consists of bronchopulmonary secretions covered by upper respiratory secretions, it is difficult to differentiate bacteria of bronchopulmonary origin and those of upper respiratory tract when it is cultured as is.[6–8] Washed sputum culture[8,9] and quantitative culture are used to detect the true causative bacteria of bronchopulmonary infections. In washed sputum culture, a sputum specimen is washed with sterile saline solution, airway secretions thought to originate from the lower airway based on cytological evidence are cultured, and the predominant bacterium as determined semi-quantitatively is considered as the causative bacterium.

Table 3 Clinical specimens for identifying causative bacteria for pneumonia (created with modification from Moffet[4])

1. Conclusive Culture Sources
   - blood
   - pleural fluid
   - lung puncture
2. Occasionally Significant Culture Sources
   - transtracheal aspiration
   - tracheotomy aspiration
   - bronchoscopy aspiration
   - (washed sputum) ←
3. Cultures of Dubious Significance
   - tracheal aspiration
   - sputum ——
   - throat
   - nose/nasopharynx

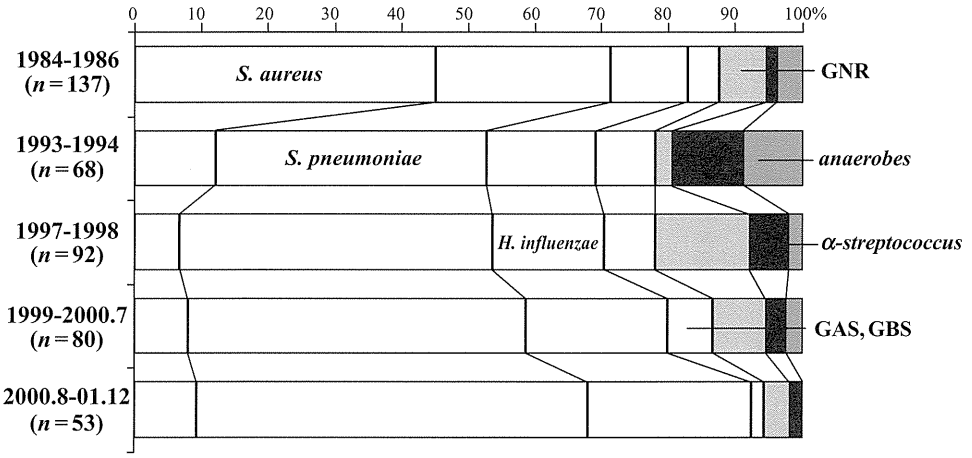

Fig. 2 Causative bacteria detected from blood, pleural fluid and/or lung tissue samples from pediatric pneumonia patients. GAS, Group A Streptococcus; GBS, Group B Streptococcus; GNR, gram-negative rods. (Reproduced from *The Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007*, Uehara and Sunakawa [eds.][2] with permission.)

Pathogenic respiratory bacteria are predominantly isolated from purulent sputum and are often the likely causative bacterium. However, if the sputum is viscous, the isolated species may be from the oral flora. Broad classification of the causative bacterium can be made by Gram staining of sputum. The classifications defined by Geckler *et al.*[10] are used for the quality control of sputum. Sputum is Gram-stained and observed under weak magnification (×100). Evaluation is based on squamous epithelial cells and neutrophil counts. The predominant organism detected in a Gram-stained smear of a washed sputum culture is of greater significance as the likely causative bacterium of a bronchopulmonary infection when found in close contact with alveolar macrophages (Fig. 3).[9]

Table 4 lists criteria for determining causative bacteria.[8] For *M. catarrhalis* to be confirmed as the causative species, the bacterium must be the predominant species in sputum culture and detected in macrophages by sputum cytology.[7]

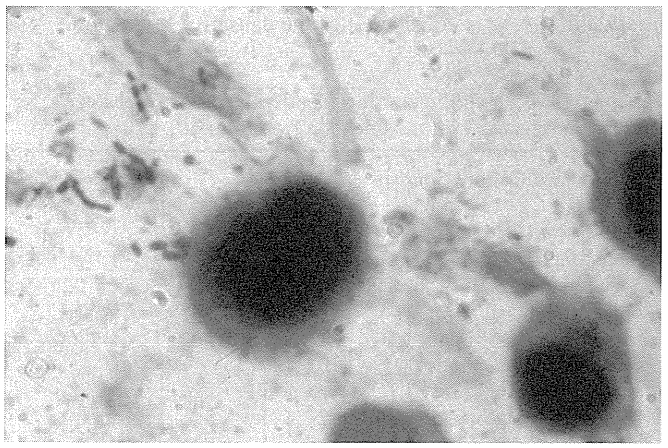

Fig. 3 Alveolar macrophage and perialveolar existence of Gram-positive diplococci (*Streptococcus pneumoniae*) and Gram-negative bacilli (*Haemophilus influenzae*) on gram-stained washed sputum.

Sputum collection in infants and children[7,8] is shown in Figure 4. Sputum collection should be attempted when the patient has a productive cough. If the patient is able to expectorate sputum, they should be instructed to discharge sputum into a sterile Petri dish with saline without contaminating the specimen by further productions of saliva, as far as possible. If the patient is an infant or preschool child who is unable to expectorate, the tongue should be depressed using a tongue depressor with a lamp to induce coughing. When the patient expectorates into the throat, a sterile swab should be promptly swiped around the sputum and placed in sterile saline. Recently, 1-mL disposable syringes have been used to aspirate specimens.[7]

8 The value of sputum washing and nasopharyngeal and pharyngeal culture:[11] Figure 5 shows the results of simultaneous culturing washed sputum, non-washed sputum, and nasopharyngeal and pharyngeal secretions for cases in which the causative bacteria was detected predominantly in washed sputum samples. Washed sputum samples showed better results than non-washed sputum samples. In the same patients, nasopharyngeal swabs showed better results than pharyngeal swabs, though detection was lower than in non-washed sputum samples.[11] Direct culturing of sputum

Table 4 Criteria for determination of causative bacteria in bronchopulmonary infection (adapted from Uehara[8] with permission)

① **Pathogens occupying more than half of the colonies in culture or presenting >10^7 cfu/mL of washed sputum were regarded as "dominant".**
② The same dominant pathogens were grown by repeated cultures.
③ The pathogens were seen perialveorally in smeared specimens.
④ Heavier growth of pathogens was observed with washed sputum than with nasopharyngeal or throat swabs.
⑤ **The pathogens in washed sputum correlated with the clinical course of the disease: signs and symptoms, acute phase reactants, and especially the purulence (neutrophilia) of the sputum.**

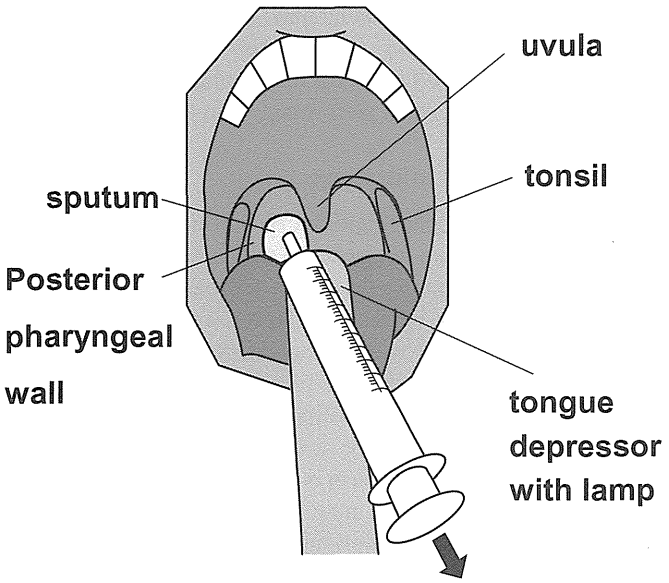

Fig. 4 Placement of instruments for the collection of sputum from pediatric patients.

(non-washed) results in inferior identification of causative bacteria, as it is covered with bacteria from upper airway secretions. Sputum specimens should be pretreated to remove contamination from the upper airway as completely as possible before culturing. Use of nasopharyngeal and pharyngeal cultures is only of limited value in etiological diagnosis of bronchopulmonary infections. Nasopharyngeal culture should therefore be conducted when sputum cannot be collected. Nasopharyngeal culture, however, should be used to postulate rather than definitively identify the bacterium responsible for pneumonia.

9 Detection of bacterial antigens in urine: Pneumococcal antigen may show false-positive results in urine because of the high prevalence of *S. pneumoniae* in the upper respiratory tract of children.[12] Urinary antigens are of excellent value in diagnosing legionellosis. Urinary antigen testing for *Legionella* spp. should be performed as a precaution in the critical cases of pneumonia.

10 Blood culture: Although sensitivity is not as high as other methods, blood cultures are of extreme value in selecting drugs for treatment when identifying the causative pathogen. Blood culture should be conducted whenever possible. Blood culture is discussed in detail in *Cumitech 1C: Blood Cultures IV*, a publication of the American Society for Microbiology.[13]

Mycoplasma, Chlamydia

Mycoplasma pneumoniae and *Chlamydia* infections are diagnosed by: (i) confirming significantly elevated or abnormally high serum antibody titers; and (ii) performing isolation culture, antigen detection, and nucleic acid detection on specimens from the infection site.

1 *Mycoplasma*: *M. pneumoniae* is the only significant pathogen involved in childhood respiratory infections. *Mycoplasma* infections are diagnosed by detection of *Mycoplasma* from the infection site and confirmation of increased antibody titers. *Mycoplasma* is detected in nasopharyngeal swab specimens, sputum, and pleural fluid. Detection is accomplished with direct fluorescent antibody assay, isolation culture, enzyme immunoassay, DNA probe assay, polymerase chain reaction (PCR), and other methods. Liquid pleuropneumonia-like organism (PPLO) media and other special media are used for isolation culture, which typically requires at least 7 days. PCR features excellent sensitivity and specificity. Serological diagnosis is accomplished with methods including particle agglutination (PA), cold agglutinin titer, complement fixation, indirect hemagglutination assay, and enzyme immunoassay.[14] Although serum antibody titer is at least fourfold higher in the acute and convalescent phases, increased immunoglobulin (Ig)M antibody levels must be identified to reach a definitive diagnosis. Infection may be strongly suspected if a PA titer of at least 320 or a complement fixation titer of at least 64 is detected in single serum. Infections in infants show poor antibody response.

2 *Chlamydia*: The three species *Chlamydophila pneumoniae*, *Chlamydophila psittaci*, and *Chlamydia trachomatis* are the causes of childhood *Chlamydia* respiratory infections. *Chlamydia* infections are diagnosed by detection of *Chlamydia* from

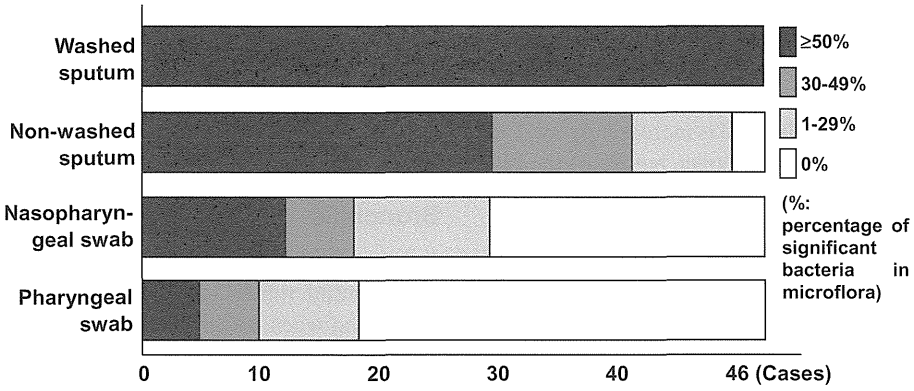

Fig. 5 Simultaneous culturing of washed and non-washed sputum specimens and nasopharyngeal and pharyngeal swabs from cases in which causative bacteria could be identified from washed sputum cultures. (Reproduced from Takeda *et al.*,[11] with permission.)

the infection site and confirmation of significantly increased antibody titer. *Chlamydia* is detected in nasopharyngeal swab specimens, sputum, and pleural fluid. Direct fluorescent antibody, enzyme immunoassay, PCR, and other techniques are used for detection. Isolation culture in cell culture requires at least 7 days. PCR offers good sensitivity and specificity. The Committee on Serological Diagnosis of *Chlamydophila pneumoniae* infection (chaired by Toshio Kishimoto) sets related diagnostic standards in Japan.[15]

Although serum antibody titer is at least fourfold higher in the acute and convalescent phases, increased IgM antibody levels must be identified to achieve serological diagnosis. For initial infections, a diagnosis can be reached in a relatively early stage using IgM antibody assay. Infections in infants show poor antibody response.

It should be noted that legionellosis is attributable to aspiration of *Legionella pneumophila* and other *Legionella* spp. from water coolers and other climate-control equipment. Only a few infants have acquired legionellosis in a neonatal intensive care unit. Legionellosis is more often diagnosed through rapid antigen diagnostics of urine specimens (61%) than it is from serum antibody titers. Rapid antigen diagnostics should therefore be attempted in cases of critical pneumonia. Isolation culture requires special media (B-CYEα medium, World Health Organization [WHO] agar medium).

Viruses

The characteristics of the viruses often isolated in childhood respiratory tract infections differ according to the infection site. Determining causative microorganisms according to symptoms alone is often difficult. The flow of testing is presented in the original *Guidelines*.

Medical staff collecting specimens for testing must be careful to perform collection at initial presentation in the early stage of the disease and to place specimens in a preserving solution for specimens for isolation (such as those designated by testing facilities). Specimens should be stored at low temperature (often 4°C). Specimens should be promptly shipped refrigerated to the testing facility. Serum specimens must be collected as paired sera once during the acute phase and again during the convalescent phase, 14–21 days after onset. A definitive diagnosis is reached when antibody titer is increased at least fourfold. The microplate method used by Numazaki *et al.* at the Virus Research Center of Sendai National Hospital[16] is well suited for the co-detection of viruses, is recommended by the WHO, and is increasingly used at Prefectural Institutes of Public Health in Japan, but the method is not feasible in all cases and must be selected according to the reason for culturing. The *2007 Guidelines* refined the list of rapid diagnostic testing, isolation culturing, nucleic acid detection testing, and serological detection methods for influenza virus, respiratory syncytial virus (RSV), and adenovirus pathogens.

Testing for rapid diagnosis of childhood respiratory infections

The *Guidelines* summarize: (i) trends in testing for the rapid diagnosis of childhood respiratory infections; (ii) the strengths and limits of immunochromatography; (iii) reagents for blood assay for *Mycobacterium tuberculosis* (BAMT), including whole-blood interferon-γ assay for diagnosing tuberculosis; and (iv) points to consider when performing rapid diagnostic testing.

Upper respiratory infections

1 Common cold (nasopharyngitis): Colds, which are caused primarily by viruses, are not treatable with antimicrobials. Antimicrobials fail to improve the course or prognosis of colds and have been found not to protect against lower respiratory tract infections. Fever alone with no respiratory symptoms is differentiated based on the presence of occult bacteremia, urinary tract infections, and other conditions.

2 Pharyngitis/tonsillitis: These conditions are often of viral origin. Antimicrobial treatment is indicated for primarily GAS infections. The *Guidelines* now recommend penicillin (PC)-based antimicrobials[17] as first-line treatments for GAS based on the discussions of GAS treatment that have taken place since 2004, but also list cephem antimicrobials for short-term therapy. Cephem or macrolide antimicrobials are recommended for children with penicillin allergies, but some children are also allergic to cephem antimicrobials. Not a few GAS isolates in Japan show resistance to macrolide antimicrobials, making cross-resistance a concern.

3 Croup syndrome
   (1) Viral croup: Viral croup is to be treated symptomatically. Dexamethasone therapy is an option for severe cases.
   (2) Acute epiglottitis: The course of this serious disease can include asphyxiation occurring 10 h after onset. A tongue depressor must not be used. Securing the airway is an urgent priority. Lateral radiography of the neck can show any epiglottic enlargement. *H. influenzae* type b (Hib) is the causative microorganism in ≥90% of all cases. The disease is treated with the antimicrobials: ceftriaxone, cefotaxime, meropenem, or tazobactam/piperacillin. Now that the Hib vaccine (approved in January 2007 in Japan) has been found to be safe and effective, Hib epiglottitis can be almost completely prevented through vaccination.[18]
   (3) Laryngeal diphtheria: Although very rare (only one case has been officially reported over the past several years), the possibility of laryngeal diphtheria must be kept in mind in unvaccinated and older children. Antitoxin therapy should be administered first and foremost.
   (4) Bacterial tracheitis: Although very rare, bacterial tracheitis can cause asphyxiation. *S. aureus* and other organisms cause this disease.

Bronchitis

1 Acute bronchitis:[19] Although acute bronchitis is usually viral, oral antimicrobials (consistent with those used for pneumonia) are used when bacterial bronchitis (*H. influenzae*, *S. pneumoniae*) is suspected based on fever, productive cough, or purulent sputum.

2 Protracted bronchitis (protracted, recurrent, and chronic bronchitis):[7] If infection is confirmed, the causative bacteria (*H.*

influenzae > *Streptococcus pneumoniae*) should be identified from the sputum and treated with the appropriate antimicrobial(s). Any underlying diseases (e.g. sinusitis, immunodeficiency) must be identified and superinfection by *Pseudomonas aeruginosa* or other organisms must be avoided.

Bronchiolitis

Acute bronchiolitis[20] is common in infants and is primarily caused by RSV (45–75%). Fever infrequently exceeds 38.5°C, and chest radiography often shows hyperinflated lungs. Some serious cases in infants under 3 months old require respiratory management. Antigen testing is useful. Some infections are caused by the human metapneumovirus, which has become a recent focus of attention.[21] No consensus has been reached on the value of PCR detection of the human bocavirus. Palivizumab is an effective prophylactic for RSV infection in high-risk infants.

Pneumonia

1 The definition of pneumonia: This acute respiratory infection is characterized by fever, rhinorrhea, and cough. Chest radiography, computed tomography (CT), and other imaging modalities show acute new infiltration in the lungs. Adventitious breath sounds and decreased respiratory sounds on chest auscultation can be observed in pneumonia.

2 Diagnosis of pneumonia: Patients suffering primarily from fever, cough, and dyspnea and who are suspected of having pneumonia based on chest findings should undergo chest radiography. Viral and *Mycoplasma* pneumonia are characterized primarily by interstitial lesions, and may show no abnormalities on chest auscultation. Once a definitive diagnosis of pneumonia is made based on imaging, the causative microorganisms should be identified in the blood and sputum (or in nasopharyngeal secretions). The need for antimicrobial(s) is determined in reference to pulmonary radiographs, acute phase reactants, and in consideration of the presumed causative microorganism. It must be remembered that infants and preschool children often cannot report dyspnea. When evaluating severity, features to check in addition to chest imaging include tachypnea (≥50 breaths/minute in children 1 year old and younger and ≥40 breaths/minute in children aged 2–5 years old) and retractions, nasal alar breathing, shoulder breathing, grunting, and cyanosis as signs of dyspnea (discussed later).

3 Causative microorganisms and examination

(1) Incidence of causative microorganisms: Based on the limited number of pneumonia cases for which the causative bacterium was confirmed through blood or pleural fluid culture in a nation-wide survey, the incidence of infections caused by *S. pneumoniae* and *H. influenzae* have exceeded those caused by *S. aureus* since the 1990s (Fig. 2).[6] Trends in *S. aureus* infection must be monitored.

Of the washed sputum cultures from bronchopulmonary infections, predominant bacteria were identified in about 30% of cases, and recent trends show that *H. influenzae* became more common than *S. pneumoniae*, and *M. catarrhalis*, in that order. *S. pneumoniae* pneumonia has been increasing since 1995 and accounted for about 30% of cases in which a causative organism was identified in 2005 (Fig. 6). For cases in which the causative pathogen of pneumonia was identified by washed sputum culture, about 30% of cases were attributed to bacterial pneumonia, 10–20% were attributed to *M. pneumoniae*, about 20% were viral, and the cause of the remaining 30% could not be determined. Trends in causative pathogens identified in washed sputum culture at three medical institutions associated with Chiba University showed *H. influenzae* and *S. pneumoniae* to be the major culprits since 1965, with some cases attributed to *M. catarrhalis*.

(2) Causative microorganisms and age distribution: The *Guidelines* summarize evidence about the age distributions associated with the causative microorganisms of pneumonia from the publication of McIntosh.[22] The Japanese evidence is similar. Although *C. pneumoniae* is well characterized, the data on other microorganisms do not differ substantially from those listed in medical texts, and no frequencies are stated. An investigation of the relationship of age in childhood pneumonia at Chiba Kaihin Municipal Hospital (1998–99) showed that of the 634 cases of childhood pneumonia treated, 170 (26.8%) were in 1-year-old children, 115 (18.1%) were in 2-year-old children, and 84 (13.2%) were in 4- to 11-month-old infants. A total of 512 (80.8%) were in children 4 years old and younger. Bacterial pneumonia was confirmed in washed sputum culture in 163 cases (25.7%). All cases were attributable to *H. influenzae*, *S. pneumoniae*, or combinations of these two, with the exception of three cases caused by *M. catarrhalis*, one caused by *Bordetella pertussis*, and two caused by GAS. Pneumonia was more commonly of bacterial origin in the younger age groups of hospitalized patients at Chiba Children's Hospital, while the incidence of *Mycoplasma* pneumonia increased with age (Fig. 7).

Although *C. pneumoniae* infections are relatively common beginning at young ages outside Japan, the prevalence of *C. pneumoniae* IgG antibody in Japanese children increases with age starting with an increased prevalence in 4–7-year-olds, a sharp increase to 44% in 8–11-year-olds, and about 50% above the age of 11 years.[23] The data provided by Kishimoto[24] on antibody incidence similarly indicate an increase in prevalence beginning at 6 years old. Grayston,[25] who stated that the incidences of bronchitis and pneumonia are about equal from 5 to 9 years old and that pneumonia is more common from 10 years old, reported that most cases of pneumonia are attributed to *C. pneumoniae* in older children.

4 Clinical symptoms, laboratory test findings, and antimicrobial selection

(1) Clinical symptoms and physical findings encountered with different causative pathogens: Investigation of many

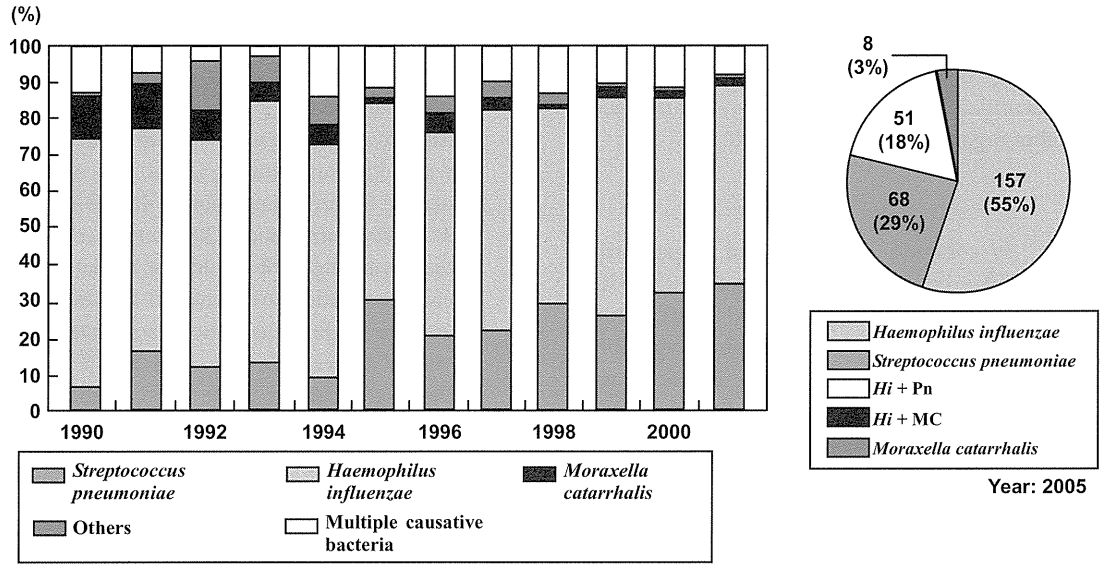

Fig. 6 Trends in causative bacteria in childhood bronchopulmonary infections based on washed sputum culture (percentages among cases of known pathogens). MC, *Moraxella catarrhalis*; Pn, *pneumococcus*. (Prepared from data provided by Dr. Kurosaki of Chiba Municipal Kaihin Hospital; reproduced from *The Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007*, Uehara and Sunakawa [eds.][2] with permission.)

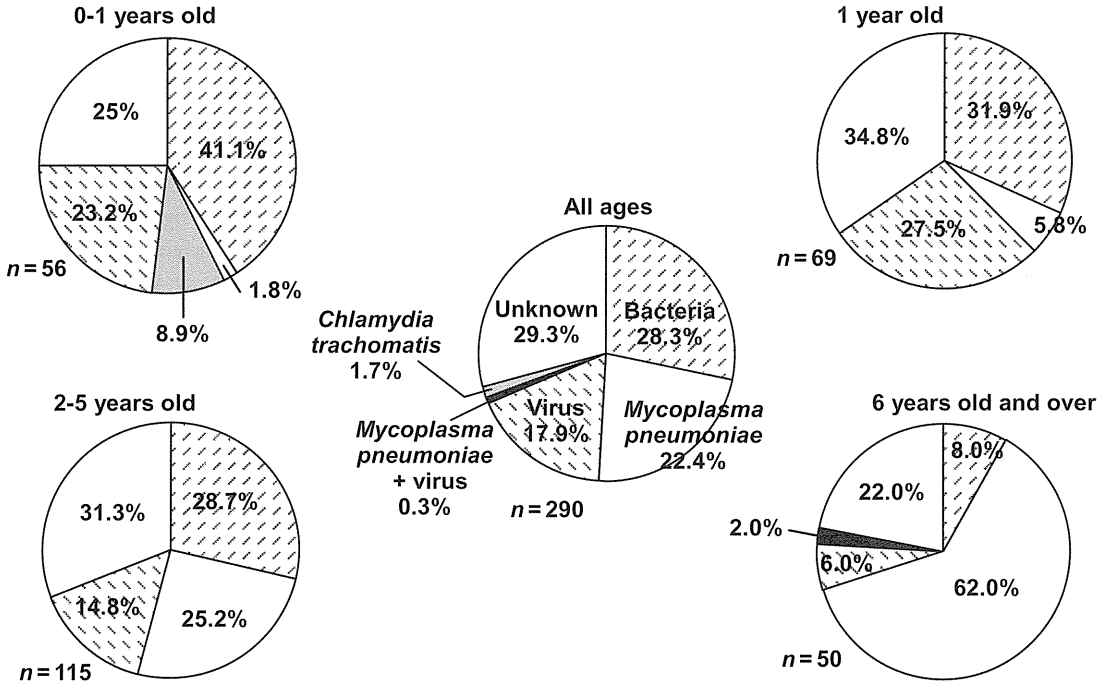

Fig. 7 Causative bacteria of community-acquired pneumonia in children. (Data collected October 1988–March 2002 by A. Nakamura of Chiba Children's Hospital; reproduced from *The Guidelines for the Management of Respiratory Infectious Diseases in Children in Japan 2007*, Uehara and Sunakawa [eds.][2] with permission.)

Table 5 Community-acquired pneumonia: determining severity through physical and laboratory observations

	Mild	Moderate	Severe	Critical
General condition	Good		Poor	
Cyanosis	Absent		Present	
Respiratory rate†	Normal		Rapid (over normal range)	
Forced respiration (grunting, nasal alar breathing, retraction)	Absent		Present	
Extent of infiltration on chest X-ray examination	≤1/3 of one lung		≥2/3 of one lung	
Pleural effusion	Absent		Measurable quantity	
SpO_2	>96%		<90%	
C-reactive protein (mg/dL)	<3.0		>15	
Neutrophils: Infant	4000–8000		<500 or >10 000	
Preschool-age child	2500–5500		<500 or >10 000	
School-age child	3000–5000		<500 of >10 000	
Criteria	All of the above criteria are met	Not mild or extreme	Any one of the above conditions are met	Accompanied by circulatory failure or when artificial respiratory care is required

†Respiratory rate by age: (breaths/min): neonate, <60; infant, <50; preschool-age child, <40; school-age child, <30.

cases in which the causative pathogen has been identified has revealed that bacterial pneumonia often involves productive cough and that *M. pneumoniae* disease often lacks labored breathing and abnormalities on auscultation. *C. pneumoniae* infections result in low-grade fever and prolonged coughing. Diagnostics for causative organisms, however, are required because postulating the causative microorganism according to symptoms is difficult in individual patients.[26,27]

(2) Causative microorganisms and laboratory test findings on admission: Bacterial and viral pneumonia had been considered distinguishable by the intensity of the inflammatory response. In blood culture-negative bacterial pneumonia, although white blood cell counts, C-reactive protein levels, and erythrocyte sedimentation rates were significantly different from those of viral pneumonia ($P < 0.01$), overlap is seen in about one-third of patients, making differentiation of cause impossible in individual cases.[27] Bacterial culture is therefore necessary before antimicrobial treatment. The possibility of *Mycoplasma* pneumonia should be considered when C-reactive protein levels and erythrocyte sedimentation rates are high, but white blood cell counts are not elevated.

(3) Causative pathogens and findings from chest radiography: The cause of pneumonia cannot be clearly differentiated based on chest radiography performed on admission using the differentiation methods of Swischuk and Hayden[28] or the scoring method of Khamapirad and Glezen.[29]

(4) Classifications of pneumonia severity: Tachypnea: The WHO established management criteria for pneumonia in developing countries, with a focus on tachypnea and labored breathing. Kurosaki[27] compared respiratory rates (≥50 breaths/minute in children 1 year old and younger and ≥40 breaths/minute in children under 5 years old) to findings from washed sputum cultures and reported that tachypnea can be used as an index for determining the appropriateness of antimicrobial treatment before culture results become available for 1–4-year-old children.[30] Assessing the severity of pneumonia is a first step toward determining whether the patient should be treated as an outpatient or admitted, whether antimicrobials should be administered, and whether oral or intravenous (i.v.) administration is appropriate. Criteria for assessing pneumonia severity are shown in Table 5.

(5) Hospitalization eligibility criteria: Patients with a severity classification of mild should be treated on an outpatient basis, while patients with moderate or severe infections should be admitted for treatment.

(6) Important factors when considering initial antimicrobial therapy

(i) Intensity of bacterial pathogenicity: *S. pneumoniae* has the strongest pathogenicity of the three causative organisms of bronchopulmonary infections: *S. pneumoniae*, *H. influenzae* and *M. catarrhalis*. Antimicrobial therapy that considers *S. aureus* is occasionally recommended for infants and children with underlying diseases.

(ii) Relationship between age and causative organism: The organisms primarily responsible for pneumonia differ with age of children as follows.

Neonates: Group B Streptococcus, *Escherichia coli*, and other intestinal flora.

Infants to children aged 5 years old: viruses, *H. influenzae*, and *S. pneumoniae*.

Children 6 years of age and older: *M. pneumoniae*, *C. pneumoniae*, *H. influenzae*, and *S. pneumoniae*.

Macrolide antibiotics should be considered first in children at least 6 years old who do not exhibit productive cough.

(iii) Pharmacokinetics of oral antibiotics: The pharmacokinetics-pharmacodynamics (PK-PD)

theory indicates that new cephem antibiotics, when recommended, should be administered at a high dose.

(iv) Minimizing drug resistance: Care must be taken to use antibiotics appropriately (particularly oral cephem antibiotics).

(v) Synthetic penicillin therapy for *S. pneumoniae* and *H. influenzae*: The Drug-Resistant *Streptococcus pneumoniae* (DRSP) Therapeutic Working Group of the Centers for Disease Control and Prevention of the USA, reasoning that pneumonia in children under 5 years of age is often bacterial in origin, advocates a β-lactam antibiotic (amoxicillin, amoxicillin/clavulanic acid, or cefuroxime for outpatient cases) for the initial treatment of pneumonia.[31,32] We set dosages for the treatment of cases for which *S. pneumoniae* was the predominant organism isolated from washed sputum based on the breakpoint for i.v. ampicillin defined by the Japanese Society of Chemotherapy of 2 μg/mL. Treatment with oral amoxicillin (30–40 mg/kg/day) and i.v. ampicillin (80–150 mg/kg/day) showed no significant differences for pneumonia with the following: penicillin-susceptible *S. pneumoniae* (PSSP), penicillin-intermediate resistant *S. pneumoniae* (PISP), and penicillin-resistant *S. pneumoniae* (PRSP).[32] (Note: The 2007 edition of the *Guidelines* lists penicillin G [PcG] resistance criteria that were revised in 2008. Following are the criteria in the 2007 edition of the *Guidelines*: PSSP, PcG-minimum inhibitory concentration [MIC] ≤ 0.06 μg/mL; PISP, PcG-MIC, 0.12–1 μg/mL; and PRSP, PcG-MIC ≥ 2 μg/mL). The *H. influenzae* ampicillin resistance criteria of the Clinical Laboratory Standards Institute (CLSI) of the USA[33] defines sensitivity as ≤1 μg/mL, moderate resistance as 2 μg/mL, and resistance as ≥4 μg/mL by the broth microdilution method. Most bronchopulmonary infections caused by β-lactamase-non-producing ampicillin-resistant (BLNAR) strains in Japan are treatable with i.v. ampicillin. Piperacillin, cefotaxime, and ceftriaxone offer reliable antibiotic activity against BLNAR strains. The response rate to piperacillin was 95%.[34] There are few patients with pathology caused by β-lactamase-producing *H. influenzae* strains that have shown clinical deterioration when treatment is initiated with oral amoxicillin or i.v. ampicillin. There is still time to switch antibiotics if resistance is identified after treatment is initiated.

(vi) Synthetic penicillin therapy for *M. catarrhalis*: Synthetic penicillin is clinically effective in treating *M. catarrhalis* infections even though the microorganism produces β-lactamase and is bacteriologically resistant to amoxicillin[35,36] because the produced β-lactamase has low activity.

(vii) Penicillin-binding protein (PBP) mutations: PBP of *S. pneumoniae* readily mutate in the presence of cephem antibiotics.[37] Mutation leads to increased resistance to β-lactam antibiotics and consequently DRSP strains. PBP mutations also underlie BLNAR and β-lactamase-producing amoxicillin-clavulanate resistant (BLPACR) *H. influenzae* strains. The increase in the prevalence of BLNAR strains is attributable to the widespread use of oral cephem antibiotics, which reaches a concentration that is only a fraction of that of amoxicillin.[37]

(7) Initial antimicrobial therapy when etiological pathogen is unknown: Antimicrobial agents recommended for initial treatment when the pathogen is unknown are shown for different age groups and for hospitalized patients and outpatients in Table 6. Agreement has been reached[38,39] on the appropriateness of the selections of initial antimicrobial agents given in the 2004 edition of the *Guidelines*. These selections must be continuously evaluated to take trends in causative microorganisms and drug resistance into account.

(8) Selection of antimicrobial agents when the etiological pathogen of pneumonia is known (monotherapy as a starting point): When the pathogen responsible for the pneumonia is known, the antimicrobial agent is selected in consideration of drug susceptibility and pharmacokinetics. Macrolide-resistant *Mycoplasma* strains have been increasing since 2000 (this is discussed later).

(9) Assessment of antimicrobial agent efficacy and duration of use: Antimicrobial agents for treating community-acquired pneumonia are normally sufficiently effective when administered for 3 to 7 days. Efficacy is assessed after 2 or 3 days (48–72 h after start of administration). Efficacy should be initially assessed after 2 days in younger children and severe cases. Assessment is performed to determine whether the initial antimicrobial agent is effective and whether the drug should be continued or switched. The duration of use will vary among individual patients. For common bacteria, use can be discontinued 3 days after the patient's fever breaks. A longer duration is required for *S. aureus* pneumonia. For *Mycoplasma* and *Chlamydia* infections, 10 days of new macrolide (clarithromycin) treatment or 3 days of azithromycin treatment (5 days in the USA) is recommended.

(10) Actions to take and selections to make when no response is achieved

(i) Actions to take when the patient does not respond to antimicrobial therapy: The correctness of the pneumonia diagnosis and the possibility of another disease producing pneumonia-like findings on imaging should be considered in order to distinguish pneumonia cases due to causative microorganisms other than common causative bacteria, such as viruses, tuberculosis, and fungi.

Table 6 Initial antimicrobial therapy in children for unknown etiological pathogen

	Severity	2 months to 5 years old*1*2*5	≥6 years old
Outpatient	Mild	AMPC ± CVA or SBTPC p.o. or Broad-spectrum cephem p.o.*3	Macrolide p.o. or Tetracyclin p.o.*4
Inpatient	Moderate to Severe	ABPC ± SBT i.v. or PIPC i.v. or Broad-spectrum cephem i.v.*3	ABPC ± SBT i.v. or PIPC i.v.*2 or Broad-spectrum cephem i.v.*3 ± Macrolide p.o./d.i.v. or Tetracycline p.o./d.i.v.*4
	Critical	Carbapenem d.i.v. ± Macrolide p.o./d.i.v.*6	

When the causative pathogen has been identified, change to the appropriate antimicrobial agent.
*1: With concomitant macrolide when *Chlamydia trachomatis* infection is identified.
*2: With concomitant macrolide when *Mycoplasma/Chlamydophila pneumoniae* infection is strongly suspected.
*3: The following offer superior antibacterial activity against *S. pneumoniae* and *H. influenzae*: Representative oral drugs: CDTR-PI; CFPN-PI; CFTM-PI. Representative intravenous drugs: CTRX; CTX.
*4: Use in children <8 years old only when other agents are ineffective or cannot be used.
*5: In principal, children <1 year old are hospitalized.
*6: With concomitant macrolide when Legionellosis cannot be ruled out.
AMPC, amoxicillin; CDTR-PI, cefditoren pivoxil; CFPN-PI, cefcapene pivoxil; CFTM-PI, cefteram pivoxil; CTRX, ceftriaxone; CTX, cefotaxime; CVA, clavulanic acid; d.i.v., drip intravenous; i.v., intravenous; PIPC, piperacillin; p.o., per os; SBTPC, sultamicillin.

(ii) Selection of antimicrobials when the patient does not respond to antimicrobial therapy:
- If a β-lactam antibiotic was initially used: Pneumonia is often caused by *H. influenzae* and *S. pneumoniae*, against which ampicillin and amoxicillin are recommended. These drugs are reportedly effective even against BLNAR and PRSP. For mild and moderate non-responsive cases, *Mycoplasma* or *Chlamydia* infection should be suspected, and the initial antimicrobial agent should be switched to or used in combination with a macrolide. A broad-spectrum i.v. cephem antibiotic or i.v. carbapenem antibiotic should be used when response is insufficient. For rapidly progressive, severe cases and critical cases, a carbapenem antibiotic and macrolide antibiotic should be used in combination. Addition of an anti-methicillin-resistant *Staphylococcus aureus* (MRSA) agent is to be considered.
- If a macrolide antibiotic was initially used: Treatment should be switched to a β-lactam antibiotic to treat macrolide-resistant *S. pneumoniae* and *H. influenzae*. Treatment should be switched to the optimal antimicrobial agent once the causative pathogen is identified. Table 6 provides recommendations for critical cases. When the condition of the patient is good and a *Mycoplasma* infection is suspected, switching to a tetracycline antibiotic should be considered to treat possible macrolide-resistant *Mycoplasma* infection.

(11) Outpatient parenteral antimicrobial therapy (OPAT): OPAT is sometimes used to treat patients with moderate pneumonia who are unable to be admitted. Such patients must visit the medical institution daily and be carefully monitored. Once-daily ceftriaxone has a long half-life and is commonly used.[40] A first-line treatment for bacterial meningitis, ceftriaxone should not be used readily and widely until the Hib vaccine has substantially reduced the prevalence of meningitis.

Pleurisy and pyothorax

Although pyothorax prevalence in Japan has decreased with the waning incidence of *S. aureus* pneumonia, vigilance is required because the disease is still on the increase in countries outside Japan, despite widespread use of the pneumococcal conjugate vaccine.

Pneumonia in patients with underlying diseases

The 2007 edition discusses pneumonia with accompanying underlying conditions (blood diseases, immunodeficiency, neonates, and cardiac diseases).

Nosocomial pneumonia

Nosocomial pneumonia is defined as pneumonia acquired after a hospital stay of at least 48 h. Measures must be taken to prevent children from becoming infected due to the hospital environment and medical acts (including those leading to ventilator-associated pneumonia) as well as from other patients, attendants, visitors, and medical personnel. The *Guidelines* present measures for preventing respiratory infections acquired through different routes and discuss the person-to-person transmission of respiratory infections. The *Guidelines* also recommend the vaccination of medical personnel.