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An outbreak of *Burkholderia stabilis* colonization in a nasal ward

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SUMMARY

Objective: The aim of this study was to describe an outbreak of *Burkholderia stabilis* colonization among patients in a nasal ward.
Methods: Multilocus sequence typing (MLST) was used for the molecular typing of *B. stabilis* isolates. Microbiological records were reviewed to delineate the colonization outbreak period. One hundred seventy-one cultures of environment and equipment samples from the nasal ward were performed to trace the source of contamination. Infection control measures were taken in order to end the outbreak.
Results: All *B. stabilis* isolates were identified as a new MLST type, ST821. A total of 53 patients carried this *B. stabilis* in the nasal ward between March and September 2013, which was defined as the outbreak period. The source of the colonization was not determined because all environment cultures were negative for *Burkholderia cepacia* complex. No further *B. stabilis* carriers have been found in the ward since the implementation of interventions.
Conclusions: Attention must be paid to asymptomatic colonization in order to identify outbreaks early.

1. Introduction

The *Burkholderia cepacia* complex (Bcc) comprises at least 17 distinct species, including *Burkholderia stabilis*, *Burkholderia cepacia*, *Burkholderia cenocepacia*, and *Burkholderia multivorans*, which are widely distributed in the natural environment, such as soil, water, and the rhizosphere.

Bcc species are particularly virulent, devastating pathogens for individuals suffering from cystic fibrosis (CF)[1,2] or chronic granulomatous disease, but are also important opportunistic pathogens that can cause nosocomial infections.[3,4] So far, many genomovars have been isolated from hospital outbreaks. Medina-Pascual et al. reported that *B. cenocepacia* was the most prevalent genomovar found in patients with CF, whereas *B. cepacia* was the most common among non-CF patients. *B. stabilis* was the most common environmental genomovar.[5] Furthermore, reported outbreaks of Bcc species infections have been linked to the contamination of medical devices, disinfectants, and medical solutions.[4,6]

Various epidemiological typing methods are currently utilized for infection outbreak investigations, such as multilocus restriction typing,[7] pulsed-field gel electrophoresis (PFGE), and random amplified polymorphic DNA (RAPD). PFGE is used widely, but is not a transferable technique. The multilocus sequence typing (MLST) method is a means to assist in species identification as well as unambiguous strain discrimination of the Bcc, and can facilitate global epidemiological investigations of Bcc.[8,9]

In the present study, we describe a 7-month outbreak of *B. stabilis* colonization among hospitalized patients with nasal disease. An environmental investigation was implemented to trace the source of the colonization, and infection control measures were implemented to terminate the outbreak. To our knowledge, this is the first report of an outbreak of *B. stabilis* colonization from a nasal ward in China.

2. Materials and methods

2.1. Clinical characteristics

This study took place in a 500-bed tertiary hospital in Beijing, China, ophthalmology and otolaryngology. The colonization

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outbreak was only identified in a 45-bed nasal ward with 14 rooms.

Samples were obtained from the middle meatus under nasal endoscopic guidance from all patients on the day subsequent to admission to the ward and were submitted to culture and pathogen diagnosis. Almost all patients underwent an endoscopic sinusotomy as part of the therapeutic management.

All microbiological records of the hospital laboratory information system were reviewed to establish the colonization outbreak period. 'Colonization' was defined as *B. stabilis* isolated from any body site without any apparent infection. 'Outbreak' was defined as the simultaneous presence of two patients with positive cultures for *B. stabilis*.

2.2. Microbiological studies

Clinical swabs were cultured on Columbia agar plates supplemented with 5% sheep blood and incubated at 37 °C for 24–48 h. The initial identification was done using a matrix-assisted laser desorption/ionization time-of-flight mass spectrometry assay (MALDI-TOF MS). Environmental sample swabs were cultured in trypticase soy broth, and then subcultured after 24 h and processed as above. Disinfectants and water were cultured on Columbia agar plates supplemented with 5% sheep blood.

The MALDI-TOF MS assay was performed using a MALDI-TOF Autoflex III (Bruker Daltonics) operating in linear positive ion mode at a laser frequency of 200 Hz in a mass range of 2–20 kDa. Spectra were analyzed using MALDI Biotyper software (Bruker Daltonics) and using the Biotyper Library (Bruker Daltonics) for database searching and identification.[10,11] *rec*A sequencing and MLST were performed at the species-level for Bcc isolates.[12]

Minimum inhibitory concentration (MIC) results for trimethoprim–sulfamethoxazole, ceftazidime, meropenem, and levofloxacin were determined by Vitek 2 system (bioMérieux Inc., Durham, NC, USA). The results were interpreted according to the criteria of the Clinical and Laboratory Standards Institute (CLSI) for Bcc species. The control strains used were *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922.

2.3. Multilocus sequence typing (MLST)

MLST of the isolates was done using seven standard housekeeping genes (*atp*D, *glt*B, *gyr*B, *lep*A, *pha*C, *trp*B, *rec*A) according to the protocol and primers specified in a public database of MLST sequence data (http://pubmlst.org/bcc/).[8] DNA sequencing was performed commercially by SinoGenoMax Co. Ltd (Beijing, China). Clustering analysis of the MLST association with profiles identified worldwide was performed using BURST software (http://eburst.mlst.net/v3).

2.4. Environmental screening

As well as the middle meatus specimen cultures obtained under nasal endoscopic guidance, surveillance cultures were also done in association with endoscope-related procedures. On the ward, nasal endoscopes were disinfected in stages, in accordance with the manufacturer's recommendations, including cleaning, disinfecting in sodium dichloroisocyanurate–synergist HZ704 solution for 5 min, and rinsing.

Epidemiological environmental sampling was carried out in every ward room and examination room by swab-rinse sampling method, including tabletops ($n = 48$), bed rails ($n = 45$), knobs ($n = 48$), taps ($n = 11$), hand lotion ($n = 3$), nasal endoscope ($n = 6$), and physician's hands ($n = 4$). All disinfectants ($n = 6$) used in the ward were also subjected to culture.

2.5. Infection control measures

General interventions used to control the spread of multidrug-resistant organisms include hand hygiene measures and environmental cleaning.[13] Bcc nosocomial outbreaks are typically associated with water and medical products.[14,15] Therefore, the nurses began to implement the following infection control measures on September 15, 2013. First, environmental surfaces, taps, and medical devices were disinfected with 75% alcohol. Second, the nasal endoscope disinfection time was extended to 10 min with the use of a high-level disinfectant, sodium dichloroisocyanurate–synergist HZ704. Third, healthcare hand hygiene was strengthened in the ward.

3. Results

3.1. Epidemiological features

After reviewing the medical records, the first appearance of *B. stabilis* in the ward occurred on March 19. From March to September 2013, a total of 740 patients suffering from chronic bacterial or fungal sinusitis were admitted to the ward and had microbiological cultures done on admission. Fifty-eight sample cultures from 53 patients, including five nasal pyogenic excretions obtained surgically during operations and 53 middle meatus swabs, were positive for *B. stabilis* between March and September 2013. None of the patients developed *B. stabilis* infections. Colonization was defined as Bcc isolation from any body site without any apparent infection; in this way the outbreak of *B. stabilis* colonization was identified. The colonized patients were distributed across every ward room. The outbreak period was defined as the time between March 19 and September 20, 2013. The pre-outbreak period was defined as June 2012 through February 2013. The post-outbreak period was defined as October 2013 through March 2014. The peak colonization occurred in July and August (Figure 1). The last isolate of *B. stabilis* was obtained on September 20. No cultures were positive for *B. stabilis* during the pre-outbreak and post-outbreak periods in the nasal ward.

Two isolates of *B. multivorans* were isolated from a patient in the intensive care unit (ICU) in December 2012 – one from sputum and the other from hydrothorax.

None of the environment cultures, including disinfectants, surfaces, and equipment, grew *B. stabilis*. The source of the colonization was not determined.

3.2. Isolate features

All clinical isolates were identified as *B. stabilis* by *rec*A gene. Two isolates were resistant to meropenem and three isolates were resistant to ceftazidime. Other isolates were susceptible to meropenem, ceftazidime, trimethoprim–sulfamethoxazole, and levofloxacin.

3.3. MLST

Epidemiological typing of all *B. stabilis* isolates showed them to be identical, belonging to sequence type ST821, which was uploaded to the database (http://pubmlst.org/bcc/). ST821 is a new type and has no correlation with any other profile worldwide (Figure 2).

3.4. Infection control

After control measures were implemented in September 2013, *B. stabilis* colonization declined. So far as we know, no further clinical cultures in the nasal ward have grown *B. stabilis*. The