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Antibiotic resistance of *Bacillus* species isolated in foodstuff samples that were collected in Babylon (Iraq)

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Abstract

A total of 164 food samples were collected from various supermarkets and farmers in various areas of Babylon, were placed in plastic containers, and were transported to the laboratory. The isolation of *Bacillus* species was performed by culture in blood agar, chromogenic agar, and the absence of growth on McConkey agar, by staining with Gram stain, and through molecular identification of the species' 16SrRNA gene and sequencing. The antibiotic susceptibility test used eight types of antibiotics. The undertaken sequencing identified the *Bacillus* isolates to belong to the following species: *B. subtilis* (2), *B. cereus* (6), *B. thuringiensis* (1), *B. anthracis* (1), and *B. spizizenii* (1). The susceptibility test of the six *B. cereus* isolates revealed that 5 (83.3%), 4 (66.6%), 3 (50%), 2 (33.3%), 2 (33.3%), 2 (33.3%), 1 (16.6%), and 0 (0%) were resistant to rifampicin, clindamycin, erythromycin, tetracycline, trimethoprim, nitrofurantoin, gentamicin, and ciprofloxacin, while the respective resistance numbers for the two isolates of *B. subtilis* were 2 (100%), 2 (100%), 2 (100%), 0 (0%), 1 (50%), 0 (0%), 0 (0%), and 0 (0%). One isolate of *B. thuringiensis* presented resistance to erythromycin, erythromycin, trimethoprim, and rifampicin, while one isolate of *B. anthracis* was found to be resistant to gentamicin, erythromycin, nitrofurantoin, and rifampicin. The *B. spizizenii* isolate was resistant to all antibiotics except gentamicin and trimethoprim.

KEYWORDS
Bacillus spp., antibiotics, sequencing, foodstuff, antibiotic resistance

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1. INTRODUCTION

Bacterial infections transmitted through food have become one of the most prevalent global public health issues. The *Bacillus* species are Gram-positive, rod-shaped, motile (flagellate) bacteria that can be aerobic or anaerobic by choice. They are known for generating spores and biofilms, and are often found in nature, having been isolated from both fermented and unfermented diets and foodstuff [1,2].

Many *Bacillus* species, such as *B. cereus*, *B. subtilis*, and *B. licheniformis*, demonstrate resistance to antibiotics and can transmit genes for resistance to antibiotics. This suggests that residues of antibiotics could find their way into consumer food items and, subsequently, into the food chain that humans are part of [3]. This may result in the development of bacteria resistant to multiple drugs, accompanied by the likelihood of transferring resistance genes to pathogenic and non-pathogenic bacteria [4]. The purpose of this study was to examine the antibiotic susceptibility of *Bacillus* species that have been isolated from food samples in Babylon (Iraq).

2. MATERIALS AND METHODS

Sample collection: In this study, a total of 164 food samples were collected; of these, 19 were of meat products (chicken, beef, minced meat), 77 were of dairy product (pasteurized milk, raw milk, cheese, and yogurt), and 68 were of other foods. The samples were collected from September 2023 to November 2023 from different supermarkets and farmers in various regions of Babylon (Iraq).

Bacterial identification: The food samples were measured, labelled distinctly, gathered individually in plastic containers, and then transported to the laboratory for suspension in brain heart infusion broth and incubation at 37°C for 24 h. The samples were then culture in blood agar, chromogenic agar, and MacConkey agar. The form, size, texture, and colony arrangement were then examined. Each colony was picked up, stained with Gram stain, and checked under the light microscope (100x) by using oil emersion.

DNA extraction and polymerase chain reaction (PCR): The DNA extraction was done according to the manufacturer's protocol (Geneaid, Taiwan). The PCR mixture for the 16SrRNA genes in this study was set up for each gene alone in a final volume of 25 µL. The PCR is an enzymatic reaction used for the *in vitro* amplification of target DNA with specific primers and a DNA polymerase. The extracted DNA, primers, and master mix were vortexed and centrifuged briefly to bring the contents to the bottom of the tubes, then placed in a PCR thermocycler in order to amplify the 16SrRNA genes (1,500 bp; F - AGA GTT TGA TCC TGG CTC AG; R - GGT TAC CTT GTT ACG ACT T [4].

Sequencing of 16SrRNA gene: The PCR products of 17 specimens of *Bacillus* species were stored at -20°C, and then the nucleotide sequences of their 16SrRNA genes were carried out by sending the specimens and primer to Macrogen Inc. (South Korea).

Antibiotic susceptibility test: The Kirby-Bauer method was used in order to carry out the antibiotic susceptibility test. The inhibition zones around the disks were measured in millimetres (mm) by using a metric ruler [5].

3. RESULTS AND DISCUSSION

A total of 164 different food samples were collected from September 2023 to November 2023 from various supermarkets and farmers in various areas of Babylon (Table 1). The prevalence of *Bacillus* species according to the undertaken cultures and the biochemical and molecular identification was 7.5% (11 samples). The *Bacillus* isolates belonged to the following species: *B. subtilis* (2), *B. cereus* (6), *B. thuringiensis* (1), *B. anthracis* (1), and *B. spizizenii* (1). Hornik *et al*. [6] have found that the percentage of *Bacillus* species is 10%. Another study has found that 35% of different food samples were positive for *B. cereus* [7]. In China, a recent study has found that 50% of all vegetable samples contained *B. cereus* [8], whereas in Thailand the percentage was approximately 21% [9]. The results differ depending on the origin and number of the sample collection.

The antibiotic susceptibility test used eight types of antibiotics. The undertaken sequencing identified the *Bacillus* isolates to belong to the following species: *B. subtilis* (2), *B. cereus* (6), *B. thuringiensis* (1), *B. anthracis* (1), and *B. spizizenii* (1). The susceptibility test of the six *B. cereus* isolates revealed that 5 (83.3%), 4 (66.6%), 3 (50%), 2 (33.3%), 2 (33.3%), 2 (33.3%), 1 (16.6%), and 0 (0%) were resistant to rifampicin, clindamycin, erythromycin, tetracycline, trimethoprim, nitrofurantoin, gentamicin, and ciprofloxacin, while the respective resistance numbers for the two isolates of *B. subtilis* were 2 (100%), 2 (100%), 2 (100%), 0 (0%), 1 (50%), 0 (0%), 0 (0%), and 0 (0%). One isolate of *B. thuringiensis* presented resistance to erythromycin, erythromycin, trimethoprim, and rifampicin, while one isolate of *B. anthracis* was found to be resistant to gentamicin, erythromycin, nitrofurantoin, and rifampicin. The *B. spizizenii* isolate was resistant to all antibiotics except gentamicin and trimethoprim.

Fiedler *et al*. [10] have found that all *Bacillus* species isolates were resistant to penicillin and cefotaxime, while the resistance to amoxicillin/clavulanic acid and ampicillin was 99.3%. They have also found that the percentage of sensitivity was 99.3%, 98.6%, 98.0%, 93.9%, 91.8%, 76.2%, 88.4%, and 52.4% against ciprofloxacin, chloramphenicol, amikacin, imipenem, erythromycin, gentamicin, tetracycline, and the trimethoprim / sulfamethoxazole combination, respectively [10].

Both public health officials and food processors are now concerned about the rising incidence of multiple antibiotic resistance. Since fresh food doesn't need to be heated further before consumption, there is worry that bacteria resistant to antibiotics might make it through the gastrointestinal tract and complicate therapy for elderly or very young patients, as well as for those with compromised immune systems [10].

Table 1. Overview of the foodstuff samples assessed and of the isolates identified.

Types of samples			**Positive samples** (number and %); **isolates**	**Negative samples** (number and %)	**Total samples** (number and %)
Milk products	yogurt		0 (0%)	11 (6.7%)	11 (6.7%)
	milk	raw	1 (0.6%); *B. cereus*	21 (14.3%)	22 (13.4%)
		canned	1 (0.6%); *B. cereus*	5 (3%)	6 (3.6%)
	cheese	raw	0 (0%)	11 (6.7%)	11 (6.7%)
		canned	0 (0%)	12 (7.3%)	12 (7.3%)
	cream	raw	1 (0.6%); *B. spizizenii*	4 (2.4%)	5 (3%)
		canned	0 (0%)	10 (6%)	10 (6%)
Meat products	beef meat	raw	0 (0%)	4 (2.4%)	4 (2.4%)
		cooked	0 (0%)	7 (4.2%)	7 (4.2%)
	chicken meat	raw	0 (0%)	4 (2.4%)	4 (2.4%)
		cooked	0 (0%)	4 (2.4%)	4 (2.4%)
	fish meat	raw	0 (0%)	1 (0.6%)	1 (0.6%)
		cooked	0 (0%)	2 (1.2%)	2 (1.2%)
Other products	vegetable		1 (0.6); *B. subtilis*	9 (5.4%)	10 (6%)
	rice	cooked	0 (0%)	7 (4.2%)	7 (4.2%)
		raw	1 (0.6); *B. subtilis*	3 (1.8%)	4 (2.4%)
	macaroni		0 (0%)	6 (3.6%)	6 (3.6%)
	bulgur		3 (1.8); *B. cereus* (2x), *B. thuringiensis*	5 (3%)	8 (4.8)
	flour		1 (0.6); *B. cereus*	4 (2.4%)	5 (3%)
	starch		1 (0.6); *B. anthracis*	3 (1.8%)	4 (2.4%)
	wheat		0 (0%)	2 (1.2%)	2 (1.2%)
	barley		0 (0%)	2 (1.2%)	2 (1.2%)
	honey		0 (0%)	5 (3%)	5 (3%)
	bread		1 (0.6); *B. cereus*	4 (2.4%)	5 (3%)
	juice		0 (0%)	7 (4.2%)	7 (4.2%)
Total samples (number and %)			**11 (7.5%)**	**153 (92.4%)**	**164 (100%)**

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

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