

Original Article

Investigating a pulmonary *Mycobacterium abscessus* infection outbreak among elderly inpatients in the intensive care wardWenya Hua¹ #, Kunyang Wu² #, Yelei Zhu², Zhengwei Liu², Yu Zhang², Jiazhen Chen¹¹ Department of Infectious Diseases, Shanghai Key Laboratory of Infectious Diseases and Biosafety Emergency Response, National Medical Center for Infectious Diseases, Huashan Hospital, Shanghai Medical College, Fudan University, Shanghai, China² Zhejiang Provincial Center for Disease Control and Prevention, Hangzhou, China

Authors contributed equally to this work.

Abstract

Introduction: *Mycobacterium abscessus* is an opportunistic nontuberculous mycobacteria pathogen; however, the prevalence of nosocomial and community infections is increasing. In January 2016, several bedridden inpatients in the intensive care unit of a hospital had positive sputum smears for acid-fast bacilli, suggesting a mycobacteria outbreak.

Methodology: Acid-fast bacilli smear microscopy, isolation, and culturing were performed twice using sputa from each suspected intensive care unit inpatient (n = 13); in addition, medical history was obtained for each inpatient with suspected infection. Furthermore, environmental specimens were surveyed, collected, and cultured. We used DNA microarray chip analysis to identify positive mycobacterial isolates at the species level and performed whole-genome sequencing and phylogenetic tree construction.

Results: Seven inpatients had *M. abscessus* pulmonary infection, confirmed by 2 positive cultures; five of the inpatients had only one positive culture, while one had two negative cultures. Six of 13 ventilator condensate samples were mycobacterial culture-positive, identified as *M. abscessus*; the other environmental samples were negative. The *M. abscessus* isolates (15 sputa and 4 environmental samples) clustered together in the phylogenetic analysis with only one single-nucleotide polymorphism difference. All patients were symptom-free after 8 months of multi-drug treatment.

Conclusions: We confirmed a pulmonary *M. abscessus* outbreak among 12 bedridden patients in the intensive care unit through microbiological, molecular epidemiological, and environmental investigations. The possible infection source was contaminated ventilator condensate. This outbreak reemphasizes the importance of standardized ventilator maintenance and disinfection for preventing ventilator-associated pneumonia and is a reminder that nontuberculous mycobacteria-related ventilator-associated pneumonia is possible.

Key words: *Mycobacterium abscessus*; pulmonary infection; hospital outbreak; ventilator-associated pneumonia.*J Infect Dev Ctries* 2023; 17(12):1732-1739. doi:10.3855/jidc.17395

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Copyright © 2023 Hua *et al.* This is an open-access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.**Introduction**

Mycobacterium abscessus (MAB) is a rapidly growing mycobacterium intrinsically resistant to many antibiotics and disinfectants [1]. Nontuberculous mycobacteria (NTM) are opportunistic pathogens, generally only infecting immunocompromised patients [2]. However, the possibility of cross-transmission among humans of MAB has been reported [3–5], particularly in skin and soft tissue infections [6] and in cystic fibrosis patients [3]. Furthermore, NTM exhibits a strong survival tendency in aquatic environments [7–9]. Therefore, improper community use of tap water, such as for drinking, swimming, and bathing, may cause NTM infections [10]. Cutaneous MAB infection outbreaks in community settings have been reported in

an indoor wading pool [11], at tattoo studios [12–14], and in a spa worker exposed to MAB contamination in the tap water [15]. Furthermore, reports of NTM outbreaks in healthcare settings are increasing, most of which are MAB-associated. For example, outbreak investigations have identified MAB in heater-cooler units in the operating room [16] and biofilm colonization on disinfectant trays [17], causing postoperative infection. In addition, MAB has been isolated from peritoneal dialysis-associated catheters [18,19] or exit sites [20,21], resulting in blood or cutaneous infections.

Hospital-acquired infection is a common problem in hospitals around the world. Nosocomial infection causes serious health problems, including prolonged

hospital stays and increased mortality of affected patients, especially in developing countries [22]. Since the healthcare environment seems to play an important role in the spread of diseases, high adherence to infection prevention and control measures is essential. In this case, knowing which implementation strategies based on dissemination intervention are most effective is important [23]. Previous studies have shown that the educational program on promoting preventive behaviors of nosocomial infections in nurses is effective [24]. In addition, a thorough understanding of the occurrence and transmission of hospital infection is essential.

This study aimed to analyze a series of MAB pulmonary disease cases possibly transmitted through the environment in a hospital's intensive care unit (ICU) in Southeast China. The objective was to use a combination of epidemiological investigations, traditional *Mycobacterium* identification methods, and whole-genome sequencing (WGS) to investigate the transmission of the cases. Over the past decade, infections by MAB isolates have been increasingly reported nationwide [25,26]. However, there were few studies on MAB pulmonary infection in hospitals in China with the sample size found in our study. Our results potentially illustrate the role of the transmission mode of MAB in nosocomial infections and improve the effectiveness of infection prevention and control efforts.

Methodology

Patients and Samples

In total, 17 ICU inpatients had suspected pulmonary infections. Thus, we obtained their medical histories, including sex, age, admission time, clinical symptoms, laboratory results, chest computed tomography (CT) examination, and treatment. Additionally, a questionnaire was used to evaluate the potential risk factors for infection, including airway conditions, basic pulmonary conditions, and possible water-related exposures in the hospital. Case definitions were based on China's diagnostic criteria for NTM pulmonary disease [27]. Briefly, a confirmed case was a patient who met the clinical and radiologic criteria and had appropriate exclusion of other diagnoses. Furthermore, a positive NTM culture result from at least two separate expectorated sputum samples was necessary.

The study was approved by the ethics committee of Huashan Hospital affiliated to Fudan University. Data collection was conducted according to regulations covering information collection.

Acid-fast Bacilli (AFB) Smear Microscopy

Each suspected patient underwent AFB smear microscopy twice [28]. The smears were prepared and stained using the Ziehl–Neelsen method [29].

Isolates, Culturing, and Species identification

Mycobacterial culturing was performed using the sputa of the suspected patients in Mycobacteria Growth Indicator Tube (MGIT) liquid media (BD, Franklin Lakes, NJ, USA) following the manufacturer's protocol [30]. Briefly, specimens for culture were decontaminated with N-acetyl-L-cysteine and sodium hydroxide and centrifuged for 15 minutes at $3000 \times g$. After discarding the supernatant, the pellet was resuspended in 1 mL of phosphate-buffered saline, and then 0.5 mL of this resuspension was incubated in an MGIT medium. The cultures were then incubated at 37 °C and monitored hourly for up to 42 days in an automated Mycobacterium liquid culture system (BACTEC™ MIGHT™ 320, BD) [30,31]. Cultures with growth within 42 days were considered positive, and cultures without growth were considered negative.

Growing isolates were identified at the species level using a DNA microarray chip from the Mycobacterial Species Identification Array Kit (CapitalBio Technology Inc., Beijing, China) based on previous studies [32–35]. The chip can identify 17 *Mycobacterium* species: *M. tuberculosis* complex and *M. chelonae*-*M. abscessus*, *M. fortuitum*, *M. intracellulare*, *M. avium*, *M. kansasii*, *M. gordonae*, *M. terrae*, *M. smegmatis*, *M. szulgai*/*M. malmoense*, *M. nonchromogenicum*, *M. scrofulaceum*, *M. xenopi*, *M. aurum*, *M. marinum*/*M. ulcerans*, *M. gilvum*, and *M. phlei*.

Environmental Investigations

Eight days after the first sampling, ICU maintenance was evaluated through interviews with the ICU staff, a review of the ICU maintenance records, and direct observation by the local Centers for Disease Control. Environmental specimens were sampled, including the ventilator surface and air conditioner using dry sterile swabs and the ventilator condensate after each use in a sterile sampling tube without additives. Environmental samples were cultured in MGIT medium immediately after collection. The species of the positive mycobacterial isolates were identified using the DNA microarray chip, as described above.

WGS and Phylogenetic Tree Construction

Genomic DNA extraction, library construction, and WGS of MAB were performed as previously described for *Mycobacterium tuberculosis* strains [36]. The whole genomes of all 19 strains are available at GenBank under the BioProject ID: PRJNA861738. De novo analysis of MAB strain No. 20160611 was performed using SPAdes 3.13 with default parameters [37]. A single nucleotide variant (SNV) analysis was performed on 19 clinical MAB isolates using SOAPaligner v2.21 as previously described [38], except that the de novo genomic sequence of MAB No. 20160611 was used as a template. SNVs ranging from 1 to 5 bp were sorted and called at a minimum depth of 10 without strand bias. If the mutation ratio of a specific site was > 85%, it was considered a fixed mutation. A phylogenetic tree was constructed by the maximum likelihood method using MEGA (version 10.0) software [39] based on the SNV with MAB ATCC 19977 as the root.

Results

Epidemiologic and Clinical Investigations

In January 2016, patient number 12 tested AFB-positive with hemoptysis in the ICU of a hospital in Southeast China. Considering the tuberculosis transmission risk among the patients, all 17 ICU patients underwent a sputum AFB smear microscopic examination; 10 were AFB positive, and 7 were AFB negative. Four AFB-negative inpatients were discharged with the hospital's consent. The remaining 13 inpatients underwent a second round of sputa sampling eight days after the first sampling, for AFB smear microscopy. The two sputum samples from the 13 inpatients were cultured, and the positive isolates were identified at the species level. Seven inpatients (numbers 1, 2, 4, 6, 7, 9, and 11) had two positive

cultures, and the isolates were the same species (*M. abscessus*-*M. chelonae*), confirming them as MAB pulmonary cases (Table 1). Five inpatients (numbers 3, 5, 8, 12, and 13) had only one positive culture, and the isolates were identified as *M. abscessus*-*M. chelonae* (Table 1). The other inpatient, number 10, was admitted to the ICU due to "head injury and fracture" without basic lung disease and had two negative cultures. Thus, MAB infection was ruled out. Overall, 19 positive isolates from inpatients were collected, and 15 were preserved.

Of the 13 inpatients, 7 were men, and 6 were women. The median age was 78 (range, 60–92) years. The median admission date was December 2015 (range, July 2015–January 2016). All inpatients used a ventilator to assist with breathing; 11 (84.6%) underwent a tracheotomy, and 2 (15.4%) underwent endotracheal intubation. All 12 patients with MAB infection reported coughs and expectorations. Furthermore, 1 patient (8.3%) had hemoptysis, 7 (58.3%) had a fever, and 4 (33.3%) had lung cavities on chest CT images.

Of these 13 elderly patients, 12 were immunocompromised and long-term bedridden with no direct contact with other patients in the same ward. Thus, these patients were possibly infected with MAB during airway opening interventions. One case (patient number 5, male, 70 years old) was diagnosed with suspected pulmonary tuberculosis in June 2009 and had undergone six months of treatment.

Environmental Survey and Remediation

The survey indicated that ultraviolet radiation disinfection equipment was present in the ICU but rarely used. In addition, the disinfection equipment included a plasma air purifier, used for two hours per

Table 1. The result of Epidemiologic, Clinical, Laboratory and Environmental Investigation.

| Patient ID | Age (Years) | Sex | January, 2016 | | 8 days after | | |
|------------|-------------|-----|---------------|--|--------------|--|--|
| | | | sputum | | sputum | Ventilator condensate | |
| | | | AFB Stain | Culture Result | AFB Stain | Culture Result | Culture Result |
| 1 | 92 | M | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> ¹ | Negative |
| 2 | 78 | F | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> ² | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> ³ | <i>M. abscessus</i> - <i>M. chelonae</i> ⁴ |
| 3 | 81 | F | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> ⁵ | Positive | Negative | <i>M. abscessus</i> - <i>M. chelonae</i> ⁸ |
| 4 | 60 | M | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> ⁶ | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> ⁷ | <i>M. abscessus</i> - <i>M. chelonae</i> ⁸ |
| 5 | 70 | M | Negative | Negative | Negative | <i>M. abscessus</i> - <i>M. chelonae</i> ⁹ | Negative |
| 6 | 65 | F | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> | Negative | <i>M. abscessus</i> - <i>M. chelonae</i> ¹⁰ | Negative |
| 7 | 80 | F | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> ¹¹ | Negative |
| 8 | 85 | F | Negative | Negative | Negative | <i>M. abscessus</i> - <i>M. chelonae</i> ¹² | Negative |
| 9 | 83 | M | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> ¹³ | <i>M. abscessus</i> - <i>M. chelonae</i> ¹⁴ |
| 10 | 78 | M | Negative | Negative | Negative | Negative | <i>M. abscessus</i> - <i>M. chelonae</i> ¹⁵ |
| 11 | 75 | F | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> ¹⁶ | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> ¹⁷ | <i>M. abscessus</i> - <i>M. chelonae</i> |
| 12 | 78 | M | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> ¹⁸ | Positive | Negative | Negative |
| 13 | 79 | M | Positive | <i>M. abscessus</i> - <i>M. chelonae</i> ¹⁹ | Negative | Negative | Negative |

AFB: acid-fast bacteria; ID: identification. ¹⁻¹⁹ Successfully preserved strains.

day. This ICU contained 20 beds, including eight beds in the hall, four single rooms, and four double rooms. Only the south of the ward had windows capable of opening for ventilation.

The environmental samples tested negative for mycobacteria. However, six of thirteen ventilator condensate samples tested positive, and four strains were successfully preserved (Table 1). Thus, ventilators were the most likely infection source as these instruments were shared by the inpatients, although the tubes were used independently. The ventilator tube was routinely sent to the supply room for disinfection with 1000 mg/L of chlorine for 30 minutes after each use. All ventilator tubes were sealed and unpolluted during storage and transportation.

Nonetheless, tube disinfection was possibly invalid owing to bubbles inside the tubes. Thus, the disinfectant could not completely cover the tube’s surface during disinfection. In addition, the ICU maintenance records were inconsistent. Therefore, regular cleaning and disinfection of the ventilator tube could not be confirmed, and it could not be verified whether the liquid in the ventilator atomizer was distilled or tap water.

The ICU was closed 7 days after the first sampling, and the ICU environment was thoroughly disinfected. The entire ICU area, including the hospital beds and body of the ventilators, was disinfected using a hydrogen peroxide generator. In addition, the ventilator’s surface was wiped with a chlorine-containing disinfectant, and the ventilator tube was disinfected with ethylene oxide. Environmental

samples after disinfection were all Mycobacteria-negative, and no more transmitted cases were found. Therefore, the preliminary suspicion was that the MAB contamination occurred in the ICU ventilator equipment, indicating the equipment to be a possible source of the MAB outbreak.

WGS Single Nucleotide Variation (SNV) Analysis and Phylogenetic Tree Construction

WGS analysis revealed that the 19 clinical isolates were *M. abscessus* subsp. *abscessus*. A large genetic distance was observed between the MAB clinical strains and the MAB ATCC 19977 reference strain (GenBank: NC_010397). Phylogenetic analysis of all 19 MAB isolates was conducted using fixed SNVs (mutation frequency > 85%), and these isolates clustered together with one SNV difference among them. Ten isolates (numbers 1, 9, 10, 11, 13, 16, 17, 18, and 19 and ventilator condensate number 14) clustered together with no SNV, while nine isolates (sputum numbers 2, 3, 5, 6, 7, and 12 and ventilator condensate numbers 4, 8, and 15) clustered together (Figure 1), demonstrating that this outbreak’s infection source was one predominant strain. The only SNV among these isolates was C702G in MAB_2374c, the function of which is unknown. The genetic distance between the cluster and the control strain, *M. abscessus* ATCC 19977, reached 8984 SNVs (Figure 1)

Treatment and Outcomes

All 12 patients with MAB infection were treated with a regimen that included oral clarithromycin (500 mg) twice daily, an intravenous infusion of amikacin (15 mg/kg/d), once daily, an intravenous infusion of cefoxitin (200 mg/kg/d), and oral ciprofloxacin (1000 mg/d), as recommended by the guidelines on the diagnosis and treatment of NTM pulmonary disease in China [40]. After eight months of treatment, all patients were symptom-free. Furthermore, all patients

Figure 1. Phylogenetic analysis of the Mycobacterium abscessus-related samples (clinical specimens from patients, and ventilator condensate). Numbers of single-nucleotide polymorphisms among isolates are shown within both clusters, and number above branch represent bootstrap probability based on 1000 replicates. Abbreviations: ATCC, American Type Culture Collection; SNV: single-nucleotide variation. Bar: 1000 SNVs.

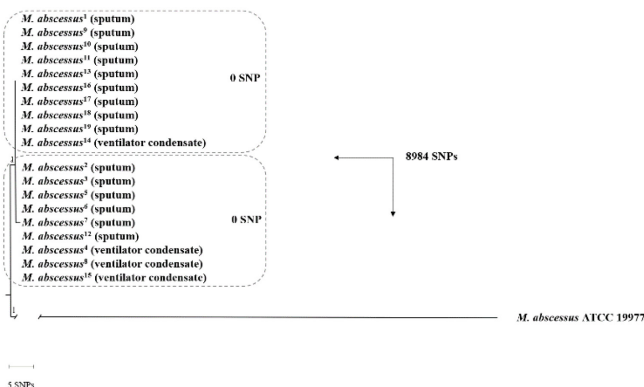
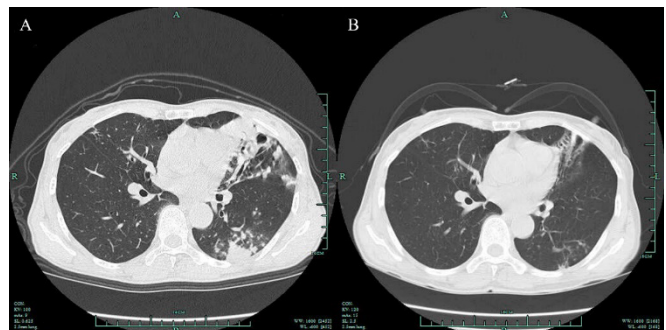


Figure 2. CT images of one patient before (A) and after (B) treatment.



underwent a chest CT examination, which showed focal absorption and no lung cavities (Figure 2), and they had negative sputum cultures.

Discussion

NTM is an opportunistic pathogen common in natural environments, such as water, soil, and dust [41]. Patients with chronic respiratory diseases or immune insufficiency are susceptible to NTM infection [2]. Furthermore, most nosocomial NTM infections are rapidly growing mycobacteria, specifically MAB [42,43]. Recently, MAB has been associated with various clinical manifestations, including pulmonary infection and common cystic fibrosis [44,45]. Moreover, multiple healthcare-associated MAB outbreaks among patients with cystic fibrosis have been reported within medical centers [44]. For example, one study analyzed the recent emergence and transmission of MAB in the global cystic fibrosis population, finding that person-to-person transmission remains indirect [46]. Therefore, early treatment and cross-infection control are necessary to restrict the transmission of mycobacterial pathogens, particularly MAB-specific measures, to limit future outbreaks.

Although medical supplies and equipment are the main vectors of hospital infection [22], disinfection and sterilization have always been a huge challenge in hospitals and clinics. Ventilator-associated pneumonia (VAP), defined as pneumonia occurring at least 48 hours after introducing mechanical ventilation [47], is one of the most common healthcare-associated infections [48]. However, the microorganisms associated with VAP differ based on the geographic area [49]. In China, the most frequent isolates were *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, followed by *Staphylococcus aureus* and *Stenotrophomonas maltophilia* [48]. Furthermore, ST11 carbapenem-resistant hypervirulent *Klebsiella pneumoniae* strains caused a fatal outbreak of VAP in Hangzhou, China [50]. However, only a few have reported MAB-induced VAP in the ICU in China.

Condensate often forms in ventilator tubes. Here, bacteria can grow and reproduce, leading to VAP. The guidelines for the diagnosis and treatment of hospital-acquired pneumonia and VAP in adult hospitals in China (2018 Edition) [51] emphasize the necessity of avoiding condensate-containing bacteria flowing directly into the lower respiratory tract, which can cause VAP. Moreover, these guidelines suggest avoiding backflow to the humidification tank, which can suck humidified aerosol-containing bacteria into the lower respiratory tract. Moreover, the condensate collection

bottle should always be at the lowest position of the tube, kept upright, and regularly cleaned. Sterilized water should be used in the humidification tank and the atomizer and should be replaced every 24 hours. In our cases, the ventilator's external tubes and accessories were disinfected or sterilized after each patient. However, for patients requiring long-term mechanical ventilation, replacing the ventilator tubes once per week and when stains are visible to the naked eye is generally recommended [52]. In this study, the environmental investigation identified some nonstandard operations during the disinfection process. Once the ventilator tube disinfection method was improved, transmission ceased. Thus, we infer that ineffective pipeline disinfection caused MAB transmission in the ICU of the hospital.

In China, the prevalence of nosocomial infection among ICU patients is high, which increases the hospitalization expenses of patients, reduces the health-related quality of life, leads to a notable impact on incidence rate and mortality, and extends the duration of hospitalization [53]. Among nosocomial infections, VAP is common. In recent years, due to the prevalence of COVID-19, even young patients may suffer severe nosocomial infections due to prolonged mechanical ventilation [54]. To control infection, molecular epidemiology, and rapid diagnosis is important to quickly determine the source of infection. Previous studies have shown that with the gradual implementation of *Mycobacterium tuberculosis* nucleic acid amplification tests and cultures in intensive care practice, the duration of the hospital transmission period could be shortened [55].

The treatment of pulmonary infection caused by MAB is difficult given its high toxicity, long treatment course, serious side effects, and scarcity of new antibiotics available [56]. Although the treatment was based on drug sensitivities, there was a case report with a fatal outcome caused by MAB endocarditis of the native aortic valve in an immunocompetent patient after coronary angiography in the presence of renal failure [57]. In this regard, an urgent need exists to develop an alternative treatment strategy aimed at improving the current management of patients with chronic MAB infection. Besides antibiotics, phage therapy has been described as a successful regimen in combating MAB in advanced lung diseases [58]. In our case, the MAB strain was not drug-resistant and the treatment outcome was satisfying.

However, our study has certain limitations. First, we could not confirm the validity of the ventilator tube disinfections. Second, we could not rule out the

possibility of water pollution in the ventilator's humidification tank since we could not confirm whether the water was distilled or from the tap. Third, due to the long duration of the study and the restrictions of local hospital policies, our study had some missing patient data, including height, weight, body mass index (BMI), occupation, degree of education, income level, marriage status, and clinical and radiological records. Finally, only 19 of the 25 MAB isolates were well-preserved and successfully recovered; we failed to recover the other six isolates. Therefore, we could not obtain all the strain information to trace the source completely.

Conclusions

This study used mycobacterial smear, culturing, species identification, environmental investigations, WGS, and comparative genomics analyses to confirm an outbreak of MAB among bedridden patients in the ICU. Furthermore, the possible infection source was the ventilator condensate, which allowed MAB growth. These cases emphasize the importance of standardized ventilator maintenance and disinfection to prevent VAP. In addition, these cases are a reminder that, albeit rare, NTM-related VAP is a potential threat in hospitals.

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Authors' contributions

WH: performed the WGS experiment and co-writing the manuscript; KW: collection of clinical sample and strain; YZ and ZL: assisting the clinical samples collection; YZ: designed the study and in charge of the environmental sampling; JC: statistics analysis and in charge of co-writing of this manuscript.

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Corresponding authors

Yu Zhang
 Zhejiang Provincial Center for Disease Control and Prevention
 No.3399 Binsheng Rd.
 Hangzhou 310051, China.
 Email: yzhang@cdc.zj.cn
 Tel: +86 0571 87115111

Jiazhen Chen
 Department of Infectious Diseases
 Huashan Hospital, Fudan University
 No.12 Middle Wulumuqi Rd.,
 Shanghai 200040, China.
 Email: jiazhen_chen@163.com
 Tel: +86 021 52887961

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