Mechanism of Formation of the Orange-Colored Sputum in Pneumonia Caused by *Legionella pneumophila*

Jiro Fujita¹, Masato Touyama², Kenji Chibana², Michio Koide¹, Shusaku Haranaga¹, Futoshi Higa¹ and Masao Tateyama¹

Abstract

It has been said that the color of sputum from patients with *Legionella pneumophila* pneumonia is orange. However, why the color is orange has not been clarified. First, orange-colored sputum obtained from a patient with *L. pneumophila* pneumonia is presented. Then, the formation of an orange-colored pigment in a culture medium (without charcoal) after the growth of *L. pneumophila* is demonstrated.

Key words: *Legionella pneumophila*, pneumonia, sputum, orange-colored

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Introduction

*Legionella pneumophila* is the cause of both community-acquired and nosocomial pneumonia. Pneumonia caused by *L. pneumophila* cannot be differentiated from other types of pneumonia by clinical, radiographic or laboratory findings. Therefore, a specific etiologic diagnosis is performed on the basis of the results of a culture growth, direct immunofluorescence, serologic testing, and antigen detection in the urine. Of these, the advantages of diagnosing legionellosis by urinary antigen detection are widely recognized, and include early detection, testing rapidity, and ease of specimen collection. Lipopolysaccharides (LPS) are considered to be the major bacterial components excreted in urine, and the sensitivity and specificity of this method are good (1-8).

Radiologically, it is important that pneumonia caused by *L. pneumophila* shows the pattern of lobar pneumonia (non-segmental distribution) (9-11), and this pattern is also observed in pneumonias caused by *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Mycoplasma pneumoniae* with a Th2 type of host reaction (12).

In addition, it has been clinically suggested that the color of sputum obtained from patients with pneumonia caused by *L. pneumophila* is orange. However, there have been no previous reports that demonstrate the color of sputum obtained from patients with *L. pneumophila*.

In the present report, we demonstrate a case of *L. pneumophila* pneumonia in which the color of sputum was clearly observed during a bronchofiberscopic examination. In addition, we also demonstrated a change in the color of a culture medium using an inoculation of *L. pneumophila*.

Case Report

A 52-year-old man complained of anorexia, diarrhea and fever. He was referred to a private hospital. His chest x-ray showed infiltrative shadow. He had a history of smoking (20 cigarettes/day for 32 years) and drinking alcohol (160 g/day on a daily basis). The physical examination demonstrated an elevated fever (38.4°C), normotensive (114/64 mmHg) with a regular heart rate of 92 beats per minute, but an increased respiratory rate (35/min). His height was 169 cm and his weight was 65 kg. He had clear consciousness, and meningeal signs were absent. The cranial nerve examination was normal and sensory functions appeared unremarkable. There was no edema of the lower extremities. Examination of the oral cavity, lymphoreticular system, chest, and abdomen was unremarkable. By auscultation, rhonchi and inspiratory coarse crackles were demonstrated. Saturation of hemoglobin was 94% (with nasal O₂ 3 L/min).

Laboratory findings were as follows; erythrocyte sedimentation rate 97 mm/hr, white blood cell count 12200/µl (neutrophils 87.4%), hemoglobin 15.1 g/dl, platelet count 18.4×

¹Department of Medicine and Therapeutics, Control and Prevention of Infectious Diseases (The First Department of Internal Medicine), Faculty of Medicine, University of the Ryukyus, Okinawa and ²Department of Internal Medicine, Yonabaru Central Hospital, Okinawa

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Correspondence to Dr. Jiro Fujita, fujita@med.u-ryukyu.ac.jp
Figure 1. (A) Chest X-ray findings of a patient with *L. pneumophila* pneumonia. Consolidation of the right lower lobe is clearly demonstrated. (B) Chest CT findings of the same patient. Consolidation of the right lower lobe with air bronchogram is observed. Ground-glass opacities are also demonstrated around the consolidation.

Figure 2. (A) Bronchofiberscopic findings of the patient with *L. pneumophila* pneumonia. Orange-colored sticky sputum is observed. (B) Sputum obtained through a bronchofiberscope. Orange-colored sputum is observed.

10^4 μl, total protein 6.7 g/dl, albumin 3.6 g/dl, total bilirubin 0.5 mg/dl, aspartate aminotransferase 48 U/L, alanine aminotransferase 83 U/L, lactic dehydrogenase 360 U/L, blood urea nitrogen 21.2 mg/dL, creatinine 0.9 mg/dL, and uric acid 6.7 mg/dl. Serum electrolytes were as follows: Na 130 mEq/L, K 3.4 mEq/L, and Cl 94 mEq/L. C-reactive protein was 35.9 mg/dL. Blood gas analysis showed that pH was 7.53, pCO2 27.9 torr, pO2 64.0 torr, and HCO3^- 22.8 mEq/L (with O2 3 L/min nasal, and a respiratory rate of 35/min).

Plain chest x-ray showed consolidation of the right lower lobe (Fig. 1A). Chest CT also demonstrated consolidation of the right lower lobe with air bronchogram accompanying ground-glass infiltrations around the consolidation (Fig. 2B).

Since no sputum was obtained, bronchofiberscope was performed (Fig. 2A), and an orange-colored sticky sputum was observed (Fig. 2B). The urine sample showed a positive result with the Binax NOW Immunochromatographic Test kit (Binax, Portland, ME, USA). In addition, the *L. pneumophila* serogroup 1 was cultured in a BCYEα medium. After diagnosis, he was treated with ciprofloxacin followed by oral moxifloxacin, and his symptoms gradually improved.

**Mechanism of Formation of Orange-Colored Sputum**

Based on the referenced studies, we hypothesized that orange pigment might be excreted by *L. pneumophila*, and attempted to corroborate this hypothesis *in vitro* (13, 14). To observe the color produced by *L. pneumophila*, we used...
Figure 3. (A) The BYE agar medium (without charcoal), and no inoculation of L. pneumophila. (B) In the BYE agar medium, moderate growth was observed at 4 days after inoculation of L. pneumophila. Brown-orange pigment was observed. Since distribution of brown-orange color was homogenous throughout the medium, it was thought that this color was produced by the chemicals contained in this medium and not produced by the L. pneumophila itself.

BYE agar (BCYEα agar without charcoal, Fig. 3A). The formula for this medium was as follows (L): 10 g of yeast extract (Becton-Dickinson, Sparks, MD, USA.), 17 g of agar (Becton-Dickinson, Bacto agar), 10 g of N-(2-acetamido)-2-aminoethanesulfonic acid (ACES) buffer (Dojin chemical, Kumamoto, Japan), 3.5 g of potassium hydroxide (Wako Chemical, Osaka, Japan), 1 g of α-ketoglutarate (ICN Biomedicals, Aurora, OH, USA), 0.4 g of L-cysteine hydrochloride (Nacalai Tesque, Kyoto, Japan), and 0.25 g of ferric pyrophosphate (Wako chemical). We also made BYE-tyrosine agar [BYE agar added by 0.2 g/L of L-tyrosine (Wako chemical)] (15, 16) and compared the strength of pigmentation to the BYE agar. L. pneumophila serogroup 1 Philadelphia-1 strain (type strain ATCC33152) was streaked on the BYE agar and BYE-tyrosine agar, and incubated at 37°C for up to 10 days.

The result was the observation of a slight growth of L. pneumophila on the BYE agar at 3 days of incubation and the observation of moderate growth at 4 days. A slight amount of brown-orange pigment was observed at 4 days of incubation, which gradually strengthened during the period up to 6 days (Fig. 3B). Pigment production was more enhanced on the BYE-tyrosine agar in comparison to the BYE agar (data not shown). Since the distribution of the brown-orange color was homogenous, it was supposed that this color was produced by the chemicals contained in this medium and not produced by the L. pneumophila itself.

Discussion

It is well known that the color of sputum is sometimes an important clue when speculating about pathogens. For example, a yellow-colored sputum suggests that the infection was caused by Staphylococcus aureus, and a green-colored sputum suggests infection caused by Pseudomonas aeruginosa. Among those pathogens causing a pattern of lobar pneumonia, it is well known that sputum obtained from pneumonia caused by S. pneumoniae shows a red-iron color and that sputum obtained from lobar pneumonia caused by K. pneumoniae shows a strawberry-jelly color. In addition, it is known that sputum obtained from pneumonia caused by L. pneumophila shows an orange color as demonstrated in the present case.

The mechanism as to why L. pneumophila produces an orange-colored sputum was evaluated in this study. To culture L. pneumophila, a specific medium called a BCYEα medium is usually used. Since this medium contains a charcoal, the color of this medium is black. Therefore, it is very difficult to evaluate any color change caused by the growth of L. pneumophila.

In the present study, we made a new medium which does not contain a charcoal to culture L. pneumophila. Using this new medium, it was very easy to evaluate any color change in the medium after the growth of L. pneumophila. As demonstrated in the present study, the color of the medium changed diffusely after the growth of L. pneumophila, suggesting that factors released from L. pneumophila caused the color of the medium to change.

It has been suggested that tyrosine included in the medium produced the orange color after exposure of the culture supernatant of L. pneumophila (15, 16). Such evidence suggests that factors released from L. pneumophila play an important role in changing the color of the medium. Since tyrosine is included in epithelial lining fluids, the orange color of sputum will be produced by L. pneumophila using tyrosine in the body.

Although the exact mechanism is still unclear, physicians should be aware of the color of sputum obtained from patients with pneumonia caused by L. pneumophila.

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