J Infect Chemother 22 (2016) 515–520

Contents lists available at ScienceDirect

Journal of Infection and Chemotherapy

journal homepage: http://www.elsevier.com/locate/jic

Original article

Clinical and microbiological characterization of serotype 6D pneumococcal infections in South Korea

Hee Jin Cheong, Joon Young Song*, Min Joo Choi, Ji Ho Jeon, Seong Hee Kang, Eun Joo Jeong, Ji Yun Noh, Woo Joo Kim

Department of Internal Medicine, Korea University Guro Hospital, Korea University College of Medicine, Seoul, Republic of Korea

ARTICLE INFO

Article history:
Received 26 January 2016
Received in revised form
9 April 2016
Accepted 21 April 2016
Available online 24 May 2016

Keywords:
Streptococcus pneumoniae
Capsular serotype
Multilocus sequence typing
Serotyping

ABSTRACT

Background: The prevalence of Serotype 6D *Streptococcus pneumoniae* was reported relatively high in South Korea. Since the introduction of 7-valent pneumococcal conjugate vaccine (PCV7), serotype replacement was observed. This study was designed to better clarify genetic diversity of pneumococcal serotype 6D and its clinical characteristics after introduction of PCV7 in 2000.

Methods: We performed serotyping analysis with 1298 pneumococcal isolates from clinical specimens in South Korea from 2004 to 2011. Multilocus sequence typing was performed, and minimal inhibitory concentration was determined for the available serotype 6D and nontypeable (NT) pneumococcal isolates during the 2006–2007 period.

Results: The proportion of serotype 6D pneumococci increased from 0.8% (2004–2007) to 2.9% (2008–2011) of all clinical pneumococcal isolates, accounting for 14.9% of serogroup 6 pneumococci in South Korea. NT pneumococci markedly increased to 13.3% during 2006–2007 in advance of the increase in serotype 6D. Among the 26 available serotype 6D pneumococcal isolates, ST282 was predominant (23 isolates, 88.5%). The STs of NT pneumococci (26 isolates) were diverse, but clonal complex 271 was the dominant clone. The oral penicillin non-susceptibility rate was 92.3% (24 among 26 isolates) for both serotype 6D and NT pneumococci. The ceftriaxone non-susceptibility rates of serotype 6D and NT pneumococci were 7.7% and 3.8%, respectively.

Conclusion: ST228^{6D} strain expanded, particularly among old adults with comorbidities in South Korea. Both antibiotic and PCV7 pressure might have contributed to the selective increase of NT and serotype 6D pneumococci.

1. Introduction

Serotype 6D *Streptococcus pneumoniae* isolates have been reported with genetic diversity in several countries, including Korea, Japan, China, Hong Kong, Fiji, Australia, Finland, Poland, Peru, and Canada [1]. However, serotype distributions of pneumococcal capsules vary geographically, so serotype 6D has been rarely detected in large parts of the world [1]. In fact, the US Centers for Disease Control and Prevention (CDC) identified only two 6D isolates in its extensive multi-year surveys [2]. Although the clinical significance has been unclear, invasive serotype 6D pneumococcal diseases have been reported [3,4]. Moreover, some of serotype 6D pneumococci showed multidrug resistance [1]. Thus, pneumococcal serotype 6D needs to be investigated further in genetic and clinical aspects.

Since the introduction of 7-valent pneumococcal conjugate vaccine (PCV7) in South Korea, serotype replacement was observed [5]. PCV7 includes serotypes 4, 6B, 9V, 14, 18C, 19F and 23F. Although serotype 6D has similar capsular structures with serotype 6B, cross-protection was considered insufficient [5].

This study was designed to better clarify genetic diversity of pneumococcal serotype 6D and its clinical characteristics. First, we evaluated the proportion of serotype 6D pneumococci after the

* Corresponding author. Division of Infectious Disease, Department of Internal Medicine, Korea University Guro Hospital, 148 Gurodong-ro, Guro-gu, Seoul 08308, Republic of Korea. Tel.: +82 2 2626 3052; fax: +82 2 2626 1105.
E-mail address: infection@korea.ac.kr (J.Y. Song).

http://dx.doi.org/10.1016/j.jiac.2016.04.005
1341-321X/

introduction of PCV7, using both serotyping and molecular typing methods. We hypothesized that serotype 6D pneumococci might increase with capsular switching through nontypeable, null capsule step after PCV7 introduction. Second, we compared the clinical characteristics of serotype 6D with other serogroup 6 pneumococcal infections.

2. Methods

2.1. Pneumococcal isolates

Since 2004, hospital-wide surveillance has been conducted to monitor pneumococcal diseases as a part of routine clinical care at Korea University Guro Hospital (KUGH), a 1000-bed teaching hospital. KUGH expanded from 600 beds to 1000 beds in 2008. Pneumococcal isolates were collected from patients with clinically diagnosed pneumococcal diseases: pneumonia (sputum or nasotracheal aspirate), sinusitis (nasal discharge), otitis media (ear discharge), meningitis (cerebrospinal fluid), empyema (pleural fluid) and bloodstream infection (blood). For each patient, only initial pneumococcal isolates were used for the analysis; repeated isolates from the same patient were excluded from the study.

This study was intended to evaluate changes in the serological and molecular epidemiology of pneumococci, particularly serotype 6D in South Korea after the introduction of PCV7. In South Korea, the coverage rate of PCV7 was estimated to be over 50% among pediatric population around 2007 years [5]. Based on the coverage rate of PCV7, the study period was divided into two periods: period 1 (early post-PCV7 period, 2004–2007) versus period 2 (late post-PCV7 period, 2008–2011).

This study was approved by the ethics committee of KUGH (IRB No. KUGH14106) and was conducted in accordance with the Declaration of Helsinki and Good Clinical Practice. The institutional review board of KUGH waived written informed consent. Patient records/information was anonymized and de-identified prior to analysis.

2.2. Serotyping

Serotyping was carried out using the multibead serotyping assay methods as described previously [6], which included a multibead assay with monoclonal antibodies (reaction A) and a multibead assay with multiplex PCR (reactions B and C).

2.3. Multilocus sequence typing analysis

Multilocus sequence typing (MLST) was performed for the available serotype 6D isolates (N = 26) and nontypeable (NT) pneumococcal isolates (N = 26) as described previously [7–9]. NT isolates were selected from the surging periods between 2006 and 2007 years (Fig. 1). New alleles and STs were submitted to the *S. pneumoniae* MLST database (http://pubmlst.org/spneumoniae). Clonal complexes were determined using the eBURST program.

2.4. Antimicrobial susceptibility test

Minimal inhibitory concentration (MIC) was determined for each pneumococcal isolate by the broth microdilution method in accordance with the Clinical and Laboratory Standards Institute (CLSI) guidelines [10]. *S. pneumoniae* ATCC 49619 was used as a control strain. Interpretive breakpoints included the following values: oral penicillin, ≤0.06 μg/mL (susceptible), 0.12–1.0 μg/mL (intermediate), and ≥2.0 μg/mL (resistant); parenteral penicillin (non-meningitis *S. pneumoniae* isolates), ≤2.0 μg/mL (susceptible), 4.0 μg/mL (intermediate), and ≥8.0 μg/mL (resistant); ceftriaxone, ≤1.0 μg/mL (susceptible), 2.0 μg/mL (intermediate), and ≥4.0 μg/mL (resistant); and for levofloxacin, ≤ 2.0 μg/mL (susceptible), 4.0 μg/mL (intermediate), and ≥8.0 μg/mL (resistant).

2.5. Clinical data collection and analysis

For the cases with serogroup 6 infections, data regarding patient demographics, co-morbidities, primary infection sites, presence of bloodstream infection (BSI), and 30-day case fatalities were collected retrospectively from medical records. The demographic and clinical characteristics of serotype 6D cases were compared to those of the other serogroups (serotypes 6A, 6B and 6C).

2.6. Statistical analysis

Statistical analysis was performed using SPSS software version 13.0 (SPSS, Chicago, IL, USA). Serotype proportion in each period was compared using the χ^2 or Fisher exact test, as appropriate.

3. Results

3.1. Changes in serotype distribution

During study periods, 1298 pneumococcal isolates were collected from diverse clinical specimens: 362 isolates in 2004–2007 years and 936 isolates in 2008–2011 isolates. Among them, 202 (15.6%) isolates belonged to serogroup 6, in which 30 isolates were serotype 6D. Serotype 6D pneumococci increased from 0.8% (2004–2007) to 2.9% (2008–2011) of all clinical pneumococcal isolates, accounting for 14.9% of serogroup 6 pneumococci infections (Fig. 1 and Table 1). Nontypeable (NT) pneumococci markedly increased to 13.3% during 2006–2007 in advance of the increase in serotype 6D (Fig. 1). Over the duration of the study, the proportions of serotypes 9V/9A, 19F, and 23F decreased, whereas those of serotypes 3, 6D, and 19A increased.

The PCV7 coverage rate for pneumococcal isolates decreased from 36.7% in 2004–2007 to 24.3% in 2008–2011 ($p < 0.01$), while those of PCV13 and the 23-valent pneumococcal polysaccharide vaccine (PPV23) did not change significantly (Table 1).

3.2. MLST analysis and antimicrobial susceptibility test

Among the 26 available serotype 6D pneumococcal isolates, four sequence types (STs) were identified: ST282, ST2778, ST447, and ST166 (Fig. 2). Most of the isolates (23 isolates, 88.5%) were ST282. The STs of NT pneumococci were diverse, but clonal complex 271, including ST271, ST320, and ST1464, was the dominant clone. Each one of the NT isolates belonged to ST282 and ST447, respectively.

Results of the antimicrobial susceptibility tests for the 26 6D serotypes and the 26 NT pneumococcal isolates are shown in Table 2. The oral penicillin non-susceptibility rate was 92.3% (24 among 26 isolates) for both serotype 6D and NT pneumococci. However, when analyzed according to the parenteral non-meningitis breakpoints, most serotype 6D isolates were susceptible to penicillin (24, 92.3%), but only 69.2% (18 isolates) of the NT pneumococci were susceptible. In particular, a higher proportion of the ST320 (4, 66.7%) and ST1464 (4, 66.7%) NT isolates were not susceptible to penicillin compared to the other isolates. The ceftriaxone non-susceptibility rates of serotype 6D and NT pneumococci were 7.7% and 3.8%, respectively. All pneumococcal isolates were susceptible to levofloxacin.