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Brief Report

Extended drip infusion of peripheral parental nutrition containing amino acids might be associated with *Bacillus cereus* bloodstream infection

Arufumi Shiota PhD [a,b], Nobuhiro Asai MD, PhD [a,c], Yusuke Koizumi MD, PhD [a,c], Hiroki Watanabe MD, PhD [a,c], Miki Sakata RN [a], Ai Kurumiya RN [a], Tomoko Takahashi RN [a], Yuki Muramatsu RN [a], Mao Hagihara PhD [d], Hiroyuki Suematsu [a], Yuka Yamagishi MD, PhD [a,c], Hiroshige Mikamo MD, PhD [a,c,*]

[a] *Department of Infection Control and Prevention, Aichi Medical University Hospital, Japan*
[b] *Department of Pharmacy, Aichi Medical University Hospital, Japan*
[c] *Department of Clinical Infectious Diseases, Aichi Medical University, Japan*
[d] *Department of Molecular Epidemiology and Disease Control, Aichi Medical University, Japan*

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In some Japanese hospitals, patients using infusion bags for parenteral nutrition containing amino acids have developed *Bacillus cereus* bloodstream infections. We considered that proliferation of contaminated *B cereus* in the bag during prolonged drip infusion might be one of the causes of infection. This study indicated that 8 h is the maximum appropriate drip infusion time for peripheral parental nutrition containing amino acids to prevent *B cereus* bloodstream infections.

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Patients who receive peripheral parenteral nutrition containing amino acids have a higher risk of developing *Bacillus cereus* bloodstream infections than those who receive other parenteral nutritional solutions.[1] In some Japanese hospitals, patients who were administered parenteral nutrition using infusion bags have developed *B cereus* bloodstream infections.[2,3] It has also been previously reported that long-term infusions of large volumes of preparations correlated with the onset of bloodstream infections by *Bacillus* species.[4] This suggests that prolonged drip infusions (eg, 24 h) might be 1 of the causes of *B cereus* bloodstream infection due to contaminated *B cereus* proliferating in the bag.

This study aimed to determine the appropriate drip infusion time for peripheral parental nutrition containing amino acids, to prevent *B cereus* bloodstream infection. We investigated whether contamination of the infusion bag is caused by injection needle for mixing or infusion setting and what drip infusion time could prevent *B cereus* proliferation.

METHODS

Investigation of contamination by injection needle

We inoculated 5 μL of *B cereus* culture with a cell density of 10^8 colony-forming units (CFU)/mL onto the rubber stopper site of a premixed infusion bag of peripheral parental nutrition containing amino acids (BFLUID; Otsuka Pharmaceutical Factory Co, Ltd, Tokushima, Japan). BFLUID contains mainly glucose, vitamin B1, and amino acids (Table 1). After 30 min, we injected 10 mL of saline using an 18-G needle from the inoculated rubber stopper site. We used 5 strains of *B cereus* in each bag: 1 ATCC strain (ATCC11778) and 4 clinical isolates. The infusion bag was cultured at 25°C. We aspirated 1 mL of solution from the infusion bag 0 and 48 h after contamination. The aspirate was subjected to quantitative assay using sheep blood agar plates, and the number of viable bacteria was calculated after 18 h of cultivation at 37°C.

Investigation of the starting time for the proliferation of B cereus

We inoculated different cell densities of *B cereus* (10^3, 10^4, 10^5, and 10^6 cells in 500 mL) into BFLUID solution in infusion bags. The infusion bags were cultured at 25°C. We aspirated 1 mL of solution from each infusion bag every 3 h, from 0 to 24 h, and every 12 h, from 24 to 48 h. The aspirate was subjected to a quantitative assay using the method mentioned earlier.

* Address correspondence to Hiroshige Mikamo, MD, PhD, Department of Infection Control and Prevention, Aichi Medical University Hospital, 1-1 Yazakokarimata, Nagakute, Aichi 480-1195, Japan
E-mail address: mikamo@aichi-med-u.ac.jp (H. Mikamo).
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Table 1
Composition of BFLUID (premixed infusion bag of peripheral parental nutrition containing amino acids)

Composition per 500 mL					
L-Leucine	2.100	g	Total amino acids	15.00	g
L-Isoleucine	1.200	g	Glucose	37.50	g
L-Valine	1.200	g	Na^+	17.5	mEq
L-Lysine HCL	1.965	g	K^+	10	mEq
(as L-Lysine)	1.573	g	Mg^{2+}	2.5	mEq
L-Threonine	0.855	g	Ca^{2+}	2.5	mEq
L-Tryptophan	0.300	g	Cl^-	17.5	mEq
L-Methionine	0.585	g	SO_4^{2-}	2.5	mEq
Acetyl Cysteine	0.202	g	$Acetate^-$	8	mEq
(as L-Cysteine)	0.150	g	$L\text{-}Lactate^-$	10	mEq
L-Phenylalanine	1.050	g	$Citrate^{3-}$	3	mEq
L-Tyrosine	0.075	g	P	5	mmol
L-Arginine	1.575	g	Zn	2.5	μmol
L-Histidine	0.750	g	Thiamine (VB1)	0.75	mg
L-Alanine	1.200	g			
L-Proline	0.750	g			
L-Serine	0.450	g			
Glycine	0.885	g	Characteristics		
L-Aspartic acid	0.150	g	pH		~6.7
L-Glutamic acid	0.150	g	OPR		~3

OPR, osmotic pressure ratio to physiological saline.

Statistical analysis

Results are expressed as the mean ± SE. Means of 5 strains to be compared were analyzed using paired Student t tests. Significance was set at $P < .05$. Statistical analysis was performed with SPSS software version 23.0 (IBM Corporation, Armonk, NY).

RESULTS

During the investigation into whether contamination occurs by injection needle, the number of viable *B cereus* cells in the infusion bag at 0 h was $1.3 \times 10 \pm 0.9 \times 10$ CFU/mL. The number of viable *B cereus* cells increased to $1.5 \times 10^7 \pm 0.7 \times 10^7$ CFU/mL after 48 h. The increase was statistically significant ($P < .05$).

In the investigation of the starting time for *B cereus* proliferation, the number of viable *B cereus* cells did not increase until 9 h after inoculation, regardless of the inoculation size. It then increased significantly after 12 h ($P < .05$). The number of viable *B cereus* cells remained at $1.5–2.5 \times 10^7$ CFU/mL from 36 to 48 h (Fig 1).

DISCUSSION

B cereus is a gram-positive, aerobic, and spore-forming bacterium, ubiquitous in the environment, and well known to cause nosocomial infections.[5] Although the frequency is not high, there are several reports of nosocomial infections in Japan. Since *B cereus* forms spores, complete disinfection is difficult, even if the rubber stopper site of an infusion bag is disinfected with alcohol.[6] Therefore, a constant possibility of contamination with *B cereus* exists during mixing and infusion setting. *B cereus* may become unresponsive to antibiotics when bacteremia occurs, and only limited drugs, such as vancomycin, are then effective.[7,8] To investigate the possibility of developing bacteremia due to proliferation of *B cereus* in the infusion bag, we examined whether the bag can be contaminated with *B cereus* by injection needle, and whether it can then grow inside the bag. Causes of infection not only include contaminated infusion bags but also other factors, such as contaminated catheter and hospital linen.[9]

The source of the *B cereus* contamination in the infusion bags was the rubber stopper site. Even though the initial number of *B cereus* cells was low, the number of cells had increased greatly after 48 h, an extended period of time. Therefore, it is considered necessary to limit the infusion time of peripheral parental nutrition containing amino acids.

In Japanese Society for Parenteral & Enteral Nutrition guidelines, total parenteral nutrition is also recommended to be 12 or 24 h of infusion time. Compared with total parenteral nutrition, peripheral parenteral nutrition frequently takes more than 12 h of infusion time. It has previously been reported that BFLUID, extracted from a bag, showed *B cereus* proliferation after 24 h.[10] We contaminated *B cereus* into an infusion bag and, for the first time, examined them periodically within the first 24 h, revealing that they did not proliferate until 9 h had passed. A possible reason that there was no significant increase in cell number for 9 h, is that the *B cereus* cells in the infusion bags might have been in the lag phase. Therefore, a certain amount of time may be necessary for the cells to shift to the logarithmic growth phase in the infusion bag. The time required for this transition was as long as 9 h because the infusion used was not a complete medium. It is possible that the sodium bisulfite in the infusion preparation affected the outcome, or that a portion of the *B cereus* cells were spores.

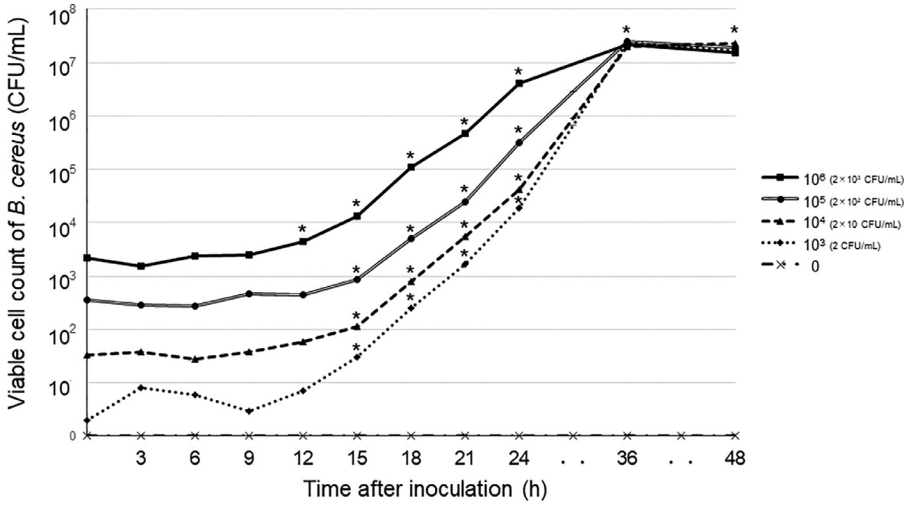

Fig 1. *Bacillus cereus* proliferation in BFLUID. This graph shows *B cereus* proliferation after inoculation at each concentration (mean, respectively, N = 5). *B cereus* did not increase until 9 hours after inoculation. BFLUID, premixed infusion bag of peripheral parental nutrition containing amino acids. CFU, colony-forming units. *Paired Student t test; $P < .05$ (vs 0 h).

In conclusion, this study suggests that, to prevent *B cereus* bloodstream infections from the perspective of infection control and prevention, the appropriate drip infusion time for peripheral parental nutrition containing amino acids might not exceed 8 h, taking into account the preparation time.

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