

BIOCHEMICAL CHARACTERIZATION AND ANTIMICROBIAL SUSCEPTIBILITY OF *Bacillus cereus* ISOLATES FROM SOME RETAILED FOODS IN OGUN STATE, NIGERIA

Titilayo O. Adesetan,^{1,2} Prof. Moses O. Efuntoye¹ and Prof. Olubukola O. Babalola^{2*}

Address(es): Prof. Olubukola O. Babalola,

¹Department of Microbiology, Olabisi Onabanjo University, Ogun State, PMB 2002, Ago – Iwoye, Nigeria.

²Food Security and Safety Niche, Faculty of Natural and Agricultural Sciences, North-West University, Mafikeng Campus, Private Bag X2046, Mmabatho 2735, South Africa.

*Corresponding author: olubukola.babalola@nwu.ac.za

doi: 10.15414/jmbfs.2019/20.9.3.616-621

ARTICLE INFO

Received 20. 9. 2018
Revised 13. 6. 2019
Accepted 13. 6. 2019
Published 1. 12. 2019

Regular article



ABSTRACT

Food borne disease caused by *Bacillus cereus* has been a major health issue because of its ability to cause two syndromes: diarrhoeal and emetic which sometimes lead to death. Six hundred (600) samples of some retailed foods: cooked rice, jollof rice, fried rice, meat pie, smoked fish: African chad, Titus, blue whiting, fried meat, smoked hide, carrot, runner beans, cabbage and raw green pea were collected from the eight main markets in Ogun State. Serial dilutions of the samples were carried out and cultured on Mannitol Egg Yolk Polymyxin Agar (MYP) using the spread plate technique. *B. cereus* was confirmed with standard biochemical methods. Antibiotic susceptibility was performed by the Kirby – Bauer disc diffusion method with nine antibiotics. The mean microbial load in the retailed food was in the range of $1.00 \times 10^4 - 8.92 \times 10^4$ cfu/g. All the isolates were gram positive rods, catalase and citrate positive. Most of the isolates were motile (97.7%). Two hundred and twenty one (221) isolates were confirmed as *B. cereus* with biochemical tests. They were 100% sensitive to gentamicin and 100% resistant to penicillin and ampicillin which are β – lactam antibiotics. All the isolates showed resistance to more than two antibiotics. This study has clearly revealed the presence of *B. cereus* in some retailed foods sold in Ogun State, Nigeria in which all the isolates were resistant to β -lactam antibiotics. Therefore, extreme caution should be taken when handling foods to avoid contamination by *B. cereus* and prevent any future food-borne outbreak by *B. cereus*.

Keywords: Antibiotics, *Bacillus cereus*, biochemical tests, Mannitol Egg Yolk Polymyxin Agar (MYP), retailed foods

INTRODUCTION

Food is essential for man's sustenance. Microorganisms attack our food for survival thereby causing deterioration and contamination of the food. Therefore, the food we eat can be beneficial to our body and at the same time cause harm when microorganisms in interaction with the food release their metabolites thereby causing illness. One of such bacteria is *Bacillus cereus*. Members in the *B. cereus* group are gram positive, ubiquitous rod shaped spore former. They are known to survive virtually in all environments because of their ability to form spores that are resistant to heat and acid. There are seven members in the group namely: *B. anthracis*, *B. cereus sensu stricto*, *B. thuringiensis*, *B. mycoides*, *B. pseudomycoides*, *B. weihenstephanensis* and *B. cytotoxicus*. The first three are the most important within the group (Lapidus *et al.* 2008; Logan and de Vos, 2009). The economic situation of some countries has encouraged the consumption of ready-to-eat foods and establishment of more fast foods restaurant, canteens and road side food outlets. These foods are believed to be affordable and easily accessible. Food-borne pathogens may multiply in foods that are not well handled, prepared or stored due to lack of hygiene and poor sanitation (Angelidis *et al.*, 2006; Desai and Varadaraj, 2009).

B. cereus causes two types of poisonings namely: diarrhoeal with abdominal cramp and diarrhoea as the symptom and emetic with nausea and vomiting. Diarrhoeal poisoning is associated with proteins such as meat while starchy foods such as rice and pasta have been linked to the emetic poisoning. It had been reported to cause illnesses (Ghelardi *et al.*, 2002; Martinelli *et al.*, 2013; Zhou *et al.*, 2014) or death (Dierick *et al.*, 2005; Naranjo *et al.*, 2011).

B. cereus can survive an array of stress situations including those found in some foods because of its ability to form spores. It has been isolated from a wide variety of foods, including milk and dairy products (De Jonghe *et al.*, 2008; Arslan *et al.*, 2014), rice and pasta (Rajkovic *et al.*, 2013), infant foods (Organji *et al.*, 2015), spices (Iurlina *et al.*, 2006), meat product (Gueven *et al.*, 2006), seafood (Das *et al.*, 2009), fresh vegetables (Valero *et al.*, 2002), vegetable salad (Valero *et al.*,

2007) and ready to eat foods (Fang *et al.*, 2003; Samapundo *et al.*, 2011). *Bacillus* infections can be treated with antibiotics such as vancomycin, clindamycin, ciprofloxacin, and gentamicin. Penicillins and ampicillins are not effective (Lindback and Granum, 2006; Chon *et al.*, 2012) because they produce β -lactamase that hydrolyses the β -lactam ring of the antibiotics.

Review of literatures in Nigeria showed that there is scarcity of information on characterization of *B. cereus* in retailed foods in Ogun State; therefore, there is need to characterize these food pathogens using both morphological and biochemical properties and determine their susceptibility to common antibiotics to forestall any future occurrence of food borne outbreak.

MATERIALS AND METHODS

Sample Collection

Six hundred (600) samples of ready-to-eat foods and vegetables such as cooked white rice (*Oryza sativa*), jollof rice, fried rice, meat pie, cooked spaghetti, smoked African chad (*Etmalosa fimbriata*), smoked Titus (*Scumber scumbrus*), smoked blue whiting (*Micromesistius poutassou*), smoked hide, fried meat (*Bos taurus*), green pea (*Pisum sativum*), sweet pepper (*Capsicum annum*), runner bean (*Phaseolus vulgaris*), cabbage (*Brassica oleracea*) and carrot (*Daucus carota*) (Table 1) were collected randomly inside sterile sampling bags/plastics from food outlets/canteens in the markets namely: Oke-Aje and Ago-Iwoye markets (Ijebu), Sagamu and Ikenne markets (Remo), Kuto and Omida markets (Egba) and Ayetoro and Imeko markets (Yewa) in the four geographical regions in Ogun State, they were sealed to prevent contamination and transported to the laboratory for analysis. Forty samples were collected from each food type. The samples were collected in the morning and it was ensured that the vegetables were fresh ones without any sign of deterioration. The samples were collected between September 2013 and April 2015. The analyses were carried out at the Laboratories of the Department of Microbiology, Olabisi Onabanjo University, Ago – Iwoye, Nigeria and Microbial

Biotechnology Laboratory of North – West University, Mafikeng Campus, South Africa.

Table 1 Number of food samples collected from each division

Food groups	Food name	Food Code	Egba	Ijebu	Remo	Yewa	Total
Starch	Cooked Rice	WR	10	10	10	10	40
	Jollof Rice	JR	10	10	10	10	40
	Fried Rice	FR	10	11	11	8	40
	Meat pie	MP	10	10	10	10	40
	Spaghetti	SG	10	10	10	10	40
Protein	Fried Meat	MT	10	10	10	10	40
	Smoked Titus	TT	10	10	10	10	40
	Smoked African chad	SW	10	10	10	10	40
	Smoked Blue whiting	PN	10	10	10	10	40
	Smoked Hide	PM	10	10	10	10	40
Vegetable	Green pea	GP	12	12	12	4	40
	Sweet pepper	SP	10	10	10	10	40
	Cabbage	CB	11	12	11	6	40
	Carrot	CR	10	10	10	10	40
	Runner Bean	RB	10	10	10	10	40
	Total		153	155	154	138	600

Sample preparation

Samples of meat, runner beans, carrot, cabbage, smoked hide and sweet pepper were chopped into pieces with sterile knife after which 10g of each sample was added to 100 ml sterile peptone water (1:10 dilution) and diluted up to 10⁻⁵.

Isolation of *B. cereus*

Mannitol Egg Yolk Polymyxin (MYP) agar (Oxoid, UK) plates were inoculated with 0.1ml of appropriate dilution and spread evenly onto surface of each plate with sterile bent glass rod. Plates were incubated at 30°C for 18hrs and observed for colonies surrounded by precipitate zone, which indicated that lecithinase was produced (Tallent et al., 2001).

Microbial load

After incubating for 24hrs, the plates with distinct colonies were counted and then multiplied with the dilution factor to get the total bacteria count in colony forming unit per gram (cfu/g).

Confirmation of *B. cereus*

The colonial morphology of the isolates was first recorded after which the biochemical tests based on Food and Drug Administration (FDA) methods as described by Tallent et al. (2001) and Cheesebrough (2000) were carried out. Briefly, five (5) or more eosin pink, lecithinase-positive colonies from MYP agar (Oxoid, UK) plates were transferred to nutrient agar plates (MAST, Merseyside UK) and incubated for 24 h at 30°C. The isolates were gram stained and confirmed using the following tests: glucose fermentation, nitrate reduction, motility test, catalase test, citrate utilization, starch hydrolysis, casein hydrolysis, rhizoid growth, haemolysis and growth at 42°C.

Antimicrobial susceptibility test

This test was performed using Kirby-Bauer Disc Diffusion method (Bauer et al. 1966). Nine (9) different antibiotics (Bio-Rad, USA) were employed: penicillin (PEN) 10IU, ciprofloxacin (CIP) 5 µg, tetracycline (TET) 30µg, erythromycin (ERY) 15 µg, gentamicin (GMN) 10 µg, amoxicillin-clavulanic acid (AMC) 30 µg, vancomycin (VAN) 30 µg, ampicillin (AMP) 10 µg and clindamycin (CMN) 2 µg (Oxoid, UK). The results were interpreted using Clinical Laboratory Standard Institute CLSI (2013) guideline for Gram positive and /or aerobic bacteria.

Statistical Analysis

Data were analyzed using the Statistical Package for Social Sciences (SPSS) version 20.0 (IBM Corp, 2011). Mean values were compared using Analysis of Variance (ANOVA). Results were presented as Mean±Standard deviation. Post hoc test was done using the Student-Newman-Keuls (SNK). p < 0.05 was considered to be statistically significant.

RESULTS

Microbial load of retailed foods

The microbial load in the food samples is presented on Table 2. Food with the highest microbial load was spaghetti from Yewa with mean bacterial load of 8.92 x 10⁴cfu/g, followed by White rice from Yewa with 4.18 x 10⁴cfu/g while the food with the least count was green pea from Ijebu –Ode with mean bacterial load of 1.00 x 10⁴cfu/g. The One Way Analysis of Variance (ANOVA) revealed a significant difference (p ≤ 0.05 in the microbial load of the food samples SP and SW collected from all the locations. There was also a significant difference (p ≤ 0.05) between the locations where the samples were collected.

Table 2 Mean Microbial load in food samples collected from different divisions (x10⁴cfu/g)

Location / Food code	Egba	Ijebu	Remo	Yewa
WR	3.42±1.22 ^a	2.82±0.83 ^a	3.92±0.76 ^a	4.18±0.80 ^a
JR	3.70±0.20 ^a	3.00±0.57 ^a	4.90±0.10 ^a	0.00±0.00 ^b
FR	3.10±1.27 ^b	6.85±0.64 ^a	1.20±0.20 ^c	3.10±0.10 ^b
MP	1.90±0.28 ^a	2.03±1.33 ^a	1.60±0.14 ^a	1.60±0.56 ^a
SG	5.28±3.18 ^a	5.68±4.17 ^a	4.08±1.81 ^a	8.92±2.60 ^a
MT	2.78±1.59 ^a	2.00±0.72 ^a	2.70±1.44 ^a	2.62±0.60 ^a
TT	3.48±0.94 ^a	3.64±1.55 ^a	3.00±0.96 ^a	5.23±0.42 ^a
SW	6.03±3.46 ^a	2.86±1.49 ^b	2.38±0.54 ^b	4.70±1.04 ^a
PN	3.65±4.31 ^b	2.90±0.28 ^b	6.50±0.50 ^a	3.10±2.12 ^b
PM	4.00±0.50 ^a	4.37±1.21 ^a	4.35±0.21 ^a	4.70±0.20 ^a
GP	1.35±0.21 ^a	1.00±0.50 ^a	1.38±0.28 ^a	2.00±0.60 ^a
SP	1.57±0.40 ^b	2.00±0.60 ^b	2.18±0.17 ^b	3.90±0.28 ^a
RB	4.04±0.57 ^a	4.10±0.96 ^a	2.88±1.04 ^a	3.96±1.68 ^a
CB	2.77±0.68 ^b	2.53±0.51 ^b	1.70±0.26 ^b	3.30±0.30 ^a
CR	2.00±0.60 ^b	3.70±0.20 ^a	2.65±0.92 ^b	3.40±0.40 ^a

^{abc}Means (±Standard deviation) in the same row having similar superscripts are not significantly different at p < 0.05. n = 181

WR – White Rice JR – Jollof Rice FR – Fried Rice TT – Smoked Titus GP – Green Pea SG – Spaghetti RB – Runner Bean MP – Meat pie CR – Carrot MT – Meat CB – Cabbage SW – Smoked African chad SP – Sweet Pepper PN – Smoked blue whiting PM – Smoked hide

Colonial morphology of *B. cereus*

The isolates formed opaque, irregular, smooth/rough, creamish colonies on Nutrient Agar. Some also showed waxy growth while others had short hair-like growth around the colony. Most of the colonial characteristics exhibited were typical of *Bacillus cereus*.

Gram stain reaction and biochemical characterization

All the isolates were Gram positive rods. Many were in chains while few were single rods. Some have central to sub-terminal spores which do not swell the sporangium. All the isolates were catalase and citrate positive. 95.9% of the isolates were haemolytic, 97.7% were motile, 77.4% and 86.0% hydrolysed starch and casein respectively. They grew well at 42°C (Table 3).

Table 3 Biochemical tests pattern for *B. cereus* isolates from some retailed foods

Pattern/Test	Glucose fermentation	Motility	Rhizoidal growth	Haemolysis	Nitrate Reduction	Catalase	Starch hydrolysis	Casein hydrolysis	Citrate utilization	Growth at 42°C	Egba	Ijebu	Remo	Sagamu
I	+	+	-	-	+	+	+	+	+	+	1	0	0	1
II	+	+	-	β	+	+	+	+	+	+	32	41	40	31
III	+	+	-	α	+	+	-	+	+	+	1	0	0	0
IV	+	+	-	β	+	+	-	+	+	+	9	6	7	13
V	+	+	-	α	+	+	-	-	+	+	0	0	1	2
VI	+	+	-	β	+	+	+	-	+	+	7	5	3	7
VII	+	+	-	-	+	+	-	+	+	+	1	1	1	1
VIII	+	+	-	-	+	+	+	-	+	+	0	1	0	0
IX	+	-	-	-	+	+	+	-	+	+	0	1	0	0
X	+	-	-	β	+	+	-	+	+	+	0	2	0	0
XI	+	+	-	-	+	+	-	-	+	+	0	1	0	0
XII	+	+	-	α	+	+	+	+	+	+	0	0	1	0
XIII	+	+	-	β	+	+	-	-	+	+	0	0	2	0
XIV	+	-	-	β	+	+	+	+	+	+	0	0	1	0
XV	+	-	-	α	+	+	-	-	+	+	1	0	0	0
TOTAL	100%	97.7%	100%	95.9%	100%	100%	77.4%	86.0%	100%	100%	52	58	56	55

α alpha haemolysis, β beta haemolysis, + positive - negative

Occurrence of *B. cereus* isolates in food samples

The occurrence of *B. cereus* in the retailed food sample collected from the four geographical divisions in the state is presented on Table 4. The highest occurrence of *B. cereus* was from food samples from Ijebu while the least was from Egba division.

Table 4 Occurrence of *B. cereus* in some retailed food sample

Location/ Food code	No of isolates	Egba	Ijebu	Remo	Yewa
WR	33	8	7	8	10
JR	4	1	2	1	-
FR	6	2	2	1	1
SG	34	8	9	7	10
MP	10	2	3	2	3
MT	18	4	6	3	5
SW	27	4	8	8	7
PN	8	2	2	1	3
PM	7	1	3	2	1
TT	16	4	5	4	3
GP	8	2	1	4	1
RB	25	7	5	6	7
CR	5	1	1	2	1
CB	10	3	3	3	1
SP	10	3	1	4	2
	221	52 (23.5%)	58 (26.2%)	56 (25.3%)	55 (24.0%)

WR – White Rice JR – Jollof Rice FR – Fried Rice TT – Smoked Titus GP – Green Pea SG – Spaghetti RB – Runner Bean MP – Meat pie CR – Carrot MT – Meat CB – Cabbage SW – Smoked African chad SP – Sweet Pepper PN – Smoked blue whiting PM – Smoked hide

Antimicrobial Susceptibility Test

All the isolates were resistant to penicillin and ampicillin while 99.5% were resistant to amoxicillin-clavulanic acid. They were all susceptible to gentamicin (Table 5). The resistance pattern of the *B. cereus* isolates from the retailed food is presented on Table 6. Some of the isolates were resistant to more than one antibiotic. Resistance to penicillin, ampicillin and amoxicillin-clavulanic acid was displayed by 7.7% of the isolates, 22.6% showed resistance to penicillin, amoxicillin-clavulanic acid, erythromycin and ampicillin while 5.4% were resistant to all the antibiotics except gentamicin.

Table 5 Antibiotic Resistance of *B. cereus* from each location

Class of Antibiotics	β-lactam	Aminoglycosides	β-lactam	Lincosamide	Macrolides	Quinolones	Tetracyclines	Glycopeptide	β-lactam
Antibiotics/ Location	PEN (10IU)	GMN (10µg)	AMC (30µg)	CMN (2µg)	ERY (15µg)	CIP (5µg)	TET (30µg)	VAN (30µg)	AMP (10µg)
EGBA	52	0	52	23	33	19	17	25	52
IJEBU	58	0	57	23	37	15	18	16	58

REMO	56	0	56	25	45	15	19	24	56
YEWA	55	0	55	19	33	17	13	21	55
TOTAL	221	0	220	90	148	66	69	86	221
(%)	100%	0%	99.5%	40.7%	67%	29.9%	30.3%	38.9%	100%

PEN – Penicillin GMN – Gentamicin AMC – Amoxicillin-clavulanic acid CMN – Clindamycin ERY- Erythromycin CIP – Ciprofloxacin TET- Tetracycline VAN – Vancomycin, AMP – Ampicillin
 I – β-Lactam, II – Aminoglycosides, III – Lincosamide, IV – Macrolides, V – Quinolones, VI – Tetracycline, VII - Glycopeptides

Table 6 Resistance pattern of *B. cereus* isolates from retailed foods

Resistance pattern	Antibiotic class	No of isolates	Resistance (%)
PEN, AMC, AMP	I	17	7.7
PEN, AMC, ERY, AMP	I,IV	50	22.6
PEN, AMC, CIP, AMP	I,V	7	3.2
PEN, AMC, TET, AMP	I,VI	8	3.6
PEN, AMC, VAN, AMP	I,VII	4	1.8
PEN, AMC, CMN, AMP	I,III	11	5.0
PEN, AMC, ERY, VAN, AMP	I,IV,VII	9	4.1
PEN, AMC, CMN, ERY, AMP	I,III,IV	14	6.3
PEN, AMC, CMN, VAN, AMP	I,III,VII	5	2.3
PEN, AMC, CIP, TET, AMP	I,V,VI	5	2.3
PEN, AMC, ERY, CIP, AMP	I,IV,V	4	1.8
PEN, AMC, TET, VAN, AMP	I,VI,VII	1	0.5
PEN, AMC, ERY, TET, AMP	I,IV,VI	3	1.4
PEN, AMC, CMN, ERY, AMP	I,III,IV	1	0.5
PEN, CMN, TET, VAN, AMP	I,III,VI,VII	1	0.5
PEN, AMC, CMN, TET, AMP	I,III,VI	1	0.5
PEN, AMC, CMN, CIP, AMP	I,III,V	2	0.9
PEN, AMC, CMN, ERY, TET, AMP	I,III,IV,VI	1	0.5
PEN, AMC, CMN, CIP, TET, AMP	I,III,V,VI	1	0.5
PEN, AMC, ERY, CIP, VAN, AMP	I,IV,V,VII	10	4.5
PEN, AMC, CMN, ERY, CIP, AMP	I,III,IV,V	1	0.5
PEN, AMC, CMN, TET, VAN, AMP	I,III,VI,VII	2	0.9
PEN, AMC, ERY, CIP, TET, AMP	I,IV,V,VI	3	1.4
PEN, AMC, ERY, TET, VAN, AMP	I,IV,VI,VII	3	1.4
PEN, AMC, CMN, ERY, VAN, AMP	I,III,IV,VII	12	5.4
PEN, AMC, CMN, CIP, VAN, AMP	I,III,V,VII	3	1.4
PEN, AMC, CMN, ERY, TET, AMP	I,III,IV,VI	3	1.4
PEN, AMC, CIP, TET, VAN, AMP	I,V,VI,VII	2	0.9
PEN, AMC, ERY, CIP, TET, VAN, AMP	I,IV,V,VI,VII	5	2.3
PEN, AMC, CMN, ERY, TET, VAN, AMP	I,III,IV,VI,VII	9	4.1
PEN, AMC, CMN, ERY, CIP, TET, AMP	I,III,IV,V,VI	3	1.4
PEN, AMC, CMN, CIP, TET, VAN, AMP	I,III,VI,VII	3	1.4
PEN, AMC, CMN, ERY, CIP, VAN, AMP	I,III,IV,V,VII	5	2.3
PEN, AMC, CMN, ERY, CIP, TET, VAN, AMP	I,III,IV,V,VI,VII	12	5.4

PEN – Penicillin GMN – Gentamicin AMC – Amoxicillin-clavulanic acid CMN – Clindamycin ERY- Erythromycin CIP – Ciprofloxacin TET- Tetracycline VAN – Vancomycin, AMP – Ampicillin
 I – β-Lactam, II – Aminoglycosides, III – Lincosamide, IV – Macrolides, V – Quinolones, VI – Tetracycline, VII - Glycopeptides

Table 7 showed the percentage resistance for each food sample. All the isolates from each food sample showed 100% resistance to penicillin, ampicillin and amoxicillin-clavulanic acid except for isolates from PM which showed 85.7%

resistance to amoxicillin-clavulanic acid. Isolates from CB and JR showed 100% resistance to erythromycin. PM isolates showed the highest resistance of 71.4% to tetracycline.

Table 7 Percentage (%) resistance of *B. cereus* isolates in each food sample

Food code	No of Isolates	PEN (%)	AMC (%)	CMN (%)	ERY (%)	CIP (%)	TET (%)	VAN (%)	AMP (%)
WR	33	100	100	33.3	81.8			57.6	100
SG	34	100	100	41.2	76.4	48.5	24.2	47.1	100
SW	27	100	100	29.6	74.1			29.6	100
MT	18	100	100	61.1	22.2	29.4	35.3	38.9	100
RB	25	100	100	32.0	32.0			48.0	100
TT	16	100	100	50.0	68.8	33.3	29.6	37.5	100
CB	10	100	100	30.0				10.0	100
SP	10	100	100	60.0	100.0	33.3	22.2	30.0	100
CR	5	100	100	40.0	70.0			20.0	100
FR	6	100	100	33.3	80.0	28.0	40.0	33.3	100
GP	8	100	100	37.5	33.3			37.5	100
JR	4	100	100	50.0	62.5	12.5	18.8	25.0	100
PM	7	100	85.7	28.6				42.9	100
PN	8	100	100	25.0	100.0	10.0	10.0	25.0	100
MP	10	100	100	40.0	85.7	62.5	60.0	40.0	100
					80.0		20.0	40.0	
						0.0	0.0		
						12.5	37.5		
						0.0	0.0		
						14.3	71.4		
						37.5	25.0		
						30.0	50.0		

PEN – Penicillin GMN – Gentamicin AMC – Amoxicillin-clavulanic acid CMN – Clindamycin ERY- Erythromycin CIP – Ciprofloxacin TET- Tetracycline VAN – Vancomycin, AMP – Ampicillin WR – White Rice JR – Jollof Rice FR – Fried Rice TT – Smoked Titus GP – Green Pea SG – Spaghetti RB – Runner Bean MP – Meat pie CR – Carrot MT – Meat CB – Cabbage SW – Smoked African chad SP – Sweet Pepper PN – Smoked blue whiting PM – Smoked hide

DISCUSSION

Six hundred (600) different food samples were analysed for the presence of *Bacillus cereus*. The microbial load in the retailed food sample was high. Fang et al. (2003) also reported *B. cereus* load in 18°C ready-to-eat food products in the range of 10⁴ to ≥ 10⁵cfu/g. According to Gilbert et al. (2000) *B. cereus* count in food greater than 10⁴ – <10⁵ is unsatisfactory while >10⁵ is unacceptable. There were significant differences in the microbial load of the food samples FR, SW, PN, SP, CB and CR from the different locations at p ≤ 0.05.

Two hundred and twenty one (221) *B. cereus* isolates were recovered from the retailed foods analyzed. Ninety three point two (93.2%) formed β – haemolysis on sheep blood agar. Six strains were α haemolytic and nine were non-haemolytic. This is in line with the work of Chaves et al., (2011) who reported 81.4% of their *B. cereus* to be β – haemolytic, six were non haemolytic while seven were α – haemolytic. Chon et al., (2012) reported that 89% of their strains were haemolytic. Eighty five point one (86.0%) and seventy seven point four (77.4%) of the strains hydrolysed skim milk, and starch agar. Chon et al. (2012) reported that 84% and 89% each of their strains hydrolysed lecithinase and starch. They all grew very well at 42°C. This is in line with the findings of Lindbäck and Granum (2006) who reported that *B. cereus* grows fast at 42°C. Detection of *B. cereus* in retailed foods in this research is in line with the work of Desai and Varadaraj (2009); Al-Abri et al. (2011); Naranjo et al. (2011); Martinelli et al. (2013); Lopez et al. (2015).

The rate of occurrence of *B. cereus* from the retailed food samples showed that samples from Ijebu division had the highest occurrence while Egba had the least occurrence. Sixteen percent (16%) of *B. cereus* isolates were recovered from spaghetti. This may be as a result of poor hygiene practised by the food handlers.

The food handlers put spaghetti in small transparent plastics buckets instead of hot food containers that can keep them hot. Also, most of them use their bare hands to support the spaghetti when serving it with fork to the consumers. These same hands were used to receive money and handle their food utensils. These can cause cross contamination from the money to the food because currency notes are known to harbour pathogenic microorganisms including *Bacillus* species (Uneke and Ogbu, 2007; Awe et al., 2010; Orukotan and Yabaya, 2011).

All the *B. cereus* strains were 100% sensitive to gentamicin. This compared well with the works of Chaves et al. (2011); Chon et al. (2012); Mohammed et al. (2012) and Organji et al. (2015) who reported 100% sensitivity to gentamicin by *B. cereus* strains recovered from food. Also, 62% of the strains in this study were sensitive to vancomycin, while 38% were resistant to the drug. Ankolekar et al. (2009) reported that 44% of the isolates were resistant to vancomycin, 56% were intermediate strains and none was sensitive to the drug while Organji et al. (2015) reported 100% sensitivity to vancomycin. WR strains showed the highest resistance of 57.6% to the drug.

All the *B. cereus* strains were 100% resistant to penicillin and ampicillin while 99.5% were resistant to amoxicillin-clavulanic acid. This conforms to the work of Park et al. (2009), Chon et al. (2012) and Savic et al. (2016) who reported that their strains displayed 100% resistance to ampicillin and penicillin. It was reported by Arslan et al. (2014) that *B. cereus* strains were resistant to ampicillin and penicillin G with the equal rate of 89.7% while 27.6% and 13.8% showed resistance and intermediate resistance respectively to amoxicillin-clavulanic acid. This is in contrast with the result in this work where higher percentage (99.5%) of the strains was resistant to amoxicillin-clavulanic acid. Penicillin, ampicillin and amoxicillin-clavulanic acid are β -lactam antibiotics and resistance to these drugs is as a result of synthesis of β -lactamase by *B. cereus*.

Sensitivity to ciprofloxacin was 70.1%, 29.0% showed intermediate resistance while 0.9% was completely resistant to the antibiotic. Jawad et al. (2016) reported that 42% of *B. cereus* strains from fried rice showed resistant to the antibiotic. This result contradicts the work of Luna et al. (2007); Chon et al. (2012); Savic et al. (2016) who reported that *B. cereus* isolates were 100% sensitive to ciprofloxacin. SP isolates showed 48.5% resistance to the drug.

Resistance to clindamycin was 9.1%; intermediate resistance was 30.7% while 60.2% were sensitive to the drug. This is in contrast to the work of Luna et al. (2007) and Park et al. (2009) who reported that 15% and 72% of *B. cereus* strains were resistant to clindamycin. Likewise, Arslan et al. (2014) reported 6.9% resistant to the drug while Chaves et al. (2011) recorded ten intermediate strains among *B. cereus* strains. However, some authors like Ankolekar et al. (2009), Chon et al. (2012), Organji et al. (2015) reported 100% sensitivity to the drug by *B. cereus*. MT strains showed the highest resistance of 61.1% to clindamycin.

Comparatively, the *B. cereus* strains in this research were highly resistance to erythromycin. Isolates from CB and JR demonstrated the highest resistance of 100% to erythromycin. Resistance to erythromycin was 38%, Intermediate resistance was 29% while 33% were sensitive to the drug. However, the result of our study is supported by the work of Jawad et al. (2016) who reported that 42% of strains from fried rice were resistant to the drug. Other workers like Park et al. (2009), Chon et al. (2012) and Arslan et al. (2014) had reported 82%, 93.1% and 97% sensitivity to the drug respectively. Although, Ankolekar et al. (2009) reported that 58% of *B. cereus* strains from rice were resistant to erythromycin. Authors like Organji et al. (2015) and Savic et al. (2016) reported 100% sensitivity to the drug. A higher sensitivity to tetracycline (69%) was obtained in this study compared to 54.8% and 54% sensitivity reported by Mohammed et al. (2012) and Chon et al. (2012) respectively. Ankolekar et al. (2009) reported that 98% of their *B. cereus* strains were resistant to tetracycline. Park et al. (2009) and Jawad et al. (2016) stated that 85% and 86% of their isolates were sensitive while Savic et al. (2016) reported 100% sensitivity to the drug. Resistance to the drug was displayed by 71.4% of PM strains while the least resistance was 10.0% by CB isolates. PM is animal hide and the high resistance may be as a result of misuse of antibiotics.

More than 50% of the isolates displayed multiple drug resistances i.e. resistance to three or more antimicrobial classes. The highest resistance was displayed by fourteen strains which were resistant to three classes of antibiotics followed by twelve strains that were resistant to all the antibiotics except gentamicin.

CONCLUSION

This research work has revealed that *B. cereus* contaminated some retailed foods sold in Ogun State especially the samples from Ijebu division and are multi-resistant to common antibiotics. Therefore extra caution should be taken when handling foods to avoid contamination of the food from the handler and the environment.

Acknowledgement: The authors appreciate the financial support provided by the University management through the Tertiary Education Tax Fund (TETFUND) and the Microbial Biotechnology Laboratory, NWU, South Africa for hosting the first author.

REFERENCES

- Al-Abri, S.S., Al-Jardania, A.K., Al-Hosnia, M.S., Kurupb, P.J., Al-Busaidi, S., & Beechingd, N.J. (2011). A hospital acquired outbreak of *Bacillus cereus* gastroenteritis, Oman. *Journal of Infection and Public Health*, 4,180-186. <http://dx.doi.org/10.1016/j.jiph.2011.05.003>.
- Angelidis, A., Chronis, G., Papageorgiou, D., Kazakis, I., Arsenoglou, K., & Stathopoulos, G. (2006). Non-lactic acid, contaminating microbial flora in ready-to-eat foods: A potential food-quality index. *Food Microbiology*, 23,95-100. <http://dx.doi.org/10.1016/j.fm.2005.01.015>.
- Ankolekar, C., Rahmati, T., & Labbé, R.G. (2009). Detection of toxigenic *Bacillus cereus* and *Bacillus thuringiensis* spores in U.S. rice. *International Journal of Food Microbiology*, 128(3),460-466. <http://dx.doi.org/10.1016/j.ijfoodmicro.2008.10.006>.
- ARSLAN, S., EYL, A., & KÜÇÜKSARI, R. (2014). Toxigenic genes, spoilage potential, and antimicrobial resistance of *Bacillus cereus* group strains from ice cream. *Anaerobe*, 25,42-46. <http://dx.doi.org/10.1016/j.anaerobe.2013.11.006>.
- Awe, S., Eniola, K.I.T., Ojo, F.T., & Sani, A. (2010). Bacteriological quality of some Nigerian currencies in circulation. *African Journal of Microbiology Research*, 4(21), 2231- 2234.
- Bauer, A.W., Kirby, W.M., Sherris, J.C., & Turck, M. (1966). Antibiotic susceptibility testing by a standardized single disk method. *American Journal of Clinical Pathology*, 45(4), 493-496.
- Chaves, J.Q., Pires, E.S., & Vivoni, A.M. (2011). Genetic diversity, antimicrobial resistance and toxigenic profiles of *Bacillus cereus* isolated from food in Brazil over three decades. *International Journal of Food Microbiology*, 147(1), 12-16. <http://dx.doi.org/10.1016/j.ijfoodmicro.2011.02.029>.
- Cheesebrough, M. (2000). District Laboratory Practice in Tropical countries Part 2. London: Cambridge University Press.
- Chon, J.W., Kim, J.H., Lee, S.J., Hyeon, J.Y., & Seo, K.H. (2012). Toxin profile, antibiotic resistance, and phenotypic and molecular characterization of *Bacillus cereus* in Sunsik. *Food Microbiology*, 32, 217-222. <http://dx.doi.org/10.1016/j.fm.2012.06.003>.
- CLSI (2013). Performance standards for antimicrobial disc susceptibility tests; Twenty third informational supplement M100 - S23 (M02- A11). Clinical and Laboratory Standards Institutes, Wayne, Pennsylvania.
- Das, S., Surendran, P., & Thampuran, N. (2009). PCR-based detection of enterotoxigenic isolates of *Bacillus cereus* from tropical seafood. *Indian Journal of Medical Research*, 129, 316-320.
- De Jonghe, V., Coorevits, A., Vandromme, J., Heyrman, J., Herman, L., De Vos, P., & Heyndrickx, M. (2008). Intraspecific genotypic diversity of *Bacillus* species from raw milk. *International Dairy Journal*,18, 496-505. <http://dx.doi.org/10.1016/j.idairyj.2007.11.007>.
- Desai, S.V., & Varadaraj, M.C. (2009). Prevalence of toxigenic traits in native food isolates of *Bacillus cereus* in the city of Mysore, Southern India. *Journal of Microbiology and Antimicrobials*, 1, 027-034.
- Dierick, K., Van Coillie, E., Swiecicka, I., Meyfroidt, G., Devlieger, H., Meulemans, A., Hoedemaekers, G., Fourie, L., Heyndrickx, M., & Mahillon, J. (2005). Fatal family outbreak of *Bacillus cereus*-associated food poisoning. *Journal of Clinical Microbiology*, 43(8), 4277-4279. <http://dx.doi.org/10.1128/JCM.43.8.4277-4279.2005>.
- Fang, T.J., Wei, Q.K., Liao, C.W., Hung, M.J., & Wang, T.H. (2003). Microbiological quality of 18°C ready-to-eat food products sold in Taiwan. *International Journal of Food Microbiology*, 80(3), 241-250.
- Ghelardi, E., Celandroni, F., Salvetti, S., Barsotti, C., Baggiani, A., & Senesi, S. (2002). Identification and characterization of toxigenic *Bacillus cereus* isolates responsible for two food poisoning outbreaks. *FEMS Microbiology Letters*, 208, 129-134.
- Gilbert, R.J., De Louvois, J., Donovan, T., Little, C., Nye, K., Ribeiro, C.D., Richards, J., Roberts, D., & Bolton, F.J. (2000). Guidelines for the microbiological quality of some ready-to-eat foods sampled at the point of sale. *Commun. Dis. Public Health*, 3, 163-167.
- Gueven, K., Mutlu, M.B., & Avci, O. (2006). Incidence and characterization of *Bacillus cereus* in meat and meat products consumed in Turkey. *Journal of Food Safety*, 26,30-40. <http://dx.doi.org/10.1111/j.1745-4565.2005.00031.x>.
- IBM Corporation (2011). IBM SPSS statistics for Windows version 20.0. Armonk, NY:IBM Corp.
- Iurlina, M.O., Saiz, A.I., Fuselli, S.R., & Fritz, R. (2006). Prevalence of *Bacillus* spp. in different food products collected in Argentina. *LWT - Food Science and Technology*, 39,105-110. <http://dx.doi.org/10.1111/j.1750-3841.2010.01566.x>.
- Jawad, N., Abd Mutalib, S., & Abdullah, A. (2016). Antimicrobial resistance pattern of *Bacillus cereus* strains isolated from fried rice samples. *International Journal of ChemTech Research*, 9(1), 160-167.
- Lapidus, A., Goltsman, E., Auger, S., Galleron, N., Ségurens, B., Dossat, C., Land, M., Broussolle, V., Brillard, J., Guinebretiere, M.H., Sanchis, V., Nguyen-The, C., Leredus, D., Richardson, P., Wincker, P., Weissenbach, J., Ehrlich, S.D., & Sorokin, A. (2008). Extending the *Bacillus cereus* group genomics to putative

- food-borne pathogens of different toxicity. *Chemico-Biological Interactions*, 171(2), 236-249. <http://dx.doi.org/10.1016/j.cbi.2007.03.003>.
- Lindbäck, T., & Granum, P.E. (2006). Detection and Purification of *Bacillus cereus* Enterotoxins. In C.C. Adley (Ed.), *Food-Borne Pathogens: Methods and Protocols* (pp. 15-26). New Jersey: Humana Press Inc.
- Logan, N.L., & De Vos, P. (2009). Genus I. *Bacillus*. In P. de Vos, G.M. Garrity, D. Jones, N.R. Krieg, W. Ludwig, F.A. Rainey et al. (Eds.), *Bergey's Manual of Systematic Bacteriology* (pp. 21-128). New York: Springer.
- López, A.C., Minnaard, J., Pérez, P.F., & Alippi, A.M. (2015). A case of intoxication due to a highly cytotoxic *Bacillus cereus* strain isolated from cooked chicken. *Food Microbiology*, 46, 195-199. <http://dx.doi.org/10.1016/j.fm.2014.08.005>.
- Luna, V.A., King, D.S., Gulledd, E. J., Cannons, A.C., Amiso, P.T., & Cattani, J. (2007). Susceptibility of *bacillus anthracis*, *Bacillus cereus*, *Bacillus mycoides*, *Bacillus pseudomycoloides* and *B. thuringensis* to 24 antimicrobials using sensititre automated microbroth dilution and Etest agar gradient diffusion methods. *Journal of Antimicrobial Chemotherapy*, 60, 555-5670.
- Martinelli, D., Fortunato, F., Tafuri, S., Cozza, V., Chironna, M., Germinario, C., Pedalino, B., & Prato, R. (2013). Lessons from a birthday party: a *Bacillus cereus* outbreak, Bari, Italy, January 2012. *Ann 1st Super Sanita*, 49 (4), 391 -394. http://dx.doi.org/10.4415/ann_13_04_12.
- Mohammed, K.H., Basil, A.A., & Ban, M.S. (2012). Molecular detection of Enterotoxin (*CytK*) Gene and Antimicrobial susceptibility of *B. cereus* isolates from milk and milk products. *Basrah Journal of Veterinary Research*, 11(1), 164-173.
- Naranjo, M., Denayer, S., Bottledorm, N., Delbrassinne, L., Veys, J., Waegenaere, J., Sirtaine, N., Driesen, R.B., Sipido, K.R., Mahillon, J., & Dierick, K. (2011). Sudden Death of a Young Adult Associated with *Bacillus cereus* Food Poisoning. *Journal of Clinical Microbiology*, 49(12), 4379-4381. <http://dx.doi.org/10.1128/JCM.05129-11>.
- Organji, S.R., Abulreesh, H.H., Elbanna, K., Osman, G.E.H., & Khider, M. (2015). Occurrence and characterization of toxigenic *Bacillus cereus* in food and infant feces. *Asian Pacific Journal of Tropical Biomedicine*, 5, 515-520. <http://dx.doi.org/10.1016/j.apjtb.2015.04.004>.
- Orukotan, A.A., & Yabaya, A. (2011). Microbial Contamination of Naira Notes in Circulation within Kaduna Metropolis. *Journal of Medical and Applied Biosciences*, 2, 20-27.
- Park, Y.B., Kim, J.B., Shin, S.G., Kim, J.C., Cho, S.H, Lee, B.K., Ahn, J., Kim, J.M., & Oh, D.H. (2009). Prevalence, Genetic Diversity, and Antibiotic Susceptibility of *Bacillus cereus* Strains from Rice and Cereals collected in Korea. *Journal of Food Protection*, 72(3), 612-617.
- Rajkovic, A., Kljajic, M., Smigic, N., Devlieghere, F., & Uyttendaele, M. (2013). Toxin producing *Bacillus cereus* persist in ready-to-reheat spaghetti Bolognese mainly in vegetative state. *International Journal of Food Microbiology*, 167, 236-243. <http://dx.doi.org/10.1016/j.ijfoodmicro.2013.09.001>.
- Samapundo, S., Heyndrickx, M., Xhaferi, R., & Devlieghere, F. (2011). Incidence, diversity and toxin gene characteristics of *Bacillus cereus* group strains isolated from food products marketed in Belgium. *International Journal of Food Microbiology*, 150(1), 34-41. <http://dx.doi.org/10.1016/j.ijfoodmicro.2011.07.013>.
- Savic, D., Miljkovic-Selimovic, B., Lepsanovic, Z., Tambur, Z., Konstantinovic, S., Stankovic, N., & Ristanovic, E. (2016). Antimicrobial Susceptibility and β -lactamase production in *B. cereus* isolates from stool of patients, food and environment samples. *Vojnosanit Preg*, 73(10), 904-909. <http://dx.doi.org/10.2298/VSP150415134S>.
- Tallent, S.M., Jeffery Rhodehamel E.J., Harmon, S.M., & Bennett, R.W. (2001). *Bacillus cereus*. *Bacteriological Analytical Manual*. Chapter 14.
- Uneke, C.J., & Ogbu, O. (2007). Potential for parasite and bacterial transmission by paper currency in Nigeria. *Journal of Environmental Health*, 69, 54- 60.
- Valero, M., Hernández-Herrero, L.A., Fernández, P.S., & Salmerón, M.C. (2002). Characterization of *Bacillus cereus* isolates from fresh vegetables and refrigerated minimally processed foods by biochemical and physiological tests. *Food Microbiology*, 19, 491-499. <http://dx.doi.org/10.1006/yfmic.507>.
- Valero, M., Hernández-Herrero, L.A., & Giner, M.J. (2007). Survival, isolation and characterization of a psychrotrophic *Bacillus cereus* strain from a mayonnaise-based ready-to-eat vegetable salad. *Food Microbiology*, 24, 671-677. <http://dx.doi.org/10.1016/j.fm.2007.04.005>.
- Zhou, G., Bester, K., Liao, B., Yang, Z., Jiang, R., & Hendriksen, N.B. (2014). Characterization of Three *Bacillus* Strains Involved in a Major Outbreak of Food Poisoning After Consumption of Fermented Black Beans (Douchi) in Yunan, China. *Food Borne Pathogens and Disease*, 11(10), 69-774. <http://dx.doi.org/10.1089/fpd.2014.1768>.