Table 1. *Outcome based on presence or absence of underlying disease**

	Children			Adults		
	Underlying disease			Underlying disease		
Outcome	(+)	(−)	Subtotal	(+)	(−)	Subtotal
Fatality	2	0	2 (1·4)	37	6	43 (22·1)
Sequelae (+)	1	3	4 (2·9)	13	4	17 (8·7)
Sequelae (−)	17	115	132 (95·7)	85	50	135 (69·2)
Total	20	118	138 (100·0)	135	60	195 (100·0)

* Patients with unknown status concerning underlying disease and outcome were excluded from analysis.

conditions consisted of 30 cycles at 94 °C for 15 s, 53 °C for 15 s, and 72 °C for 15 s and amplified using a Takara PCR Thermal Cycler (Model TP600; Takara Bio, Japan). Amplified DNA fragments were analysed by electrophoresis on a 3% agarose gel. In the presence of all three DNA fragments corresponding to *pbp1a*, *pbp2x*, and *pbp2b*, the PBP genes were regarded as having essentially the same sequences as the sensitive R6 strain (PEN-susceptible *S. pneumoniae*, PSSP). We regarded the absence of DNA fragments as indicative of sequences other than those in PSSP. Genotypic determination is indicated by adding 'g' to designations as follows: gPSSP, gPISP (*pbp2x*), gPISP (*pbp2b*), gPISP (*pbp1a+2x*), gPISP (*pbp2x+2b*), and gPRSP (*pbp1a+2x+2b*).

Pulsed-field gel electrophoresis (PFGE)

PFGE was performed using a modification of a method described previously [12]. For digestion, DNA plugs were incubated in 1 ml restriction enzyme buffer with 100 U of *Apa*I at 37 °C for 16 h. Electrophoresis was performed with a CHEF Mapper (Bio-Rad Laboratories, USA) at 5·7 V/cm at 14 °C for 18 h.

RESULTS

IPD

IPD was classified into five groups as follows: septicaemia and bacteraemia (including two cases of bacterial endocarditis); pneumonia, where *S. pneumoniae* was isolated from blood cultures; meningitis diagnosed by clinical findings, where *S. pneumoniae* was isolated from CSF or blood; suppurative arthritis or osteomyelitis; and others. In 193 children aged ⩽17 years, septicaemia was predominant with 114 (59·1%) cases, followed by pneumonia with 44 (22·8%) cases, and meningitis with 30 (15·5%) cases; other diseases were rare. Almost 92% of IPD cases in children were aged ⩽4 years. In the 303 adults, septicaemia and pneumonia predominated with 115 (38·0%) cases and 112 (37·0%) cases, respectively, followed by meningitis with 57(18·8%) cases. The median age of adults with septicaemia and meningitis was 66 years, but was somewhat higher in patients with pneumonia (73 years).

Outcomes and underlying diseases

Table 1 shows outcomes and underlying diseases in 138 children (71·5% of those studied), and 195 adults (64·4%), according to reports returned by collaborating institutions. In children, 20 (14·5%) had underlying diseases, mostly congenital abnormalities. Adverse outcomes for children included death in two (1·4%) cases and neurological sequelae in four (2·9%) cases.

In adults, 135 (69·2%) had underlying diseases, the most common being cancer surgery (38), diabetes (30), cardiovascular disease (18), hepatic disease (16), kidney disease (9), immunological deficiency (3), and splenectomy (2). Deaths were numerous [43 (22·1%)], but 37 of those patients had underlying diseases, and the cause of death was not considered in detail. The median hospital stay in adults who did not survive was 2 days. Seventeen patients, including 13 with underlying disease, had severe neurological sequelae. When outcomes in cases with underlying diseases and those without underlying diseases were compared separately for children and adults, the mortality and sequelae rates were statistically higher in both children and adults having underlying

Table 2. *Clinical laboratory findings associated with fatal outcome in adults with invasive pneumococcal disease*

	Median or % (25/75 percentiles) and [no./total]			
	Non-survivors ($n=43$)	Survivors ($n=147$)	Univariate analysis OR (95% CI)	P value
WBC (10^9 cells/l)	5·1 (2·3–8·8) [37/43]	13·2 (8·2–19·1) [136/147]		
$<5{\cdot}0\times10^9$ cells/l	48·6% [18/37]	11·0% [15/136]	7·64 (3·30–17·68)	$P<0{\cdot}0001$
C-reactive protein (mg/dl)	24·8 (16·3–31·7) [36/43]	20·6 (8·9–33·6) [131/147]		
⩾15 mg/dl	77·8% [28/36]	65·6% [86/131]	1·83 (0·77–4·35)	$P=0{\cdot}1661$
PLT (10^9 cells/l)	119 (69–171) [36/43]	197 (130–262) [134/147]		
$<130\times10^9$/l	55·6% [20/36]	23·1% [31/134]	4·15 (1·92–8·97)	$P=0{\cdot}0002$

OR, Odds ratio; CI, confidence interval; WBC, white blood cell count; PLT, platelet count.

Table 3. *MIC_{90} and resistance genes identified by PCR in* S. pneumoniae

		MIC_{90} (μg/ml)					
Resistance class	n	PEN	AMP	CTX	MEM	PAM	VAN
gPSSP	101	0·031	0·031	0·125	0·016	0·004	0·5
gPISP (*pbp2b*)	38	0·125	0·031	0·063	0·031	0·008	0·5
gPISP (*pbp2x*)	124	0·063	0·125	0·5	0·016	0·008	0·5
gPISP (*pbp1a+2x*)	54	0·5	0·5	1	0·125	0·031	0·5
gPISP (*pbp2x+2b*)	35	0·5	0·5	2	0·125	0·031	0·5
gPRSP (*pbp1a+2x+2b*)	140	2	4	2	0·5	0·125	0·5

Each *pbp* gene alteration detected by PCR appears within parentheses.
MICs were determined for the following antibiotics: PEN, penicillin; AMP, ampicillin; CTX, cefotaxime; MEM, meropenem; PAM, panipenem; VAN, vancomycin.
Strains tested MICs: 492 isolates grown on sheep blood agar plate from stock at −80 °C.

diseases (Fisher's test: children, $P=0{\cdot}0395$; adults, $P=0{\cdot}0043$).

Haematological findings and outcomes in adults

We compared WBC, CRP, and PLT at time of admission between the non-surviving and surviving adults. Analysis was carried out using a non-parametric Kruskal–Wallis test and the results are shown in Table 2. The median WBC in non-survivors and survivors was $5{\cdot}1\times10^9$ and $13{\cdot}2\times10^9$ cells/l, respectively; the odds ratio between patients with WBC below and above $5{\cdot}0\times10^9$ cells/l was calculated as 7·64. A clear difference in the PLT was also noted between the two groups; and the odds ratio for mortality between patients with PLT below and above 130×10^9 cells/l was 4·15. No significant difference in CRP was evident between non-survivors and survivors. In addition, no significant difference in resistance type of gPSSP, gPISP, and gPRSP or in serotype (PPV23) was found between the non-survivors and survivors ($P=0{\cdot}1200$, $P=0{\cdot}9891$, respectively).

PBP gene alterations and β-lactam susceptibility

Table 3 shows results of MIC_{90} of PEN, AMP, CTX, MEM, and VAN. Genotype was based on PCR results for the *pbp1a*, *pbp2x*, and *pbp2b* genes. PEN susceptibility declined according to addition of altered *pbp* genes, from a MIC_{90} of 0·063 μg/ml for gPISP (*pbp2x*) to 2 μg/ml for gPRSP (*pbp1a+2x+2b*). In particular, susceptibility to CTX was affected by alterations of *pbp2x*, a pattern markedly different from that of susceptibility to PEN. In contrast, although susceptibility to MEM was affected by the gene alterations, the effect was much less. The MIC_{90} of VAN for all *S. pneumoniae* strains was 0·5 μg/ml.

Relationship between serotype and resistance genotype for β-lactams

The serotypes of *S. pneumoniae* isolates from children, classified as either PCV7 or non-PCV7 types, in decreasing order of prevalence are shown in Figure 1 and the percentage rate of resistance genotypes for β-lactams is also given for each serotype. Serotype 6B

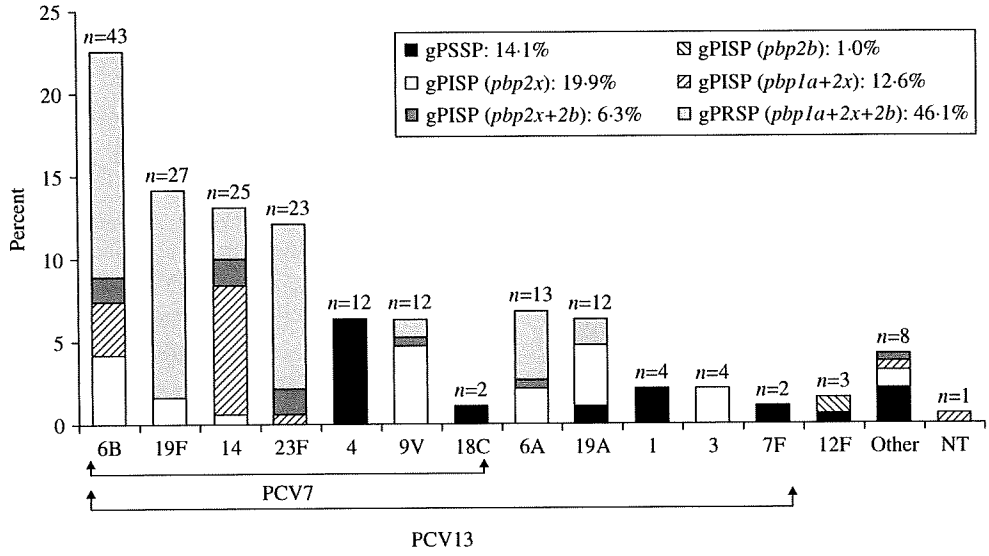

Fig. 1. Serotype distribution and resistance genes identified by PCR in *S. pneumoniae* isolated from children. 'Other' category includes serotypes 15B, 23A, 8, 24, 34, 35, and 38.

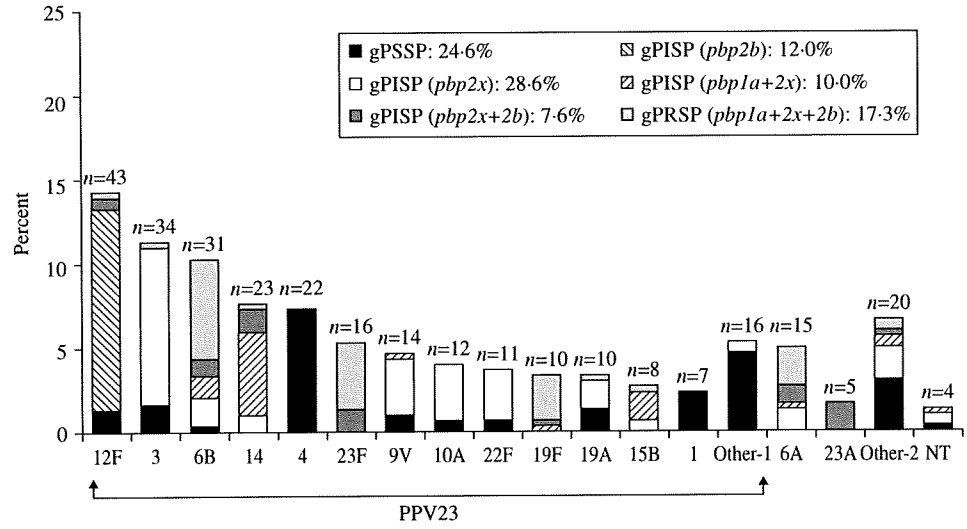

Fig. 2. Serotype distribution and resistance genes identified by PCR in *S. pneumoniae* isolated from adults. 'Other-1' category includes serotypes 9N, 11A, 33, 18C, 20, 2, 7F, 8. 'Other-2' category includes serotypes 35, 7C, 15A, 38, 15C, 31, 16, and 36.

predominated in the PCV7 types, followed in order by 19F, 14, and 23F. Coverage by PCV7, to which types 9V, 4, and 18C were added, was calculated as 75·4%. PCV7 covered types 6B, 19F, 14 and 23F, all of which showed high rates of gPRSP. In addition, coverage by PCV13 was calculated as 93·7%. The resistance rate of gPRSP (*pbp1a+2x+2b*) was highest, at 46·1%, followed by gPISP (*pbp2x*) at 19·9%, gPISP (*pbp1a+2x*) at 12·6%, gPISP (*pbp2x+2b*) at 6·3%, and gPISP (*pbp2b*) at 1·0%. The rate of gPSSP was only 14·1%.

The serotypes of *S. pneumoniae* isolates from adults that were covered by PPV23 are shown in Figure 2, in decreasing order of prevalence. These results differed markedly from those for children. The most prevalent type, 12F, accounted for 14·3% of the total; interestingly, almost all had gPISP (*pbp2b*). Serotype 3 (11·3%), with a high incidence of gPISP (*pbp2x*), was second only to 12F. Other common serotypes were, type 6B (10·3%), with a high frequency of gPRSP (*pbp1a+2x+2b*), while type 14 (7·6%) showed a high frequency of gPISP (*pbp1a+2x*). PPV23 and PCV13

Fig. 3. PFGE patterns of *Apa*I digests of chromosomal DNA from serotype 12F isolates. A, gPISP (*pbp2b*) (lanes 1–19); B, gPSSP (lanes 20–24); C, gPISP (*pbp2x*+*2b*) (lanes 25, 26); D, gPRSP (*pbp1a*+*2x*+*2b*) (lane 27).

provided coverage in 85·4% and 61·5%, respectively. Non-survivors and patients with sequelae had developed IPD involving strains of various serotypes. The predominant resistance genotype in adults was gPISP (*pbp2x*) at 28·6%, followed by gPSSP at 24·6%, gPRSP (*pbp1a*+*2x*+*2b*) at 17·3%, gPISP (*pbp2b*) at 12·0%, gPISP (*pbp1a*+*2x*) at 10·0%, and gPISP (*pbp2x*+*2b*) at 7·6%. The serotype and the resistance genotype of strains differed significantly between children and adults (both $P<0{\cdot}0001$).

PFGE pattern of strains serotyped 12F

Figure 3 shows PFGE patterns of *Apa*I DNA digests of serotype 12F strains. The 27 strains pictured, namely five gPSSP, two gPISP (*pbp2x*+*2b*), one gPRSP, and 19 gPISP (*pbp2b*), were selected randomly from 38 strains which were isolated from patients throughout Japan. DNA restriction patterns of strains with the same resistance genotype were homogeneous, suggesting that *S. pneumoniae* strains possessing the same *pbp* alterations had spread widely. There has been a rapid increase in the prevalence of serotype 12 in Japan and this serotype is present in 18% of cases with a poor prognosis in adults. This increase is therefore considered to be of clinical significance.

DISCUSSION

S. pneumoniae is a major causative agent of diseases such as pneumonia, meningitis, and acute otitis media (AOM), as well as various other serious invasive infections. In the USA, the PCV7 vaccine was developed for children and approved in 2000, and has been incorporated into the paediatric vaccination schedule [14]. Immunization programmes using PCV7 have spread widely, and are presently conducted in almost 70 countries worldwide [27]. The incidence of IPD involving vaccine-type *S. pneumoniae* has been reported to have decreased significantly [15, 17, 18], and a related decrease in IPD in adults has been noted [16]. However, the incidence of IPD caused by non-vaccine-type *S. pneumoniae* has increased; particularly type 19A [19–21]. In order to provide increased coverage, a new vaccine, PCV13, is being developed, which will include types 19A, 6A, and 3 [28].

Much clinical attention has been drawn to a rapid increase in PRSP in *S. pneumoniae* isolates. These strains have been causative agents of paediatric AOM [29] and meningitis [22] in Japan since 1990 and this increase is strongly related to a shift from prescribing oral penicillins for outpatients to using oral cephalosporins. The increase may also be related to use of macrolides, considering that most PRSP are multidrug-resistant *S. pneumoniae* (MDRSP) also resistant to macrolides [30]. In addition, Japan's high population density tends to accelerate increases in resistant organisms.

We previously compared *pbp* gene alterations in *S. pneumoniae* strains that had been isolated in the same time period from the USA and Japan [10]. In the USA, where use of penicillins predominated, increases were evident in resistant strains with the *pbp2b* gene alteration whereas in Japan, where cephalosporins predominated, many strains characteristically had the *pbp2x* gene alteration. As shown in this study, the latter pattern still persists in Japan.

According to USA guidelines [31], the use of third-generation cephalosporins – CTX, CRO, or either of these in combination with VAN – is recommended for meningitis caused by PRSP. In Japan, however, carbapenems such as PAM and MEM are recommended as first-choice antibiotics in this situation. A major reason for this practice is that 60% of Japanese paediatric meningitis cases are caused by *Haemophilus influenzae* type b (Hib), of which about 36·2% show resistance to AMP and CTX, reflecting β-lactamase non-producing and AMP-resistant *H. influenzae* as the causative pathogens [32]. Therefore, in Japan, the preferred paediatric treatment increasingly involves concomitant use of a carbapenem, with its superior bactericidal effect against *S. pneumoniae*, plus CTX or CRO, with superior activity

against *H. influenzae*; treatment now is basically the same for adults.

As for vaccines against *S. pneumoniae*, PPV23 has been introduced in Japan, where it is used mainly on a voluntary basis for elderly people as well as adults and children with underlying diseases. The PCV7 vaccine is currently under review by the Japanese Ministry of Health, Labour and Welfare, and approval is expected soon. Nevertheless, one needs to know the extent to which PCV7 covers IPD. According to our epidemiological surveillance in the current study, PCV7 covers 75·4% of strains isolated from children with IPD. However, the incidence of types 6A and 19A, which are non-vaccine types, is significant, so the introduction of PCV13 will be beneficial.

In Japan, a recent rapid increase in IPD in adults may reflect the rapid ageing of society and an increase of lifestyle-related diseases in the adult population. The current situation whereby PPV23 vaccination is voluntary, limits its effectiveness against this increase. Development of disease caused by *S. pneumoniae* in adults with underlying disease often triggers disseminated intravascular coagulation (DIC), leading to death or serious sequelae for which the prognosis is extremely poor. Also of concern is the poor prognosis for adults who develop IPD caused by *S. pneumoniae* with intermediate PEN resistance. In addition, serotype 12F was very rare in 2000, but in the current study accounted for 12·0% of IPD cases and strains show essentially the same PFGE pattern as gPISP (*pbp2b*). The reason why this type of *S. pneumoniae* has increased so rapidly in adults is unknown, and requires further investigation. Finally, but importantly, the impact of the forthcoming introduction of PCV7 will need to be assessed by continued epidemiological surveillance of IPD throughout Japan.

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DECLARATION OF INTEREST

None.

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cific. The high plasma creatinine level in the newborn sometimes reflects the mother's plasma creatinine level (*9*). However, kidney function of the mother of the newborn was within normal limits at the time of Caesarean section; plasma creatinine level of 0.7 mg/dL. An elevated plasma creatinine level is observed frequently in premature infants due to immaturity of the kidney tissue and will usually decrease within a few weeks. Oseltamivir was administered with dose adjustment based on the infant's estimated glomerular filtration rate. The recommended dose of oseltamivir for glomerular filtration rate <30 mL/min/1.73 m^2 is 2–3 mg/kg/day, based on preliminary data obtained by a National Institutes of Health–funded Collaborative Antiviral Study Group (*10*). The success of our management strategy for this case suggests early treatment with oseltamivir can prevent severe illness in newborns with perinatal influenza A pandemic (H1N1) 2009 infection.

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Bronchial Casts and Pandemic (H1N1) 2009 Virus Infection

To the Editor: In the late 1990s, triple-reassortant influenza A viruses containing genes from avian, human, and swine influenza viruses emerged and became enzootic in swine herds in North America (*1*). The first 11 human cases of novel influenza A virus infection were reported to the Centers for Disease Control and Prevention (CDC; Atlanta, GA, USA) from December 2005 through February 2009 (*1*). In response to those reports, surveillance for human infection with nonsubtypeable influenza A viruses was implemented.

In the spring of 2009, outbreaks of febrile respiratory infections caused by a novel influenza A virus (H1N1) were reported among persons in Mexico, the United States, and Canada (*2*). Patient specimens were sent to CDC for real-time reverse transcription–PCR (RT-PCR) testing, and from April 15 through May 5, 2009, a total of 642 infections with the virus, now called pandemic (H1N1) 2009 virus, were confirmed. Of those 642 patients, 60% were ≤18 years of age, indicating that

children may be particularly susceptible to pandemic (H1N1) 2009 (*2*).

Children and adults with preexisting underlying respiratory conditions, such as asthma, are at increased risk for complications from infection with pandemic (H1N1) 2009 virus. One possible complication is plastic bronchitis, a rare respiratory illness characterized by formation of large gelatinous or rigid branching airway casts (*3*). Plastic bronchitis is a potentially fatal condition induced by bronchial obstruction from mucus accumulation resulting from infection, inflammation, or vascular stasis (*4*). We report a case of bronchial casts that caused atelectasis of the right lung of a child infected with influenza A pandemic (H1N1) 2009 virus.

A 6-year-old boy with asthma and a 1-day history of fever and cough was referred to a hospital pediatrics department because of dyspnea. Clinical examination at hospital admission found respiratory distress, as shown by tachypnea (respiratory rate 66 breaths/min) and inspiratory retraction, deficient vesicular sounds over the right lung field, elevated blood levels of immunoglobulin E (1,770 IU/mL) and a reduced number of lymphocytes (483 cells/μL), and radiographic evidence of atelectasis of the right lung and hyperinflation of the left lung without air leakage (Figure, panel A). Pandemic (H1N1) 2009 virus infection was confirmed by real-time RT-PCR, as described (*5*), of an endotracheal aspirate. Real-time PCR ruled out *Streptococcus pneumoniae, Haemophilus influenzae, Mycoplasma pneumoniae, Legionella pneumophila, Chlamydophila pneumoniae, S. pyogenes*, respiratory syncytial viruses A and B, seasonal influenza viruses A and B, parainfluenza viruses 1–3, rhinovirus, enterovirus, human metapneumovirus, human bocavirus, and adenovirus (*6*). While the patient was breathing room air, his percutaneously monitored oxygen saturation was 86%; respiratory support by mechanical ventilation was then initiated. Mucus casts were extracted by intratracheal suction (Figure, panel B). The patient was treated with an inhaled bronchodilator, intravenous methylprednisolone (20–60 mg/day for 7 days), and antiviral (oseltamivir) and antimicrobial (ampicillin/sulbactam) drugs.

On hospital day 2, chest radiographs showed that atelectasis of the right lower lobe had partially resolved (Figure, panel C). A histologic examination of casts (May-Giemsa stain; Figure, panel D) indicated a mucoid substance containing a predominantly eosinophilic infiltrate (>90% of cells). The patient's respiratory condition during 11 days of oxygen supplementation gradually improved, and he was discharged on hospital day 18.

Plastic bronchitis is related mainly to respiratory, cyanotic cardiac (post-Fontan), and hematologic (sickle cell anemia) diseases. A diagnosis of plastic bronchitis is determined on the basis of clinical findings (pointing to allergic and asthmatic, cardiac, or idiopathic etiologies) and pathologic findings (inflammatory vs. noninflammatory) on examination of casts (*3*). Inflammatory casts contain fibrin, eosinophils, and Charcot-Leyden crystals; noninflammatory casts contain mucin and exhibit vascular hydrostatic changes. The case presented here was the allergic-inflammatory type of plastic bronchitis.

Various treatments for plastic bronchitis have been described and vary from cast removal by expectoration or by bronchoscopy (*7,8*). Other interventions involve cast disruption by tissue plasminogen activator or urokinase and prevention of cast formation by use of mucolytic agents, steroids, or anticoagulants. However, evidence remains anecdotal because too few plastic bronchitis patients are available for clinical trials. Details of steroid dosage will need to be clarified for pandemic (H1N1) 2009 virus–infected children with respiratory distress from bronchitis and pneumonia.

In Iran during 1998–2001, avian influenza (H9N2) infection among broiler chickens resulted in 20%–60% mortality rates on affected farms (*9*). Macroscopic examination of specimens from infected chickens showed extensive hyperemia of the respiratory tract, followed by exudate and casts extending from the tracheal bifurcation to the secondary bronchi. Light microscopy indicated severe necrotizing tracheitis. Pandemic (H1N1) 2009

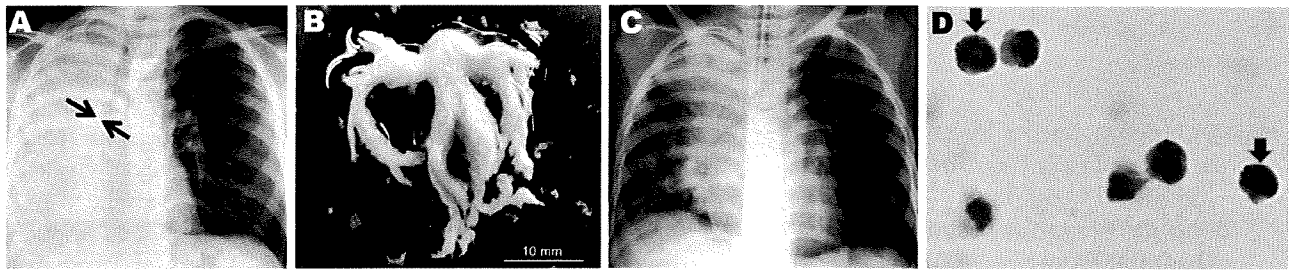

Figure. A) Chest radiograph obtained at hospital admission from a child infected with influenza subtype H1N1 virus. The image shows atelectasis of the right lung and hyperinflation of the left lung; arrows indicate obstruction of the right main bronchus. B) Macroscopic bronchial casts extracted by intratracheal suction. C) Chest radiograph obtained on hospital day 2, indicating partial resolution of atelectasis of the right lower lobe. D) Light micrograph of casts, characterized by predominant eosinophil infiltration (>90% of cells) (May-Giemsa stain, original magnification ×1,000). Arrows indicate typical eosinophil granules. A color version of this figure is available online (www.cdc.gov/EID/content/16/2/344-F.htm).

can produce similar airway cast formation in humans; severe respiratory distress reflects extensive obstruction of the respiratory system.

Healthcare providers should be aware of the possibility of bronchial casts when examining children with influenza (H1N1) infection accompanied by atelectasis. Steroids can be administered early in infection to avoid cast formation, and antiviral drug therapy and respiratory support can be used for influenza (H1N1)–infected children in whom airway casts have developed.

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Methicillin-Resistant *Staphylococcus aureus* ST398, Italy

To the Editor: It has recently become apparent that livestock can constitute a new methicillin-resistant *Staphylococcus aureus* (MRSA) reservoir and be a source of a novel and rapidly emerging type of MRSA. These livestock-associated MRSA clones are nontypeable by use of pulsed-field gel electrophoresis with *Sma*I and belong to sequence type (ST) 398 (*1*). MRSA ST398 clones account for 20% of all MRSA in the Netherlands (*2*), but the emergence of such clones has been described worldwide (*3*). Although ST398 transmission has been reported primarily between animals, persons with occupational exposure to livestock are at higher risk for MRSA carriage than the general population. Even though MRSA ST398 usually causes colonization, several cases of infections of variable clinical relevance, varying from skin and soft tissue infections (*4*) to endocarditis (*5*) and pneumonia (*6*), have been described over the past few years. Most instances of ST398 human carriers have been identified among persons who work at pig farms (*7*). Data regarding MRSA colonization of dairy farmers are less exhaustive and, to our knowledge, only 1 instance of direct transmission between cattle and humans has been proven. MRSA isolates from cows with subclinical mastitis in 2007 in Hungary were indistinguishable from MRSA isolates from the tonsil swab of a farmer who worked with these animals (*8*). We report a case of MRSA ST398 invasive disease in a cattle farmer, as well as a case of MRSA ST398 necrotizing fasciitis.

In early April 2008, a 52-year-old man was admitted to an intensive care unit in Manerbio, Italy, because of severe sepsis and a large ulcerative and

preceding viral illness [2,3]. Leucopenia has also been a finding in cases during the current pandemic of influenza A (H1N1) infection [8–10]. Our patient had leucopenia with mild neutropenia and lymphopenia.

The disease is self-limiting and, when the myositis develops, the patient is already at the early convalescent phase of the viral illness. Therefore, antivirals are not usually indicated. Only supportive treatment and follow-up aiming to monitor full recovery of the patient are required [17]. In our case, the patient was already afebrile at the time of admission and he recovered fully in less than 48 h.

BACM is an acute, self-limiting condition with an excellent prognosis, which occurs during the acute convalescent phase of viral illnesses, mainly influenza A and influenza B infections, and requires no therapeutic intervention. Correct diagnosis by considering the characteristic clinical and laboratory findings as well as the history of the preceding viral illness can prevent unnecessary diagnostic procedures and reassure both the parents and the patient of the excellent prognosis.

Transparency Declaration

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Spontaneous pneumomediastinum complicating pneumonia in children infected with the 2009 pandemic influenza A (H1N1) virus

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Abstract

We report two occurrences of spontaneous pneumomediastinum (SPM) complicating pneumonia in Japanese children infected with the novel influenza A (H1N1) virus (IV). General practitioners especially should suspect possible SPM when examining and treating children with the novel influenza accompanied by status asthmaticus or wheezing. The presented patients illustrate the specific clinical and radiological signs associated with SPM complicating pneumonia in children infected with A(HINI)v.

Keywords: A (H1N1) virus, children, influenza, pneumonia, spontaneous pneumomediastinum

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A novel influenza A (H1N1) virus (IV), was identified as the cause of outbreaks of febrile respiratory infection ranging from self-limiting to severe illness in Mexico, the USA, Canada, and elsewhere in the spring of 2009 [1]. Triple-reassortant IV containing genes from avian, human and swine influenza viruses emerged and became enzootic among swine herds in North America during the late 1990s [2]. Clinical aspects of the first 11 patients sporadically infected with IV were reported to the CDC in the USA, from December 2005 to February 2009 [2]. Accordingly, surveillance was implemented for human infections with influenza A viruses that could not be subtyped. Specimens were sent to the CDC RT-PCR confirmatory testing for IV. A total of 642 confirmed cases of IV infection were identified from 15 April to 5 May. Sixty percent of patients were aged 18 years or younger, indicating that children may be particularly susceptible to IV [1].

Eighteen cases of pneumonia and confirmed IV infection were identified among 98 patients hospitalized for acute respiratory illness from 24 March to 24 April in Mexico, including five patients aged 15 years or younger [3]. All patients had fever, cough, dyspnoea, increased serum lactate dehydrogenase levels, and bilateral patchy pulmonary shadows on roentgenograms. Other common findings were increased creatine kinase levels and lymphopaenia. Mechanical ventilation was required for 12 patients, and seven patients died.

We report two IV-infected Japanese children with spontaneous pneumomediastinum (SPM) complicating pneumonia.

IV was confirmed in nasopharyngeal-swab specimens using real-time RT-PCR as previously described [4–6]. IV-specific primers for amplification (87 bp) of the nonstructural protein 1 gene (accession number FJ966086) included a sense primer, 5′- GCGAACTTCAGTGTAATC-3′, and a reverse primer, 5′- AATTTCTCCAACTATTGCTC-3′; the specific molecular beacon probe used was 6-carboxyfluorescein-CGCGATGACCTTGATACTACTAAGGGCTTATCGCG-black hole quencher 1. IV-associated pneumonia was based on evidence of both influenza-like illnesses with opacities on chest radiographs and laboratory-confirmed IV infection [3].

Case 1

A previously healthy 6-year-old girl with a 2-day history of cough was referred because of fever and dyspnoea. Respiratory distress was evident from tachypnoea (respiratory rate, 48/min) and supraclavicular and intercostal inspiratory retraction (Table 1). Bilateral expiratory rhonchi were apparent on auscultation, together with insufficient vesicular sounds. The patient required oxygen supplementation (5 L/min) because percutaneous oxygen saturation (SpO_2) was 84% when breathing room air. A chest roentgenogram on admission indicated air leakage into the mediastinum, together with bilateral hyperaeration and a right middle-lobe infiltrate (Fig. 1). She was treated with continuous inhalation of a bronchodilator and intravenous prednisolone (10–30 mg daily for 4 days), as well as antiviral therapy. On day 3 after admission, the radiographic SPM findings had resolved.

Case 2

An 8-year-old boy with a past history of allergic rhinitis had fever and cough for 1 day, and presented with chest pain and dyspnoea. Tachypnoea (36/min) was accompanied by supraclavicular and intercostal inspiratory retraction (Table 1). Vesicular sounds were insufficient over the left hemithorax, with neither rhonchi nor rales. The patient required oxygen supplementation (3 L/min) because SpO_2 was 91% when breathing room air. Chest roentgenography on admission indicated air leakage into the mediastinum and neck, as well as bilateral hyperaeration and a left lower lobe infiltrate (Fig. 1). The patient was treated with continuous inhalation of a bronchodilator and intravenous administration of both aminophylline (0.6 mg/kg/h for 7 days) and prednisolone (10–30 mg daily for 3 days), together with antiviral therapy. On day 4 after admission, there was no evidence of SPM.

Nineteen IV-infected children with pneumonia (n = 16) or bronchitis (n = 3) were admitted to our department from 9 August to 25 September. Clinical and laboratory features, treatment, and outcomes are shown in Table 1. The two

TABLE 1. Clinical and laboratory features, treatments, and outcomes of pneumonia or bronchitis in 19 children infected with 2009 pandemic influenza A (H1N1) virus

Patient no.	Age (years)	Gender	Underlying disease	Time from onset to admission (days)	Main symptom	Body temperature (°C)	Heart rate (beats/min)	Respiratory rate (/min)	SpO_2 (%)	Inspiratory (%) retraction	Auscultation finding	Respiratory disease	WBC (lymphocyte count) (× 10^9 cells/L)	CRP (mg/dL)	LDH/CK (IU/L, IU/L)	Antiviral therapy	Anti-biotic	Duration of oxygen supplementation (days)	Duration of admission (days)	Outcome
1 (SPM)	6	F	Healthy	3	Respiratory distress	39.2	156	48	84	Remarkable	Rhonchi, poor vesicular sounds	Pneumonia	10.9 (0.4)	4.72	193/86	Oseltamivir	MINO	6	13	Recovered
2 (SPM)	8	M	Allergic rhinitis	2	Chest pain	39.9	132	36	91	Remarkable	Poor vesicular sounds	Pneumonia	9.2 (0.6)	3.65	200/75	Oseltamivir	CEZ	5	10	Recovered
3	1	M	Healthy	2	Cough	38.9	126	48	ND	Negative	Normal	Bronchitis	15.6 (6.9)	6.88	424/126	Oseltamivir	PIPC	0	4	Recovered
4	4	F	Healthy	7	Cough	39.7	144	42	96	Negative	Rales	Pneumonia	7.7 (1.9)	1.03	309/45	None	CEZ	0	4	Recovered
5	9	M	Healthy	2	Respiratory distress	40.1	153	60	92	Positive	Rales	Pneumonia	9.7 (0.6)	3.15	236/110	Oseltamivir	CEZ	2	6	Recovered
6	8	F	Healthy	2	Cough	39	150	42	94	Positive	Rales, poor vesicular sounds	Pneumonia	8.7 (0.4)	2.48	252/112	Oseltamivir	ABPC/SBT	2	8	Recovered
7	10	M	Asthma	1	Respiratory distress	38.8	102	54	84	Positive	Rhonchi, poor vesicular sounds	Pneumonia	10.2 (0.8)	3.64	218/108	Oseltamivir	ABPC/SBT	4	8	Recovered
8	8	M	Healthy	2	Cough	39.9	108	36	93	Negative	Poor vesicular sounds	Pneumonia	5.0 (0.3)	2.43	340/71	Oseltamivir	ABPC/SBT	2	7	Recovered
9	6	M	Cough-variant asthma	2	Respiratory distress	39.8	153	66	86	Positive	Poor vesicular sounds	Pneumonia, atelectasis	16.1 (0.5)	2.82	276/57	Oseltamivir	ABPC/SBT	11	18	Recovered
10	11	F	Healthy	2	Cough	39.6	146	48	94	Positive	Poor vesicular sounds	Pneumonia	7.1 (0.6)	0.38	184/55	Oseltamivir	CTX	3	6	Recovered
11	12	F	Healthy	2	Respiratory distress	38.3	134	48	92	Positive	Rhonchi, poor vesicular sounds	Pneumonia	11.3 (0.4)	2.99	215/ND	Oseltamivir	CEZ	4	6	Recovered
12	6	F	Asthma	2	Respiratory distress	39.6	157	28	90	Positive	Poor vesicular sounds	Pneumonia	10.0 (0.8)	1	288/208	Oseltamivir	None	5	8	Recovered
13	12	F	Allergic rhinitis	2	Respiratory distress	39.6	159	36	92	Positive	Poor vesicular sounds	Pneumonia	14.5 (0.3)	3.26	253/137	Oseltamivir	CEZ	3	6	Recovered
14	8	M	Asthma	2	Respiratory distress	38.7	124	42	87	Positive	Rhonchi, poor vesicular sounds	Pneumonia	6.1 (0.3)	0.75	275/87	Oseltamivir	CEZ	2	6	Recovered
15	12	F	Asthma	1	Respiratory distress	39.1	170	48	91	Positive	Rales, poor vesicular sounds	Bronchitis	22.7 (0.3)	1.17	245/86	Oseltamivir	CEZ	5	12	Recovered
16	8	M	Asthma	2	Respiratory distress	39.4	150	52	89	Positive	Rales, poor vesicular sounds	Pneumonia	17.2 (0.5)	1.18	242/249	Oseltamivir	CEZ	7	10	Recovered
17	4	M	Asthma	3	Respiratory distress	37.3	138	48	93	Positive	Rhonchi	Bronchitis	7.9 (2.8)	2.47	221/ND	Oseltamivir	CEZ	3	6	Recovered
18	8	M	Healthy	5	Cough	38.1	102	30	96	Negative	Normal	Pneumonia	3.4 (1.4)	0.1	307/64	Oseltamivir	CAM	0	5	Recovered
19	13	M	Healthy	4	Cough	38	104	24	ND	Negative	Normal	Pneumonia	7.2 (1.0)	6.78	384/1931	Oseltamivir	ABPC/SBT	0	7	Recovered

ABPC, ampicillin; CAM, clarithromycin; CEZ, cefazolin; CK, creatine phosphokinase; CRP, C-reactive protein; CTX, cefotaxime; F, female; LDH, lactate dehydrogenase; M, male; MINO, minocycline; ND, not determined; PIPC, piperacillin; SBT, sulbactam; SPM, spontaneous pneumomediastinum; SpO_2, percutaneous saturation of oxygen under room air; WBC, white blood cell count. Clinical and laboratory findings were recorded on admission.

patients with SPM represented 10.5%. To the best of our knowledge, this is the first report of SPM complicating pneumonia in IV-infected children.

A rare disorder, SPM typically is triggered by respiratory infection and inflammation. It follows intrathoracic pressure increases leading to rupture of alveoli or pneumatoceles near the mediastinal pleura with air leakage along vessels. Additionally, SPM can complicate pulmonary emphysema, with air drainage through the interstitium to the hilum, mediastinum, neck, and skin [7].

Pneumonias complicated by SPM most notably include *Pneumocystis jirovecii* pneumonia (PCP) [8]. High-resolution chest computed tomography in a PCP patient was reported to show SPM associated with peripheral air-trapping in the right middle lobe [8]. Two patients presenting with tension SPM, which is often fatal, both had AIDS-related PCP [9]. SPM subsequent to seasonal influenza is relatively rare, even though a patient with influenza virus bronchiolitis complicated by SPM has been described [10]. Our observations suggest a possible high prevalence of SPM in IV-infected children that may reflect a characteristic pathology in the respiratory tract.

In children, SPM is observed most commonly in status asthmaticus, bronchiolitis or bronchitis with casts [11,12], but it may also occur in any patient during a Valsalva manoeuver related to coughing, forceful vomiting or wheezing [13]. Interestingly, SPM has occurred in status asthmaticus associated with influenza [14]. Clinicians, therefore, should consider the possibility of SPM when examining and treating children with both novel influenza and wheezing or status asthmaticus.

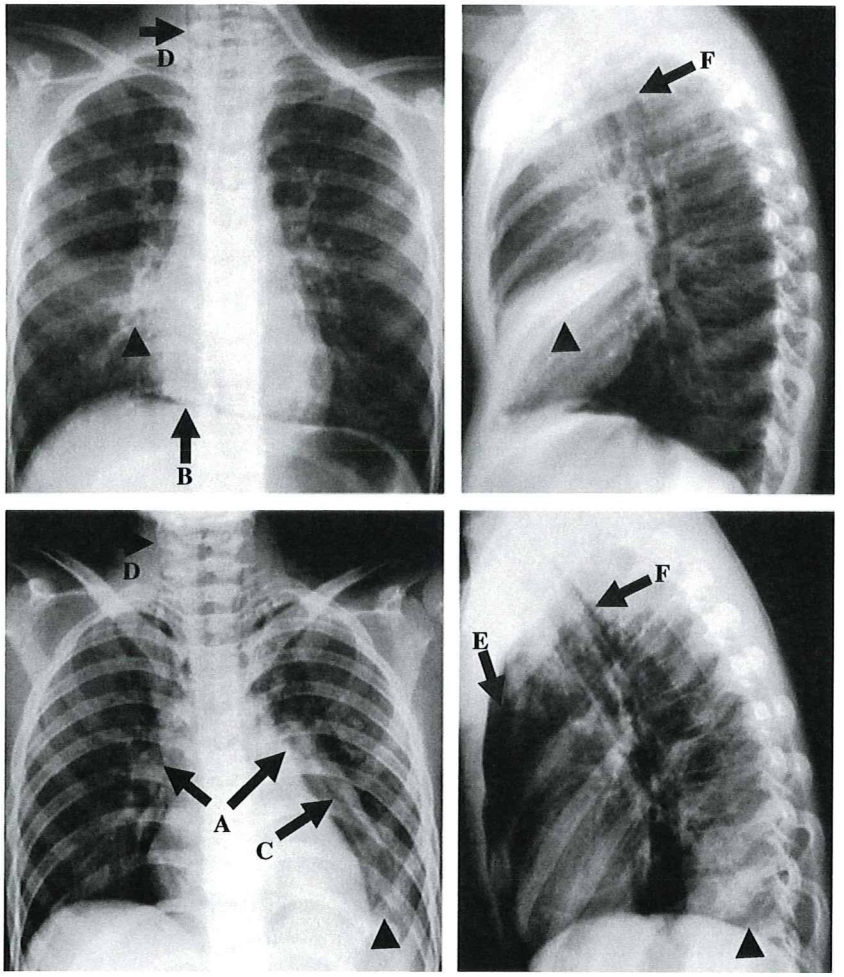

FIG. 1. Chest roentgenograms showing spontaneous pneumomediastinum (arrows) complicating pneumonia in two children (case 1, upper row; case 2, lower row) infected with influenza A (H1N1) virus. The left images are frontal (posterior-anterior) views, and the right images are lateral views. Arrowheads indicate pulmonary infiltrates in each patient. A, spinnaker sail sign (angel wing sign); B, continuous diaphragm sign; C, vertical lucent streak on the left side of the heart; D, subcutaneous emphysema; E, retrosternal emphysema; F, posterior superior mediastinal emphysema.

In summary, SPM in children generally resolves spontaneously with aggressive supportive care. Our patients illustrate the specific clinical and radiologic signs associated with SPM complicating pneumonia in IV-infected children. Surveillance results concerning paediatric deaths (n = 36) associated with the 2009 pandemic IV infection have been reported for April to August in the USA [15]. Clinicians should also be aware that an early diagnosis of influenza can allow for prompt initiation of antiviral therapy for children with an increased risk of severe illness.

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Transparency Declaration

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REVIEW ARTICLE

Macrolide-resistant *Mycoplasma pneumoniae*: characteristics of isolates and clinical aspects of community-acquired pneumonia

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Abstract *Mycoplasma pneumoniae* is one of the main pathogens causing community-acquired respiratory tract infections in children and adults. Macrolide (ML) antibiotics are recognized generally as first-choice agents for *M. pneumoniae* infections, and these antibiotics were thought to have excellent effectiveness against *M. pneumoniae* for many years. In 2000, however, *M. pneumoniae* showing resistance to macrolides was isolated from clinical samples obtained from Japanese pediatric patients with community-acquired pneumonia (CAP). Since then, prevalence of ML-resistant *M. pneumoniae* isolates in pediatric patients has increased rapidly. In 2007, ML-resistant *M. pneumoniae* isolates were obtained from Japanese adults with CAP; numbers of such isolates also have gradually increased in Japan. Recently, similar antimicrobial resistance in *M. pneumoniae* has begun to emerge worldwide. In this review, we focus on changes of ML-resistant *M. pneumoniae* from year to year and consider resistance mechanisms as well as clinical features of patients with resistant *M. pneumoniae* infection.

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Introduction

Sixteen *Mycoplasma* species have been isolated from human respiratory and urogenital specimens [1]. One of these species, *M. pneumoniae*, is a main pathogen in respiratory tract infections (RTI) acquired in the community. In school-aged children and young adults with community-acquired pneumonia (CAP), *M. pneumoniae* accounts for as many as 10–30% of cases [2–8]. In distinction, *M. pneumoniae* pneumonia in younger children and in the elderly is infrequent but not rare [9–15].

M. pneumoniae pneumonia is diagnosed based on characteristic chest radiographic abnormalities, patient symptoms, and clinical laboratory data. Diagnosis ultimately is confirmed using serologic tests performed upon paired sera obtained during both acute and convalescent phases. Conventional culture using pleuropneumonia-like organism (PPLO) broth, which requires more than 2 weeks, has not been carried out routinely. As a consequence, antibiotic choice usually is empirical.

Development of molecular methods such as polymerase chain reaction (PCR) assays to be used in combination with conventional diagnostic tests using serology and culture have contributed to improved diagnosis and characterization of *M. pneumoniae* infection in pediatric and adult patients [16–28]. Macrolides (ML) such as clarithromycin (CAM) and azithromycin (AZM) generally are recognized as first-choice agents against *M. pneumoniae* [29–33]. However, in parallel with increased use of oral ML with a

14-membered ring (14-ML) and AZM for RTI, *M. pneumoniae* showing resistance to ML has been isolated increasingly in clinical samples from Japanese pediatric patients with CAP [31, 34, 35]. Prevalence of ML-resistant (ML^r) *M. pneumoniae* in pediatric patients has increased rapidly [35, 36] and ML^r *M. pneumoniae* is beginning to be isolated worldwide [37–41].

In this review, we summarize the emergence and increase of ML^r *M. pneumoniae* isolates, resistance mechanisms, and clinical features of ML^r *M. pneumoniae* infection.

Diagnosis of infection

M. pneumoniae infection tends to show cyclic epidemics every 3–5 years; these outbreaks are particularly likely to occur in summer or early fall [42–46]. Although the incidence of *M. pneumoniae* pneumonia is highest in children 5–9 years old, such pneumonia also occurs frequently in younger children and in the elderly. Prevalence of *M. pneumoniae* infection also may vary according to population and diagnostic methods used.

Isolation of *M. pneumoniae* by culture from clinical samples such as throat or nasopharyngeal swabs was considered standard for diagnosis several years ago. However, *M. pneumoniae* is difficult to grow in liquid culture; cultures using PPLO broth require at least 2 weeks for completion. Compared with serologic tests or molecular techniques, including PCR, sensitivity of cultures may be <60% or 70%, even in laboratories with expertise [6, 18, 20]. Therefore, culture methods are only used rarely for routine diagnosing *M. pneumoniae* infection.

Serologic methods are used most frequently to diagnose *M. pneumoniae* infection. Frequently used, widely available serologic tests for *M. pneumoniae* include complement fixation (CF), enzyme immunoassay (EIA), and particle agglutination (PA) assays. However, these ordinarily require paired sera obtained during acute and convalescent phases in order to demonstrate rises in antibody titers; fourfold increase is thought to be significant. The second sample should be obtained 7–14 days after the first, provided that *M. pneumoniae* infection still is suspected.

Although one alternative, the ImmunoCard based on an immunoglobulin M (IgM) assay, is rapid and easy to perform, reliable diagnosis of *M. pneumoniae* infection still cannot be made on the basis of single acute-phase sera; confirmation using convalescent-phase samples still is necessary, as otherwise, both false-positive and false-negative results are frequent [24, 47, 48]. Additionally, sensitivity of IgM assays tends to be low, particularly for adult patients who are known to have weak IgM responses during primary infection or reinfection [49, 50]. Neither culture nor serologic testing can provide timely information for guiding choice of chemotherapeutic agents to use for early intervention. Serologic methods generally have only low sensitivity during the acute phase of disease.

Development of molecular methods (e.g. PCR) has lessened the importance of culture as a way to detect *M. pneumoniae* directly. Two specific targets in the P1 adhesion gene and the 16S ribosomal RNA (rRNA) gene are the main ones used in PCR assays to detect *M. pneumoniae* DNA. Various PCR methods have been developed in several laboratories. *M. pneumoniae* detection by conventional PCR or real-time PCR has been examined as an approach to early diagnosis. Many studies have described application of multiplex PCR, hybridization assays, nucleic acid sequence-based amplification (NASBA), real-time PCR using a molecular beacon probe or TaqMan probe, or cycling probe, to the identification of *M. pneumoniae* DNA [16–28]. Among these methods, real-time PCR has both high sensitivity and high specificity and can detect pathogen DNA even when damaged by empirical administration of antibiotics. Sensitivity (60–100%) and specificity (96.7–100%) of real-time PCR are both higher than those of serologic assays for *M. pneumoniae* [18, 19, 22, 24–26]. Almost all PCR-positive cases (>90%) also were confirmed serologically [22, 24]. In addition, real-time PCR using a fluorescent probe allows continuous monitoring of in vitro DNA amplification, eliminating nonspecific amplification product using fluorescence; no gel electrophoresis is needed. This makes the method suitable for clinical laboratory settings. A molecular approach including real-time PCR is useful for confirming *M. pneumoniae* and providing rapid diagnosis of CAP, thus permitting rational choice of effective antimicrobial agents.

Mechanisms of ML resistance in *M. pneumoniae*

Intrinsically, absence of cell walls in *M. pneumoniae* confers resistance to β-lactams, and indeed to all antibiotics that inhibit synthesis of cell walls. In *M. pneumoniae* infections, 14-ML and 15-membered ring ML (15-ML) usually are considered the first-line agents. *M. pneumoniae* ordinarily are susceptible to all ML, including ketolides.

Main mechanisms of microorganism resistance to ML identified in previous studies include modification of the target sites in 23S rRNA by methylation or mutation, as well as on efflux pump [51–53]. ML act on the 50S ribosomal subunit to inhibit protein synthesis. The site of peptide bond formation on the large 50S ribosomal subunit is associated with the central loop in domain V of 23S rRNA. Nucleotides present in the central loop are necessary constituents of the ML binding site, which implies that all ML bind to the same site (Fig. 1a). A loop of hairpin 35

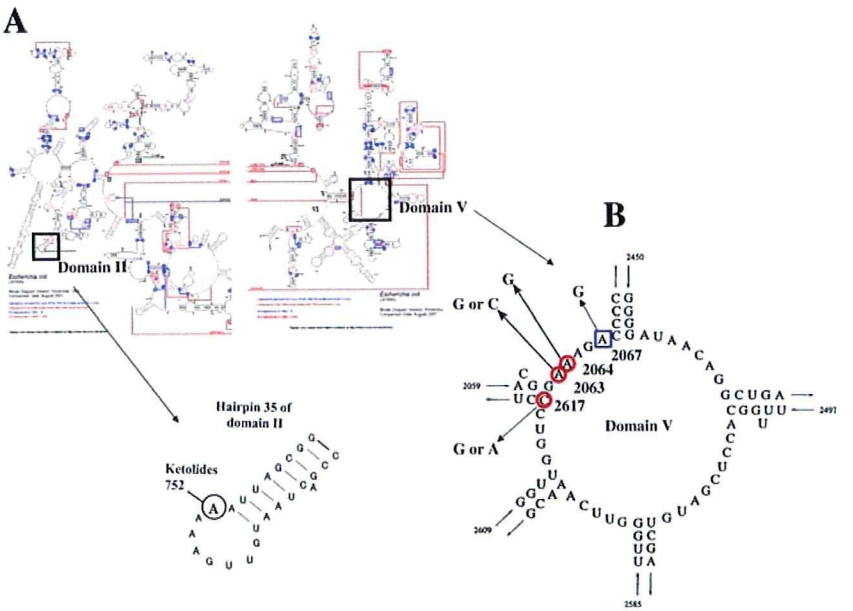

Fig. 1 Binding site of macrolides on the 23S ribosomal RNA. **a** Secondary structure of the large subunit of ribosomal RNA (*Escherichia coli*; http://www.rna.ccbb.utexas.edu/). **b** Central loop in domain V (*Mycoplasma pneumoniae* numbering) and the loop of hairpin 35 in domain II (*E. coli* numbering) of 23S rRNA as modified according to [34, 51]. *Circles* indicate positions of mutations associated with macrolide resistance in clinical isolates. The *square* shows the position of a substitution associated with 16-membered-ring macrolide resistance in a mutant selected in vitro

in domain II also may be part of binding site of ketolides; mutations involving ribosomal proteins L4 and L22 may contribute as well [51–54].

ML inhibit protein synthesis mainly by binding to domain V of 23S rRNA at nucleotide positions 2063 and 2064 (*M. pneumoniae* numbering); these positions appear essential for binding (Fig. 1b) [55]. Mutations at A2063 or A2064 confer the highest resistance to these antimicrobials. A lower level of antibiotic resistance is caused by mutations at positions A2067 and C2617, which are near A2063 and A2064 in the secondary structure, although slightly present outside the central point of ML interaction. The A2067G mutation was reported to cause highest levels of resistance only for 16-membered-ring ML (16-ML), as it interferes with formation of the covalent bond specific to 16-ML [56].

Ketolides are a new class of antimicrobial agent characterized by the substitution at position 3 from L-cladinose to keto group. These antibiotics are active against *Streptococcus pneumoniae* strains resistant to ML because of interaction at both A2058/2059 (*Escherichia coli* numbering) in domain V and A752 in domain II [51, 54, 57]. Substitutions in ribosomal proteins L4 and L22 have been found to confer resistance in *S. pneumoniae* [58, 59], but mutations in domain II of 23S rRNA and in ribosomal proteins L4 and L22 are not involved in ML resistance of clinical *M. pneumoniae* isolates [60]. Furthermore, neither plasmids nor *erm* genes mediating ribosomal modification have been described with respect to *M. pneumoniae*.

Fluoroquinolone (FQ)-resistant *M. pneumoniae* mutants may be obtained in vitro by exposure to subinhibitory concentrations of FQ [61]. These mutants possess mutations in *gyrA* and *gyrB* genes, which express topoisomerase II, and in *parC* and *parE* genes, which express topoisomerase IV; both of these enzymes are essential for bacterial DNA replication. FQ often are chosen as empiric treatment of respiratory tract infection in adults, as they are active against the main pathogens, including *S. pneumoniae*, *Haemophilus influenzae*, and *M. pneumoniae*. In contrast to in vitro findings, no FQ-resistant strains have been observed in clinical settings.

Tetracyclines inhibit protein synthesis by preventing association of aminoacyl-tRNA to the acceptor (A) site of the ribosome [62, 63]. These antibiotics bind to the ribosomal 30S subunit involving 16S rRNA and several ribosomal proteins. Only the *tetM* determinant associated with conjugative transposon Tn*916*, which confers resistance to the tetracyclines in *M. hominis*, has been documented so far [64]. These agents are administered for treatment of *M. pneumoniae* infection in adults and in pediatric patients >8 years, but development of resistance to tetracyclines has not yet been reported.

Association of ML resistance and mutation site in 23S rRNA from ML^r *M. pneumoniae*

Specific sites of the 23S rRNA mutation contribute to ML resistance in *M. pneumoniae*. In actuality, based on our results, clinical isolates with mutations at positions A2063 and A2064 show high resistance to 14-ML and 15-ML, with minimal inhibitory concentration (MIC) $\geq$ 32 μg/ml (Table 1). Especially, the A2063G mutation conveys the highest level of resistance to 14-ML, 15-ML, and telithromycin (TEL) but intermediate resistance to josamycin and rokitamycin belong to 16-ML. The A2063C and

Table 1 Antimicrobial activity of macrolides, fluoroquinolones, and minocycline against *Mycoplasma pneumoniae* strains isolated from clinical samples

ML^r class		MIC range (μg/ml)				
Antimicrobial agent	Susceptible strains (n = 423)	A2063G (n = 96)	A2064G (n = 7)	A2063C (n = 1)	C2617A (n = 1)	C2617G (n = 1)
Erythromycin	0.00195–0.0313	32 to >64	64 to >64	>256	1	8
Clarithromycin	0.00049–0.0313	32 to >64	16 to >64	>256	0.5	1
Azithromycin	0.00012–0.00195	16 to >64	16–64	16	0.0313	0.0313
Telithromycin	0.00024–0.0039	16 to >64	1–16	ND	0.0625	ND
Josamycin	0.0156–0.0625	0.0625–64	64 to >64	64	0.0625	0.25
Midecamycin	0.0625–0.25	2 to >64	>64	64	ND	0.25
Rokitamycin	0.0039–0.0313	0.0156–16	8–16	4	0.0313	0.0625
Levofloxacin	0.125–1	0.5–1	0.5–1	ND	1	ND
Moxifloxacin	0.0625–0.125	0.0625–0.125	0.0625–0.125	ND	0.125	ND
Sitafloxacin	0.0313–0.0625	0.0313–0.0625	0.0313–0.0625	ND	0.0625	ND
Garenoxacin	0.0313–0.0625	0.0313–0.0625	0.0313–0.0625	ND	0.0313	ND
Minocycline	0.0313–2	0.0625–1	0.0313–1	ND	1	ND

MICs of susceptible strains and resistant strains with A2063G, A2064G, and C2617A mutations were determined in our laboratory [31, 36]

MIC data for strains with A2063C and C2617G mutations were adapted from [35, 65]

MIC minimal inhibitory concentration, *ML*^{r}macrolide resistance, *ND* not determined

A2064G mutations result in a high level of resistance to all ML [36, 65]. The ketolide TEL is affected by both mutations, although A2063G was associated with MICs higher than those associated with A2064G.

MICs for minocycline (MINO) and FQ in ML^r strains were equivalent to those in susceptible strains. No strains having resistance to MINO and FQ have been observed among clinical isolates.

Several strains with the C2617 to G or A mutations show slight increases in MIC of ML compared with those with mutations at positions 2063 and 2064. A2063G in domain V of 23S rRNA is the most frequent mutation association with ML resistance, followed by A2064G; others (A2063C, C2617G, and C2617A) are rare.

Ribosomal protein L4 and L22 mutants with a few amino acids deleted or inserted have been described, but roles of these mutations in resistance to ML are uncertain [60]. No mutation has been detected in domain II of 23S rRNA.

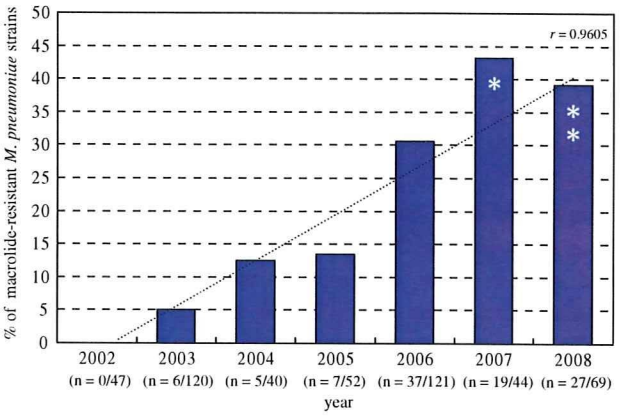

Fig. 2 Increases in numbers of macrolide-resistant *Mycoplasma pneumoniae* strains from 2002 to 2008 in Japan according to our analyses. *Asterisks* indicate macrolide-resistant strains isolated from adult patients. The *dotted line* can be expressed by the equation $y = 7x - 8.62$ ($r = 0.9605$)

Emergence and increase of ML^r strains

ML^r *M. pneumoniae* isolates possessing a nucleotide mutation in 23S rRNA first were isolated from pediatric patients with CAP, as reported by Okazaki and colleagues in 2001 [34].

Together with growing use of ML in Japan, resistant strains increased rapidly year by year (Fig. 2) [36]. Along with greater overall prevalence of *M. pneumoniae* infection in 2006, ML^r strains increasingly were isolated and identified in diverse regions across Japan.

In Japanese adult CAP patients, ML^r strains first were isolated in 2007; since then, occasional isolations have continued [66]. An increase in ML^r strains among adult patients, las in pediatric patients, may occur in the future. However, FQ are recommended for CAP treatment in adults, and no FQ-resistant strains have been observed among isolates from adult patients with CAP.

Emergence of ML^r isolates having either an A2063G or an A2064G mutation has been reported not only in Japan but also in other countries, including France, the USA, Denmark, and China [37–41]. A notably high isolation rate (92%) of ML^r strains was reported in China [38, 40].

The emergence and increase of ML^r *M. pneumoniae* has attracted the attention of microbiologists worldwide. Several recent studies demonstrated direct detection of ML^r isolates using real-time PCR [37, 41, 67].

DNA profiles of ML^r- and ML-susceptible (ML^s) strains obtained by pulsed-field gel electrophoresis (PFGE) have suggested classification into two groups [36, 68]. Significant differences in these DNA patterns were not recognized between strains, geographic locations, or years.

PCR-based restriction fragment length polymorphism (RFLP) types of the P1 adhesin gene also were used to divide strains into two types. ML^r isolates were present in both types [38, 39, 69, 70], showing no predominance.

Clinical aspects of ML^r *M. pneumoniae* infection

The clinical course of patients with ML^r *M. pneumoniae* infection appears to be prolonged [36, 66, 71, 72]. Patients infected with ML^r strains mostly had been treated with ML previously [36, 40]. Among CAP patients infected with ML^r, treatment frequently was changed from ML to MINO or levofloxacin (LVFX) because of either persistent symptoms (i.e., fever and cough) or unresolved or worsening chest radiographic abnormalities. In our observations, treatment was changed from ML to MINO or LVFX in 8.2% of patients with ML^s strains and in 39.7% of patients with ML^r strains, representing a significant difference between the two patient groups ($P < 0.01$). ML also was more frequently discontinued in favor of MINO among ML^r patients than ML^s patients in a report by Suzuki et al. [71]. Neither LVFX nor MINO are recommended for pediatric patients. However, when ML are ineffective against *M. pneumoniae* infection, pediatricians have little choice of antimicrobials except for MINO. In adult inpatients infected with ML^r strains, antibiotic agents were changed from CAM to intravenous pazufloxacin when symptoms did not improve [66]. Orally administered respiratory FQ such as moxifloxacin, sitafloxacin, and garenoxacin may be selected for adult outpatients with ML^r strains.

Among clinical features of ML^r *M. pneumoniae* infection, fever duration has been significantly longer than for ML^s-strain infection. In our data, fever persisted after ML initiation for 1.6 ± 0.8 days in ML^s *M. pneumoniae* infection and for 4.1 ± 2.3 days in ML^r-strain infection, showing a significant difference ($P < 0.01$). In other studies, febrile days during ML administration also were significantly greater in ML^r-infected patients than in ML^s-infected patients (3.5–4.0 days vs. 1.0–1.5 days) [71, 72]. Furthermore, the mean duration of persistent cough after ML administration was 7.0 days in ML^s-infected patients and 11.4 days in ML^r-infected patients [72]. Finally, the efficacy rate of ML therapy was 91.5% and 22.7% in ML^s- and ML^r-strain infections, respectively [72].

Fever may have resolved spontaneously in some patients with continuing ML treatment for ML^r infections; *M. pneumoniae* infection is associated with occasional spontaneous symptomatic recovery. According to previous reports, fever duration was 1 week in symptomatically treated *M. pneumoniae* infection [73, 74]. In another study, mean fever duration in hospitalized patients receiving AZM or EM was 2.1 days, whereas fever persisted for about 1 week in patients not receiving ML [75]. Similarly, mean cough duration was reported as 8.5 days [76]. Although treatment failure or serious illness has not yet been attributed to ML^r-strain infection, the clinical course of patients infected with ML^r strains may be prolonged.

A brief consideration of the host response is helpful here. *M. pneumoniae* resides on the surface of ciliated human respiratory epithelial cell. Several membrane proteins in *M. pneumoniae* have high affinity for various surface receptors on host cells [77]. Through interaction with recognition receptors, including toll-like receptors 2 and 6, mycoplasma membrane lipoproteins are able to induce host immune responses [77, 78]. Adherence of *M. pneumoniae* to host cells in the respiratory tract is mediated by the P1 adhesin protein and accessory proteins [79–82]. Following adherence, macrophages become activated and release cytokines, and a mononuclear cell inflammatory response is established. Human lung epithelial cells infected with *M. pneumoniae* induce expression of interleukin (IL)-8, which is both a potent chemoattractant and an activator of neutrophils, monocytes, and T lymphocytes [83–85]. In particular, significant production of IL-8 and IL-18, a cytokine that induces interferon gamma (IFN-γ) production and promotes a type 1 cytokine response, is associated with the severity of *M. pneumoniae* pneumonia in children [86–88] and adults [89].

As previously described, the inflammatory response to *M. pneumoniae* infection is considered to play a crucial role in the pathogenesis of the ensuing clinical disease. ML possess antimicrobial activity against *M. pneumoniae*, and also have anti-inflammatory effects. For example, CAM is able to suppress IL-8 induction, even in ML^r *M. pneumoniae* infection [78, 86, 90]. Thus, clinical efficacy of ML in treating *M. pneumoniae* infection may reflect not only direct antimicrobial activity but also anti-inflammatory effects of inhibition of production of cytokines, including IL-8.

Symptoms and severity of illness due to *M. pneumoniae* were similar in younger and older patients, and the mortality rate was low, even in the elderly [13, 91, 92]. This disease ordinarily is mild, and reinfection may occur in adults who already experienced *M. pneumoniae* infection, as protective immunity usually is not established following initial infection [93]. However, symptoms appear to be

prolonged in certain clinical situations regardless of antimicrobial resistance [15, 94]. Takahashi and colleagues reported *M. pneumoniae* DNA in sputum after 2 weeks of MINO therapy. Elderly persons with *M. pneumoniae* pneumonia may have a longer clinical course than younger persons. Further observations from various patients infected with *M. pneumoniae* are necessary.

In the future, alternative treatment strategies, such as promptly initiated steroid therapy, might be considered for patients with ML^r strains as a symptomatic measure [95–97].

Conclusions

In pediatric patients with CAP, ML^r *M. pneumoniae* possessing a 23S-rRNA mutation was first isolated in 2000; numbers of these isolates have increased rapidly year by year in Japan. In other countries, ML^r *M. pneumoniae* also is emerging. Symptoms often are relatively prolonged in patients with ML^r *M. pneumoniae* infection. ML^r *M. pneumoniae* should be considered an emerging issue for not only pediatric patients but also adult patients with CAP.

No FQ-resistant *M. pneumoniae* strain has been observed thus far among specimens from adult patients with CAP. However, emergence of infections with FQ-resistant *M. pneumoniae* might occur, considering the increasing FQ prescription rate among adult patients. Further studies are needed to clarify the prevalence of ML^r *M. pneumoniae* in pediatric and adult patients with RTI and to establish clinical guidelines regarding the most appropriate antimicrobial agents to use against these strains. Interventions to prevent emergence and increases of novel antibiotic-resistant strains are needed, including rapid identification of pathogens by methods such as real-time PCR, as well as antimicrobial treatments capable of eliminating the organisms.

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