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High prevalence of multi-drug resistant *Streptococcus pneumoniae* among healthy children in Thailand

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Summary Antibiotic resistance in *Streptococcus pneumoniae* is an emerging health problem worldwide. The incidence of antimicrobial-resistant *S. pneumoniae* is increasing, and nasal colonization of *S. pneumoniae* in children increases the risk of pneumococcal infection. In this study, the prevalence of *S. pneumoniae* nasal colonization was studied in Thai children from three different districts. *S. pneumoniae* nasal colonization was found in 38 of 237 subjects (16.0%). The carriage rate indicated higher rates in two rural districts (18.2% and 29.8%) than in the urban district (2.8%). The antibiotic susceptibility pattern was determined using the disk diffusion method. Prevalence of multi-drug resistance *S. pneumoniae* (MDR-SP) was 31.6%. Resistance to commonly prescribed antibiotics was found for ampicillin (5.3%), azithromycin (26.3%), cefepime (2.6%), chloramphenicol (18.4%), clindamycin (18.4%), erythromycin (21.1%), oxacillin (44.7%), trimethoprim/sulfamethoxazole (78.9%) and tetracycline (15.8%). All isolates were sensitive to ceftriaxone. The pulsed-field gel electrophoresis pattern was used to compare genetic diversity of the *S. pneumoniae* isolates. PFGE demonstrated the variation in genotypes of *S. pneumoniae* from different areas. High prevalence of multi-drug

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resistance *S. pneumoniae* nasal colonization in healthy Thai children was indicated. Effective strategies for appropriate use of antibiotics are therefore needed in the community.

Introduction

Streptococcus pneumoniae is a medically important pathogen that causes a number of community-acquired infections, including chronic bronchitis, otitis media, acute bacterial sinusitis and pneumonia. Pneumococcal pneumonia is a major cause of morbidity and mortality in developing countries [1,2]. The emergence of drug-resistant *S. pneumoniae* has occurred around the world including Thailand [3—6]. Infections caused by resistant *S. pneumoniae* can be difficult to treat, resulting in greater risk of death. Pneumococci resistant to more than three separate classes of antibiotics are considered to be multidrug resistant [7]. To date, multidrug-resistant *S. pneumoniae* (MDR-SP) have been isolated from both adults and children around the world [7—11]. They are resistant to penicillin, clindamycin, cotrimoxazole and erythromycin [9,10,12—14]. *S. pneumoniae* colonization rates are high in children aged less than 5 years [9,12,13]. The carrier state is asymptomatic, and transmission of pneumococci in children can occur from any individual colonized with the microorganisms. The incidence of antimicrobial-resistant *S. pneumoniae* in the nasopharynx of children increases the risk of resistant strains that cause *S. pneumoniae* infection [12]. The evolution of antibiotic-resistant strains is attributed to antimicrobial acquisition or inappropriate use of antibiotics in the community [15,16]. In addition, *S. pneumoniae* is a bacterium that possesses horizontal gene transfer. This mechanism allows the acquisition of antibiotic-resistant genes which increases the resistance to a variety of antibiotics. Little is known about the epidemiology and antibiotic resistance pattern of nasal colonization *S. pneumoniae* in healthy children in Thailand. The aim of this study was to determine the prevalence of carriage rate, the antibiotic sensitivity profile and the genetic diversity of *S. pneumoniae* isolated from Thai children.

Materials and methods

Population studied

From October 2012 to March 2013, samples were collected from nasal swabs of 237 healthychildren from 4 schools in 3 different districts (one urban area and two rural areas designated as Rural 1 and Rural 2 districts) in Phitsanulok province, Thailand. The schools in each district were randomly selected. The children were attending nursery (2—3 years), kindergarten (4—6 years) or elementary level (7—10 years) in each school. Informed consent was obtained from parents/guardians prior to participation in the study. After receiving informed consent, questionnaires were sent to parents/guardians to determine risk factors for carriage of *S. pneumoniae*. Ethical approval was granted by the Naresuan University Ethics Committee.

Bacterial isolation and identification of *S. pneumoniae*

Swab samples were collected in skim-milk tryptone glucose glycerol (STGG) transport medium [17] and stored at −20 °C until used. Broth enrichment swab culture for enhanced pneumococcal growth was prepared by transferring 200 μl of the STGG sample to 5 ml of Todd Hewitt broth (Himedia, India) containing 0.5% yeast extract (THY) and 1 ml of fetal calf serum. THY broth samples were incubated overnight at 37 °C in a CO_2 incubator. Pneumococcal isolation was performed by inoculating one loop (10 μl) of the THY enriched culture on CVNG media [18] and streaking in four quadrants for colony isolation and incubation for 18—24 h at 37 °C in a CO_2 incubator. Typical pneumococcal colonies were small and grayish surrounded by a greenish zone of alpha-hemolysis. The suspected pneumococcal colonies were collected and identified by Gram stain and optochin test.

Detection of 16S rRNA and *lytA* gene by PCR

All *S. pneumoniae* isolates were confirmed by detection of 16S rRNA and *lytA* genes using the PCR method [19,20]. The sequences of primers used for PCR are as follows: 16S: 5′-AGTCGGTGAGGTAACCG-TAAG-3′, 5′-AGGAGGTGATCCAACCGCA-3′ and *lytA*: 5′-CAACCGTACAGAATGAAGCGG-3′, 5′-TTATTCGTG-CAATACTCGTGCG-3′. PCR reactions were performed with genomic DNA of *S. pneumoniae* as the