Prevalence of *Streptococcus Pneumonia* and *Haemophillus Influenza* in primary school children that diagnosed acute otitis media

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Abstract. Acute otitis media (AOM) remains a disease that cause major public health problem worldwide. Less information about its prevalence in Indonesia, especially in Java. The most common bacterial causes of AOM are *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*, and *Moraxella catarrhalis*. There is increasing evidence that the predominant causative pathogen in AOM is changing from *Streptococcus pneumoniae* to non-typeable *Haemophilus influenza* since the introduction of pneumococcal conjugate vaccines. This study aims to determine the prevalence of *Streptococcus pneumonia* and *Haemophillus influenzae* in AOM in primary school children in Banyumas Regency. The design of this study was cross sectional and we conducted multistage random sampling to recruit the subject. Approximately 3,574 school children in Urban Banyumas Regency were screened based on the diagnose of AOM by Otolaryngologist. The resultof this study showed that the prevalence of AOM was 4,64 % (166 children were diagnosed with AOM). In AOM samples, the prevalence of *Streptococcus pneumonia* was found in 78,4 % by optochin test, while that of *Haemophillus influenzae* was found in 70,4% based on the need of X and V factor.

Keyword : Streptococcus *pneumonia* and *Haemophillus influenzae,* prevalence, Acute Otitis Media

1. Introduction

Acute Otitis media (AOM) is one of the most common infections in early childhood and can be considered as a complication of upper respiratory tract infections [1]. This disease is characterized by the presence of middle-ear effusion and the acute onset of signs and symptoms caused by middle-ear inflammation [2]. Symptoms of AOM include earache in older children; or pulling, tugging, or rubbing of the ear or non-specific symptoms such as fever, irritability, or poor feeding in younger

children. Signs of AOM involve a distinctly dark, yellow, or cloudy tympanic membrane [3]. The detection of AOM is improved by the presence of a bulging tympanic membrane, air-fluid level behind the tympanic membrane, tympanic membrane perforation, and/or discharge into the ear canal. Pneumatic otoscopy and/or tympanometry can be used to determine the presence (or absence) of middle ear effusion (MEE).For children with breathing tubes (grommets) in place, the discharge of the ear is a symptom of AOM, where the fluid that has collected in the middle ear flows through the tube into the child's ear canal [4].

The most common bacterial causes of AOM are *Streptococcus pneumoniae*, non-typeable *Haemophilus influenzae*, and *Moraxella catarrhalis*. There is increasing evidence that the predominant causative pathogen in AOM is changing from *Streptococcus pneumoniae* to non-typeable *Haemophilus influenza*since the introduction of pneumococcal conjugate vaccines. Group childcare outside the home and passive smoking is thought to be the most important risk factor for AOM [5]. OM covers a diverse group of diseases, which contain some degree of inflammation or infection located in the middle ear and the tympanic membrane. OM is also the primary cause of narrow and broad-spectrum antibiotic prescriptions for children in developing countries, including Indonesia.In Indonesia, the prevalence of CSOM is relatively high, with the majority of diseases occurring in rural areas. High rates in rural Bali with early progression to tympanosclerosis suggest a significant burden of potentially vaccine-preventable disease [6].

AOM is still considered a neglected disease in Indonesia since the community seems not to have enough awareness of this disease. The previous study stated that 38 children from Surabaya, Indonesia, who suffered from chronic suppurative otitis media (CSOM) were studied using strict microbiological methodology [7]. Information on prevalence of *Streptococcus pneumonia* and *Haemophillus influenzae* related to AOM in children also very limited particularly in Indonesia. An increased risk of otitis media recurrences were observed in the presence of allergy, persistent cough and runny nose [8]. The objective of this study is to determine prevalence of *Streptococcus pneumonia* and *Haemophillus influenzae* in primary school children in Banyumas Regency. Results of this study will provide valuable information related to incidence of AOM.

2. Methods

2.1. Sample collection

The study site used in this analysis is the Banyumas Regency, located in the southwest of Central Java Province, Indonesia. A cross-sectional study was conducted in the selected primary school of 6 regions in Banyumas Regency, namely in Region 1: Pekuncen, Ajibarang, Region 2: Karanglewas and Cilongok, Region 3: Rawalo and Kebasen, Region 4: Sumbang and Kembaran Region 5 = North Purwokerto, South, West Purwokerto and Region 6 = Kalibagor, Banyumas and Kemranjen. The selection of primary schools in the chosen areas was made based on inclusion criteria. Screening for AOM was carried out bya co-assistant doctor, Faculty of Medicine, Jenderal Soedirman University. The inclusion criteria for OMA sampling were patients who experiencedcough and cold for approximately one week and experiencing pain in the middle ear. Informed consent was obtained from the parent of children, which was also signed by the witness. Based on screening tests 987 children from 3,574 were classified as the suspect of AOM. Then confirmed diagnosis was determined by Otolaryngologist and 166 children diagnosed as positive cases of AOM. From 166 positive cases, only 125 participants who willing to take part in this study. This study was approved by Health Research of Ethics Committee, Faculty of Medicine, Jenderal Soedirman University, Purwokerto, Indonesia (No 4015/KEPK/FK/2018). The specimens were placed on 1 ml of STGG (Skim milk, Tryptone, Glucose and Glycerine) media and stored at -80°C.

2.2. Isolation and Identification of Streptococcus pneumonia

The 200 µl of swab-inoculated STGG media was transferred into 5.0 ml Todd Hewitt broth containing 0.5 % yeast extract (THYB), and 1 ml of rabbit serum as enrichment media, then it was incubated at 35-37°C for 5 hours [9]. One loop of cultured broth (10 µl) was plated onto Blood Agar Plate (BAP) media 8% and incubated at 35-37°C with 5% CO_2. After 18-24 hours of incubation, *S. pneumonia* were examined as a transparent, moist, watery, and surrounded by a green zone colony on BAP media indicating α-hemolysis. Colonies with S. pneumoniae character were distreaked into 8% BAP media. Then, a 5 µg optochin disk with a diameter of 6 mm was placed over the reacted area. After incubation at 37oC, 5% CO2 for 18-24 hours, if the isolate is sensitive to optochin (inhibition zone ≥14 mm), it is suspected that the isolate is S. pneumonia [10]

2.3. Isolation and Identification of Haemophillus influenzae

One loop of enriched THYB media containing 200 µl of swab-inoculated STGG were cultured onto *chocolate agar plate* (CAP) media as a selective agar for the identification of *H. influenzae*, then incubated at 37°C for 24-48 hours with 5% CO_2 [11]. *H. influenzae* appeared as transparent, slightly brownish, wet [12], grayish, opaque, smooth surface, convex colonies with a diameter of 1-2 mm, after being incubated for 24 hours on CAP media [13]. *H. influenzae* isolates that had been identified based on their macromorphological test were then inoculated on filter paper that had been dripped with Kovac oxidase reagent (0.1 gr tetramethyl-p-phenylenediamine dihydrochloride in 10 mL of distilled water). Positive interpretation is indicated by the color of the filter paper which changes to purple [14]. Identification of *H. influenzae* based on the need for factor X (hemin) and factor V (NAD) was performed using the paper disc method. Isolates identified through the Kovac oxidase assay were made cell suspensions (1.0 McFarland standard) in soy broth trypticase (TSB) and homogenized completely using a vortexer. A total of 10 µL of suspension was inoculated on soy agar trypticase (TSA) using a sterile loop or swab and allowed to dry. After drying, paper discs containing hemin, NAD, hemin + NAD were placed on the inoculum, then incubated at 35-370C with 5% CO2 for 18-24 hours. After incubation, the growth of bacteria around the paper discs was observed. *H. influenzae* will grow around disc paper containing hemin+NAD [14].

3. Results

From 3.574 children, 166 were diagnosed with AOM, so the prevalence of AOM in the Banyumas District was 4.64%. Nevertheless, only 125 supporting participants are able to continue engaging in this prevalence study. The features of the participants can be seen in Table 1.

Table 1. Characteristic of AOM samples

Characteristic	n	%
AOM	125	100.0
Age (year)		
6-7	33	26.4
8-9	51	40.8
10-11	35	28.0
>11	6	4.8
Gender		
Male	61	48.8
Female	64	51.2

A total of 125 AOM samples that were enriched in THYB + rabbit serum were cultured on 8% BAP media. The culture results showed that 78.4% (98/125) had characters similar to *S. pneumoniae*.

According to the CDC (2014), *S. pneumoniae* is round, grayish in color, wet or mucoid, small in size and the surface of the colony is convex at the beginning of growth, the same as S. viridans. However, *S. pneumoniae* colonies will form a hollow in the middle or called a flattened and depressed center (FDC) after an incubation time of 18-24 hours. In addition, the most important characteristic of *S. pneumoniae* is to have a green zone around the colony which indicates the occurrence of alpha hemolytic. By, optochin test *S. pneumoniae* colonies appeared an inhibition zone with a diameter of ≥ 14 mm (Figure 1).

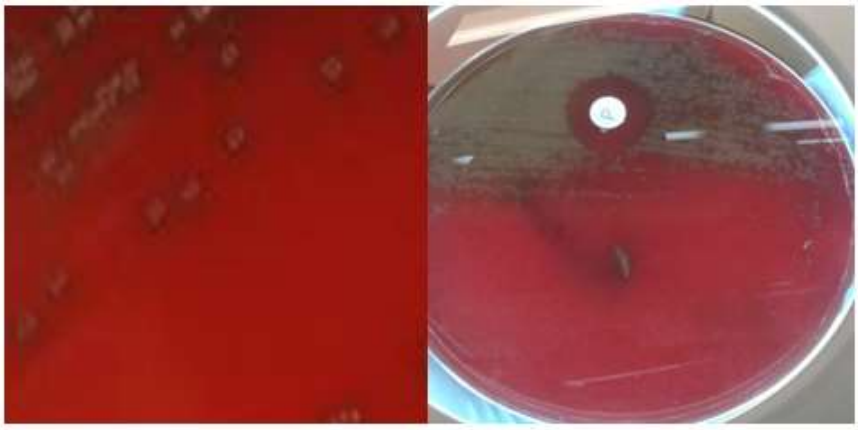

Figure 1. Morphology of *S. pneumoniae* colony on BAP media which appeared alpha hemolyt around the colony. (II) The results streak of the optochin sensitive test

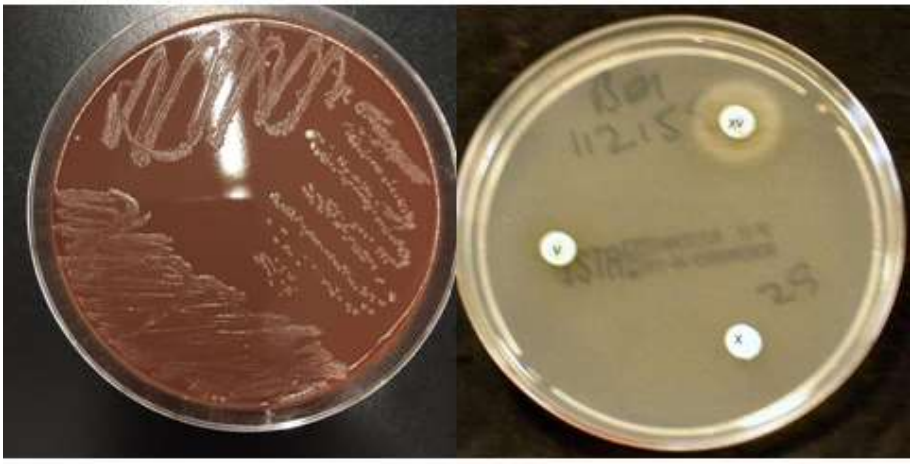

Figure 2. Morphology of *H. influenzae* colony on BAP media which appeared alpha hemolyt around the colony. (II) The results streak of the optochin sensitive test

The prevalence of *H. influenzae* was found in 70.4% (88/125) in AOM samples. *H. influenzae* is transparent, slightly brownish, wet, greyish, opaque, smooth surface, convex colonies on CAP media. While, *H. influenzae* is determined by the colour of the filter paper which changes to purple. According to WHO (2011), *H. influenzae* will grow around disc paper containing hemin + NAD based on the need for factor X (hemin) and factor V (NAD) (Figure 2.)

4. Discussion

As the AOM case number report in health institutions in Banyumas Regency is inadequate, we perform a clinical examination in children in selected primary schools by ear, nose, and throat/ENT specialist who has been involved in the study. Based on the results of the examination, the prevalence of OMA in the Banyumas Regency was 3.%. This percentage is quite high because some previous studies in Indonesia such as in North Sumatra province recorded a prevalence of 2.1 [15]. A previuous study in 7005 public school children (6–15 years) in Indonesia found that the prevalence of OM was 2.5% [16]. A total of 125 patients with tubotympanic CSOM were included in this study. There were no differences in AOM infection within age and gender status (Table 1).

Our finding was higher than previous studies that observed *S. pneumoniae* (78.4%) and *H. influenzae* (70.4%), respectively, were dominant bacterial species isolated from patients with AOM infection. This can be a mutualistic association between *S. pneumoniae* and *H. influenzae* to cause AOM infection. According to the CDC, *S. pneumoniae* is found in AOM (28-55%). The highest OM prevalence rate occurs in Alaska, Australian Aboriginal tribes, and America (12-46%) [17], while the prevalence rate that occurs in New Zealand, Nepal, and Malaysia (4-8%) [18]. About 2% of children aged less than 6 years in Europe experience more than three times cases of AOM per year [19]. *H. influenzae* infection is indicated to occur in 16-61% of cases of AOM [20]. *H. influenzae* is found in the nasopharynx and oropharynx, as many as 26.3% of children under 5 years of age with respiratory disease were found to be infected with *H. influenzae* [21]. H. influenzae infection in children suffering from AOM is 16-52% with a case rate of 27% [22]. In previous study, 30-52% of AOM cases in children are caused by *H. influenzae* [23]. These bacteria attack children a lot, because the immune system of the children is still very vulnerable [24]. *H. Influenzae* is often found in the nasopharynx of children and is rarely found in adults [25]. However, after running the program using the pneumococcal conjugate vaccine (PCV), the predominance of the bacteria that cause AOM has now shifted from S. pneumoniae to *H. influenzae* [26]. In Indonesia, the prevalence of CSOM is relatively high, with the majority of diseases occurring in rural areas. High rates in rural Bali with early progression to tympanosclerosis suggest a significant burden of potentially vaccine-preventable disease [6]. AOM is still considered a neglected disease in Indonesia since the community seems not to have enough awareness of this disease. The previous study stated that 38 children from Surabaya, Indonesia, who suffered from chronic suppurative otitis media (CSOM) [7]. Information on risk factors related to AOM in children also very limited. A previous study identifying risk factors for chronic and recurrent Otitis Media and reveal that snoring, previous history of AOM/ROM, second-hand smoke, and low social status are important risk factors for COM/ROM [27]. Another study also found that low parental educational attainment, exposure to smoke, indoor exposure to mold, laryngopharyngeal reflux disease,and the lack of breastfeeding; an increased risk of otitis media recurrences were observed in the presence of allergy, persistent cough and runny nose [8].

5. Conclusion

We successfully describe prevalence of *S. pneumoniae* and *H. influenzae* from children with AOM, which remains limited in Indonesia. Our study will give a recommendation for appropriate risk factor among children with AOM. However, further study should be conducted with risk factor, serotype dan antibiotic profile to elucidate the role of of *S. pneumoniae* and *H. influenzae* as causative agents of AOM accurately.

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