Fig. 1 Pediatric age distribution in bacterial meningitis

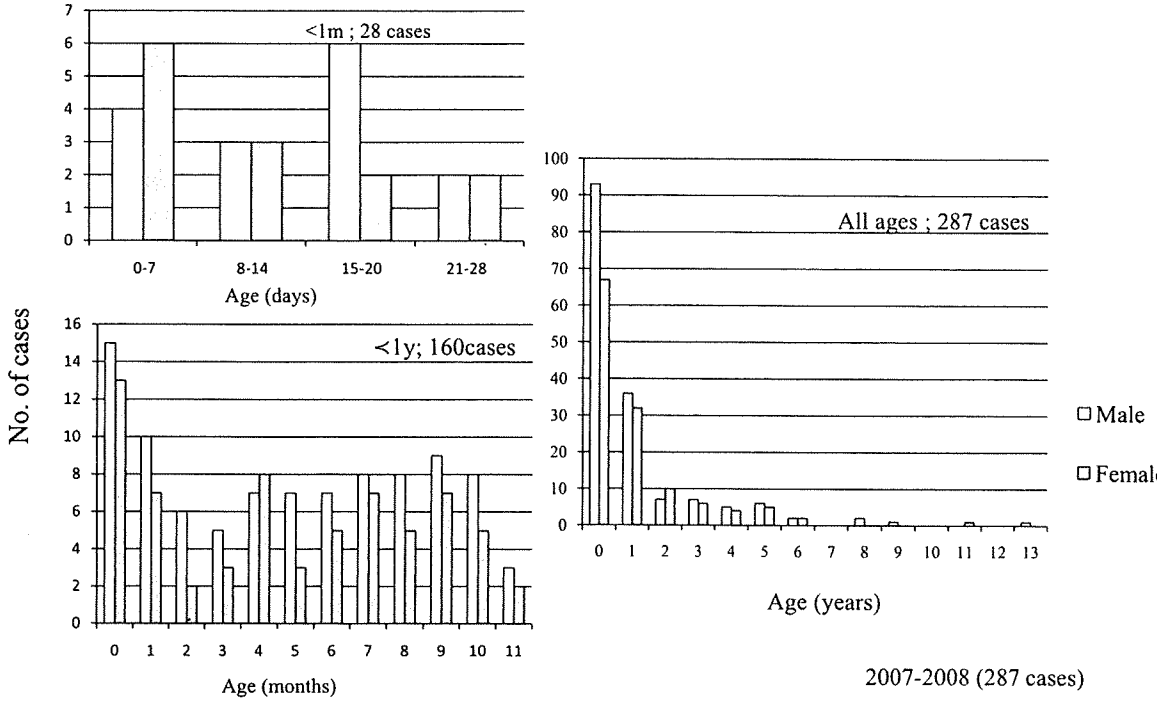
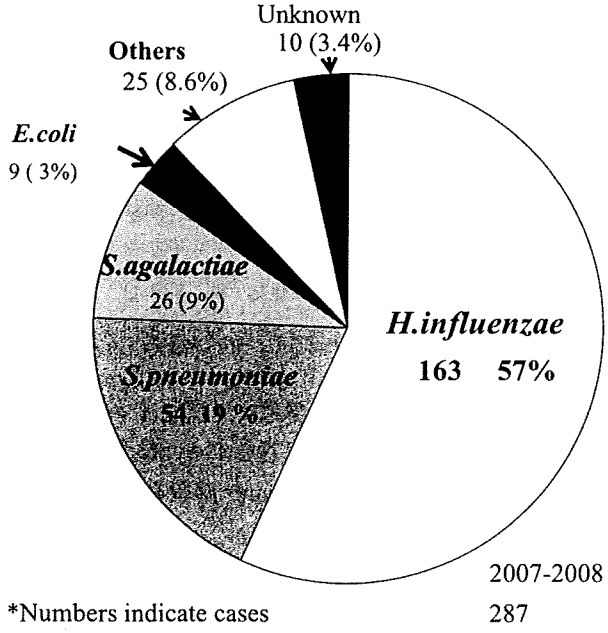


Fig. 2 Causative frequency



2. 年齢分布（Fig. 1）

年齢分布は，男児では1カ月未満が15例，1カ月～1歳未満が78例，1歳以上が67例，女児では1カ月未満が13例，1カ月～1歳未満が54例，1歳以上が60例であり，過去の報告[7]~[9]と同様であった．

3. 原因菌の種類（Fig. 2）

原因菌としては *H. influenzae* が163例（57%）と最も多く，次いで *S. pneumoniae* が54例(19.0%)，*Streptococcus agalactiae*（GBS）は26例（9%），*Escherichia coli* 9例（3.0%）の順であった．その他の25例としては，*Staphylococcus aureus* 5例，*Enterococcus faecalis* 3例，*Klebsiella pneumoniae* 2例，*Enterobacter cloacae* 2例，*Listeria monocytogenes* 2例，*Staphylococcus epidermidis* 1例，*Streptococcus pyogenes* 1例，*Psudomonas aeruginosa* 1例，*Staphylococcus capitis* 1例，*Streptococcus gallolyticus* 1例，*Streptococcus parasangius* 1例，*Campylobacter* sp. 1例，*Micrococcus luteus* 1例，*Bacillus cereus* 1例，グラム陽性菌1例，*K. pneumoniae*，*Neisseria meningitis*，*Pediococcus* 3菌種同時検出1例が含まれていた．原因菌が不明であった症例は10例であった．この割合は1997年に調査を開始して以来大きな差は認めなかった．

4. 原因菌別年齢分布（Fig. 3）

上位4菌種の年齢分布は，GBSは26株が分離され，22株は2カ月未満で，残りは3，4，5，7カ月に各1例見られた．*E. coli* は9株で，全例4カ月以下の乳児であった．

最も分離が多い *H. influenzae* は163株で，1カ月で3例の症例が認められ，その後6歳まで分布したが，1歳台に最も多いとの結果であった．一方，*S. pneumoniae* は54株で2カ月～13歳に分布しており，*H. influenzae* と異なり，6歳以上の年長児にも3例の報告があった．

5. *H. influenzae*，*S. pneumoniae* の薬剤感受性（Fig. 4）

薬剤感受性は，各施設で実施した成績，すなわち

Fig. 3　Major cause by age

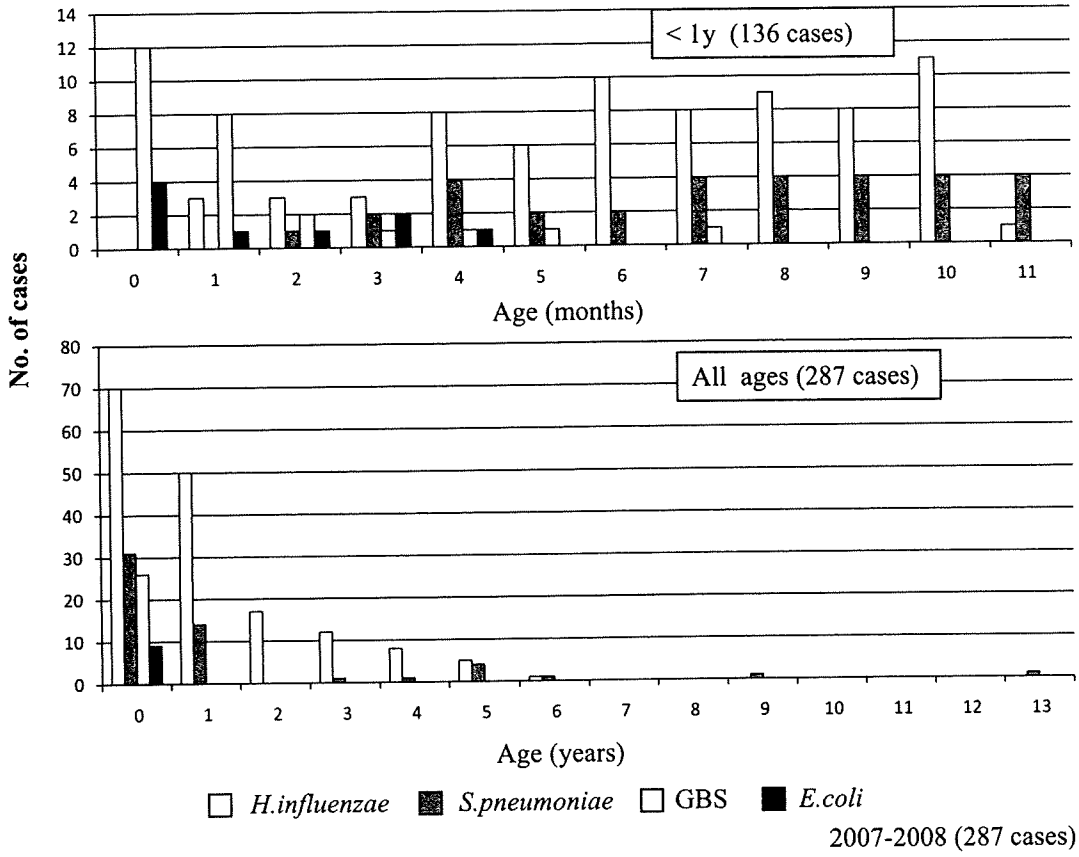
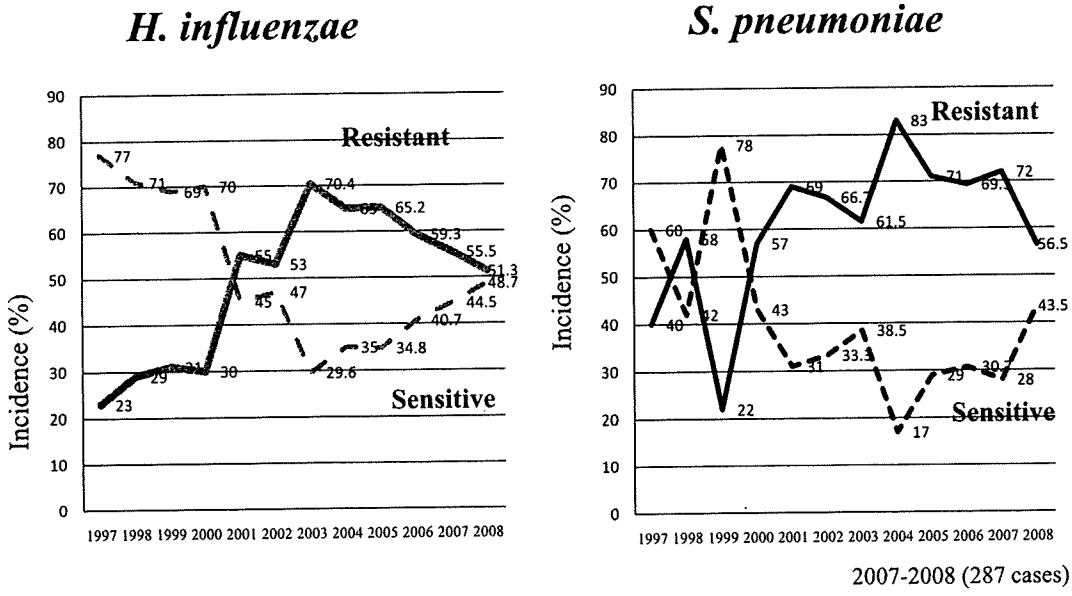


Fig. 4　Annual ratio of resistant strains



H. influenzae は ampicillin：ABPC に対する感受性，*S. pneumoniae* はペニシリン G に対する感受性で分類した．2001 年に *H. influenzae* の耐性菌の占める割合が半数を越え，2003 年には 70.4% と高い値を示したが，その後耐性率は減少し，2008 年には 51.3% となっている（$p<0.05$）．*S. pneumoniae* は，2000 年に耐性の割合が既に半数を越え，2004 年に 83.0% と最悪の事態となったが，2008 年は 56.5% であり，*H. influenzae* 同様減少の方向にある．

6. 死亡例及び菌種別の予後（Table 1，Fig. 5）

平成22年 1 月20日

Fig. 5 Prognosis by causative bacteria. (*Mortality)

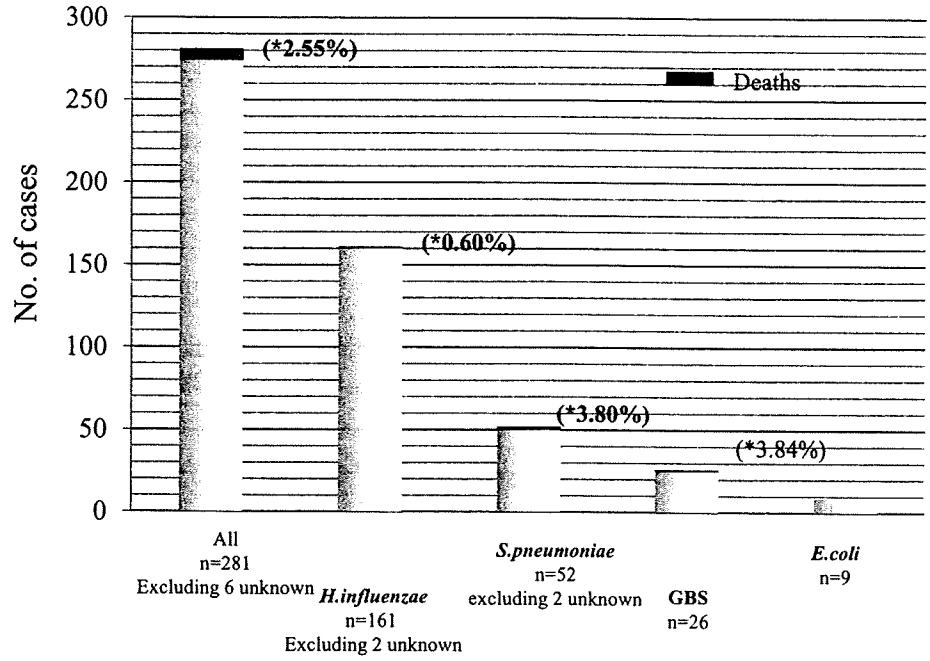
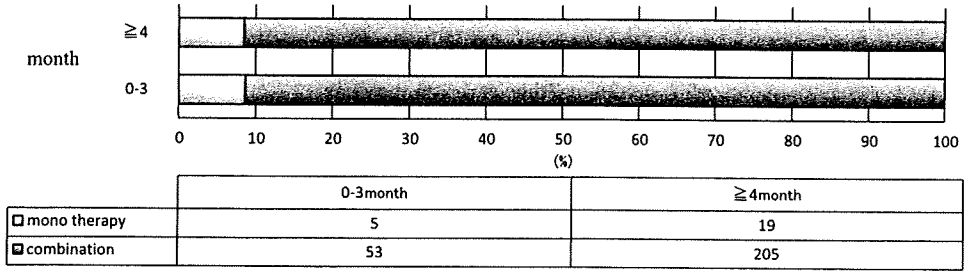
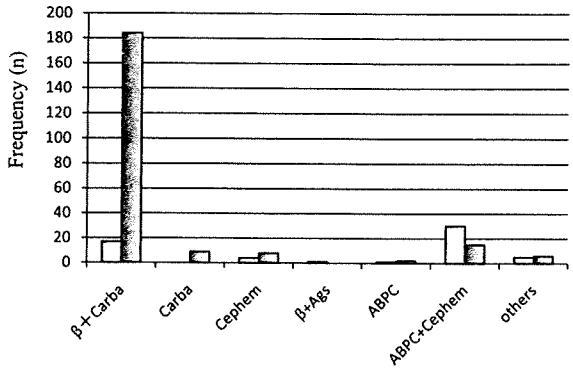


Fig. 6 Antibiotic use by age in initial treatment (2007-2008)

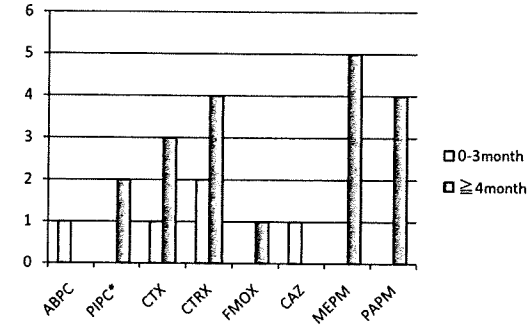


	0-3month	≧4month
mono therapy	5	19
combination	53	205

Primary care antibiotics setting



Monotherapy antibiotics



死亡は転医などの理由で予後が不明の6例を除く281例中7例（2.55%）で，年齢，性別，原因菌はTable 1に示した通りであった．死亡例の原因菌としては *S. pneumoniae* 2例（ペニシリン感性・耐性各1例），*H. influenzae* 1例(BLNAR)，GBS，*S. epidermidis*，*P. aeruginosa*，*B. cereus* 各1例であった．死亡例のうち基礎疾患の有無については *S. epidermidis* の症例は生後9日で脳腫瘍（基礎疾患が原因で死亡），*B. cereus* の症例は5歳3カ月で急性リンパ性白血病であったが，その他5例に基礎疾患は認められなかった．

Fig. 7　Comparison initial and second-line treatments by age

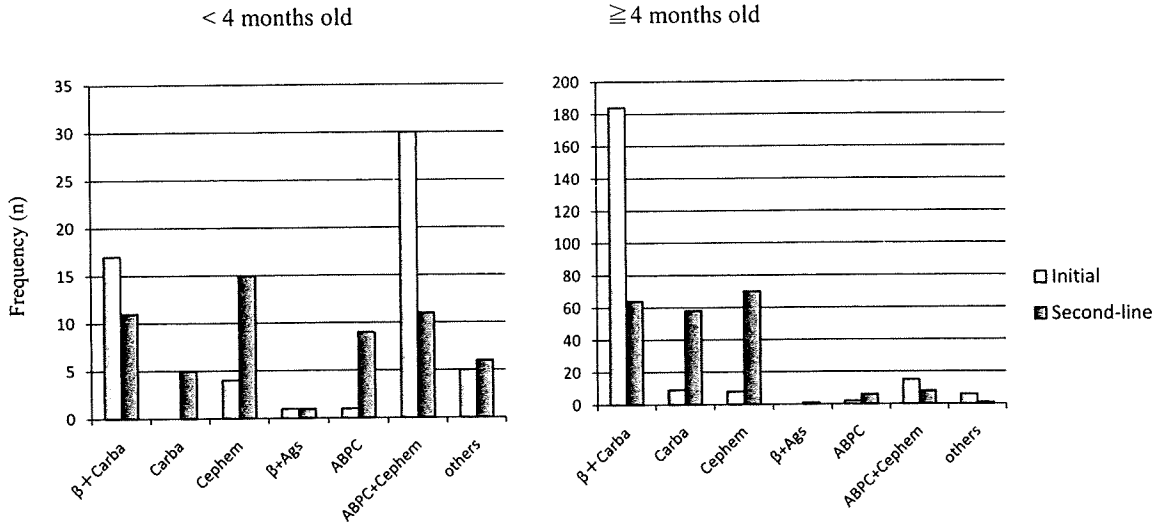


Fig. 8　Frequency of causative organisms by year

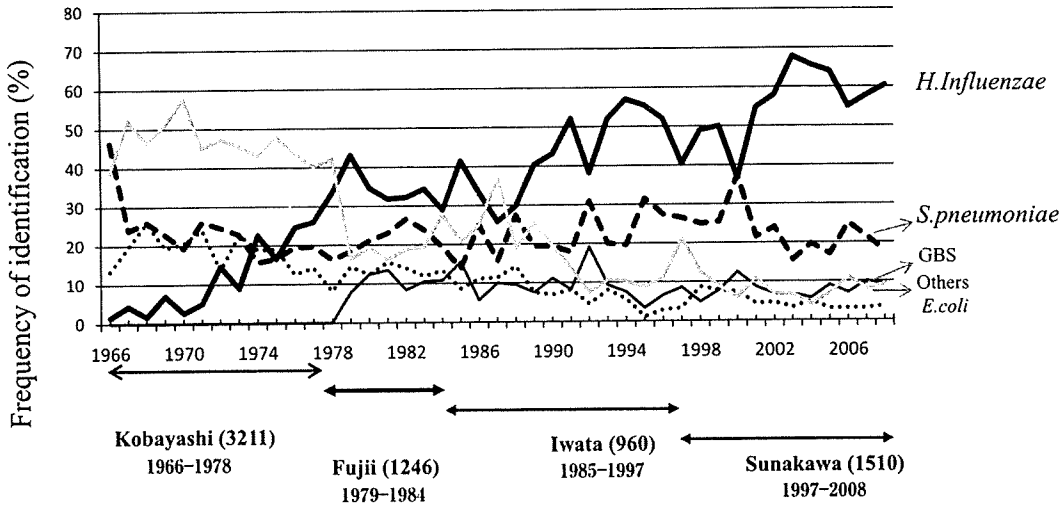


Table 1　Mortality

Age	Gender	Causative organism	Underlying disease
7 days	M	*P. aeruginosa*	
9 days	M	*S. epidermidis*	Brain tumor
1 month	F	GBS	
11 months	M	*S. pneumoniae* (Resistant strain)	
1.08 years	M	*H. influenzae* (BLNAR)	
3.1 years	F	*S. pneumoniae* (Sensitive strain)	
5.25 years	M	*Bacillus cereus*	ALL

2007-2008 (287 cases)

菌種別に死亡，後遺症の有無について解析を行ったが，後遺症については長期間観察している施設と入院期間だけの記載の施設があり，菌種別の死亡率のみを記した．全体の死亡率は 2.5% であり，*H. influenzae* は 0.6%，*S. pneumoniae* は 3.8%，GBS は前回の 10.5% から減少して 3.8% であった．菌種間に死亡率に差は認められなかった．

7．使用抗菌薬

1）初期治療抗菌薬の種類（Fig. 6）

初期治療に使用した抗菌薬の種類は，原因菌の頻度を考慮して 4 カ月未満と 4 カ月以上の年齢群で集計した．

平成22年 1 月20日

アンケート調査協力施設（回答時の施設名で記載）

所在地	病院名
＜北海道＞	旭川医科大学　市立札幌病院　網走厚生病院　市立小樽病院
＜青森県＞	青森県立中央病院
＜秋田県＞	秋田大学医学部
＜岩手県＞	盛岡赤十字病院
＜宮城県＞	仙台市立病院
＜山形県＞	山形県立中央病院
＜長野県＞	国立病院機構長野病院
＜新潟県＞	新潟大学医歯学総合病院
＜栃木県＞	国立病院機構栃木病院　足利赤十字病院　小山市立病院
＜群馬県＞	国立病院機構高崎病院　国立病院機構沼田病院　前橋赤十字病院　総合太田病院
＜茨城県＞	日立総合病院　水府病院
＜千葉県＞	千葉大学医学部
＜埼玉県＞	国立病院機構埼玉病院　埼玉社会保険病院
＜東京都＞	東京大学医学部　慶應義塾大学医学部　東京慈恵会医科大学　順天堂大学医学部　昭和大学病院 東邦大学医療センター大森病院　国立病院機構東京医療センター　都立大塚病院　済生会中央病院 公立福生病院　共済立川病院　東京女子医科大学東医療センター
＜神奈川県＞	横浜市立大学医学部　北里大学医学部　聖マリアンナ医科大学横浜市西部病院　東海大学医学部 昭和大学藤が丘病院　国立病院機構相模原病院　川崎市立川崎病院　横浜市立市民病院 伊勢原協同病院　平塚市民病院　平塚共済病院 海老名総合病院　横浜南共済病院　横須賀共済病院
＜静岡県＞	国立病院機構静岡医療センター　静岡県立総合病院　浜松赤十字病院
＜愛知県＞	名古屋市立大学医学部　藤田保健衛生大学　名鉄病院 国立病院機構名古屋医療センター　厚生連安城厚生病院
＜三重県＞	市立伊勢総合病院　山田赤十字病院
＜岐阜県＞	岐阜大学医学部　高山赤十字病院
＜石川県＞	金沢大学医学部
＜福井県＞	福井赤十字病院
＜京都府＞	京都大学医学部（小児科・ICU）国立病院機構舞鶴医療センター　京都第一赤十字病院　日本バプテスト病院
＜奈良県＞	大和高田市立病院
＜和歌山県＞	日本赤十字社和歌山医療センター
＜大阪府＞	大阪医科大学　大阪市立総合医療センター 住友病院　淀川キリスト教病院
＜兵庫県＞	国立病院機構姫路医療センター　兵庫県立塚口病院　神戸市立医療センター中央市民病院 公立八鹿病院明石市立市民病院　公立豊岡病院　赤穂市民病院　姫路赤十字病院　神鋼加古川病院
＜岡山県＞	川崎医科大学　川崎医科大学付属川崎病院
＜鳥取県＞	鳥取大学医学部
＜広島県＞	県立広島病院　広島市立舟入病院　吉田総合病院 広島赤十字・原爆病院　庄原赤十字病院 マツダ株式会社マツダ病院　尾道総合病院　広島通信病院　広島鉄道病院　JA 府中総合病院
＜島根県＞	国立病院機構浜田医療センター　島根県立中央病院　松江赤十字病院
＜愛媛県＞	愛媛大学医学部　愛媛県立中央病院
＜徳島県＞	徳島大学医学部
＜高知県＞	国立病院機構高知病院
＜福岡県＞	久留米大学医学部　産業医科大学　国立病院機構九州医療センター
＜佐賀県＞	国立病院機構嬉野医療センター
＜大分県＞	国立病院機構別府医療センター
＜熊本県＞	熊本大学医学部
＜長崎県＞	長崎大学医学部・歯学部
＜鹿児島県＞	鹿児島市立病院
＜沖縄県＞	沖縄県立中部病院　沖縄赤十字病院

初期治療薬剤の選択にあたっては，髄液の塗沫・染色やメニンギートキット（日本ビオメリュー）などの原因抗原検出のための迅速診断キットを用いて原因菌を推定して治療を開始する施設と，複数の抗菌薬を組み合わせて広域に対応する施設が見られたが，併用で開始する施設が多く見られた．原因菌としてGBS，*E. coli* が多い4カ月未満では，従来の標準的治療法とされているABPC＋セフェム（cefotaxime：CTX：ceftriaxone：CTRX）を組み合わせた使用が29/53（54.7%）と半数を占め，続いてカルバペネム＋β-lactamの併用が16/53(30.1%)であった．ABPC＋aminoglycosideは1例であった．*H. influenzae* や *S. pneumoniae* が原因として多くなる4カ月以降に関しては，PRSPを考慮したカルバペネム＋β-lactamの併用が184/205（89.7%）と前回の73%に比べさらに増加し，標準的治療とされてきたABPC＋セフェムの併用は15/205（7.3%）であった．セフェム単独は8/205（3.9%）と半減し，PRSPに効果が優れているカルバペネム単独は9/205（4.4%）であった．

2）最終治療（Fig. 7）

最終治療は原因菌の種類・薬剤感受性が判明した後に選択する例が多く，その結果に従って投与することから，併用→単独への変更が多く見られた．今回の調査では4カ月未満ではABPC＋セフェム併用例の約2/3がABPCまたはセフェムの単独使用に，4カ月以上ではカルバペネム＋β-lactam併用例の約2/3がセフェムまたはカルバペネムの単独に変更されていた．

8．ステロイド薬の併用

ステロイド薬の併用の有無について，記載がされていた3カ月未満の症例50例中20例が併用あり(40%)，3カ月以上の症例230例中205例が併用あり(91.4%)で，3カ月以上の症例でステロイド薬を使用する例が有意に多かった．ステロイド使用の有無による死亡，後遺症の有無と種類，原因菌別効果を調べたが，特に特徴は認められなかった．ステロイド薬の投与日数は1日投与5例，2日120例（60.6%），3日32例，4日36例，5日以上5例で，2日投与が主流であった．

考　察

今回287例の症例が集積された．小児髄膜炎の発生頻度について，年間の小児入院症例数1,000に対する割合は，2007年1.54，2008年1.62人で，1997年調査開始以降増減はみられないが，約20年前の藤井の報告[3]の3.1～4.0に比べ明らかに減少した（$p<0.001$）．細菌性髄膜炎の定点報告数も，過去9年間定点あたり0.01～0.03を前後し，増減がみられていない[11]．男女比は1.26：1であり，従来[1)~10)]の報告と大きな違いはみられず，年齢分布についても1997年以降大きな変化はみられなかった．

原因菌については，過去の報告と同様に生後3カ月まではGBS，*E. coli*が多く，その後*H. influenzae*，*S. pneumoniae*が主要原因菌となっていた．

我が国の小児細菌性髄膜炎の原因菌の変遷を見ると(Fig. 8)，小林の調査[1]の初期には*H. influenzae*の占める割合が極めて低い数字であり，1970年代に入り増加に転じ，その後年々比率が増し[1)~10)]，現在では約2/3を占めている．*S. pneumoniae*は1966年に約50%を占めていたが，翌年以降20～30%を維持している．

GBSは検査が一般化した1979年以降から報告が見られ1992年の19.1%が最高で，その後は10%前後を推移している．*E. coli*は1990年代以降10%以下の数値であった．

Hibワクチンの導入以降の海外で，原因菌として頻度の少なくなった*H. influenzae*は，ワクチンの未承認であった我が国では2005～2006年の調査で全体の55%を占め，相変わらず第一位であった．その上BLNARなどの耐性菌が増加している点[12]からも，2007年承認されたHibワクチンのリスクの高い乳幼児に対する早期の普及が望まれる．

薬剤耐性化が問題となっている*H. influenzae*と*S. pneumoniae*の各施設で実施した薬剤感受性は，ABPC耐性の*H. influenzae*は1997年に比べ有意に増加し，今回調査の2007年55.5%，2008年51.3%で，最も高かった2003年の70.4%に比べ年々減少の方向にあるといえる．ペニシリン耐性の*S. pneumoniae*も2007年72%，2008年56.5%であり，*H. influenzae*と同様に2004年の83%に比べ年々減少の方向にあった．現在我々が実施している，全国の小児由来*H. influenzae*と*S. pneumoniae*の薬剤感受性サーベイランスでも2007年にはPRSP，BLNARの割合が減少の方向にあり，今後抗菌薬の適正使用と耐性菌の出現についての検討を行っていきたい．

治療に関して，抗菌力の面からは耐性菌を含めて*H. influenzae*に対してはセフェム系のCTRXまたはCTXおよびカルバペネム系のmeropenem（MEPM）が，*S. pneumoniae*に対してはカルバペネム系で髄膜炎に適応を有するpanipenem/betamipron（PAPM/BP）とMEPMが最も優れておりガイドラインにも記載されている[13)14)]．

初期治療について原因菌の頻度から4カ月未満，4カ月以上に分けて検討したところ，1997年～2000年は当時標準的治療とされていたABPC＋CTX or CTRXが最も好んで使用されていた．PRSPやBLNARなどの耐性菌の蔓延が小児科医の間で広く認識されて以来，治療に関して変化がみられるようになった．*H. influenzae*や*S. pneumoniae*の分離頻度が少ない4カ月未満ではβ-ラクタム＋カルバペネムの組み合わせは増加の傾向にあるものの，2007～2008年で17例（29.3%）であったが，ABPC＋セフェムは1997～2000年51例（61%），2003～2004年には19例（43%）に減少したものの，2007～2008年再び30例（51%）となり，最も好まれる初期治療の組み合わせとなっている．一方*H. influenzae*や*S. pneumoniae*の多い4カ月以上の小児に対しては，これら耐性菌を念頭に置いたカルバペネム＋セフェムの組み合わせが年々増加し，2007～2008年には184例（82%）となっていた．*S. pneumoniae*に対して抗菌力が若干劣るABPC＋セフェムの組み合わせは1997～2000年の202例（59.6%）から2007～2008年には15例（7.5%）に減少していた．セフェム単独使用も1997～2000年の50例（14.7%）から2007～2008年には8例（3.6%）と減少した．

初期治療が併用で開始されても，その約2/3の症例では原因菌の薬剤感受性の結果ABPC，セフェム，カルバペネムのいずれかの薬剤単独投与に変更されており，抗菌薬の適正使用の考えが浸透しつつあることが

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うかがえた.

ステロイド薬併用の有無に関する調査では，3カ月未満では併用は40%と低いが3カ月以降はほとんどの症例がステロイドが併用され，2日間投与が標準的な治療として広く知られていることがうかがわれた．残念ながら今回の調査からステロイド併用の意義に関する結果は得られなかったが，アンケートの質問内容について再度検討する必要があると考えられた．

今後は *H. influenzae* や *S. pneumoniae* に対してより有効な薬剤の開発が望まれるとともに，我々自身が抗菌薬の投与量，投与間隔，投与時間などPK-PD理論に基づいた投与法について検証し，有効性・安全性はもとより耐性菌発現防止にも注意をはらう必要性を痛感した．更に，これら耐性菌の伝播が保育園を介して行われていること，髄膜炎の発症年齢を考慮すると，我が国においても，既に海外で感染予防効果が十分に確認されている *H. influenzae*，*S. pneumoniae* のワクチン[15)~17)]による予防法が早期に確立されるべく努力していく必要がある．

文　献

1) 小林　裕，春田恒和，森川嘉郎，藤原　徹：本邦における1966年以降13年間の小児化膿性髄膜炎の動向．127施設におけるアンケート調査成績．Jap. J. Antibiotics. 1979；2：795—805.
2) 藤井良知，平岩幹男，野中千鶴，小林　裕：本邦における1979年以降6年間の小児細菌性髄膜炎の動向．第1報　起炎菌について．感染症誌 1986；60：592—601.
3) 藤井良知，平岩幹男，小林　裕：本邦における1979年以降6年間の小児細菌性髄膜炎の動向．第2報　予後について．感染症誌　1987；61：849—57.
4) 藤井良知，平岩幹男，小林　裕：本邦における1979年以降6年間の小児細菌性髄膜炎の動向．第3報　抗生剤の単独または併用療法と予後について．Jap. J. Antibiotics. 1987；40：284—94.
5) 藤井良知，平岩幹男，小林　裕：本邦における1979年以降6年間の小児細菌性髄膜炎の動向．第4報　原因菌別抗菌剤療法と予後．Jap. J. Antibiotics. 1987；40：812—22.
6) 岩田　敏：ペニシリン耐性 *S. pneumoniae*—臨床の立場から．小児感染免疫　1987；10：139—46.
7) 砂川慶介，野々山勝人，高山陽子，山口禎夫，大石智洋，岩田　敏，他：本邦における1997年7月以降3年間の小児化膿性髄膜炎の動向．感染症誌　2001；78：931—8.
8) 砂川慶介，野々山勝人，大石智洋，岩田　敏，秋田博伸，佐藤吉壮，他：本邦における小児化膿性髄膜炎の動向(2000～2002)．感染症誌　2004；78：879—90.
9) 砂川慶介，野々山勝人，大石智洋，岩田　敏，秋田博伸，佐藤吉壮，他：本邦における小児化膿性髄膜炎の動向(2003～2004)．感染症誌　2006；80：27—38.
10) 砂川慶介，生方公子，千葉菜穂子，長谷川恵子，野々山勝人，岩田　敏，他：本邦における小児細菌性髄膜炎の動向（2005～2006)．感染症誌 2008；82：187—97.
11) 国立感染症研究所IDWR（感染症発生動向調査週報)，http://idsc.nih.go.jp/idwr/kanja/weeklygraph/16bacmen.html.
12) Hasegawa K, Kobayashi R, Takada E, Ono A, Chiba N, Morozumi M, *et al.*：High prevalence of type β-lacatmase-non-producing ampicillin-resistant *Haemophilus influenzae* in meningitis: the situation in Japan where Hib vaccine has not been introduced. J. Antimicrob. Chemother. 2006；57：1077—82.
13) 春田恒和：中枢神経系感染症　髄膜炎—小児，日本感染症学会．日本化学療法学会編，抗菌薬使用のガイドライン．協和企画，東京，2005；p. 94—6.
14) 細菌性髄膜炎の診療ガイドライン作成委員会：小児，日本神経治療学会，日本神経学会．日本神経感染症学会編，細菌性髄膜炎の診療ガイドライン．医学書院，東京，2007；p. 52—65.
15) Olowokure B, Spencer NJ, Hawker JI, Blair I, Smith RL：Invasive *Haemophilus influenzae* disease: an ecological study of sociodemographic risk factors before and after the introduction of Hib conjugate vaccine. Eur J Epidemiol 2003；18：363—7.
16) Poehling KA, Talbot TR, Griffin MR, Craig AS, Whitney CG, Zell E, *et al.*：Invasive pneumococcal disease among infants before and after introduction of pneumococcal conjugate vaccine. JAMA 2006；295：1668—74.
17) Black S, France EK, Isaacman D, Bracken L, Lewis E, Hansen J, *et al.*：Surveillance for Invasive Pneumococcal Disease During 2000-2005 in a Population of Children Who Received 7-Valent Pneumococcal Conjugate Vaccine. Pediatr Infect Dis J 2007；26：771—7.

Childhood Bacterial Meningitis Trends in Japan from 2007 to 2008

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We surveyed pediatrics bacterial meningitis epidemiology from January 2007 to December 2008 in Japan, with the following results: Cases numbered 287-160 male and 127 female-equivalent to 1.54-1.62 of 1,000 pediatric hospitalization per year. Children under 1-year-old accounted for the highest number of cases, which decreased with increasing age. *Haemophilus influenzae* was the most common cause of infection, followed by *Streptococcus pneumoniae*, group B streptococcus (GBS), and *Escherichia coli*. GBS and *E. coli* were major pathogens in children under 4 months of age, while *H. influenzae* and *S. pneumoniae* mainly accounted for those over 4 months of age. Susceptibility tests showed that 51% of *H. influenzae* isolates and 56.5% of *S. pneumoniae* isolates in 2008 were drug-resistant.

Ampicillin combined with cephem antibiotics effective against GBS, *E. coli*, and Listeria, were mainly used to initially treat those under 4 months of age. In those over 4 months of age, carbapenem antibiotics are effective against PRSP and cephem antibiotics against *H. influenza*.

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ORIGINAL ARTICLE

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Rapid optimization of antimicrobial chemotherapy given to pediatric patients with community-acquired pneumonia using PCR techniques with serology and standard culture

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Abstract Children (n = 117; mean age 2.4 ± 2.9 years) were diagnosed as having community-acquired pneumonia (CAP) using clinical symptoms, chest X-rays, and hematological data. The causative pathogen was determined using real-time polymerase chain reaction (PCR) (6 bacteria), multiple reverse transcription-PCR (MPCR; 11 viruses), bacterial culture, and serology. The initial chemotherapy was evaluated based on the pathogens identified using PCR. We found 27 viral cases (23.1%), 25 bacterial cases (21.4%), 45 mixed infections with virus and bacteria (38.5%), 10 *Mycoplasma pneumoniae* (8.5%), 7 mixed infections with *M. pneumoniae* and another pathogen (6.0%), 1 *Chlamydophila pneumoniae* (0.9%), and 2 unknown pathogens (1.7%). *Streptococcus pneumoniae* and *Haemophilus influenzae* accounted for 58 (49.5%) and 27 (23.0%) of the cases, respectively. The median values (50%) of the white blood cell count (WBC) and C-reactive protein (CRP) using the box-and-whisker and plot method, respectively, were 11.7 × 10^3 mm^{-3} and 1.4 mg/dl in viral infections, 15.6 × 10^3 mm^{-3} and 4.8 mg/dl in mixed infections with virus and bacteria, 17.8 × 10^3 mm^{-3} and 6.3 mg/dl in bacterial infections, 6.7 × 10^3 mm^{-3} and 1.4 mg/dl in *M. pneumoniae* infections, and 21.5 × 10^3 mm^{-3} and 6.4 mg/dl in mixed infections with *M. pneumoniae* and other bacterial infections. Sulbactam/ampicillin (n = 61), carbapenems (n = 12), and ceftriaxone (n = 7) were selected for the patients suspected of having bacterial infections alone or mixed infections with bacterial and viruses in accordance with our criteria defined tentatively. For those with *M. pneumoniae* and *C. pneumoniae* infections, azithromycin or minocycline was initially used. Treatments averaged 3–5 days. The empirical chemotherapy was improper in 9.4% of cases in relation to the etiologic agents finally identified. We conclude that rapid and comprehensive identification using PCR can provide optimal antimicrobial chemotherapy for CAP patients.

Key words Community-acquired pneumonia · Child · C-Reactive protein · Antimicrobial chemotherapy

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Introduction

Community-acquired pneumonia (CAP) is one of the most common infections occurring in children.[1–3] CAP is caused by multiple etiologic agents including viruses, *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, and other agents.[4–6] The rates of these causative microorganisms are quite different depending on many factors including the detection methods, seasonal epidemics, and the antibiotics predominantly used.[7–9]

In Japan, antimicrobial chemotherapy for patients with CAP is begun empirically based on (1) chest X-rays, (2) clinical findings including respiratory status, (3) age, and (4) laboratory tests such as white blood cell count (WBC) and C-reactive protein concentration (CRP). Recently, the guidelines to optimize empirical chemotherapy for CAP patients were published.[10] However, we believe the goal of chemotherapy is to select the most appropriate antibiotic for every patient within a short time after admission, based on laboratory results and immediately determining the causative agent.

Recently, the detection of viruses and bacteria using polymerase chain reaction (PCR) in addition to serological diagnosis has determined etiologic agents with high precision.[11–16] In children with CAP, the determination of the causative pathogen is difficult because of the difficulty in collecting direct clinical samples from alveoli, unlike in

adults. Because the pathogens must be identified using indirect nasopharyngeal samples that have low invasiveness, the physician has to determine whether the isolated microorganism is the etiologic agent or not. Therefore, a system to estimate the causative pathogens using obtainable clinical samples on the day of hospitalization is needed to quickly select the appropriate chemotherapy.

Our aim here was to use a multiplex PCR (MPCR) for viruses in parallel with bacterial detection using real-time PCR[17] in nasopharyngeal samples that were obtained from patients with pediatric CAP. Conventional bacterial cultures using the same samples and serological diagnosis with paired sera from the patient were performed to verify the results of the PCR. The clinical findings and laboratory test results from the patient were compared for every causative pathogen, and the appropriateness of the antimicrobial agents selected empirically according to the clinical findings was evaluated.

Patients and methods

Patients

Pediatric patients with CAP (male: $n = 59$; female: $n = 58$) were admitted to the Pediatric Department of Hakujikai Memorial Hospital, Tokyo, from May 2004 to April 2005. The criteria for hospitalization were: (1) the presence of pulmonary infiltrates found in the chest X-ray, (2) acute respiratory symptoms (e.g., tachypnea), and (3) deterioration of the general clinical state. We excluded patients requiring intensive therapy including artificial ventilation, those with chronic respiratory disease, those with congenital heart disease, those hospitalized with the same disease within a 1-month period, and patients with congenital or acquired immunosuppressive conditions.

Identification of the causative pathogens

After informed consent obtained from the child's parents or guardians, blood samples were taken to determine WBC, CRP, and serum antibody titers for several pathogens. Nasopharyngeal samples were also collected to determine the causative pathogens.

Each sample was used for: (1) real-time PCR screening for six bacterial pathogens, (2) multiple-reverse transcription PCR (MPCR) screening for 11 viral pathogens, and (3) conventional bacterial culture. These techniques were performed immediately after collection of the samples at the Laboratory of Molecular Epidemiology for Infectious Agents, Kitasato Institute for Life Sciences.

Real-time PCR

Streptococcus pneumoniae, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Streptococcus pyogenes*, and *Legionella pneumophila* were identified within 1.5 h after using the real-time PCR with the RTI kit[17] (Takara Bio, Kyoto, Japan) and Stratagene Mx3000P (Stratagene, La Jolla, CA, USA). The sensitivity and specificity were high at 95% and 98%, respectively, as compared with standard culture as previously described.[17,18]

Multiple-reverse transcription PCR

For the identification of viruses, an MPCR kit (Maximbio, San Francisco, CA, USA) was used according to the manufacturer's instructions. The MPCR kit identifies seven viruses: respiratory syncytial virus (RSV), adenovirus (Adeno), influenza virus A (FluA), influenza virus B (FluB), and parainfluenza virus-1, -2, and -3 (PIV-1, PIV-2, PIV-3). In addition, four primer sets to identify rhinovirus (Rhino),[19] human metapneumovirus (hMPV),[20] human bocavirus (hBoV),[21] and coronavirus[19] were prepared and the PCRs were performed using the same conditions as used for the MPCR kit. MPCR required 5 h.

Bacterial cultures

Bacterial culture was performed according to the *Manual of Clinical Microbiology*.[22] Serotyping of *S. pneumoniae* was performed using antiserum purchased from Statens Serum Institut (Denmark).

Serological test

Antibody titers against *M. pneumonia*, *C. pneumonia*, RSV, Adeno, FluA and FluB, and PIV-1, -2, and -3 were determined in paired sera from the acute and convalescent phase using the complement fixation (CF) test, hemagglutination inhibition (HI) test, or enzyme-linked immunosorbent assay (ELISA). When a significant rise in antibody titer was noted in the convalescent phase, the corresponding microorganism was considered to be the causative pathogen. A four-fold rise in titer for *M. pneumoniae* and Adeno; for RSV using the CF assay; and for PIV-1, -2, and -3, and FluA and FluB using the HI assay were used as indicators. *Chlamydophila pneumonia* was diagnosed using ELISA and the identified patient had an index value (ID) of 1.35 for IgG in the paired sera.

Clinical criteria to begin antimicrobial chemotherapy

The decision to begin antimicrobial chemotherapy was based on four conditions described previously;[23] clinical course, chest X-ray findings, age, and the laboratory findings. For clinical observations we used: (1) the presence or absence and timing of fever and respiratory symptoms such as tachypnea, wheezing, and retractive breathing; (2) presence or absence of nasal discharge and its properties; and (3) recurrent fever during the period of recovery of common cold-like symptoms. The diagnosis of tachypnea used World Health Organization (WHO) criteria.[24] Chest X-rays were divided into typical pneumonia (segmental or bronchial pneumonia), atypical pneumonia (ground-glass appear-

ance, skip lesion, pleurisy, and segmental), and viral pneumonia (bronchial pneumonia and interstitial shadow).

With regard to age, patients aged 5 years or older were usually thought to have *M. pneumoniae* infection and those aged 4 years or younger to have viral or mixed viral and bacterial infections. Bacterial infection and mixed infection were suspected in patients aged 4 years or younger with a WBC count of $13 \times 10^3 mm^{-3}$ or greater, or a CRP value of 3.8 mg/dl or greater. These values may be low in some cases in the early stage after onset and the WBC may readily fluctuate for various reasons in infants. However, ultimately the estimation of infection to be bacterial, viral, mycoplasmal, or mixed was determined using a composite of the clinical course, chest X-rays, age, and the laboratory data.

Criteria for selection of antimicrobial agent and treatment period

Of the four parenteral antibiotics of ampicillin–sulbactam (SBT/ABPC), panipenem (PAPM), meropenem (MEPM), and ceftriaxone (CTRX), a single agent was selected for the patients suspected of having a bacterial infection alone or a viral–bacterial mixed infection from four conditions described above. The respective doses were as follows: SBT/ABPC 100–120 $mg\,kg^{-1}\,day^{-1}$ (q.i.d.), PAPM and MEPM 60 $mg\,kg^{-1}\,day^{-1}$ (t.i.d.), and CTRX 40–60 $mg\,kg^{-1}\,day^{-1}$ (b.i.d.). The antibiotics were administered for 3 days after defervescence (37.5°C).

The febrile period varied considerably in patients with viral and bacterial mixed infections, so the antibiotic was withdrawn 1–2 days after defervescence where we judged the antibiotic had been effective against the bacterial pathogen. For patients suspected of having *M. pneumoniae* or *C. pneumoniae* infection from the four conditions, azithromycin (AZM) or minocycline (MINO) was used. We did not use antimicrobial agents for patients where viral infection alone was strongly suggested from the four conditions described above.

Criteria for etiological classification

Table 1 shows tentative criteria for the etiological classification in CAP patients. Seven categories are as follows: (1) bacterial, (2) mixed infection of viral and bacterial, (3) viral, (4) mycoplasmal, (5) mixed infection of mycoplasmal (chlamydial) and bacterial, (6) mixed infection of mycoplasmal (chlamydial) and viral, and (7) unknown that could not identify any etiological agent.

Statistical analysis

Statistical analysis of the difference in clinical findings in relation to the causative pathogens was performed using the Fisher's exact test. WBC and CRP values on the day of admission were analyzed using the box-and-whisker plot method. The lower hinge, median, and upper hinge of the box corresponded to the 25%, 50%, and 75% percentiles, respectively; half of the cases were included in the box. The dotted line in each box is 1.5 times the quartile deviation.

Results

Patients

One hundred and seventeen CAP cases were 59 male and 58 female patients during May 2004 to April 2005. The

Table 1. Tentative criteria for the etiological classification of pediatric community-acquired pneumonia (CAP)

Diagnosis	Criteria
Bacterial	(i) CRP ≧3.8 mg/dl or WBC ≧$13 \times 10^3 mm^{-3}$ (ii) Blood culture positive (iii) *S. pneumoniae* and/or *H. influenzae* ≧10^4 CFU in nasopharyngeal (iv) Inflammation findings of PMN in nasopharyngeal (v) Antibacterial agent was effective
Mixed infection Viral–bacterial	In addition to the above (i)–(iv) criteria for "Bacterial" (i) Significant rise of virus antibody (ii) PCR positive for virus
Viral	(i) Significant rise of virus antibody (ii) PCR positive for virus (iii) Antibacterial agent was not effective (iv) Improved without an antibacterial agent
Mycoplasmal (chlamydial)	(i) Significant rise of antibody (ii) PCR positive for *M. pneumoniae* (*C. pneumoniae*)
Mixed infection Mycoplasmal (chlamydial)–bacterial	In addition to (i)–(iv) criteria for "Bacterial" (i) Significant rise of antibody against *M. pneumoniae* (*C. pneumoniae*) (ii) PCR positive for *M. pneumoniae* (*C. pneumoniae*)
Mixed infection Mycoplasmal (chlamydial)–viral	In addition to the two criteria for "Mycoplasmal" (i) Significant rise of virus antibody (ii) PCR positive for virus
Unknown	Not detected any etiologic agent

CRP, C-Reactive protein; WBC, white blood cell count; CFU, colony-forming units; PMN, polymorphonuclease leukocyte; PCR, polymerase chain reaction

patients' ages were <1 year for 26 cases (22.2%), 1–2 years for 49 (41.9%), 3–5 years for 30 (25.6%), and ≥6 years for 12 cases (10.3%).

As described previously, the decision to admit was determined using chest X-rays (i.e., segmental pneumonia, bronchial pneumonia, atypical pneumonia, or interstitial shadows), acute respiratory symptoms, and deterioration in their general state. Fifty-eight cases (49.6%) had previously received oral antimicrobial agents within the 2 weeks prior to their hospitalization.

Viral pathogens

MPCR with 11 viruses were correlated with serological test results (Table 2). All PCR positive cases for RSV, Adeno, FluA, FluB, PIV-1, and PIV-3 showed significantly high antibody titers to the corresponding virus. The specificity of PCR for these agents was 100%; however, the sensitivity of the PCR was 63.6%–100% for viral infections alone and only 21.1%–75.0% for mixed infections. Simultaneous infections with two viruses with Adeno and RSV or hMPV and PIV-3 were identified in two patients each.

The cumulative positive cases determined by serology and PCR were 23 RSV (19.7%) cases, 23 PIV1-3 (19.7%), 13 Adeno (11.1%), 9 FluA (7.7%), 9 FluB (7.7%), 6 Rhino (5.1%), and 3 hMPV (2.6%) cases. No cases having Corona and hBoV infection were identified.

Bacterial pathogens

The bacteria suspected to be the causative pathogens was determined by standard culture and real-time PCR for six pathogens: *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Mycoplasma pneumoniae*, *Chlamydophila pneumoniae*, *Streptococcus pyogenes*, and *Legionella pneumophila* (Table 3). *Streptococcus pneumoniae* was suspected in 47 (40.2%) cases, *H. influenzae* in 16 (13.7%), *M. pneumoniae* in 17 (14.5%), *C. pneumoniae* in 1 (0.9%), and *Moraxella catarrhalis* in 1 case (0.9%). Mixed infections with both *S. pneumoniae* and *H. influenzae* were suspected in 11 (9.4%) cases. In total, *S. pneumoniae* and *H. influenzae* CAP infection was found in 58(49.5%) and 27(23.0%) cases, respectively.

In the patients suspected of having an infection caused by *S. pneumoniae* and *H. influenzae*, the real-time PCR results were all positive with early threshold cycles of 15–25 that indicated more than 1000 CFU per sample. Direct bacterial count showed more than 10^4 CFU per sample using standard culture and was found 83.3% and 84.6% of the time, respectively, for *S. pneumoniae* and *H. influenzae*.

Seventeen cases identified as *M. pneumoniae* infection showed single (ten cases; 8.5%) and mixed (seven cases; 6.0%) infections with other microorganisms. Fourteen (82.3%) of these cases were identified rapidly by real-time PCR.

Table 2. Viruses identified as etiologic agents by PCR and/or serologic test results in CAP patients

Virus	No. of patients[a]	Viral infection		Mixed infection[b]	
		Serological positive	PCR positive	Serological positive	PCR positive
Respiratory syncytial virus	23 (19.7)	11	7 (63.6)	12	6 (50.0)
Adenovirus	13 (11.1)	9	8 (88.9)	4	3 (75.0)
Influenza virus A and B	9 (7.7)	3	3 (100)	6	4 (66.7)
Parainfluenza viruses 1-3	23 (19.7)	4	4 (100)	19	4 (21.1)
Human metapneumovirus	3 (2.6)	ND	0	ND	3
Rhinovirus	6 (5.1)	ND	1	ND	5
Total	77 (65.8)	27	23	41	25

Data shown in parentheses are percentages
ND, Not determined
[a] Percentage for the total number of patients
[b] Mixed infections means viral and bacterial coinfections

Table 3. Bacterial pathogens suspected as etiologic agents with high probability in pediatric patients with CAP

Bacterial pathogens	No. of patients[a]	Bacterial infection alone	Mixed infection with viruses	Mixed infection with *M. pneumoniae*
Streptococcus pneumoniae	47 (40.2)	18	27	2
Haemophilus influenzae	16 (13.7)	3	12	1
S. pneumoniae and *H. influenzae*	11 (9.4)	4	5	2
Moraxella catarrhalis	1 (0.9)		1	–
Mycoplasma pneumoniae	17 (14.5)	10	2	–
Chlamydophila pneumoniae	1 (0.9)	1	–	–
Total	88 (75.2)[b]	36	47	5

[a] Percentages given in parentheses for the total number of 117 patients
[b] Total cases excluded mixed infection of *M. pneumoniae* and other bacteria

Chlamydophila pneumoniae infection was identified in only one (0.9%) case using ELISA serology and PCR. In the 58 isolates of *S. pneumoniae*, 12 isolates belonged to capsule serotype 6B, 8 to 19F, 7 to 6A, 14 to 23F, 2 to serotype 19A, and 15 to other types.

Etiologic agents and distribution by patient age

The etiologic agents found in the 117 patients are shown in Fig. 1. Bacterial infection caused by *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis* was identified in 27 (21.4%) of the patients, viral-mixed and bacterial-mixed infection in 45 (38.5%), *M. pneumoniae* infection in 10 (8.5%), *M. pneumoniae* and viral mixed infections in 2 (1.7%), *M. pneumoniae* and bacterial mixed infections in 5 (4.3%), *C. pneumoniae* in 1 (0.9%), and viral infections in 27 (23.1%) cases. Infections where etiologic agent was unknown occurred in only 2 (1.7%) of all the patients examined here.

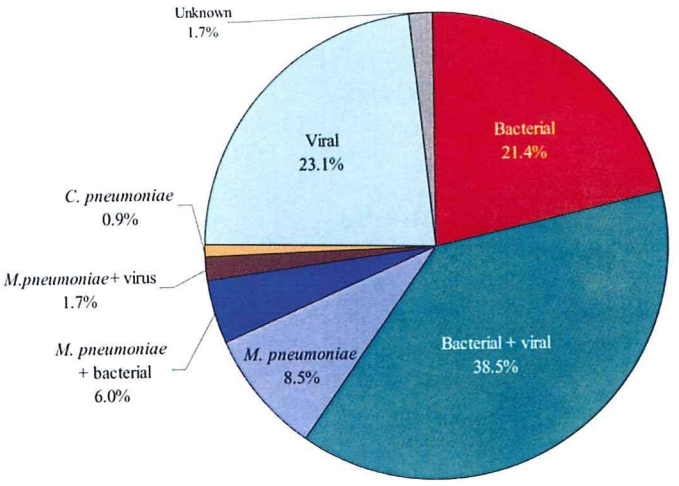

Fig. 1. Pathogens suspected with high probability as the etiologic agents in 117 hospitalized children with community-acquired pneumonia

The rate of individual pathogens in the patients distributed by age is shown in Fig. 2. Viral infection and mixed infection with viruses and bacteria were the most frequent among children 6 months to 3 years of age, bacterial infection alone occurred in those aged 1–6 years, and *M. pneumoniae* occurred in those aged 3 years or older.

Clinical characteristics according to etiologic agents

Table 4 shows the differences in clinical findings among patients classified into groups according to the etiologic agents: viral infections (n = 27, Group A), viral-mixed and bacterial-mixed infections (n = 45, Group B), bacterial infections (n = 25, Group C), *M. pneumoniae* infections (n = 10, Group D), and *M. pneumoniae* and bacterial mixed infections (n = 5, Group E).

The following clinical findings on admission were classified according to the respective criteria: (1) chest X-ray findings, (2) with/without asthma, (3) respiration rate, (4) WBC, and (5) CRP. They were analyzed statistically using the Fisher's exact test. As expected, significant differences were noted in chest X-rays among the five groups with signs of interstitial pneumonia being frequently found in patients with viral infection, segmental pneumonia in those with bacterial infections, bronchial or segmental pneumonia in those with mixed infections, and with atypical pneumonia in patients having *M. pneumoniae* infections.

Viral infection and viral and bacterial mixed infections were more frequent in children with asthma compared with other infections. With regard to respiratory rate, tachypnea was not observed in *M. pneumoniae* infections. WBC and CRP differed significantly between viral and bacterial infection, and between bacterial or mixed and *M. pneumoniae* groups.

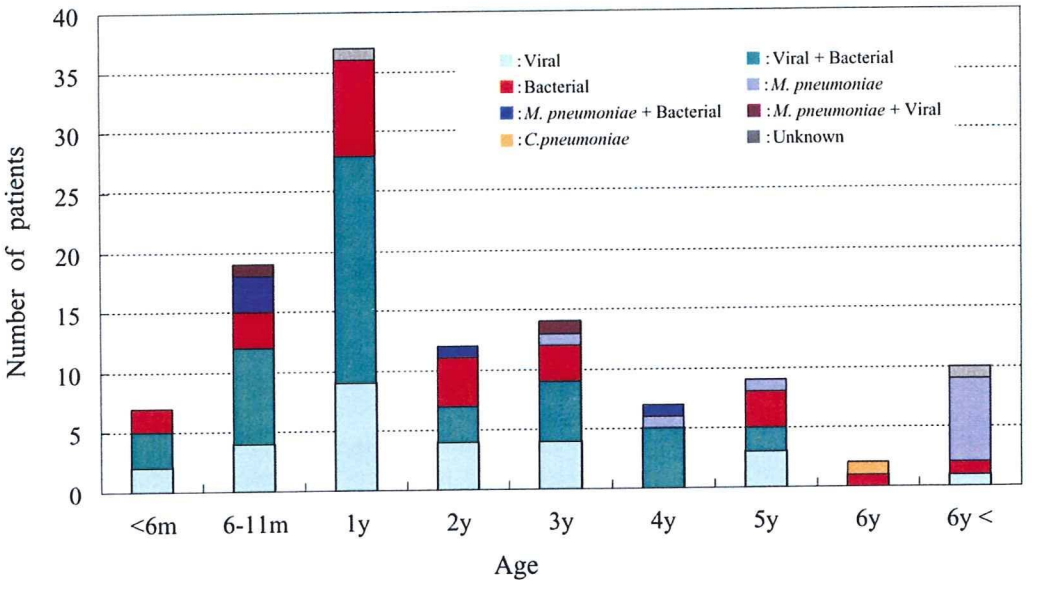

Fig. 2. Causative pathogens in 117 hospitalized children with community-acquired pneumonia distributed by age

Table 4. Characteristics of some clinical findings among 112 hospitalized children with CAP classified into etiologic agents

Category		$n = 112$	Group A	Group B	Group C	Group D	Group E	Fisher's exact test
			viral ($n = 27$)	mixed ($n = 45$)	bacterial ($n = 25$)	mycoplasmal ($n = 10$)	mycoplasma and bacterial ($n = 5$)	
Chest X-ray finding	Interstitial	21	21 (100)[a]	0	0	0	0	
	Bronchial	38	5 (13.1)	27 (71.0)	6 (15.8)	0	0	$P < 0.001$
	Segmental	47	0	18 (38.3)	19 (40.4)	6 (12.8)	4 (8.5)	
	Atypical	6	1 (16.7)	0	0	4 (66.7)	1 (16.7)	
Asthma	–	88	14 (15.9)	35 (39.8)	24 (27.2)	10 (11.4)	5 (5.7)	$P < 0.001$
	+	24	13 (54.2)	10 (41.7)	1 (4.2)	0	0	
Respiratory rate	20–29	17	6 (35.3)	2 (11.8)	0	8 (47.0)	1 (5.9)	
	30–39	32	8 (25.0)	13 (40.6)	8 (25.0)	2 (6.3)	1 (3.1)	$P < 0.001$
	40–49	26	4 (15.4)	14 (53.8)	7 (26.9)	0	1 (3.8)	
	≥50	37	9 (24.3)	16 (43.2)	10 (27.0)	0	2 (5.4)	
WBC (mm^{-3})	$<10 \times 10^3$	26	11 (42.3)	6 (23.1)	0	9 (34.6)	0	
	$10–15 \times 10^3$	31	8 (25.8)	13 (41.9)	8 (25.8)	1 (3.2)	1 (3.2)	$P < 0.001$
	$\geq 15 \times 10^3$	55	8 (14.5)	26 (47.3)	17 (30.9)	0	4 (7.3)	
CRP (mg/dl)	<1.9	21	15 (71.4)	2 (9.5)	0	4 (19.0)	0	
	1.9–3.8	24	3 (12.5)	13 (59.1)	4 (16.7)	4 (16.7)	0	
	3.8–5.7	23	3 (13.0)	13 (56.5)	6 (26.1)	0	1 (4.3)	$P < 0.001$
	≥5	44	6 (13.6)	17 (38.6)	15 (34.1)	2 (4.5)	4 (9.1)	

[a]The values in parentheses show percentage in each category

WBC and CRP values compared with causative pathogens

WBC and CRP values of 112 patients on the day of admission were plotted according to the respective causative pathogens (Figs. 3 and 4). Patients with an unclear causative pathogen ($n = 2$), *C. pneumoniae* ($n = 1$), and mixed infections with *M. pneumoniae* and virus ($n = 2$) were excluded. The data were analyzed using the box-and-whisker plot method where each box encompasses 50% of the cases.

As shown in Fig. 3, the median WBC values in the patients with viral, viral and bacterial, bacterial, *M. pneumoniae*, and *M. pneumoniae* and bacterial infections were 11.7×10^3, 15.6×10^3, 17.8×10^3, 6.7×10^3, and $21.5 \times 10^3 mm^{-3}$, respectively. In patients with defined viral and bacterial mixed infections, the 50% box of WBC values was located between those of the viral and bacterial cases.

As shown in Fig. 4, the median values of the CRP in patients with viral, viral and bacterial, bacterial, *M. pneumoniae*, and *M. pneumoniae* and bacterial infections were 1.4, 4.8, 6.3, 1.4, and 6.4 mg/dl, respectively. The 50% box of cases having bacterial infection show clearly the highest CRP values and do not overlap with the box in cases with *M. pneumoniae* infection. The median CRP value in the case of mixed infections with virus and bacteria was intermediate between the viral and bacterial infections similar to the WBC.

Evaluation of antimicrobial agents selected empirically

The relation between the empirically selected antibiotic for 117 cases and the causative pathogens is shown in Table 5. The decision to use antimicrobials and the selective criterion for the initial treatment were as outlined in Patients and methods. Antibiotics were used in 97 patients (82.9%), namely SBT/ABPC in 61 (52.1%); CTRX in 8 (6.8%); a carbapenem, either MEPM or PAPM, in 12 (10.3%); AZM in 10 (8.5%); MINO in 3 (2.6%); and SBT/ABPC and AZM in 2 (1.7%) patients. No antibiotic was used in 20 (17.0%) patients.

The initially selected antibiotic was retrospectively considered inappropriate in 9 patients with viral infection (Adeno, 4; RSV, 3; InfA, 1; PIV-3, 1), 1 with *M. pneumonia* and viral mixed infection, and 1 patient with an undetermined causative pathogen. Out of the 117 cases, antimicrobials were inappropriately used in 11 (9.4%) cases.

Clinically, defervescence was achieved within 24 h after admission in all the patients with bacterial infections. The average duration of antimicrobial treatment was 3–5 days. The duration of antibiotic therapy was also 4 days in four patients in whom *S. pneumoniae* or *H. influenzae* was isolated from blood cultures taken at admission.

After the termination of treatment, no patient experienced a relapse or was refractory to treatment. However, we experienced four cases having recurrent fever whose hospitalization was slightly prolonged, but this was not due to relapse of pneumonia and all of them recovered spontaneously without further antimicrobial use.

Discussion

Identification of the causative pathogens in children with CAP is not always easy because sputum or bronchoalveolar lavage (BAL) samples cannot be obtained as routinely performed in adults. To diagnose children, the causative pathogen is suspected from the history, chest X-ray, and blood examination data while taking into account the patient's age, and then the antibiotic is selected empirically. Recently

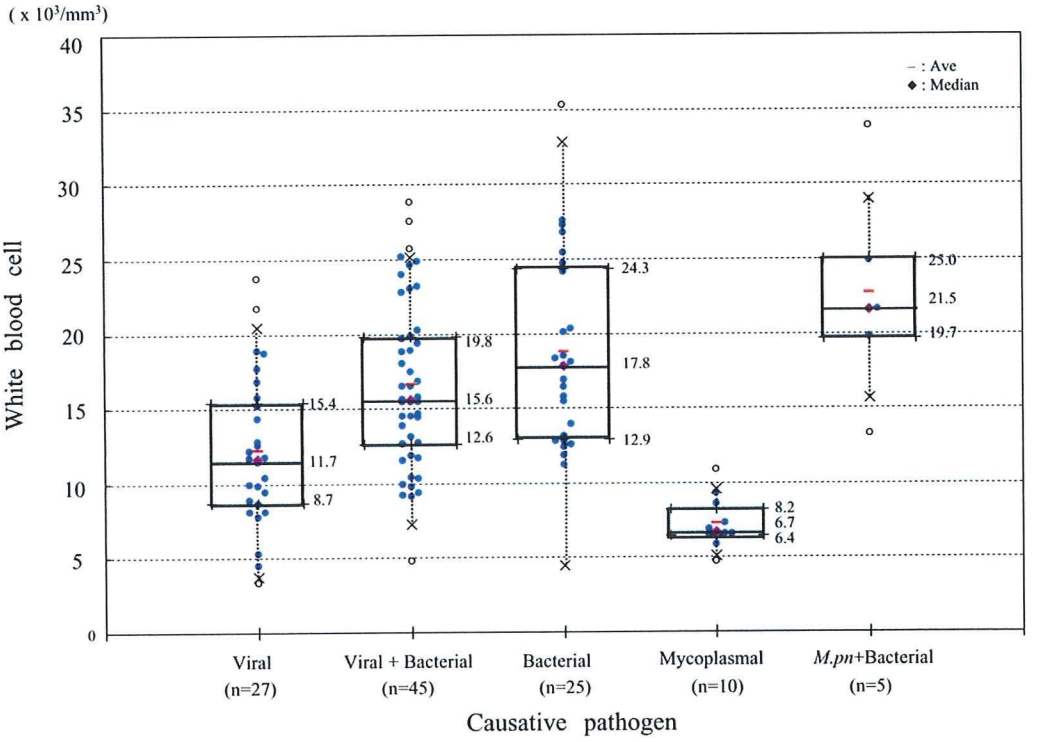

Fig. 3. Characteristics of white blood cell counts analyzed by box-and-whiskers plot method by causative pathogens

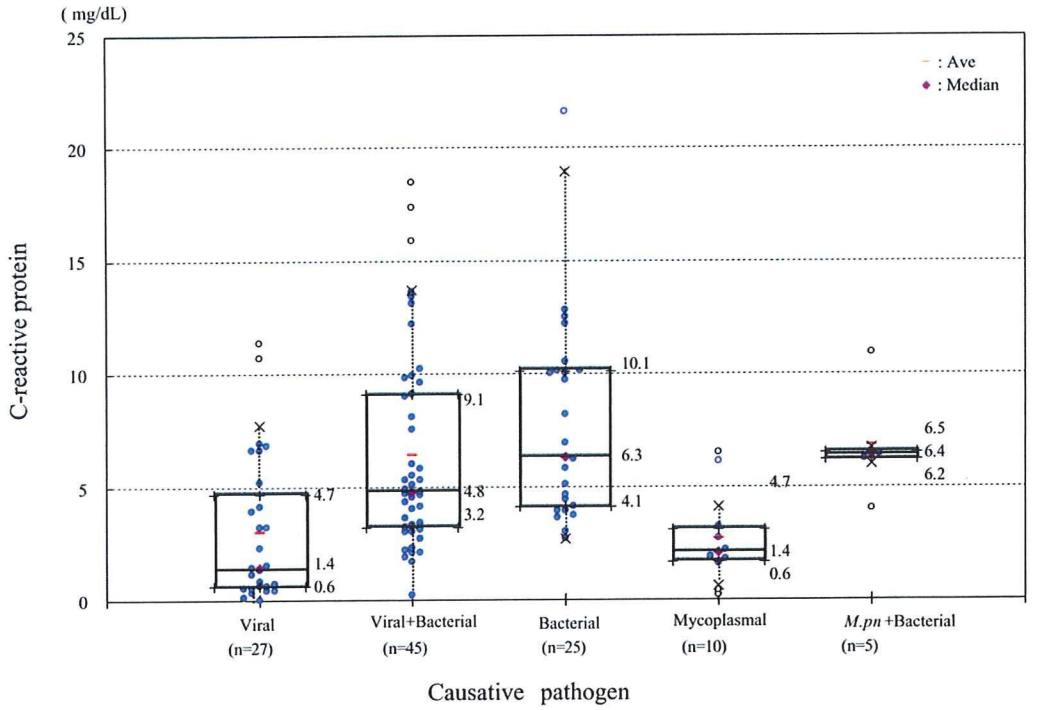

Fig. 4. Characteristics of C-reactive protein values analyzed by the box-and-whiskers plot method by causative pathogens

in Japan,[10] the guidelines for the treatment and management of respiratory tract infection in pediatric patients have been proposed to promote optimal chemotherapy, and similar guidelines are found in other countries.[25,26] However, the incidence rate of pathogenic microorganisms in pediatric CAP in other countries likely differs due to many factors such as the health insurance system, vaccination programs, kinds of antibiotics predominantly used, and population density. There are published studies of the use of PCR methods to determine the pathogens in CAP,[11–16] and the simultaneous detection of both bacteria and viruses is expected to enhance the accuracy of CAP diagnosis.

Here our aim was to identify bacteria and viruses using PCR within a short time frame and to evaluate the PCR results in relation to clinical findings. As previously described,[17] DNA/RNA samples were extracted from clini-

Table 5. Retrospective interpretation for dosing of antimicrobial agents for 117 patients with CAP

Causative pathogen	No. of patients	Not used	Antibiotics used					
			SBT/ampicillin	Ceftriaxone	Panipenem or meropenem	Azithromycin	Minocycline	Azithromycin + SBT/ampicillin
Viral alone	27	18	6[a]	1[a]	0	1[a]	1[a]	0
Viral + bacterial	45	0	32	6	7	0	0	0
Bacterial alone	25	0	19	1	5	0	0	0
Mycoplasma pneumoniae alone	10	0	0	0	0	8	2	0
Mycoplasma pneumoniae + bacterial	5	0	4	0	0	0	0	1
Mycoplasma pneumoniae + viral	2	1	1[a]	0	0	0	0	0
Chlamydophila pneumoniae alone	1	0	0	0	0	1	0	0
Unknown	2	1	0	0	0	0	1[a]	0
Total	117	20 (17.1%)	62 (53.0%)	8 (6.8%)	12 (10.3%)	10 (8.5%)	4 (3.4%)	1 (0.8%)

SBT, Sulbactam
[a]Antibiotics were used improperly

cal specimens using the EXTRAGEN II kit and bacterial detection was performed using real-time PCR; MPCR was performed for viral detection. Although, the results for bacterial analysis by the real-time PCR constructed by us can be rapidly obtained within 1.5 h, MPCR for viruses requires 5 h. In the near future, it is anticipated we will also be able to accomplish viral identification with real-time PCR.

We used nasopharyngeal samples as a source to identify the causative pathogens. These materials are appropriate to identify viruses, *Mycoplasma pneumoniae*, and *Chlamydophila pneumoniae*, but this sample type is questionable for *Streptococcus pneumoniae* and *Haemophilus influenzae* because they can be present in normal individuals. A positive result for *S. pneumoniae* and *H. influenzae* by real-time PCR should be carefully considered as to whether it indicates their causal relation with the infection or not where the physician should consider the chest X-rays, clinical symptoms, clinical laboratory findings such as WBC and CRP, bacterial amounts, and inflammation findings of leucocytes in nasopharyngeal samples.

The rate of viruses and bacteria identified in this study as the causative pathogens was similar to the data reported by Michelow et al.[12] and Juvèn et al.,[5] although the rate of *H. influenzae* differed. This was presumably because Hib vaccination has not been approved in Japan[27] where there are cases of pneumonia due to Hib and nontypable *H. influenzae*. Three CAP cases with Hib having positive blood cultures and positive real-time PCR were found among our 117 cases. *Chlamydophila pneumoniae* was detected in only one patient possibly because there were few children older than 6 years in this study.

As for the relation between the diagnosis of CAP and blood examination test, although some studies have only concluded that CRP and WBC can provide useful information for pneumococcal pneumonia,[28–31] these values are used by Japanese pediatricians as useful references in addition to routine chest X-ray to diagnose pneumonia. In clinical practice, we have observed these values do not fluctuate for about half a day after the onset of bacterial infection. In addition, in the cases of adenovirus infection, and virus or *M. pneumoniae* infection in school-age children, the values of WBC and CRP are relatively high. The physician must be mindful of these kinds of exceptional cases; however, as shown in our data, significant differences in these values are found and are correlated to the causative pathogens. At present, we believe the time from onset to presentation in hospital is relatively uniform in Japan as compared with other countries because of our universal health insurance system.

In this study, the antimicrobial agent that was empirically selected was found to have been inappropriately administered to 9.4% of the 117 cases. Using our techniques, we found the treatment for most cases was completed within 3–5 days. In general, the dosing period recommended in the guidelines is 7–10 days; however, we consider this is somewhat long because the antibiotics acted against the causative bacteria within a shorter period. Of the 58 strains of *S. pneumoniae*, 25 were Penicillin resistant streptococcus pneumonia (PRSP), and none of the patients experienced a relapse after treatment with SBT/ABPC and a carbapenem antibiotic (data not shown).

Viral and bacterial mixed infections were identified in 40.2% of the cases in addition to viral infection in 23.1%. Thus, involvement with the viral-related cases was 74 (63.2%) cases in total. Furthermore, cases relating to virus infections may be present, which could not be demonstrated when 5 days or more had elapsed after onset. Timing of acquiring the clinical samples is also very important to demonstrate the causative viruses.

In the future, we expect comprehensive and rapid identification of the causative pathogens outlined here will become routine in clinical practice.

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References

1. Bartlett JG, Mundy LM. Community-acquired pneumonia. N Engl J Med 1995;333:1618–24.
2. McCracken GH. Etiology and treatment of pneumonia. Pediatr Infect Dis J 2000;19:273–7.
3. McCracken GH. Diagnosis and management of pneumonia in children. Pediatr Infect Dis J 2000;19:924–8.
4. McIntosh K. Community-acquired pneumonia in children. N Engl J Med 2002;346:429–37.
5. Juvèn T, Mertsola J, Waris M, Leinonen M, Meurman O, Roivainen M, et al. Etiology of community acquired pneumonia in 254 hospitalized children. Pediatr Infect Dis J 2000;19:293–8.
6. Bradley JS. Management of community-acquired pediatric pneumonia in an era of increasing antibiotic resistance and conjugate vaccines. Pediatr Infect Dis J 2002;21:592–8.
7. Block S, Hedrick J, Hammerschlag MR, Cassell GH, Craft JC. *Mycoplasma pneumoniae* and *Chlamidia pneumoniae* in pediatric community-acquired pneumonia: comparative efficacy and safety of clarithromycin vs erythromycin ethylsuccinate. Pediatr Infect Dis J 1995;14:471–7.
8. Heiskanen-Kosma T, Korppi M, Jokinen C, Kurki S, Heiskanen L, Juvonen H, et al. Etiology of childhood pneumonia: serologic results of a prospective, population-based study. Pediatr Infect Dis J 1998;17:986–91.
9. Clements H, Stephenson T, Gabriel V, Harrison T, Millar M, Smyth A, et al. Rationalised prescribing for community-acquired pneumonia: a closed loop audit. Arch Dis Child 2000;83:320–4.
10. Uehara S, Sunakawa K (editors). Practice guidelines for respiratory tract infections in childhood (in Japanese). Tokyo: Kyowakikaku; 2004.
11. Wubbel L, Muniz L, Ahmed A, Trujillo M, Carubelli C, McCoig C, et al. Etiology and treatment of community-acquired pneumonia in ambulatory children. Pediatr Infect Dis J 1999;18:98–104.
12. Michelow IC, Olsen K, Lozano J, Rollins NK, Duffy LB, Ziegler T, et al. Epidemiology and clinical characteristics of community-acquired pneumonia in hospitalized children. Pediatrics 2004;113: 701–7.
13. Gröndahl B, Puppe W, Hoppe A, Kühne I, Weigl JAI, Schmitt HJ. Rapid identification of nine microorganisms causing acute respiratory tract infections by single-tube multiplex reverse transcription-PCR: feasibility study. J Clin Microbiol 1999;37:1–7.
14. Henrickson KJ, Hoover S, Kehl KS, Hua W. National disease burden of respiratory viruses detected in children by polymerase chain reaction. Pediatr Infect Dis J 2004;23:S11–18.
15. Weigl JAI, Puppe W, Grondahl B, Schmitt HJ. Epidemiological investigation of nine respiratory pathogens in hospitalized children in Germany using multiplex reverse-transcriptase polymerase chain reaction. Eur J Clin Microbiol Infect Dis 2000;19:336–43.
16. Weigl JAI, Puppe W, Belke O, Neususs J, Bagci F, Schmitt HJ. Population-based incidence of severe pneumonia in children in Kiel, Germany. Klin Pädiatrie 2005;217:2111–219.
17. Morozumi M, Nakayama E, Iwata S, Aoki Y, Hasegawa K, Kobayashi R, et al. Simultaneous detection of pathogens in clinical samples from patients with community-acquired pneumonia by real-time PCR with pathogen-specific molecular beacon probes. J Clin Microbiol 2006;44:1440–6.
18. Morozumi M, Ito A, Murayama SY, Hasegawa K, Kobayashi R, Iwata S, et al. Assessment of real-time PCR for diagnosis of *Mycoplasma pneumoniae* pneumonia in pediatric patients. Can J Microbiol 2006;52:125–9.
19. Coiras MT, Aguilar JC, Garcia ML, Casas I, Perez-Brena P. Simultaneous detection of fourteen respiratory viruses in clinical specimens by two multiplex reverse transcription nested-PCR assays. J Med Virol 2004;72:484–95.
20. Ebihara T, Endo R, Kikuta H, Ishiguro N, Ishiko H, Hara M, et al. Human metapneumovirus infection in Japanese children. J Clin Microbiol 2004;42:126–32.
21. Ma X, Endo R, Ishiguro N, Ebihara T, Ishiko H, Ariga T, et al. Detection of human bocavirus in Japanese children with lower respiratory tract infections. J Clin Microbiol 2006;44: 1132–4.
22. Murray PR, Baron EJ, Jorgensen JH, Pfaller MA, Yolken RH (editors). Manual of clinical microbiology, 8th ed. Washington DC: American Society for Microbiology; 2003.
23. Tajima T, Nakayama E, Kondo Y, Hirai F, Ito H, Iitsuka T, et al. Etiology and clinical study of community-aquired pneumonia in 157 hospitalized children. J Infect Chemother 2006;12:372–9.
24. World Health Organization. Geneva. [cited March 2007]. Communicable disease toolkit. Available from: http://www.who.int/infectious-disease-news/IDdocs/whocds200317/5casedefns.pdf.
25. British Thoracic Society of Standards of Care Committee. BTS guidelines for the management of community acquired pneumonia in childhood. Thorax 2002;57:1–24.
26. Jadavji T, Law B, Lebel MH, Kennedy WA, Gold R, Wang EE. A practical guide for the diagnosis and treatment of pediatric pneumonia. Can Med Assoc J 1997;156:S703–11.
27. Hasegawa K, Kobayashi R, Takada E, Ono A, Chiba N, Morozumi M, et al. High prevalence of type b β-lactamase-non-producing ampicillin-resistant *Haemophilus influenzae* in meningitis: the situation in Japan where Hib vaccine has not been introduced. J Antimicrob Chemother 2006;57:1077–82.
28. Ponka A, Sarna S. Differential diagnosis of viral, mycoplasmal and bacteraemic pneumococcal pneumonias on admission to hospital. Eur J Respir Dis 1983;64:360–3.
29. Korppi M, Heiskanen-Kosma T, Leinonen M. White blood cells, C-reactive protein and erythrocyte sedimentation rate in pneumococcal pneumonia in children. Eur Respir J 1997;10:1125–9.
30. Korppi M, Kronger L. C-Reactive protein in viral and bacterial respiratory infection in children. Scand J Infect Dis 1993;25: 207–13.
31. Toikka P, Virkki R, Mertsola J, Ashorn P, Eskols J, Ruuskanen O. Bacteremic pneumococcal pneumonia in children. Clin Infect Dis 1999;29:568–72.

小児医学
最近の進歩

肺炎球菌の耐性化とワクチン

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要旨

全国規模で収集した侵襲性感染症由来の肺炎球菌における薬剤耐性化の現況とワクチネーションの基盤となる莢膜型について述べた.

肺炎球菌のβ-ラクタム系薬剤耐性は,その作用標的である細胞壁合成酵素のPBP1A, PBP2X, およびPBP2Bをコードする遺伝子変異から解析すると,PBP変異のないgPSSPは19.9%, 1~2遺伝子が変異したgPISPは48.3%, 3遺伝子が変異したgPRSPは31.8%の割合であった.またそれらの株の80%はマクロライド系薬剤耐性遺伝子も保持していた.

莢膜型は多岐にわたっていたが,7価コンジュゲートワクチン(7PCV)型に含まれる4, 6B, 9V, 14, 18C, 19F, 23F型の菌は全体の73.8%を占めていた.それらの型にはgPRSPが圧倒的に多く,また2歳以下の症例が80%以上を占めており,乳幼児への7PCVの導入は肺炎球菌による重症感染症例の減少に寄与するものと考えられた.

はじめに

肺炎球菌は,小児あるいは成人が市中において罹患する呼吸器感染症の原因菌としてもっとも検出頻度の高い細菌である.特に,抗菌薬が発達した現代においても,本菌によって敗血症や化膿性髄膜炎を惹起した場合には,しばしば重篤な後遺症の残存や致命的となりうる[1].このような臨床的に重要な肺炎球菌において,本菌による疾患に対する基本的な治療薬であるβ-ラクタム系薬剤耐性菌(PRSP)が注目されて久しい[2][3].

そもそも,PRSPに関する最初の報告は1970年代に遡るが,それ以降,この耐性菌が臨床的に注目されたのは治療薬の主体がペニシリン系薬剤を処方している欧米からであり,セフェム系薬剤が主体のわが国においては長い間ほとんど問題にならなかった耐性菌である.

本邦においてPRSPが臨床上の関心事となったのは,1988年の本菌による小児化膿性髄膜炎例の報告に始まる[4].筆者らは1993年から全国規模で肺炎球菌を収集し,薬剤感受性や耐性遺伝子レベルでの疫学調査,病原性にかかわる莢膜の血清型別を含めた疫学研究を開始し,その成績を報告してきている[5].

ここでは,肺炎球菌による感染症の予防策となるワクチネーションの疫学的基礎となる薬剤耐性化の現況と莢膜型の成績について,小児の侵襲性感染症由来株の成績を中心に述べる.

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表 ワクチネーションの基盤となる肺炎球菌の莢膜型

Type	グループ	Type	グループ
1			24（24F，24A，24B）
2			25（25F，25A）
3		27	
4			28（28F，28A）
5		29	
	6（6A，**6B**）	31	
	7（7F，7A，7B，7C）		32（32F，32A）
8			33（33F，33A，33B，33C，33D）
	9（9A，9L，9N，**9V**）	34	
	10（10F，10A，10B，10C）		35（35F，35A，35B，35C）
	11（11F，11A，11B，11C，11D）	36	
	12（12F，12A，12B）	37	
13		38	
14		39	
	15（15F，15A，15B，15C）	40	
	16（16F，16A）		41（41F，41A）
	17（17F，17A）	42	
	18（18F，18A，18B，**18C**）	43	
	19（**19F**，19A，19B，19C）	44	
20		45	
21		46	
	22（22F，22A）		47（47F，47A）
	23（**23F**，23A，23B）	48	

注：太字は7PCVに含まれる型，下線はさらに13PCVに追加されている型を示す．

Ⅰ．病原因子としての莢膜型と7PCV

肺炎球菌の病原因子としては多くの菌体成分が知られている[6]が，そのなかでも抗原特異性も含めて重要なのは莢膜である．莢膜は菌の最外層に存在し，その構成成分は多糖体（polysaccharide）である．この厚い莢膜の存在によってオプソニゼーションにかかわる補体の活性化が阻害され，菌は多核白血球による貪食作用に抵抗することとなる．

一方，肺炎球菌は，乳幼児の鼻咽腔内に常在菌として棲息していることも多いが，そのことによってヒトに本質的に備わる免疫機構を成熟させていく．細菌感染はウイルス罹患後の続発感染として重要であるが，肺炎，敗血症，化膿性髄膜炎，あるいは急性中耳炎などの原因菌は，ほぼ100％の株が莢膜を有している．

莢膜は**表**に示すように，21のグループに属する65型と25の型の計90型のいずれかに分類される．これらの型の中から，世界的な疫学データに基づいてコンジュゲートワクチン用抗原として選択されたのが，4，6B，9V，14，18C，19F，23F型の7種である．このワクチンを7PCV（7価 Pneumococcal Conjugate Vaccine：Prevenar™）とよぶ．このワクチンは，各多糖体に無毒化したジフテリアトキシン CRM_{197} を結合させ，混合されている．

欧米においては，7PCVは2000年から臨床へ導入され，その効果が盛んに報告されている[7)~9)]．わが国においても7PCVの治験は終了しており，カバーする型がさらに追加された13PCV（1，3，5，6A，7F，19Aを追加）の治

験中とのことである.

その他に，インフルエンザ菌の表層リポ蛋白の protein D を担体として莢膜多糖体に結合させ，インフルエンザ菌に対する抗体獲得もねらった 11 価ワクチン（7 価に 1，3，5，7F を追加）も開発されつつある.

Ⅱ. 莢膜型別

肺炎球菌の莢膜型は，古くから抗血清（Pneumococcus antiserum）を作製している Statens Serum Institut（デンマーク）より購入して用いている．その方法は，被験菌と抗血清を反応させ，抗原抗体反応として莢膜膨化が生じているか否かを顕微鏡下に判定する方法である.

図 1 には明らかな陽性反応を示した肺炎球菌を示すが，莢膜部分は細胞質と同じ幅に膨化してみえる．このような反応を示す抗血清の型を被験菌株の莢膜型とする.

高価な抗血清をすべて保持する研究施設は日本には恐らくなく，きわめてまれな型を除いた，臨床的に重要な約 40 の型が識別できる筆者らの研究室が，もっとも多種類の莢膜型を揃えていると思われる.

Ⅲ. 薬剤耐性化にかかわる遺伝子

肺炎球菌の薬剤耐性化の中で，抗菌薬選択上その動向を常に把握しておかねばならないのは，β-ラクタム系薬剤耐性，マクロライド系薬剤耐性，そしてニューキノロン系薬剤耐性である．ちなみにミノサイクリンには 80% 以上の株が耐性である.

β-ラクタム系薬剤の選択にあたっては，まずペニシリン系薬剤，セフェム系薬剤，そしてカルバペネム系薬剤に分け，さらに経口薬と注射

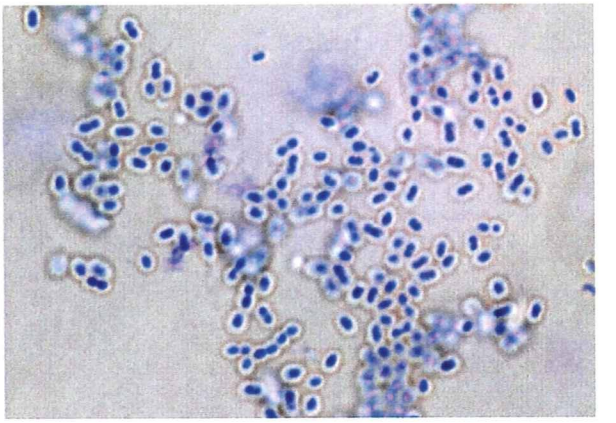

図 1　肺炎球菌の莢膜膨化試験
メチレンブルーで濃青色に染まっている部分が菌体，その外側にハローを形成している部分が対応する抗血清によって膨化した莢膜である.

薬とに区別し，それぞれの薬剤の抗菌活性のみならず，殺菌性の優劣，吸収・排泄などの体内動態についての優劣も考慮する必要のあることはいうまでもないが，紙面の関係でここでは耐性化状況と主要な抗菌薬の成績のみを示す.

β-ラクタム系薬剤の耐性化には，その作用標的で，菌の生存・発育に必須の細胞壁を合成する酵素群（penicillin-binding protein：PBP）の変化がかかわっている．**図 2** に示すように，肺炎球菌に見出される 6 種の PBP のうち，長軸方向への壁合成を行う PBP1A，隔壁合成酵素の PBP2X，そして溶菌・殺菌と密接に関連する PBP2B である．耐性菌ではこれらの酵素をコードする遺伝子が変異している.

わが国で多く処方されているセフェム系薬剤は，隔壁合成酵素の PBP2X を他の PBP よりも低濃度で強く阻害し，抗菌力を発揮しているが，溶菌性は弱い．それに対し，カルバペネム系薬剤は PBP2B と PBP1A を強く阻害し，顕微鏡下に観察すると菌は短時間で溶菌に至り，殺菌性の強いことがわかる．ペニシリン系薬剤は，一般的にはセフェム系薬剤とカルバペネム系薬剤のちょうど中間程度の殺菌性を示す.

耐性菌ではこのような PBP 遺伝子が変化しているので，抗菌力の低下と同時に殺菌性も低