Detection of genes conferring resistance on multi antibiotic resistance bacteria isolated from ready to eat foods sold in the Nigerian defence academy, Kaduna, Nigeria

Ayuba-Buhari, SB[1], Egbe, NK[1], Dibal, DM[2], Haroun, AA[1], Oaikhena , EE[1], Ozojiofor, UO*[1], Onuh, KC[1], Hassan, AU[1], Umar, Z[1].

[1]Department of Biotechnology, Nigerian Defence Academy, Kaduna, Nigeria.

[2]Department of Biology, Nigerian Defence Academy, Kaduna, Nigeria.

Submitted: 5th Nov., 2023; Accepted: 9th Dec., 2023; Published online: 31st Dec., 2023
DOI: https://doi.org/10.54117/jcbr.v3i6.1
**Corresponding Author:* *Ugochukwu Okechukwu Ozojiofor; ozojiofor.uo@nda.edu.ng*

Abstract

Food has always been the means by which disease-causing organisms are transmitted to man. This study sought to isolate multi-antibiotic resistant bacteria from ready to eat food (RTE) sold within Nigerian Defence Academy. Eight (8) food samples were collected from five (5) vendors and spread plate technique was adopted for bacteria isolation from the food samples. Twenty-five (25) bacteria isolates were obtained from the food samples and subjected to antibiotic sensitivity assay using Kirby Bauer disc diffusion method. Eight (8) of the isolates exhibited various degrees of resistance to the tested antibiotics. All the isolated bacteria in this study were susceptible to the effect of Quinolones antibiotics and were resistant to cefuroxime, cefotaxime and Ampiclox. Six (6) of the isolates that showed multi-antibiotic resistance were characterized by 16SrRNA gene sequencing as *Bacillus cereus* strains (0083 and HYM76), *Staphylococcus aureus* strain MRSA252, *Lactobacillus murinus* strain AMB_10 and *Listeria Spp*. Polymerase chain reaction (PCR) and agarose gel electrophoresis detection of four (4) antibiotics resistance genes – Aminoglycoside adenylyltransferase (AADA1), Acridine (ACRA), Chloramphenicol Resistance (CMLA) and Erythromycin (ERM(C)) – produced the following results: AADA1 genes were amplified in *Staphylococcus aureus* and *Bacillus cereus*; ACRA gene was detected in all the selected isolates; CMLA gene was detected in *Staphylococcus aureus*, *Lactobacillus murinus* and *Bacillus cereus* while ERM(C) gene was present in *Staphylococcus aureus*.

Keywords: Quinolones, antibiotics, *Staphylococcus aureus*, isolation, strain

Introduction

Food vendors abound in both urban and rural population globally, and they are generally and culturally accepted as provider of foods that are appealing, convenient and most often inexpensive (Namugumya and Muyanja, 2011). According to World Health Organisation (WHO), street foods are foods and beverages that are prepared and sold in streets and other public places by vendors, these foods are available for consumption immediately or later without further processing (WHO, 2023). The diversity that exists among food vendors is related to the kind of food prepared and sold by them, their business size, their mode of operation and location in which they prepare and sell the food, likewise the type of consumers to which they sell the food (Khairuzzaman *et al*., 2014). Consumption of food from food vendors cut across people of all socio-economic groups which include hawkers, students, industrial and office workers in various line of services. Consumers in selecting a street food vendor to patronize often attach importance to hygienic environment, but most food vendors have no knowledge of what causes food -borne diseases hence patronizing street food vendors have great public health implications (FAO, 2017). Unsanitary environment and unhygienic handling practices during the preparation, packaging or selling of such food are sources of pathogenic bacteria contamination of food sold by food vendors.

The most common cause of illness and death in developing countries is attributed to food poisoning, which occur when foods that are contaminated with pathogenic organisms or their toxins are ingested (Sapkota *et al*., 2012). Most cases of food poisoning are attributed to consumption of food from food vendors. According to Nonato *et al*. (2012) Street food from food vendors have nutritional components of an unhealthy diet; they also provide an avenue for the contamination of foods by physical, chemical and/or biological agents which are a cause for concern in food safety.

Bacteria develop mechanisms to protect themselves against drugs thereby rendering these drugs ineffective against the infections caused by such organisms. Antibiotics resistance by bacteria lead to difficulties in handling and curing of diseases caused by such bacteria invariably causing prolonged hospital admission as well as increased morbidity and mortality rates in affected patients, resulting in great financial burden on the patient and economic loss to the nation (Nonato *et al*., 2012). Antibacterial drug resistance is a major cause for concern that is rising daily. Development of antimicrobial resistant

bacteria could be due to the inappropriate use of antibiotics, use of antibiotics in animal feeds as an enhancer of growth in the animal and lack of knowledge on antibiotic consumption. The bacteria adaptation is manifested by their ability to appropriate new properties or by modification of their genome (mutation) by acquisition of genetic information through mobile genetic elements such as plasmid and transposable elements (Cardinale *et al*., 2005). The current trend of multiantibiotic resistance by bacteria is one of the most sudden and striking clinical and biological occurrence. There is dearth of data on multiantibiotic resistant bacteria associated with ready-to-eat food, especially within the Nigerian Defence Academy, Kaduna.

Materials and Methods

Study Site

The study site was Nigerian Defence Academy (NDA) premises. Nigerian Defence Academy comprises of Ribadu cantonment (old site) in Kaduna North and Afaka (New Site).

Sample Collections

Food Samples were bought from various food vendors within NDA premises. The food samples were allowed to cool down and immediately refrigerated to preserve for further analysis. Sample collection was done twice from the same food vendors.

Sample Preparation

One gram of each sample was placed into a test tube containing 5 ml of sterile distilled water. The food sample and distilled water mixture were agitated vigorously to ensure thorough mixing, after this a five-fold serial dilution of the food sample homogenate was carried out by sequentially transferring 1 ml from the test tube into another test tube containing 9 ml of sterile distilled water.

Isolation of bacteria

Spread plate method was adopted for culturing the diluted food sample. One hundred microlitre (100µl) of dilutions 10^{-2} through 10^{-5} of the homogenates were introduced in triplicates into sterile nutrient agar (NA) plates. Then sterile bent glass rod spreader was used to evenly spread the inoculum on the surface of the solid agar. Cultured plates were allowed to dry on the bench after which the plates were incubated aerobically at 37^{o}C for 24hours.

After the incubation period, cultured plates were examined for bacterial growth/colonies and counted under illuminated colony counter (BOECO, Germany). The microbial counts were expressed as colony forming unit per gram (CFU/g) of the sample homogenate (Olutiola *et al*., 2000).

Isolation of Pure Culture

Isolation of pure culture was affected by aseptically picking distinct colonies from other

colonies in the primary culture plates and streaking them on nutrient agar plates using a sterile inoculating loop. The plates were incubated at 37^{o}C for 24hrs. After repeated sub-culturing, pure colonies of bacterial isolates were inoculated in sterile agar slants for storage (at 4^{o}C), until needed for identification and further studies (Akindele *et al*., 2017).

Identification of bacterial isolates from Food

Cultural and morphological characterization of bacterial isolates

The cultural and morphological characterization was carried out in accordance with the methods described by Holt *et al*. (1994). The cultural characteristics of the bacterial isolates examined include: their sizes, shapes, colours, surface margins, and form. The morphological properties of the bacterial isolates were also examined by Gram reaction, and slides were observed under oil immersion (X100 mag.) objective lens of the microscope (Yu *et al*., 2011).

Biochemical Tests

The following biochemical characteristics of the bacterial isolates were carried out: catalase, citrate, oxidase, coagulase, mannitol salt agar, and triple sugar ion test (Olutiola *et al*., 2000). The results were interpreted in accordance with Bergey's Manual of Determinative Bacteriology Edition ,8.0 (Holt *et al*., 1994).

Multiantibiotic Resistance Test

Commercially available antibiotics test discs (Maxicare medical laboratory) were used to test for the resistance/susceptibility profile of the pure isolates obtained from the food purchased from food vendors. The antibiotics/ concentrations used are Pefloxacin (PEF30µg), Gentamycin (CN 10µg), Ampiclox (APX30µg), Zinnacef (Z20µg), Amoxacillin (AM30µg), Rocephin (R25µg), Ciprofloxacin (CPX30µg), Streptomycin (S30µg), Septrin (SXT30µg), Erythromycin (E10µg), Nitrofurantoin (NF300µg), Cefexime (ZEM5µg), CefriaxaneSulbactarm (CRO45µg), Cefuroxime (CXM30µg), Levofloxacin (LBC5µg), Amoxicillin/cluvulanate (AUG30µg), Cefotaxime (25µg), Imipenem/cilastatin (IMP10/10µg), Ofloxacin (OFX5µg), Nalidixic Acid (NA30µg). Augmentin (AU10µg), Chloramphenicol (CH30µg). Antibiotics resistance was determined using Kirby-Bauer disc diffusion method on Mueller Hinton agar (Hudzicki, 2012). Gram negative and gram-positive standard test discs were aseptically placed on prepared agar plates which had been streaked with isolates and incubated at 37^{o}C for 24hrs. Antibiotic resistance and susceptibility were noted for

each of the implanted plate. An isolate will be considered multiantibiotic resistance if the isolate shows resistance to more than two antibiotics on the strip.

DNA Isolation

Genomic DNA of Multi Antibiotic Resistant bacterial isolates were extracted using Fungi/Bacterial Miniprep Kit (Zymo Research, USA). This was done by strictly following the manufacturers protocol. Briefly, 50-100mg of the bacterial cells which have been suspended in 200µl water were added to a Zymo Research (ZR) bashing bead lysis tube. The tube was processed at maximum speed using a bench top Vortex for 10 minutes. Afterwards, the ZR bashing bead lysis tube (0.1mm & 0.5mm) was transferred to a microcentrifuge and centrifuged at a speed of 10000xg for 1 minute. 400 µl of the supernatant was measured and transferred to Zymo spin III Filter fitted in a collection tube and centrifuged at 8000xg for 1 minute, 1200 µl of genomic lysis buffer was added to the filtrate in the collection tube and 800 µl of the mixture was transferred to a Zymo spin 11c column fitted with a collection tube and then centrifuged at 10,000xg for 1 min. The flow through in the collection tube was discarded and this step was repeated. The Zymo- spin 11c column was now fitted with a new collection tube, and 200 µl of DNA pre-wash buffer was measured and dispensed into the spin 11c column, the tube was centrifuged at 10,000xg for 1 min. Then 500 µl genomic DNA wash buffer was added to the Zymo spin 11C column and centrifuged again at 10000xg for 1 min. Afterwards 100 µl of DNA elution buffer was added directly to the column matrix of the Zymo spin 11C column which has been fitted to a clean 1.5 ml microcentrifuge tube and centrifuged at 10000xg for 30 sec to elute the DNA.

Amplification of 16S rRNA gene and MAR gene from MAR bacterial DNA

The following primer pairs was used- 5'16S rRNA: CCAGACTCCTACGGGAGGCAGC, 3'-16S rRNA: CTTGTGCGGGCCCCCGT CAATTC for the partial amplification of 16S rRNA gene. Amplification of the 16S rRNA gene was carried out by using Peltier thermal cycler (PTC- 200, USA). PCR master mix (New England Biolabs,USA) which has been reconstituted with 13µl of molecular grade water, then 1µl of forward primer, 1µl of reverse primer and 5µl of DNA template (extracted DNA) were pipetted into a PCR tube. The PCR mixture were arranged inside the thermocycler. The reaction conditions used were as follows, an initial denaturation at 95ºC for 5 mins., denaturation at 94ºC for 1 min., annealing at 55ºC for 30 sec, extension at 72ºC for 1 min., Cycle 11 – 1V (35 times) and final extension was at 72ºC for 4 mins. After

completion of cycling program, the reactions were held at 4°C

Gel Electrophoresis

The amplified products were loaded on 1.5% agarose gel against molecular marker and electrophoresed. The gel was prepared by dissolving 1.5 g of agarose powder in 100ml of tris acetate EDTA (TAE) buffer, this was microwaved to ensure complete dissolution of agarose powder. After cooling of the gel, 5 µl of safe stain (EZ-Vision, USA) was added and the mixture was poured into a gel tray which has been prepared by sealing ends of gel chamber and inserting a gel comb. The gel was allowed to solidify, combs and sealing ends were removed, then the gel was submerged in the electrophoresis chamber covered with TAE buffer. Electrophoresis conditions were 100 volts for 40 mins. DNA bands were observed in an UV-transilluminator and photographed using a gel documentation system (Microdoc DI-HD, MUV21-254/365, Cleaver Scientific).

DNA Sequencing

The 16S rRNA gene of the MAR bacteria was sequenced. The sequencing reaction was prepared according to the procedure of dye cycle sequencing with quick start kit. The sequencing reaction was prepared in 2ml tube and all reagents in the following order viz: dH2O 0-9.5 µl, DNA template 0.5-10 µl, primers 2 µl, DTCS Quick start master mix 8.0 µl. Reaction was setup in the PCR machine using the program: 96oC for 20sec, 50^{o}C for 30secX30cycle and 60^{o}C for 4mins. A labeled sterile 0.5ml tube was prepared for each sample, 5ul of freshly prepared stop solution/glycogen mixture was added to each labelled tube (2ul of 3M Sodium acetate, 2 ul of 100mM Na2-EDTA and 1ul of 20 mg/ml of glycogen - provided in the kit-), and 60 ul cold 95%(v/v) ethanol from -20 freezer was added and mix thoroughly. This was immediately centrifuged at 14,000 rpm at 4^{0}C for 15min.

The nucleotide sequencesobtained were compared with the sequences in National Centre for Biotechnology Information database (NCBI) using the Basic Local Alignment Search Tool (BLAST) in order to confirm the identity of the bacteria. Highest similarity was the basis used for the identification of the bacteria.

Polymerase Chain Reaction (PCR) Detection of Genes Encoding Resistance to Selected Antibiotics

The isolates in this study were subjected to PCR amplification for the detection of the following resistance genes: AadA1,CMLA, ACRA and Erm(C)

Selected Antibiotics Target Gene Sequence in Bacteria Isolates

Antibiotic	Target Gene	Primer Sequence	Annealing Temperature
Streptomycin	AadA1	TATCCAGCTAAGCGCGAACT(F) ATTTGCCGACTACCTTGGTC(R)	58^0C
Ciprofloxacin	ACRA	CTCTCAGGCAGCTTAGCCCTAA(F) TGCAGAGGTTCAGTTTTGACTGTT (R)	52^0C
Chloramphenicol	CMLA	TACTCGGATCCATGCTGGCC(F) TCCTCGAAGAGCGCCATTGG(R)	65^0C
Erythromycin	ERM(C)	ATCTTTGAAATCGGCTCAGG(F) CAAACCCGTATTCCACGAATT(R)	48^0C

Results

Bacteria Isolates from Food Samples within NDA Premises and Antibiotics Sensitivity test of Bacteria Isolates

A total of eight (8) food samples which comprises of rice and stew, rice, beans and stew; cassava meal (Eba) and soup were purchased from five (5) different food vendors within NDA premises. Six of the samples were from NDA permanent site in Afaka while two (2) were purchased from NDA Ribadu campus. Twenty-five (25) bacteria were isolated from all the food samples and stored in refrigerator prior to further analysis (Table 1). As shown in Table 1, Food with Sample Id, PB had the highest level of bacterial loads or contamination, followed by PE and then PF, while PD had the lowest bacterial count

Antibiotics susceptibility testing revealed that eight (8) of the isolates exhibited various degrees of resistance as shown in Table 2. While all the isolates in this study exhibited resistance to cefuroxime, cefotaxime and ampiclox, none of the isolates showed resistance to the quinolones (Ofloxacin, Levofloxacin, Pefloxacin and Ciprofloxacin).

Table 1: Bacteria Isolated from Food Obtained from Food Vendors

S/N	Sample ID	Serial Dilution Factor		
		10^1	10^2	10^5 (CFU/ML)
1	PF	2.2×10^2	1.4×10^3	0.4×10^6
2	PB	TNC	TNC	0.8×10^6

3	PC	TNC	TNC	0.2×10^6
4	PA	3.4×10^2	1.6×10^3	0.2×10^6
5	PE	TNC	TNC	0.5×10^6
6	PD	TNC	TNC	0.1×10^6
7	PI	2.0×10^2	0.5×10^3	0.3×10^6

Key

TNC: Too Numerous to Count

P: point of collection and Alphabets stand for various food vendors

Table 2: Antibiotics Sensitivity Profile of Eight Multiantibiotic resistant Bacteria Isolates

ANTIBIOTICS	PD1	PD3	AS	PE2	PF2	PF3	OQR	BS
AM								
APX								
SXT								
R								
CH								
AU								
E								
S								
CN								
NA								
CXM								
AUG								
CTX								
IMP								
CRO								
ZEM								
Z								
NF								

CPX								
LBC								
PEF								
OFX								



Key

AM: Amoxacillin
APX: Ampiclox
SXT: Septrin
R: Rocephin
CH: Chloramphenicol
AU: Augmentin
E: Erythromycin
S: Streptomycin
CN: Gentamycin
NA: Nalidixic Acid
CXM: Cefuroxime
AUG: Amoxicillin /cluvalanate
CTX: Cefotaxime
IMP: Impenem /cilastatin
CRO: Cefriaxone/Sulbactarm
ZEM: Cefexime
Z: Zinnacef
NF: Nitrofurantoin
CPX: Ciprofloxacin
LBC: Levofloxacin
PEF: Pefloxacin
OFX: Ofloxacin

Sample ID	**Gram Stain**	**Catalase**	**Oxidase**	**Coagulase**	**Citrate**	**MSA**	**TSIA**	Probable Organism
PD1	-	+	+	_	+	-	-	*E coli*
PD3	+	+	-	+	+	+	-	*S. aureus*
PE2	-	-	+	+	-	-	-	Bacillus
PF2	+	+	-	-	-	+	-	*S. aureus*
PF3	+	-	+	+	+	+	-	*Lactobacillus*
AS	+	+	+	-	+	-	-	*S. aureus*
OQR	+	-	+	+	-	-	-	*B cereus*
BS	+	+	-	+	+	-	-	*Listeria*

Table 3: Cultural and Biochemical Characterisation of Bacteria Isolates

Molecular Characterization of the Isolates

The results from the sequencing of the 16SrRNA genes of six multi-antibiotics resistance isolates identified OQR and PE2 as *Bacillus cereus*, F2 as *Staphylococcus aureus* and F3 as Lactobacillus murinus. AS and BS produced no sequence results but the biochemical identification showed that probable organism for AS is Staphylococcus aureus and that of BS as Listeria species. The gel pictures showing 800bp of the 16SrRNA gene amplicon are presented in Plates 1-3 and the BLAST result is shown in Table 4

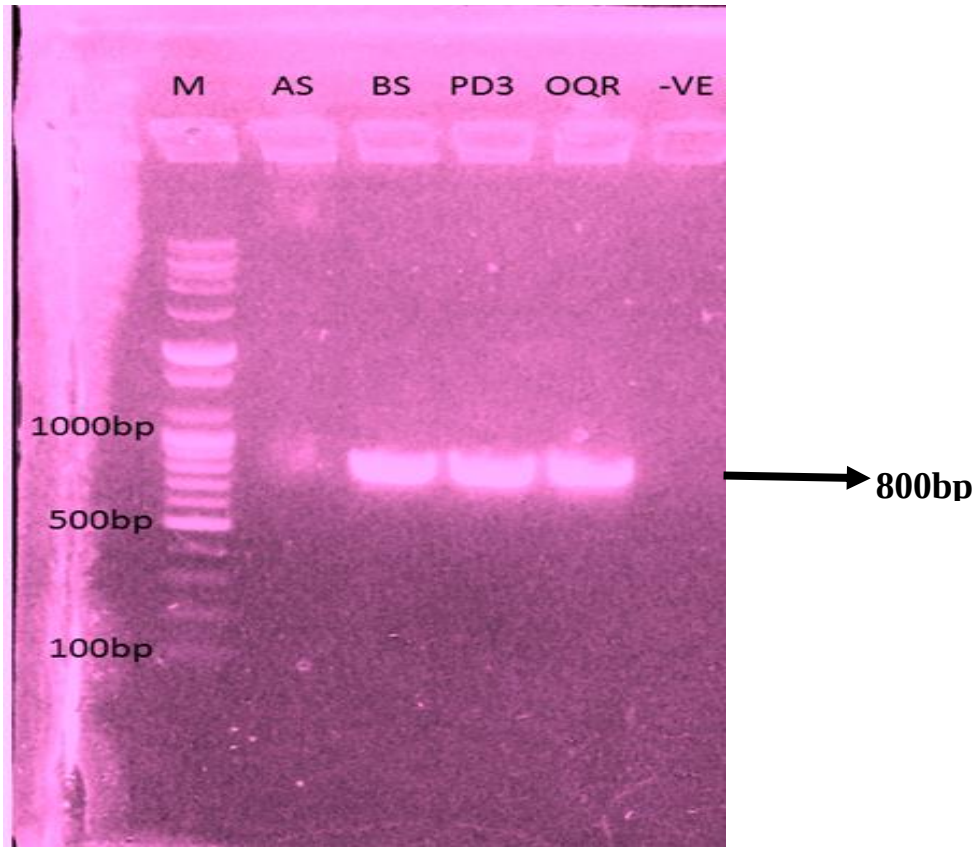


Plate 1: 16S rRNA gene Amplification of Isolates
M: Molecular Marker; -ve: Negative Control

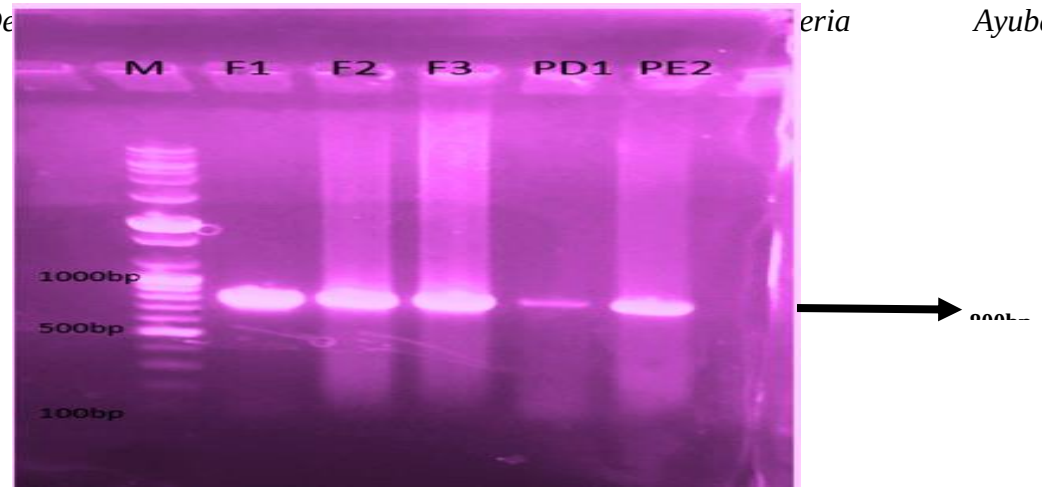


Plate 2: 16SrRNA gene Amplification of Isolates

M: Molecular Marker

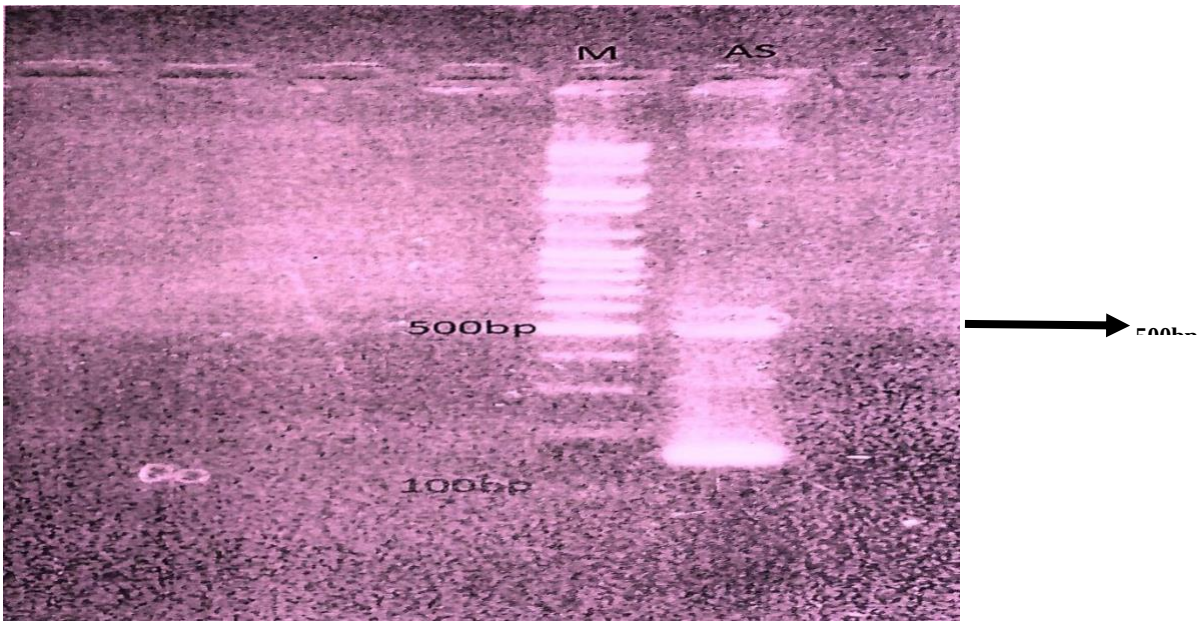


Plate 3: 16S rRNA gene Amplification of Isolate AS

M: Molecular Maker

Table 4: Blast Result for the Identification of Bacteria Isolates

Isolate Code	Query Id/ Length	Query Cover	E Value	Per Identity	Accession Number	Organism
OQR	2609/780	99%	0.0	99.49%	KP236219.1	*Bacillus cereus*

F2	18015/701	99%	0.0	92.88%	BX571856.1	*S. aureus*
F3	29517/706	100%	0.0	99.72%	JX971527.1	*Lactobacillus murinus*
PE2	14187/701	99%	0.0	100%	KT982233	*Bacillus cereus*
AS		NO	RESULT			
BS		NO	RESULT			

Amplification of AADA1, ACRA, CMLA and ERM Genes from Isolated Bacteria

Amplification of antibiotic resistance genes in the bacterial isolates showed that all the isolates tested AS (S. aureus), F2 (S. aureus), PE2 (B. cereus), OQR (B. cereus), F3 (Lactobacillus murinus) and BS (Listeria) harboured ACRA gene. AADA1 and CLMA genes were detected in isolate AS (S. aureus) and PE2 (B. cereus), as showed in plates 4 – 9.

The band sizes for each of the genes vary and are indicated in the plate 4 – 9.

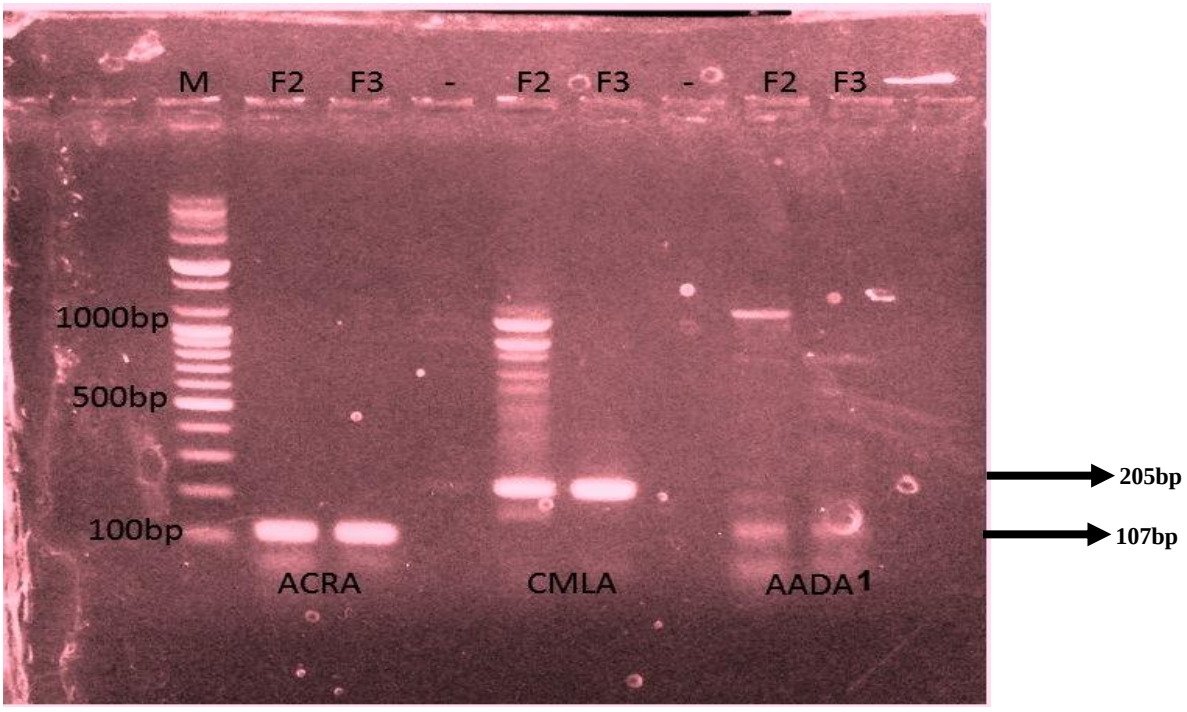


Plate 4: Amplification of antibiotic resistance genes (ACRA, CMLA, AADA1)

[ACRA: (107bp), **CMLA: (**205bp)]

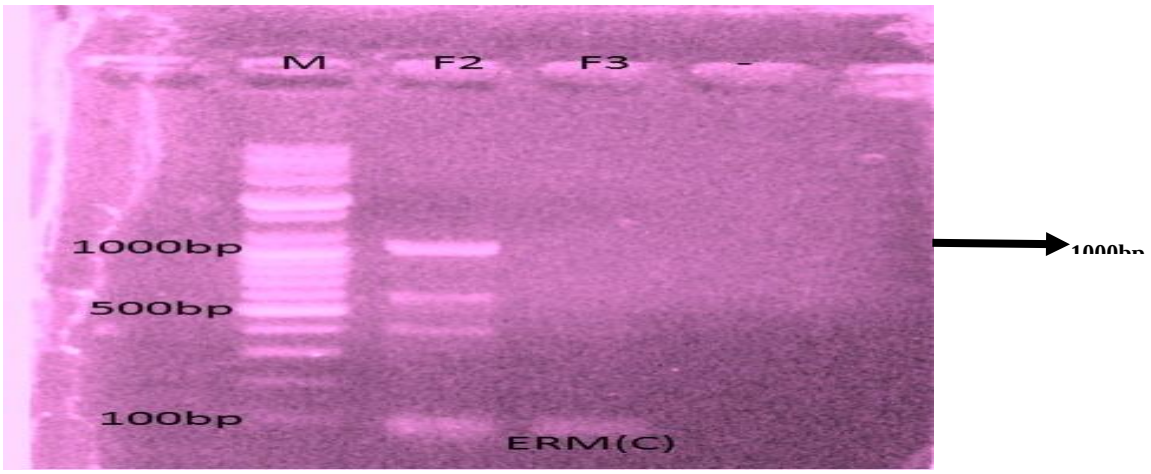


Plate 5: Amplification of antibiotic resistance gene (ERM(C))

[ERM(C) (1000bp)]

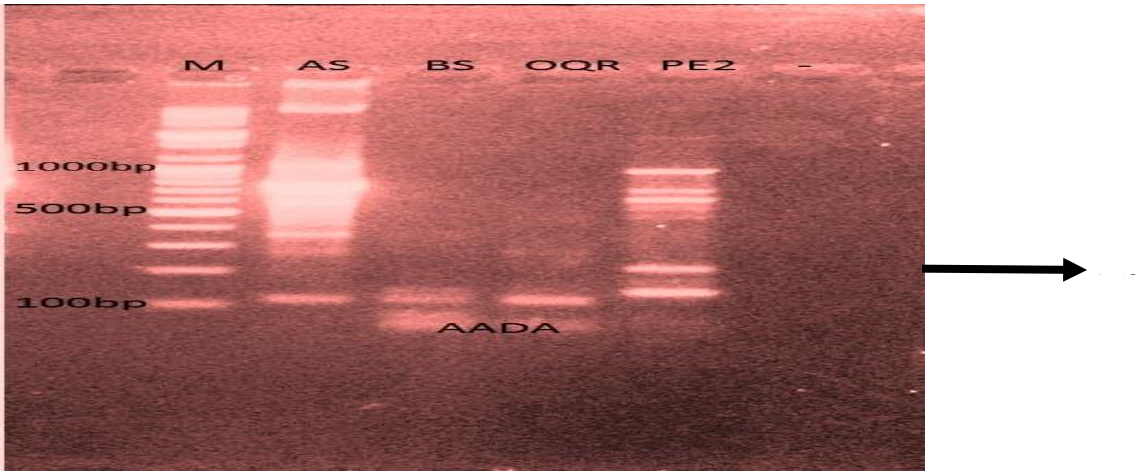


Plate 6: Amplification of antibiotic resistance gene (AADA)

[AADA: (200bp)]

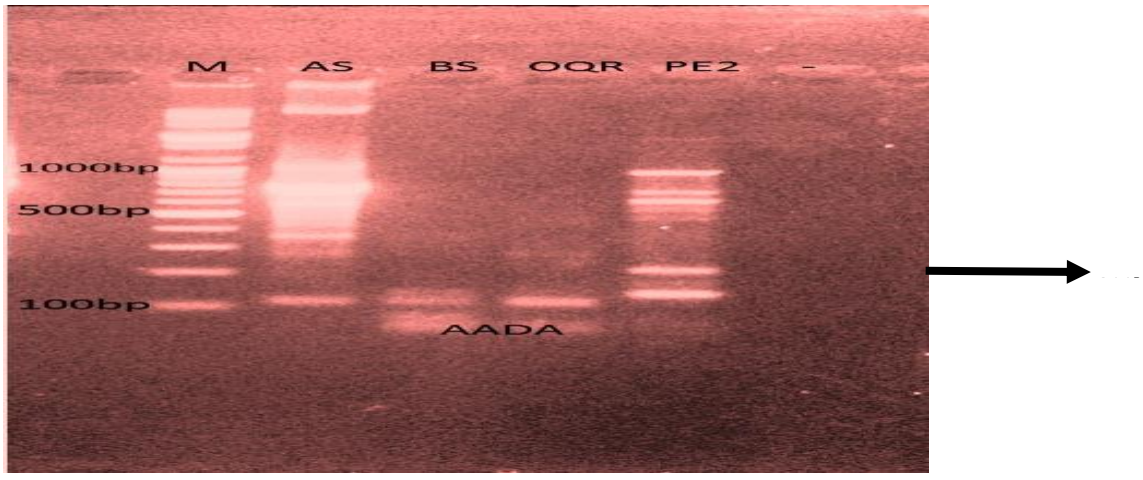


Plate 7: Amplification of antibiotic resistance gene (ACRA)

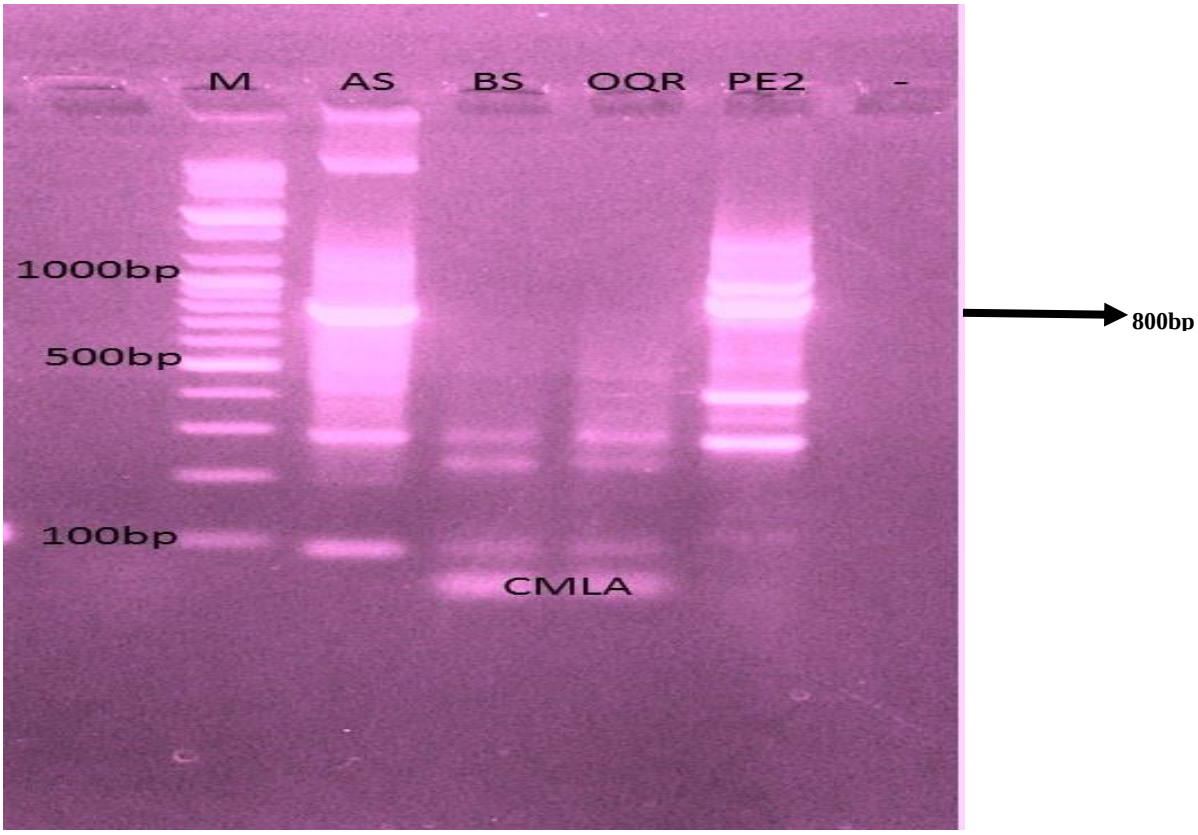
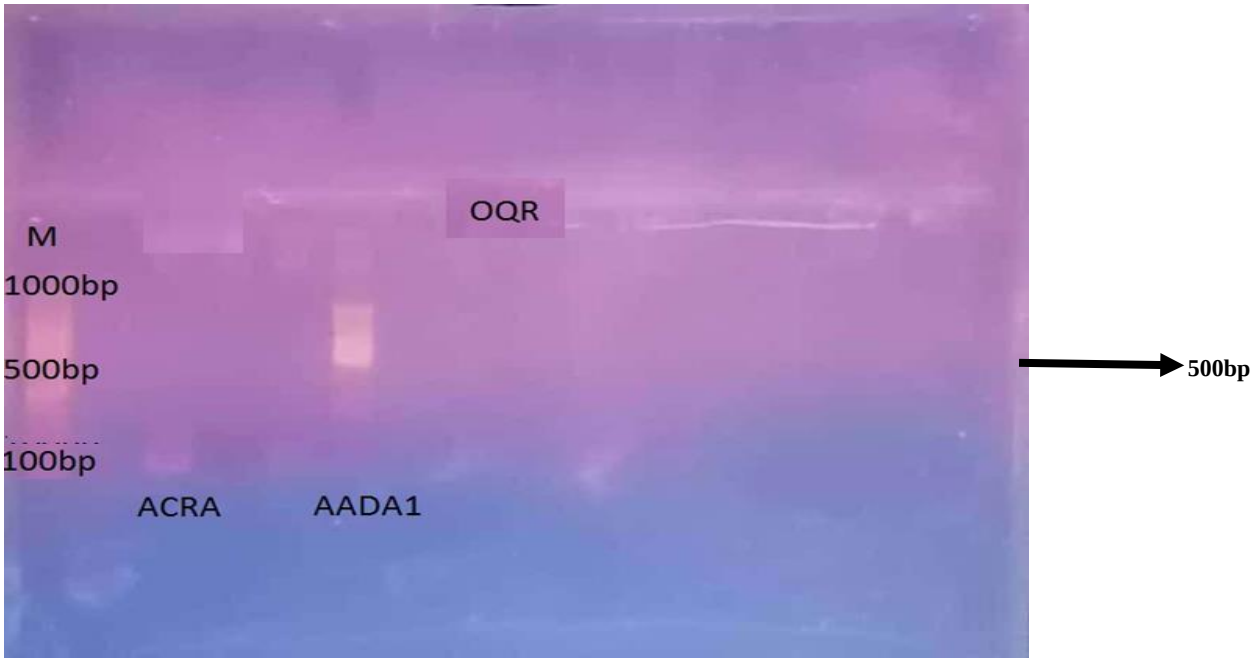


Plate 8: Amplification of antibiotic resistance gene (CMLA)

[CMLA: (800bp)**]**



Discussion

Foodborne infections are life threatening and also common problems for millions of people around the world (WHO, 2015). WHO reported that high resistance rates to major antibiotics used in human medicine have been observed in bacteria isolated from food producing animals (WHO, 2015).

The bacteria isolated in this study are *B. cereus, Staphylococcus aureus,* Listeria and Lactobacillus. In a review by Thomas Bintsis (2017), these bacteria isolates were classified as common and important foodborne pathogens. France classified Staphylococcus aureus and Bacillus cereus as the causative agents of food borne outbreaks (Dahl *et al*., 2007). *Bacillus cereus* is one of the most prevalent human pathogens among the Bacillus group (Bernard *et al*., 2005). It has over the years become an important food borne pathogen with presence in almost all types of foods, like Rice, foods of animal origin, vegetables as well as desserts (Jay, 2005). Contamination of food by *Bacillus cereus* is most often due to unhygienic handling, improper storage as well as insufficient reheating of food in restaurants and other ready to eat food outlets (Health Protection Surveillance Centre, 2012).

Drug resistance in bacteria could be due to the presence of plasmid, transposons or a gene coding for resistance to a specific agent and also by the action of multidrug resistance efflux pumps. Development of antibiotics resistance within bacteria are attributed to inappropriate use of antibiotics, extensive use of antibiotics as growth enhancers in animal feeds and increased transboundary passage of antibiotic resistance bacteria (Lowg, 2003). Antimicrobial resistant bacteria exist everywhere in the environment, also the transfer of antimicrobial resistance bacteria occurs through contact with animals and in the food chain which invariably enhances the spreading of antimicrobial resistance genes (ARGS) which could be transferred to bacteria from other sources (Slovis *et al*., 2014; Agga *et al*., 2019; Wichmann *et al*., 2014).

Staphylococcus aureus isolates exhibited resistance to Erythromycin and Chloramphenicol, similar result was reported by Fawzy *et al*. (2017). Argudin *et al*. (2012) also reported isolation of Staphylococcus aureus strains that were resistant to three or more classes of antibiotics from food, similar trend was observed in this study. *S. aureus* in this study exhibited high susceptibility to fluoroquinolones, several studies have equally reported susceptibility of Staphylococcus aureus isolated from other sources to

fluoroquinolones (Onanuga *et al*., 2005; Oguzkaya *et al*., 2008).

In agreement with the findings of this research, Edward (2010) reported that *Bacillus cereus* isolated from different sources as well as those implicated in food poisoning showed constant resistance to ampicillin and cephalosporins and uniform susceptibility to Erythromycin and ciprofloxacin.

Listeria monocytogenes isolated in this study exhibited susceptibility to Erythromycin, Streptomycin, Chloramphenicol and Gentamycin. Similar trend was observed by Pinar *et al*., (2018) in their study of the Prevalence and antibiotic resistance of Listeria monocytogenes isolated from ready to eat foods in Turkey. There was however, divergence as they reported isolation of strains that exhibited resistance to Levofloxacin and Ciprofloxacin. Listeria Spp. isolated in this study are fully susceptible to the antibiotics. Listeria susceptibility to Ciprofloxacin observed in our work is similar to the observations made by Al-Nabulis *et al*., (2015), Byrne *et al*., (2016) and Chemben and Puchooa (2015). *Listeria monocytogenes* in this research exhibited resistance to Imipenem in contrast to the report of Pinar *et al*., (2018). Resistance to Amoxicillin/clavulanic acid, and resistance to seven antibiotics was reported by Pinar *et al*., (2018), this was in accordance with this study.

Lactobacillus murinus was reported by Is-ani *et al*. (2018) as a relatively understudied species that gained attention as a probiotic candidate; and can also be applied for pathogen antagonism (Vasconcelos *et al*., 2003), antimicrobial production (Nardi *et al*., 2005). food allergy (Hang *et al*., 2016), age associated inflammation (Pan *et al*., 2018) and bacteria translocation (Fine *et al*., 2020). Researchers have isolated various strains of *Lactobacillus* species from ready to eat food Resistance of isolates to Cephalosporins, sulphonamides, and Penicillin observed in the study have been previously reported by Kathline and Arthur (2000) whose Lactobacillus isolates were resistant to cephalosporins. Cephalosporins are broad spectrum antibiotics which act by inhibiting cell wall synthesis.

Ciprofloxacin, Levofloxacin, Ofloxacin and Pefloxacin are the effective drugs in this study while Amoxicillin and Ampiclox are the least effective, this result is similar to that reported by Ibanga *et al*., (2020).

In this study ACRA gene was detected in all the isolates. ACRA is a linker protein in the tripartite ACRAB- TOLC system, this is a well-studied MDR pump system in which an inner membrane efflux transporter (ACRB) removes the antibiotics from the cytoplasm to

the periplasm and ACRA (Linker protein) directs the antibiotics via the inter membrane transport to the outer membrane channel – TOLC- and then to the surrounding environment (Nikaido, 2001; Page *et al.*,2005). Active efflux effected by efflux pump which expunges a wide variety of detergents and antibiotics from bacteria cells was first discovered in 1980 when it was linked to resistance of enterobacteria to tetracycline (Barbara and Ilaria, 2009). Over the years, efflux mechanism has been implicated in bacteria resistance to almost all antimicrobial agents, and over expression of efflux pump proteins makes the bacterium less susceptible to antimicrobial agent.

Going by the explanation above this may likely be the reason why all the bacteria isolated in the work were resistant to wide varieties of antibiotics. This is also supported by Piddock (2006) who explained that additional resistance is gained by bacteria due to action of over expressed efflux pumps as a result of their ability to extrude a wide variety of correlated antibiotics. According to Nikaido (2001), ACRA is one of the essential components of the ACRAB-TOLC multidrug efflux pump system.

Kenneth *et al.*, (2005), in their study of multidrug resistance in swine *Escherichia coli* detected thetheprescence of chloramphenicol resistance gene, CMLA on large (> 1000kbp) plasmids. The presence of this gene in *Staphylococcus aureus* and *Bacillus cereus* isolated in this study might be the reason why they showed resistant to chloramphenicol. Antibiotics resistance in bacteria can be due to dynamism and acquisition of antibiotics resistance gene that can be transferred horizontally from one bacterium to another (Graziani *et al.*, 2008). In our study staphylococcus aureus and Lactobacillus murinus exhibited phenotypic resistance to Erythromycin but ERM (C) gene which confers resistance to Erythromycin was amplified only in S. aureus. This might be an indication of the presence of other Erythromycin resistance gene(s) in the *Lactobacillus murinus* aside from ERM(C). A particular antibiotic resistance gene could be present in two or more strains of bacteria yet their phenotypic resistance may differ remarkably, this may be an indication of an association between Antibiotic Resistant Genes and high mutation rate. Many researchers have reported that acquiring a mutation confers antibiotics resistance (Sheikheldin *et al.*, 2018).

Conclusion

Our study showed presence of bacteria, which have been classified in several researches as

important foodborne pathogens in the food samples studied. The study revealed that the bacterial isolates exhibited multiantibiotic resistance traits. Sequencing of a 16SrRNA gene from the multiantibiotic bacterial isolates confirmed the the isolates as Bacillus cereus strains (0083 and HYM76), Staphylococcus aureus strain MRSA252, *Lactobacillus murinus* strain AMB_10 and *Listeria Spp*. The study also detected the presence of antibiotic resistant genes in MAR bacteria isolates.. Our findings could be an indication that RTE foods can serve as repository for nurturing multiantibiotic resistant bacteria.

References

Agga, G.E, Cook, K,L., Netthisinghe, A.M.P., Gilfillen, R.A., Woosley, P.B., Sistanic, K.R. (2019). Persistence of antibiotic resistance genes in beef cattle backgrounding environment over two years after cessation of operation. *Plos ONE* 15: e0212510. http://DOI.ORG/10.1371/Journal.pone.0212510.

Al- Nabulis, A.A., Osuilli, T.M., Shaker, R.R. (2015). "Effects of *Listeria monocytogenes*spp and antibiotics susceptibility of *Listeria monocytogenes*." *Food microbiology*. Vol. 46: pp. 154 – 160.

Alcaine, S.D., Lorin, D.W., Martin, W. (2007). Antimicrobial resistance in Nontyphoidal *Salmonella. Journal of food protection* 70(3), 780 – 790.

Argudina, M.A., Mendoza, M.C., Gonzalez-Hezia, M.A., Banca, M., Guerra, B., Rodicioa, M.R. (2012). Genotype, exotoxin gene content, and antimicrobial resistance of *Staphylococcus aureus* strain recovered from foods and food handlers. *Appl. Environ. Microbiol.* 78(8): 2930 – 2935.

Barbara, Z., and Illaria, V. (2009). Inhibition of multidrug resistant efflux systems in Bacteria. Recent patents on anti-infective drug discovery. 4: 37 -50

Bernard, R., Ghachi, M.E., Mengin-lecreulx, D., Chippaux, M., and Denizot, F. (2005). Berc from *Bacillus subtilis* act as an undecaprenyl pyrophosphate phosphatase in bacitracin resistance gene *J. Biol. Chem.* 280: 28852 – 7 \

Cardinale, E., Claude, J.D., Tall, F., Gueye, E.F., and Salva, G. (2005). Risk factors for contamination of ready-to-eat street vended poultry dishes in Dakar, Senegal . *International Journal Food Microbiology*. Vol. 103: 157-165.

Chembem, S.S., and Puchooa, D. (2015). "Isolation and characterization of Listeria monocytogenes species from ready to eat raw pork sausages processed in Mauritius" *Asian Journal of Agriculture and Food Science,* Vol. 3(3). 312 – 320.

Dahl, K.H., Yvon-Lewis, S.A., and Saltzman, E.S. (2007). Transfer of plasmid and chromosomal gylcopeptide resistance determinants occurs more readily in the digestive tract of mice than invitro and ex conjugants can persist stably invivo in the absence of glycopeptide selection. *J. Antimicrob. Chemother* 59: 478 – 486.

Edward, J.B. (2010). *Bacillus cereus*, a versatile human pathogen. Clinical Microbiology Review. Vol. 23 NO.2 pp. 382 – 398

Fawzy, R., Elseedy, A.A., Samyhala, S.H., Eman, A., Khany-Aya, A., Koraney, B. (2017). Polymerase chain reaction and detection of genes responsible for multiple antibiotic resistance *Staphylococcus aureus* Isolated from food of Animal Origin in Egypt. *Veterinary World*, ELSSN: 2231 – 0916 pp. 1205 -1211.

Gutierrez, R.M., Michell, S., Solis, R.V. (2008). *Psidium guajava:* A review of its traditional uses, phytochemistry and pharmacology. *Journal of Ethnopharmacol.* 117(1), 1 – 27

Hang, C.H., Shen, C.C., Liang, Y.C., and Jan, T.R. (2016). The probiotic activity of *Lactobacillus murinus* against food allergy. *J. Funct. Foods* 25: 231 – 241.

Health Protection Surveillance Center (HPSC) (2012). "Bacillus cereus food borne illness" Infectious intestinal disease: public health and clinical guidance.

Ibanga, I.A., Akan, O.D., Uyanga, F.Z., Asuquo, N. (2020). Multidrug resistance pattern of *Staphylococcus aureus* from paediatric ward, General Hospital, Ikot -Ekpaw, Mkpat LGA, Akwa – ibom State, Nigeria. *Archives of clinical microbiology* 25: 21 – 29.

Is-ani., M., Bell, B.A., Delaplain, P.T., Bowling, J.D., Golden, J.M., Elizee, M. (2018). *Lactobacillus murinus* HF 12 colonizes neonatal gut and protects rats from necrotizing enterocolitis. Plos One 13(6), e0196710Vasconcelos ALS., Jacques RN., Regina MDN. (2003). Antagonistic and Protective Effects against *Salmonella Enterica* Serovar Typhimurium By*Lactobacillus Murinus* In The Digestive Tract Of Gnotobiotic Mice, *Brazilian Journal of Microbiology* 34 (1):21-24.

Jay, M. (2005). Modern Food Microbiology 4th ed. CBS publishers and Distributors put Ltd. Pp. 501-503.

Kenneth, M.B., David, G.W., Michael, E.H., Toni, L.P., David, J.N. (2005). The Chloramphenicol resistance gene CMLA is disseminated on transferable plasmids that confer multidrug resistance Swine Escherichia coli. *FEMS Microbiology Letters* 243: 285 – 291.

Khairuzzaman, M., Chowdhury, F.M., Zaman, S., Manun, A., and Bari, M.I. (2014). Food safety challenges towards safe, healthy and nutritious food in Bangladesh. *International Journal of food science.* 2014: 483519.

Onuh**,** K., Dikwa, K.B., Dan, V.M.Y., Abdulsalami, M.S., Ozojiofor, U.O., Oaikhena, E.E., Onuh, K., Kereakede, E., Asabe, D. (2022) Molecular detection of transgenes in improved varieties of cowpea. *International Journal of Biochemistry Research & Review pp 1-9*

Lowg, F. (2003). Antimicrobioal resistance (AMR): the example of *Staphylococcus aureus. J. clin. Invest* 111: 1265 – 73.

Fine, R.L., Manfredo, V.S., Gilmore, M.S, Kriegel, M.A. (2020). Mechanisms and consequences of gut commensal translocation in chronic diseases. Gut Microbes;11(2):217-230. doi: 10.1080/19490976.2019.1629236.

Maclean, R.C., and San-Millan, A. (2015). Microbial Evolution: Towards Resolving the Plasmid Paradox.*Current Biology* : CB, 25(17):R764-7

Namugumya, B.S., and Muyanja, C. (2011). Contribution of street foods to the dietary needs of street food vendors in Kampala, Jinja and Masaka districts, Uganda. *Public Health Nutrition*. Vol. 15: 1503-1511.

Nardi, R.M., Santoro, M.M., Oliveira, J.S., Pimenta, A.M., Feraz, V.P.,

Benchetrit, L.C., Nicoli, JR. (2005). Purification and Molecular characterization of antibacterial compounds produced by *Lactobacillus murinus* strain L1. *J. Appl. Microbiol*. 99(3), 649 – 659.

Nikaido, H. (2001). Preventing drug access to targets: Cell Surface permeability barriers and active efflux in Bacteria. Sem. in cell Dev. Biolo. 12: 215 – 223.

Nonato, I.L., Fonseca, V.R.S., Paz, J.G. (2012). Sanitary-hygienic quality of sale points and microbiological analysis of Street Food Sold in the Umuarama Campus of the Federal University of Uberlandia. *Bioscience Journal*, 28:1061-1071.

Onanuga, A., Oyi, A.R., Olayinka, B.O., Onaolapo, J.A. (2005). Prevalence of community associated multi – resistant *Staphylococcus aureus* among healthy women in Abuja – Nigeria. *Afr. J. Biotech.* 4: 942 – 945

Oguzkaya-Artan, M., Bakyan, Z., Artan, C. (2008). Nasal carriage of *Staphylococcus aureus* in healthy preschool children. *Jpn. J. Infect, Dis*. 61: 70 – 72.

Page, J.M., Masi, M., Barbe, J. (2005). Inhibition of efflux pumps in Gram-negative bacteria. *Trends Mol. Med*. 11: 382 – 389

Pan, W.H., Xin-Ya, X., Ni, S., Siu, W.T., and Hong-Jie, Z. (2018). Antimalarial Activity of Plant Metabolites, *Int. J. Mol. Sci*. 19(5), 1382.

Uymaz-Tezel, P.S.B., and Cakmak, G.A. (2018). Prevalence and Antibiotic Resistance of *Listeria monocytogenes* isolated from ready to eat foods in Turkey. *Hindawi journal of food quality*, vol. 2018 Article ID 107693782 pp. 1-9.

Sapkota, R., Dasgupta, B., Nancy, A., and Rawat, D.S. (2012). Anti-bacterial effects of plant extracts on human microbial pathogens and microbial limit tests. *International Journal of Research Pharmacological Chemistry.* Vol 2(4): 926 – 936.

Sheikheldin, A.A., Shi, D., Chen, M., Zhang, L., Hejair, H.M.A., Muhammad, U., Yao, H., Zhang, W.(2018). Antibiotic resistance genes screening and comparative genomic analysis of commensal Escherichia coli Isolated from poultry farms between China and Sudan. *Hindawi Biomed Research International* Vol. 2018 Article ID 5327450.

Slovis, N.M., Elam, J., Estrada, M. (2014). Infectious agents associated with diarrhoea in neonatal foals in central Kentucky: a comprehensive molecular study. *Equine Vet. J*. 46: 311 – 316.

Srivastava, J., Chandra, H., Nautiyal, A.R., and Kalra, S.J.S. (2013). Antimicrobial resistance (AMR) and plant-derived antimicrobials (PDAms) as an alternative drug line to control infections. *Biotech*. Vol 4: 451-460.

Bintsis, T. (2017). Food borne pathogens. A review. *Aims Microbiology*. Vol. 3 ISSN.3 pp. 529 – 563.

World Health Organisation (2023). Essential safety requirements for street vended foods. Food safety Unit Division of food and Nutrition.