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Original article

Isolation frequency and antimicrobial susceptibilities of *Bacillus* species in a tertiary care hospital in Korea in the past four years (2020–2024): a retrospective surveillance study

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Abstract

Background: Identifying *Bacillus* spp. and performing antimicrobial susceptibility testing (AST) is challenging because of their diversity and limited clinical laboratory resources. We investigated the isolation frequency and antimicrobial susceptibility of *Bacillus* spp. over a 4-year period.

Methods: *Bacillus* isolates collected between 2020 and 2024 were identified using matrix-assisted laser desorption ionization-time of flight mass spectrometry, and AST was performed using the Pos Breakpoint Combo Panel Type 28 (Beckman Coulter).

Results: Species among total isolates (n = 432) were *B. cereus* (25.1%), *B. subtilis* (11.8%), *B. licheniformis* (10.8%), *B. pumilus* (7.4%), *B. simplex* (6.9%), *B. circulans* (6.4%), and *B. amyloliquefaciens* ssp. *plantarum* (5.9%). Overall, 65% of all *Bacillus* isolates were obtained from patients aged ≥ 60 years. The isolation ratios of sterile body fluids, including blood, to non-sterile specimens, in decreasing order, were: *B. licheniformis*, 4.5; *B. subtilis*, 2.4; *B. pumilus*, 2.0; *B. amyloliquefaciens* ssp. *plantarum*, 2.0; *B. circulans*, 1.2; *B. thuringiensis*, 1.0; *B. cereus*, 0.76; *B. simplex*, 0.56; and *B. infantis*, 0.43. The overall antimicrobial resistance rates were as follows: penicillin, 57.1%; ampicillin, 52.4%; clindamycin, 31.6%; erythromycin, 9.0%; cotrimoxazole, 6.4%; tetracycline, 2.3%; ciprofloxacin, 1.9%; rifampicin, 1.1%; levofloxacin, 0.9%; vancomycin, 0.4%; gentamicin, 0.4%; and imipenem, 0.4%. Penicillin resistance was particularly high in *B. thuringiensis* (100%), *B. cereus* (93.5%), and *B. licheniformis* (71.4%). Clindamycin resistance was high in *B. circulans, B. licheniformis,* and *B. pumilus* at 81.8%, 71.4%, and 64.3%, respectively.

Conclusion: Accurate identification and AST of *Bacillus* spp. are essential when they are isolated from invasive infections, as resistance profiles and isolated species vary significantly depending on the specimens.

Keywords: *Bacillus* species, Bacteriological techniques, Microbial sensitivity tests

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Introduction

Backgrounds

Aerobic endospore-forming *Bacillus* species are widely distributed in nature and are commonly found in food and environmental sources [1]. The spores of these species are resilient and can spread through the air, dust, and aerosols, thereby contributing to the contamination of hospital environments. Clinical *Bacillus* isolates were initially considered mere contaminants and non-pathogenic. However, its pathogenic potential has been recognized, particularly in the *Bacillus cereus* group, which includes *B. cereus*, *B. anthracis*, and *B. thuringiensis* [2]. Patients with intravenous drug abuse, trauma, implanted medical devices, malignancy, neutropenia, or corticosteroid therapy are vulnerable to severe *Bacillus* infections [3].

Although most *Bacillus* spp. are considered contaminants, these organisms should be identified at the species level when they are isolated from sterile body fluids, including blood, and are predominantly isolated from adequately collected clinical specimens. The accurate identification and antimicrobial susceptibility testing (AST) of *Bacillus* species are difficult because of the extensive diversity of these organisms and the limited commercial identification and AST systems available in clinical laboratories [4,5]. Among *Bacillus* spp., the most clinically relevant is *B. cereus*, a gram-positive spore-forming bacterium that is widely distributed in the environment. Although primarily associated with foodborne illnesses, its pathogenicity, which is related to the production of tissue-destructive exotoxins, has been reported to cause fatal infections [6]. Various *Bacillus* species cause infections in extra-intestinal organs and are frequently detected in wounds [7].

With the widespread use of matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF MS) in clinical laboratories, the identification of various *Bacillus* spp. that were previously unidentifiable at the species level has become possible, making it difficult to determine their clinical significance. As *Bacillus* strains can be reported to the species level, clinical laboratories are faced with the challenge of whether to perform AST for *Bacillus* species. The Clinical and Laboratory Standards Institute (CLSI) recommends AST for *Bacillus* isolates from normally sterile sources, such as deep tissue, cerebrospinal fluid, and multiple positive blood cultures, particularly in patients with an implanted prosthetic device, immunosuppression, or a history of intravenous drug abuse [8]. The interpretive criteria for minimal inhibitory concentration (MIC) values of *Bacillus* species are adapted from those for *Staphylococcus* species as published in the CLSI document M100 [9]. The key citations used in the derivation of interpretive criteria are based on previously reported researches on AST of *Bacillus* spp. [9-11].

An increasing prevalence of multidrug-resistant *Bacillus* species isolated from the stool samples of patients with gastrointestinal infections has been reported, and multidrug resistance has been observed in *B. cereus* isolates identified in freshly collected food samples, including milk [1,12]. Additionally, certain species such as *B. coagulans* and *B. subtilis* have been utilized as probiotics [13,14]. These bacteria may possess intrinsic antimicrobial resistance or have the potential to horizontally transfer resistance genes [15–17]. As *Bacillus* spp. are increasingly associated with normal human life, there is a growing need for more clinical data on these organisms.

Aims

This study aimed to investigate the isolation frequency by age group, specimen type, and antimicrobial susceptibility of *Bacillus* isolates over the past four years (2020–2024).

Methods

Study design

It is a retrospective surveillance study based on the laboratory records. It was described according to the Microbiology Investigation Criteria for Reporting Objectively: a framework for the reporting and interpretation of clinical microbiology data available at: https://bmcmedicine.biomedcentral.com/articles/10.1186/s12916-019-1301-1.

Setting

From August 2020 to July 2024, *Bacillus* isolates identified during routine clinical practice at Wonju Severance Christian Hospital (WSCH) were collected from the laboratory information system, and all data were automatically de-identified.

Laboratory work

Bacillus species were identified by Gram staining, colony morphology analysis, and MALDI-TOF MS using a Bruker Biotyper (Bruker Daltonics GmbH & Co.). When species-level identification could not be clearly determined, further identification was not performed using additional methods. In such cases, the isolates were classified and analyzed as "*Bacillus* species." Specimens were classified as either sterile body fluids (blood, cerebrospinal fluid, peritoneal fluid, pleural fluid, and synovial fluid) or non-sterile (catheter, drain, and wound). AST was performed using the Pos Breakpoint Combo Panel Type 28 (Beckman Coulter), and the results were interpreted according to CLSI recommendations [8]. Ampicillin, penicillin, ciprofloxacin, levofloxacin, clindamycin, erythromycin, tetracycline, cotrimoxazole, gentamicin, imipenem, rifampicin, and vancomycin have been reported previously. As of February 1, 2024, the AST for *Bacillus* species transitioned from the MicroScan WalkAway 96 plus system (Siemens Healthcare Diagnostics Inc.) to the DxM 1096 MicroScan WalkAway system (Beckman Coulter). For the AST of gram-positive bacteria using these systems, modifying the organism designation to *Bacillus* species is necessary during the electronic data entry process after generating the predefined barcode required for recognition of the AST testing panel. If the organism designation is missed, AST results for ampicillin, imipenem, and levofloxacin may not be reported. Data were analyzed using IBM SPSS Statistics (version 25.0; IBM Corp.).

Quality assurance

The Department of Laboratory Medicine at WSCH consistently participates in Proficiency Testing and External Quality Assurance programs organized by the Korean Association of External Quality Assessment

Services. Additionally, the department holds accreditation through the Outstanding Laboratory Accreditation Program administered by the Laboratory Medicine Foundation and Korean Society for Laboratory Medicine.

Bias

Only one of the initially isolated *Bacillus* strains was included in the data analysis if the same *Bacillus* species was identified in multiple blood culture bottles from the same patient. If *Bacillus* species were identified in different specimen types from the same patient, each case was analyzed separately.

Results

Basic characteristics and specimen types of isolated *Bacillus* species

After excluding duplicates, a final set of 432 *Bacillus* isolates (502) were identified. Among the *Bacillus* isolates identified to the species level, the most common *Bacillus* species were *B. cereus* (25.1%), *B. subtilis* (11.8%), *B. licheniformis* (10.8%), *B. pumilus* (7.4%), *B. simplex* (6.9%), *B. circulans* (6.4%), *B. amyloliquefaciens* ssp. *plantarum* (5.9%), *B. infantis* (4.9%), *B. thuringiensis* (4.9%), and *B. sonorensis* (4.4%). The male-to-female isolation ratio of all *Bacillus* isolates was 1.17:1, with no statistically significant differences according to sex for any species (chi-square test, $P = 0.492$) (Table 1). The isolation frequency of *Bacillus* spp. by age group, in decreasing order, were 60s (23.8%), 70s (20.8%), ≥ 80s (20.4%), and 50s (14.8%), showing a higher isolation rate in older individuals. However, the Kruskal–Wallis rank sum test revealed no statistically significant differences in the age of the patients across the identified species ($P = 0.222$) (Table 1). The ratio of sterile to non-sterile specimens isolated from all *Bacillus* spp. was 0.58 (159/273). Among the *Bacillus* spp. with at least 10 isolates, the ratios of sterile to non-sterile specimens were as follows: *B. licheniformis*, 4.5; *B. subtilis*, 2.4; *B. pumilus*, 2.0; *B. amyloliquefaciens ssp. plantarum*, 2.0; *B. circulans*, 1.2; *B. thuringiensis*, 1.0; *B. cereus*, 0.76; *B. simplex*, 0.56; and *B. infantis*, 0.43 (Table 2).

Antimicrobial resistance of isolated *Bacillus* species

The overall rates of antimicrobial resistance decreased in the following order: penicillin G, 57.1%; ampicillin, 52.4%; clindamycin, 31.6%; erythromycin, 9.0%; cotrimoxazole, 6.4%; tetracycline, 2.3%; ciprofloxacin, 1.9%; rifampicin, 1.1%; levofloxacin, 0.9%; vancomycin, 0.4%; gentamicin, 0.4%; and imipenem, 0.4%. The only isolate showing vancomycin resistance was *B. circulans* strain isolated from the pleural fluid of a 59-year-old man with a vancomycin MIC of 32 mg/L, which was resistant to all tested antimicrobial agents except rifampicin (MIC 2 mg/L, intermediate). Penicillin resistance rates were particularly high in *B. thuringiensis* (100%), *B. cereus* (93.5%), and *B. licheniformis* (71.4%), with the ampicillin resistance rates similar to those of penicillin. Clindamycin resistance was high in *B. circulans*, *B. licheniformis*, and *B. pumilus* at rates of 81.8%, 71.4%, and 64.3%, respectively. Among the two *B. cereus* isolates resistant to ciprofloxacin, one exhibited intermediate resistance, and the other was resistant to levofloxacin (Table 3).

Table 1. Basic characteristics of isolated *Bacillus* species

Isolates	Patients' information		Total No. (%)[b]
	Sex female:male	Age (yr)[a] M (IQR)	
Bacillus amyloliquefaciens	0:2	82 (80–85)	2 (0.5/1.0)
Bacillus amyloliquefaciens ssp. *plantarum*	8:4	74 (64–78)	12 (2.8/5.9)
Bacillus asahii	0:1	76	1 (0.2/0.5)
Bacillus cereus	23:28	61 (45–76)	51 (11.8/25.1)
Bacillus circulans	7:6	61 (53–74)	13 (3.0/6.4)
Bacillus clausii	1:0	47	1 (0.2/0.5)
Bacillus cytotoxicus	0:1	75	1 (0.2/0.5)
Bacillus flexus	2:2	60 (31–79)	4 (0.9/2.0)
Bacillus gibsonii	2:0	68 (51–84)	2 (0.5/1.0)
Bacillus halosaccharovorans	0:3	52 (33–62)	3 (0.7/1.5)
Bacillus idriensis	1:1	65 (55–75)	2 (0.5/1.0)
Bacillus indicus	1:0	78	1 (0.2/0.5)
Bacillus infantis	2:8	74 (62–83)	10 (2.3/4.9)
Bacillus licheniformis	12:10	74 (67–80)	22 (5.1/10.8)
Bacillus mojavensis	0:3	66 (63–76)	3 (0.7/1.5)
Bacillus oceanisediminis	1:0	82	1 (0.2/0.5)
Bacillus pumilus	7:8	69 (56–77)	15 (3.5/7.4)
Bacillus simplex	7:7	67 (60–74)	14 (3.2/6.9)
Bacillus sonorensis	4:5	65 (59–84)	9 (2.1/4.4)
Bacillus subtilis	11:13	66 (52–74)	24 (5.6/11.8)
Bacillus thuringiensis	4:6	58 (43–66)	10 (2.3/4.9)
Bacillus vallismortis	0:1	80	1 (0.2/0.5)
Bacillus velezensis	1:0	81	1 (0.2/0.5)
Bacillus species	105:124	66 (53–77)	229 (53.0)
Total	199:233	67 (53–77)	432 (100.0)

[a]If the number of isolation is one, only the patient's age is presented. If the number of isolation is two, a mean of ages is indicated with the two patients as an age range. For three or more isolations, median (M) and interquartile range (IQR) are reported.

[b]The number before the slash in parentheses is the frequency percentage including *Bacillus* species, and the number after the slash in parentheses is the frequency percentage excluding *Bacillus* species.

Table 2. Isolation frequency of *Bacillus* species according to specimen type

Bacillus species (No. of isolates)	Blood	CSF	Peritoneal F	Pleural F	Synovial F	Catheter	Drain	Wound	Others[a]
B. amyloliquefaciens (2)	2 (100.0)	0	0	0	0	0	0	0	0
B. amyloliquefaciens ssp. *plantarum* (12)	8 (66.7)	0	0	0	0	0	3 (25.0)	1 (8.3)	0
B. asahii (1)	0	0	0	0	0	0	1 (100.0)	0	0
B. cereus (51)	13 (25.5)	0	7 (13.7)	2 (3.9)	0	0	4 (7.8)	24 (47.1)	1 (2.0)
B. circulans (13)	4 (30.8)	1 (7.7)	1 (7.7)	1 (7.7)	0	1 (7.7)	1 (7.7)	4 (30.8)	0
B. clausii (1)	1 (100.0)	0	0	0	0	0	0	0	0
B. cytotoxicus (1)	0	0	0	0	0	0	1 (100)	0	0
B. flexus (4)	1 (25.0)	0	0	0	0	1 (25.0)	1 (25.0)	1 (25.0)	0
B. gibsonii (2)	2 (100.0)	0	0	0	0	0	0	0	0
B. halosaccharovorans (3)	0	0	0	0	0	0	0	2 (66.7)	1 (33.3)
B. idriensis (2)	1 (50.0)	0	0	0	0	0	0	1 (50.0)	0
B. indicus (1)	0	0	0	0	0	0	0	1 (100.0)	0
B. infantis (10)	3 (30.0)	0	0	0	0	4 (40.0)	2 (20.0)	0	1 (10.0)
B. licheniformis (22)	16 (72.7)	0	0	1 (4.5)	1 (4.5)	1 (4.5)	2 (9.1)	1 (4.5)	0
B. mojavensis (3)	2 (66.7)	0	0	0	0	0	1 (33.3)	0	0
B. oceanisediminis (1)	0	0	0	0	0	0	0	1 (100.0)	0
B. pumilus (15)	7 (46.7)	0	0	2 (13.3)	1 (6.7)	0	0	5 (33.3)	0
B. simplex (14)	5 (35.7)	0	0	0	0	2 (14.3)	2 (14.3)	3 (21.4)	2 (14.3)
B. sonorensis (9)	2 (22.2)	0	0	1 (11.1)	0	1 (11.1)	3 (33.3)	2 (22.2)	0
B. subtilis (24)	15 (62.5)	0	0	2 (8.3)	0	0	4 (16.7)	3 (12.5)	0
B. thuringiensis (10)	4 (40.0)	0	0	1 (10.0)	0	0	0	5 (50.0)	0
B. vallismortis (1)	0	0	0	1 (100.0)	0	0	0	0	0
B. velezensis (1)	1 (100.0)	0	0	0	0	0	0	0	0
Bacillus species (229)	44 (19.2)	1 (0.4)	4 (1.7)	1 (0.4)	0	44 (19.2)	48 (21.0)	86 (37.6)	1 (0.4)
Total	131 (30.3)	2 (0.5)	12 (2.8)	12 (2.8)	2 (0.5)	54 (12.5)	73 (16.9)	140 (32.4)	6 (1.4)

Values are presented as n or n (%).

[a]Urine (2), bile fluid (2), bronchial washing (1), and liver abscess (1) are included.

Abbreviations: CSF, cerebrospinal fluid; F, fluid.

Table 3. Resistance rate of antimicrobial agents according to *Bacillus* species

Bacillus species[b]	VAN (266)[a]	PEN (266)	TET (266)	CLI (266)	ERY (266)	SXT (266)	CIP (266)	GEN (266)	RIF (266)	IMP (231)	AMP (231)	LEV (231)
B. amyloliquefaciens (2/2)	0	0	0	0	0	0	0	0	0	0	0	0
B. amyloliquefaciens ssp. *plantarum* (11/9)	0	9.1	9.1	9.1	0	18.2	0	0	0	0	0	0
B. asahii (1/1)	0	0	0	0	0	0	0	0	0	0	0	0
B. cereus (46/36)	0	93.5	4.3	0	0	17.4	4.3	0	0	2.8	91.7	2.8
B. circulans (11/7)	9.1	27.3	9.1	81.8	27.3	18.2	9.1	9.1	0	0	0	0
B. clausii (1/1)	0	0	0	0	0	0	0	0	0	0	0	0
B. cytotoxicus (1/1)	0	0	0	0	0	0	0	0	0	0	0	0
B. flexus (1/0)	0	0	0	0	100	0	0	0	0	-	-	-
B. gibsonii (2/2)	0	0	0	50.0	0	0	0	0	0	0	0	0
B. halosaccharovorans (1/0)	0	0	0	0	0	0	0	0	0	-	-	-
B. idriensis (1/1)	0	0	0	0	0	0	0	0	0	0	0	0
B. infantis (4/4)	0	0	0	0	0	0	0	0	0	0	0	0
B. licheniformis (21/19)	0	71.4	0	71.4	28.6	0	0	0	0	0	73.7	0
B. mojavensis (3/2)	0	0	0	0	0	0	0	0	0	0	0	0
B. oceanisediminis (1/1)	0	100	0	100	0	0	0	0	0	0	0	0
B. pumilus (14/13)	0	0	7.1	64.3	7.1	0	0	0	0	0	0	0
B. simplex (7/7)	0	28.6	0	0	14.3	0	0	0	0	0	28.6	0
B. sonorensis (8/7)	0	75.0	0	25.0	12.5	0	0	0	0	0	14.3	0
B. subtilis (21/16)	0	4.8	0	28.6	0	0	0	0	4.8	0	6.3	0
B. thuringiensis (9/8)	0	100	0	0	11.1	33.3	11.1	0	0	0	100	0
B. vallismortis (1/1)	0	0	0	0	0	0	0	0	0	0	0	0
B. velezensis (1/1)	0	0	0	0	0	0	0	0	0	0	0	0
Bacillus species (98/92)	0	72.4	1.0	40.8	10.2	2.0	1.0	0	2.0	0	67.4	1.1
Total	0.4	57.1	2.3	31.6	9.0	6.4	1.9	0.4	1.1	0.4	52.4	0.9

Values are presented as %.

[a]Number in parenthesis refers to the tested number of isolates.

[b]The number before the slash in parentheses is the number of 266 strains tested against nine antimicrobial agents excluding imipenem, ampicillin, and levofloxacin, and the number after the slash in parentheses is the number of strains tested against all 12 antimicrobial agents.

Abbreviations: VAN, vancomycin; PEN, penicillin G; TET, tetracycline; CLI, clindamycin; ERY, erythromycin; SXT, cotrimoxazole; CIP, ciprofloxacin; GEN, gentamicin; RIF, rifampicin; IMP, imipenem; AMP, ampicillin; LEV, levofloxacin.

Discussion

Interpretation and comparison with previous studies

In this study, the most frequently isolated *Bacillus* species was *B. cereus* (25.1%), followed by *B. subtilis*, *B. licheniformis*, *B. pumilus*, *B. simplex*, *B. circulans*, *B. amyloliquefaciens* ssp. *plantarum*, *B. infantis*, *B. thuringiensis*, and *B. sonorensis* (ranging from 4.4% to 11.8%).

B. cereus is a well-known cause of foodborne illness outbreaks because its spores can survive at cooking temperatures and cleaning procedures. Recently, *B. cereus* has been increasingly recognized as an opportunistic pathogen that causes localized wounds, eye, and systemic infections [18]. The pathogenicity of *B. cereus*, whether intestinal or non-intestinal, is closely associated with adhesion and tissue destructive/reactive secreted toxins, such as hemolysins, phospholipases, proteases, emesis-inducing toxins, and pore-forming enterotoxins [18,19]. *B. thuringiensis* strains are invertebrate pathogens that have been used as biopesticides,

and some strains have been reported to cause bacteremia, mainly in immunocompromised individuals [20].

The *Bacillus subtilis* group included *B. subtilis, B. licheniformis, B. pumilus, B. amyloliquefaciens,* and *B. sonorensis. B. subtilis* is generally considered non-pathogenic and harmless to human health. It has also been used as a probiotic [21]. However, true infections, such as ocular infections, traumatic wound infections, meningitis, and bacteremia have also been reported [22]. *B. licheniformis* bacteremia is associated with the presence of long-term central venous catheters, especially in immunocompromised patients [23]. Although rare, *B. pumilus* has been reported to cause food poisoning, septic arthritis, cutaneous infections, and bacteremia [24]. Fan et al. [25] proposed that the *B. amyloliquefaciens* clade should be considered as a taxonomic unit above the species level, designated as "operational group *B. amyloliquefaciens*". This group consists of soil-borne *B. amyloliquefaciens*, and plant associated *B. siamensis* and *B. velezensis*, whose members are closely related. These species undergo genomic changes as they adapt to the plant-associated lifestyles. *B. sonorensis* is closely related to *B. licheniformis* [26]. *B. simplex* is an environmental microorganism primarily found in soil and rarely causes infections in humans [27].

The *Bacillus circulans* group included *B. circulans, B. firmus,* and *B. coagulans. B. circulans* is commonly found in soil, and its non-pathogenic strains are utilized in industrial enzyme production, particularly of proteinases [28]. However, pathogenic strains of *B. circulans* have been implicated in multiple human infections, such as septicemia, wounds, and abscesses, particularly in immunocompromised individuals [28]. *B. infantis* was first isolated from a patient with neonatal sepsis and was closely related to *B. firmus* (98.2% sequence similarity) [29].

Identification of *Bacillus* species was based on Gram staining, an examination of colony morphology, and MALDI-TOF MS analysis. When MALDI-TOF MS was used to identify the *Bacillus* species, the most commonly isolated species in clinical laboratories were *B. cereus, B. pumilus, B. subtilis, B. licheniformis,* and *B. simplex* [30]. Although there are over 280 species in the *Bacillus* genus [19], the number of *Bacillus* species in the MALDI-TOF MS database library is much smaller, even though protein profile codes (universally unique identifiers) are continuously being added. Therefore, *Bacillus* spp. that can be identified by MALDI-TOF MS may vary depending on the version of the database library.

Bacillus species were detected in a range of specimen types. With respect to analysis based on the ratio of sterile to non-sterile specimens, although we obtained an average value of 0.58, there was a considerable variation among species, with ratios ranging from 0.43 to 4.5 among species for which more than 10 isolates were obtained. The reporting criteria for the identification level and AST of *Bacillus* species in clinical microbiology laboratories may vary depending on the type of specimen, colony count, and the clinical condition of the patient. When *Bacillus* species are isolated from non-sterile sites with low colony counts, descriptive identification based on Gram staining may be sufficient. In contrast, when numerous *Bacillus* spp. are isolated from open wounds with ground contamination, species-level identification and AST are required [31]. Cotton et al. [32] reported a significant difference in the frequency of recurrent bacteremia between 9.2% (positive in two of two bottles) and 3.3% (positive in one of two bottles) according to the degree of initial blood culture bottle positivity. *Bacillus* bacteremia occurs frequently in

immunosuppressed patients, such as those with cancer or leukemia, and is considered an independent risk factor in patients with a central venous catheter and the use of extended-spectrum cephalosporins within one month [33].

When *Bacillus* species are isolated from clinical specimens, distinguishing between contamination and true infection is challenging for the following reasons: First, no clear criteria have been established based on specimen type to differentiate contamination from infection. Second, testing for the diverse toxins produced by *Bacillus* species in clinical laboratories is impractical. Third, the clinical characteristics of most *Bacillus* species remain poorly understood, with the exception of well-known pathogens, such as *B. anthrax* and *B. cereus*.

Our study revealed a marked variability in antimicrobial susceptibility patterns among *Bacillus* species. Therefore, when clinicians suspect a true infection due to *Bacillus* spp., the AST results can be of great help in treatment. Most *B. cereus* strains are intrinsically resistant to penicillin, cephalosporins, and β-lactamase inhibitor combinations because they produce three different β-lactamases (penicillinase, cephalosporinase, and metallolactamase) [34–36]. Therefore, β-lactam agents should be avoided in the empirical coverage of patients, especially in immunocompromised patients who have had *Bacillus* spp. isolated, until AST results are available. Additionally, in this study, *B. licheniformis, B. pumilus, B. subtilis*, and *B. sonorensis*, which belong to the *B. subtilis* group, were more resistant to clindamycin than other *Bacillus* species. *B. circulans* strains resistant to common antibiotics pose a threat as they can lead to the formation of more dangerous multidrug-resistant strains. Ligozzi et al. [37] reported that a vancomycin-resistant clinical isolate of *B. circulans* was associated with the acquisition of a *vanA* gene cluster located on the chromosome, showing a high degree of homology with that of enterococci. However, the cluster was not carried by Tn*1546* and was borne by the chromosome.

Limitations

This study has several limitations. First, 53.0% of the isolates could not be identified at the species level. Although molecular methods provide the most accurate identification, clinical laboratories often lack standardized AST guidelines and interpretation criteria for newly identified or rare organisms. As our laboratory prioritized rapid reporting to support patient care, the results were communicated to physicians even when full species-level identification was unavailable. Second, the AST results were not uniformly reported. The AST system requires manual organism designation to ensure proper reporting of key antibiotics, such as imipenem, ampicillin, and levofloxacin. Owing to this limitation, 13.2% of the 266 isolates tested lacked AST results for these antibiotics (Table 3), highlighting the need for improved system integration.

Conclusion

Bacillus species are increasingly integrated into various aspects of human life, leading to more frequent human contact. Most *Bacillus* species exhibit antibiotic resistance and have the potential to transfer antibiotic resistance genes to humans, suggesting that antibiotic residues may enter consumer food products and the

human food chain. Therefore, continuous monitoring of the isolation frequency and AST results of *Bacillus* species isolated from clinical specimens is necessary.

Ethics statement

This study focused on the analysis of previous identification records of *Bacillus* and did not involve human participants or the collection of individual patient information. Given the study design, the Institutional Review Board (IRB) waived the requirement for informed consent. The study was conducted in compliance with the principles of the Declaration of Helsinki and was approved by the IRB of WSCH (approval no. CR324123) on February 4, 2025.

Conflicts of interest

Young Uh has been a statistical editor of the *Annals of Clinical Microbiology* since 2024. However, he was not involved in the review process of this article. No other potential conflicts of interest relevant to this article were reported.

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Data availability

The datasets generated during the current study are available from the corresponding author upon request.

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