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Undetected Bacillus Pseudo-Outbreak after Renovation Work in a Teaching Hospital

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Abstract

A 602-bed capacity hospital underwent complete renovation from 1999 to 2004. In April 2005, the Infection Control Team was informed of the occurrence of three consecutive cases of *Bacillus cereus* bacteremia in a ward for patients with hematologic malignancies. A retrospective analysis of patients with *Bacillus* isolates was initiated. We found more *Bacillus cereus* isolates from blood samples in 2004 compare to the preceding years. Swab samples were collected in the particular ward from the surface of a working desk, filter unit of the air-conditioners, entrance of air inlet ducts, exit of the air outlet ducts and three-way valves of the particular ward under the consideration of iatrogenic contamination. Towels and gowns used in the ward were examined. Dens dust was noted in the filter of the air-conditioner and inlets/outlets of the air-ventilation system of the ward. *Bacillus cereus* was isolated from the dust, and from cleaned towels and gowns. PFGE fingerprinting differed among four patients' sample. We considered the present case as an undetected *Bacillus cereus* pseudo-outbreak that lasted for about one year after the renovation work of the hospital. We also considered that filters of the HVAC-system and towels and gowns were probable sources of the outbreak.

Key words: *Bacillus cereus*, outbreak, pseudo-outbreak, air-conditioner, air-ventilation, bacteremia, laundry.

Introduction

Bacillus cereus, a gram-positive, rod-shaped, spore-forming aerobe is widely distributed in nature, and is a well-recognized cause of toxin-mediated food poisoning.[1] It also causes rare systemic infections usually in immunocompromised patients.[2] These rare non-gastrointestinal infections are associated with wound injuries and burns, hemodialysis, immunocompromise, parenteral drug abuse, blood transfusion, and spinal anesthesia.[3]

There have been few reported outbreaks of non-gastrointestinal *Bacillus cereus* infections, including two in neonatal intensive care units (NICU)[4, 5] and one in an adult intensive care unit (ICU).[6] An unusual outbreak of *Bacillus* species in which calcium gluconate vials were implicated has also been reported.[7] A pseudo-outbreak is a situation in which an organism is recovered from culture at a rate that is greater than expected and that cannot be clinically correlated with the supposed infection indicated by the results of culture.[8] This situation may result from systemic extrinsic contamination during specimen collection or processing or intrinsic contamination at the time the culture medium is manufactured or prepared.[9] Dissemination of *Bacillus* species in hospitals has been reported,[10] however, most of the episodes were pseudo-outbreaks. Recognizing and tracking the source of such pseudo-outbreaks can be a difficult task.[8, 11]

Evidence obtained from epidemiological studies and laboratory measurements supports the hypothesis that a contaminated heating, ventilation and air-conditioning (HVAC)-system may be a source of pollutants. Humidifiers, dirty air duct, and dirty filters could all be the perfect sites for amplification and dissemination of indoor fungi. Some investigations have suggested that microbial contamination might be present in

construction materials, ventilation systems, or results from human activities.[12, 13] However, contamination of the HVAC-system has not been recognized as a cause of *Bacillus* outbreak.

In the present report, we describe an increase of *Bacillus cereus* isolates from blood samples in a teaching hospital after general renovation work of the building. The HVAC-system and the laundry process were considered as the probable sources by tracking the incidence.

Background

The teaching hospital

An eleven-story teaching hospital with 602-bed capacity was built in 1976. Each floor was divided into two segments, an east ward and a west ward, by an elevator hall. Each ward has around 48 beds. Maintenance of the air-ventilation system and cleaning of the hospital was in accordance with the national guidelines established by the Japanese Government.

In this hospital, blood culture was not included among routine examinations. Usually it was performed when patients presented a sudden bout of high fever or showed signs of severe infection especially these with an indwelling continuous intravenous line for infusions. Blood samples were usually taken by veno-puncture or arterial puncture. An environmental survey was not carried out on a regular basis in the hospital.

General renovation of the hospital

General renovation of the teaching hospital began in 1999 and finished in 2004. A new building was added to the hospital on the east side to increase the floor surface by 150%. The renovation was accomplished in three phases. First, the new eastern building was built between 1999 and August 2001. Renovation of the old eastern part, which became the central part, started after the extension work. During this period of time the new eastern part and the old western part were used. Then, the old western part followed by the first, second and third floor of the central part were renovated using the eastern part and above the forth floor of the central part. After the renovation work, the building was divided into western ward and eastern ward again. All renovation work of the wards finished by March 2004. During the renovation, each working part

was tightly separated from those under construction by temporary walls.

The old hospital had a heat air-ventilation system but did not have an air-conditioning system. The renovated hospital is equipped with an HVAC-system, which includes an air-ventilation system and a heat and cool air-conditioning system. There are air inlet ducts, air outlet ducts of the air-ventilation system and units of circulating air-conditioner in the ceiling of each room and corridor. Outside air enters through an air intake with air-filters located on the roof of the hospital and is distributed through the air-ventilation system. Each air-conditioner is equipped with a simple one-layer air filter made of a plastic-fiber net. The laundry is located in the first basement floor of the eastern part.

***Bacillus cereus* bacteremia cases**

In April 2005, the Infection Control Team of the hospital was informed of the detection of three consecutive *Bacillus cereus* positive blood cultures from febrile patients with severe blood stream infection in a ward for patients with hematologic malignancies which located on the forth floor of the western part. White blood cells counts in these cases were low due to anti-malignancy chemotherapy and they had an indwelling continuous venous line. These three cases were experienced during an interval of three weeks. Although sporadic cases of blood culture positive for *Bacillus cereus* had been found all around the hospital, positive cultures from three consecutive patients had hardly been experienced. We first considered these three cases as an iatrogenic infection, therefore an investigation by the Infection Control Team was launched.

Methods

Retrospective analysis of isolates

The hospital has a reporting system for the detection of major pathogens such as multi-drug resistant *Staphylococcus aureus* (MRSA) and multi-drug resistant *Pseudomonas aeruginosa* (MDRP). *Bacillus* species were not under surveillance although it had been recovered from a blood sample. However, all data of bacteria isolated from most samples were recorded in the main database. A retrospective analysis of cases of *Bacillus* species isolated from blood samples was initiated using the database.

Tracking source of Bacillus contamination

Under the consideration of iatrogenic introduction of *Bacillus* into the blood stream, swab samples were collected from the surface of working desks for the preparation of drop intravenous infusion and three-way valves used in the venous route of several patients in the particular ward. Swab samples were also collected from filter unit of the air-conditioners, entrance of the air inlet ducts, exit of the air outlet ducts which located just above the working desk because dense dust were noted.
The towels and gowns used in the ward were also examined for *Bacillus cereus*. Information about contamination of infusion drugs, cottons, alcoholic solutions as well as culture bottles was searched. *Bacillus* was identified according to standard reference methods[14] using Vitek automated system (Biomerieux, France) as well as by the lecitho-vitellin reaction.

Pulsed field gel electrophoresis fingerprinting analysis

Restriction fragment length polymorphism (RFLP) analysis was performed using *Bacillus* species isolated from the above samples to determine whether DNA typing was similar among the isolates. DNA was extracted from the isolates and

digested with *Sma I.* [15] Then the digested DNA was analyzed using pulsed field gel electrophoresis (PFGE) to compare the DNA fingerprinting of the isolates. This RFLP analysis was done in an independent laboratory.

Results

Distribution of Bacillus cereus isolates in the hospital

We found *Bacillus cereus* species had been isolated from 60 blood samples in this hospital between May, 2004 and April, 2005. More than two samples were positive for *Bacillus cereus* in 7 patients; the number of sample was 18. Among these 7 patients, 4 patients were diagnosed to have bacteremia and contamination was considered in 3 patients. The number was 20 between May 2003 and April 2004 and less in the preceding years (Figure 1), although there was an increasing trend since 2002. Usually, 0 to 3 *Bacillus cereus* isolates were found in one month between 2000 and 2003 (average 1.08, range 0-8, median 1), however, the average monthly number of isolates was 5.00 (range 1-7, median 6) between May, 2004 and April, 2005 (Figure 1).

We reviewed the distribution of *Bacillus cereus* isolated from blood samples in the hospital. Isolates were not distributed to a particular ward, although more isolates were found in the 4th, 5th and 6th floors (Table 1). *Bacillus cereus* was isolated from blood samples in 3/4 of the wards in 2004, in contrast, the microorganism was isolated in less than 1/2 of the wards in the preceding years. Although information about patients with skin infection, blood stream infection and infectious arthritis was searched, increase of such patients had not been noticed.

Environmental screening

During the environmental screening, we noticed dense dust in the filters of the air-conditioner and inlets/outlets of the air-ventilation system of the 4F-west ward. *Bacillus cereus* was also isolated from the dust (Table 2). There was a working desk for preparation of drop intravenous infusions just under the air-conditioner. *Bacillus cereus* was isolated from the dust and two of 10 used three-way valves for injection. A

number of *Bacillus cereus* colonies were also isolated from cleaned towels and gowns, suggesting the existence of a source of *Bacillus* contamination in the laundry process. We found that sodium hypochlorite was not appropriately used to reach the adequate final concentration. However, as *Bacillus cereus* is widely distributed in nature as well as in the normal flora of humans, the routes of the current *Bacillus cereus* contamination could not be confirmed even with these results.

PFGE fingerprinting

PFGE fingerprinting analysis was performed using isolates from two three-way valves (3-way valve 1 and 2), first three cases (Pt A, Pt B and Pt C) and filters of the air-conditioning system above the DIV preparation desk (air-con 1, 2 and 3, Figure 2). Two isolates from patients who were in the ward just before this outbreak were noticed (Pt D and Pt E) and added to the analysis. Although, fingerprinting analysis was not possible in Pt A and air-con 1 as shown in figure 2, the fingerprinting differed among Pt B, Pt C, Pt D and Pt E suggesting that this case was not an iatrogenic contamination from a single source.

Decontaminating procedures

We considered the present case as an undetected pseudo-outbreak of *Bacillus cereus* that lasted for about one year after the renovation work of the hospital, because the number of *Bacillus cereus* isolates increased after the renovation work. We also considered that filters of the HVAC-system and towels and gowns were probable sources of the *Bacillus* contamination. We therefore recommended cleaning of the filters of the air-conditioning system and inlets and outlets of the air-ventilation system of the renovated hospital at intervals of four weeks, and using autoclaved towels and gowns particularly for immunocompromised patients. We also recommended using

enough sodium hypochlorite to obtain the recommended final concentration in the laundry process. We increased amount of 12% NaOCl solution to reach 0.46% (v/v); residual chloride concentration was 192 ppm. Since then, the number of *Bacillus cereus* isolates from blood samples returned to the levels observed before the renovation of the hospital (Figure 1).

Discussion

In the present case, more isolates were found in 4F-west, 5F-west and 6F-east wards. 4F-west is a ward for pediatrics, 5F-west for head and neck disease and 6F-east for adult hematology. Final renovation work was performed on the third floor of the central part i.e. a part of the new western section. We considered this was a reason why more *Bacillus cereus* isolates were fond in 4F-west and 5F-west wards. During the renovation work, room air was repeatedly dusty in the 4F-west ward. The renovation work probably caused accumulation of dusts containing *Bacillus cereus* in these wards then caused the present undetected pseudo-outbreak.

Two probable sources of the *Bacillus* contamination were suspected in the present investigation. The first probable source was the filters of the air-conditioning system and inlets and outlets of ducts of the air-ventilation system. Accumulation of dust containing *Bacillus cereus* in the air-conditioner filters and air outlets in the wards had probably progressed during and after the renovation of the hospital.
HVAC-systems have been shown to contribute to the increase of fungal contamination in indoor air through various of their components.[16-18] Liu *et al.*[15] speculated that the epidemic strain of *Bacillus cereus* had contaminated the unit's air filtration system in an epidemic of *Bacillus cereus* they had experienced. Although they suspected the unit's air filtration system as the primary source, they were not able to confirm the primary source of the organism. The isolation of *Bacillus cereus* decreased after they cleaned the ventilation system, and encouraged hand washing and the aseptic procedures. Youngs *et al.* [19] reported a similar *Bacillus cereus* outbreak in a maternity unit.

The second probable route in the present case was *via* the towels and gowns of the patients. We speculated that *Bacillus cereus* in the environment contaminated

towels and gowns. This was suggested because a number of *Bacillus cereus* colonies were found in towels and gowns just after washing with hot water in the laundry. The laundry process in this hospital included rinsing using 80°C water, however, *Bacillus cereus* is resistant to heat. Those bacteria were probably selected during the laundry process and contaminated other cloths. Unfortunately, we did not perform swabs sampling upon patient's skin, bed and linens. Although these two routes were considered as sources of contamination in the present case, it was difficult to determine them as the origin of the pseudo-outbreak because *Bacillus cereus* can be found nearly everywhere in the environment.

PFGE analysis of *Bacillus cereus* isolated from four patients revealed that the gene fingerprinting was different in the patients. Although quality of PFGE analysis was not satisfactory probably because *Bacillus* species isolates from the patients were not monoclonal, this result suggested that *Bacillus cereus* bacteremia in those patients was not an iatrogenic infection. If *Bacillus cereus* had been from a single origin, the isolates would have been monoclonal. Previous analysis by amplified fragment length polymorphism of *Bacillus cereus* from cases of food poisoning suggested considerable variation of this bacterium that can be detected by this technique.[20] Dubouix *et al.* reported distinct fingerprinting patterns in all 30 *Bacillus cereus* strains isolated from infections in a traumatology-orthopedics department.[21]

As previously reported, recognizing and tracking the source of such pseudo-outbreaks is a difficult task.[8, 11] Loeb *et al.* reported pseudobacteremia following hospital construction. They speculated that *Bacillus* species acting as airborne contaminants from the construction site likely seeded the plastic lids or rubber septa of the stored blood culture bottles.[22] In the present case, contamination of

infusion drugs or culture medium for blood culture had not been reported, and culture positive patients were not distributed to one particular ward.[16] Therefore, the possibility of a pseudo-outbreak due to contamination of culture medium or procedures could be excluded.

Bacillus cereus has been associated with environmental reservoirs such as contaminated air filtration system, ventilator equipment, dressing, gloves, hands of healthy staff, intravenous catheters, alcohol-based hand-wash solutions, specimen collection tubes, blood culture media and linens. It has been speculated that construction work increases the amount of bacteria in the air. *Bacillus species* has been reported to cause serious infection among hospitalized patients with hematological malignancies.[23] Our results suggest that environmental contamination by *Bacillus cereus* may last and may cause epidemic until the source is identified and removed. Increase of *Bacillus* isolates from blood sample may help early detection of such epidemic, especially under circumstances of possible environmental contamination.

Table 1. Y. Ohsaki *et al.* Undetected *Bacillus* pseudo-outbreak after renovation work in a teaching hospital.

Distribution of patients whose blood culture was positive for *Bacillus cereus*

Floor#	West	East
10F	0	4
9F	3	1
8F	3	0
7F	1	2
6F	5	16
5F	9	0
4F	14	0
ICU	1	
NICU	1	

May, 2004 – April, 2005

Table 2. Y. Ohsaki *et al.* Undetected *Bacillus* pseudo-outbreak after renovation work in a teaching hospital.

Bacteria isolated in the 4F-west ward

Location	Bacteria
Air-conditioner above the DIV desk	*Bacillus cereus*
	CNS
Outlets of the air-ventilation system	Negative
Surface of the cleanbench	Negative
Surface of the DIV desk	*Bacillus subtilis*
	CNS
Sink	CNS
	Sphingomonas paucimobilis
	Agrobacterium radiobacter
	Rhodotorula spp.
Personal humidifier	Negative
3-way valves	*Bacillus cereus* in 2/10
	CNS in 4/10

DIV desk: working desk for the preparation of drop intravenous infusions. CNS: coagulase negative *staphylococci*.

Figure legends

Figure 1. Monthly number of blood samples positive for *Bacillus cereus*. Bar A indicates the period when the new eastern building was built, B: renovation of the old eastern part and C: renovation of the western part followed by first, second and third floor of the central part.

Figure 2. Result of PFGE fingerprinting analysis. PFGE fingerprinting DNA from *Bacillus cereus* isolates was compared among isolates from different sources.

Figure 1. Y. Ohsaki *et al.* Undetected *Bacillus* pseudo-outbreak after renovation work in a teaching hospital.

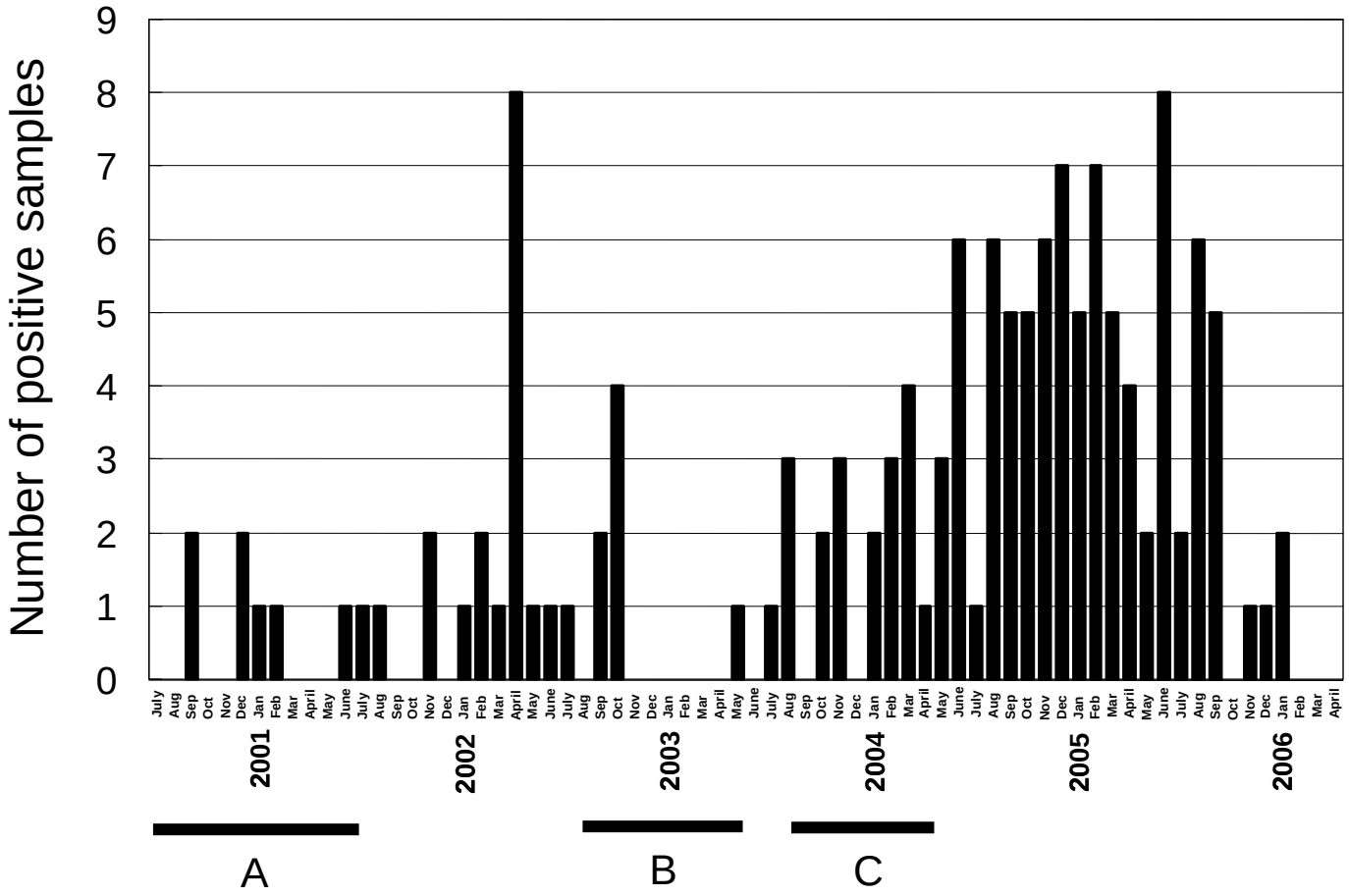
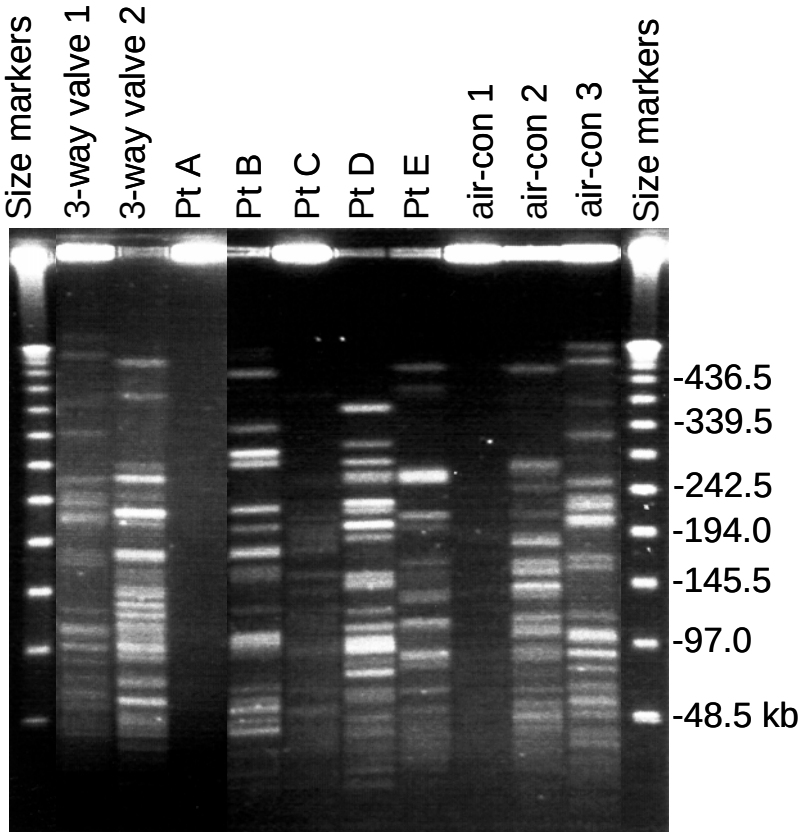


Figure 2. Y. Ohsaki *et al.* Undetected *Bacillus* pseudo-outbreak after renovation work in a teaching hospital.



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