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Baseline and seasonal trends of *Bacillus cereus* and *Bacillus subtilis* from clinical samples in Japan

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SUMMARY

Background: Outbreaks of *Bacillus cereus* bloodstream infections (BSIs) are a concern in Japanese medical settings.
Aim: This study determined baseline values for *B. cereus* detection in clinical samples that are useful as reference values for hospitals when assessing the need for intervention.
Method: A retrospective analysis of *B. cereus* detection in the Japan Nosocomial Infections Surveillance data from 2008 to 2014 was performed; it included 950 individual hospitals across the country.
Findings: *Bacillus* spp. were detected in 0.54% of the clinical specimens submitted for bacteriological testing. Specimens positive for *Bacillus* spp. were mainly blood (24.6%), stool (26.5%), and respiratory specimens (23.3%). Identification of *Bacillus* spp. at the species level (i.e., *B. cereus* or *B. subtilis*) was reported in 55.3%, 14.7%, and 15.4% of cases, of which 88.9%, 48.3%, and 33.1% were *B. cereus* in blood, stool, and respiratory specimens, respectively. Of the 4105 hospital-years, 75.7% had blood specimens with *Bacillus* spp., with a median of 0.85 blood specimens/100 beds annually (interquartile range, 0.17—2.10). The *B. cereus* detection showed significant summer seasonality, regardless of specimen type or geographic distribution. The *B. subtilis* detection did not show seasonality, and its detection remained constant throughout the year. The seasonality of *Bacillus* spp. reflects the high proportion of *B. cereus*.
Conclusions: The increased detection rate of *Bacillus* spp. during summer should be interpreted as a risk factor for *B. cereus* BSIs. A post-summer decrease in *Bacillus* spp. should not be interpreted as an effect of interventions.

Introduction

Bacillus cereus is a known cause of food poisoning illness [1]. However, bloodstream infections (BSIs) caused by *B. cereus*, including fatalities, do occur among hospitalised patients [2—4]. Commonly reported risk factors for *B. cereus* BSIs

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include haematological malignancies [3], recent antimicrobial use [5], intravenous catheter placement [6], administration of peripheral nutritional solutions [7,8], and use of alcohol prep pads [9]. Particularly, an association between the use of recyclable linens and *B. cereus* BSIs has been reported in several hospitals in Japan, where it is customary to give patients a bed bath with hot, steamed towels [10–12]. Investigation of these outbreak cases suggested that the towels were highly contaminated with *B. cereus* during the washing and storage processes and acted as a source of infection. In studies following these outbreaks, it was revealed that enhanced hygiene of reusable towels [12], the introduction of disposable towels [13,14], and hand hygiene with soap and running water for healthcare workers who handle towels [15] are key methods to prevent *B. cereus* BSIs in healthcare settings. Nevertheless, outbreaks of *B. cereus* BSIs were reported in teaching hospitals [16–19]. One of the possible reasons why outbreaks of *B. cereus* BSIs continue to occur is that *Bacillus* spp., including *B. cereus*, are ubiquitous in the environment and cannot be eliminated in totality. Moreover, the detection of *Bacillus* spp. in blood culture tends to be classified as contamination [20]. This may result in missed opportunities for professionals, such as epidemiologists, to intervene at an optimal time to control infection. Therefore, the aim of this study was to obtain baseline data on the detection of *B. cereus* in hospitals by analysing a nationwide nosocomial infection database and to clarify the status of *B. cereus* detection that would require intervention.

The Japan Nosocomial Infections Surveillance (JANIS) system is a nationwide hospital-based surveillance system established in 2000 by the Japanese Ministry of Health, Labour, and Welfare [21]. The clinical laboratory (CL) division of JANIS is designed to collect all clinical microbiological test results, including culture-negative results, from participating hospitals electronically. With approximately 1000 voluntarily participating hospitals as of 2014, the JANIS CL division is the largest database of clinically isolated bacteria in Japan. Using this database, we described the prevalence of *B. cereus* in blood specimens from hospitals across Japan. In addition, because non-sterile specimens are likely to reflect environmental contamination, we described the prevalence of *B. cereus* in non-sterile specimens as a potential indicator for risk of *B. cereus* BSI in healthcare settings.

Methods

Data source and inclusion criteria

The clinical microbiological test results of the hospitals participating in JANIS and submitted to the JANIS CL division between January 2008 and December 2014 were extracted with the approval of the Japanese Ministry of Health, Labour, and Welfare. We limited our analysis to data to the end of 2014 when JANIS covered only large hospitals (200 beds or more), primarily acute care, and teaching hospitals because in 2015, JANIS expanded its target to hospitals with less than 200 beds, which included hospitals providing long-term care. The number of hospitals was counted on a single year basis, and hospitals that participated in JANIS for more than two years were independent facilities each year (hospital-years). The JANIS CL division system accepts clinical microbiological test results data, electronically, from JANIS participating hospitals [21,22]. The method of isolation and identification of *Bacillus* spp. from non-sterile samples was left partly to the discretion of each hospital, but it can be assumed that detection of *Bacillus* spp. has been reported when *Bacillus*-like colonies were dominant. The following hospital-based information was required from the JANIS CL division: hospital ID, patient ID, type of specimen, date of specimen submission, and patient hospitalisation status (inpatient or outpatient). Hospitals that reported data lacking this information were excluded from this study. In addition, hospitals that reported isolation of *B. anthracis* were excluded from this study because they were deemed to have submitted unreliable data, and probably misplaced the bacterial code. Incidentally, there were no reports on anthrax in Japan since 1994. Data from hospitals that did not report the number of beds were also excluded.

Table I
Number of samples tested and number of samples positive for *Bacillus* (*B. cereus*, *B. subtilis*, and *Bacillus* sp.) by sample types collected from the Japan Nosocomial Infection Surveillance Clinical Laboratory Division between January 2008 and December 2014

Sample type	Number of samples tested	Number of samples positive for *Bacillus*	Positivity for *Bacillus* (%)
Respiratory	7,516,401	30,633	0.41
Blood	6,558,062	32,275	0.49
Urine	2,925,701	1,920	0.07
Stool	2,021,788	34,736	1.72
Others	5,223,137	31,782	0.61
Total	24,245,089	131,346	0.54

Definitions and data processing

JANIS provided four categories for reporting *Bacillus* detection: *B. cereus*, *B. subtilis*, *B. anthracis*, and *Bacillus* sp. when the isolate was not identified to the species level. In this study, we set an additional category of ‘Bacillus’ to represent the detection of *B. cereus*, *B. subtilis*, and *Bacillus* sp. together. The monthly detection rate was defined as the proportion of samples that tested positive for the target pathogen over the total samples tested for a particular month. The period from July to September was defined as the summer season. A three-month moving average was used to visualise seasonal trends in the detection rate. Relevant geographical and weather data were obtained from published data [23,24].

Statistical analysis

Pearson’s correlation analysis was used to examine the relationship between two variables. The chi-square test was used to examine the statistical significance among categorical variables. Statistical significance was set at $P < 0.05$. All statistical analyses were performed using STATA version 13.1 (StataCorp, College Station, TX, USA).

Results

Microbiological test results of 24,245,089 clinical samples reported from 4105 hospital-years originating from 950

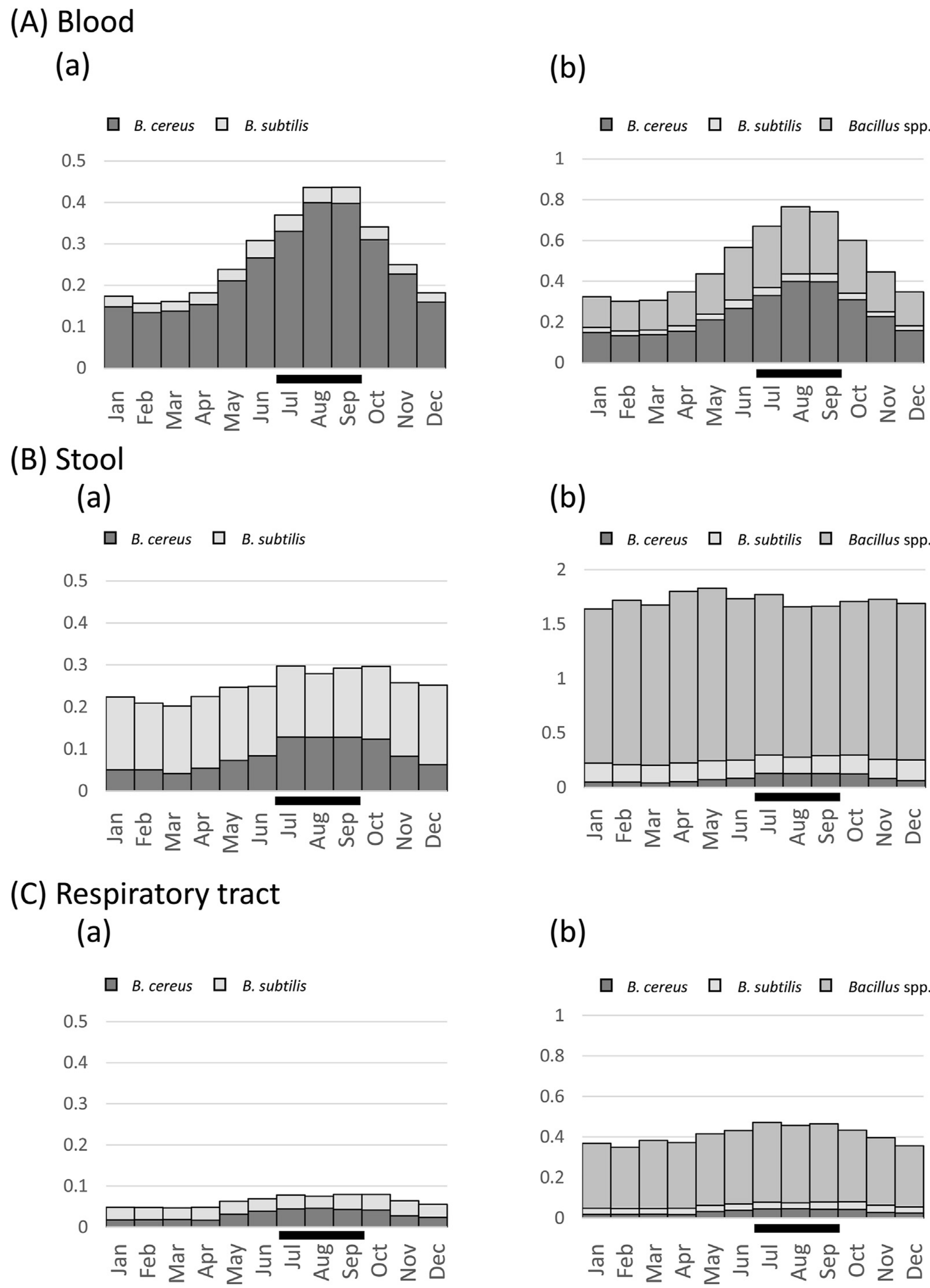

Figure 1. Monthly average detection rates of *Bacillus* spp. in blood (A), stool (B), and respiratory (C) specimens from January 2008 to December 2014 are shown, along with a breakdown of the species, which included *B. cereus*, *B. subtilis*, or *Bacillus* spp. Summer months (July to September) are indicated by the black bars.

individual hospitals were eligible for this study. The median number of beds was 404 (interquartile range [IQR], 310−541; range, 44−1435). As shown in Table I, respiratory specimens were the most common type of clinical samples, followed by blood, urine, and stool samples. *Bacillus* (i.e., *Bacillus* sp., *B. cereus*, and *B. subtilis*) was positive in 0.54% of all samples tested and the highest proportion of *Bacillus* positivity was reported in stool samples (1.72%). Since *Bacillus* were mainly detected in blood, stool, and respiratory specimens, we further investigated these samples by classifying them into blood and non-blood (stool and respiratory specimens) samples. Among blood samples, *Bacillus* was reported in 75.7% (3109/4105) hospital-years, with an annual median number of 0.85 blood samples/100 beds (IQR, 0.17−2.10 blood samples/100 beds). The upper 90th tile of that number was 3.9 samples/100 beds, with a maximum of 33.25 blood samples/100 beds, suggesting

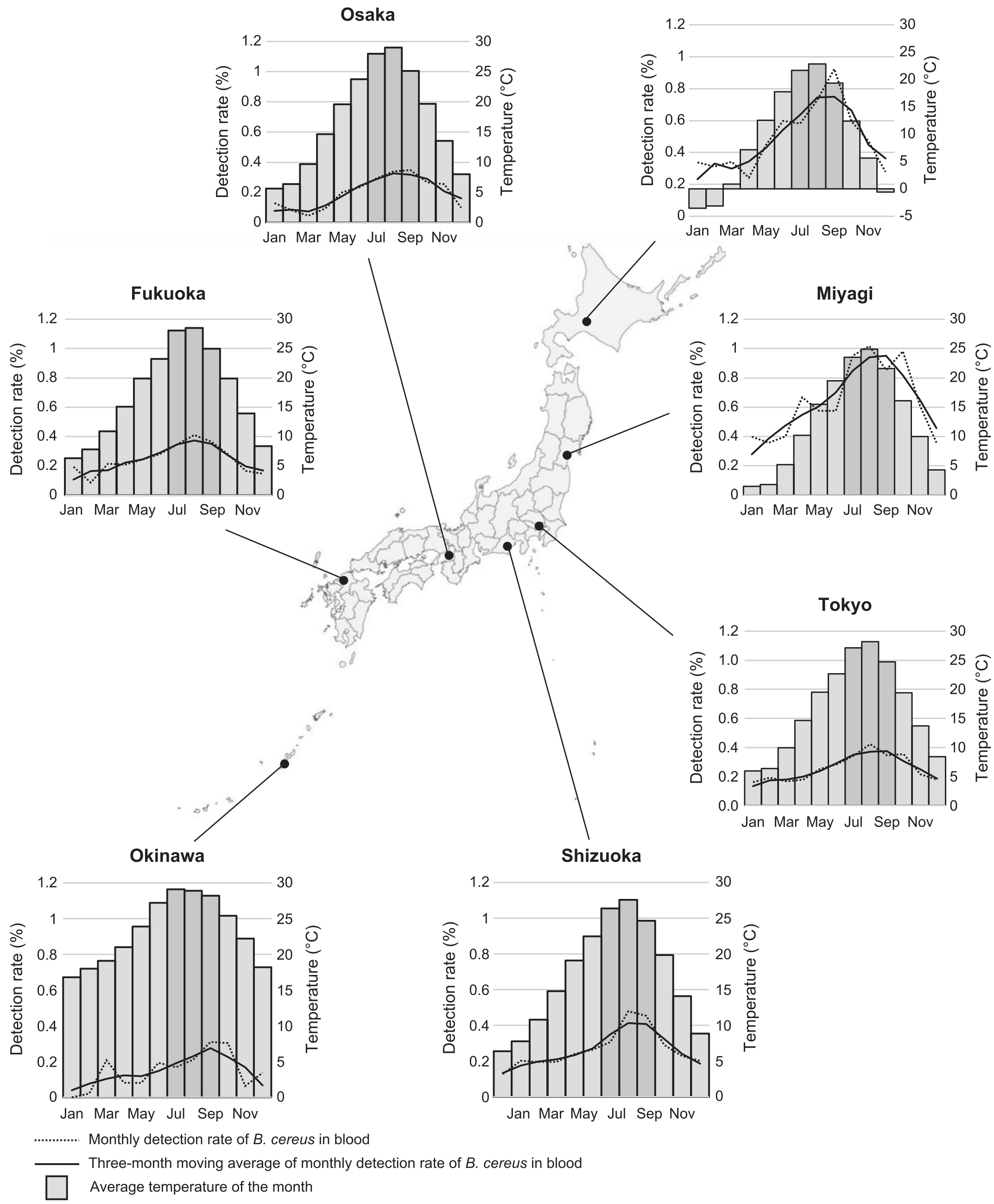

Figure 2. The monthly detection rate of *B. cereus* along with its three-month moving average in seven prefectures of Japan (Hokkaido, Miyagi, Tokyo, Shizuoka, Osaka, Fukuoka, and Okinawa) between January 2008 and December 2014. Dashed line indicates *B. cereus*, and solid line indicates the three-month moving average. The bar graph shows the average monthly temperatures over a seven-year period (2008–2014), with the summer months filled in dark grey.

that those hospitals may have experienced an outbreak of *B. cereus* BSI or at least *Bacillus* BSI. In non-blood samples, *Bacillus* was detected in 80.9% (3320/4105) hospital-years with an annual median number of 1.50 samples/100 beds (IQR, 0.31–5.56 samples/100 beds). Pearsons correlation analysis showed a very weak positive correlation between the number of bed-adjusted annual numbers of *Bacillus* in blood and non-blood samples (r=0.27, P <0.001).

Overall, 28.9% isolates were reported at the species level among *Bacillus*. By sample type, species level identification was seen in 55.3% of blood, 14.7% of stool, and 15.4% of respiratory specimens. Among the *Bacillus* isolates reported at the species level, the proportion of *B. cereus* was 72.7% overall, and by sample type, blood had the highest proportion of *B. cereus*, 88.9%, followed by respiratory specimens (48.3%) and stool (33.1%).

The monthly detection rates of those identified as *B. cereus* or *B. subtilis* in the blood, stool, and respiratory specimens are shown in Figure 1. The detection rate of *B. cereus* clearly increased in summer for all three sample types. In contrast, the detection rate of *B. subtilis* was nearly stable throughout the year. Comparing January and August as representative months of winter and summer in Japan, respectively, the detection rate of *B. cereus* in blood was 0.15% in January and 0.40% in

August (relative risk [RR], 2.69; 95% confidence interval [CI], 2.48−2.92; P <0.001). The detection rate of *B. cereus* in stool was 0.05% in January and 0.13% in August (RR, 2.55; 95% CI, 1.99−3.26; P <0.001), and that in respiratory specimens was 0.02% in January and 0.05% in August (RR, 2.66; 95% CI, 2.14−3.31; P <0.001). In contrast, the monthly detection rates of *Bacillus*, showed a clear and significant seasonality that was observed only in blood samples, 0.30% in January and 0.76% in August (RR, 2.36; 95% CI, 2.24−2.50; P <0.001). The detection rate of *Bacillus* in both respiratory and stool samples did not show notable seasonality. In other words, summer seasonality in *Bacillus* detection rate was primarily due to a higher proportion of *B. cereus* identified at the species level.

Since Japan is spread lengthwise from north to south and has a diverse climate, we also examined the seasonality of *B. cereus* detection in blood by region. Seven prefectures, from north to south, were extracted, including Hokkaido, the northmost region (capital location, lat. 43°03′51″ N.), and Okinawa, the south-most region (capital location, lat. 26°12′45″ N.) [24]. In the monthly average temperatures for seven years (2008−2014), the largest difference between winter and summer temperatures was observed in Hokkaido (January, −3.5 °C; August, 22.9 °C; difference of 26.4 °C) and minimal difference in Okinawa (January 16.8 °C, August 28.9 °C; difference 12.1 °C). As shown in Figure 2, summer seasonality in *B. cereus* detection rate was observed in all seven regions examined. The difference in monthly detection rate of *B. cereus* between winter and summer seasons was larger in Hokkaido (0.51%) than Okinawa (0.18%). Interestingly, although not statistically significant, there was a trend toward greater increases in *B. cereus* detection rate during the summer months at sites with greater temperature differences between winter and summer (r=0.56, P =0.2; Figure 3).

Discussion

The purpose of this study was to describe the prevalence of *B. cereus* in clinical samples in Japan and provide baseline data

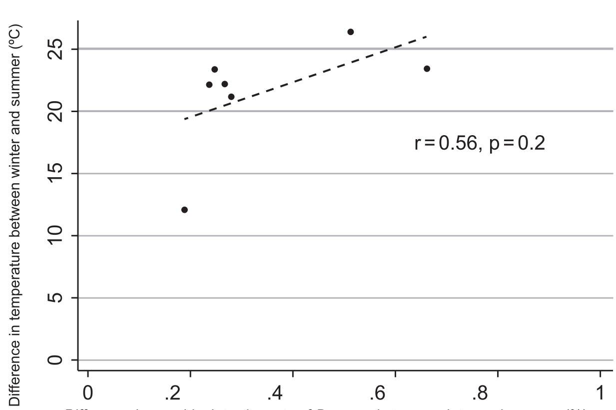

Figure 3. Correlation between difference in temperature and difference in *B. cereus* detection rate between 2008 and 2014. Difference in temperature calculated as the difference in mean monthly temperature in August and mean monthly temperature in January. Difference in detection rate calculated as difference in mean monthly detection rate in August and mean monthly detection rate in January.

for *B. cereus* detection in blood samples. A broader purpose of this study was to inform hospitals with a high prevalence of *B. cereus* detected in blood samples compared to the national baseline, and to encourage epidemiologists and infection control practitioners in hospitals to implement countermeasures to prevent *B. cereus* BSI outbreak.

Nationwide surveillance data revealed that approximately only half of the *Bacillus* isolates from blood samples were identified at species level. However, almost 90% of *Bacillus* species detected in blood samples were *B. cereus*. Thus, prevalence of *Bacillus* detections in blood samples could be an alternate indicator of prevalence of *B. cereus*. The national baseline values for *Bacillus* detections were annually 0.85 blood samples/100 beds (IQR, 0.17−2.10 blood samples/100 beds). Furthermore, only *B. cereus* detection showed clear seasonality, with peaks in summer, while *B. subtilis* detection remained constant throughout the year.

From an observational study at a teaching hospital in Japan, *B. cereus* detections during a non-outbreak period is calculated to be 0.4 blood samples/100 beds annually [25]. In contrast, in hospitals that experienced *B. cereus* BSI outbreaks, the positivity of blood samples was calculated to be 3 to 10 blood samples/100 beds during the outbreak period [6,11]. Notably, a retrospective review of hospitals with these outbreaks showed an increasing trend in *B. cereus* detection, exceeding the upper 75th percentile value (2.1 samples/100 beds) in our analysis, at least one year before the outbreak [6,11,19]. One such report pointed out that the number of *B. cereus* detections remained consistently high in hospitals reporting constant renovation work [11], suggesting that an increased detection of *B. cereus* in the blood samples may reflect the amount of *B. cereus* in the hospital environment increasing the risk of a *B. cereus* outbreak.

The summer seasonality in the detection of *B. cereus* in blood has been reported earlier [25,26]. This study demonstrated a seasonal trend that was common throughout Japan irrespective of climatic variations. Laboratory experiments have shown that the optimal temperature for *B. cereus* growth is 25−37 °C [27], and growth drastically decreases at temperatures below 15 °C [28]. Indeed, temperatures in most parts of Japan are optimal for the growth of *B. cereus* in summer than in winter [23]. A retrospective analysis of several outbreaks of *B. cereus* as BSIs indicated that there were signs of increased detection of *B. cereus* prior to outbreaks. Unfortunately, increased detection did not result in implementation of appropriate interventions, such as improving cleaning measures of linens, introducing disposable towels, and enhancing hand hygiene and maybe let the outbreaks occur [6,11,19]. It should be recognised that the decreased detection rate of *Bacillus* spp. after the summer can be due to seasonality of *B. cereus* and not due to the infection control procedure. Therefore, the assessment should be conducted not only by short-term trends in the detection rates of *Bacillus* spp., but also by its annual trends using data from several years. Interestingly, in countries with tropical or subtropical climates where average temperatures for most parts of the year are optimal for *B. cereus* growth, *B. cereus* detection in hospitals still showed summer seasonality and the annual detections were two−ten times higher than those observed in this study [29,30]. Our analysis showed that the greater the difference between summer and winter temperatures, the greater the difference in *B. cereus* detection rates. However, this difference was not statistically significant. These findings suggest that the detection rate of *B. cereus* and its seasonality are related to both the absolute temperature and degree of seasonal variations. Studies focusing on specific localities would provide more insights on the association between temperature and *B. cereus* detection.

Our study has some limitations. First, since only about half of the Bacillus detections, including those in blood samples, were reported at species level, the baseline value for the number of *B. cereus* detections could not be calculated directly. A possible reason for such low proportions of species identification is that *B. cereus* can be visually distinguished from non-cereus *Bacillus*, especially from *B. subtilis*, by its characteristic colony properties and haemolytic findings on blood agar medium. A widespread, species level identification with better documentation practices would increase the accuracy of surveillance. A major challenge is to balance clinical needs with the cost of testing. Second, participation in the JANIS surveillance is voluntary and can be biased toward hospitals with resources for infection control. Such hospitals are likely to be more active in bacterial culture testing, and this could have affected the detection rates of pathogens. In addition, the timing and number of sets of cultures collected were at the clinician's discretion. This may have influenced the pathogen detection rates. Third, due to limitations of the information available through JANIS, this study does not distinguish true infection and contamination. However, since it is not always easy to identify true infections in clinical practice, using the total number of detections as an indicator would be practical.

In summary, using the JANIS database, we clarified the distribution of *Bacillus* spp. in the clinical samples of hospitalised patients. These results will serve as indicators for infection control measures in each hospital.

Conclusions

The summer seasonality in *B. cereus* detection rates was found to be consistent nationwide. This seasonal trend, together with the baseline values of the annual *Bacillus* spp. detection rate per hospital bed obtained in this study, should be referred to in determining when to implement infection control interventions and in evaluating their effectiveness. Species level identification of *Bacillus* spp. was low in non-sterile specimens, and the association between *B. cereus* detection in blood and non-sterile specimens could not be fully elucidated. Future studies focusing on whether the detection of *B. cereus* in non-sterile specimens could be an indicator of contamination levels in the hospital environment, and thus a risk for *B. cereus* BSI are warranted.

Ethical considerations

Not required.

Data availability

The datasets analysed during the current study are available on the JANIS official website in Japanese (https://janis.mhlw.go.jp/index.asp) and partly in English (https://janis.mhlw.go.jp/english/index.asp).

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Author contributions

A.K and S.S designed the study; A.K, supervised by S.S, performed the analysis, interpreted the results, and worked on the manuscript. H.H and T.S. contributed to the design of the study and aided in interpreting the results. All authors have approved the final version of the article.

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Conflict of interest

None declared.

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