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Original Article

Investigation of a cluster of *Bacillus cereus* bacteremia in neonatal care units

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KEYWORDS
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Abstract *Background: Bacillus cereus* is a well-known pathogen for self-limited foodborne illness, and rarely an opportunistic pathogen associated with invasive infections among immunocompromised patients. Nosocomial outbreaks have been rarely reported.
Methods: Between August and November 2019, four preterm neonates in neonatal care units of a medical center developed late-onset *B. cereus* bacteremia. An investigation was carried out. Forty-eight environmental specimens were obtained from these neonatal units, skin surface and environmental objects of Patient 4 for the detection of this organism 19 days after the onset of illness of Patient 4. *B. cereus* isolates from Patient 4, five unrelated patients and environmental objects if identified were further characterized by pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST).
Results: All four infants survived after vancomycin-containing treatment. Patient 4 developed diffuse cerebritis, brain abscess with severe neurologic sequelae. Of the 48 environmental samplings, 26 specimens showed positive for *B. cereus*, with one major clone (sequence type 365) accounting for 73%. The isolate from Patient 4 (ST427) was identical to one isolate collected from environmental objects in the same unit. After extensive cleaning of the environment and re-institution of the sterilization procedure of hospital linens, which was ceased since two months before the outbreak, no more cases was identified in these units for at least one year.
Conclusions: We documented a cluster of *B. cereus* bacteremia involving four preterm infants, which might be associated with cessation of the procedure for linen sterilization and was successfully controlled by re-institution of this procedure.

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Introduction

Bacillus cereus is a motile, aerobic or facultatively anaerobic, spore-forming gram-positive bacterium. *Bacillus cereus* is a ubiquitous environmental bacterium, which is found worldwide in dust, air, and water. The *B. cereus* spore is refractory to extreme environmental conditions inclusive of heat, freezing, drying, and radiation and may be regarded as the infective agent for this bacterium.[1]

Organisms of the genus Bacillus, excluding the highly virulent anthrax bacillus, are mainly ubiquitous saprophytes possessing weak pathogenicity for human. When identified from clinical specimens, even blood, they are almost dismissed as harmless contaminants. Since 1960s, *B. cereus* was found to be able to cause a variety of intestinal and extra-intestinal diseases.[2] The clinical manifestations include gastroenteritis, vomiting, endophthalmitis, respiratory tract infections, and infections similar to gas gangrene.[3]

Nowadays *B. cereus* is best known for self-limited foodborne disease, which can present with emetic or diarrheal illness.[4] The emetic disease is a food intoxication caused by cereulide, a small ring-formed dodecadepsipeptide. The diarrheal syndrome of *B. cereus* is an infection caused by vegetative cells, ingested as viable cells or spores, thought to produce protein enterotoxins in the small intestine. Three pore-forming cytotoxins have been associated with diarrheal disease: hemolysin BL (Hbl), nonhemolytic enterotoxin (Nhe) and cytotoxin K.[5,6] *B. cereus* is now recognized as an infrequent cause of serious non-gastrointestinal infection, particularly in drug addicts, the immunosuppressed, neonates, and postsurgical patients, especially when prosthetic implants such as ventricular shunts are inserted.[7]

B. cereus infection in neonates or infants can present as bacteremia as well as multifocal brain abscess and hemorrhage.[7—11] Outbreaks due to *B. cereus* were ever reported to be associated with reusable ventilator equipments,[12] contaminated alcohol prepa pads,[13] and contaminated hospital linens. Removal of the contaminated medical items and modification of the laundering process of hospital linens can control these outbreaks.[14—18]

Here we report a cluster of *Bacillus cereus* bacteremia in neonatal care units of a medical center. The investigation and infection control measurements are presented.

Methods

The outbreak

Chang Gung Memorial Hospital (CGMH) is a tertiary care hospital in northern Taiwan. There are 144 beds in the neonatal care units (NCUs), that include 50 intensive care beds (NICU1, NICU2, NICU3), 54 step-down intermediate care beds (NBC1, NBC2), and 40 baby-room beds (BR). Most patients in the NICUs are premature infants. NICU1, NICU3, NBC1 and milk supply room are located in 3rd floor of the building. NICU2 and NBC2 are located in the 4th floor.

Between August 18 and November 7, 2019, four premature infants hospitalized in the NCUs of CGMH developed *Bacillus cereus* sepsis, which was identified as isolation of *Bacillus cereus* from the bloodstream in a patient with clinical symptoms and signs of infection. An outbreak was therefore suspected because there had not been *B. cereus* -associated bacteremia in these units during the previous 3 years. The timeline of the onset of *B. cereus* bacteremia in these four cases is illustrated in Fig. 1.

Patient 1 was a late-preterm neonate born at gestational age (GA) of 36 weeks with a birth body weight (BBW) of 3000 gm. The neonate was intubated since delivery with the initial impression of transient tachypnea of newborn. High frequency oscillatory ventilation and nitric oxide inhalation were applied soon due to secondary respiratory distress syndrome (grade II to III) and pulmonary hypertension. The neonate was empirically administered with ampicillin and gentamicin since birth due to maternal group B streptococcus colonization without adequate intrapartum prophylaxis. His hemogram and biochemistry data were within normal limit at birth and at 24-h-old. He developed fever on August 18, when he was 4 days old. Septic work-up revealed white blood cell count (WBC) 6200/uL, hemoglobin (Hb) 13.0 g/dL, platelet 129000/uL, C-reactive protein (CRP) 6.2 mg/L (normal, $<$5 mg/L). Blood culture from a peripheral blood vessel yielded *B. cereus* and sputum culture yielded *Pseudomonas aeruginosa* while cerebral spinal fluid was sterile. Clinical illness well improved after treatment with vancomycin for 14 days and anti-pseudomonas antimicrobial agent for 7 days.

Two additional extremely preterm neonates, Patient 2 and Patient 3, had similar septic illness on August 31st and September 19th, respectively. Patient 2 was born at gestational age of 24 weeks with a birth body weight of 673 gm. The baby was intubated since birth. Chest X-ray disclosed respiratory distress syndrome at birth. He received two courses of oral ibuprofen for patent ductus arteriosus. Ventilator-associated pneumonia with positive sputum culture for *Pseudomonas aeruginosa* was impressed and ceftazidime was administered since 15-day-old, then his clinical status was stabilized. At 20 days of age, the infant developed an illness with petechiae, bradycardia and desaturation. Blood tests revealed WBC 7200/uL, Hb 12.0 g/dL, platelets 54000/uL, and CRP 10.89 mg/L. Two sets of blood culture yielded *B. cereus*, and additional two sets of positive blood culture were found at day 3 and day 6 after antibiotic treatment, respectively. Blood culture at day 9 post vancomycin therapy was sterile. Patient 3 was born at gestational age of 23 weeks with a BBW of 540 gm. He had bronchopulmonary dysplasia, intraventricular hemorrhage and neonatal seizure with ventilator support. The infant had clinical illness at 50 days of age with decreased activity, and tachycardia. Blood tests revealed WBC 12700/uL, Hb 15.0 g/

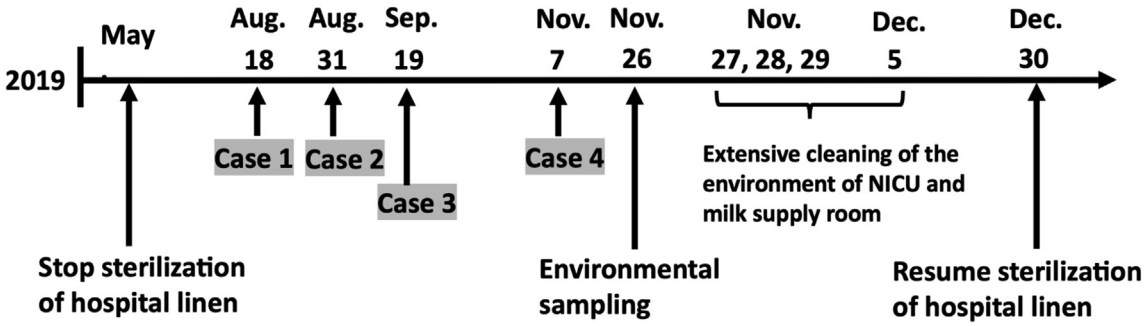

Figure 1. Timeline for the onsets of *Bacillus cereus* bacteremia, outbreak investigation, and interventions.

dL, thrombocytopenia (platelets 102000/uL), and elevated CRP level (CRP 10.89 mg/L). One set of blood culture yielded *B. cereus*. The central line was removed and sent for tip culture, which showed negative for bacteria. He received antimicrobial treatment with vancomycin for 7 days.

Patient 4 was a preterm neonate born at gestational age of 32 weeks, with a BBW of 2125 gm. He was admitted with a diagnosis of apnea of prematurity and neonatal hyperbilirubinemia under nasal cannula airflow and phototherapy treatment. Neither central venous catheter nor intubation were used. He started enteral feeding since 1-day-old and advanced feeding amount gradually. On November 7, the infant aged 10 days developed seizure, sepsis-like illness, and fever. Blood tests revealed WBC 20000/uL, band 4%, Hb 15.5 g/dL, platelets 184000/uL, and CRP 51.7 mg/L. Two sets of blood culture yielded *B. cereus*. Cerebrospinal fluid (CSF) analysis showed pleocytosis (sugar 103 mg/dL, protein 109 mg/dL, leukocyte count 37, neutrophil 96%) but negative culture result for bacteria. Computed tomography and sonography of brain showed diffuse cerebritis, meningitis, ventriculitis, and grade 1 intraventricular hemorrhage. Due to hemodynamic instability, antimicrobial agents with vancomycin, meropenem, and acyclovir were given initially and then adjusted to vancomycin only for a total of 8 weeks. Fig. 2 shows the neuroimages of Case 4.

Clinical and laboratory investigation

Although the four infants were located in four different neonatal units, we still highly suspected it was a cluster due to the rarity of this pathogen, which was surprisingly identified from the bloodstream of four infants during a 3-month period. On November 26, 19 days after the onset of Case 4, we conducted an investigation for the presence of *B. cereus* in the local environment of these four neonatal units and milk supply room. Totally, forty-eight specimens were collected with damp sterile swabs. The inanimate objects swabbed included baby linen, cabinet for linen, bedside cabinet, incubators, new diapers, pacifier, container of pacifier, bed rails, sonography probe and jelly, milk-warming instruments, formula milk, bottom of water dispenser, tap water, handle of refrigerator, barcode machine, handle of milk trolley, hand-washing soap fluids, alcohols, and the surfaces of computer devices (Table 1). Swabs from the skin and the umbilicus of Case 4 were also collected.

The swabs were seeded in 5% Sheep blood, and then in Thio Medium. The *Bacillus* species isolated from blood and environmental samples were identified by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) using the ethanol-formic acid extraction method according to the Bruker protocol. Once identified from the survey, the isolates of *B. cereus* were stored for further molecular characterization to compare the genetic relatedness with the clinical isolates. However, of the four cases, only one clinical isolate from Case 4 was reserved and available for molecular analysis because before we commenced this investigation, the identification of this microorganism from clinical specimens were classified as contaminants and the isolates of *B. cereus* were discarded by the microbiology laboratory staff. Subsequent 5 isolates from unrelated patients identified as *B. cereus* in the microbiology laboratory, including one isolate from a pediatric patient and four from adult patients, were collected as control strains and were characterized together.

The molecular methods used in this study included pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST). PFGE with *Sma*I digestion was performed according to the procedure described previously.[19] Strains with banding patterns identical in size and number of bands were considered genetically indistinguishable and assigned into the same type; strains with banding patterns that differed by only three or fewer bands were considered closely related and described as subtypes of a given pulsotype; strains with banding patterns that differed by four or more bands were considered different and assigned into separate pulsotypes. MLST was performed according to the procedure described previously to characterize 7 alleles (glpF, gmk, ilvD, pta, pur, pycA, tpi)[20] and their phylogenetic lineage based on sequence type, using the *B. cereus* MLST website (https://pubmlst.org/B.cereus). This study was approved by the Institutional Review Board 202100220B0.

Results

Characterization of environmental *Bacillus cereus* and the genetic relatedness between environmental and clinical isolates

The four infants had a gestational age ranging from 23 to 36 weeks and a BBW ranging from 540 to 3000 gm. All the infants were male. The age of onset of *B. cereus* sepsis ranged from 4 to 55 days. Except Patient 4, the other three infants were intubated and had central venous catheters

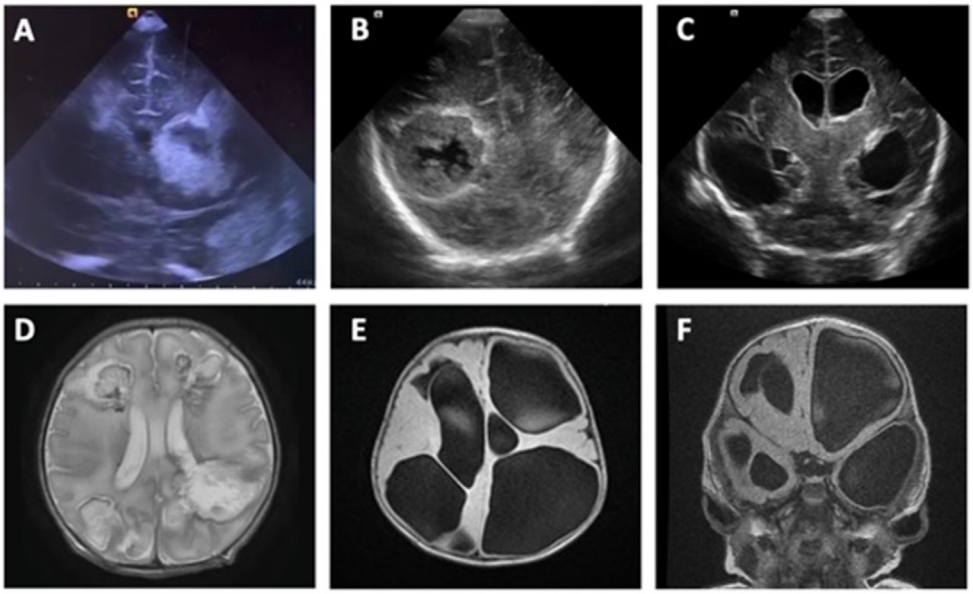

Figure 2. Brain images of Patient 4 (A) Bedside sonography at 10-day-old revealed diffuse hypo- and hyper-density lesions (B) Sonography at 11-day-old (C) Sonography at 30-day-old revealed diffuse ventriculomegaly and encephalomalacia (D) Brain CT at 11-day-old showed suspected cerebritis, meningitis and ventriculitis (E) (F) MRI T2 at 66-day-old showed diffuse encephalomalacia and hydrocephalus.

for parenteral nutrition before the onset of sepsis. Patient 4 only had peripheral IV catheter for low-dose TPN use, oral-gastric tube for enteral feeding, and nasal cannula airflow before onset. After antimicrobial treatment with vancomycin, all the four infants survived. Patient 4 had severe neurologic sequelae of encephalomalacia and hydrocephalus. Demographics, clinical manifestations, neonatal diseases, treatment, and outcomes of these 4 infants are summarized in Table 2.

Twenty-six (54.1%) of the 48 environmental specimens were positive for *B. cereus*. The bacterial growth was either rare or enriched for these positive specimens and was relatively higher from the specimens of linens and cabinets for linens. The specimens with positive results included those collected from a bed rail, incubator, bedside cabinets, linens, keyboards, computer mouses, milk warmers, button of water dispenser, and handles of a formula milk cart and a refrigerator. Specimens obtained from the skin and the umbilicus of Case 4 were also positive for *B. cereus*. No bacteria was identified from sterile region in the milk supply room, alcohol, and dis-infective agent (Aq-BI). The detailed survey data are shown in Table 1.

Totally, 32 isolates were characterized, including one isolate from Case 4, five control isolates from unrelated patients and 26 environmental isolates from this investigation. Of these 32 isolates, a total of 11 pulsotypes were identified, with four types being singletons (named as type A, E, J and K). Seven isolates, including the isolate from Case 4, were not successfully typed by PFGE. All 5 control strains belonged to 5 different pulsotypes. Of the 20 environmental isolates typable by PFGE, nine pulsotypes were identified, with 5 pulsotypes shared by two or more environmental isolates. For MLST, we initially characterized 10 isolates of 8 pulsotypes. One pair of isolates with pulsotype G shared a same sequence type (ST365) and the other pair of isolates with pulsotype H also shared a sequence type (also ST365). We then presumed that the isolates of same pulsotype shared a same sequence type. Totally, all 7 untypable isolates, 4 singleton isolates and 9 representative isolates of seven pulsotypes with multiple isolates subsequently underwent MLST analysis. A total of nine STs were identified. One common type, namely ST365, was shared by 20 isolates of 7 pulsotypes and one untypable isolate. Detailed distribution of pulsotypes and STs for these 32 isolates is shown in Table 1.

Phylogenetic relatedness of 19 isolates analyzed according to MLST analysis is shown in Fig. 3. According to the phylogenic tree, 18 of 19 isolates analyzed were clustered into two groups. All the four linen-associated isolates were included in one cluster, which comprised ST177, ST73, ST1969, and ST427. The other cluster comprised ST365, ST1428, and ST2184 and none of the linen-associated isolates were included in this cluster. The isolate from Case 4 shared identical characteristics (a unique sequence type, ST427, and untypable by PFGE) with an isolate identified from the cabinet for linens storage in NBC2, where Case 4 developed the illness. Of the 26 environmental isolates, ST365, the major clone, accounted for 19 isolates (73%) and distributed in all four units and regions surveyed (Table 3).

Interventions

Between 2019 November 27th and November 29th, several infectious control measurements were performed, including extensive cleaning of all objects in the neonatal units with detergents and disinfectants, using plastic wrap cover the keyboards and mouses of computers in the nursing stations and changing three times per day, and sending all unused

Table 1 Microbiological results of environmental sampling 19 days after onset of *B. cereus* infection of case 4.

Samples	Obtained Units	Bacterial load	PFGE	MLST
Case 4's blood culture	NBC2[a]	—	UT	ST427
Blood culture control 1[e]	ward	—	A	ST73
Blood culture control 2	ward	—	J	ST2363
Blood culture control 3	ward	—	B	ST365
Blood culture control 4	ward	—	C	ST2184
Blood culture control 5	ward	—	D	ST365
Baby linen(1)	NICU1	R[c]	D2	(ST365)[b]
Baby linen(2)	NBC1	R	UT	ST177
Baby linen(3)	NBC2	R	H	ST365
Cabinet for linen(1)	NICU1	E[d]	UT	ST177
Cabinet for linen(2)	NICU1	R	I1	(ST365)[b]
Cabinet for linen(3)	NBC1	E	UT	ST1969
Cabinet for linen(4)	NBC2	R	UT	ST427
Cabinet for linen(5)	NBC2	E	I1	(ST365)[b]
Bedside cabinet(1)	NICU1	E	G	(ST365)[b]
Bedside cabinet(2)	NBC1	E	B2	(ST365)[b]
Bedside cabinet(3)	NBC2	E	G3	(ST365)[b]
Incubator	NICU1	R	UT	ST365
Keyboard(1)	NBC1	R	G2	(ST365)[b]
Keyboard(2)	NBC2	R	H	ST365
Mouse(1)	NICU1	—	—	—
Mouse(2)	NBC1	R	C	(ST2184)[b]
Mouse(3)	NBC2	R	D1	(ST365)[b]
Mouse(4)	Milk supply room	E	F	ST365
Milk warmer(1)	NICU1	E	G	(ST365)[b]
Milk warmer(2)	NBC1	E	F1	(ST365)[b]
Milk warmer(3)	NBC2	R	I	ST365
Bedrail of case 4	NICU1	R	UT	ST1428
Skin of case 4	NICU1	E	D1	(ST365)[b]
Umbilicus of case 4	NICU1	E	G1	ST365
Sonography probe		—	—	—
Sonography jelly		—	—	—
Water dispenser bottom	Milk supply room	E	K	ST1477
Handle of refrigerator	Milk supply room	E	G	ST365
Handle of formula milk	Milk supply room	E	E	ST365
Cart	Milk supply room	—	—	—
Formula milk				
Water in bottle(1)	Milk supply room	—	—	—
Water in bottle(2)	Milk supply room	—		
Tap water	Milk supply room	—	—	—
aqua beta-iodine	Milk supply room	—	—-	—
Phone(1)	Milk supply room	—	—	—
Phone(2)	Milk supply room	—	—	—
Barcode machine	Milk supply room	—	—	—
Alcohol(1)	NICU1	—	—	—
Alcohol(2)	NBC1	—	—	—
Alcohol(3)	NBC2	—	—	—
Case 4's new diaper	NICU1	—	—	—
New diaper	NBC1	—	—	—
Case 4's Pacifier(1)	NICU1	—	—	—
Pacifier(2)	NBC1	—	—	—
Pacifier (3)	NBC2	—	—	—
Case 4's pacifier container	NICU1	—	—	—

Abbreviation.NICU: neonatal intensive care unit; NBC: newborn center; PFGE: pulsed-field gel electrophoresis; MLST: multilocus sequence typing; UT: untypable; R: rare; E: enriched

[a] Case 4 was located in NBC2 at onset of sepsis and then was transferred to NICU1 for critical care.

[b] The MLST typing in the parentheses is inferred based on the results of PFGE.

[c] "R" resembles "rare" bacterial found in 5% Sheep blood agar.

[d] "E" resembles that bacterial is not found in 5% Sheep blood agar and could only found in "enriched" Thio Medium.

[e] *Bacillus cereus* identified from adults were used as control strains.

Table 2 Demographic data, possible predisposing factors, treatment, and outcome of *Bacillus cereus* infection.

Unit	Pt	Sex	GA(week) /BBW(g)	Date of Onset /Age(days)	Duration of Usage before Sepsis (days)			Diagnosis at sepsis onset	*B. cereus* infection site	Presentation	Treatment	Outcome
					ETT	CVC	UAC/UVC					
NICU3	1	M	36+0/3000	Aug. 18/4	4	-	4/4	TTN,2nd RDS, PPHN, PDA	Bacteremia	Fever, elevated CRP, thrombocytopenia	Vancomycin 14d	recovery
NICU1	2	M	24+4/673	Aug. 31/20	20	11	no/no	RDS, PDA, VAP	Bacteremia	Petechiae, elevated CRP, thrombocytopenia	Vancomycin 14d	recovery
NICU2	3	M	23+0/540	Sep. 19/55	55	52	no/no	RDS, BPD, seizure, IVH	Bacteremia	Decreased activity, tachycardia, elevated CRP, thrombocytopenia	Vancomycin 7d	recovery
NBC2	4	M	32+6/2125	Nov. 7/10	no	no	no/no	AOP, jaundice	Bacteremia Cerebritis Meningitis Ventriculitis	Seizure, tonic movement, tachycardia/ bradycardia, leukocytosis, bandemia, elevated CRP	Vancomycin 8wk Meropenem 2wk	Epilepsy, Encephalomalacia, Ventriculomegaly, Hydrocephalus

NICU, neonate intensive care unit; NBC, newborn center; Pt, patient; M, male; GA, gestational age; BBW, birth body weight; ETT endotracheal tube; CVC, central venous catheter; UAC, umbiliical artery catheter; UVC, umbilical venous catheter; TPN, total parenteral nutrition; TTN, transient tachypnea of newborn; RDS, respiratory distress syndrome; PPHN, persistent pulmonary hypertension; VAP, ventilator associated pneumonia; PDA, patent ductus arteriosus; IVH, intraventricular hemorrhage; AOP, apnea of prematurity; VA, Vancomycin; Mero, Meropenem; IICP, increased intracranial pressure; CRP, C-reactive protein.

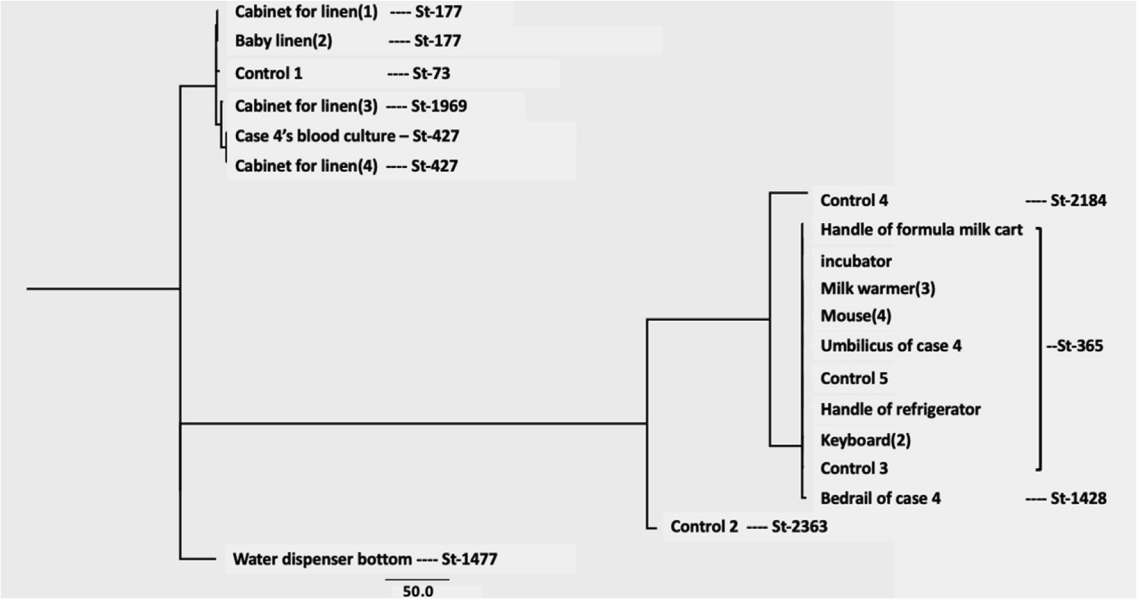

Figure 3. Multilocus sequence typing (MLST)—based phylogenetic trees of strains and STs of *Bacillus cereus* isolates. Scale bars indicates nucleotide substitutions per site. ST, sequence type.

linens to the department of laundry for washing. Milk supply room was also extensively cleaned with detergents and disinfectants on December 5th. Reviewing the cleaning processes of hospitalized babies' linens, we found that the process of sterilization was cancelled since May 2019. Since December 2019, the procedure of sterilization of the baby linens was resumed again.

No more *B. cereus* sepsis occurred in these neonatal units in the following 14 months. The timeline of the investigation and interventions is illustrated in Fig. 1.

Discussion

We presented a cluster of *Bacillus cereus* bacteremia involving four hospitalized neonates, which may be related to the contaminated hospital baby linens. The outbreak was successfully controlled by implementation of effective infection control measurements, including extensive cleaning of environmental objects in these neonatal units and resuming the sterilization procedure of hospital linens etc.

Contaminated hospital linens have been associated with Bacillus-related outbreaks and improper laundry processing was often implicated in these outbreaks.[21] Cheng et al. reported an outbreak of *Bacillus* bacteremia in Hong Kong between 2012 and 2016 with higher incidence of positive blood culture between June 2015 and July 2015. In their investigation, *B. cereus* was identified in 27 of 99 linen samples during the outbreak period. Numbers of *B. species* isolates were decreased after the cessation of contaminated linen supply from the implicated substandard laundry, and improvement of the laundry processes. Findings from the current investigation also suggest that the outbreak of *Bacillus cereus* bacteremia might be attributed to the increased bacteria or spore load in the hospital linens, which may be due to cessation of the process of sterilization of hospital linens. First, more than half of the environmental samples showed positive result for *B. cereus*, and most of them were related to hospital linens and cabinets for linens storage. Furthermore, according to the phylogenetic tree by MLST analysis, all the four linen-associated isolates were clustered with the isolate from Case 4 in one genogroup. Second, the isolate from Case 4 was genetically indistinguishable with an isolate from the cabinet for linens storage in the same unit. Though we had no direct evidence of the usage of these contaminated linens in Case 4, the baby supposed to use hospital linens as bedsheet, as nesting, and as positioning according to our neonatal care routine practice,. Third, there was one dominant strain, namely ST365, among the environmental isolates, which accounted for nearly three quarters of the isolates. Generally, *Bacillus cereus* is quite diverse in nature. This result is a strong evidence that the strain circulated and spread in these neonatal units. The last but the most important, the cessation of sterilization procedure in cleaning processes of hospital linens preceded the cluster, and after resumption of the sterilization procedure, no more cases of *B. cereus* bacteremia occurred in these neonatal units for more than one year.

Increased environmental contamination due to dust or air pollution has also been implicated in *B. cereus* bacteremia clusters.[22] Bar-Meir et al. identified three cases of *B. cereus* bacteremia in the NICU of a medical center in Israel and one also developed multiple brain abscess although no bacterial growth in CSF. An epidemiologic investigation revealed that 10 of 50 environmental specimens grew *B. cereus* with multiclonality. The cluster was coincided with the construction work so that enabled dust to be carried into the NICU. The outbreak ceased after strict sealing of the construction area.

Clinically, identification of *B. cereus* from various specimens, even blood, would be regarded as contaminant. While for immunocompromised patients and vulnerable population

Table 3 Molecular characteristics of environmental *B. cereus* isolates[a] stratified by identified units.

Units	Specimens collected	Positive No. (%)	MLST (No.)	PFGE
NICU1[b]	14	9 (64.2)	ST365 (7)	D1,D2, G, G, G1, I1
			ST177 (1)	UT[g]
			ST1428 (1)	UT
NBC2[c]	9	7 (77.8)	ST365 (6)	D1, G3, H, H, I, I1
			ST427 (1)	UT
NBC1[d]	9	6 (66.7)	ST365 (3)	B2, F1, G2
			ST177 (1)	UT
			ST1969 (1)	UT
			ST2184 (1)	C
Milk supply room[e]	14	4 (28.6)	ST365 (3)	E, F, G
			ST1477 (1)	K
Sonography[f] probe and jelly	2	0	—	—
Total	48	26 (54.1)		

Abbreviation: NICU: neonatal intensive care unit; NBC: newborn center; PFGE: pulsed-field gel electrophoresis; MLST: multilocus sequence typing; UT: untypable;

[a] Environmental sampling was conducted on November 29, 2019, 19 days after onset of *B. cereus* infection of Case 4.

[b] Case 2 was located in NICU1 at onset of symptoms with positive blood culture with *B. cereus*.

[c] Case 4 was located in NBC2 at onset of symptoms with positive blood culture with *B. cereus*.

[d] In NBC1, no case of *Bacillus cereus* infection diagnosed during this outbreak period. Environmental sampling was conducted in the unit as a presumed negative control unit.

[e] The milk supply room stored and supplied both formula milk and human milk to all neonates admitted to these neonatal units.

[f] The portable sonography used and placed in NICU1 or NICU2.

[g] Some isolates, including the isolate from Case 4′ s blood culture, were not successfully genotyped by PFGE and thus were marked as UT (untypable).

such as preterm infants in this study, the interpretation should be more meticulous. If two or more sets of blood culture all reveal positive results for this unusual pathogen, clinicians would feel more comfortable to diagnose and treat the patients as *B. cereus* bacteremia, such as Case 2 and 4 in this study. However, in routine clinical practice in most neonatal units, including ours, only one set of blood sample would be obtained for bacterial culture while performing septic work-up. For case 1 and case 3 in this study, who were symptomatic and only one set of blood culture was obtained and positive for *B. cereus*, they were diagnosed as *Bacillus cereus* sepsis by our clinical physicians and antimicrobial agents were administered accordingly.

Although there is no CLSI guideline to determine the MIC cut-off for *B. cereus*, intravenous vancomycin alone seems to be efficient for treatment for *B. cereus* bacteremia in our four cases. However, severe neurogenic sequelae developed in case 4, who had severe hydrocephalus, encephalomalacia, and suspected cortical blindness. It is worth noting that while primary blood culture reported Gram-positive bacteria, the possibility of neonatal listeriosis should not be ignored. The annual incidence of listeriosis in Taiwan is not known but one report in Northern Taiwan showed an increasing trend.[23] Group B Streptococcus (GBS) is one of the leading causes of invasive infections in neonates caused by Gram-positive bacteria, and outbreaks of nosocomial transmissions of GBS had been reported rarely.[24]

There have been scanty reports regarding *B. cereus* bacteremia from Taiwan. Hsueh et al. described a *Bacillus cereus* pseudobacteremia outbreak in 1990 that consisted of 15 in-hospital patients and resulted from the contamination of 70% ethyl alcohol used for skin disinfectant while blood drawn in the hospital.[25] Another report was also a pseudo-outbreak investigation in a pediatric unit in 1998, which was suspected to be related to the unit's air filtration system.[19] Recently, Liao SL el reported a case of *Bacillus cereus* bacteremia in a preterm infant, probably caused by consumption of contaminated breastmilk.[26]

There are several limitations in this investigation. First, the isolates of *B. cereus* from the first three cases were not available for molecular characterization and thus we cannot delineate whether the outbreak was caused by a unique strain. Second, although there are relative large amount of *B. cereus* found in linens, we have no previous environmental sampling data before the outbreak that could not confirm that bacteria load increased during the outbreak.

Author contributions

An-Li Tsai and Yhu-Chering Huang conceived and designed the study. A.-L. T, collected clinical information and samples. A.-L. T, analyzed and interpreted the data. A.-L. T and Y.-C. H wrote the manuscript. All authors critically reviewed and approved the final manuscript.

Declaration of competing interest

The authors declare that they have no competing interests.

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