protein of PspA1 and PspA2 fragment extend protection against pneumococcal infection with strains bearing diverse PspA fragments [74].

Although the immune systems are different between mice and human being, maternal intranasal immunization can induce specific immune responses in mothers and that this immunity is effectively transferred to their infants. The findings strongly suggest that maternal mucosal (intranasal) immunization would be an attractive procedure to elicit early immunity against *S. pneumoniae* infections among young children. It is anticipated that this immunity is mediated by transplacental immunoglobulin (Ig) transferred during pregnancy and via breast milk. Naturally acquired IgA and IgG antibodies to PspA have been shown to be transferred from mother to child and to protect against early pneumococcal infections. Baril et al reported that transplacental transfer of IgG antibody to PspA was more efficient with IgG1 than IgG2 [75]. This finding is not inconsistent with a potential protective role of transplacental IgG in humans since human IgG1 and IgG2 are both highly complement fixing like IgG2 in the mouse [76–78]. Mouse IgG1 is poorly complement fixing and this observation may explain its weaker association with protection in this paper and in earlier studies [70].

Thus, infants may be able to be protected by prior immunization of their mothers. If pneumococcal protein-based vaccines are found to be efficacious, the direct correlation of the magnitude of the mother's IgG to PspA and that of the infant could be an indicator that the immunization of women of childbearing age with protein pneumococcal vaccine could protect their infants from pneumococcal disease. A clinical trial could be designed to test the potential of immunization of young adult women or infants with a combination of pneumococcal proteins as an experimental vaccination approach to prevent against invasive pneumococcal disease.

In conclusion, the current findings suggest that maternal intranasal immunization would be an attractive procedure against pneumococcal infections among during childhood because the transport of the specific antibody to the neonate can be expected to occur through both the placenta and mother's milk.

Materials and Methods

Bacterial strains

S. pneumoniae strains TIGR4 (serotype 4, PspA2), D39 (serotype 2, PspA1), EF3030 (serotype 19F, PspA1), L82016 (serotype 6B, PspA1), and BG7322 (serotype 6B, PspA1) used in this study. All of *S. pneumoniae* strains were grown in Todd-Hewitt broth with 0.5% yeast extract (THY) at 37°C until mild log-phase and stocked in aliquots at known CFU concentrations in THY broth containing 10% glycerol at −80°C until use for infections.

Recombinant PspA

rPspA for immunization was PspA2/TIGR4 including α-helical region [37]. Briefly, an internal gene fragment of *pspA* was amplified by polymerase chain reaction from *S. pneumoniae* strain TIGR4. The amplified gene fragment of the expected sizes were sub-cloned by TOPO TA Cloning Kit (Invitrogen Inc., Carlsbad, CA, USA) and then cloned to the pET20b vector (Novagen Inc., Madison, WI, USA) incorporating between *Nco*I and *Xho*I sites. The pET20b vector containing *pspA* fragment was transformed into the *E. coli* strain BL21 (DE3) for protein production. Expression of rPspA was induced with 1 mM isopropylthio-ß-D-galactoside (IPTG) for 2 h. The six-histidine-tagged rPspA was purified by nickel affinity chromatography.

Immunization

Four-week-old BALB/cByJ female mice were maintained under specific pathogen-free condition. They were immunized twice each week with 1 μg of rPspA mixed with 4 μg cholera toxin B subunit (CTB) (List Biological Labs, Campbell, CA, USA) on the Mondays and Fridays of 3 consecutive weeks [32]. During the first two weeks the immunization included CTB. During the last week, the two immunizations contained antigen alone. Control mice received only CTB for the first 2 weeks and only saline for the last week. After the final immunization, the female mice were mated with male mice for two weeks. Offspring were obtained approximately 3 weeks after mating. All animal experiments were approved by the Institutional Animal Ethics Committee of the Wakayama Medical University (Project Number: 237 and 429).

Division of offspring

In order to evaluate the importance of feeding status, we further divided offspring into 4 groups as follows. In Group A, offspring were delivered form PspA-immunized mother and breast-fed by their PspA-immunized mother. In Group B, offspring were delivered from sham-immunized mother and breast-fed by PspA-immunized mother. In Group C, offspring were delivered from PspA-immunized mother and breast-fed by sham-immunized mother. In Group D, offspring were delivered from sham-immunized mother and breast-fed by sham-immunized mother.

Enzyme linked immunosorbent assay (ELISA)

Sera and milk were collected from mother mice at birth of their pups (day0), and on days 7, 14 days after their birth. Sera were also collected from offspring at birth (day 0), 7, 14 days after birth. Anti-PspA specific antibodies in milk and sera were evaluated by the solid ELISA. Briefly, 96 well microplates (MaxiSorp, Nunc, Roskilde, Denmark) were coated with 50 μl of rPspA (2 μg/ml) in phosphate buffered saline (PBS) overnight at 4°C. After washing three times with PBS containing 0.05% Tween 20 (PBS-T), the wells were blocked for 1 h with casein buffer (0.2% casein, 0.05% Tween 20 in PBS) at room temperature. Then, 50 μl of samples diluted with casein buffer were incubated at 4°C overnight. To determined PspA specific antibody isotypes, after washing with PBS-T, the plate was incubated with 50 μl of 1/3000 biotinylated antibody to mouse IgG, IgA or IgM (Southern Biotechnology Associates, Birmingham, AL, USA) diluted in casein buffer for 2 h at room temperature, respectively. Then, after washing with PBS-T, the plate was incubated with 1/4000 alkaline phosphatase conjugated streptavidin (Southern Biotechnology Associates) for 2 h at room temperature. Color was developed with p–nitrophenyl phosphate (PNPP) (Sigma Chemical Co., St. Louis, MO, USA) and the optical density of each well was measured by a spectrophotometer at 405 nm. The subclass of anti-PspA specific IgG antibody was also determined using 1/2000 biotinylated antibodies to mouse IgG1, IgG2a, IgG2b or IgG3 (Southern Biotechnology Associates).

Challenge with pneumococci in nasal carriage, pneumonia, and bacteremia models

For nasal carriage, 7-day-old offspring were given the bacteria in a 5-μl volume of sterile Ringer's solution in a single nostril without anesthesia. Two days after inoculation, offspring were euthanized by CO_2 inhalation and the nasal cavity of each offspring was washed by flushing 100 μl of Ringer's solution into the trachea and out through the nostrils. Next, the nasal tissue including nasal conchae, olfactory epithelium, and sinus mucosa

was excised and were homogenized individually in 1 ml Ringer's solution as described previously [79].

For lung infection, the bacteria were given in a 10-μl volume of sterile Ringer's solution in a single nostril to 7-day-old offspring anesthetized with diethylether (Wako Chemical Co., Japan) to facilitate aspiration. After 3 days, offspring were sacrificed and the lungs were removed. The lobes of the lungs were placed into 1 ml of Ringer's solution in a stomacher bag and homogenized. Blood from the euthanized mice was also plated to determine numbers of CFU/ml. All specimens were serially diluted and plated on blood agar plates, blood agar plates supplemented with 4 μg/ml gentamicin, and blood agar plates supplemented with 4 μg/ml gentamicin and 5 μg/ml optochin. The viable pneumococcal counts were determined after overnight incubation.

For systemic fatal infection, 10-day-old offspring were given the bacteria in 0.1 ml sterile Ringer's solution intraperitoneally with anesthesia. The offspring were observed for 5 days to determine survival or the day of death.

Statistics

The levels of PspA specific antibody in each group were compared by ANOVA test with Dunn's multiple comparison test. The IgG1/IgG2a ratio was compared by Kruskal-Wallis test with Dunn's multiple comparison test. The carriage density of challenged offspring in each group was expressed as $\log_{10}$ CFUs and compared by Kruskal-Wallis test with Dunn's multiple comparison test. Survival of challenged offspring in each group was assessed by Kaplan-Meier test with Log-rank test. Statistical values were calculated with Prism 4 (GraphPad Software, La Jolla, CA, USA). For all comparison, $p<0.05$ was considered to represent a significant difference.

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Author Contributions

Conceived and designed the experiments: MH MK. Performed the experiments: MH MK. Analyzed the data: MH MK SKH DEB NY. Contributed reagents/materials/analysis tools: MH MK SKH DEB NY. Wrote the paper: MH MK SKH DEB NY.

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Clinical Scoring System of Acute Pharyngotonsillitis

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Abstract

Objectives: The aim of this study was to evaluate clinical outcomes of acute pharyngo-tonsillitis by a scoring system and the impact of causative pathogens. **Methods:** The patients with acute pharyngotonsillitis were evaluated for causative pathogens and her clinical symptoms and pharyngotonsillar finding by a clinical scoring system were assessed. **Results:** *Streptococococcus pyogenes* were identified at 13.6%. Thirty-one viruses were also identified by PCR. The numbers of total white blood cells and the levels of C-reactive protein showed a significant positive correlation with clinical scores ($p < 0.001$) and were significantly higher in cases with *S. pyogenes*. The clinical scores rapidly improved after the antimicrobial treatments in moderate cases and severe cases. **Conclusion:** A clinical scoring system represents the severity of acute pharyngotonsillitis and is useful for evaluating clinical course of the illness.

Acute pharyngotonisillits is one of the most frequent respiratory infectious diseases. To investigate clinical and microbial features of acute pharyngotonsillitis, we organized a nationwide prospective surveillance study group named the Pharyngotonsillitis Study Group (PhaTONS) in Japan. The aims of this prospective study were to evaluate the causative pathogens of adult acute pharyngotonsillitis in comparison with clinical features of the infectious disease [1]. Some cases of pharyngotonsillitis are caused by viral infections and are self-limiting by only symptomatic treatments, whereas sometimes bacterial infection takes part in this disease resulting in severe symptoms and complications such as peritonsillar abscess which need treatment with antimicrobial agents. To save medical costs and avoid complications, the appropriate usage of antimicrobial agents against acute pharyngotonsillitis would be essential.

We conducted a prospective study between May 2004 and June 2005.

214 patients with acute pharyngo-tonsillitis were assessed for clinical symptoms and pharyngotonsillar findings by a clinical scoring system (table 1) and were also evaluated for causative pathogens.

They were 114 males and 100 females, ranging in age between 16 and 79 years, median 34 years.

Patients were classified into three groups according to their total score as mild (less than 3), moderate (4–8) and severe (higher than 9).

In this study, 33 (15.4%) cases were classified into the mild group, 121 (56.5%) into the moderate group, and 60 (28.0%) into the severe group.

Table 2. A Clinical scoring system for acute pharyngo-tonsillitis

	Clinical scores		
	0	1	2
Symptoms			
Difficulties in daily life	not so annoying	annoying, but can work	absence from work
Sore throat/swallowing pain	discomfort	sore, but can swallow	difficult to swallow
Fever	<37.5°C	37.0–38.5°C	<38.6°C
Pharyngo–tonsillar findings			
Pharyngeal erythema or swelling	mild	moderate	marked
Tonsillar erythema or swelling	mild	moderate	marked
Presence of exudates or plugs in tonsils	none	scattered	whole tonsil

A total of 234 pathogenic bacteria were identified in 157 (73.4%) of 214 patients. The microorganisms detected in patients with acute pharyngotonsillitis were *Streptococcus pyogenes* (29 isolates, 13.6%), hemolytic streptococci (35 isolates, 16.4%), *Haemophilus influenzae* (52 isolates, 24.3%), and others (71 isolates, 33.2%). There was no significant difference in distribution of bacteria between acute tonsillitis and pharyngitis.

31 viruses were identified by PCR. Major viruses were adenovirus (10 of 205 cases, 4.9%), and hMPV (human metapneumovirus) (13 of 206 cases, 6.3%). Among the 206 cases in which both bacterial culture and virus PCR were performed, bacteria alone were identified in 128 cases (62.1%), virus alone were identified in 7 cases (3.4%), and both bacteria and virus were identified in 23 cases (11.2%). In 48 cases (23.3%), neither bacteria nor virus was identified.

Both severities of disease and inflammatory parameters were compared with pathogens. Although *S. pyogenes* together with hemolytic streptococci, *H. influenza, Haemophilus haemolyticus, Staphylococcus aureus, Streptococcus pneumonia* and *Moraxilla catarrhalis* were frequently identified among patients with acute pharyngotonsillitis, we could not find any differences in the clinical scores among those pathogens.

The numbers of total WBC at the first visit were significantly higher in cases with *S. pyogenes* than in cases with other pathogens. The levels of CRP were also significantly higher in cases with *S. pyogenes* than in cases with *S. aureus* and *M. catharrhalis* ($p < 0.05$). There was no difference among other pathogens.

The total number of WBC at the first visit showed a positive correlation with clinical scores ($r = 0.482$, $p < 0.001$). The levels of CRP at the first visit also showed a positive correlation with clinical scores ($r = 0.571$, $p < 0.001$).

Of 33 mild cases, 21 (63.6%) patients were followed up without antibiotics and showed gradual improvement of their scores, whereas 12 (36.4%) patients were treated with oral antimicrobial agents (AMPC: 12.1%, LVFX: 15.2%, CFPN: 9.1%) due to exacerbation of the disease.

Of 121 moderate cases, 120 (99.2%) patients were treated with antimicrobial agents and 87 patients (71.9%) were treated with AMPC (66 patients, 54.5%) or LVFX (21 patients, 17.4%).

All severe cases were treated with antimicrobial agents. Seven patients (11.7%) were treated

with AMPC and 14 patients (23.3%) were treated with LVFX. In the severe cases, 22 patients (36.7%) were treated with parenteral antimicrobial agents. Among those 22 patients, 12 (20.0%) were treated with CTRX.

The clinical scores of moderate and severe cases decreased significantly after antimicrobial treatment ($p < 0.01$). In contrast to the moderate and severe cases, the mild cases showed a decrease in their scores regardless of the antimicrobial treatment.

The current findings strongly suggest that the clinical scoring system will be valuable to determine the severity of the disease and for the appropriate usage of antimicrobial agents.

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We greatly thank Prof. Edward L. Kaplan, MD (Department of Pediatrics, the University of Minneapolis, Minn., USA) for his superb suggestions on this paper. This work was organized by a nationwide surveillance group for adult acute pharyngotonsillitis (Pharyngotonsillitis Study Group: PhaTONS). We greatly thank all members of the PhaTONS.

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Protection of Pneumococcal Infection by Maternal Intranasal Immunization with Pneumococcal Surface Protein A

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Abstract

Streptococcus pneumoniae is one of the important causative pathogens for both upper and lower respiratory tract infections during childhood. The current study was designed to evaluate the protection against fatal pneumococcal infections during the infant period by maternal immunization with pneumococcal surface protein A (PspA). Four-week-old females BALB/c mice were immunized with PspA and cholera toxin B (CTB) intranasally twice a week for 3 weeks. After mating, the 10-day-old offspring of these mice were intraperitoneally (i.p.) infected with *S. pneumoniae* to evaluate survival. Anti-PspA-specific IgG antibody was induced in the sera of mother and offspring. The survival times to death after systemic fatal pneumococcal infections were significantly extended among offspring delivered from PspA-immunized mothers than the controls. Current findings suggest that maternal intranasal immunization with PspA is an attractive procedure against pneumococcal infections in early childhood.

Streptococcus pneumoniae is responsible for a significant proportion of both upper and lower respiratory infectious diseases [1]. Pneumococci first colonize asymptomatically in the human nasopharynx and transmission to others is thought to be primarily from carriers. Children who colonized pneumococci in their nasopharynx in the first year of life were at an increased risk of developing acute otitis media (AOM) compared with children who remained free of pneumococci.

The efficacy of polysaccharide-based pneumococcal vaccines have some limitations due to the wide variety of capsular serotypes of pneumococci, while capsular polysaccharide vaccine against type B encapsulated *Haemophilus influenzae* has achieved tremendous success [2]. Efforts have been made to develop protein-conjugated polysaccharide vaccines containing up to 11 serotypes. However, children younger than 2 years of age usually have lower levels of pathogen-specific IgG antibody in their sera due to the age-related immaturity of immune responses [3]. Otitis-prone children also showed subnormal levels of serum IgG antibody against the causative pathogens [3–5]. Thus, it is important to induce effective protective immune responses against pneumococci during early childhood.

Pneumococcal surface protein A (PspA), a surface-exposed protein antigen of pneumococci, is a virulent factor and a highly immunogenic protein [6–10]. PspA is an attractive candidate for future protein-based pneumococcal vaccines. In this study, we evaluated the protection against pneumococcal infections during infant period by maternal intranasal immunization with PspA.

Materials and Methods

Experimental Design and Immunization Schedule

Four-week-old female BALB/c bjy mice were used in this experiment. Mice were immunized intranasally with rPspA (1 μg) mixed with cholera toxin B subunit (CTB) (List Biological Labs, Campbell, Calif., USA) (4 μg) on the Mondays and Fridays of 3 consecutive weeks. These immunizations included CTB for the first 2 weeks of immunization. During the last week, the last two immunizations, mice received antigen alone. Control mice received only CTB for the first 2 weeks and only saline for the last week.

After the final immunization, the female mice were mated with male mice for 2 weeks. Approximately 3 weeks after mating, offspring were obtained. Sera and milk were collected from the mothers and sera were collected from the offspring at birth (day 0) and 7 and 14 days after birth. At 10 days of age, the offspring were used as the systemic fatal pneumococcal infection model. All animal studies were conducted in accordance with and after approval of the Wakayama Medical University Animal Care and Use Committee.

Enzyme-Linked Immunosorbent Assay

Anti-PspA-specific IgG antibody in milk and sera of mothers and offspring were evaluated by ELISA. Briefly, 96-well microplates (MaxSorp, Nunc, Roskilde, Denmark) were coated with 50 μl of rPspA (2 μg/ml) in phosphate-buffered saline (PBS) overnight at 4°C. After washing three times with PBS containing 0.05% Tween 20 (PBS-T), the wells were blocked for 1 h with casein buffer (0.2% casein, 0.05% Tween 20 in PBS) at room temperature. Then, 50-μl samples diluted with casein buffer were incubated at 4°C overnight. After washing with PBS-T, the plate was incubated with 50 μl of biotynylated antimouse IgG antibody (1:3,000) (Southern Biotechnology Associates, Birmingham, Ala., USA) diluted in casein buffer for 2 h at room temperature. Then, after washing with PBS-T, the plate was incubated with alkaline phosphatase-conjugated streptavidin (1:4,000) (Southern Biotechnology Associates) for 2 h at room temperature. Color was developed with *p*-nitorophenyl phosphate (Sigma Chemical Co., St. Louis, Mo., USA) and the optical density of each well was measured by a spectrophotometer at 405 nm.

Systemic Infection Model

At 10 days of age, the offspring were inoculated intraperitoneally (i.p.) with pneumococcal cells at 1×10^4 CFUs in 100 μl of sterile PBS under anesthesia. *S. pneumoniae* TIGR4, serotype 4 strain, was grown at 37°C in Todd-Hewitt broth with 0.5% yeast extract (THY) until the mild log phase. After the intraperitoneal infection, the offspring were monitored for their death every 6–8 h. The survival periods were expressed as times from inoculations of pneumococci to death.

Statistics

Comparisons between the two groups were calculated by Student's t test. Statistical values were calculated with Prism 4 (GraphPad Software, La Jolla, Calif., USA).

Results

Anti-PspA-Specific IgG Antibody in Sera of Mother Mice and Offspring

Anti-PspA-specific IgG antibody levels were high at birth and maintained during the nursing periods in both sera and breast milk of PspA-immunized mothers, while the sera of the control mothers did not possess any of the specific IgG antibody (fig. 1). Offspring delivered from PspA-immunized mothers had similar levels of anti-PspA-specific IgG antibody in sera at birth (fig. 2).

Survival after Pneumococcal Infection

The median, 75% value, and 25% value of survival times of offspring delivered from PspA-immunized mothers were 32, 43 and 28 h, respectively. On the other hand, survival times of offspring delivered from control mothers were 16, 18 and 16 h, respectively (fig. 3). Times to death of PspA-immunized offspring were significantly prolonged compared with the controls ($p < 0.05$).

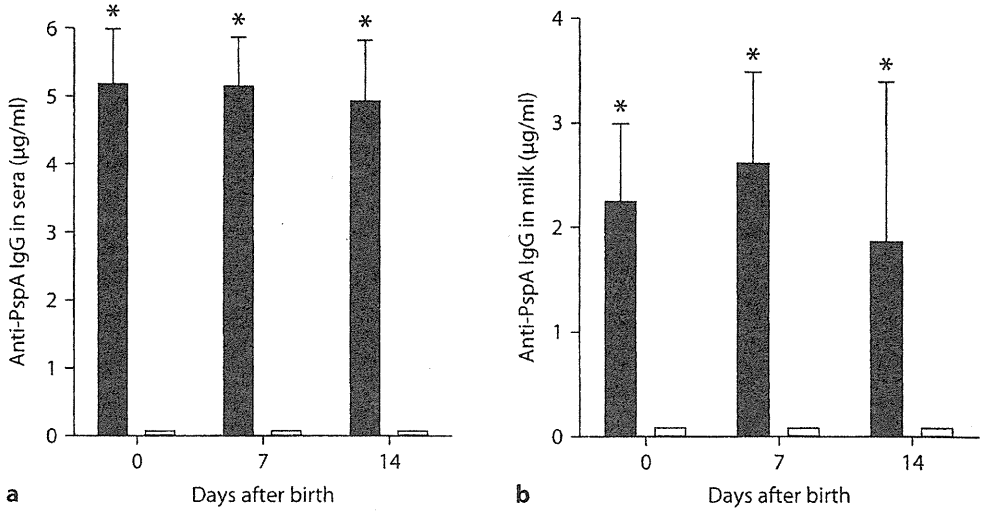

Fig. 1. Levels of anti-PspA-specific IgG antibody in mother's sera and breast milk. Results are expressed as mean ± SE of anti-PspA-specific IgG in mother's sera (**a**) and breast milk (**b**). Closed bar = Immunized mother; open bar = nonimmunized mother. * $p < 0.05$ compared with nonimmunized control by the Student's t test.

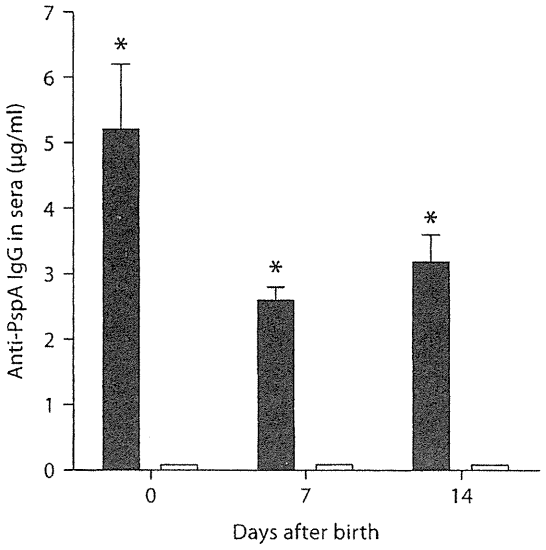

Fig. 2. Levels of anti-PspA-specific IgG antibody in offspring sera. Results are expressed as mean ± SE of anti-PspA-specific IgG in offspring sera. Closed bar = Immunized mother; open bar = nonimmunized mother. * $p < 0.05$ compared with nonimmunized control by the Student's t test.

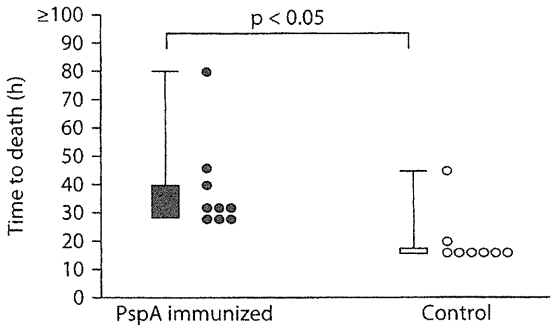

Fig. 3. Survival times of mice and offspring after systemic fatal pneumococcal infections. ● = Offspring delivered form PspA immunized mother mice; ○ = offspring delivered from nonimmunized mother mice. $p < 0.05$ compared with offspring delivered from nonimmunized control by the Student's t test.

Discussion

Recurrent bacterial infections are considered to correlate with subnormal levels of serum IgG antibody against causative pathogens due to age-related immaturity [3]. Virolainen et al. [10] showed that children who were infected most frequently with pneumococci had the lowest titer of antibody to PspA among children with invasive pneumococcal infections. In addition, young children below 2 years of age, no matter if they are healthy or not, show the lowest serum IgG levels that they will achieve throughout their lives. Maternal immunization is thought to be the most suitable approach to induce effective immune protection against pneumococcal infections.

Our previous study showed that maternal intranasal immunization with the outer membrane protein P6 of *H. influenzae* evoked anti-P6-specific IgG antibody in breast milk and sera of the mothers and this was then transferred to the offspring [11]. The present study further reports that induction of specific immune responses among offspring results in prolongation of survival times after lethal pneumococcal infections. The anti-PspA-specific IgG antibody in sera was transferred to the offspring via the placenta [12]. Differing from humans, mice colostrum/breast milk contains high amounts of IgG antibody and the IgG antibody in the mother's sera is transferred from mother to fetus through the placenta by a neonatal Fc receptor (FcRn) expressing in the yolk sac [13]. IgG antibody in breast milk is also transferred from the intestinal lumen to the systemic circulation in neonate mice by FcRn expressing in the intestine [14, 15].

Offspring delivered from PspA intranasally immunized mothers were protected from acquiring systemic pneumococcal infections. Some studies reported enhanced survival times of intraperitoneally infected mice after systemic immunization with PspA [16, 17]. PspA could also elicit protective immune responses from passive protection experiments with monoclonal antibodies to PspA [18, 19]. The current findings suggest that maternal immunization is an attractive procedure against pneumococcal infections in early childhood because transplacental immunoglobulin is transferred during pregnancy as well as after birth.

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Streptococcus pneumoniae Isolates from Middle Ear Fluid and Nasopharynx of Children with Acute Otitis Media Exhibit Phase Variation▽

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Pneumococcal phase variation of 37 middle ear and 31 nasopharyngeal isolates obtained from children with acute otitis media was examined in the absence of intervening culture. The fraction of the opaque colonies was significantly higher in middle ear isolates than in nasopharyngeal isolates. The difference is probably the result of the pneumococci adapting to differential selective environments.

Streptococcus pneumoniae first colonizes the mucosal surface of the human nasopharynx and can then infect the middle ear cavity via the Eustachian tubes to cause acute otitis media (AOM). In 1994, Weiser et al. reported spontaneous, reversible intrastrain phase variation of pneumococci in colony opacity on transparent agar surfaces (15). Transparent variants associated with low levels of capsular polysaccharide are able to most efficiently colonize the nasopharynx in animal models of pneumococcal carriage (8). In contrast, opaque variants associated with greater amounts of capsular polysaccharide colonize poorly in animals and provide greater resistance to clearance by phagocytes but at the same time decrease the adherence of opaque variants to host cells (2, 4). Thus, the intrastrain phase shifts of pneumococci are hypothesized to be adaptations to different stages in the pathogenesis of infections (1, 8, 10, 13, 14). However, the phase shifts have never been clearly demonstrated with human samples. A study in 2001 examined 19 "minimally passaged" paired nasal and blood isolates, but no statistical association was observed between the phase and body isolation site ($P = 0.51$) (12). The present study was designed to investigate the morphological phase of pneumococci by plating them on transparent agar immediately upon their isolation from the nasopharynx and middle ear of children with AOM. By avoiding any growth between isolation and plating, we hoped to minimize the effects of bacterial growth *in vitro* on bacterial phase. The study focused on samples from young children because of their high rates of pneumococcal carriage and AOM.

Middle ear and nasopharyngeal cultures were collected from all children between 5 and 70 months of age who presented at our clinic with AOM from 2008 to 2010 and who had not previously been treated with antibiotics. No subsequent cultures were collected for the study from any patients at later clinic visits. The swabs used to collect cultures were stored on transport agar with charcoal media (Eiken Chemical Co., Tokyo, Japan) at room temperature and transported to the laboratory for processing, and within 18 h of collection, they were plated on tryptic soy agar (TSA) plates for phase determination (with no intervening culture).

Samples were obtained from 42 children, 25 males and 17 females, with a median age of 21.5 months. The children were all unrelated to each other, and only one culture from the nasopharynx and one from each ear were included in the study for a single patient, when available from the patient. All together, from the 42 children, 37 middle ear and 31 nasopharyngeal cultures were examined. Eighteen patients had paired cultures in the study that were isolated on the same day, one culture from their nasopharyngeal secretions (NPSs) and the second and/or third culture from their middle ear fluids (MEFs). A total of 6 of 18 patients had cultures from all three sites. For the other 12 of 18 patients, there was an NPS culture and a culture from one ear only. There were an additional 13 patients for whom there were nasopharyngeal cultures alone, 9 patients for whom there were single MEF cultures, and 2 patients for whom there was a MEF culture from each ear. The study was approved by the institutional review board of the ethical committee of Wakayama Medical University, and informed consent for the use of the cultures was obtained from the parent or guardian of each patient.

Within 18 h of collection, each culture swab was suspended in 150 μl of sterile phosphate-buffered saline (PBS). Serial dilutions of each sample were plated on tryptic soy plates solidified with 1% agar containing 5,000 U/ml of catalase (Worthington Biochemical, Freehold, NJ) to allow for direct evaluation of individual colony morphology while limiting any growth of bacteria between extraction of the culture from the patient and the time it was plated on TSA (1, 15). Transparent and opaque bacteria identified macroscopically on the TSA plates and classified by phase were subsequently confirmed to be pneumococci by streaking representative colonies on blood agar and examining them for colony morphology, alpha hemolysis, and sensitivity to optochin. Of those that appeared to be

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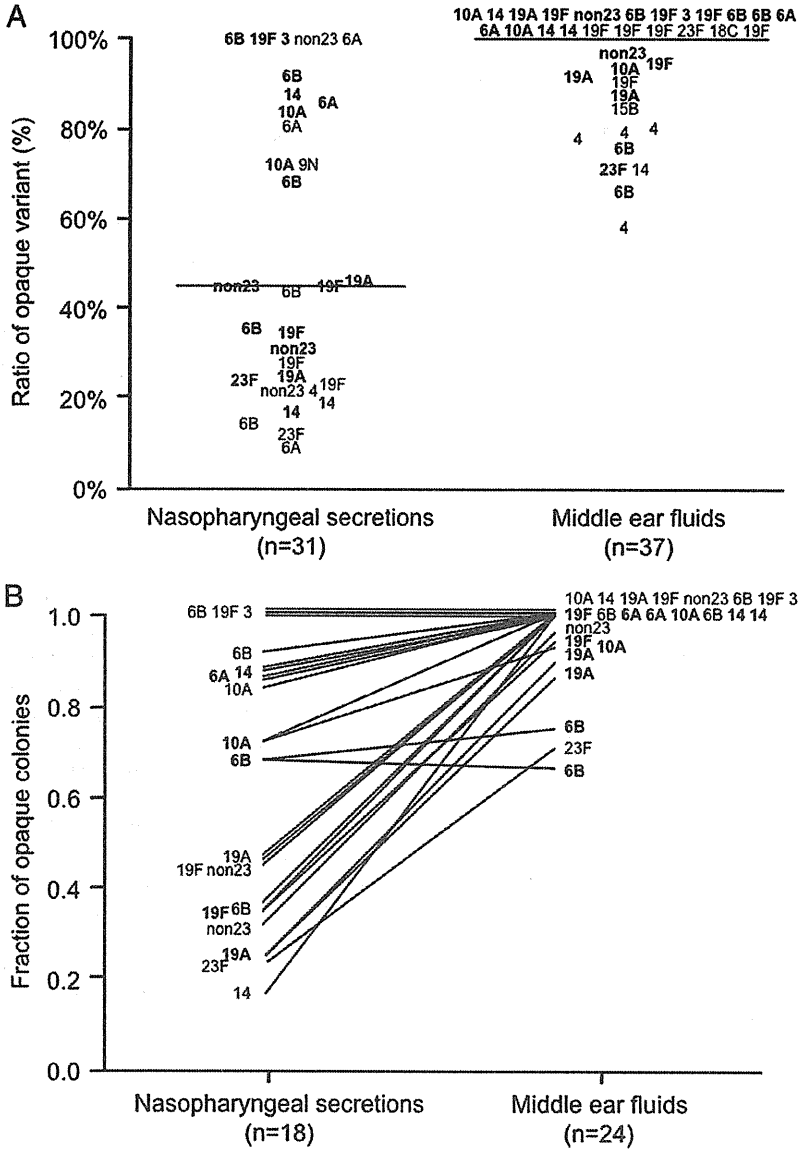

FIG. 1. Fractions of the opaque variant in pneumococcal isolates obtained from nasopharyngeal secretions and middle ear fluids. (A) Each number designates a serotype of an isolate from an individual patient. From 18 patients, cultures were obtained from both the middle ear and the nasopharynx (in boldface). The horizontal bar shows the median fraction of variants that are opaque. This difference has a P value of 0.0007 by the Mann-Whitney two-sample rank test. (B) Numbers show the serotypes of isolates from the 18 cases from which pneumococci were recovered simultaneously from MEFs and NPSs from the same patient. From 6 of these 18 patients, cultures from both the left and right ears (in boldface) were obtained. Lines connect the results obtained with cultures from NPSs and MEFs from the same patient. The trend to have a higher fraction of opaque colonies from MEF cultures than from NPS cultures was significant at P values of <0.0001 by the Wilcoxon matched-pairs signed-rank test.

pneumococci, representative colonies were examined by Gram staining, bile solubility testing, and PCR for the presence of the autolysin gene (*lytA*). The decimal fraction of total colonies that were opaque was determined for each isolate. For each isolate, at least 20 distinguishable pneumococcal colonies on TSA plates were evaluated to determine the fraction of opaque colonies. The restriction fragment patterns of genomic DNAs purified from the restreaks of one colony of each phase type isolated from each body site were analyzed by pulsed-field gel electrophoresis (PFGE) as described elsewhere (9). Statistical analysis was performed with Prism 4 (GraphPad Software, Inc.) to compare the fractions of opaque colonies. P values of <0.05 were considered statistically significant.

The fractions of opaque colonies in MEF cultures ranged from 0.58 to 1.00 (median, 1.00; 25% percentile, 0.87; and 75% percentile, 1.00). In 59% (22/37) of the MEF cultures, all of the pneumococcal colonies were opaque. In contrast, the fractions of opaque variants in the NPS cultures covered a wide range, from 0.09 to 1.00 (median, 0.45; 25% percentile, 0.23; and 75% percentile, 0.86). Only 16% (5/31) of the NPS cultures were

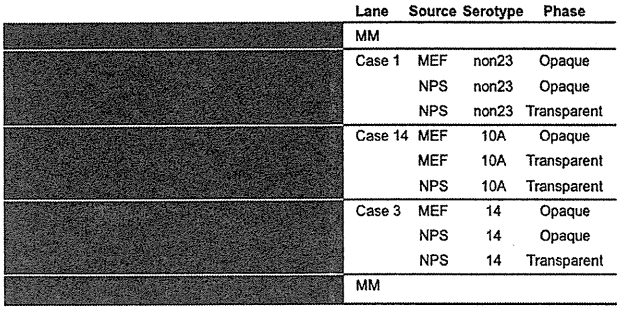

FIG. 2. PFGE patterns of SmaI digests of genomic DNA from paired nasopharyngeal and middle ear isolates of *S. pneumoniae*. PFGE patterns of paired nasopharyngeal and middle ear isolates collected from three representative patients are shown. The three different isolates examined from each of these three patients appeared identical by PFGE analysis, but each patient clearly had a different strain. In each case, the NPS and MEF isolates from each of the 15 remaining patients were also found to be identical, but differences between the isolates of the different patients were observed (data not shown). MM is molecular size marker (48.5-kb lambda DNA ladder). The non23 strain is the serotype not including the 23-valent pneumococcal capsular polysaccharide vaccine.

exclusively composed of opaque variants. The prevalence of opaque variants in MEFs was significantly higher than that in NPSs ($P < 0.01$) (Fig. 1A).

Among the 18 sets of paired samples, the fractions of opaque or transparent variants in the MEF cultures and the NPS cultures differed in the same fashion as the larger data set, with the tendency being toward exclusively opaque variants in the MEFs and mostly transparent variants in the NPSs (Fig. 1B). The fraction of opaque variants in the paired group of MEFs (range, 1.00 to 0.66; median, 1.00; 25% percentile, 0.94; and 75% percentile, 1.00) was significantly (by paired sign rank test; $P < 0.001$) higher than that in the NPSs (range, 1.00 to 0.17; median, 0.57; 25% percentile, 0.33; and 75% percentile, 0.89). The isolates collected on the same day from MEFs and NPSs of the 18 patients were found in each case to be identical to each other in the same patient, as determined by PFGE (Fig. 2). Although strains of different capsular types had varied percentages of opaque and transparent variants, there was no statistically significant correlation between capsular serotypes and pneumococcal phase variations.

Previous studies in animal models have shown that transparent variants are released when nasal tissues are washed with physiological salt solutions, while the majority of *S. pneumoniae* isolates recovered from previously washed homogenized nasal tissue, from blood specimens, or from lung aspirates were in the opaque phase (1, 12). In chinchillas pretreated with live influenza virus, inoculation with opaque pneumococci caused more severe middle ear infections than did inoculation with transparent pneumococci (10).

Relatively little is understood about the means by which pneumococci become established in the human nasopharynx and then make the transition to the middle ear (10, 12). Laboratory cultures of most of the previously obtained clinical isolates of pneumococci consist of heterogeneous populations of transparent and opaque variants, and the ability of pneumococci to switch back and forth between transparent and opaque variants is thought to represent a viable strategy for adaptation to the different local environments during the course of disease pathogenesis (13–15).

This study is the first to report on the phase variation of pneumococci obtained from MEFs and NPSs from children with AOM. The differences in the distribution of phase variants between the NPS and MEF isolates may result from differential selection within the two environments. The ability to adhere may have greater importance within the nasopharyngeal environment, thus allowing a selection toward the greater frequency of transparent variants. The ability to evade opsonophagocytosis may be a much stronger requirement of the middle ear, especially as it enters into an inflammatory state during AOM. This requirement to evade opsonophagocytosis would impose the selection of the more highly encapsulated opaque variants within the middle ear environment, as is proposed for the blood and other invasive sites (11). Li-Korotky et al. and Weiser et al. suggested that environments with lower oxygen contents, such as the middle ear space of AOM patients, promote selection for the more virulent opaque variant (7, 12). Opaque variants are more efficient at survival and multiplication in the middle ear cavity of the chinchilla (10).

Changes in the amount of cell wall teichoic acid are a second phenotype associated with the described phase variation. In this case, the transparent-phase variants are observed to have more cell-associated teichoic acid than the opaque-phase variants (5). Phosphocholine (PC) is a major determinant of teichoic acid. Since teichoic acid is a major component of the cell surface, the differences in the amount of phosphocholine (PC) that distinguish phase variations could alter the surface localization of pneumococcal proteins anchored to the PC. Evidence that expression of choline binding proteins varies in association with phase variation has been reported (4, 6, 12). The difference in surface-localized PC may also directly affect the bacterial adherence to some epithelial cells that occurs through phosphocholine (2, 3).

Overall, the distributions of phase variants that we observed are consistent with the proposed hypothesis that the transparent variant may be better adapted to adhere in the normal microenvironments of the nasopharynx and, consequently, may be a key player in the initial step of pathogenesis of pneumococcal AOM. In this study, we did not find any correlation between tympanic membrane findings or clinical outcomes of AOM and phase variation (data not shown). This may not be surprising, however, since the disease outcome may be more related to which virulence genes the bacterium is able to express in its tissue-specific phases than to the absolute percentage of bacteria in a particular phase at each site. Further investigation will be required to completely evaluate the roles of the phase variations in the clinical features of AOM.

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Review article

A review of the burden of disease due to otitis media in the Asia-Pacific

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ABSTRACT

Objective: The burden of disease due to otitis media (OM) in Asia Pacific countries was reviewed to increase awareness and raise understanding within the region.
Methods: Published literature and unpublished studies were reviewed.
Results: In school-age children, OM prevalence varied between 3.25% (Thailand) and 12.23% (Philippines) being highest (42%) in Aboriginal Australian children. OME prevalence at school age varied between 1.14% (Thailand) and 13.8% (Malaysia). Higher prevalence was reported in children with hearing impairment, HIV, pneumonia and rhinitis. CSOM prevalence was 5.4% in Indonesia (all ages), 15% in Aboriginal Australian children and 2–4% in Thailand, Philippines, Malaysia and Vietnam (WHO estimate). OM prevalence/incidence and service utilisation were highest in children 2–5 years of age. The disease burden was substantially higher in Pacific Island children living in New Zealand (25.4% with OME), and was highest in indigenous Australians (>90% with any OM). *Streptococcus pneumoniae* and *Haemophilus influenzae* dominated as primary causes of AOM in all studies. Few studies examined pneumococcal serotype distribution. Health-related cost estimates for OM, when available, were substantial. In developing countries, significant investment is needed to provide facilities for detection and treatment of ear disease in children, if long term hearing deficits and other sequelae are to be prevented.
Conclusion: The available evidence suggests an important burden of disease and economic cost associated with OM in most Asia Pacific countries and a potential benefit of prevention through vaccination. Large, prospective community-based studies are needed to better define the prevalence of ear disease in children, and to predict and track pneumococcal conjugate vaccine impacts. AOM prevention through vaccination may also provide a means of reducing antibiotic use and controlling antibiotic-resistant disease in children. This review highlights the need for additional research, and provides a basis on which to build and develop regional guidelines for OM management.

Contents

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1. Introduction

Otitis media (OM) is one of the most commonly diagnosed infections among children. In the United States, more physician visits and more antibiotic prescriptions are written for OM than for any other infectious disease in childhood [1]. By one year of age, around 60% of children have experienced at least one episode of acute otitis media (AOM) [2]. By the age of 7 years, there are few children who have not had at least one episode of AOM, and around 75% who have had at least three episodes [2]. OM represents a significant economic health burden and adversely affects the quality of life of children and their families [1,3].

AOM is frequently a self-limiting disease although around 50% of children will have a recurrent episode and as many as 26% will have a persisting middle ear effusion 3 months after an AOM episode (otitis media with effusion, OME, also know as serous OM) [4]. Tympanic membrane (TM) perforation is the most common complication of AOM [5]. A persisting discharge through a perforated TM that lasts for 6 weeks or more is known as chronic suppurative otitis media (CSOM). CSOM is a disabling outcome estimated to affect between 65 and 350 million individuals globally, causing hearing loss in around 60% [6]. Mastoiditis is the second most frequent complication of OM after TM perforation [5]. Other serious complications of OM are generally classified as being extra-cranial: including temporary or permanent hearing loss, vestibular dysfunction, facial paralysis, adhesive OM and cholesteatoma, or, intracranial: including brain abscess and sinus thrombosis [4].

AOM is a polymicrobial disease where aetiology is often difficult to establish. The most frequently identified bacterial causes are *Streptococcus pneumoniae* (Spn) and *Haemophilus influenzae* (Hi), followed to a varying degree by *Moraxella catarrhalis*, *Staphylococcus aureus* and *S. pyogenes* [7–10]. Growing antibiotic resistance of Spn and Hi to first line therapies has been documented in many countries within the Asia-Pacific region [11,12]. Prevention of infection through vaccination may have a role in reducing antibiotic use and reducing infections due to resistant bacterial strains.

The global epidemiology of OM is not uniform. In some indigenous populations such as Australian Aborigines, Maori and Pacific Islanders, AOM is characterised by early age of onset, high risk of persistent disease and high risk of subsequent development of CSOM [6,13]. The World Health Organization classifies populations by CSOM prevalence rates among children: A low prevalence is defined as CSOM prevalence between 1% and 2%, high prevalence ("avoidable disease burden that must be addressed") between 2% and 4% and highest prevalence ("urgent attention needed to deal with a massive public health problem") greater than 4% [6]. The global burden of disease due to CSOM lies in Africa, South-East Asia and the Western Pacific [6]. For many countries within the Asia-Pacific, the burden of disease due to OM is not well described and general awareness of the disease amongst physicians may be low. Countries defined as high CSOM prevalence countries include Thailand, the Philippines, Malaysia and Vietnam [6]. Aboriginal Australians are defined as a very high prevalence group [6].

Pneumococcal conjugate vaccines containing polysaccharides from 7 or 11 pneumococcal serotypes have show efficacy in preventing AOM due to pneumococcal vaccine serotypes in efficacy trials [7,14–16]. The Pneumococcal Otitis Efficacy Trial (POET) employed a vaccine using Protein D, a cell-surface protein highly conserved among strains of Hi, as carrier protein. As well as demonstrating a statistically significant protective effect of vaccination on overall AOM disease burden and on AOM caused by vaccine serotype Spn, vaccination also prevented AOM due to *Hi* [7].

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Table 1
Prevalence of OM by country.

Year of study	Population/setting	Age (years)	n	Examination method	OM Syndrome	Prevalence	Ref.
Australia							
1998–2004	Aboriginal children attending primary school in urban area	4–12 yr	119	O, T, A, AR	OM	42.0%	[24]
					OME	18.5%	
					CSOM	1.7%	
2001	Aboriginal children from 29 remote communities	6–30 mos	709	T, PO	OME	41%	[23]
					AOM	33%	
					CSOM	15%	
					Any suppurative OM	48%	
					Any perforation	24%	
					Any OM 6–18 mos	93%	
					Any OM 18–30 mos	92%	
1997	10 Day care centres	<4 yr	252	T, O	OME	9.6 per child yr (cumulative)	[88]
Indonesia							
2002	Population survey	All ages	–	–	CSOM	5.4%	[32]
Japan	No data						
Malaysia							
1993	Urban and rural kindergartens	5–6 yr	1097	T, O	All OME	13.8%	[57]
					Rural region	9.48%	
					Urban region	17.9%	
2005	Population survey	All ages	7041	T, O, OE	All OME	2.93%	Unpublished SSH and others
					Rural region	3.23%	
					Urban region	2.74%	
New Zealand							
2002–2003	Pacific Island children in NZ	2 yr	1001	ST, PO	OME	25.4%	[64]
					AOM	1.9%	
2008–2009	Retrospective primary care medical records review	<5 yr	19,146	Record review	AOM	30.2% (annual)	[62]
Philippines							
1997–1998	National survey	Children	15,381,796		OM	12.23%	[70]
2004–2005	Community survey	All ages	5953	O	Perforation	5.1%	[70]
1994	Hearing impaired	Children	665	SA	OM	18.34%	[69]
					Otorrhoea	2.2%	
					Inflammation	0.7%	
1994	District survey	School children	320	NS	Chronic OM	3.2%	[6]
Taiwan							
2001	Kindergartens & day care centres	3–6 yr	3013	T, PO	OM	9.8%	[74]
					OME	5.2%	
2006	Retrospective database review	≤12 yr	3,678,982	ICD-9 codes	AOM	64.5/1000	Unpublished PCW
1999	High schools	10–18 yr	8723	Questionnaire	OM (annual)	4.3%	[89]
Thailand							
1993–1995	Primary school	7–9 yr	2184	SA, O, T	Any OM	3.25%	[79]
					AOM	0.69%	
					OME	1.14%	
					Chronic OM	1.74%	
2004–2005	HIV+ve children	1–15 yr	76	0	Any OM	38.1%	[80]
					AOM	3.9%	
					CSOM	38.1%	
					Chronic OM	34.2%	
Vietnam							
1995	Rural and urban	6 mos–10 yr	3300	T, PO	Any OM	6.86%	[85]
					COM	2.1%	
					Sequelae otitidis	4.1%	

ST = (screening) tympanometry; SA = (screening) audiometry; PO = (pneumatic) otoscopy; OE = otoacoustic emission; NS = not specified, AR = acoustic reflectometry.

At present, pneumococcal conjugate vaccines are recommended for use in Australia, New Zealand, and Singapore, and the Western Pacific islands of Micronesia, Niue and Palau (http://apps.who.int/immunization_monitoring/en/globalsummary/ScheduleSelect.cfm). With the availability of vaccines that show efficacy in preventing AOM, baseline data on OM disease burden will be important for countries considering vaccination and for assessing vaccine effects on disease epidemiology.

A panel of ear nose and throat (ENT) experts from countries within the Asia-Pacific region met in February 2010 with the aims

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of increasing awareness and understanding of the burden of disease of OM in the region and developing regional management guidelines. The first step in the process and the purpose of this report is to review the available published and unpublished literature describing the burden of disease caused by OM within selected Asia-Pacific countries.

2. Methods

PubMed was searched using the terms "otitis media" AND (Asia OR Philippines OR Indonesia OR Singapore OR Malaysia OR Thailand OR Taiwan OR Vietnam OR Australia OR New Zealand OR Japan). Gateway to Japan Journals (J-East, http://science-links.jp/j-east/) was also searched for English abstracts of relevant articles written in Japanese. Publications were limited to human studies published between 1995 and 2010. English and non-English language literature was assessed for relevance by review of abstracts when available. Citations in published papers were also examined. Only studies in children were reviewed. In order to describe all of the available data in the region, papers were not assessed using quality criteria. Additional unpublished data made available by the expert panel were included.

3. Results

3.1. Studies identified in the literature search

There were 1004 'hits' in the initial PubMed search and 87 were selected after abstract review as being of potential interest. Searches of bibliographies yielded additional publications and datasets. Most studies were observational prevalence studies, retrospective hospital-based reviews of medical records or microbiological evaluations of flora isolated from middle ear fluid (MEF) or otorrhoea.

3.2. OM burden in the Asia-Pacific

Available OM prevalence data are summarised in Table 1. Results from studies that evaluated the bacterial aetiology of AOM and CSOM are given in Tables 2 and 3, respectively.

3.3. Australia

3.3.1. Incidence and prevalence of OM

Few studies retrieved after 1995 evaluated OM prevalence or incidence in the general population. A national report on disease burden in Australia estimated that in 2003, 1,174,267 cases of OM occurred in all age groups, of which the majority (67.6%) were in children 0–14 years of age [17].

A recent report on the economic cost of OM in Australia using US prevalence data and Australian population statistics estimated that in 2008, between 658,006 and 1,615,486 children less than 15 years of age were affected by OM [18]. By the age of 12 months, 73% of Australian children are expected to have experienced at least one OM episode. Indigenous children accounted for 12.8% of cases (the indigenous community comprises approximately 2.5% of the national population). In 2008 temporary hearing loss is likely to have affected more than 354,457 children, 87,655 children were

Table 2
Distribution of frequently isolated bacteria from culture-positive samples in children with AOM, by country.

Year of study	Population/setting	Age	*n*	Sample source	Spn (%)	Hi (%)	*M. catarrhalis* (%)	*S. aureus* (%)	Spn + Hi	PRSP	Ref.
Australia											
pre-PCV7	Aboriginal	<18 mos	53 ears	First/new AOM with perforation	38% Vaccine or vaccine related 21%	55%	–	19%	28%	–	[90]
2000	Aboriginal children	<8 ys	31	Children with AOM	29%	32%	5%	–	–	–	[22]
2001–2004	Tiwi islands post-PCV7	≤12 ys	73 ears	New TM perforations	64%	84%	8%	–	48%	–	[28]
1996–2001	Tiwi islands pre-PCV7		72 ears		59%	88%	0%	–	47%	–	[28]
Japan											
2002–2004	Retrospective. Recurrent AOM	Children	70	MEF, otorrhoea, NP	35.7%	37.1%	–	15.7%	–	40%	[91]
2000–2005	Hospital ENT Dept	Children	41	–	24.7%	24.7%	12.3%	–	–	–	[92]
2001–2002	Hospital	Infants	85	–	38.6% (PRSP)	34.3%	11.4%	–	–	–	[93]
2001–2002	Hospital ENT Dept. patients with influenza	Children	80	Otorrhoea	13.7%	8.7%	3.7%	–	–	–	[94]
–	14 General practices	Children		–	31.8%	35.8%	1.5%	–	–	42.2%	[95]
2001	Clinics	≤10 ys	123 ears	–	40.8%	30.9%	28.3%	–	–	52.6%	[96]
2004–2005	Clinics	≤10 ys		–	32.7%	30.0%	27.3%	–	–	61.6%	[96]
–	–	3 mos–11 ys	47	–	30.4%	41.1%	–	–	–	–	[97]
1998–1999	Hospital	Children	33	Otorrhoea	33%	21%	–	–	–	65%	[98]
–	–	Children	152	Otorrhoea	13.2%	27.9%	2.5%	19.1	–	44.4%	[99]
Taiwan											
2004	Hospital	<18 ys	96	MEF	32%	26%	–	14%	–	73.3%	[100]
1993–2001	Hospital	4–96 mos	18	Medically refractory AOM[a]	25%	0	0	17%	0%	100%	[101]
1997–1999	Hospital	3 m–14 ys	243	MEF[b]	21.7%	10.2%	–	7.0%	–	95.8%	[78]
Thailand											
–	Hospital	3–59 mos	112	MEF/otorrhoea	47%	37%	11%	–	0.9%	19%	[82]

PRSP – penicillin-resistant *S. pneumoniae*.
[a] AOM requiring emergency myringotomy because of toxicity following second-phase antibiotics.
[b] Persistent AOM failed AB Rx for whom myringotomy was indicated; NP nasopharyngeal sample.

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Table 3
Microbiological findings in children with CSOM (or otorrhoea) by country.

Country	Year of study	Population/ setting	Age	*n*	Examination method	Commonly identified bacteria	Antibiotic resistance	Ref.
Indonesia	1989	ENT patients	5–16y	38	Middle ear sample through perforation	60% mixed flora *Peptostreptococcus sp., P. aeruginosa, Bacteroides sp., S. aureus, Prevotella sp.,*	58% of patients had B-lactamase producing bacteria	[31]
Malaysia	1994–1995	CSOM	6m–78y	382 swabs	Otorrhoea	*P. aeruginosa* 27.2% *S. aureus* 23.6%		[59]
Philippines	2004–2005	Outpatients	Paediatric	16	Otorrhoea	*S. aureus* 52%, *P. aeruginosa* 35%	–[a]	[102]
Thailand	2004–2005	HIV+ve	1–15y	16	Otorrhoea	*P. aeruginosa* 31% *Staphylococcus* sp. 12.5% *Hi* 12.5%	–	[80]
Taiwan	2000–2001	Hospital	Outpatients	161	Otorrhoea	*S. aureus* 43.5% *Pseudomonas* sp. 28.8%	13.7% were MRSA	[103]
Vietnam	2009	Urban	1m–15y	114	?	*P. aeruginosa* 26% Spn 21% *S. aureus* 22%	93% of Spn	Dr Nguyen

n = number of patients.
[a] Not specified for paediatric samples; m = months; y = years.

likely to have been affected by TM perforation, 237 by mastoiditis and 217 by intracranial complications [18].

The epidemiology of OM is markedly different among Aboriginal and non-Aboriginal Australian children. Repeated studies have shown prevalence rates of severe OM in Aboriginal children that are among the highest reported in the world [19]. Middle ear disease in Aboriginal children may be almost universal [13,20,21].

Compared to non-Aboriginal children, OM in Aboriginal children begins very early in life, is frequently bilateral and is less likely to resolve spontaneously, establishing within the first year of life a pattern of chronic persisting disease [13,20–22]. By the age of 6 months, 14% of children have experienced TM perforation, increasing to 40% by 18 months of age [23]. In a survey of 29 remote communities conducted in 2001, OM was detected in 91% of children between 6 and 30 months of age [23]. Almost one quarter of children (24%) had TM perforation and 15% had CSOM. The results were noted to be essentially unchanged compared to a survey conducted 25 years earlier [23]. High rates of middle ear disease (42%) were also present in Aboriginal school-children children in urban locations, although CSOM rates in this setting were lower (1.7%) (Table 1) [24].

Data extracted from a national cluster survey of GP consultations was used to assess OM presentations and complications in indigenous versus non-indigenous children [25]. Over an 8-year survey period, ear problems were the fourth most frequent problem seen by GPs. The incidence of OM was 9.8 per 100 consultations in indigenous children versus 7.3 per 100 consultations in non-indigenous children. Severe OM including CSOM, COM and TM perforation were more frequent in indigenous children than non-indigenous children (7.9% of OM diagnoses versus 1.7%). Management of OM was similar in both groups of children. In a different study, CSOM was the most common reason Aboriginal children attended a Paediatric Outreach service in Far North Queensland between 2001 and 2006 [26].

3.3.2. Impact of PCV7 on OM

Infant vaccination with PCV7 was introduced in 2001 for indigenous and at-risk children, and for all children in 2005. Vaccination has been associated with a significant decrease in hospitalisations due to myringotomy and tympanostomy tube placement in children less than 3 years of age [27]. The decrease has been most marked in children less than one year of age (23% reduction) followed by 1 years olds (16%) and 2 year olds (8%) [27].

One study assessed the effects of PCV7 vaccination on OM prevention in indigenous Australians [28]. No changes in the percentage of children developing AOM, OME, TM perforation or CSOM were observed in vaccinated versus unvaccinated cohorts (Fig. 1). The occurrence of repeated TM perforation was statistically significantly lower in the vaccinated cohort at one time point (Fig. 1). The incidence of AOM in the unvaccinated cohort was 1.87 per person year, versus 2.05 per person year in the cohort who receive PCV7. Spn serotypes were different in the two cohorts, possibly reflecting an effect of vaccination. In the unvaccinated cohort vaccine serotypes including 6B, 23F, and serotypes 19A, 16F and 11A predominated. In the vaccinated cohort, serotypes 19A, 19F and 16Fpredominated. The minimal impact of PCV7 vaccination on the onset and progression of OM in this population was attributed to low serotype coverage by PCV7 and onset of disease before completion of the 3-dose primary vaccination course [28].

3.3.3. Microbiology of OM

Studies identified in the review period were all conducted in Aboriginal children with a new TM perforation. In line with reports from other countries (Table 2), Spn and Hi were the most frequently identified aetiological agents in AOM (Table). Co-infection with Spn and Hi was commonly observed, present in 28–48% of children.

Unpublished nasopharyngeal carriage data in non-Aboriginal Australian children with recurrent AOM showed that the

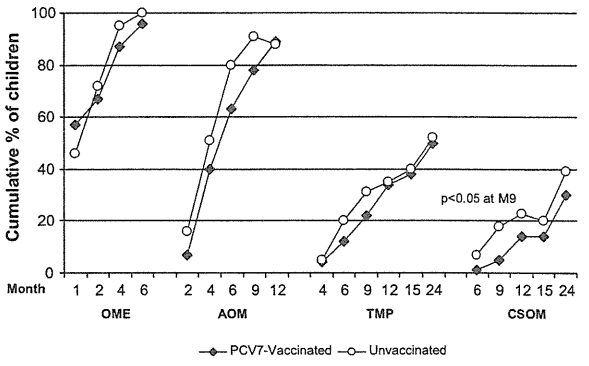

Fig. 1. Cumulative percentage of two cohorts of indigenous children (1996–2001 unvaccinated and 2001–2004 PCV7-vaccinated) with different otitis syndromes over time [28].

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