



Research Article

Toxin gene and antibiotic resistance of *Staphylococcus aureus* and *Bacillus cereus* isolated from kitchen cleaning tools in child care centers

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Abstract This study analyzed the toxin genes and antibiotic resistance of food-poisoning bacteria isolated from dishcloths and scourers in child care centers. Two *S. aureus* and five *B. cereus* strains isolated from dishcloths and scourers, respectively, were used in this study. The toxin genes of *S. aureus* and *B. cereus* were detected using polymerase chain reaction (PCR) methods. Antibiotic resistance was determined using the disc diffusion method according to the Clinical and Laboratory Standard Institute criteria. Toxin gene analysis revealed the presence of *sei* and *seg* toxin genes in *S. aureus* isolated from dishcloths and scourers. All five *B. cereus* strains possessed the *nheA*, *nheB*, *nheC*, *hblA*, *hblC*, and *hblD* enterotoxin genes that cause diarrhea. The *entFM* enterotoxin gene was detected in three of the five isolates, but the *cytK* enterotoxin gene and *ces* vomiting toxin gene were not detected. *S. aureus* and *B. cereus* isolated from kitchen cleaning tools were found to be multidrug-resistant strains that were resistant to five to eight antibiotics. Therefore, thorough sterilization and disinfection of dishcloths and scourers is required to prevent *S. aureus* and *B. cereus* from contaminating cooking utensils.

Keywords kitchen cleaning tools, child care center, food-borne pathogens, toxin characteristics, antibiotic resistance



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1. Introduction

Food poisoning is defined as “an infectious or toxin-type disease caused or judged to be caused by microorganisms or toxic substances harmful to the human body due to food intake” (MOLEG, 2022). As food poisoning occurs acutely in many people, it is important to analyze the risk factors for prevention in advance. A collective foodborne illness is identified when “the epidemiological investigation determines that food or water is the cause of the disease, and two or more people who ingest water and food from the same source have experienced similar diseases” (WHO, 2008). The sources of food poisoning in Korea are 65.8% from food service companies, 23.2% from cafeteria food services, 1.8% from homes, and 9.1% from other sources, and cafeterias are reported as the major places of occurrence

(KOSIS, 2022b).

Food poisoning bacteria include infectious and toxigenic type. Infectious food poisoning occurs when food contaminated with a large number of food poisoning bacteria is ingested and bacteria invade the intestinal mucosa of the digestive system (Ryu, 2011). Representative infectious food-poisoning bacteria include *Escherichia coli*, *Salmonella* spp., *Vibrio parahaemolyticus*, *Listeria monocytogenes*, *Campylobacter jejuni*, and *Yersinia enterocolitica* (Roh, 1997). Toxigenic food-poisoning bacteria include *Bacillus cereus*, *Staphylococcus aureus*, and *Clostridium botulinum* (KDCA, 2022b). *Bacillus cereus* is widely present in nature and can survive in extreme environments. It has been reported that *B. cereus* has diarrheal toxin genes (*hblA*, *hblC*, *hblD*, *entFM*, *nheA*, *nheB*, *nheC*, *cytK*) and emetic toxin genes (*ces*) (Park et al., 2020). As *Staphylococcus aureus* also resides on human hands and skin, cross-contamination must be avoided. *S. aureus* contains 16 types of enterotoxins: SEA, SEB, SEC, SED, SEE, SEG, SEH, SEI, SEJ, SEK, SEL, SEM, SEN, SEO, SEP, and SEQ (Cho et al., 2011; Shin et al., 2018). In the current status of the outbreak of *S. aureus*, it has been reported that the occurrence of SEA, SEB, SEC, SED, and SEE-toxins is the most common (Cho et al., 2011). Toxigenic food poisoning is acutely caused by toxin proteins produced by toxin genes, and the analysis of toxin genes in food-poisoning bacteria is important for identifying risk factors (Kim et al., 2009).

Antibiotic resistance refers to drug resistance that allows microorganisms to survive exposure to antibiotics (CDC, 2022; KDCA, 2022a). According to the WHO, antibiotic resistance is becoming an increasingly serious issue that threatens public health globally (Song, 2009). The overall antibiotic resistance rate is higher in Asian countries than in

Western countries, and recently, strains that exhibit antibiotic resistance are rapidly emerging owing to antibiotic abuse (Song, 2009). Because some antibiotic-resistant bacteria easily propagate resistance factors to other bacteria by plasmid transfer, the abuse of antibiotics is bound to lead to resistant bacteria (Yu et al., 2014). Therefore, antibiotics should only be used when appropriate. In addition, the increase in antibiotic resistance can make initial treatment difficult in the event of outbreaks, which is a threat to public health. Thus, it is necessary to analyze the antibiotic resistance of food poisoning bacteria isolated from cafeterias in childcare centers for rapid initial treatment.

According to the National Statistical Office, the rate of use of child welfare services, such as childcare facilities and centers, was 51.9% in 2019, an increase of 7.7% from 44.2% in 2015 (KOSIS, 2022c). Accordingly, meal support services are also on the rise, with 81.8% of them using meal support services as of 2018 (KOSIS, 2022a). Childcare centers manage hygiene through childcare center meal management manuals and guidelines; however, collective food poisoning accidents occur every year. In June 2020, a cross-contamination food poisoning accident occurred in more than 100 patients at a childcare center in Ansan city. Since acute food poisoning in such a large number of people can be fatal to children and infants with weak immunity and physical development, it is important to prevent food poisoning by analyzing the harmful factors affecting meals at childcare facilities. Previous research on toxic genes and antibiotic resistance at cafeterias in childcare centers have focused on airborne microorganisms and bacteria identified in the hands working in cafeterias (Kim et al., 2012). However, research on food poisoning bacteria isolated from washing

tools, such as dishcloths and scourers, which are the main sources of food poisoning due to cross-contamination, is insufficient.

Therefore, this study analyzed the toxin gene and antibiotic resistance of food-poisoning bacteria isolated from dishcloths and scourers used in childcare centers.

2. Materials and methods

2.1. Food poisoning bacteria

The food poisoning bacteria (two isolates of *S. aureus* and five isolates of *B. cereus*) used in this study were isolated from dishcloths and scourers in 89 childcare centers located in Jeollanam-do. *B. cereus* was isolated in 2 dishcloths and 3 scourers. *S. aureus* was isolated in 1 dishcloth and 1 scourer. The isolates were inoculated with tryptic soy broth (TSB; MBcell, KisanBio, Seoul, Korea), cultured at 35°C for 24 h, subjected to tryptic soy agar (TSA; MBcell), and reactivated at 35°C for 24 h for use in this study.

2.2. Biochemical identification

Reactivated *S. aureus* and *B. cereus* were cultured on nutrient agar (NA; MBcell) and incubated at 35°C for 18 h. Cultured *S. aureus* and *B. cereus* were biochemically re-identified using API Staph (Bio Merieux, Marcy-l'Etoile, France) and API 50CHB (BioMerieux, Marcy-l'Etoile).

2.3. DNA extraction

One colony each of *S. aureus* and *B. cereus* was inoculated into 10 mL of tryptic soy broth (TSB; MBcell) and cultured at 35°C for 24 h. One milliliter of the culture solution was transferred to an Eppendorf tube and centrifuged (Smart R17; Hanil Scientific, Gimpo, Korea) at 12,000 rpm for 10 min.

After this process was repeated three times, the supernatant of the Eppendorf tube was removed, 1 mL of sterilized distilled water was added, and the reaction was conducted in a heating block (HB-96D, Daihan Scientific, Wonju, Korea) at 99°C for 10 min. The heated Eppendorf tubes were centrifuged at 12,000 rpm for 10 min to separate the supernatant. The supernatant was used as the template DNA for polymerase chain reaction (PCR).

2.4. PCR reaction

PCR for the detection of toxin genes in *S. aureus* was conducted using a *Staphylococcus aureus* toxin ID detection kit (Kogenbiotech, Seoul, Korea). The PCR conditions were as follows: 32 cycles of pre-denaturation at 95°C for 10 min, denaturation at 95°C for 30 s, annealing at 58°C for 30 s, extension at 72°C for 30 s, and a final extension at 72°C for 10 min. *B. cereus* toxin gene PCR was performed with each toxin gene primer (*hblA*, *hblC*, *hblD*, *entFM*, *nheA*, *nheB*, *nheC*, and *cytK*) (Kim et al., 2013). PCR reaction conditions were as follows: pre-denaturation at 94°C for 2 min, denaturation at 94°C for 60 s, annealing (*hblA* 56°C, *hblC* 58°C, *hblD* 58°C, *entFM* 72°C, *nheA* 56°C, *nheB* 54°C, *nheC* 54°C, *cytK* 48°C), 60 s, and final extension at 72°C for 5 min. To confirm the specific toxin genes of *S. aureus* and *B. cereus*, 5 µL of the PCR product was loaded on a 1.2% agarose gel, and electrophoresis was performed at 220 V for 30 min.

2.5. Antibiotic resistance

Antibiotic resistance tests of food-poisoning bacteria were conducted using the disc diffusion method (Baik et al., 2011). The discs used in this study were ampicillin 10 µg, cefepime 30 µg, cefotetan 30 µg, ciprofloxacin 5 µg, chloramphenicol 30 µg, clindamycin 2 µg, erythromycin 15 µg,

gentamicin 10 μg , oxacillin 1 μg , penicillin 10 U, rifampicin 5 μg , tetracycline 30 μg , and sulfamethoxazole/trimethoprim 1.25/23.75 μg . As a test method, the bacteria were incubated on Mueller Hinton agar (MHA; Mbccl, Seoul, Korea) at 35°C for 24 h, and then one colony on MHA was inoculated into 3 mL of Mueller Hinton broth (MHB; Oxoid, England) at 35°C for 24 h. Cultured MHB broth was adjusted to 0.5 McFarland. After smearing the bacterial solution on MHA using a sterile cotton swab, an antibiotic disk was inoculated on the surface of the medium and incubated at 35°C for 24 h. Antibiotic susceptibility was determined according to the Clinical and Laboratory Standard Institute (CLSI) criteria as: susceptibility (S), intermediate (I), and resistance (R). The antibiotic susceptibility for *B. cereus* was not established in CLSI, so it was judged by applying the standard of *S. aureus*.

3. Results and discussion

3.1. Distribution of toxin genes in food poisoning bacteria

To analyze the possibility of food poisoning, the distribution of toxin genes in food poisoning bacteria isolated from dishcloths and scourers in childcare centers was analyzed. The distribution of the toxin genes in *S. aureus* is presented in Table 1. There are currently 16 types of toxin genes reported in *S. aureus*, but the main detection toxin

genes are reported as *sea*, *seb*, *sec*, *sed*, and *see* (Cho et al., 2011). Therefore, we used a kit containing these 5 toxin genes to check the toxin gene distribution of *S. aureus*. In this study, *sei* and *seg* toxin genes were detected in *S. aureus* isolated from dishcloths and scourers. According to a domestic *S. aureus* enterotoxin study, *sea*, *seb*, *sec*, *sed*, and *see* toxin genes account for 95% of the incidence of food poisoning, and among them, the *sea* toxin gene is reported as the main cause of food poisoning (Cho et al., 2011). As a result of analyzing 129 strains with toxin genes among *S. aureus* isolated from domestic *sushi* and *sashimi*, the distribution of *S. aureus* toxin genes showed a high detection rate with 107 strains of *sea* toxin type (Lee, 2011). In addition, it has been reported that 75 of the 76 strains of *S. aureus* isolated from various foods have the *sea* toxin gene (Cho et al., 2011). The *sei* and *seg* toxin genes in *S. aureus* isolated from kitchen cleaning tools at childcare centers were detected in this study, and these results were somewhat different from previous reports that the *sea* toxin gene was the dominant toxin gene. However, analysis of 72 strains of *S. aureus* isolated from agricultural production environments revealed *sei* and *seg* toxin genes in all 72 strains (Park et al., 2013). In addition, *sei* and *seg* toxin genes were judged to be the major cause of food poisoning, as *sei* and *seg* enterotoxin were detected in 11 of the 16 strains of *S. aureus* (Oh et

Table 1. Distribution of toxin genes in *Staphylococcus aureus* isolated from dishcloths and scourers

Isolates	Toxin gene							
	<i>sea</i>	<i>seb</i>	<i>sec</i>	<i>sed</i>	<i>see</i>	<i>seh</i>	<i>seg</i>	<i>sei</i>
Dishcloth C2	- ¹⁾	-	-	-	-	-	+ ²⁾	+
Scourer C11	-	-	-	-	-	-	+	+

¹⁾-, not detected.

²⁾+, detected.

al., 2021). Since the enterotoxin of *S. aureus* is not destroyed even when heated at 100°C for 30 min, care must be taken to avoid contamination and cross-contamination of the raw materials (MFDS, 2022). In particular, it is judged that if *S. aureus* contaminates washing tools such as dishcloths and scourers being used in childcare, it can imply the possibility of food poisoning.

The distribution of toxin genes in *B. cereus* isolated from childcare center kitchen cleaning tools is shown in Table 2. Diarrheal enterotoxin genes, such as *nheA*, *nheB*, *nheC*, *hblA*, *hblC*, and *hblD*, were detected in all five isolates. The *entFM* enterotoxin gene was detected in three of the five isolates, but the *cytK* enterotoxin gene and *ces* emetic toxin gene were not detected. *B. cereus* causes two major symptoms after infection in the human body. A diarrheal type causing diarrhea and an emetic type causing vomiting have been reported, which may cause both symptoms at the same time (Kim et al., 2009). Diarrhea-type infection causes abdominal pain and diarrhea after an incubation period of 8-16 h, and vomiting-type infection causes nausea and vomiting due to the emetic toxin produced by *B. cereus* (Agata et al., 2002). The diarrheal and emetic toxin genes of *B. cereus* have been reported as the main toxin genes (Kim

et al., 2011). *B. cereus* isolated from domestic medical environments and food mainly contains *nheABC* and *hblCDA* diarrhea toxin genes (Kim et al., 2015). As a result of this study, it was determined that *S. aureus* and *B. cereus* contaminated in dishcloths and scourers in childcare centers have toxin genes, which could cause food poisoning. Therefore, thorough sterilization and disinfection of dishcloths and scourers is required to prevent *S. aureus* and *B. cereus* from contaminating cooking utensils.

3.2. Antibiotic resistance

The antibiotic resistance of *S. aureus* and *B. cereus* was analyzed using the disc diffusion method. The antibiotic susceptibility of *S. aureus* isolated from childcare center kitchen cleaning tools is shown in Table 3. *S. aureus* isolated from dishcloths showed resistance to antibiotics such as ampicillin, cefotetan, cefepime, rifampicin, sulfamethoxazole/trimethoprim, oxacillin, and penicillin. *S. aureus* isolated from the scourer showed resistance to antibiotics, such as ampicillin, cefotetan, oxacillin, and penicillin. Because *S. aureus* is highly resistant, many strains have been reported to show resistance to various antibiotics, such as penicillin, gentamicin, and erythromycin (MFDS, 2022). *S. aureus* isolated from kitchen cleaning tools showed resistance to β -

Table 2. Distribution of toxin genes in *Bacillus cereus* isolated from dishcloths and scourers

Isolates	Toxin gene								
	<i>nheA</i>	<i>nheB</i>	<i>nheC</i>	<i>hblA</i>	<i>hblC</i>	<i>hblD</i>	<i>entFM</i>	<i>cytK</i>	<i>ces</i>
Dishcloth B7	+ ¹⁾	+	+	+	+	+	+	- ²⁾	-
Dishcloth D3	+	+	+	+	+	+	-	-	-
Scourer A14	+	+	+	+	+	+	+	-	-
Scourer C13	+	+	+	+	+	+	-	-	-
Scourer E8	+	+	+	+	+	+	+	-	-

¹⁾+, detected.

²⁾-, not detected.

Table 3. Antibiotic resistance of *Bacillus cereus* and *Staphylococcus aureus* isolated from dishcloths and scourers

Antimicrobial agent	<i>Bacillus cereus</i>					<i>Staphylococcus aureus</i>	
	Dishcloth B7	Dishcloth D3	Scourer A14	Scourer C13	Scourer E8	Dishcloth C2	Scourer C11
Ampicillin	R ¹⁾	R	R	R	R	R	R
Cefepime	R	R	R	R	R	R	I
Cefotetan	R	R	R	R	R	R	R
Ciprofloxacin	S ²⁾	S	S	I	S	S	S
Chloramphenicol	S	S	S	S	I	S	S
Clindamycin	S	S	S	S	S	S	S
Erythromycin	I ³⁾	S	S	I	I	S	I
Gentamicin	S	S	S	S	S	S	S
Oxacillin	R	R	R	R	R	R	R
Penicillin	R	R	R	R	R	R	R
Rifampicin	R	R	R	R	R	R	S
Tetracycline	S	S	S	I	R	I	S
Sulfamethoxazole/trimethoprim	R	R	R	R	R	R	S

¹⁾R, resistance.

²⁾S, susceptibility.

³⁾I, intermediate.

lactam antibiotics, such as ampicillin and cefotetan. These results were consistent with the previous reports; one report showed that resistance to β -lactam antibiotics was caused by an enzyme produced by the plasmid of *S. aureus*, while the other report showed that *S. aureus* isolated from crops showed high resistance to oxacillin and sensitivity to ciprofloxacin and gentamicin antibiotics (MFDS, 2013; Park, 2013).

The antibiotic susceptibility of *B. cereus* isolated from kitchen cleaning tools is shown in Table 3. All five strains of *B. cereus* showed resistance to antibiotics such as ampicillin, cefepime, penicillin, sulfamethoxazole/trimethoprim, and rifampicin. Clindamycin or gentamicin antibiotics are used for treatment because *B. cereus* is resistant to mycosis when using β -lactam antibiotics (Kim and Choi, 2009). All five strains of *B. cereus* isolated from the dishcloths and scourers of childcare centers showed

sensitivity to clindamycin and gentamicin antibiotics and resistance to β -lactam antibiotics. These results were consistent with a report that *B. cereus* produces β -lactamase and therefore has no effect on β -lactam antibiotics (Yoon et al., 2012). *S. aureus* and *B. cereus* isolated from kitchen cleaning tools were found to be multidrug-resistant strains that were resistant to five to eight antibiotics. Therefore, the thorough sterilization of dishcloths and scourers is necessary so that they do not become contaminated by *B. cereus* and *S. aureus*.

4. Conclusions

Toxin gene analysis revealed the presence of *sei* and *seg* toxin genes in *S. aureus* and *B. cereus* strains possessed the *nheA*, *nheB*, *nheC*, *hblA*, *hblC*, and *hblD* enterotoxin genes isolated from dishcloth and scourers in Jeollanam-do. *S. aureus* and *B. cereus*

were found to be multidrug-resistant strains that were resistant to five to eight antibiotics. Therefore, thorough sterilization and disinfection of dishcloths and scourers is required to prevent *S. aureus* and *B. cereus* from contaminating cooking utensils.

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Conflict of interests

The authors declare no potential conflicts of interest.

Author contributions

Conceptualization: Kim CY, Kim JB. Formal analysis: Kim CY, Im JY, Kim E. Methodology: Kim CY, Im JY, Kim E. Writing - original draft: Kim CY, Kim M, Kim JB. Writing - review & editing: Kim M, Kim JB.

Ethics approval

This article does not require IRB/IACUC approval because there are no human and animal participants.

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