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Biofilm Production and Antimicrobial Susceptibility Profiles of *Bacillus* spp. from Meats

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ABSTRACT

The genus *Bacillus* is frequently found in soil, water, and food. *Bacillus cereus* and *Bacillus anthracis* are the main pathogens causing foodborne diseases and serious infections in humans. A total of 52 *Bacillus* spp. from meat samples was tested for determination of biofilm production, antimicrobial resistance pattern, and beta-lactamase activity. The 24 (46.1%) *Bacillus* isolates were found to be for biofilm production. Of the 24 (46.1%) biofilm producer *Bacillus* isolates, 13 (25%), 6 (11.5%) and 5 (9.6%) were considered as strong, moderate and weak biofilm producer, respectively. The most common species for the production of biofilm was *Bacillus thuringiensis* (80%). Antimicrobial disk susceptibility tests of *Bacillus* spp. revealed high resistance to ampicillin (84.6%) followed by penicillin (75%), cefepime (34.6%), and cefoxitin (26.9%). A multidrug resistance to at least 3 or more antimicrobials was observed in the 25 isolates (48.1%). All *Bacillus* spp. were sensitive to vancomycin, gentamicin, amikacin, ciprofloxacin, and imipenem. The susceptibility rate to streptomycin, chloramphenicol, and trimethoprim-sulphamethoxazole was 94.2%. Among the isolates, the 6 (11.5%) isolates were found to be sensitive to all antimicrobial agents tested. Besides, only one isolate from meat was found to be positive for beta-lactamase test. The existence of biofilm production as a virulence factor and of multidrug resistance in bacteria isolated from food should not be underestimated in terms of food safety, public health, and economic concerns.

Keywords: *Bacillus* spp., biofilm production, antimicrobial resistance, beta-lactamase, meat

1. INTRODUCTION

The *Bacillus* genus are rod-shaped and endospore forming organisms that are widely distributed in the natural environment due to their many physiological properties such as endospore formation and nutritional versatility. Endospores readily survive and are being contaminants in environments and foods due to resistance to heat, radiation, disinfectants, and desiccation. Therefore, the presence of *Bacillus* species such as *B. cereus*, *B. subtilis*, *B. licheniformis*, and *B. pumilus* in foods is inevitable and undesirable due to considered as foodborne pathogens and spoilage-associated species. The contamination of food with pathogenic and spoilage strains of *Bacillus* is a major concern for human health and food safety [1, 2]. Among the *Bacillus* species, mostly *B. cereus* and *B. anthracis* are known as the most frequent human pathogens which cause a wide range of infections including

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food poisoning, anthrax, bacteremia, pneumonia, endocarditis, meningitis, endophthalmitis, respiratory, and soft tissue infections [1, 3]. Many *Bacillus* species are able to produce a wide variety of enterotoxins, emetic toxins, extracellular enzymes, and biofilms which are considered as major contributing factors in the establishment of infections by these pathogenic bacteria [2].

Biofilms considered as a potential virulence factor by bacteria including *Bacillus* species are microbial communities embedded in an extracellular matrix consisted of polysaccharide [4, 5]. Most bacteria are able to form biofilms on abiotic surfaces in food processing facilities, thereby being a major source of food contamination. Besides, biofilms by pathogenic bacteria may easily attach to surfaces such as living tissues, indwelling medical devices and industrial or natural aquatic systems under suitable conditions. Therefore, biofilms play a significant role in the transmission of pathogens, microbial contamination and colonization that cause to infections [5, 6]. Moreover, biofilm producing bacteria can be responsible for development of some biomaterial-associated infections such as cystic fibrosis, native valve endocarditis, otitis media, periodontitis, and chronic prostatitis. However, bacteria within biofilms on medical devices as a cause of infection dramatically reduce antimicrobial susceptibility to antimicrobial agents [4, 7, 8]. In addition to the decrease in antibiotic susceptibility, the biofilm producing bacteria has an increased resistance to extreme temperatures, light, drying, cleaning agents [4].

Antimicrobial resistance has been increasing public health problem worldwide due to misuse or overuse of antimicrobial agents in aquaculture, agriculture, and human medicine [9, 10]. Resistant bacteria can be transmitted from food such as fish and ground beef to human. Infections caused by these resistant pathogens can be treated with difficulty. Although *Bacillus* species are an unusual source of human infection, they can cause mild to severe infections in immunocompromised individuals. Systemic antimicrobial therapy is usually required in the treatment of most serious *Bacillus* infections [1, 11]. Vancomycin, clindamycin, ciprofloxacin, and gentamicin can be used successfully in the treatment of most serious *Bacillus* infections [1]. Anthrax caused by *B. anthracis* generally is treatable with penicillin. Nevertheless, most strains of *B. anthracis* are resistant to many cephalosporins. Furthermore, a broad-spectrum beta-lactamase produced by *Bacillus* species inactivates the penicillins and cephalosporins thus make the organism resistant to penicillins and cephalosporins [2, 3].

Determination of biofilm production as an important virulence trait and screening antimicrobial resistance in bacteria from food are important for recognition of their pathogenic potential. Therefore, this study aims to determine the biofilm production, antimicrobial resistance profiles, and beta-lactamase activity of the *Bacillus* spp. isolated from meat samples.

2. MATERIALS AND METHODS

2.1. Bacterial isolates

A total of 52 *Bacillus* spp. comprising 24 *B. cereus*, 2 *B. anthracis*, 10 *B. thuringiensis*, 9 *B. subtilis*, 3 *B. licheniformis*, 2 *B. pumilus*, 1 *B. firmus*, and 1 *B. coagulans* from fish and ground beef were performed in this study. All isolates were grown in Brain Heart Infusion (BHI) broth (Merck, Darmstadt, Germany) at 37°C for 24 h.

2.2. Biofilm production

The adherence of *Bacillus* spp. was tested using a microtiter plate assay previously described by [12] with some modifications. Briefly, *Bacillus* isolates were grown in Tryptic Soy broth (TSB) (Merck) overnight at 37°C. The overnight culture was diluted with TSB in order to obtain optical density (OD) at approximately $1.5x10^8$ CFU per mL. The 96 well flat bottom tissue culture plates were filled with 200 µL of *Bacillus* culture in TSB. Negative control wells contained TSB only. The plates were incubated at 30°C for 48 h in a static condition. At the end of incubation, the contents of the plates were removed by inverting the plates, and then the wells were washed five times with sterile distilled water. The plates were air-dried for 45 min and each well was stained with 200 µL of 1% crystal violet solution for 45 min. After staining, the plates were washed five times with sterile distilled water. For the quantitative analysis of biofilm formation, 200

µL of ethanol-acetone solution (4:1) was added to the wells. The OD of each well was measured at 570 nm using a microtiter plate reader (Thermo Electron Corporation Multiskan Spectrum, Vantaa, Finland). Isolates were classified into the four following categories based upon the absorbance: no biofilm producer (OD ≤ ODc), and weak (ODc <OD ≤ 2XODc), moderate (2XODc < OD ≤ 4XODc), or strong (OD > 4XODc) biofilm producer [13], where ODc is the optical density measured for the negative control. Six replicate wells were performed for each experimental parameter and each data point was averaged from these six.

2.3. Antimicrobial susceptibility test

Bacillus spp. isolates from fish and ground beef were examined for evaluation of antimicrobial resistance patterns using the disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) [14]. Twenty antimicrobial agents (Oxoid, Basingstoke, UK) were chosen according to their common use. They belonged to the following groups: penicillins (penicillin -10 units, ampicillin-10 µg), beta-lactams (amoxicillin-clavulanic acid - 30 µg, cephalothin - 30 µg, cefoxitin - 30 µg, ceftriaxone - 30 µg, cefepime - 30 µg), carbapenems (imipenem - 30 µg), glycopeptides (teicoplanin - 30 µg, vancomycin - 30 µg), aminoglycosides (gentamicin - 10 µg, streptomycin - 10 µg, amikacin - 30 µg), macrolides (erythromycin - 15 µg), tetracyclines (tetracycline - 30 µg), fluoroquinolones (ciprofloxacin - 5 µg), phenicols (chloramphenicol - 30 µg), miscellaneous (trimethoprim-sulfamethoxazole - 25 µg, clindamycin - 2 µg, rifampin - 5 µg). The turbidity of bacterial suspension was adjusted to 0.5 McFarland standard on Mueller Hinton broth (Merck). Then the suspensions were spread on Mueller Hinton agar (Merck) and the antibiotic disks were placed on the agar surface. The inhibition zone of each bacterium was measured after incubation on Mueller Hinton agar (37°C /18 h). The results were interpreted as susceptible, intermediate or resistant with respect to the CLSI [14] guideline for *Staphylococcus* spp.

2.4. Beta-lactamase activity

The production of beta lactamase was determined by the acidimetric strip method. This method was done as previously described [15]. Penicillin and bromocresol purple were dissolved in NaOH solution. A filter paper (Whatman No: 1) was placed in a Petri dish. A few drops of the solution were then added on to the filter paper until the filter strips was almost saturated. A loopfull of bacteria was kept in the center of the filter paper. The presence of purple color in the paper around the bacterial mass indicated positive reaction for beta-lactamase.

3. RESULTS

3.1. Biofilm production of *Bacillus* spp.

In the Table 1, the biofilm producing ability of the *Bacillus* spp. from meats is given. Of the 52 *Bacillus* spp. tested, the 24 (46.1%) isolates were considered as biofilm producers. The incidence of biofilm production in the *Bacillus* isolates from fish and ground beef was 45% and 50%, respectively. The biofilm producing isolates were categorized as strong (13 isolates), moderate (6 isolates), weak producers (5 isolates). The most common *Bacillus* species was *B. thuringiensis* (80%) regarded as biofilm producers. The biofilm production of the other *Bacillus* species was as follows: *B. licheniformis* 66.7%, *B. anthracis*, *B. thuringiensis* 50%, *B. pumilus* 50%, *B. subtilis* 33.3%, and *B. cereus* 12.5%. None of the *B. coagulans* and *B. firmus* was able to form biofilm. The distribution of the 24 biofilm producing *Bacillus* isolates from fish and ground beef and their antimicrobial resistance profiles and beta-lactamase activity is presented in Table 2. Most *Bacillus* isolates were resistant to ampicillin (95.5%) and penicillin (91.7%). A multidrug resistance was observed in 14 (58.3%) of the biofilm producing isolates to at least three or more antimicrobials. All of the 7 isolates from seawater fish were strong biofilm producer which had resistance to at least two antimicrobial agents. Moreover, *B. licheniformis* from seawater fish was resistant to eight antimicrobials. Only one isolate recognized as *B. cereus* from freshwater fish was sensitive to all antimicrobials tested.

Table 1. Biofilm production of *Bacillus* species by microtiter plate technique

		Interpretation of biofilm			
Bacillus species	No.of isolates	No adherence	Weak adherence	Moderate adherence	Strong adherence
B. cereus	24	16[1] (66.7%)	3 (12.5%)	3 (12.5%)	2 (8.3%)
B. anthracis	2	1 (50%)	-	1 (50%)	-
B. thuringiensis	10	2 (20%)	2 (20%)	1 (10%)	5 (50%)
B. subtilis	9	5 (55.6%)	-	1 (11.1%)	3 (33.3%)
B. licheniformis	3	1 (33.3%)	-	-	2 (66.7%)
B. pumilus	2	1 (50%)	-	-	1 (50%)
B. coagulans	1	1 (100%)	-	-	-
B. firmus	1	1 (100%)	-	-	-
Total	52	28 (53.9%)	5 (9.6%)	6 (11.5%)	13 (25%)

[1] Number of positive isolates

Table 2. The distribution of the 24 biofilm producing *Bacillus* isolates from fish and ground beef and their antimicrobial resistance profiles and beta-lactamase activity

Isolate	*Bacillus* spp.	Origin	Biofilm production	Antimicrobial resistance	Beta-lactamase
F1	*B. cereus*	Freshwater fish	Moderate	AMP, P, FEP	-
F2	*B. cereus*	Freshwater fish	Moderate	-	-
F3	*B. cereus*	Freshwater fish	Strong	AMP, P, FEP, DA	-
F4	*B. cereus*	Freshwater fish	Weak	AMP, P, TE	-
F5	*B. cereus*	Freshwater fish	Moderate	AMP, P, RD	-
F6	*B. cereus*	Freshwater fish	Weak	AMP, P, RD	-
F7	*B. thuringiensis*	Freshwater fish	Strong	AMP, P	-
F8	*B. thuringiensis*	Freshwater fish	Moderate	AMP	-
F9	*B. thuringiensis*	Freshwater fish	Strong	AMP, P, E	-
F10	*B. thuringiensis*	Freshwater fish	Weak	AMP, P	-
F11	*B. licheniformis*	Freshwater fish	Strong	AMP, P, AMC, DA	-
S1	*B. cereus*	Seawater fish	Strong	AMP, P	-
S2	*B. thuringiensis*	Seawater fish	Strong	AMP, P	-
S3	*B. thuringiensis*	Seawater fish	Strong	AMP, FEP	-
S4	*B. thuringiensis*	Seawater fish	Strong	AMP, P, TE	-
S5	*B. subtilis*	Seawater fish	Strong	AMP, P, AMC, DA	-
S6	*B. subtilis*	Seawater fish	Strong	AMP, P	-
S7	*B. licheniformis*	Seawater fish	Strong	AMP, P, FEP, FOX, S, E, TE,C	+
G1	*B. cereus*	Ground beef	Weak	AMP, P, FEP, FOX	-
G2	*B. anthracis*	Ground beef	Moderate	AMP, P	-
G3	*B. thuringiensis*	Ground beef	Weak	AMP, P, TE	-
G4	*B. subtilis*	Ground beef	Strong	AMP, P	-
G5	*B. subtilis*	Ground beef	Moderate	AMP, P, FEP, DA	-
G6	*B. pumilus*	Ground beef	Strong	AMP, P, FEP, FOX	-

Abbreviations of antimicrobial agents are listed in alphabetical order. AMC, amoxicillin-clavulanic acid; AMP, ampicillin; C, chloramphenicol; DA, clindamycin; E,erythromycin; FEP, cefepime; FOX, cefoxitin; P, penicillin; RD, rifampin; S, streptomycin; TE, tetracycline

3.2. Antimicrobial resistance profiles of *Bacillus* spp.

The antimicrobial susceptibility of 52 *Bacillus* spp. from fish and ground beef samples to various antimicrobial agents was examined. The antimicrobial resistance pattern of *Bacillus* spp. is given in Table 3. The most common resistance to ampicillin and penicillin G was detected in 84.6% and 75% of the *Bacillus* isolates, respectively. Among the cephalosporins tested, the isolates were resistant to cefepime (34.6%), followed by cefoxitin (26.9%), cephalothin (13.5%), and ceftriaxone (13.5%).

Resistance to rifampin and clindamycin was 13.5%. Furthermore, all *Bacillus* spp. were sensitive to imipenem, vancomycin, amikacin, gentamicin, and ciprofloxacin. Multidrug resistance pattern was observed in 25 (48.1%) of the isolates to at least three or more antimicrobials (Table 4). Only six isolates had resistance to one antimicrobial. Resistance to two antimicrobials was also detected in 15 (28.8%) of the isolates.

Table 3. Antimicrobial resistance patterns of *Bacillus* spp. from meat

Antimicrobial Class	Antimicrobial agents	Conc.[1] (µg/disk)	Number of isolates (%)		
			Resistant	Intermediate	Susceptible
Penicillins	Ampicillin	30	44 (84.6)	0 (0)	8 (15.4)
	Penicillin G	10	39 (75)	0 (0)	13 (25)
B-lactams	Amoxicillin-clavulanic acid	30	11 (21.2)	0 (0)	41 (78.8)
Cephems	Cefepime	30	18 (34.6)	3 (5.8)	31 (59.6)
	Cephalothin	30	7 (13.5)	2 (3.8)	43 (82.7)
	Ceftriaxone	30	7 (13.5)	15 (28.8)	30 (57.7)
	Cefoxitin	30	14 (26.9)	0 (0)	38 (73.1)
Carbapenems	Imipenem	10	0 (0)	0 (0)	52 (100)
Glycopeptides	Teicoplanin	30	1 (1.9)	1 (1.9)	50 (96.2)
	Vancomycin	30	0 (0)	0 (0)	52 (100)
Aminoglycosides	Amikacin	30	0 (0)	0 (0)	52 (100)
	Gentamicin	10	0 (0)	0 (0)	52 (100)
	Streptomycin	10	1(1.9)	2 (3.9)	49 (94.2)
Macrolides	Erythromycin	15	2 (3.9)	8 (15.4)	42 (80.7)
Tetracyclines	Tetracycline	10	6 (11.5)	7 (13.5)	39 (75)
Fluoroquinolones	Ciprofloxacin	5	0 (0)	0 (0)	52 (100)
Lincosamides	Clindamycin	2	7 (13.5)	22 (42.3)	23 (44.2)
Folate pathway inhibitors	Trimethoprim/sulfamethoxazole	25	3 (5.8)	0 (0)	49 (94.2)
Phenicols	Chloramphenicol	30	1 (1.9)	2 (3.9)	49 (94.2)
Ansamycins	Rifampin	5	7 (13.5)	22 (42.3)	23 (44.2)

[1] Concentration of disk

Table 4. Multiple antimicrobial-resistant *Bacillus* spp. in meats

Antimicrobial resistance profiles	Number of antimicrobials	Number of resistant *Bacillus* spp. (%)
AMP, P, E	3	1 (1.9)
AMP, P, FEP	3	1 (1.9)
AMP, P, RD	3	1 (1.9)
AMP, P, TE	3	4 (7.6)
AMP, P, AMC, DA	4	2 (3.9)
AMP, P, AMC, RD	4	1 (1.9)
AMP, P, FEP, DA	4	2 (3.9)
AMP, P, FEP, FOX	4	2 (3.9)
AMP, P, FEP, FOX, DA	5	2 (3.9)
AMP, P, AMC, FEP, KF, CRO, FOX	7	2 (3.9)
AMP, P, AMC, KF, FOX, TEC, DA	7	1 (1.9)
AMP, P, AMC, FEP, CRO, FOX, SXT, RD	8	1 (1.9)
AMP, P, AMC, FEP, KF, CRO, FOX, RD	8	1 (1.9)
AMP, P, FEP, FOX, E, TE, C, S	8	1 (1.9)
AMP, P, AMC, FEP, KF, CRO, FOX, SXT, RD	9	2 (3.9)
AMP, P, AMC, FEP, KF, CRO, FOX, TE, RD	9	1 (1.9)
Total		25 (48.1)

Abbreviations of antimicrobial agents are listed in alphabetical order. AMC, amoxicillin-clavulanic acid; AMP, ampicillin; C, chloramphenicol; CRO, ceftriaxone; DA, clindamycin; E, erythromycin; FEP, cefepime; FOX, cefoxitin; KF, cephalothin; P, penicillin; RD, rifampin; S, streptomycin; SXT, trimethoprim/sulfamethoxazole; TE, tetracycline; TEC, teicoplanin

3.3. Beta-lactamase production

In this study, only one isolate identified as *B. licheniformis* from seawater fish was found to be positive for beta-lactamase production (Table 2). Nevertheless, the 39 isolates were resistant to penicillin and none of them were positive for beta-lactamase.

4. DISCUSSION

Bacillus spp. as spore-forming bacteria are widely distributed in nature and isolated from the environment, food, animals, and humans. The resistance of spores to heat, radiation, disinfectants, and desiccation results in *Bacillus* species being frequent contaminants in foods. The pathogenic bacteria such as *B. cereus* and *B. anthracis* can be directly or indirectly transmitted through food to human and causes serious threat for public health and food safety [3].

Numerous studies have shown that *Bacillus* species known as the most common bacteria which are capable of adhering and have a high tendency to form a biofilm on various surfaces in food industry, medical field, and water systems [4, 6, 16]. Biofilm production by *Bacillus* species from different sources using microtiter plate assay has been investigated [6, 16, 17]. The present data indicated that the biofilm production by microtiter assay was predominant in 8 (80%) of the *B. thuringiensis* isolates (Tables 1, 2). Of the 24 *B. cereus*, 8 (33.3%) were found to be positive for biofilm production in this study. Biofilm forming capability of *B. cereus* from a milk-processing dairy plant was documented [6]. In this study, among the *Bacillus* isolates, biofilm producers were commonly found in the ground beef isolates (50%), followed by the freshwater fish isolates (45.8%) and the seawater fish isolates (43.8%). In this study, among the biofilm producer *Bacillus* spp., the proportion of resistance to three or more antimicrobials was 58.3% while 35.7% in the non-biofilm producing isolates that it may be indication of a relationship between biofilm production and antimicrobial resistance. Indeed, *Bacillus* species in biofilms can generate highly resistant and

adhesive spores that will increase the resistance of the bacteria to antimicrobial agents or to disinfectants [4, 18]. The extensive use of antimicrobials in food animals and aquaculture for growth enhancement or treatment purposes has contributed to the emergence and development of antimicrobial resistance [3, 19]. Common antibiotic classes including penicillins, aminoglycosides, macrolides, quinolones, sulfonamides, and tetracyclines on the World Health Organization list are regularly used in agriculture and aquaculture [19]. Penicillin is the oldest and widely used in the treatment of *Bacillus* infections such as anthrax [1, 3]. Resistance among the *Bacillus* spp. was in particular seen to penicillin and cephalosporins [3]. Our results were in close agreement with previous studies reported a high resistance to penicillin and ampicillin in *Bacillus* spp. [20, 21, 22].

In this study, many of the isolates were susceptible to trimethoprim/sulfamethoxazole (94.2%), erythromycin (81%) and tetracycline (75%) that these antimicrobials have been used as alternative drugs for patients allergic to penicillin [1]. Furthermore, in a study conducted by Yim et al. [21], the results related to erythromycin, tetracycline, and trimethoprim/sulfamethoxazole were in agreement with our results. In our study susceptibility of the isolates to erythromycin and tetracycline was 80.7% and 75%, respectively. Similarly, Yim et al. [21] reported that isolates were susceptible to tetracycline (90.8%) and erythromycin (78.2%). Chaabouni et al. [22] also documented that susceptibility of isolates to tetracycline was 97% and to erythromycin 88%.

Chloramphenicol, clindamycin, tetracycline, and erythromycin have activity against *Bacillus* species [11]. On contrast, the isolates in our study were sensitive to clindamycin (44.2%), tetracycline (75%), erythromycin (80.7%), and chloramphenicol (94.2%). Compared to our results, Noor Uddin et al. [23] reported resistance to clindamycin (38.3%), chloramphenicol (30%) and erythromycin (16.7%) in *Bacillus* spp. from probiotic products used in aquaculture. A high level of resistance to chloramphenicol (61.5%) among *Bacillus* strains isolated from Mbuja was reported by Mohammadou et al. [24]. Infections associated with *Bacillus* have been treated successfully by both vancomycin and clindamycin [3, 11] that this data agree with our results related resistance to vancomycin (100%) and clindamycin (86.5%). Besides, previous studies indicated that all *Bacillus* isolates were sensitive to vancomycin [20, 22, 24]. A study by Yim et al. [21] reported that vancomycin susceptibility rate was 86.2%. Noor Uddin et al. [23] reported that clindamycin resistance in the *Bacillus* strains from probiotic products used in aquaculture was 38.3% which were higher than our result (13.5%). Moreover, high levels of clindamycin resistance (65.5%) have been reported by Ikeda et al. [25] in *B. cereus* from blood stream infections. On the other hand, in this study, resistance to three or more antimicrobials was 48.1% in *Bacillus* spp. from meat when this rate was 20% in *Bacillus* spp. from probiotic products used in aquaculture reported by Noor Uddin et al. [23].

In conclusion, this study provides substantial information on the production of biofilms and antimicrobial resistance pattern in *Bacillus* spp. from fish and ground beef. The presence of biofilm producing bacteria and the emergence of antimicrobial resistant bacteria in certain fields including food, aquaculture, and medical may be considered as a major threat to public health and food safety.

REFERENCES

[1] W. E. Farrar and A. C. Reboli, “The Genus *Bacillus*-Medical”. In: Dworkin, M, Falkow S. Rosenberg E, Stackebrandt E, Schleifer KH. (Eds.), The Prokaryotes, vol.4. Springer, Minneapolis, pp. 609-630, 2006.

[2] A. K. Bhunia, “Foodborne microbial pathogens: mechanisms and pathogenesis”, Springer, New York, 2008.

[3] N. A. Logan, A. R. Hoffmaster, S. V. Shadomy, K. E. Stauffer, “*Bacillus* and other aerobic endospore forming bacteria”. In: Versalovic J, Carroll, KC, Funke G, Jorgensen JH, Landry, ML, Warnock DW. (Eds.), Manual of Clinical Microbiology, 10th edn, Vol. 1, Washington DC, American Society for Microbiology, pp. 381–402. 2011.

[4] L. V. Poulsen, "Microbial biofilm in food processing", *Lebensmittel-Wissenschaft and Technologie*, vol. 32, pp. 321-326, 1999.

[5] R. M. Donlan and J. W. Costerton, "Biofilms: survival mechanisms of clinically relevant microorganisms", *Clinical Microbiology Reviews*, vol. 15, pp. 167-193, 2002.

[6] A. Cherif-Antar, B. Moussa Boudjemaa, N. Didouh, K. Medjahdi, B. Mayo, and A. B. Florez, "Diversity and biofilm-forming capability of bacteria recovered from stainless steel pipes of a milk-processing dairy plant", *Dairy Science and Technology*, vol. 96, pp. 27–38, 2016.

[7] T. F. Mah and G. A. O'Toole, "Mechanisms of biofilm resistance to antimicrobial agents", *Trends in Microbiology*, vol. 9, pp. 34-39, 2001.

[8] J. D. Brooks and S. H. Flint, "Biofilms in the food industry: problems and potential solutions", *International Journal of Food Science and Technology*, vol. 43 no. 12, pp. 2163–2176, 2008.

[9] P. F. McDermott, S. Zhao, D. D. Wagner, S. Simjee, R. D. Walker, and D. G. White, "The food safety perspective of antibiotic resistance", *Animal Biotechnology*, 13:71–84. 2002.

[10] J. Romero, C. G. Feijoó, and P. Navarrete, "Antibiotics in Aquaculture – Use, Abuse and Alternatives", *Health and Environment in Aquaculture*, 10.5772/28157, 2012.

[11] C. U. Tuazon, H. W. Murray, C. Levy, M. N. Solny, J. A. Curtin, and J. N. Sheagren, "Serious infections from *Bacillus* sp.", *Journal of the American Medical Association*, 241:1137-1140, 1979.

[12] D. Djordjevic, M. Wiedmann, and L. A. McLandsborough, "Microtiter plate assay for assessment of *Listeria monocytogenes* biofilm formation", *Applied and Environmental Microbiology*, vol. 68, pp. 2950-2958, 2002.

[13] S. Stepanovic, D. Vukovic, I Dakic, B. Savic, and M. Svabic-Vlahovic, "A modified microtiter-plate test for quantification of staphylococcal biofilm formation", *Journal of Microbiological Methods*, vol.40, pp. 175–179, 2000.

[14] Clinical and Laboratory Standards Institute. "Performance standards for antimicrobial disk susceptibility tests; approved standard-11th ed CLSI document M02-A1", vol. 32, no.1, *Clinical and Laboratory Standards Institute*, Wayne, PA, 2012.

[15] T. R. Oberhofer and D. W. Towle, "Evaluation of the rapid penicillinase paper strip test for detection of beta-lactamase" *Journal of Clinical Microbiology*, vol.15, pp. 196-199, 1982.

[16] R. Kuroki , K. Kawakami , L. Qin, C. Kaji, K. Watanabe, Y. Kimura, C. Ishiguro, S. Tanimura, Y. Tsuchiya, I. Hamaguchi, M. Sakakura, S. Sakabe S, K. Tsuji, M. Inoue, and H. Watanabe, "Nosocomial bacteremia caused by biofilm-forming *Bacillus cereus* and *Bacillus thuringiensis*", *Internal Medicine Journal*, vol. 48, pp. 791–796, 2009.

[17] M. Morikawa, S. Kagihiro, M. Haruki, K. Takano, S. Branda, R. Kolter, and S. Kanaya, "Biofilm formation by a *Bacillus subtilis* strain that produces ɣ-polyglutamate", *Microbiology*, vol.152, pp. 2801-2807, 2006.

[18] R. Majed, C. Faille, M. Kallassy, and M. Gohar, "*Bacillus cereus* biofilms-same only different", *Frontiers in Microbiology*, vol.7, 1054. doi: 10.3389/fmicb.2016.01054, 2016.

[19] H. Y. Done, A. K. Venkatesan, and R. U. Halden, "Does the recent growth of aquaculture create antibiotic resistance threats different from those associated with land animal production in agriculture?", *Journal of the American Association of Pharmaceutical Scientists*, vol.17, no.3, pp. 513-24, 2015.

[20] O. Kursun, A. Guner, and G. Ozmen, "Prevalence of *Bacillus cereus* in rabbit meat consumed in Burdur-Turkey, its enterotoxin producing ability and antibiotic

susceptibility", *Kafkas Universitesi Veteriner Fakultesi Dergisi*, vol. 17 (Suppl A): S31-S35, 2011.

[21] J. H. Yim, K. Y. Kim, J. W. Chon, D. H. Kim, H. S. Kim, D. S. Choi, I. S. Choi, and K. H. Seo, "Incidence, antibiotic susceptibility, and toxin profiles of *Bacillus cereus* sensu lato isolated from Korean fermented soybean products", *Journal of Food Science*, vol.80, no.6, M1266-67, 2015.

[22] I. Chaabouni, I. Barkallah, C. Hamdi, A. Jouii, M. Saidi, J. Mahillon, and A. Cherif, "Metabolic capacities and toxigenic potential as key drivers of *Bacillus cereus* ubiquity and adaptation", *Annals of Microbiology*, vol. 65, pp. 975-983, 2015.

[23] G. M. Noor Uddin, M. H. Larsen, H. Christensen, F. M. Aarestrup, T. M. Phu, and A. Dalsgaard, "Identification and antimicrobial resistance of bacteria isolated from probiotic products used in shrimp culture", *Plos One* vol. 10, no. 7, e0132338. https://doi.org/10.1371/journal.pone.0132338, 2015.

[24] B. A. Mohammadou, G. Le Blay, C. M. Mbofung, and G. Barbier, "Antimicrobial activities, toxinogenic potential and sensitivity to antibiotics of *Bacillus* strains isolated from Mbuja, an Hibiscus sabdariffa fermented seeds from Cameroon", *African Journal of Biotechnology*, vol.13, no. 35, pp. 3617-3627, 2014.

[25] M. Ikeda, Y. Yagihara, K. Tatsuno, M. Okazaki, S. Okugawa, and K. Moriya, "Clinical characteristics and antimicrobial susceptibility of *Bacillus cereus* blood stream infection", *Annals of Clinical Microbiology and Antimicrobials*, vol.14, 43, doi 10.1186/s12941-015-0104-2, 2015.