CAUSATIVE ORGANISMS OF ACUTE RESPIRATORY INFECTIONS IN NORTHERN THAILAND

TSUYOSHI NAGATAKE¹, ATSUSHI TAKAHASHI¹, MISAO TAO¹, HIDEHIKO HIROSE², HIRONORI MASAKI¹, KIWAO WATANABE¹, KAMURUDDIN AHMED¹, NAOTO RIKITOMI¹, KEIZO MATSUMOTO¹, PRASTH THARAVICHITKUL², THIRA SIRISANTHANA³, RONATRAI RUENGVERAYUDH⁴, TAWORN KASOMSON⁵, SATID CHARUOTESKULCHAI⁶, SONBOON JIAMTAVEEVIBOON⁷, CHINTANA UTHAISILP⁸, WITAYA SWADDIWUDHIPONG⁹ AND CHARUNEE CHANDOUN⁴

Received March 5 1993/Accepted March 30 1993

Abstract: We examined the causative organisms of respiratory infections from 1989 to 1990 at Mae Sot General Hospital in collaboration with Chiang Mai University, Thailand. We collected sputum from patient with acute bronchitis and pneumonia to identify the causative organisms by sputum culture and inflammatory sputum cytology. We experienced 72 cases (97 strains of bacteria) of acute bronchitis and 17 cases of pneumonia (20 strains of bacteria) in Mae Sot General Hospital. The most frequently identified pathogens in respiratory infections were *Haemophilus influenzae*, *Streptococcus pneumoniae* and *Branhamella catarrhalis*. This study shows that Gram-stained smears and quantitative sputum culture together have a significant role in enhancing the diagnostic value of expectorated sputum specimens. The causative organisms between Thailand and Japan were very similar in acute respiratory infections. The most important outcome of this study was the detection of *Branhamella catarrhalis* in many adult cases with acute respiratory infection.

INTRODUCTION

It is well recognized that the majority of acute upper respiratory infections are caused by viral infections which are sometimes followed by bacterial infections. Bacterial infections cause complications of common cold: acute sinusitis, otitis media, acute bronchitis and pneumonia. It is sometimes dangerous for young children and aged people to catch cold because of various complications occur due to secondary bacterial infections. Respiratory infections cause much mortality and morbidity (Murray, 1982). This is true both for the developed and developing countries. From different hospitals of developed countries data on bacteria causing respiratory infections are regularly published. On the other hand, such data are not regularly obtained from developing countries. Moreover the foundation of chemotherapy of respiratory infections is first of all to determine pathogens (Matsumoto, 1963). Therefore it is important to study the bacterial pathogens causing respiratory infections. It is not known which pathogenic organisms predominate in Thailand. Therefore, this study was carried out to identify the causative organisms of respiratory infections in a rural district of Thailand. We examined the causative organisms of respiratory infections from 1989 to 1990 at Mae Sot General Hospital in collaboration with Chiang Mai University, Thailand. This type of studies are helpful to understand about the incidence of bacteria causing acute respiratory infection between the two countries, which has difference in climate, nutritional status, economy etc.

MATERIALS AND METHODS

Location: This study was carried out in Mae Sot General Hospital in collaboration with Chiang Mai University (Fig. 1). In Mae Sot, from November to February is winter, from March to May is summer and from June to October is rainy season. This investigation

1 Department of Internal Medicine, Institute of Tropical Medicine, Nagasaki University, 1-12-4 Sakamoto, Nagasaki 852, Japan
2 Department of Microbiology, Chiang Mai University, Chiang Mai 50002, Thailand
3 Faculty of Medicine, Chiang Mai University, Chiang Mai 5002, Thailand
4 Mae Sot General Hospital, Mae Sot, Tak 63110, Thailand
was done at three different time interval, i.e. from December 1989 to January 1990 (winter), from July to August 1990 (rainy) and from November to December 1990 (winter).

Collection of sputum samples: Fresh expectorated sputum, from patients with respiratory infections submitted to the Department of Internal Medicine, Mae Sot General Hospital, were used in this study.

Sputum smear: Gram staining and Acid fast staining were done of sputum smear to observe the inflammatory cytology and bacteria. The method for Gram staining of sputum specimens was based on Hucker’s modification (Sonnenwirth, 1980). Ziehl-Neelsen’s staining was done to recognize acid-fast bacilli (Cowan and Steel, 1974).

Sputum culture: Sputum was cultured either quantitatively or semi-quantitatively on TSA agar containing 7% human blood and on chocolate agar. Agar plates were incubated at 37°C for 18 hr in ordinary incubator. Bacteria were identified by standard methods (Cowan and Steel, 1974) at the Department of Microbiology, Chiang Mai University, Thailand.

Clinical studies: Our standardized criteria of respiratory infection with causative organisms are as follows; (1) Gram stain of sputum smear show plenty of polymorpho-nuclear leukocytes or macrophages and dominant bacteria located intracellularly and extracellularly, (2) much growth of these bacteria in semi-quantitative culture or more than 10^7 CFU/ml in quantitative culture (Matsumoto et al., 1978), (3) increase in number of these bacteria coincides with clinical and laboratory findings. Clinical data corresponding to the specific data of sputum collection were obtained from patient case records at Mae Sot General Hospital.

RESULTS

Time period: The first and third study period were in the winter and the second study period was in the rainy season.

Patients: There was a total 392 patients and their mean age was 43 yrs. A total 249 patients were male with a mean age of 43.4 yrs and 143 patients were female with a mean age of 42.5 yrs. Details of age, sex and number of patients are shown in Table 1.

Identification of bacterial pathogen: From a total 72 cases of acute bronchitis 97 strains of causative pathogenic bacteria were isolated. The main pathogenic bacteria were *Haemophilus influenzae* (*H. influenzae*) (44.5%), *Streptococcus pneumoniae* (*S. pneumoniae*) (26.8%) and *Branhamella catarrhalis* (*B. catarrhalis*) (14.3%). Main causative bacteria, number of cases, strain isolated in different period of time are shown in Figure 2.

From a total 17 cases of pneumonia, 20 strains of pathogenic bacteria were isolated. These bacteria were, *H. influenzae* (45%), *S. pneumoniae* (40%), *Klebsiella pneumoniae* (*K. pneumoniae*) (10%) and *B. catarrhalis* (5%). The results are shown in Figure 3.

Comparison of causative bacteria of acute bronchitis between Mae Sot General Hospital, Mae Sot,

Clinical studies: Our standardized criteria of respiratory infection with causative organisms are as follows; (1) Gram stain of sputum smear show plenty of polymorpho-nuclear leukocytes or macrophages and dominant bacteria located intracellularly and extracellularly, (2) much growth of these bacteria in semi-quantitative culture or more than 10^7 CFU/ml in quantitative culture (Matsumoto et al., 1978), (3) increase in number of these bacteria coincides with clinical and laboratory findings. Clinical data corresponding to the specific data of sputum collection were obtained from patient case records at Mae Sot General Hospital.

RESULTS

Time period: The first and third study period were in the winter and the second study period was in the rainy season.

Patients: There was a total 392 patients and their mean age was 43 yrs. A total 249 patients were male with a mean age of 43.4 yrs and 143 patients were female with a mean age of 42.5 yrs. Details of age, sex and number of patients are shown in Table 1.

Identification of bacterial pathogen: From a total 72 cases of acute bronchitis 97 strains of causative pathogenic bacteria were isolated. The main pathogenic bacteria were *Haemophilus influenzae* (*H. influenzae*) (44.5%), *Streptococcus pneumoniae* (*S. pneumoniae*) (26.8%) and *Branhamella catarrhalis* (*B. catarrhalis*) (14.3%). Main causative bacteria, number of cases, strain isolated in different period of time are shown in Figure 2.

From a total 17 cases of pneumonia, 20 strains of pathogenic bacteria were isolated. These bacteria were, *H. influenzae* (45%), *S. pneumoniae* (40%), *Klebsiella pneumoniae* (*K. pneumoniae*) (10%) and *B. catarrhalis* (5%). The results are shown in Figure 3.

Comparison of causative bacteria of acute bronchitis between Mae Sot General Hospital, Mae Sot,
Figure 2  The distribution of pathogenic bacteria isolated from sputum of patients with acute bronchitis in three different study period.

Figure 3  The distribution of pathogenic bacteria isolated from sputum of patients with pneumonia. This represent the combined results of three different study period.

Figure 4  The distribution of pathogenic bacteria isolated from sputum of patients with acute bronchitis. Left column and right column reprensent the result obtained from Iki Island, Nagasaki and Mae Sot, Thailand respectley.
Thailand and Iki Public Hospital, Nagasaki, Japan are shown in Figure 4. The percentage of causative organisms are similar.

**DISCUSSION**

In regard to the causative organisms of respiratory infection some studies of developing countries showed that the major bacterial pathogens were *S. pneumoniae* and *H. influenzae* (Ghafoor et al., 1990; McIntosh, 1990; Vathanophas et al., 1990).

In Mae Sot General Hospital we experienced a total 72 cases of acute bronchitis and 17 cases of pneumonia. From sputum of acute bronchitis and pneumonia, 97 strains and 20 strains of pathogenic bacteria were isolated respectively. The main pathogenic bacteria were *H. influenzae*, *S. pneumoniae* and *B. catarrhalis*.

Previously we reported in Japan, *B. catarrhalis* is one of the major pathogenic organism in respiratory infection (Matsumoto et al., 1981; Nagatake, 1985). Subsequently we reported various important aspect of this bacteria to elucidate its mechanism of pathogenicity (Ahmed et al., 1990; Rikitomi et al., 1991; Ahmed et al., 1992). In this study we found in Mae Sot, Thailand, *B. catarrhalis* is one of the major pathogen in respiratory infection. These data show that *B. catarrhalis* infection increased simultaneously in different parts of the world (Mcleod et al., 1986; Bartos et al., 1988). The present data also shows that there is a high incidence of *B. catarrhalis* in winter. This is also similar to our previous observation and we also showed that this increase incidence of *B. catarrhalis* infection in winter is due to the increase attachment of *B. catarrhalis* to respiratory epithelium in winter (Mbagi et al., 1987).

Although *H. influenzae* is usually regarded as a lower respiratory tract pathogen in patients with chronic lung disease. *H. influenzae* was the main pathogen of acute bronchitis and pneumonia in Mae Sot General Hospital. The incidence of *H. influenzae* pneumonia in adults have been increasing in many countries (Levin et al., 1977; Woodhead et al., 1987; Maniji et al., 1990).

It is very interesting that the causative organisms of acute respiratory infections between Thailand and Japan were similar. In Mae Sot, doctors had conception that in that area, respiratory infection occured mostly by *S. pneumoniae*. Because in Mae Sot General Hospital, instead of rabbit blood human blood is used in agar, on which it is difficult to recognize *H. influenzae*. As *B. catarrhalis* is a newly recognized pathogen, in Mae Sot they did not have experience to recognize the bacteria.

Our experience showed that in most of the developing countries have similar misconception about the recognition of pathogenic bacteria. This collaboration study helped the recognition of pathogenic bacteria in Mae Sot. We also found that Gram-stained sputum smears can greatly aid clinicians to make presumptive etiological diagnosis, and permit the laboratory to enhance the isolation of pathogenic bacteria by selective culture methods (Odhiambo et al., 1990). This study shows that 'big three' pathogens (*H. influenzae*, *S. pneumoniae* and *B. catarrhalis*) of the community acquired respiratory infections are observed commonly in the world (Nagatake, 1991; Davies et al., 1990). The criteria used in this study would appear to be more adaptable for routine use in developing countries. Similar type of collaboration studies are very helpful for the developing countries.

**ACKNOWLEDGMENTS**

This study was partly supported by the Grant for Scientific Research for the “Project on the diagnosis and treatment of acute respiratory tract infection” from WHO.

**REFERENCES**


12) McIntosh, K. (1990): Creation of a research program to determine the etiology and epidemiology of acute respiratory tract infection among children in developing countries, Rev. Infect. Dis., 12 (Suppl. 8), 861-869


