

## Upper respiratory tract bacteria in Influenza-like illness cases in Indonesia using multiplex PCR method

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### Abstrak

**Latar belakang:** Walaupun surveilans ILI di Indonesia sudah berlangsung sejak tahun 2006, namun belum diketahui data mengenai bakteri-bakteri yang dapat dideteksi dari kasus ILI dan kemungkinan menyebabkan ILI.

**Metode:** Penelitian dilakukan pada bulan Maret – Desember tahun 2012, laboratorium Virologi Pusat Biomedis dan Teknologi Dasar Kesehatan menerima spesimen apus tenggorokan dan apus hidung dari 9 puskesmas yang menjadi sentinel surveilans ILI. Spesimen diperiksa menggunakan multiplex PCR untuk mendeteksi 6 panel bakteri.

**Hasil:** Sebanyak 175 spesimen kasus ILI bakteri yang paling banyak ditemukan pada spesimen ini adalah *Streptococcus pneumoniae* (n=163), *Haemophilus influenzae* (n=146) dan *Legionella pneumophila* (41).

**Kesimpulan:** Pada pasien ILI bakteri yang biasa terdeteksi pada saluran pernapasan bagian atas adalah *Streptococcus pneumoniae*, *Haemophilus influenzae* dan *Legionella pneumophila*. (*Health Science Indones 2013;2:83-6*)

**Kata kunci:** infeksi bakteri, ILI, PCR multiplex

### Abstract

**Background:** ILI surveillance in Indonesia has been conducted since 2006, but no data on the bacteria can be detected and caused ILI has been obtained.

**Method:** From March to December 2012, Center for Research on Biomedic and Basic Health Technology's laboratory was receiving throat and nose swab specimens from nine Public Health Centers appointed as the sentinels for ILI surveillance. These specimens were analyzed using multiplex PCR method, which detected six panels of bacteria.

**Results:** 175 specimens taken from ILI patients contained three most frequently found species of bacteria: *Streptococcus pneumoniae* (n=163), *Haemophilus influenzae* (n=146) and *Legionella pneumophila* (41).

**Conclusion:** In ILI patients, there are several bacteria most frequently detected in upper respiratory tract such as *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Legionella pneumophila*. (*Health Science Indones 2013;2:83-6*)

**Key words:** bacterial infection, ILI, multiplex PCR

Influenza Like Illness (ILI) can be caused not only by influenza virus but also by a number of microbes. The symptoms of influenza viral infection are unspecific and resemble clinical features of other diseases caused by other types of pathogens. ILI is defined as acute respiratory infection which is characterized by fever more than 38°C and coughing with an onset of less than 7 days.<sup>1</sup>

The most common symptoms of upper respiratory tract infection are cold, sinusitis, pharyngitis, and laryngitis. As a comparison, the symptoms of lower

respiratory tract infection are bronchitis, bronchiolitis, and pneumonia which is usually characterized by coughing.<sup>2</sup>

Most cases of upper respiratory tract infection are caused by virus, but it can also be triggered by bacterial infection. Types of virus associated with upper respiratory tract infection are rhinovirus, parainfluenza virus, corona virus, respiratory syncytial virus (RSV), and various other types of influenza viruses.<sup>3</sup> Upper respiratory tract infection is associated with some species of bacteria such as *S.*

*pneumoniae*, *H. influenzae*, *Mycoplasma pneumoniae*, *legionella* species, and *C. pneumoniae*. Among those species, *S. pneumoniae* and *H. influenzae* are normal flora in human upper respiratory tract.<sup>4,5</sup>

In a number of cases, bacterial infections are caused not only by a single infection but also by a coinfection.<sup>6</sup> Complication caused by bacterial co infections has been identified in almost all cases of death in the 1918 influenza pandemic. The number of co-infection cases increased by 29% in a recent A(H1N1) influenza pandemic throughout the world.<sup>7</sup>

Some studies on the distribution of influenza virus have been conducted in Indonesia. The results showed that influenza virus contributes only 20% of all ILI cases in Indonesia. However, there has been no research on the species of bacteria which may serve as an additional cause of ILI, especially in Indonesia. This study aimed to identify bacteria species which can cause ILI symptoms.

## METHODS

This research was carried out as a part of laboratory-based influenza surveillance on ILI patients at nine Public Health Centers conducted from January to September 2012. ILI surveillance was also conducted in 26 ILI sentinel Public Health Centers of Indonesian National Institute of Health Research and Development. 26 Public Health Centers were appointed as ILI sentinels because they are situated within urban area; such strategic locations allow easier access to the airports, making the transportation of research specimens more efficient.<sup>8</sup> Data and specimen collection including shipment and storage in the national influenza surveillance networks were adapted to international case definitions, following WHO requirements and CDC recommendations.<sup>1</sup>

Of 26 sentinels, 9 were selected as samples in this study since these sentinels contributed more specimens than other sentinel sites. ILI patients were given explanation regarding this study and were asked to sign consent forms to join this research. They were then interviewed by means of questionnaires. Next, their throat and nose swabs specimens were taken and were placed in one tube of Viral Transport Medium (VTM) and stored at 2-8°C. The questionnaires and specimens were then transported once a week to the virology laboratory of Center for Research on Biomedical and Basic Health Technology in Jakarta for further analysis.

DNA extraction by centrifugation and filtration was done as part of the preparation of the specimens

before tested by Polymerase Chain Reaction (PCR) multiplex method (developed by Seegene, Seeplex® PneumoBacter ACE detection) as per manufacturer's instructions.<sup>9</sup> PCR multiplex examination is based on real-time PCR using multiprimer which was conducted to identify and define the etiology of other potential diseases in ILI patients, in addition to influenza. Therefore, it took only one single procedure to identify the types of bacteria in the specimens under investigation. The specimens then underwent the process of DNA extraction using centrifugation and filtration methods. The extracted samples then underwent the process of PCR amplification with 6 bacteria primers which were enhanced by internal control. The amplicons were then processed by the Caliper 96 machine. The separation of amplicons for each type of bacteria was carried out by the electrophoresis principle.

## RESULTS

The number of specimens which underwent the multiplex PCR process was 175. The age of the patients participating in the research ranged from 4 month to 79 years old. The number of children cases (≤ 14 years old) was 142 out of 175 total cases.

Five out 175 cases were negative for bacteria. Table 1 shows that a majority of bacterial detected from ILI patients were *Streptococcus pneumoniae*, *Haemophilus influenzae*, and *Legionella pneumophila*. These three bacteria species were mostly found in the city of Balikpapan (East Kalimantan).

Other types of pathogens have also been found to have caused atypical pneumonia, which were *Chlamidia pneumonie* and *Mycoplasma pneumoniae*, one case each, all found in children. 115 cases (81%) of *Streptococcus pneumoniae* and *Haemophilus influenzae* together were found among children under 14 years old. Single bacteria of *Streptococcus pneumoniae* was found in 18 ILI cases, whereas single bacteria of *Haemophilus influenzae* was only found in 5 ILI cases.

The samples, which were examined to discover the species of bacteria detected from ILI patients, showed that more than one bacteria in single specimens occurred in 85% of all cases investigated, all of which detected between 2 to 4 species of bacteria. 163 specimens were positive for *Streptococcus pneumoniae*, while 146 were positive for *Haemophilus Influenzae*. Combination of two bacteria which mostly detected (almost 60%) was *Streptococcus pneumoniae* and *Haemophilus influenzae*, as shown on table 2.

Table 1. Distribution of bacteria found in ILI cases by sentinel sites

| Sentinel sites by city | Sample | Chlamydia pneumoniae | Legionella pneumo-phila | Borde-tella pertusis | Mycoplas-ma pneumoniae | Streptococ-cus pneumoniae | Haemophi-lus influenza |
|------------------------|--------|----------------------|-------------------------|----------------------|------------------------|---------------------------|------------------------|
| Bandung                | 28     | 0                    | 11                      | 0                    | 0                      | 28                        | 25                     |
| Banda Aceh             | 13     | 0                    | 2                       | 0                    | 0                      | 12                        | 10                     |
| Ambon                  | 19     | 0                    | 3                       | 0                    | 1                      | 18                        | 17                     |
| Balikpapan             | 29     | 1                    | 11                      | 0                    | 0                      | 29                        | 24                     |
| Palu                   | 22     | 0                    | 9                       | 1                    | 0                      | 20                        | 17                     |
| Riau                   | 3      | 0                    | 0                       | 0                    | 0                      | 2                         | 1                      |
| Mamuju                 | 2      | 0                    | 0                       | 0                    | 0                      | 2                         | 1                      |
| Batam                  | 29     | 0                    | 1                       | 0                    | 0                      | 25                        | 23                     |
| Banjarmasin            | 30     | 0                    | 3                       | 0                    | 0                      | 27                        | 28                     |
| Total                  | 175    | 1                    | 40                      | 1                    | 1                      | 163                       | 146                    |

Table 2. Distribution of detectable multiple bacteria by PCR multiplex

| Bacteria  | Total (n=175) |
|---|---------------|
| S. pneumoniae   | 18            |
| H. influenzae   | 5             |
| S. pneumoniae + H. influenzae                                 | 103           |
| L. pneumoniae + S. pneumoniae                                 | 6             |
| L. pneumoniae + S. pneumoniae + H. influenzae                 | 34            |
| L. pneumoniae + S. pneumoniae + H. influenzae + M. pneumoniae | 1             |
| C. pneumoniae + S. pneumoniae + H. influenzae                 | 2             |
| L. pneumoniae + S. pneumoniae + H. influenzae + B. pertusis   | 1             |
| Negative  | 5             |

## DISCUSSIONS

The examination of throat and nose swabs taken from 175 patients with ILI symptoms by multiplex PCR showed that 23 cases had single bacteria; 18 cases (10.3%) with *Streptococcus pneumoniae*, while 5 (2.9%) with *Haemophilus influenzae*. We also detect combination of more than one bacteria in one specimen. This results similar with a research conducted in the United States, in which 45 ILI cases were examined by multiplex to identify the infecting viruses and bacteria; 17 cases (37.8%) were positive for influenza viruses and 2 cases (4.4%) for RSV, while 6 patients (13.3%) carried *Streptococcus pneumoniae* and 6 patients (13.3%) *Haemophilus influenzae*.<sup>10</sup>

During the 2009 A(H1N1) pandemic in the United States, as many as 183 (67%) of 274 patients with suspected co-infection were found to have positive bacterial cultures. The types of pathogens most commonly isolated from respiratory cultures were *S. aureus* (39%), *Pseudomonas* species (16%), *Strep-*

*tococcus pneumoniae*, (8%), *Haemophilus influenzae* (7%), and *Streptococcus pyogenes* (4%).<sup>4</sup>

Another research which conducted in Enugu and Onitsha, Southern Nigeria, examined school children for potential bacterial infections in upper respiratory tracts. The results of bacterial isolation showed that *H. Influenzae* was the most common pathogen, followed by *S. Pyrogenes* (13.9%), *K. Pneumonia* (12.5%), *Streptococcus pneumoniae* (6.8%), *Staphylococcus aureus* (5.4%), and *Clostridium diphtheriae* (2.5%).<sup>11</sup> These statistics correspond to the current results of ILI-related cases examination conducted at nine Public Health Centers in Indonesia.

In addition to confirm the prevalence of the most dominant types of bacteria, the examination of 175 specimens has also identified two other types of bacteria responsible for causing atypical pneumonia among children, which are *Chlamydia pneumoniae* and *Mycoplasma pneumoniae*.<sup>3</sup>

Most upper respiratory tract infections are caused by viruses and develop into diseases which end with full natural recovery. Viral infection in upper respiratory tract may serve as a predisposition factor in the prevalence of bacterial infection in children.<sup>3,12</sup> *Streptococcus pneumoniae* and *Haemophilus influenzae* are species of bacteria most commonly found in cases of upper respiratory tract infection. Infection of influenza virus or RSV in respiratory tract can enhance the connection between *Streptococcus pneumoniae* and *Haemophilus influenzae* on nasopharyngeal lining cell.<sup>3,13-15</sup>

*Streptococcus pneumoniae* is a pathogenic bacterium which can cause severe respiratory tract infection with initial symptoms similar to influenza's. Bacterial infection found in influenza cases is associated with increasing death rate. A research in Mexico found

that co infections increased the number of patients treated at the ICU (75%), while the death rate reached 16.23%.<sup>16</sup> Some studies on seasonal influenza showed that *Streptococcus pneumoniae* was the leading cause of bacterial confection (16.6%), followed by *Staphylococcus spp.* (6.2%), *Haemophilus influenzae* (5.2%), and *Streptococcus spp.* (1.8%).<sup>17</sup>

Few cases of ILI showed positive influenza virus infections (20%), most of which exhibited only light symptoms, and the patients did not require hospitalization.<sup>8</sup> Multiplex PCR examination can detect genetic materials of bacteria which can trigger pneumonia, but actual samples of bacteria obtained from upper respiratory tracts suggested that the infections of these bacteria did not always lead to pneumonia (severe lower respiratory tract infection)<sup>3</sup>. Thus, further examination is needed to confirm the PCR multiplex results.

In conclusion, in patients with ILI symptoms, using the PCR multiplex method, we found the most common types of bacteria in human upper respiratory tract are *Streptococcus pneumoniae*, *Haemophilus influenzae* and *Legionella pneumophila*.

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