表1　GAS，SDSE，GBSの抗菌薬感受性

抗菌薬	GAS		SDSE		GBS	
	MIC range (μg/mL)	MIC_{90} (μg/mL)	MIC range (μg/mL)	MIC_{90} (μg/mL)	MIC range (μg/mL)	MIC_{90} (μg/mL)
経口薬						
penicillin G	0.004～0.016	0.016	0.008～0.016	0.016	0.016～**0.5**	0.063
ampicillin	0.008～0.031	0.031	0.016～0.031	0.031	0.031～**0.25**	0.125
amoxicillin	0.008～0.031	0.031	0.016～0.031	0.031	0.031～**0.25**	0.125
cefdinir	0.002～0.016	0.016	0.008～0.031	0.016	0.016～**0.25**	0.063
cefditoren	0.002～0.016	0.016	0.008～0.016	0.016	0.016～**0.25**	0.031
levofloxacin	0.5～>16	4	0.5～>16	2	1～>16	>64
clarithromycin	0.063～>64	>64	0.063～>64	>64	0.063～>64	>64
clindamycin	0.063～>64	>64	0.063～>64	>64	0.063～>64	>64
注射薬						
cefotaxime	0.002～0.031	0.016	0.008～0.031	0.016	0.016～**0.125**	0.063
panipenem	0.001～0.008	0.008	0.004～0.016	0.008	0.008～0.063	0.031
meropenem	0.004～0.031	0.031	0.008～0.016	0.031	0.031～**1**	0.063
vancomycin	0.25～1	0.5	0.25～1	0.5	0.25～0.5	0.5

太字で示すMICの菌株は*pbp2x*に変異を有する

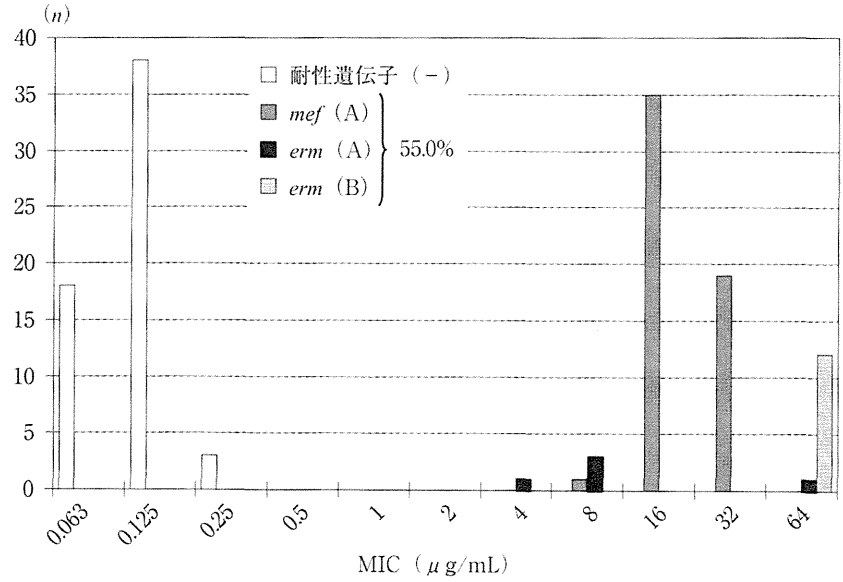

図5　GASのクラリスロマイシン感受性と耐性遺伝子（$n=131$）

GBSでは21%が耐性である．いずれも4年前と比較すると，耐性菌は増加傾向にある．

これら3菌種におけるML系薬に対する耐性化機構には，①ML系薬排出蛋白をコードする*mef*（A）遺伝子，②23S rRNAメチラーゼをコードする誘導型の*erm*（A）遺伝子，③構成型の*erm*（B）遺伝子のいずれかがかかわっている[15]．図5には，ML系薬耐性菌が増加しているGASの成績を示したが，*mef*（A）保持株はML系薬にのみ耐性，*erm*(A) あるいは*erm*(B) 保持株は，リンコマイシン系薬にも耐性を示す．Erm（A）は誘導型酵素であるため，ML系薬に触れさせる

と誘導がかかり，Erm（B）と同レベルの高度耐性を示すので注意しなければならない[16]．

●フルオロキノロン（FQ）系抗菌薬

FQ系薬の耐性化は，標的部位であるDNAジャイレースをコードする*gyrA*，*gyrB*遺伝子，トポイソメラーゼⅣをコードする*parC*，*parE*遺伝子のキノロン耐性決定領域に生じた変異で起こる[17]．特に，*gyrA*遺伝子のこの領域に変異が起こると高度耐性化することが知られている．筆者らの成績では*parC*または*gyrA*遺伝子に変異を持つFQ系薬耐性菌はGASで15%，SDSEで14%，GBSで53%であった．

耐性率からもわかるように，GBSの急速なFQ耐性化が臨床的に問題となっている．特に，成人由来株の62%が耐性であり，さらにその72%が高度耐性株である．これらの株の莢膜型はほとんどがⅠb型であるが，今後はⅢ型株の耐性化動向に注意が必要である．

おわりに

分子生物学の進歩とともに，近年比較的容易に遺伝子レベルでの解析ができるようになっている．それとともに，世界的には菌の型別も抗血清を用いた古典的手法から遺伝子を直接検出する手法へと急速にシフトしてきている．特に，欧米ではワクチン開発を志向し，このような遺伝的手法を用いた疫学解析が盛んに行われている．

一方，本邦ではこのような手法の基盤が不十分であるといわざるを得ない．サーベイランスの結果を諸外国の成績と比較し共有できる点，流行菌型や耐性遺伝子などを正確に把握できる点からも，遺伝学的手法による疫学解析の基盤づくりは急務である．

*β*溶血性レンサ球菌に関しても，その病原性や疾患を理解するには，菌を分離するだけではなく，遺伝子型やその疫学の変化に敏感に対応していく環境づくりが必要であろう．

謝　辞：厚生労働科学研究「新型インフルエンザ等新興・再興感染症研究事業(H22-新興-一般-013)，研究課題：重症型のレンサ球菌・肺炎球菌感染症に対するサーベイランスの構築と病因解析，その診断・治療に関する研究」にご協力いただいております検査技師各位に感謝いたします．

文　献

1) 生方公子（研究代表者）：重症型のレンサ球菌・肺炎球菌感染症に対するサーベイランスの構築と病因解析その診断・治療に関する研究．厚生労働科学研究費補助金：新型インフルエンザ等新興・再興感染症研究事業，平成22年度総括研究報告書．
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J Infect Chemother (2012) 18:853–857
DOI 10.1007/s10156-012-0426-3

ORIGINAL ARTICLE

Evaluation of intrapartum antibiotic prophylaxis for the prevention of early-onset group B streptococcal infection

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Received: 28 February 2012 / Accepted: 25 April 2012 / Published online: 22 May 2012

Abstract We retrospectively assessed the medical records of pregnant women who delivered at Asahikawa Kosei Hospital during a period of 3 years between January 2009 and December 2011 and their neonates. Our prophylactic measures against group B *Streptococcus* (GBS) infection are based on the Japanese guidelines. More specifically, we performed screening by examining bacterial cultures of vaginal–perianal swabs from pregnant women between gestational weeks 33 and 37. Then, sulbactam/ampicillin (SBT/ABPC) was given at a dose of 1.5 g through a drip intravenous infusion at delivery if pregnant women were screened positive for GBS. For neonates born to GBS carrier women, bacterial cultures of pharyngeal swabs, vernix caseosa, stool, and gastric juice were performed at birth. There were 2,399 deliveries and 2,499 births at our hospital. In 169 of the deliveries (175 of the births), GBS was isolated from specimens obtained from gestational weeks 33–37. According to delivery mode, there were 42 cases of cesarean section (45 births) and 127 cases of vaginal delivery (130 births). The GBS-positive neonates accounted for 4.1 % of all deliveries in pregnant women who tested positive for GBS at gestational weeks 33–37. In neonates born by vaginal delivery, the GBS-positive rate was 5.5 %. Of the 2,499 neonates born at our hospital during a period of 3 years, early-onset GBS infection occurred in 1 neonate. The incidence of early-onset GBS infection was 0.40 per 1,000 live births. From 1997 to 2001 (routine GBS screening of mothers was not performed), there were 2,097 deliveries and 2,166 births. Early-onset GBS infection occurred in 1 neonate during this period; thus, the incidence of early-onset GBS infection was 0.46 per 1,000 live births. There were no significant differences in the two periods. The present prophylactic measures such as screening of maternal GBS carriers and intrapartum antibiotic administration are inadequate to decrease the occurrence of early-onset GBS infection.

Keywords Group B *Streptococcus* · Neonatal sepsis · Antibiotic prophylaxis · Intrapartum antibiotic · Prenatal culture

Introduction

Group B *Streptococcus* (GBS) is the major causative agent of sepsis, pneumonia, and meningitis in neonates. GBS infection is classified as "early-onset," occurring within 3 days of birth, and "late-onset," occurring more than 3 days after birth. Early-onset GBS infection is frequently transmitted vertically via the maternal birth canal. If GBS infection has manifested, the prognosis is frequently poor despite adequate treatment. Thus, the Centers for Disease Control and Prevention (CDC) of the United States published guidelines for prophylactic measures against neonatal GBS infection in 1996 [1]. Specifically, the primary measure is to intravenously administer penicillin antibiotics at delivery to pregnant women with risk factors for GBS infection such as fever and prolonged rupture of the membranes. In 2002, the guidelines were revised, stating that indications for antibiotic therapy should be determined on the basis of vaginal culture screening results in pregnant women [2]. In the United States, the incidence of early-onset GBS infection in the early 1990s was 1.5–1.8 per 1,000 live births [3]. Since CDC published the guidelines for the prevention of GBS infection in 1996, the incidence

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decreased to approximately 0.5 per 1,000 live births in 2000 and has remained constant at 0.34–0.37 since 2002 [3]. The Japan Society of Obstetrics and Gynecology and the Japan Association of Obstetricians and Gynecologists published "guidelines for obstetrical practice in Japan 2008" [4] (this guideline was revised in 2011 [5]), in conformity with the CDC guidelines, which were revised in 2002. In the United States, surveillance for GBS infection is performed every year, and the contents of the guidelines are evaluated [3]. In Japan, however, there are very few reports on neonatal GBS infection and the efficacy of prophylactic measures. Thus, we conducted a screening study of GBS infection at our hospital over a period of 3 years from 2009 through 2011. Herein, we report the results of the present screening study, including the incidence of neonatal GBS infection, with a discussion of problems with the Japanese guidelines.

Subjects and methods

We retrospectively assessed the medical records of pregnant women, who delivered at Asahikawa Kosei General Hospital during the 3-year period from January 2009 through December 2011, and their neonates.

Our prophylactic measures against GBS infection are based on the Japanese guidelines [4, 5]. More specifically, we performed screening by examining bacterial cultures of vaginal–perianal swabs from pregnant women between gestational weeks 33 and 37. Sulbactam/ampicillin (SBT/ABPC) was then given at a dose of 1.5 g through a drip intravenous infusion at delivery to any pregnant woman who had screened positive for GBS, those who screened negative for GBS but had previously delivered a neonate with GBS infection, or those for whom screening had not been conducted or the result was unknown. When delivery was prolonged, additional SBT/ABPC was administered over a period of 8–12 h at the attending physician's discretion. If multiple screening tests for GBS were performed before delivery, the latest result was used to determine whether antibiotic therapy was necessary.

For neonates born to GBS carriers, bacterial cultures of pharyngeal swabs, vernix caseosa, stool, and gastric juice were performed at birth. If neonates had signs of infection, such as respiratory disorder, fever, and asphyxia, we performed blood as well as spinal fluid cultures, as necessary. Neonates born to mothers negative for GBS were subjected to bacteriological culture examination only when there were signs of infection in either the mother or the neonate. The incidence of GBS infection was calculated as the rate per 1,000 live births. For comparison with past results, we determined the incidence rate of GBS infection during the following two consecutive periods: one from 1997 to 2001, when routine GBS screening tests on pregnant women were not undertaken, and the other from 2002 to 2008, when GBS screening tests were routinely performed on pregnant women yet without standardized measures to cope with GBS-positive pregnant women and their neonates. During the period from 1997 to 2011, one child in the years 1998, 2004, 2006, and 2009 developed early-onset GBS infection.

Results

During the 3-year period from 2009 to 2011, there were 2,399 deliveries (inclusive of 633 cesarean deliveries) and 2,499 births at our hospital. In 169 of these deliveries (175 of the births), GBS was isolated from specimens obtained at gestational weeks 33–37. None of the women who had not undergone screening tests for GBS and were examined immediately before delivery were positive for GBS.

One premature infant who was found to be positive for GBS was excluded from the present analysis because the mother had not been examined between weeks 33 and 37 of gestation. The mother of this baby became GBS positive at week 23 of gestation, and the baby was delivered by cesarean section at week 26. Vernix caseosa at birth was positive for GBS, but there were no signs of infection.

According to delivery mode, there were 42 cesarean sections (45 births) and 127 vaginal deliveries (130 births). GBS was detected in 7 neonates delivered vaginally, as summarized in the table, whereas no GBS was detected in neonates born by cesarean section. The GBS-positive case rate of newborns from mothers who were found to be GBS positive between gestational weeks 33 and 37 was 7 of 169 neonates (4.1 %) overall and, when limited to vaginal births, 7 of 127 neonates (5.5 %) (Table 1).

Seven neonates were positive for GBS, all of whom had been born within 90 min after administration of antimicrobial agents. An infant delivered vaginally at a gestational age of 39 weeks (case 5) was noted to have signs of infection such as asphyxia and respiratory disorder, and GBS was demonstrated by blood cultures. Because cerebrospinal fluid examination revealed no onset of meningitis (cell counts, 19/μl; glucose, 26 mg/dl; protein, 111 mg/dl), GBS sepsis was diagnosed. The infant was treated with ampicillin, to which the organism was sensitive, resulting in a cure without sequelae. For the other six neonates, there were no risk factors, such as premature rupture of membranes or maternal fever, or any signs of infection. Among the 2,499 neonates born at our hospital during the 3-year period of this study, 1 neonate had early-onset GBS infection. The incidence rate of early-onset GBS infection, therefore, was 0.40 (95 % CI, 0.01–2.23) per 1,000 live births. From 1997 to 2001, there were 2,097 deliveries and 2,166 births. Early-onset GBS infection occurred in 1

Table 1 Characteristics of newborns from whom GBS was isolated from their mothers by screening by bacterial cultures of vaginal–perianal swabs between gestational weeks 33 and 37

Case no.		1	2	3	4	5	6	7
Gestational age (weeks + days)	At screening	34 weeks + 1 day	36 weeks + 6 days	34 weeks + 0 day	35 weeks + 0 day	35 weeks + 6 day	36 weeks + 4 days	33 weeks + 6 days
	At birth	38 weeks + 2 days	37weeks + 4 days	38 weeks + 5 days	39 weeks + 0 day	39w + 0d	37w + 5 days	40 weeks + 3 days
Intrapartum antibiotics	Drug	SBT/ABPC	SBT/ABPC	SBT/ABPC	SBT/ABPC	SBT/ABPC	SBT/ABPC	SBT/ABPC
	Dose (g)	1.5	1.5	1.5	1.5	1.5	1.5	1.5
	Time from administration to delivery (min)	90	20	42	49	83	69	80
Specimen isolated GBS		Gastric fluid	Vernix caseosa	Vernix caseosa	Vernix caseosa	Blood, vernix caseosa	Feces	Vernix caseosa
Diagnosis		Carrier	Carrier	Carrier	Carrier	Sepsis	Carrier	Carrier

GBS group B *streptococcus*, *SBT/ABPC* sulbactum/ampicillin

neonate during this period; thus, the incidence of early-onset GBS infection was 0.46 (95 % CI, 0.03–2.57) per 1,000 live births. During the period from 2002 to 2008, there were 3,746 births with 3,930 deliveries. Two of those neonates developed early-onset GBS infection; thus, the incidence rate was 0.51 (95 % CI, 0.06–1.84) per 1,000 live births. The incidence did not differ significantly between these periods.

Discussion

There have been very few reports on the incidence of early-onset GBS infection in Japan. Matsubara and Yamamoto [6] performed a questionnaire survey among institutions providing neonatal care, mainly in the Kinki and Chugoku regions, between 2000 and 2004. Based on the data on the numbers of deliveries and patients with GBS infection derived from the responses of 28 institutions, the incidence of early-onset GBS infection was 0.10 per 1,000 live births, which was lower than that in the United States [3]. According to our results, the incidence of GBS infection was 0.46 per 1,000 live births during the period when no specific prophylactic measures were taken and 0.40 during the recent 3-year period with the implementation of prophylactic measures; i.e., there was no marked difference between the two periods. These incidence rates were approximately the same as that in the United States and were higher than the figure reported by Matsubara et al. [6]. Their results were derived from a questionnaire survey involving multiple institutions in different regions of Japan, whereas our results were obtained only from a single hospital. Thus, a simple comparison of these results is difficult.

One of the cornerstones of the CDC guidelines for the prevention of GBS infection is screening. More specifically, GBS screening by culturing vaginal–anal specimens from all pregnant women at gestational weeks 35–37 should be performed to identify GBS-positive women.

The prevalence of GBS carriers among pregnant women is recognized as ranging from 10 % to 37 % [3]. An important issue is to determine the appropriate timing of screening. Although GBS is essentially considered to be an indigenous bacterium of the vagina and rectum, it does not exist persistently; instead, it is considered to repeatedly appear and disappear. Thus, a pregnant woman who is negative for GBS at screening may well be positive for GBS at delivery. Lin et al. [7] investigated the GBS carrier trend among 5,497 pregnant women at 32 gestational weeks or later. The results of their study showed that, among 1,172 women who were positive for GBS during pregnancy, 592 (approximately 50 %) were also positive immediately before delivery. On the other hand, 291 of the 3,524 GBS-negative women were positive immediately before delivery. Thus, the results differed markedly depending on the timing of screening. Another important issue in the prevention of infection is how to identify pregnant women who are negative for GBS at screening but positive immediately before delivery. According to the Japanese guidelines [4, 5], screening should be performed between gestational weeks 33 and 37, i.e., earlier than recommended by the CDC guidelines [2, 3]. However, because the presence of these bacteria is not constant, a second test may be required if the screening is performed relatively early in pregnancy.

Moreover, it remains unknown whether the aforementioned changes can be attributed to physiological

alterations in the bacteria, differences among sampling sites and methods, or the sensitivities of various bacterial culture methods. Although GBS are always present, specimens may be negative as a result of problems with testing methods. Recently, polymerase chain reaction (PCR), which is used in a wide variety of tests, has been shown to be superior to culture in terms of sensitivity and speed [8]. However, PCR has not been widely used in Japan because of the high cost of testing and the requirements for special equipment. If antibiotic therapy can be administered based on the results of PCR, it will be possible to identify pregnant women requiring antibiotic therapy with greater accuracy and to avoid unnecessary therapy. Further dissemination of PCR is awaited.

Another cornerstone of the CDC guidelines [3, 4] for the prevention of early-onset GBS infection is prophylactic intravenous administration of antibiotics to pregnant women at delivery. Lin et al. [9] reported that the mother-to-infant transmission rate of GBS is 16.1 %. The transmission rate from mothers not receiving antibiotics during delivery to neonates was 38.2 %, whereas the transmission rate from mothers receiving antibiotics to their neonates was 6.3 %, revealing a significant difference between the two groups (with versus without antibiotic therapy). The CDC guidelines recommend that penicillin G (PCG) or ABPC be administered at delivery to pregnant women with a positive screening result or risk factors for GBS infection [2, 3]. The Japanese guidelines [4, 5], which adhere to the CDC guidelines [2, 3], recommend ABPC as the first choice of antibiotic therapy, because the intravenous formulation of PCG is not available in Japan. The CDC guidelines recommend an initial dose of 2 g, followed by a dose of 1 g repeated every 4 h, although the Japanese guidelines ambiguously state that doses should be determined according to healthcare conditions. One report states that the use of ABPC leads to an increased rate of neonatal infection with β-lactamase-producing *Escherichia coli* [10]; thus, we administer SBT/ABPC as the first choice at conventional doses approved for adults in Japan.

The time from administration of antimicrobial agents until delivery is also a problem. It has been described as taking more than 4 h for the plasma ABPC concentration to reach sufficient bactericidal levels against GBS in maternal blood and fetal cord blood when the antibiotic is administered to pregnant women [11]. In a study reported by Lijiroi et al. [12], the incidence of GBS-positive newborns was 5 of 136 (3.7 %) for live births occurring $\geq$4 h after administration of antimicrobial agents, differing significantly from the 9 of 73 (12.3 %) for live births occurring <4 h after administration of antimicrobial agents. All seven neonates found to be positive for GBS in the present series had been born within 4 h after administration of antimicrobial agents. In the present study, all our seven neonates positive for GBS were born less than 4 h after administration of antibiotics.

Furthermore, because the incidence of early-onset GBS infection was originally not as high as that in the United States, a definitive effect of prophylactic measures based on the CDC guidelines could not be confirmed. Although our administration dosage (i.e., 1.5 g SBT/ABPC) is different from that recommended by the CDC, we believe that our treatment strategy is valid because there are no differences in either the mother-to-infant transmission rate of GBS or in the incidence of GBS infection, and these are indicators for therapeutic efficacy of antibiotics at delivery.

In the future, it will be necessary to adopt prophylactic measures for early-onset GBS infection. To this end, examination procedures, such as PCR, that provide prompt and highly sensitive/specific results should be introduced as appropriate screening tests for GBS-positive women, and such procedures should be performed at a time as close to delivery as possible. It is also important to set the types and doses of antibiotics to be administered at delivery. However, it should be kept in mind that GBS infection can still occur because, for example, false-negative tests cannot be completely avoided even with improved test accuracy, and there may be only a short time from administration of antimicrobial agents to parturition. Future studies should examine alternative strategies, such as conducting GBS tests on all neonates and using antimicrobial agents in carrier cases.

Acknowledgments This work was supported by grants for a Research Project for Emerging and Reemerging Infectious Diseases (H-22-013) from the Japanese Ministry of Health, Labour and Welfare.

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原　著

侵襲性肺炎球菌感染症の小児から分離された *Streptococcus pneumoniae* の抗菌薬感受性

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（平成24年7月26日受付）
（平成24年8月14日受理）

Key words: *Streptococcus pneumoniae*, bacteremia, drug susceptibility, penicillin resistant

要　旨

2006年から2011年までに侵襲性肺炎球菌感染症の小児から検出された *Streptococcus pneumoniae* 76株について最小発育阻止濃度（MIC）と最小殺菌濃度（MBC）を測定した．患者の年齢は生後4カ月から6歳までに分布し，1歳が50名（65.8%），1歳未満が10名（13.2%）と1歳以下で60名（79.1%）を占めていた．疾患ではoccult bacteremia 38例(50.0%)，肺炎34例(44.7%)，髄膜炎3例(3.9%)，敗血症1例(1.3%)であった．敗血症1例が死亡し，他は後遺症なく治癒した．莢膜の血清型は6Bが最も多く20株(26.3%)，ついで19Fが13株（17.1%），14が9株（11.8%）であった．7価結合型ワクチンに含まれる血清型は55株（72.4%）であった．ペニシリン結合蛋白遺伝子変異による耐性分類ではpenicillin resistant *S. pneumoniae*（PRSP）株32株（42.1%），penicillin intermediate-resistant *S. pneumoniae*（PISP）35株（46.1%），penicillin susceptible *S. pneumoniae*（PSSP）株11株（11.8%）であった．MIC_{90}/MBC_{90}はampicillin 4/4μg/mL，vancomycin 0.5/0.5μg/mL, cefotaxime 1/2μg/mL, ceftriaxone 1/2μg/mL, panipenem 0.125/0.125μg/mL, meropenem 0.5/0.5μg/mL，doripenem 0.25/0.25μg/mLであった．

〔感染症誌　87：1～5，2013〕

はじめに

Streptococcus pneumoniae は小児に髄膜炎，菌血症，肺炎などの侵襲性肺炎球菌感染症（invasive pneumococcal disease，IPD）をきたす，小児科領域では重要な菌の一つである．近年，*S. pneumoniae* はpenicillinやcephem薬に感受性が低下した株が増加し，治療に難渋する症例も少なくない．IPDに対する適切な抗菌薬を選択するために，2006年～2011年に当院を含めて北海道内の小児科施設で診療したIPDの小児から分離された株における抗菌薬感受性を検討したので報告する．

対象と方法

2006年1月から2011年12月までの6年間にIPDのために当院を含めた北海道内の小児科施設で診療した小児から検出された *S. pneumoniae* 76株を対象とした．IPDの定義は，本来無菌であるべき部位から *S. pneumoniae* を分離した感染症とし，76株は73株が血液，3株が髄液から分離された．

最小発育阻止濃度（minimum inhibitory concentration，MIC）は日本化学療法学会標準法[1]に準じた微量液体希釈法でpenicillinG（PCG），ampicillin（ABPC），cefotaxime（CTX），ceftriaxone（CTRX），panipenem（PAPM），meropenem（MEPM），doripenem（DRPM），vancomycin（VCM）の8薬剤について測定した．PCGに対するMICが2μg/mL以上をpenicillin resistant *S. pneumoniae*（PRSP），0.1μg/mL未満をpenicillin susceptible *S. pneumoniae*（PSSP），中間をpenicillin intermediate resistant *S. pneumoniae*（PISP）とした．

最小殺菌濃度（minimum bactericidal concentration，MBC）はMIC測定を行ったプレートよりMIC以上の濃度のウェルから培養液10μLを非選択培地に塗布し，35℃で20～24時間好気培養後，コロニー数を計測し，発育菌量を99.9%以上減少させる濃度とした．

血清型はPneumococcal antisera（Statens Serum

別刷請求先：（〒078-8211）旭川市1条通24丁目
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平成25年1月20日

Institute, Copenhagen, Denmark)で判定した．PBP遺伝子解析はPCRキットである肺炎球菌遺伝子検出試薬（湧永製薬）を用いて行った．その成績から生方らの報告[2]に基づいて，gPRSP，gPISP，gPSSPに分類した．

成　績

IPDの患者の年齢は生後4カ月から6歳までに分布していたが，1歳が50名（65.8%），1歳未満が10名（13.2%），2歳が6名（7.9%），3歳が5名（6.6%），4歳が4名（5.3%），6歳が1名（1.3%）と乳幼児に多く，1歳以下は60名（79.1%）であった．疾患ではoccult bacteremia 38名(50.0%)，肺炎34名(44.7%)，髄膜炎3名（3.9%），敗血症1名（1.3%）であった．死亡は1名で，敗血症の3歳女児がショックをきたして死亡した．75名は後遺症なく治癒した．

PBP遺伝子変異による耐性分類ではgPSSPが9株(11.8%)，gPISPは35例(46.1%)，そのなかでpbp1a+2xに変異が認めたのは8名（10.5%），pbp2x+2aに変異があったのは1例（1.3%），pbp2xのみに変異があったのは26例（34.2%），pPRSPは32例（42.1%）であった．MICによる分類ではPSSPは35株(46.1%)，PISPは22株（28.9%），PRSPは19株(25.0%）と遺伝子変異による分類とは乖離が認められた．

Fig. 1に示したように血清型で最も多かったのは6Bで20株（26.3%），ついで19Fが13株（17.1%），14が9株（11.8%），23Fが8株（10.5%）であった．7価結合型ワクチンに含まれる血清型(4，6B，9V，14，18C，19F，23F）は55株（72.4%），13価結合型ワクチンに含まれる血清型(1，3，4，5，6A，6B，7F，9V，14，18C，19A，19F，23F）は65株（85.5%）であった．7価結合型ワクチン血清型55株とそれ以外の血清型21株の耐性頻度を比較した．ワクチンの血清型株ではgPSSP 2株（3.6%），gPISP 23株（41.8%），gPRSP 30株（54.5%），ワクチンに含まれる血清型以外の株ではgPSSP 7株（33.3%），gPISP 12株(57.1%），gPRSP 2株（9.5%）とワクチンの血清型株の方が有意に耐性率は高かった（$p<0.05$）．MICでもPSSP 18株(32.7%），gPISP 19株(34.5%），gPRSP 18株(32.7%），ワクチン血清型以外の株ではPSSP 17株(81.0%），gPISP 3株(14.3%），gPRSP 1株(4.8%）とワクチンの血清型株の方が有意に高かった（$p<0.05$）．

各薬剤に対するMICとMBCの分布をFig. 2-1と2-2に，MIC_{90}とMBC_{90}をTable 1に示した．測定した薬剤の中で最も低値であったMIC_{90}はPAPMで0.125μg/mL，ついでDRPMが0.25μg/mL，MEPMとVCMの0.5μg/mLの順であった．ABPCは4μg/mLと最も高かった．MBCでも，MICとほぼ同様な成績であり，PAPMが最も優れた値であった．Carbapenem薬のMICとMBCはすべての菌で一致していたが，penicillin薬，cephem薬ではMBCが2倍ほど高くなる株が多かった．

考　案

今回の検討で，gPSSPが11.8%であったのに対しPSSPは46.1%と遺伝子変異による分類とMICによる分類において乖離が認められた．生方[3]はpbp2xに変異があると，penicillin薬よりcephem薬での感受性の低下が著しいとしていることから，このような結果が生じたと考えられた．

Chibaら[4]は2006年8月～2007年7月までの1年間にIPDから分離された*S. pneumoniae*を全国各地の

Fig. 1　Serotype distribution of *Streptococcus pneumoniae* isolated from children with invasive pneumococcal disease

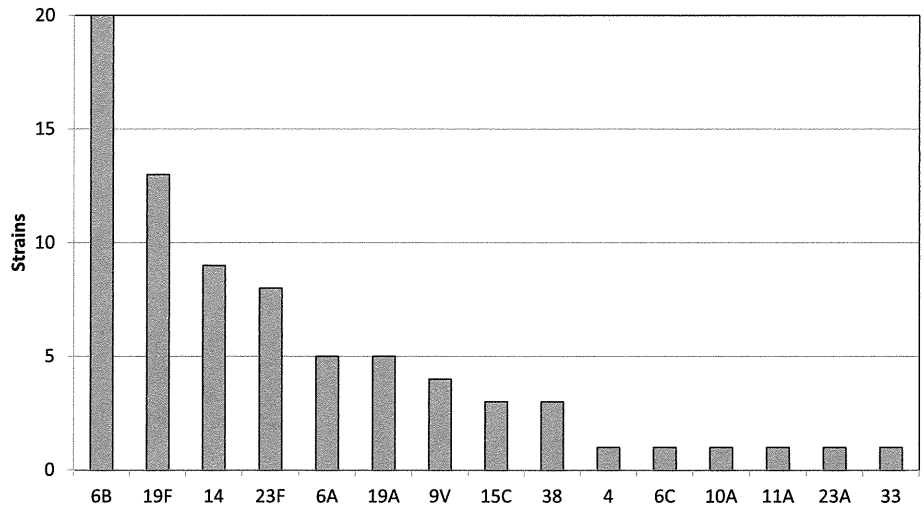


Fig. 2-1　MIC and MBC distribution in *Streptococcus pneumoniae* isolated from children with invasive pneumococcal disease

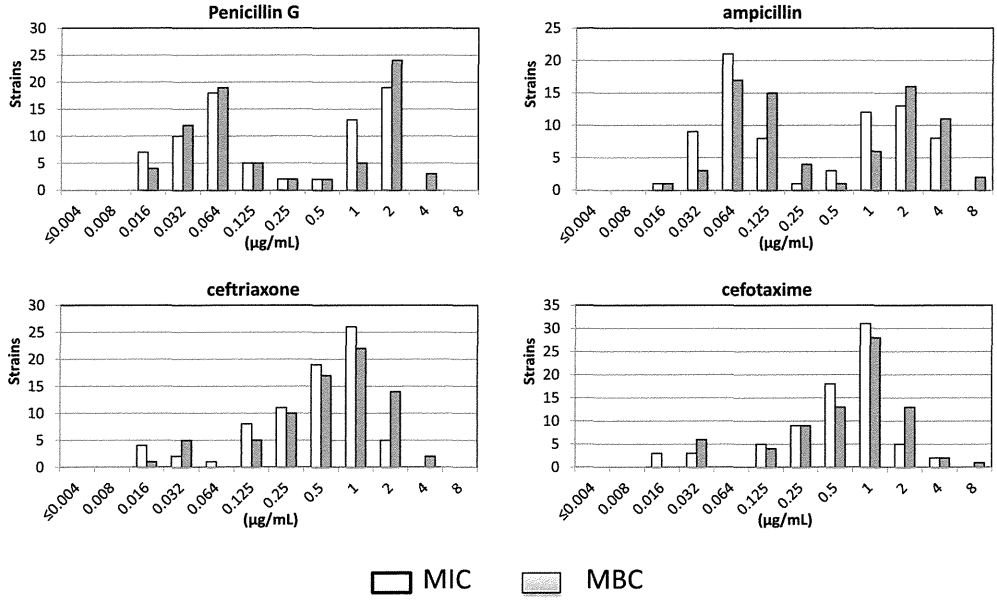
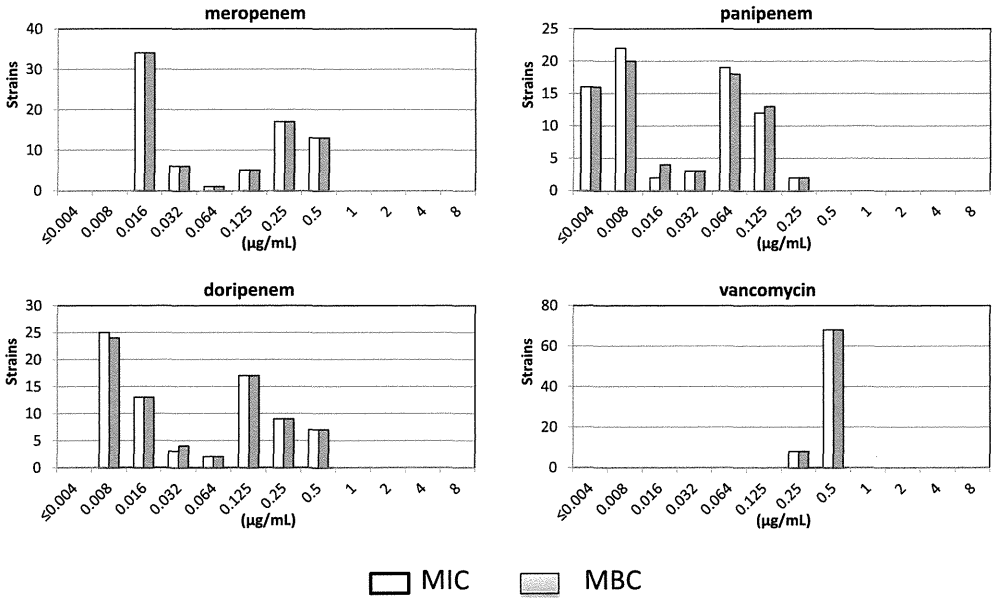


Fig. 2-2　MIC and MBC distribution in *Streptococcus pneumoniae* isolated from children with invasive pneumococcal disease



186 施設から *S. pneumoniae*　496 株を集積して，血清型と耐性遺伝子について検討している．小児からの分離株は 193 株で，4 歳以下の乳幼児からの株が 90% 以上を占めており，疾患は敗血症が 114 例 (59.1%)，肺炎が 44 例 (22.8%)，髄膜炎が 30 例 (15.5%) であった．この頻度からは敗血症に occult　bacteremia がかなり含まれていると思われる．血清型は 191 株で測定し，6B が最も多く 43 株 (22.5%)，次いで 19F が 27 株(14.1%)，14 が 25 株(13.1%)，23F が 23 株(12.0%) であり，PCV-7 のカバー率は 144 株 (75.4%)，PCV-13 のカバー率は 179 株 (93.7%) であったと報告している[3]．さらに，耐性遺伝子の検討では gPRSP は 88 株 (46.1%)，gPISP は 76 株 (39.8%)，gPSSP は 27 株 (14.1%) であった．これらは，著者らの報告とほぼ一致する成績であった．

著者の検討では，7 価結合型ワクチンに含まれる血清型の株における gPISP と gPRSP の比率は 90.3%，それ以外の株 72.3% であり，7 価結合型ワクチンに含まれる株に耐性の頻度が高かった．Chiba ら[5]はさらに，集積した株の中から 300 株について主要な抗菌薬

平成25年 1 月20日

Table 1 MIC and MBC in *Streptococcus pneumoniae* isolated from children with invasive pneumococcal disease

	MIC (μg/mL)			MBC (μg/mL)		
	range		MIC_{90}	range		MBC_{90}
penicillinG	0.016	2	2	0.016	4	2
ampicillin	0.016	4	4	0.016	8	4
ceftriaxone	0.016	2	1	0.016	4	2
cefotaxime	0.016	4	1	0.032	8	2
meropenem	0.016	0.5	0.5	0.016	0.5	0.5
panipenem	≦0.004	0.25	0.125	≦0.004	0.25	0.125
doripenem	0.008	0.5	0.25	0.008	0.5	0.25
vancomycin	0.25	0.5	0.5	0.25	0.5	0.5

に対するMICを測定し，MIC_{90}はABPCが2μg/mL，CTXが1μg/mL，MEPMが0.5μg/mL，PAPMが0.063μg/mLと，著者の成績とほぼ一致していた．以上のように，著者の結果は北海道という限局した地域の成績ではあるが，全国的に株を集めたChibaらの成績[4)5)]と同様な傾向を示していた．

IPDをきたした株が，上咽頭や喀痰から検出される*S. pneumoniae*と耐性状況が異なっているかであるが，2006年から2010年までに当院を受診した小児の臨床材料から分離された*S. pneumoniae* 2,407株の耐性率を検討した成績[6)]では，PSSPが1,099株(45.7%)，PISPが856株（35.6%），PRSPが452株（18.8%）であり，今回の成績とほぼ一致しており，IPDをきたす*S. pneumoniae*において耐性の面からは通常の感染症の小児から検出される株と異なる傾向は得られなかった．

著者は以前，2003年から2005年までにIPDの小児から分離された*S. pneumoniae*16株について今回と同様な検討を行っている[7)]．その時のMIC_{90}はABPCが4μg/mL，CTXが1μg/mL，CTRXが1μg/mL，MEPMが0.5μg/mL，PAPMが0.25μg/mL，VCMが0.5μg/mLと今回とほぼ同様の成績であり，特にそれぞれの薬剤の感受性が低下している所見は認められなかった．

IPDでも，最も臨床的に問題となるのは髄膜炎である．髄膜炎に対する抗菌薬の選択の条件としては，MBCが低いこと，髄液への移行性が良いこと，蛋白結合率が低いことなどがあげられる．抗菌薬が有効性を示すには原因菌のMBCの10倍以上の髄液濃度が必要とされている．今回MBCを測定した抗菌薬は，主に髄膜炎の治療に用いられる薬剤であるが，そのなかで最もMBCが低かったのはPAPMであった．米国ではPAPMが販売されていないこともあり，髄膜炎の治療にはVCMが用いられることが多いが，VCMのMBC_{90}は0.5μg/mL，PAPMのMIC_{90}は0.125μg/mLと大きな差が認められ，抗菌力からはPAPMを優先すべきと思われた．

謝辞：菌の分与をいただいた，遠軽厚生病院，名寄市立病院，旭川医科大学病院，札幌徳州会病院，富良野協会病院，網走厚生病院の各小児科の先生方に感謝いたします．

血清型を判定していただいた北里大学北里生命科学研究所生方公子先生に深謝します．

利益相反自己申告：申告すべきものなし

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Susceptibility in Parenteral Antibiotics in *Streptococcus pneumoniae* Isolated from Children with Invasive Pneumococcal Disease

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Minimum inhibitory concentration (MIC) and minimum bactericidal concentration (MBC) of commonly used clinical antimicrobial drugs were examined for 76 strains of *Streptococcus pneumoniae* isolated between 2006 and 2011 from pediatric patients with invasive pneumococcal infection. Patients from whom bacterial strains were isolated ranged from 4 months to 6 years old and included 50 infants (65.8%) 1 year old and 10 (13.2%) less than 1 year old, i.e., 79.1% of all patients studied. In diagnosis, 38 (50.0%) had occult bacteremia, 34 (44.7%) pneumonia, 3 (3.9%) meningitis, and 1 (1.3%) sepsis. Infections in all but one case who died of sepsis were treated without sequelae. The most frequent capsular serotype among isolates was 6B (20 strains, 26.3%), followed by 19 F (13 strains, 17.1%) and 14 (9 strains, 11.8%). Serotypes for 55 strains (72.4%) corresponded to those contained in heptavalent pneumococcal conjugate vaccine. In classification by resistance based on mutations in penicillin-binding protein genes, 32 were penicillin-resistant *S. pneumoniae* (42.1%), 35 penicillin intermediate-resistant *S. pneumoniae* (46.1%), and 11 penicillin-susceptible *S. pneumoniae* (11.8%). MIC_{90}/MBC_{90} of drugs were as follows: ampicillin 4/4μg/mL, cefotaxime 0.5/0.5μg/mL, ceftriaxone 1/2μg/mL, panipenem 0.125/0.125μg/mL, meropenem 0.5/0.5μg/mL, and doripenem 0.25/0.25μg/mL.

平成25年1月20日

Vaccine 31 (2013) 845–849

Contents lists available at SciVerse ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

Low opsonic activity to the infecting serotype in pediatric patients with invasive pneumococcal disease

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ARTICLE INFO

Article history:
Received 17 July 2012
Received in revised form 8 October 2012
Accepted 4 November 2012
Available online 12 November 2012

Keywords:
Invasive pneumococcal disease
Serotype-specific IgG
Opsonophagocytic activity
Pneumococcal vaccine
Children

ABSTRACT

Serotype-specific protective immunity in pediatric patients with invasive pneumococcal disease (IPD) has not been fully investigated. To determine the protective immunity to the infecting serotype, the serotype-specific immunoglobulin G (IgG) levels and opsonization indices (OIs) were examined in 24 Japanese pediatric patients whose serum was collected within one month of an IPD episode between May 2008 and June 2011. The median age (range) of IPD patients was 17 (10–108) months and 63% were boys. In all 17 patients tested, the levels of serotype-specific IgG to the infecting serotype were higher than 0.2 μg/ml, but the OIs to the infecting serotype were <8. The avidities of 19F- or 6B-specific IgG in patients with levels higher than 5.0 μg/ml, but with undetectable OIs, were confirmed to be lower than those in patients with high OIs. Our data demonstrated that although the levels of serotype-specific IgG to the infecting serotype were higher than 0.2 μg/ml in sera of pediatric patients with IPD, the OIs were low one month after the IPD episode. Low opsonic activities in these patients may, in part, be explained by the low avidity of serotype-specific IgG.

1. Introduction

Streptococcus pneumoniae is a leading human pathogen that causes a wide variety of diseases, ranging from otitis media to pneumonia, bacteremia, and meningitis in both children and adults [1]. Antibodies to pneumococcal capsular polysaccharide (CPS) and complement provide protection against pneumococcal strains with homologous or cross-reactive capsular serotypes [2]. Seven-valent pneumococcal conjugate vaccine (PCV7; Prevnar®, Pfizer) has been used for children in the USA since 2000 [3], and the incidence of invasive pneumococcal disease (IPD) caused by the seven vaccine serotypes (VTs) has declined markedly, although the incidence of non-VT infection has not declined [4–6]. A recent study reported that the incidence rate of IPD in children less than 5 years old was 12.6–13.8 per 100,000 in Chiba prefecture, Japan, before the introduction of PCV7 [7]. However, no information is available regarding a possible high-risk population for IPD in Japan, as was reported for Navajo children in the United States [8].

PCV7 was licensed in Japan in October 2009, and a 3 + 1 schedule (three doses for the primary series and one booster) was approved and implemented (http://idsc.nih.go.jp/vaccine/dschedule.html). Further, the Japanese government decided in November 2010 to subsidize PCV7 for children below 5 years of age.

Vaccine-induced protective immunity is currently estimated by measuring the concentrations of serotype-specific immunoglobulin G (IgG) using enzyme-linked immunosorbent assay (ELISA) [9] and the opsonization index (OI) using a multiplex-opsonophagocytic assay (MOPA) [10]. The World Health Organization working group suggested a serotype-specific IgG of

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[1] See Appendix A.

0264-410X/$ – see front matter © 2012 Elsevier Ltd. All rights reserved.
http://dx.doi.org/10.1016/j.vaccine.2012.11.010

concentration of 0.35 μg/ml as a putative measure of protection at a population level against invasive disease in infants after immunization with pneumococcal conjugate vaccine [11]. This working group also reported that antibody concentrations of 0.2–0.35 μg/ml measured with the ELISA using serum without serum absorption with 22F polysaccharide correlated best with an OI of 8, which in turn correlates best with protective efficacy. Henckaerts et al. proposed a protective threshold concentration of 0.20 μg/ml assessed with ELISA using serum absorption with 22F polysaccharide as a measure of the serotype-specific IPD efficacy for the pneumococcal conjugate vaccine [12], with the exception of serotype 19F [13]. A recent study also reported that the serological response rate following a three-dose PCV7 primary vaccination as determined using a threshold of ≥0.2 μg/ml IgG and an OI ≥ 8 corresponded well with overall effectiveness against IPD [14]. Although this threshold may not be necessarily applicable to individual patients, it is of interest to determine the protective immunity to the infecting serotype in sera collected during the acute phase in pediatric patients with IPD.

In this study, we therefore examined the IgG levels and OIs to the infecting serotype in sera of pediatric patients within one month of an IPD infectious episode. We report that the opsonic activity to the infecting serotype is low in sera obtained within one month of an episode of IPD.

2. Materials and methods

2.1. Patients

Thirty-two pediatric patients, whose cultures from sterile sites, such as blood or cerebrospinal fluid, were positive for *S. pneumoniae* between May 2008 and January 2012 at 22 hospitals in Japan, were investigated in this study. All patients were enrolled in this study when their attending doctors requested the measurement of the antipneumococcal antibodies in their sera. Sera were obtained from these 32 patients after the episode of IPD. All of the pneumococcal isolates were serotyped using coagglutination tests with rabbit antisera (Statens Serum Institute, Copenhagen, Denmark) at the Department of Bacteriology I, National Institute of Infectious Diseases. Serotype 6C was confirmed by an in-house factor antiserum [15]. All eight patients were excluded from our studies of the protective immunity to the infecting serotype: six patients for whom sera were collected more than one month after the onset of the IPD, one patient who received intravenous immunoglobulin as a treatment of IPD, and one patient with an underlying hypogammaglobulinemia. Consequently, we evaluated antipneumococcal IgG and the OIs to the infecting serotype in 24 pediatric patients with IPD. This study was reviewed and approved by the Ethics Committee of the RIMD, Osaka University, and conducted according to the principles expressed in the Declaration of Helsinki.

2.2. ELISA

Antipneumococcal IgG antibodies were measured with the WHO approved ELISA using a standard reference serum (89-SF) and C-polysaccharide and 22F polysaccharide absorptions as previously described [9,16]. The levels of serotype-specific IgG for the infecting serotypes including 6B, 9V, 14, 19F and 23F were determined according to the WHO protocol [a detailed protocol is available at www.vaccine.uab.edu/ELISAProtocol (89SF)].

2.3. MOPA

A multiplexed opsonophagocytic killing assay (MOPA) for the infecting serotype based on antibiotic-resistant target bacteria was performed at the Research Institute for Microbial Diseases, Osaka University, as previously described [10]. The quality control serum was prepared from pooled sera of adults vaccinated with the 23-valent pneumococcal polysaccharide vaccine (PPV23; Pneumovax®, MSD), and this was used in each assay. The OI was defined as the serum dilution that killed 50% of bacteria, and the OIs were determined using opsotiter3 software according to the WHO protocol (at www.vaccine.uab.edu/UAB-MOPA). Only the OI results for the infecting serotypes including 6B, 6C, 14, 19A, 19F and 23F were used in this study.

2.4. Measurement of protective immunity

Neither the serotype-specific IgG nor the OI was available in one patient with serotype 15B and another with serotype 24F infection. Only the OI was available in three patients with serotype 19A and two patients with serotype 6C infection. The OIs were not determined in another five patients because their sera contained antibiotics. Consequently, the level of serotype-specific IgG or OI to the infecting serotype was measured in 17 patients, and both the levels of serotype-specific IgG and OIs were measured in only 14 patients.

2.5. Avidity of serotype-specific IgG

The avidity of the serotype-specific IgG in sera was evaluated using ELISA by the previously published method with a minor modification [17]. Serum samples that had been preadsorbed C-polysaccharide and 22F CPS were added to the coated microtiter plates, and the plates were incubated for 1 h at 37 °C. After washing the plates, sodium thiocyanate (NaSCN) at concentrations from 0 to 1.0 M was added to each well and the plates were incubated for 15 min at room temperature. After washing of the plates, diluted goat anti-human IgG HRP-conjugate was added to each well. After incubation for 1 h at room temperature, the substrate solution was added to the plates, followed by incubation for 20 min at room temperature. The optical density at 405 nm was measured. The avidity of serotype-specific IgG was expressed as the percentage of absorbance remaining after treatment with different concentrations of NaSCN.

3. Results

The clinical characteristics of the 24 pediatric patients with IPD are shown in Table 1. The diagnosis of these patients included meningitis ($n = 11$), bacteremia ($n = 10$), and bacteremic pneumonia ($n = 2$) and septic arthritis ($n = 1$). The median age (range) was 17 (10–108) months, and 63% were boys. Four patients (17%) had associated comorbid conditions including immune thrombocytopenia and splenectomy, meningoencephalocele, asplenia and single ventricle, and hydrocephalus (V-P shunt). In the 24 examined, the most common infecting serotype was 6B (9 isolates, 38%), followed by 19F (4 isolates, 17%), 19A (3 isolates, 13%), 6C and 14 (2 isolates each 8%) and one isolate each of 9V, 15B, 23F and 24F (4%). The median (range) period from the onset of IPD to the time of serum collection was two (0–23) days.

Three patients received PPV23 due to pre-existing medical conditions (Table 1). Before their episode of IPD, two patients infected with serotype 19F and one patient infected with serotype 9V received PPV23. Because PPV23 contains serotypes 19F and 9V, all three cases were considered PPV23 vaccine failure (VF). Ten patients received one to three doses of PCV7 at various ages as shown in Table 1. Only one patient (Case 18) completed a course of three doses of PCV7 between 2 and 6 months of age. The other nine patients were immunized with PCV7 during the catch-up phase. PCV7 breakthrough infection (BTI) was defined where a patient who received at least one dose of PCV7 had an episode

Table 1
Clinical characteristics of 24 pediatric patients with invasive pneumococcal disease (IPD).

No.	Age (months)	Sex	Diagnosis	Comorbid condition	Infecting serotype	Serum obtained days after IPD	Antibody to the infecting serotype		Vaccination before IPD (doses)	Age at each dose (month)	Category of IPD after PPV23	Category of IPD after PCV7	Outcome
							IgG (μg/ml)	OI					
1	108	M	Meningitis	ITP, splenectomy	19F	10	6.53	2	PPV23(1)	62	Vaccine failure	NA	Alive
2	50	M	Meningitis	Meningoencephalocele	19F	17	5.1	2	PPV23(1)	42	Vaccine failure	NA	Alive
3	75	M	Bacteremia	Asplenia, single ventricle	9V	1	0.57	NT	PPV23(1)	24	Vaccine failure	NA	Dead
4	14	M	Bacteremia	None	6B	11	0.34	2	None	–	NA	NA	Alive
5	38	M	Meningitis	None	19F	4	1.08	2	None	–	NA	NA	Alive
6	14	M	Bacteremia	None	14	5	2.1	5	None	–	NA	NA	Alive
7	13	M	Bacteremia	None	6B	4	2.25	NT	None	–	NA	NA	Alive
8	12	M	Meningitis	None	6B	20	1.81	7	PCV7(1)	10	NA	Breakthrough infection	Alive
9	10	M	Meningitis	None	19F	0	0.85	NT	None	–	NA	NA	Alive
10	17	M	Bacteremic pneumonia	None	19A	2	NA	NT	None	–	NA	NA	Alive
11	30	M	Bacteremic pneumonia	None	6B	0	0.53	2	PCV7(1)	28	NA	Vaccine failure	Alive
12	17	F	Meningitis	None	24F	1	NA	NA	PCV7(1)	16	NA	Non-VT infection	Alive
13	12	F	Meningitis	None	6B	12	0.78	2	None	–	NA	NA	Alive
14	10	M	Meningitis	None	15B	2	NA	NA	None	–	NA	NA	Alive
15	30	F	Bacteremia	None	6B	0	1.18	2	PCV7(1)	26	NA	Vaccine failure	Alive
16	26	F	Bacteremia	None	19A	1	NA	2	None	–	NA	NA	Alive
17	15	F	Bacteremia	None	14	0	1.75	2	None	–	NA	NA	Alive
18	10	M	Bacteremia	None	19A	0	NA	2	PCV7(3)	4, 5, 6	NA	Non-VT infection	Alive
19	30	F	Meningitis	Hydrocephalus (V-P shunt)	6B	23	0.92	2	PCV7(1)	28	NA	Vaccine failure	Alive
20	17	F	Meningitis	None	6B	0	1.38	2	PCV7(2)	9, 11	NA	Breakthrough infection	Alive
21	11	F	Septic arthritis	None	23F	0	0.55	2	PCV7(3)	7, 8, 9	NA	Breakthrough infection	Alive
22	16	F	Bacteremia	None	6B	0	5.62	2	None	–	NA	NA	Alive
23	49	M	Meningitis	None	6C	1	NA	2	PCV7(1)	36	NA	Non-VT infection	Alive
24	14	M	Bacteremia	None	6C	7	NA	NT	PCV7(2)	9, 10	NA	Non-VT infection	Alive

OI, opsonization index; ITP, immune thrombocytopenia; PPV23, 23-valent pneumococcal polysaccharide vaccine; PCV7, 7-valent pneumococcal conjugate vaccine; NA, not applicable; NT, not tested because of antibiotic use; VT, vaccine type.

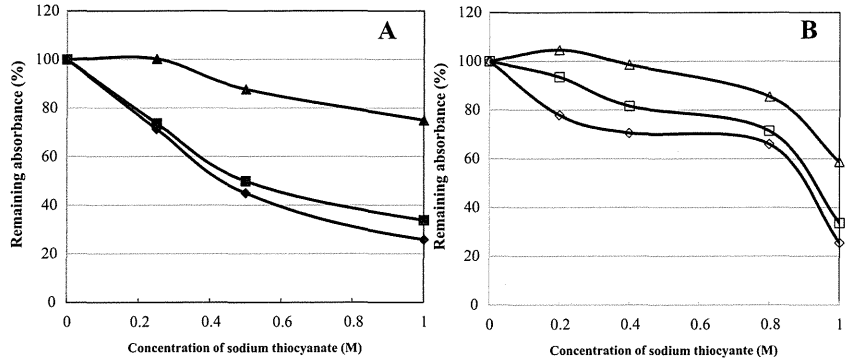

Fig. 1. Avidity of serotype 19F-specific IgG (A) and serotype 6B-specific IgG (B) in sera from pediatric patients with invasive pneumococcal diseases. Two serum samples from Case 1 (closed diamond) and Case 2 (closed square), and the positive control serum (closed triangle) from Case 6 (four months after the episode of IPD and one month after two doses of PCV7 vaccination) were examined for the avidity of serotype 19F-specific IgG. Two serum samples from Case 22 before (open diamond) and after (open square) two doses of PCV7 vaccination, and a positive control serum (open triangle) collected from Case 6 after two doses of PCV7 were used to test the avidity of serotype 6B-specific IgG.

of IPD for which the pneumococcal isolate was a PCV7 serotype, and PCV7 VF was defined as the subset of BTI in which the patient had completed the Advisory Committee on Immunization Practice (ACIP)-recommended PCV7 vaccine schedule at least two weeks before the IPD [18,19]. An instance of an IPD patient who had had at least one dose of PCV7 and for whom the pneumococcal isolate was not a PCV7 serotype was defined as PCV7 non-VT infection. Of 10 patients who received PCV7 previously, three cases (Cases 11, 15 and 19) were classified as PCV7 VF, and three cases (Cases 8, 20 and 21) were classified as PCV7 BTI. The other four cases (Cases 12, 18, 23 and 24) were classified as PCV7 non-VT infection.

The level of serotype-specific IgG or the OI for the infecting serotype was determined for 17 of 24 cases. The levels of specific IgG for the infecting serotype ranged widely from 0.34 to 6.53 μg/ml. In all 17 cases, the level of specific IgG for the infecting serotype was higher than 0.20 μg/ml, the putative threshold for preventing IPD [12,14]. The geometric mean concentration for the 17 cases was 1.35 μg/ml. In contrast, the OI for the infecting serotype was <8 in all of 17 cases. In particular, obvious discrepancies were found in two patients with serotype 19F (Cases 1 and 2) and one patient with serotype 6B (Case 22) who had serotype-specific IgG higher than 5 μg/ml and undetectable OI.

To investigate these discrepancies, we next examined the avidities of serotype 19F-specific IgG in sera from Cases 1 and 2, and the avidities of serotype-6B specific IgG in sera from Case 22. The percentages of remaining absorbance to 19F CPS of the positive control serum (IgG 7.25 μg/ml, OI 2336) collected from a patient (Case 6) after two doses of PCV7 vaccination were 100–75% at concentrations of 0.25–1.0 M of NaSCN (Fig. 1A). In contrast, the percentages of remaining absorbance to 19F CPS of sera from Cases 1 (IgG 6.53 μg/ml, OI 2) and 2 (IgG 5.10 μg/ml, OI 2) to 19F CPS were 74–44% and 71–26% at concentrations of 0.25–1.0 M of NaSCN, respectively.

The percentages of remaining absorbance to 6B CPS of the positive control serum (IgG 4.16 μg/ml, OI 4626) collected from Case 6 after two doses of PCV7 99–59% at concentrations of 0.4–1.0 M of NaSCN (Fig. 1B). In contrast, the percentages of remaining absorbance of serum from Case 22 before PCV7 vaccination (IgG 5.62 μg/ml, OI 2) and after two doses of PCV7 vaccination (IgG 2.37 μg/ml, OI 562) were 71–25% and 81–34% at concentrations of 0.4–1.0 M of NaSCN.

4. Discussion

In pediatric patients with IPD, the serum OIs for the infecting serotype within one month after the infectious episode were <8 in all 17 patients tested for OI, although the levels of IgG for the infecting serotype were higher than 0.2 μg/ml in all 17 patients tested for serotype-specific IgG. Undetectable OIs suggest that the serotype-specific IgG in their sera are largely nonfunctional. Soininen et al. similarly reported that sera from unimmunized children without nasopharyngeal carriage contained serotype-specific IgG, but infrequently had serotype-specific opsonic activity [20].

Three patients received PPV23 before PCV7 was licensed in Japan in 2009 because they were at increased risk for pneumococcal disease. Although the current guideline of the ACIP recommends that children aged 2–18 years with underlying medical conditions should receive PPV23 after completing all recommended doses of PCV13 [21], pediatricians should be aware of the possible induction of nonfunctional IgG by PPV23 in high-risk children aged >2 years. Two patients with PCV7 BTI received one or two doses of PCV7 9–11 months after birth, and two patients with PCV7 VF received only one dose of PCV7 26–28 months after birth. All four of these patients comprised the catch-up cases for PCV7. Interestingly, all cases with BTI or VF were caused by serotype 6B. A recent study from the US reported that 155 of 753 (21%) pediatric IPD cases were PCV7 BTIs caused predominantly by serotypes 6B (32%) and 19F (29%) [18]. The PCV7 BTIs caused by serotype 6B were more likely to have occurred in children who received only one or two PCV7 doses (84%) compared with infections caused by other VTs (61%). Rennels et al. also reported a low immune response to 6B and other serotypes, including 9V and 18C in children who received fewer than three doses of PCV7 [22].

Our data demonstrated that sera collected from Cases 1, 2 and 22 containing 19F- or 6B-specific IgG levels higher than 5.0 μg/ml, but lacking opsonic activity, contained lower avidity of serotype-specific IgG than the positive control sera with high OIs. An improvement of the avidity of 6B-specific IgG was confirmed in the sera with a high OI from Case 22 by two doses of PCV7 vaccination. Two previous studies using sera from healthy adults with or without vaccination with PPV23 demonstrated that higher avidity antibodies were more effective than lower avidity antibodies in in vitro complement-dependent opsonophagocytosis and for in vivo protection against pneumococcal infection in mice [23,24]. These data are, partially, in agreement with our findings of high levels of serotype-specific IgG with low avidity in serum from pediatric patients within one month after IPD. The low avidity of serotype-specific IgG levels may explain the undetectable OIs in sera collected from Cases 1, 2 and 22 within one month of an IPD episode.

O'Brien et al. recently reported the pneumococcal antibody status in a child with of PCV7 vaccine failure caused by serotype 14

[25]. In this patient, the serotype-specific IgG and the OIs in serum were 4.98 μg/ml and 1024, respectively, 35 days after the administration of three doses of PCV7. However, this patient developed occult bacteremia at 9.6 months of age, 53 days after the third dose of PCV7. Because of a slightly decreased serotype-specific IgG (4.25 μg/ml) and a significantly decreased OI of 4 in the serum of this patient after this episode of IPD, the authors suggested that the functional antibodies existing during infection with consumed by binding to the serotype 14 antigen. This finding also suggests that the ELISA assay detected some nonspecific or nonfunctional IgG in the serum of this patient, and is in agreement with the findings in the sera of our pediatric patients with IPD.

The limitations of our study are the small number of IPD cases examined and the variable timing of serum collection, although the sera were all collected within one month after the IPD episode. These limitations meant that we were unable to compare the induction of opsonic activity to the infecting serotype between the acute phase and the convalescence phase in pediatric patients with IPD.

In conclusion, in all of 17 patients tested within one month of an IPD episode, the serum OIs to the infecting serotype were <8, whereas the levels of serotype-specific IgG were higher than 0.2 μg/ml. Low avidity of the serotype-specific IgG were confirmed in three patients associated with the serotype-specific IgG levels higher than 5 μg/ml, but with undetectable OIs.

Acknowledgements

The authors are grateful to Tao Yu, Yumi Hattori, Michiyo Hayakawa, and Yuka Koizumi for technical assistance in the measurement of the serotype-specific IgG and OIs, and to Yasuyo Uemura for analyzing the clinical and laboratory data. This work was supported by research grants from the Ministry of Health, Labor and Welfare of Japan and by US NIH contract N01-AI-30021 to MHN.

Appendix A. The Japanese IPD Study Group

In addition to TO, NI, KM, JN, BC, BC, KT, YA, KO, the members of the Japanese IPD Study Group are Kenji Okada (National Fukuoka Hospital), Takashi Nakano (Kawasaki Medical University), Hideki Akeda (Okinawa Prefectural Nanbu Medical Center), Masako Habu (Tokyo Metropolitan Bokutoh General Hospital), Eri Yamaguchi (Chidoribashi Hospital), Kei Komiya (Nihon University School of Medicine), Shinji Kido (Toyota Memorial Hospital), Takahiro Niizuma (Koshigaya Municipal Hospital), Masato Arao (Saitama Medical University), Fumie Ishiwada (Chiba Kaihin Municipal Hospital), Mai Kubota (Shizuoka Children's Hospital), Kenji Furuno (National Fukuoka-Higashi Medical Center), Yoshio Yamaguchi (National Hospital Organization Tochigi Hospital), Kaoru Obinata (Juntendo University Urayasu Hospital), Mikio Yoshioka (KKR Sapporo Medical Center), and Tomomi Naito (Saiseikai Kawaguchi General Hospital).

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Journal of Medical Microbiology (2012), **61**, 1669–1672 DOI 10.1099/jmm.0.048587-0

Fifteen *Streptococcus suis* serotypes identified by multiplex PCR

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A multiplex PCR was developed to detect 15 serotypes of *Streptococcus suis*. This multiplex PCR was separated into two reaction sets. The first set identified nine serotypes (serotypes 1/2, 1, 2, 3, 4, 7, 9, 14 and 16) and the second set identified six serotypes (serotypes 5, 8, 10, 19, 23 and 25). This assay correctly detected serotypes 2, 5 and 14 in human isolates, and serotypes 1, 2, 1/2, 3, 4, 5, 7, 9, 14, 16 and 19 in pig isolates from Thailand. No cross-reaction was observed with other streptococcal species. This assay may be useful for the serotype surveillance of human and pig isolates of *S. suis*.

Received 22 June 2012
Accepted 22 August 2012

INTRODUCTION

Streptococcus suis is a zoonotic pathogen that causes invasive infections in humans who have been in close contact with infected pigs or contaminated pork-derived products, and these infections have received increasing attention worldwide (Gottschalk *et al.*, 2010). *S. suis* serotype 2 is the most prevalent serotype in humans, but human cases of serotypes 1, 4, 5, 14, 16 and 24 have also been reported (Kerdsin *et al.*, 2009, 2011a, b; Nghia *et al.*, 2008).

Thirty-three *S. suis* serotypes have been identified based on their capsular polysaccharides (Hill *et al.*, 2005). However, the identification of *S. suis* serotypes using the antiserum of each serotype is laborious and expensive. Thus, PCR is a more attractive alternative because of its rapid analytical capacity and low cost. PCR-based identification of *S. suis* serotypes 1, 14, 1/2, 2, 7, 9 and 16 has been reported previously (Kerdsin *et al.*, 2009; Marois *et al.*, 2004; Okwumabua *et al.* 2003; Smith *et al.*, 1999a, b; Silva *et al.*, 2006; Wang *et al.*, 2011a; Wisselink *et al.*, 2002). However, the genetic sequences of 15 *S. suis* capsule loci are available in GenBank (Wang *et al.*, 2011b), so we developed a multiplex PCR to identify these 15 *S. suis* serotypes using two reactions.

METHODS

Bacterial strains. Between 2006 and 2008, we collected 33 serotypes of *S. suis* reference strains, comprising serotypes 1/2, 1–31 and 33, together with 179 human isolates (Kerdsin *et al.*, 2009, 2011a, b) and 109 *S. suis* strains isolated from the tonsils of clinically healthy pigs in Khon Kaen province in 2008 (n=47) and Phayao province in 2010 (n=62) in Thailand. The 179 human isolates have been serotyped previously (Kerdsin *et al.*, 2009, 2011a, b) and comprised 165 serotype 2 strains, 12 serotype 14 strains and one isolate each of serotypes 5 and 24. We also used the reference strains of other streptococcal species: *Streptococcus pyogenes* SF370, *Streptococcus agalactiae* ATCC 13813, *Streptococcus dysgalactiae* subsp. *equisimilis* CCUG 36637, *Streptococcus dysgalactiae* subsp. *dysgalactiae* ATCC 43078, *Streptococcus porcinus* ATCC 43138, *Streptococcus pneumoniae* ATCC 33400, *Streptococcus bovis* ATCC 33317, *Streptococcus oralis* ATCC 35037, *Streptococcus mitis* ATCC 6249, *Streptococcus sanguinis* ATCC 10556, *Streptococcus gordonii* ATCC 10558, *Streptococcus mutans* ATCC 25175, *Streptococcus constellatus* subsp. *pharyngis* CCUG 46377 and *Streptococcus anginosus* ATCC 33397.

Primer design. The sequences of the capsule (*cps*) loci of *S. suis* serotypes 1, 3, 4, 5, 7, 8, 9, 10, 14, 19, 23, 25 and 1/2 were retrieved from GenBank under accession numbers JF273644–JF273656 (Wang *et al.*, 2011b). The *cps* loci sequences of *S. suis* serotypes 2 and 16 were retrieved from GenBank under accession numbers AF118389 (Smith *et al.*, 2000) and HQ694980 (Wang *et al.* 2011a), respectively.

All *cps* loci were aligned and compared with each other using the Artemis Comparison Tool (Carver *et al.*, 2005). The specific sequences

of each serotype were identified and retrieved. These sequences were used as templates for design of primers with the Primer-BLAST program (http://www.ncbi.nlm.nih.gov/tools/primer-blast/). Specific PCR amplification of the *S. suis* glutamate dehydrogenase (*gdh*) gene, which has a 695 bp PCR product, was also carried out according to a previously published method, with the minor modification of adding nucleotides at the 5′ position of primers in the *gdh* gene to increase the melting temperature and allow the use of a higher annealing temperature during multiplex PCR (Okwumabua *et al.*, 2003). The primer sequences used for the multiplex PCR are shown in Table 1.

We also retrieved the functional information of the products of each target gene for the binding of these primers from GenBank under accession numbers JF273644–JF273656, AF118389 and HQ694980 and from previously published reports (Wang *et al.*, 2011a, b; Smith *et al.*, 2000). These products included glycosyltransferase for *cps1J, cps14J, cps1/2, cps2, cps7H, cps9H* and *cps16K*, glycosyltransferase-like family 2 for *cps3J*, polysaccharide biosynthesis protein for *cps3K, cps4M* and *cps10P*, polysaccharide pyruvyltransferase for *cps23K*, UDP-glucose/GDP-mannose dehydrogenase family for *cps4N*, and CDP-glycerol : poly(glycerophosphate) glycerophosphotransferase for *cps10Q* and *cps25N*. However, the products of the target gene for each of *cps5N, cps19L, cps23J* and *cps25M* remain unknown.

Serotyping by multiplex PCR. The multiplex PCR was separated into two primer sets. Set 1 comprised the primers for serotypes 1/2, 1, 2, 3, 4, 7, 9, 14 and 16, whilst set 2 comprised the primers for serotypes 5, 8, 10, 19, 23 and 25. The PCR mixture contained 1 × KAPA2G Fast multiplex PCR mix (Kapa Biosystems) and 0.2 μM each primer for each primer set. The PCR thermal profile was as follows: initial activation of DNA polymerase at 95 °C for 3 min, followed by 30 cycles of denaturation at 95 °C for 20 s, primer annealing at 58 °C for 30 s and extension at 72 °C for 40 s, with a final extension at 72 °C for 5 min. The PCR products were analysed by gel electrophoresis for 30 min using 2 % agarose gels and 0.5 × TBE buffer. The gels were stained with ethidium bromide and visualized under UV light (GeneGenius Bioimaging System; SynGene). The sizes of the PCR products were determined by comparison with a molecular size standard (GeneRuler 100 bp plus DNA ladder; Thermo Fisher Scientific).

Serotyping of *S. suis* isolates from pigs using antisera. The serotyping of *S. suis* strains isolated from pig tonsils was achieved by co-agglutination using rabbit antisera (Statens Serum Institut). The results were confirmed by Dr M. Gottschalk at the International Reference Laboratory, Université de Montréal, Canada.

RESULTS AND DISCUSSION

We used 33 *S. suis* reference strain serotypes to verify the multiplex PCRs. As shown in Fig. 1, only the 15 target

Table 1. Primers and target genes used in the multiplex PCRs

F, Forward; R, reverse.

Serotype(s)	Sequence (5′→3′)	Gene(s)	Primer set	PCR product (bp)
1 and 14	F: AATCATGGAATAAAGCGGAGTACAG	*cps1J*	1	550
	R: ACAATTGATACGTCAAAATCCTCACC			
2 and 1/2	F: GATTTGTCGGGAGGGTTACTTG	*cps2J*		450
	R: TAAATAATATGCCACTGTAGCGTCTC			
3	F: TGGGAGAAGGCAGAAAGTACGAGA	*cps3J–cps3K*	1	1273
	R: ACCCCCAGAAGAGCCGAAGGA			
4	F: ACTTGGAGTTGTCGGAGTAGTGCT	*cps4M–cps4N*	1	783
	R: ACCGCGATGGATAGGCCGAC			
5	F: TGATGGCGGAGTTTGGGTCGC	*cps5N*	2	166
	R: CGTAACAACCGCCCCAGCCG			
7	F: GATGATTTATGGCACCCGAGTAAGC	*cps7H*	1	150
	R: AGTCACAATTGCTGGTCCTGACACC			
8	F: ATGGGCGTTGGCGGGAGTTT	*cps8H*	2	320
	R: TTACGGCCCCCATCACGCTG			
9	F: GGGATGATTGCTCGACAGAT	*cps9H*	1	300
	R: CCGAAGTATCTGGGCTACTG			
10	F: TCGCTCTGCGTTCGTCGAGT	*cps10P–cps10Q*	2	1756
	R: GCCCACCCGCCACGAGAAAG			
16	F: TGGAGGAGCATCTACAGCTCGGAAT	*cps16K*	1	202
	R: TTTGTTTGCTGGAATCTCAGGCACC			
19	F: AGCAGGGTTGCGTATGGCGG	*cps19L*	2	1024
	R: ACAAGCACCAGCAAAGACCGCA			
23	F: GCGGGCATATGCAGTGGGCA	*cps23J–cps23K*	2	825
	R: ACCGAATGCCACATCGGGTG			
25	F: GGAGGAGCTGCGGGCTCATA	*cps25M–cps25N*	2	1211
	R: TGGCCACAACCTGGATGCGTT			
All	F: TTCTGCAGCGTATTCTGTCAAACG*	*gdh*	1 and 2	695
	R: TGTTCCATGGACAGATAAAGATGG*			

*Modified from Okwumabua *et al.* (2003).