BIU Journal of Basic and Applied Sciences 6(2): 228 – 254, 2021.

ISSN: 2563-6424

BACTERIOLOGICAL QUALITY ASSESSMENT AND MOLECULAR DETECTION OF ANTIBIOTIC RESISTANT GENES PRESENT IN BACTERIA ISOLATED FROM READY-TO-EAT RICE SOLD IN SELECTED MARKETS IN BENIN CITY, NIGERIA

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ABSTRACT

The spread of antibiotic resistance genes among foodborne pathogens and increasing number of cases of foodborne diseases is a threat to public health. In this study, bacteriological quality assessment of eighteen randomly sampled ready-to-eat rice wrapped with cellophane and leaf (Thaumatococcus daniellii) obtained from Okhra, Santana and Oba markets were carried out using Standard microbiological methods. The antibiotic resistance profile of bacterial isolates from the samples were tested using eleven commonly used antibiotics; detection of antibiotic resistant genes involved the use of Molecular methods. Total aerobic plate count, Escherichia coli and Staphylococcal plate count of the leaf-wrapped samples(LWS) is within the range of 5.38-5.56, 5.0-5.87 and 3.66-5.08 log_{10}CFU/g while the corresponding values for cellophane-wrapped samples (CWS) is 4.9-5.26, 4.46-7.15 and 4.26-5.65 log_{10}CFU/g, respectively.Percentage occurrence of bacterial isolates from LWS include Staphylococcus aureus (21.66%), Klebsiella sp. (15 %), E. coli (43.34 %) and Bacillus cereus(20%) while the corresponding values were 20.4, 8.16, 44.8 and 10.2 % for CWS, respectively.Pseudomonas sp. (16.44 %) occurred only in the rice samples wrapped with cellophane. All the isolates demonstrated antibiotic resistance to ceftazidime, augmentin, cloxacillin, erythromycin and ceftriaxone. Aminoglycoside resistant genes- aac(6) and ant(4) were detected in the isolates. Biofilm forming activity involved 37.29 % and 31.71 % of the isolates from LWS and CWS, respectively.Consumption of RTE rice sold in the three markets could result in abdominal pain, vomiting, gastroenteritis, diarrhea, dysentery and pneumonia. More worrisome is the fact that some of the antibiotics might not provide effective treatment against food-borne illness caused by the bacteria isolated from the samples. Therefore, regular monitoring of food vendors by relevant agencies, implementation of good manufacturing practices (GMPs), and increase in level of public awareness on food safety and health implications associated with indiscriminate use of antibiotics are recommended.

KEYWORDS: *Thaumatococcus daniellii, Food safety, Foodborne illnesses, Biofilm*

INTRODUCTION

Food safety is considered to be a basic right of every human being because it has a great influence on human health (Oranusi *et al*., 2013; Oje *et al*., 2016). In a bid to achieve food security, it is very important to incorporate food safety (Alum *et al*., 2006; Unnevehr, 2014). Unfortunately, a lot of issues emanating from food safety remains a big challenge especially in developing countries. Frequent outbreaks of foodborne illnesses constitutes a huge economic burden affecting many families and health care systems (Faour-Klingbeil and Todd, 2020). Over the years, underreporting the number of cases of foodborne illnesses in Africa and Asia have been observed which could be attributed to insufficient and improper monitoring of outbreaks. In both continents, it is estimated that 2.2 million persons especially children die annually due to water and foodborne diarrhoeal diseases (Nguendo, 2018). Foodborne diseases are linked to a wide range of fungi, bacteria, viruses, parasites and toxins (Okareh and Erhahon, 2015; Abdullahi *et al*., 2020).

Many pathogenic and non-pathogenic microorganisms are naturally associated with all kinds of foods. Non-pathogenic microbes that contaminate man's food chain could serve as a reservoir of genes for antimicrobial resistance found in plasmids where they are encoded by integrons (Oluyege *et al*., 2009; Daniels*et al.,* 2016). Antimicrobial-resistant encoding genes in commensals can be transferred to pathogens by natural gene transfer mechanisms. Horizontal transmission of genetic materials from one organism to another is a major mechanism for spreading antimicrobial resistance (Peterson and Kaur, 2018). Some pathogenic microorganisms have the ability to adapt to environmental stress conditions in the food chain which poses a threat to food safety (Federica *et al.,* 2020). The formation of biofilms by food-borne pathogens enhances its persistence in food and a wide range of surfaces it encounters in the food chain (Hall-Stoodley *et al*., 2004).

Ready-to-eat foods (RTEs) also known as convenience foods are shelf-stable foods sold by food vendors in public places, on the streets and gatherings. The food vendors are usually involved in preparing the dishes for sale to customers to consume immediately or at a later time without further processing (Ire and Imuh, 2016; Onilude *et al*., 2016; Giri *et al*., 2021). A wide range of RTEs available in the markets are prepared using single or mixed ingredients as well as various packaging materials (Makinde *et al*., 2020). Traditionally, ready-to-eat foods are wrapped with leaves which makes it possible for beneficial phytonutrients in the leaves to permeate into the food and give it a unique aroma, taste and natural flavour appreciated by consumers (Ire *et al*., 2020). A local meal very popular in Nigeria known as '*ofada rice*' is packaged with banana leaf which consumers also use as a serving plate (Ezeudu *et al.,* 2020).

The usefulness of *Thaumatococcus daniellii* plant which also go by the name '*miracle-fruit*, *miracle berry*, or *sweet prayer plant*' is vast. It produces a tough, broad, oval, papery, ovate-elliptic rounded leaves often used by rural dwellers to cook and wrap food.In recent times, it has been observed that

many fast food restaurants in urban areas have started appreciating the uniqueness of using leaves to wrap RTE foods. This is also obtainable in some areas in the US (Aiyeloja and Ajewole 2005; Ojekale *et al*., 2007). Due to the limitations of using leaf and other traditional packaging materials, modern packaging materials such as aluminum, laminates, plastics and others are preferable (Oladepo *et al*., 2015). Huge volumes of cellophane are used daily for packaging food products because it is cheap, has a moderate tensile strength and provides a sufficient barrier to moisture (Adegunloye *et al.,* 2006).

Among all edible grains, rice from the plant *Oryza sativa* is rated second behind maize based on world output. Rice is a staple food for many families in Nigeria. The menus in various food outlets in the country are incomplete without rice being included (Wogu *et al*., 2011; Anthony *et al*., 2020). Regardless of social status, thousands of residents in Benin city patronize ready-to-eat (RTE) rice on daily basis. However, microbial contamination of RTE rice exposes the public to high risk of foodborne illnesses.Compromising standards during preparation, cooking, storage and retailing of ready-to-eat rice could affect its microbiological quality. Other contributory factors could be the type of rice, cuisine, sanitary condition of the food outlets and personal hygienic practices of food vendors (Bukar *et al*., 2010; Wogu *et al*., 2011; Okareh and Erhahon 2015). It has been observed that the level of awareness about good hygienic practices and standard food handling practices among food vendors in many developing countries is low. Majority of the food vendors are unlicensed and their activities are usually not monitored by relevant regulatory bodies empowered to enforce food safety (Ire and Imuh, 2016; Anthony *et al*., 2020; Igbinosa *et al*., 2020). Consequently, majority of the products handled daily by the food vendors could be exposed to microbial contamination which poses a risk to public health (Wogu *et al*., 2011; Makinde *et al.*, 2020).

Wogu *et al*. (2011) and Ogunyemi *et al*. (2015) reported the presence of *Bacillus cereus, Escherichia coli*, *Staphylococcus aureus*, *Klebsiella pneumonia, Brahamella* sp. *Enterobacter aerogenes* and *Salmonella typhi* in ready-to-eat rice sold in food outlets and market places in selected cities in Nigeria. Antimicrobial susceptibility testing of the bacterial isolates revealed that many of them were resistant to commonly used drugs for the treatment of bacterial infections (Abdullahi *et al*., 2020). Many published works on microbiological quality assessment of ready-to-eat rice sold to the public in different locations; antibiotic resistant profile of bacteria isolated from the RTE rice did not provide useful information regarding the antibiotic resistant genes in the bacterial isolates (Oluyege *et al*., 2009; Majolagbe *et al*., 2011). Therefore, this study is aimed at carrying out bacteriological quality assessment, antibiotic resistance profile of the bacterial isolates as well as detect the presence of antibiotic resistant genes in bacteria isolated from ready-to-eat rice wrapped with leaf (*Thaumatococcus daniellii*) and cellophane, and sold in selected markets in Benin City.

MATERIALS AND METHODS

Description of the Study Sites

Three (3) markets in Benin metropolis were selected as the study sites. They include Okha market 6.1940° N, 5.6401° E, Santana market 6.2915° N, 5.6325° E and Oba market 6.3350° N, 5.6200° E. Depicted in Fig. 1 is the selected market sites where ready-to-eat rice were sampled in Benin city, Edo state. The state is among the six states in the South-South Geopolitical zone of Nigeria.

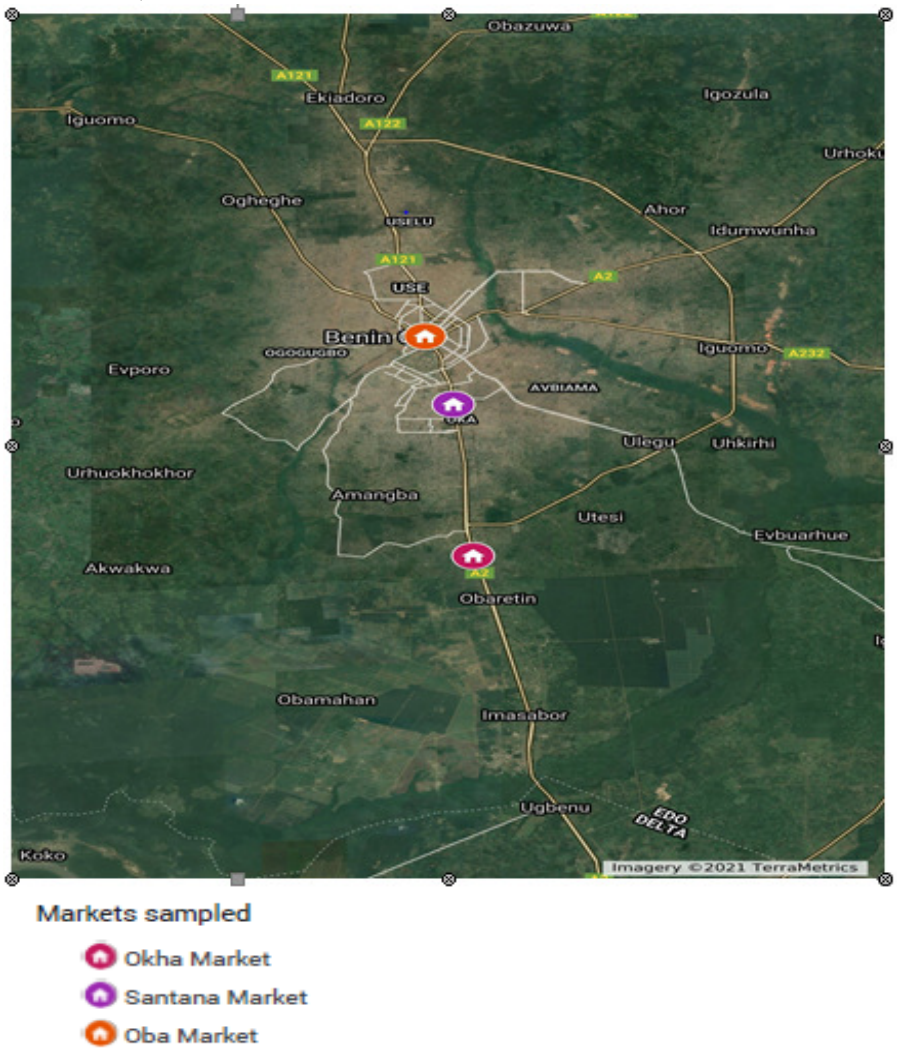


Fig 1: Selected market sites where ready-to-eat rice were sampled in Benin city, Edo State Source: Google map

Sample Collection

Three (3) samples of ready-to-eat rice wrapped with leaf (*Thaumatococcus danielli*) were purchased from different vendors in each of the selected markets. Similarly, three (3) samples of rice wrapped with transparent cellophane were purchased from different vendors in each of the selected markets. All the samples were aseptically packed inside a big sterile polythene bags, put inside a cooler

containing ice blocks and transported to Benson Idahosa University, Microbiology laboratory, for analysis within 12 hours after sampling was concluded. Molecular studies on the bacterial isolates was carried out in Molecular Biology Laboratory, International Institute of Tropical Agriculture (IITA) Ibadan, Nigeria.

Serial Dilution

Each sample was transferred into a mortar sterilized with 70% ethanol and pulverized using a sterilized pestle. One gram (1g) of the sample was homogenized in 9ml sterile peptone water. Five-fold dilutions of the homogenates were made using sterile pipette for each transfer.

Microbiological Analysis

Total Aerobic Plate count, Escherichia coli and Staphylococcal Plate Count

One milliliter (1 ml) homogenized solution from dilutions 10^{-3} and 10^{-4} was plated on nutrient agar (NA), Eosin methylene blue (EMB) and Mannitol Salt agar (MSA) plates prepared according to the manufacturer's instructions, sterilized at 121°C for 15 min at 15 psi. The plates were incubated at 37°C for 24h. The microbial growth on the culture plates were noted and the number of colonies were manually counted and reported as colony forming units per gram of food sample (CFU/g).

Obtaining Pure Cultures

Repeated subculturing of representative colonies in freshly prepared NA, EMB and MSA plates were carried out to obtain discrete colonies.The colonies were stored in slants and kept inside a refrigerator (4°C) until analyses were concluded.

Identification of the Bacterial Isolates

The cultural characteristics of the bacterial isolates on the cultures plates were noted. Gram reaction and cell morphology of the isolates were determined after examining the heat-fixed smear under the microscope. Biochemical tests carried out on the isolates include catalase, oxidase, coagulase, citrate, urease and sugar fermentation test.

Antibiotic Susceptibility Assay

The Kirby Bauer's agar disc diffusion technique method described by Onifade and Akinsola (2021) was adopted. A total of eleven (11) commonly used antibiotics; 30µg Ceftazidime, 30µg Cefuroxime, 10µg Gentamicin, 5µg Cefixime, 5µg Ofloxacin, 30µg Augmentin, 300µg Nitrofurantion, 5µg Ciprofloxacin, 5µg Erythromycin, 30µg Ceftriaxone, 5µg Cloxacillin were used in assaying antibiotic resistance of the isolates. With the aid of a flame-sterilized forceps, a commercially prepared antibiotic disc was picked and gently pressed on top of the agar surface of a 24 h culture of the test organism cultured in nutrient agar. The culture plates were kept in inverted position and incubated at 37°C for 24 h. Clear zones around each antibiotic disc was measured using a vernier caliper (Mitutoyo 530-119, Cranbury, New Jersey, United States) and interpreted as resistant (≤19mm), sensitive (≥ 23mm) and intermediate (20-22mm) in accordance with standard specified by Clinical Laboratory Standard Institute (CLSI).

Multiple Antibiotic Resistance Indices (MARI)

To calculate the MARI, the number of antibiotics a particular microorganism showed resistance is

divided by the total number of antibiotics the same microorganism was exposed to (Ekwelor *et al*., 2016).

Biofilm Forming Activity

Detection of the ability of a bacterial isolate to form biofilms was carried out using Congo red agar (CRA) method. The CRA medium was formulated by mixing 0.2g of Congo red, 9g of sucrose and 9.25g of Brain heart infusion (BHI) agar. Each isolate was plated on the medium and incubated at 37ºC for 24 h.Biofilm and non-biofilm producers were differentiated depending on the colour of the colonies. Black colonies with a dry crystalline consistency indicate biofilm producers, whereas retained pink are non-biofilm producers.

Detection of Virulence Factors

Hemolysis Test

The bacterial isolates were streaked on blood agar plate and incubated at 37ºC for 24h. The appearance of hemolysis or not on the culture plates was ascertained visually. Four types of hemolysis can be differentiated on blood agar based on the appearance. Beta hemolysis is indicated by a clear colourless zone surrounding the colonies. Alpha hemolysis is indicated by a small zone of greenish to brownish discoloration of the media. This is caused by the reduction of hemoglobin to methemoglobin and its subsequent diffusion into the surrounding `medium. Alpha prime hemolysis is indicated by a zone of complete hemolysis surrounded by a zone of partial hemolysis, a pink halo. It is easier to see it when the colony is scrapped off. Gamma hemolysis is indicated by no change in media.

Pathogenicity Test

This test is also known as Congo red binding ability test aimed at phenotypic detection of virulence factors. The ability of the bacterial isolate to bind with Congo red dye and CR+ (Congo red positive) is indicated by growth of red colonies. CR- (Congo red negative) implies that the isolate did not bind the dye and appeared as white colonies. The procedure involves preparing a media which consist of tryptone soy agar and Congo red dye and streaking the test organism on the medium.

Detection of Antibiotic Resistance Genes

The bacterial isolates selected for detection of antibiotic resistant genes demonstrated high resistance to most of the antibiotics in the sensitivity disc. Polymerase chain reaction (PCR) was performed on the DNA of the isolates for detection of antibiotic resistance genes.

Isolation of Bacteria DNA

Genomic DNA for PCR amplification were extracted from overnight tryptone soy broth (TSB) cultures at 37ºC with shaking. With the aid of heat block the cells were lysed at 100ºC for 15min. The cell fragment was thereafter retracted after centrifugation at 1100g for 2min with the aid of MiniSpin micro-centrifuge. The suspension was then kept at 20ºC until ready for use or used directly as template DNA.

Detection of Antibiotic Resistant Genes

Genes coding for AAC and ANT was carried out using polymerase chain reaction (PCR). The primers used in performing the PCR experiment involved the use of the following primers: AAC-f (5′-GATGATCTCTACTCAAACC) and

TTAGGCAGCAGGTTGAGG), ANT-f (5'-GAGAACCCATATGCAACATACTATCGCC) and ANT (5'TAGAATTCTAGCGCGCACTTCGCTCTTC). The reaction mixture contained inqaba PCR Premix (Inqaba, South Africa), which is premixed ready-to-use solution containing Taq DNA polymerase, dNTP, and $MgCl_2$. The reaction mixture was prepared in 0.2ml PCR tubes with 25µl reaction volumes (12.5µl Premix, 8.5µl nuclease free water, 0.5µl forward primer, 0.5µl reverse primer and 3.0µl template DNA) and done under the following thermocycling conditions in a GeneAmp PCR system (Geneamp, Singapore) 94°C for 4 min for initial denaturation then 31 cycles, each at 94°C for 45 s, 55°C for 1 min for *tet* M and *erm* B (60 °C for 1 min for *tet*A), 68 °C for 1 min and final extension at 68 °C for 8 min after which PCR products were separated in 1.5% agarose gel which was stained with ethidium bromide. PCR products on gel were visualized under UV trans-illuminator (Geneix biotech, Asia).

Plasmid Identification

This involves picking and inoculating a single colony from pure overnight bacteria culture grown on Luria Bertani (LB) agar into LB Broth, thereafter incubating the broth overnight. One hundred and fifty microliter (150µL) of the culture was later pelleted by centrifugation for 10 min at 1000xg. The supernatant was removed and re-suspended in 100µL of lysis buffer (3% SDS, 50mM Tris pH 12.6 with 50mM Tris adjusted by 1.6ml 2N NaOH up to 100ml final volume). The mixture was incubated at 55°C for 1 h. The resulting plasmid was extracted with 150µl of phenol: chloroform (1:1, v/v, pH 7.9) and mixed by inversion several times before spinning at highest speed for 10 min. Fifty microliter (50µL) of the supernatant was transferred into a new tube and mixed with 10µL of a loading dye. The mixture containing the plasmid was run on 1% agarose gel electrophoresis in 1X TAE buffer for more than 3 h at a voltage of 8v/cm**.**

Agarose Gel Preparation

A 0.8% agarose gel was prepared and two drops of ethidium bromide was added after which it was allowed to gel. Ten microlitres (10 µL) of the isolated plasmid with the loading dye (NO70225 Bio lab England) was loaded in each well. Then 10µl of the 1kb DNA ladder (NO4685 Bio lab England) was loaded on the last well. The electrophoresis was run at 80-150 V for about 1-1.5h after which bands were visualized with UV transilluminator.

RESULTS

Depicted in Fig. 2 is the total aerobic count of ready-to-eat rice wrapped with leaf and cellophane sampled from different market locations. The result shows that total aerobic count of rice wrapped with leaf obtained from Okha (5.56 log_{10}CFU/g), Santana (5.51 log_{10}CFU/g) and Oba (5.38log_{10}CFU/g) markets were higher than the corresponding values 4.90, 5.18 and 5.26 log_{10}CFU/g recorded for rice wrapped with cellophane, respectively.

Total *Escherichia coli* plate count of ready-to-eat rice wrapped with leaf and cellophane sampled from different market locations is depicted in Fig. 3. It shows that total *Escherichia coli* plate

count plate count of rice wrapped with leaf obtained from Okha, Santana and Oba markets is 5.38, 5.87 and 5.00log$_{10}$CFU/g while the corresponding values for cellophane-wrapped rice is 4.46, 7.15 and 6.02 log$_{10}$CFU/g, respectively.

Presented in Fig. 4 is the total *Staphylococcal* plate count of ready-to-eat rice wrapped with leaf and cellophane sampled from different market locations. The result shows that total *Staphylococcal* plate count of rice wrapped with leaf obtained from Okha, Santana and Oba markets is 3.66, 4.48 and 5.08log$_{10}$CFU/g while the corresponding values for rice wrapped with cellophane is 4.90, 4.26 and 5.65log$_{10}$CFU/g, respectively.

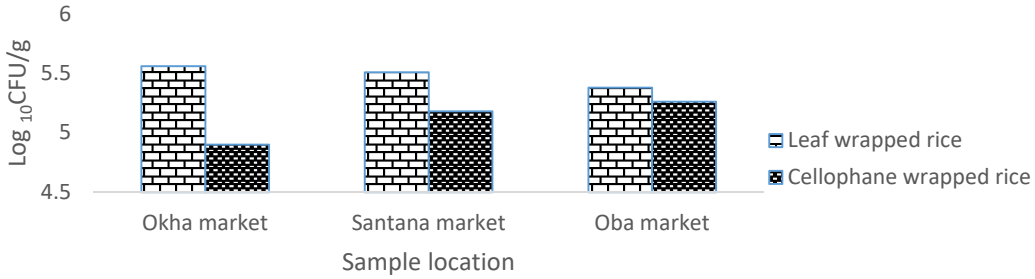


Fig. 2: Total aerobic plate count of leaf and cellophane-wrapped rice sampled from different market locations.

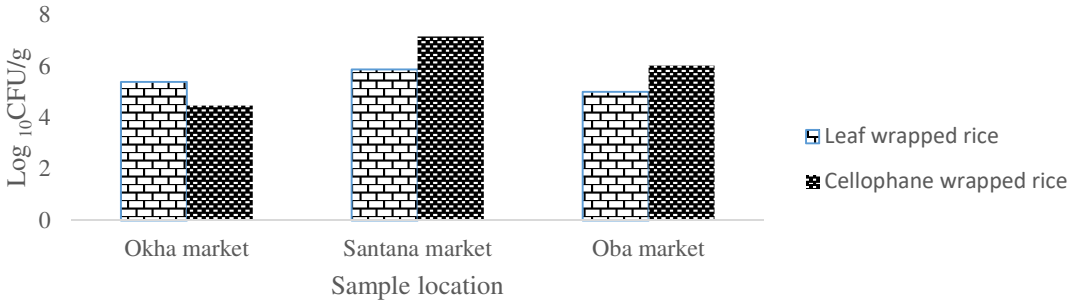


Fig. 3: Total *Escherichia coli* plate count of leaf and cellophane wrapped rice sampled from different market locations.

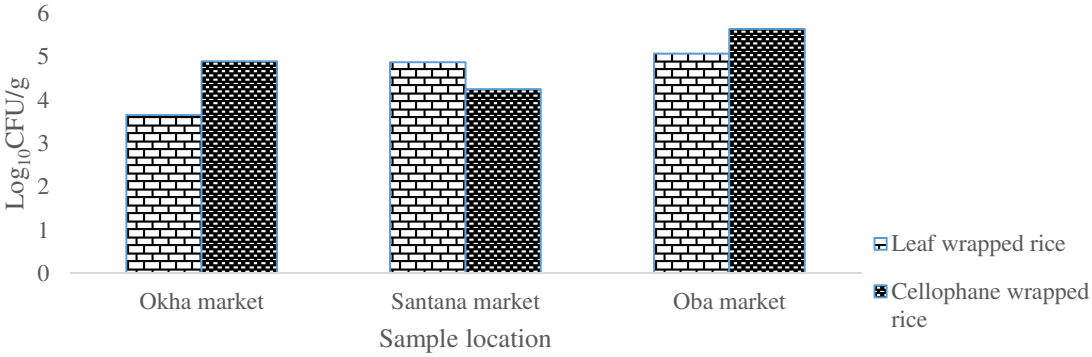


Fig. 4: Total *Staphylococcal* plate count of leaf and cellophane wrapped rice sampled from different market locations

Presented in Table 1 is the morphological and biochemical characteristics of five bacterial genera isolated from ready-to-eat rice wrapped with leaf and cellophane obtained from Okha, Santana and Oba markets. The isolates were *Staphylococcus aureus*, *Pseudomonas* sp., *Klebsiella* sp., *Escherichia coli* and *Bacillus cereus*.

Table 2 shows the distribution and proportion of antibiotic resistance among bacterial isolates from ready-to-eat rice wrapped with leaf. All the bacterial isolates demonstrated antibiotic resistance to ceftazidime, cefuroxime, cloxacillin, erythromycin and ceftriaxone. The isolates identified as *Bacillus cereus* were resistant to augmentin and nitrofurantion. *Bacillus* sp., *Klebsiella* sp. and *E. coli* also demonstrated antibiotic resistance to cefixime and augmentin. Antibiotic resistance to cefixime was also demonstrated by *S.aureus*. However, *Klebsiella* sp. did not demonstrate antibiotic resistance to ofloxacin and ciprofloxacin. Similarly, *Pseudomonas* spp. did not demonstrate antibiotic resistance to ciprofloxacin.

Presented in Table 3 is the distribution and proportion of antibiotic resistance among bacterial isolates from ready-to-eat rice wrapped with cellophane. All the isolates demonstrated antibiotic resistance to ceftazidime, cefixime, augmentin, cloxacillin, erythromycin and ceftriaxone. With the exception of *Staphylococcus aureus*, all the bacterial isolates also demonstrated antibiotic resistance to cefuroxime. *Escherichia coli* and *Klebsiella* spp. did not exhibit antibiotic resistance to ciprofloxacin. Similarly, *Pseudomonas* sp. did not exhibit antibiotic resistance to ofloxacin.

The antibiotic resistance patterns including the multiple antibiotic resistance indices (MARI) demonstrated by the bacterial isolates from ready-to-eat rice wrapped with leaf and cellophane obtained from the selected markets is presented in Table 4 and 5, respectively. The bacterial isolates encountered in ready-to-eat rice wrapped with leaf with the exception of *Klebsiella* sp. demonstrated antibiotic resistance to all the antibiotics they were subjected to. *Bacillus* and *Klebsiella* sp. isolated from ready-to-eat rice wrapped with cellophane were resistant to all the antibiotics they were subjected to. Some isolates identified as *Pseudomonas* sp, *E. coli* and *Klebsiella* sp. demonstrated resistance to all the antibiotics they were subjected to.

The multiple antibiotic resistance indices (MARI) of *B.cereus, Bacillus* sp., *Klebsiella* sp., *E. coli* and *S. aureus* isolated from ready-to-eat rice wrapped with leaf is within the range of 0.73-1.00, 0.73-1.00, 0.64-0.82, 0.64-1.00 and 0.64-1.00, respectively. As for the isolates encountered in ready-to-eat rice wrapped with cellophane which include *E. coli*, *Pseudomonas* sp., *Bacillus* sp., *S. aureus* and *Klebsiella* sp. the MARI is within the range of 0.64-1.00, 0.64-1.00, 0.73-0.91, 0.64-1.00 and 0.64-0.91, respectively.

Table 1: Morphological and biochemical characteristics of bacteria isolated from leaf and cellophane wrapped rice

Cultural characteristics	Morphology	Gram stain	Fructose	Sucrose	Lactose	Catalase	Coagulase	Urease	Citrate	Probable organism
Cocci in clusters	Cocci	+	A	A	A	+	+	+	+	*Staphylococcus aureus*
Greenish, smooth	Short rods	-	A	A	A	+	-	-	+	*Pseudomonas* spp.
Slimy, creamy	Single rods	-	A	A	A	+	-	+	+	*Klebsiella* spp.
Irregular	Single rods	-	A	A	A	+	-	-	-	*Escherichia coli*
Rhizoid, rough	Single rods	+	A	AG	A	+	+	+	+	*Bacillus cereus*

Key: +, positive; -, negative; A, acid; G, gas

Table 2: Distribution and proportion of antibiotic resistance among bacterial isolates from rice wrapped with leaf

		Total number and percentage of isolates that demonstrated resistance against the antibiotics										
Isolates identified	Total no.	Caz	Crx	Gen	Cxm	Ofl	Aug	Nit	Cpr	Cxc	Ery	Ctr
B. cereus	3	3(100%)	3(100%)	1(33.33%)	1(33.33%)	1(33.33%)	3(100%)	3(100%)	1(33.33%)	3(100%)	3(100%)	3(100%)
Bacillus sp.	9	9(100%)	9(100%)	6(66.67%)	9(100%)	3(33.33%)	9(100%)	8(88.89%)	3(33.33%)	9(100%)	9(100%)	9(100%)
Klebsiella sp.	9	9(100%)	9(100%)	5(55.56%)	9(100%)	0(0%)	9(100%)	8(88.89%)	0(0%)	9(100%)	9 (100%)	9(100%)
E. coli	25	25(100%)	25(100%)	17(68%)	25(100%)	15(60%)	25(100%)	23(92%)	6(24%)	25(100%)	25(100%)	25(100%)
S. aureus	13	13(100%)	13(100%)	9(69.23%)	9(100%)	6(46.15%)	12(92.31%)	12(92.31%)	5(38.6%)	13(100%)	13(100%)	13(100%)
Total	59	59(100%)	59(100%)	38(64.41%)	53(89.83%)	25(42.37%)	58(98.31%)	54(91.53%)	15(25.42%)	59(100%)	59(100%)	59(100%)

Key: Caz - Ceftazidime (30µg);Crx - Cefuroxime (30µg);Gen - Gentamicin (10µg); Cxm - Cefixime (5µg); Ofl - Ofloxacin (5µg); Aug - Augmentin (30µg); Nit - Nitrofurantion (300µg);Cpr - Ciprofloxacin(5µg);Cxc - Cloxacillin (5µg); Ery - Erythromycin (5µg);Ctr - Ceftriaxone(30µg).

Table 3: Distribution and proportion of antibiotic resistance among bacterial isolates from rice wrapped with cellophane

		Total number and percentage of isolates that demonstrated resistance against the antibiotics										
Isolates identified	Total no.	Caz	Crx	Gen	Cxm	Ofl	Aug	Nit	Cpr	Cxc	Ery	Ctr
E. coli	23	23(100%)	23(100%)	12(52.17%)	23(100%)	6(26.09%)	23(100%)	22(95.65%)	0(0%)	23(100%)	23(100%)	23(100%)
Pseudomonas sp.	8	8(100%)	8(100%)	6(75%)	8(100%)	0(0%)	8(100%)	7(87.5%)	2(25%)	8(100%)	8(100%)	8(100%)
Bacillus sp.	5	5(100%)	5(100%)	1(20%)	5(100%)	1(20%)	5(100%)	5(100%)	1(20%)	5(100%)	5(100%)	5(100%)
S. aureus	11	11(100%)	10(90.90%)	3(27.27%)	11(100%)	2(18.18%)	11(100%)	4(36.36%)	3(27.27%)	11(100%)	11(100%)	11(100%)
Klebsiella sp.	4	4(100%)	4(100%)	2(50%)	4(100%)	1(25%)	4(100%)	2(50%)	0(0%)	4(100%)	4(100%)	4(100%)
Total	51	51(100%)	50(98.03%)	24(47.06%)	51(100%)	10(19.61%)	51(100%)	40(78.43%)	6(11.76%)	51(100%)	51(100%)	51(100%)

Key: Caz - Ceftazidime (30µg);Crx - Cefuroxime (30µg);Gen - Gentamicin (10µg); Cxm - Cefixime (5µg); Ofl - Ofloxacin (5µg); Aug - Augmentin (30µg); Nit - Nitrofurantion (300µg);Cpr - Ciprofloxacin(5µg);Cxc - Cloxacillin (5µg); Ery - Erythromycin (5µg);Ctr - Ceftriaxone(30µg).

Table 4: Antibiotic resistance patterns and multiple antibiotic resistance indices (MARI) demonstrated by the isolates from ready to eat rice wrapped with leaf

Organism	No. of isolates that showed resistance	Antibiotic resistance pattern observed	No. of antibiotics the isolates showed resistance	MARI
Bacillus cereus	2	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr	8	0.73
	1	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr, Gen, Ofl, Cpr	11	1.00
Bacillus sp.	2	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr	8	0.73
	1	Caz, Crx, Cxm, Aug, Cxc, Ery, Ctr, Gen	8	0.73
	2	Caz, Crx, Cxm, Aug, Cxc, Ery, Ctr, Nit	8	0.73
	1	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr	8	0.73
	1	Caz, Crx, Cxm, Aug, Cxc, Ery, Ctr, Gen, Nit	9	0.82
	1	Caz, Crx, Cxm, Nit, Cxc, Ery, Ctr, Gen, Ofl	9	0.82
	1	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr, Gen, Ofl, Cpr	11	1.00
Klebsiella sp.	1	Caz, Crx, Cxm, Aug, Cxc, Ery, Ctr	7	0.64
	2	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr	8	0.73
	1	Caz, Crx, Cxm, Nit, Aug, Cxc, Ery, Ctr,	8	0.73
	5	Caz, Crx, Cxm, Aug, Gen, Nit, Cxc, Ery, Ctr	9	0.82
Escherichia coli	1	Caz, Crx, Cxm, Aug, Cxc, Ery, Ctr	7	0.64
	6	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr	8	0.73
	1	Caz, Crx, Cxm, Aug, Cxc, Ery, Ctr, Gen	8	0.73
	10	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr, Gen	9	0.82
	1	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr, Cpr	9	0.82
	1	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr, Gen	9	0.82
	1	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr, Gen, Ofl	10	0.91
	4	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr, Gen, Ofl, Cpr	11	1.00
S. aureus	1	Caz, Crx, Cxm, Nit, Cxc, Ery, Ctr	7	0.64
	1	Caz, Crx, Cxm, Aug, Cxc, Ery, Ctr,	7	0.64
	1	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr	8	0.73
	1	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr, Gen	9	0.82
	4	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr, Gen	9	0.82
	5	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr, Gen, Ofl, Cpr	11	1.00

Key: Caz - Ceftazidime (30µg);Crx - Cefuroxime (30µg);Gen - Gentamicin (10µg); Cxm - Cefixime (5µg); Ofl - Ofloxacin (5µg); Aug - Augmentin (30µg); Nit - Nitrofurantion (300µg);Cpr - Ciprofloxacin(5µg);Cxc - Cloxacillin (5µg); Ery - Erythromycin (5µg);Ctr - Ceftriaxone(30µg).

Table 5: Antibiotic resistance patterns and multiple antibiotic resistance indices (MARI) demonstrated by the isolates from ready to eat rice wrapped with cellophane

Organism	No. of isolates that showed resistance	Antibiotic resistance pattern observed	No. of antibiotics the isolates showed resistance	MARI
	1	Caz, Crx, Cxm, Aug, Cxc, Ery, Ctr	7	0.64
	6	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr	8	0.73
Escherichia coli	5	Caz, Crx, Gen, Cxm, Aug, Nit, Cxc, Ery, Ctr	9	0.82
	1	Caz, Crx, Gen, Cxm, Ofl, Aug, Nit, Cxc, Ery, Ctr	10	0.91
	10	Caz, Crx, Gen, Cxm, Ofl, Aug, Nit, Cpr, Cxc, Ery, Ctr	11	1.00
	1	Caz, Crx, Cxm, Aug, Cxc, Ery, Ctr	7	0.64
	1	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr	8	0.73
Pseudomonas sp.	4	Caz, Crx, Gen, Cxm, Aug, Nit, Cxc, Ery, Ctr,	9	0.82
	1	Caz, Crx, Gen, Cxm, Aug, Nit, Cpr, Cxc, Ery, Ctr	10	0.91
	1	Caz, Crx, Gen, Cxm, Ofl, Aug, Nit, Cpr, Cxc, Ery, Ctr	11	1.00
Bacillus sp.	4	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr	8	0.73
	1	Caz, Crx, Gen, Cxm, Ofl, Aug, Nit, Cxc, Ery, Ctr	10	0.91
	5	Caz, Crx, Cxm, Aug, Cxc, Ery, Ctr	7	0.64
	2	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr	8	0.73
Staphylococcus	1	Caz, Crx, Cxm, Aug, Cpr, Cxc, Ery, Ctr	8	0.73
aureus	1	Caz, Crx, Gen, Cxm, Aug, Cxc, Ery, Ctr	8	0.73
	1	Caz, Gen, Cxm, Ofl, Aug, Nit, Cpr, Cxc, Ery, Ctr	10	0.91
	1	Caz, Crx, Gen, Cxm, Ofl, Aug, Nit, Cpr, Cxc, Ery, Ctr	11	1.00
	1	Caz, Crx, Cxm, Aug, Cxc, Ery, Ctr	7	0.64
Klebsiella sp.	1	Caz, Crx, Cxm, Aug, Nit, Cxc, Ery, Ctr	8	0.73
	1	Caz, Crx, Gen, Cxm, Aug, Nit, Cxc, Ery, Ctr	9	0.82
	1	Caz, Crx, Gen, Cxm, Ofl, Aug, Nit, Cxc, Ery, Ctr	10	0.91

Key: Caz - Ceftazidime (30µg);Crx - Cefuroxime (30µg);Gen - Gentamicin (10µg); Cxm - Cefixime (5µg); Ofl - Ofloxacin (5µg); Aug - Augmentin (30µg); Nit - Nitrofurantion (300µg);Cpr - Ciprofloxacin(5µg);Cxc - Cloxacillin (5µg); Ery - Erythromycin (5µg);Ctr - Ceftriaxone(30µg).

Shown in Fig. 5 is the number of bacterial isolates encountered in ready-to-eat rice wrapped with leaf sold in selected markets which possess or lack biofilm forming ability. The result shows that *Escherichia coli* (17) and *Klebsiella* sp. (4) had the highest and lowest number of isolates among the bacterial genera encountered in the samples which lack ability to form biofilm, respectively. In contrast, *E. coli* (8) and *S. aureus* (3) had the highest and lowest number of isolates among the bacterial genera encountered in RTE rice wrapped with leaf which demonstrated ability to produce biofilm, respectively.

Depicted in Fig. 6 is the number of bacterial isolates encountered in ready-to eat rice wrapped with cellophane sold in selected markets which possess or lack biofilm forming ability. The bacteria specie which had the highest number of isolates that lack ability to produce biofilm is *Staphylococcus aureus* (11) whereas the lowest number of isolates involved *Bacillus* sp. (4) and *Klebsiella* sp. (4). In contrast, *Escherichia coli* (7) had the highest number of isolates which demonstrated the ability to produce biofilm whereas *S. aureus* (0) had the least number of isolates among the bacteria which produced biofilm.

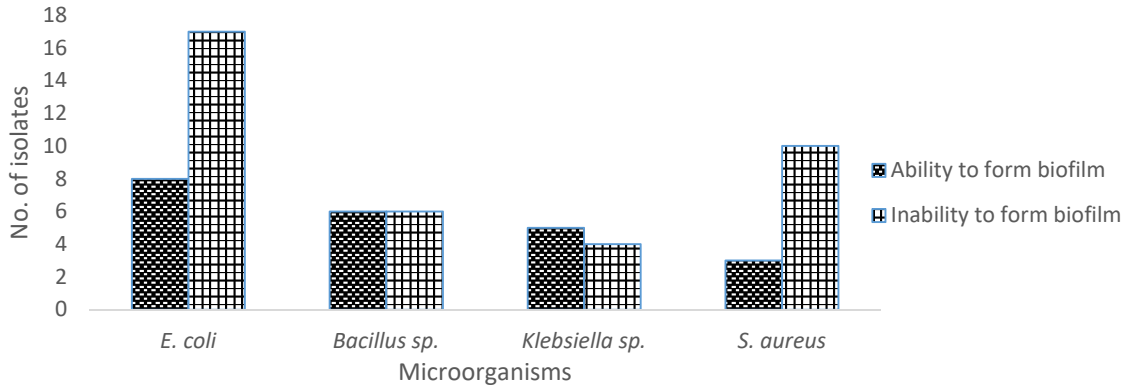


Fig. 5: Biofilm forming ability of bacterial isolates encountered in rice wrapped with leaf

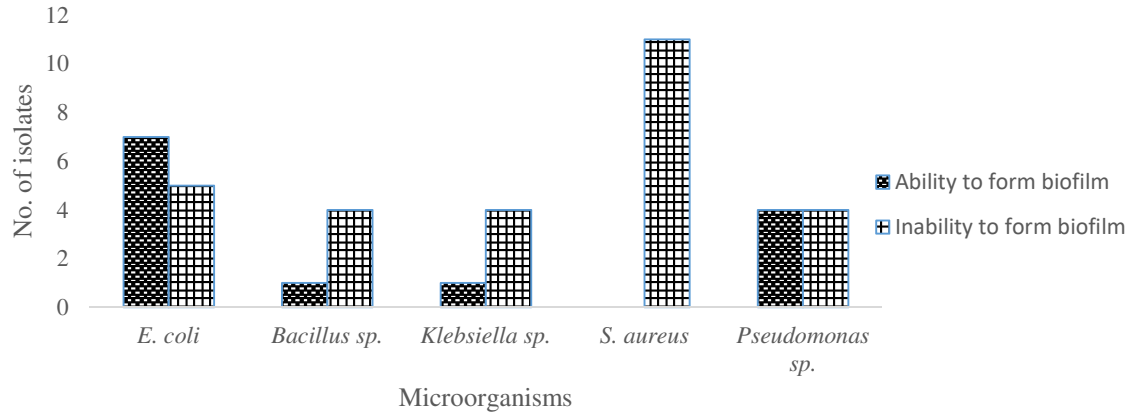


Fig. 6. Biofilm forming ability of bacterial isolates encountered in rice wrapped with cellophane

Presented in Table 6 is the result of testing for hemolysis with regards to the bacterial isolates encountered in ready-to-eat rice wrapped with leaf and cellophane. The result shows that all the isolates exhibited alpha hemolysis. Furthermore, all the bacterial isolates that exhibited alpha hemolysis were pathogenic according to the result presented in Table 7.

Table 6: Hemolysis exhibited by the isolates encountered in rice wrapped with cellophane and leaf

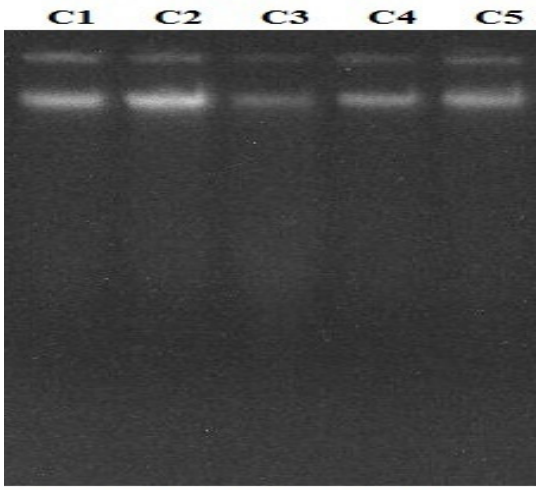
Isolates (Cellophane-wrapped rice)	Result	Isolates (Leaf-wrapped rice)	Result
$CR10^4$ M	α-hemolysis	$L10^2MO$	α-hemolysis
$CMO10^2$	α-hemolysis	$LOM10^4$	α-hemolysis
$C210^3$ ES	α-hemolysis	$L210^3ES$	α-hemolysis
$C310^5ES$	α-hemolysis	$LRM10^4$	α-hemolysis
$C2ES10^5$	α-hemolysis	$LR10^2M$	α-hemolysis

Table 7: Pathogenicity test of the isolates encountered in rice wrapped with cellophane and leaf

Isolates (Cellophane-wrapped rice)	Result	Isolates (Leaf-wrapped rice)	Result
$CR10^4$ M	+	$L10^2MO$	+
$CMO10^2$	+	$LOM10^4$	+
$C210^3$ ES	+	$L210^3ES$	+
$C310^5ES$	+	$LRM10^4$	+
$C2ES10^5$	+	$LR10^2M$	+

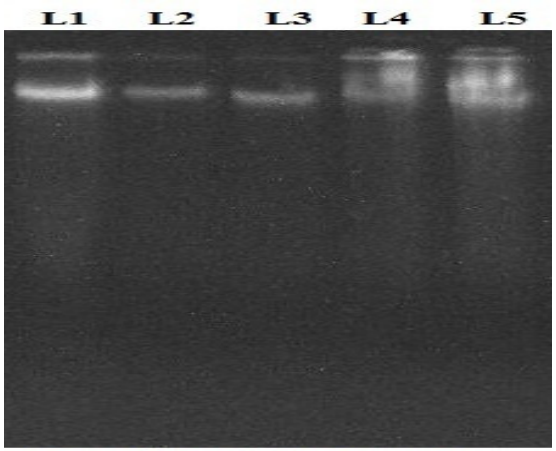
KEY= + (Positive)

Depicted in Plate 1 and 2 is the genomic DNA extracted from the bacterial isolates. Plate 3, 4, 5 and 6 shows the PCR gel image of the isolates while Plate 7 and 8 shows the plasmid DNA gel image of the isolates.



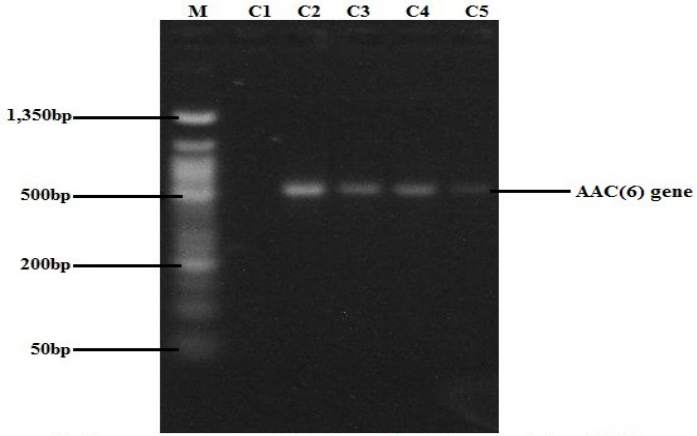
Gel image showing genomic DNA extracted from isolates.

Plate 1: Genomic DNA extracted from the isolates



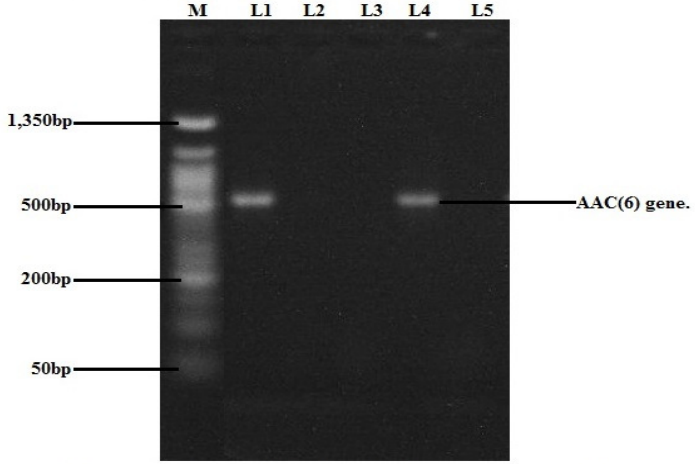
Gel image showing genomic DNA extracted from isolates.

Plate 2: Genomic DNA extracted from the isolates



Gel image showing amplification of AAC(6) gene at about 500bp.
M is 50bp ladder.
All lanes except lane C1 show amplification of the AAC(6) gene.
Lane C1 that shows no amplification indicate absence of AAC(6) gene in the genomic DNA of the sample.

Plate 3: PCR gel image of DNAfrom the isolates



Gel image showing amplification of AAC(6) gene at about 500bp.
M is 50bp ladder.
Lane L1 and L4 show amplification of the AAC(6) gene.
Other lanes showing no amplification indicate absence of AAC(6) gene in the genomic DNA of the samples.

Plate 4: PCR gel image of DNA from the isolates

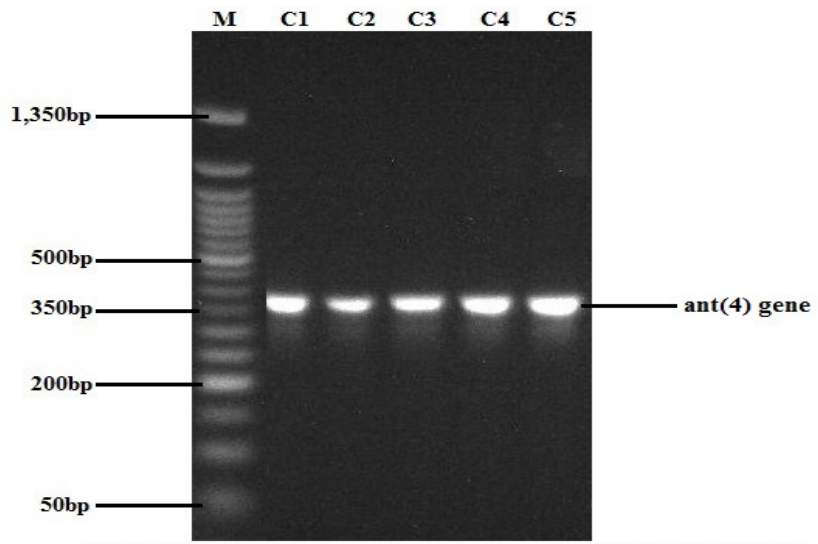


Plate 5: PCR gel image of DNA from the isolates

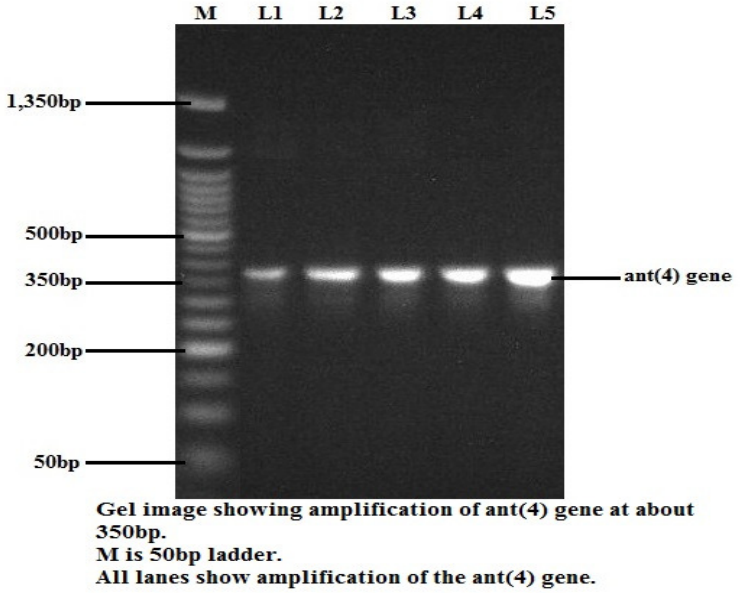


Plate 6: PCR gel image of DNA from the isolates

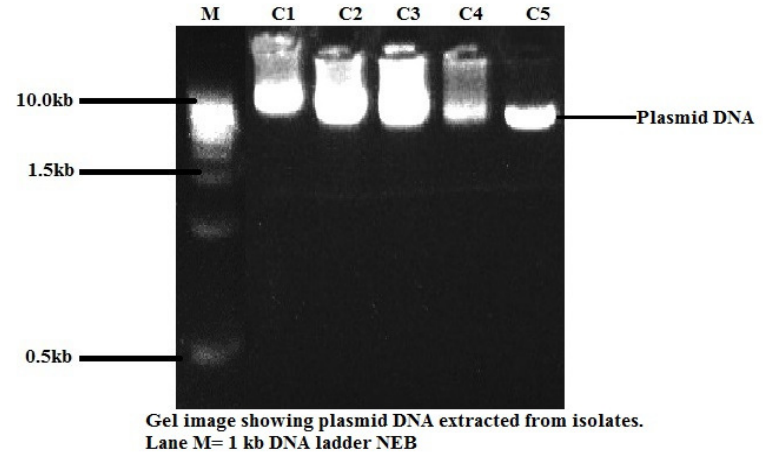


Plate 7: Plasmid DNA gel image from the isolates

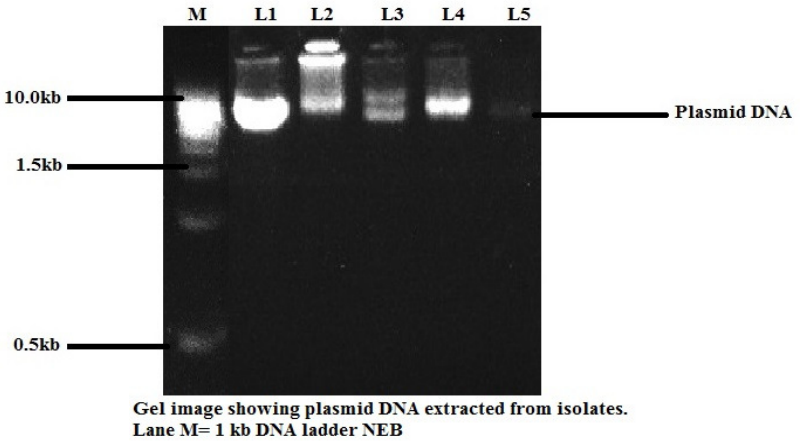


Plate 8: Plasmid DNA gel image from the isolates

DISCUSSION

This study has shown that samples of ready-to-eat (RTE) rice wrapped with leaf (*Thaumatococcus daniellii*) and cellophane obtained from three selected markets in Benin metropolis were contaminated with bacteria. The total aerobic plate count, *Escherichia coli* plate count and *Staphylococcal* plate count of rice samples wrapped with leaf is within the range of 5.38-5.56, 3.00-5.87 and 3.36-5.08 $\log_{10}$CFU/g while the corresponding values for rice samples wrapped with cellophane is 4.90-5.26, 4.46-7.15 and 4.26-5.65 $\log_{10}$CFU/g, respectively.In a related study, Ojesola *et al.* (2021) reported that total bacterial counts and total coliform counts within the range of 5.66-7.30 and 5.07-7.33 $\log_{10}$CFU/g, respectively were encountered in RTE rice samples packaged with cellophane, foam plates, leaves and sterile containers.

According to the International Commission for Microbiological Specification for Foods (ICMSF), plate counts which exceeds 10^6 is unacceptable, $10^4 - \leq 10^5$ is tolerable while $0 - 10^3$ is acceptable (Ire and Imuh, 2016). With reference to the specification, the plate count of the rice samples obtained from the markets is tolerable. As it concerns the hygiene indicator organism in RTE foods, *Escherichia coli* count <20 CFU/g is satisfactory; 20-$\leq 10^2$ is the borderline; $>10^2$CFU/g is unacceptable (Microbiological Guidelines for Food, 2014). Going by the result obtained from this study, the *Escherichia coli* plate count of the rice samples is unacceptable. According to Tsehayneh *et al.* (2021), acceptable population of *Staphylococcus aureus* in ready-to-eat foods should be lower than 10^3 CFU/g of food. It is unsatisfactory for any food sample to contain *Staphylococcus aureus* exceeding 10^4 CFU/g. Based on the result obtained from this study, the *Staphylococcal* plate count of the rice samples is unsatisfactory. Such foods if consumed poses a serious threat to public health.A serious illness could manifest after ingesting contaminated food containing staphylococcal enterotoxin (1 $ng^{-1}\mu g$)which range from minor skin infection to life threatening illnesses. Based on the standards, RTE rice samples obtained from the selected markets wrapped with leaf and cellophane is unsafe for human consumption.

Wogu *et al.* (2011) and Monday *et al*. (2014) reported the presence of *Escherichia coli*, *Salmonella* spp., S*taphylococcus aureus, Klebsiella pneumonia* and *Bacillus cereus* in ready-to-eat rice sold for public consumption. This result is in agreement with our research findings which revealed that *Escherichia coli, Bacillus cereus*, *Klebsiella* sp., *Pseudomonas* sp. and *Staphylococcus aureus* were microbial contaminants in RTE rice wrapped with leaf and cellophane.In a related study, Ogunyemi *et al*. (2015) reported the presence of *Brahamella* sp., *S. aureus, Enterobacter aerogenes* and *Salmonella typhi* in RTE cooked rice sold in parts of Lagos state, Nigeria. The sources of microbial contaminants in the rice samples wrapped with leaf and cellophane obtained from three selected markets in Benin city could be from the environment, dirty utensils, ingredients and water. Poor handling and unsanitary environment are predisposing factors for microbial contamination of the RTE

rice samples (Ire and Imuh 2016; Makinde *et al*., 2020).

A study carried out by Okareh and Erhahon (2015) reported the presence of *Staphylococcus aureus*, *S. epidermidis*, *Escherichia coli*, *Pseudomonas aeruginosa* and *Streptococcus* spp. in the hands of food handlers. In addition to food handlers being a possible source of bacterial contamination of the RTE rice samples, leaf and cellophane used in wrapping the food could also play a role. In a related study, Ojesola *et al.* (2021) isolated *Klebsiella pneumoniae*, *Staphylococcus aureus* and *Bacillus* sp. from polythene bags used in wrapping RTE rice while leaf used for the same purpose was contaminated with *Bacillus* sp. and *Klebsiella pneumoniae*. According to research findings by Ojekale *et al*. (2007), leaves of *Thaumatococcus daniellii* commonly used in wrapping RTE rice did not demonstrate a significant antimicrobial activity against food spoilage microorganisms which include *E. coli, Salmonella typhimurium, Shigella* sp., *S.dysentarieae, S. aureus, Leuconostoc* sp., *Streptococcus lactis, B. cereus*, *Pediococcus cerevisae, Candida krusei, C. albicans, Trichoderma konigii, Aspergillus niger* and *A. flavus*.

The presence of *Escherichia coli* in the rice samples wrapped with leaf and cellophane bags is an indication of direct or/and indirect feacal contamination from the food handlers, water and the use of dirty utensils. This organism is known as a normal flora in human and animal intestines (Odu and Assor, 2013). However, meningitis, urinary tract infections, diarrhea, dysentery, gastroenteritis and nosocomial pneumonia have been associated with some strains of *E. coli* (Osaenmwinda *et al*., 2019).

According to Ire and Imuh (2016), some healthy individuals are carriers of *S. aureus* in their skin, nasal passage and throat. Therefore, poor hygienic practices of the food processors and vendors could be responsible for contamination of the rice samples with *S. aureus.* Consumers of RTE rice wrapped with leaf and cellophane which were contaminated with *S. aureus* could manifest symptoms such as abdominal pain, diarrhea, vomiting and nausea depending on the population of the bacterium in the food (Osaenmwinda *et al*., 2019). Proliferation of this organism in food if allowed could bring about the release of heat-stable toxins associated with illnesses.

Isolation of *Klebsiella* spp. from the rice samples wrapped with leaf and cellophane suggests that faecal contamination of the product occurred. In a related study, Oranusi *et al*. (2013) reported the presence of *Klebsiella* sp. in fried rice sold in students' cafeterias. Obueh *et al*. (2017) stated that severe infections in humans such as bronchitis, pneumonia, meningitis and urinary tract infections are associated with *Klebsiella* sp. The presence of environmental contaminants such *Bacillus* sp. and *Klebsiella* sp. in ready-to-eat rice could be attributed to unnecessary exposure of the food to dust and air in the environment.

The source of *Bacillus cereus* in the RTE rice samples wrapped with leaf and cellophane could be from the foodstuff. According to Anthony *et al*. (2020), the organism is estimated to constitute 10 % of the soil microflora in rice paddies. *B. cereus* is implicated in 'fried rice syndrome' due to frequent

isolation of the bacterium in ready-to-eat fried rice kept for hours at room temperature. This practice is commonly seen where RTE fried rice meant for public consumption during ceremonies are prepared (Osarenmwinda *et al*., 2019). *Bacillus* sp. is widespread in the environment. According to Agwa *et al*. (2012), *Bacillus* sp. inhabits the soil, air, water and dust. The spores which usually survive harsh environmental conditions are introduced into agricultural products such as cereal crops, vegetables and others. Unnecessary exposure of RTE rice after preparation will increase the chance of food contamination by bacterial spores which will germinate into vegetative cells when the conditions are conducive.

The presence of *Pseudomonas* sp. in the RTE rice samples wrapped with cellophane could be traced to food handlers, dirty utensils, soil and water in the environment. Apart from being responsible for food spoilage, *Pseudomonas* sp. has been implicated with gastroenteritis in humans.A study carried out by Festus and Damilola (2018) reported the presence *Pseudomonas aeruginosa, P. putida, P. mendocina, P. alcaligenes, P. xiamenensis, P. fluorescens* and *P. fragi* from samples of ready-to-eat foods sold in selected markets in Ibadan, Nigeria.

The antibiotic resistance profile of bacteria isolated from RTE rice samples wrapped with leaf showed that all the isolates identified as *Bacillus* sp., *B. cereus*, *E. coli, Klebsiella* sp. and *S. aureus* were resistant to ceftazidime, cefuroxime, cloxacillin, erythromycin and ceftriaxone.Some of the bacterial isolates were resistant to some of the antibiotics used in this study with the exception of *Klebsiella* sp. which did not demonstrate resistance to ofloxacin and ciprofloxacin. All the bacterial isolates (*Pseudomonas* sp., *E. coli*, *S. aureus*, *Klebsiella* sp. and *Bacillus* sp.) encountered in RTE rice samples wrapped with cellophane showed resistance to ceftazidime, augmentin, cefixime, cloxacillin, erythromycin and ceftriaxone. However, none of the isolates identified as *E. coli* and *Klebsiella* sp. demonstrated resistance to ciprofloxacin; same with *Pseudomonas* sp. towards ofloxacin.In a related study, Ekwealor *et al*. (2006) reported that *Klebsiella pneumonia* did not show resistance to ofloxacin and ofloxacin. The antibiotic resistance profile of the bacterial isolates obtained from ready-to-eat rice wrapped with leaf and cellophane is substantially in agreement with the report of Onifade and Akinsola (2021).

The result obtained in this study support earlier assertion that ciprofloxacin is a broad spectrum antibiotic. It is regarded as the most potent antibiotics among the fluoroquinolones. Ofloxacin and ciprofloxacin are classified as second generation fluoroquinolones (Brar *et al*., 2020). Ciprofloxacin is a drug of choice in the treatment of urinary tract infections, typhoid fever, lower tract infections as well as few other bacterial infections (Sharma *et al*., 2010). However, there are concerns about indiscriminate use of ciprofloxacin in developing countries such as Nigeria (Sharma *et al*., 2017). Ali *et al*. (2010) reported that ciprofloxacin is 16.66%, 21.95 %, 27.02%, 44.44% and 72.22 % resistant to *Salmonella typhi, Staphylococcus aureus*, *Escherichia*

coli, Pseudomonas aeruginosa and *Klebsiella pneumonia*, respectively.

In this study, the minimum number of antibiotics which the bacterial isolates from leaf and cellophane-wrapped RTE rice samples showed resistance is 7 out of 11. Between 24 - 100% of the isolates belonging to different bacterial species encountered in RTE rice wrapped with leaf demonstrated resistance to the antibiotics they were exposed to. However, none of the isolates identified as *Klebsiella* sp. showed antibiotic resistance to ciprofloxacin and ofloxacin. In the case of RTE rice wrapped with cellophane, 19.61-100 % of the isolates belonging to different bacterial species isolated from the RTE rice samples were resistant to the antibiotics they were exposed to. However, none of the isolates identified as *Klebsiella* sp. and *Escherichia coli* were resistant to ciprofloxacin; the same way it is between *Pseudomonas* sp. and the antibiotic, ofloxacin. The report of antibiotic resistance of bacterial isolates from RTE rice samples wrapped with leaf and cellophane poses a threat to antibiotics therapy and public health. High incidence of antibiotic resistance bacteria could be attributed to indiscriminate, inappropriate and uncontrolled use of antibiotics. Widespread use of substandard antibiotics among the population could also play a role in the spread of antibiotic resistance (Festus and Damilola, 2018).

In a given population, the extent of spread of bacteria resistance is revealed by the multiple antibiotic resistance indices (MARI). According to Ekwelor *et al.* (2016), MARI that exceeded 0.20 is an indication that the bacteria strain involved was isolated from an environment where a lot of antibiotics have been used or misused. Since the MARI of all the bacterial isolates encountered in the ready-to-eat rice wrapped with leaf and cellophane exceeded 0.2, it raises some level of concern that all the antibiotics the bacterial isolates were exposed to in this study are used indiscriminately and inappropriately. Therefore, there is an urgent need for strict regulations in the use of antibiotics to prevent serious consequences in the healthcare system and the environment.

Findings from this study shows that *aac(6)* and *ant(4)* aminoglycoside resistant genes were present in *Staphylococcus aureus, Escherichia coli and Pseudomonas* spp. encountered in ready-to-eat rice samples wrapped with cellophane. Furthermore, 100% of the isolates encountered in the sample possess *ant(4)* gene while 60% of the isolates possess *aac(6)* gene. With regards to bacterial species isolated from ready-to-eat rice wrapped with leaf, it was revealed that *aac(6)* gene was present in *S. aureus* (40%), but absent in *Escherichia coli*. It should be noted that *S. aureus* and *E. coli* isolated from the samples possess *ant (4)* gene. However, *B. cereus* and *Klebsiella* sp. isolated from RTE rice wrapped with leaf do not possess resistant genes.

Plasmid analysis of the bacterial isolates obtained from ready-to-eat rice wrapped with cellophane revealed that all the isolates identified as S*taphylococcus aureus, Escherichia coli and Pseudomonas sp.* contain plasmids with a size of 10.0kb. With regards to ready-to-eat rice wrapped with leaf, plasmid analysis of the bacterial isolates revealed that all

isolates identified as *Escherichia coli* have plasmids, 30.76% of *Staphylococcus aureus* do not have plasmids whereas 69.23% of *S. aureus* have plasmids with size of 10.0kb.Also to be noted is the absence of plasmid in *Bacillus cereus* and *Klebsiella* spp isolated from ready-to-eat rice wrapped with leaf.

With regards to biofilm forming ability of bacterial species isolated from RTE rice wrapped with leaf, *Escherichia coli* and *Staphylococcus aureus* accounted for the highest and lowest number of isolates, respectively. *E. coli* also accounted for the highest number of isolates incapable of forming biofilm whereas the lowest number of isolates involved *S. aureus*. Among the organisms present in RTE rice wrapped with cellophane which were capable of producing biofilm, *E. coli* and *S. aureus* accounted for the highest and lowest number of isolates, respectively. *S. aureus* still accounted for the highest number of isolates incapable of producing biofilms whereas *Klebsiella* sp. *Bacillus* sp. and *Pseudomonas* sp. had the lowest number of isolates each. Overall result indicate that bacteria isolated from ready-to-eat rice wrapped with leaf demonstrated higher ability to produce biofilm compared with the isolates from RTE rice wrapped with cellophane. With regards to urinary tract infections, *Pseudomonas aeruginosa, Escherichia coli*, *Proteus mirabilis* and *Klebsiella pneumoniae* are usually characterized by their ability to form biofilm (Risal *et al*., 2018). Accorrding to Camargo *et al*. (2017), the presence of biofilm in food puts human health at risk. The level of risk is dependent on the bacterial species responsible for forming biofilm which is a tridimensional living stucture. Evidence from earlier studies suggest that biofilm contains virulence factors which helps resident bacteria attain virulent characters which were not initially present in a single bacteria (Hu and Ehrlich, 2008). Persistence of microorganisms in the environment for a long period is usually associated with the production of biofilms (Risal *et al*., 2018). This could explain the reason why larger number of isolates from RTE rice wrapped with leaf (possible source of microorganisms) were able to produce biofilms compared with isolates from RTE foods wrapped with cellophane (possible source of microorganisms). The ability of *Klebsiella* sp. to form biofilm is very important in determining the pathogenicity of the organism. It is suggested that biofilm gives protection to the organisms against antimicrobials (Gharrah *et al*., 2017).

The isolates encountered in ready-to-eat rice wrapped with cellophane and leaf displayed alpha hemolysis. Among the virulence factors present in *S. aureus,* α-hemolysin is the most characterized (Bagban *et al*., 2019). They also showed positive result for pathogenicity test. The ability of the isolates to display hemolysis is an indication that the bacterial isolates is capable of causing diseases in humans if the infective dose is ingested through consumption of contaminated ready-to-eat rice wrapped with cellophane and leaf. Hemolysins helps the bacterium to evade host immune response (Risan *et al*., 2020).

RECOMMENDATIONS

Since present day economic reality and modern lifestyle in urban and semi-

urban areas has made it nearly impossible for millions of workers both at the formal and informal sector to completely avoid patronizing ready-to-eat (RTE) foods sold in markets, restaurants and cafeterias, there is need to ensure wholesomeness of such foods meant for public consumption. To achieve this:

- Food handlers must implement good hygienic practices
- Foods meant for public consumption should be properly cooked and served in a sanitary environment
- Monitoring of facilities, food supply chain and personal hygiene of personnel involved in RTE food business by the public and environmental health inspectors and relevant regulatory agencies should be sustained
- In Nigeria, the National Agency for Food and Drug Administration and Control (NAFDAC) should continue to intensify her efforts in fighting the menace of substandard antibiotics in the healthcare system
- Strict measures must be put in place against the abuse of antibiotics by the general public without doctors' prescription.

CONCLUSION

Ready-to-eat rice wrapped with leaf and cellophane obtained from Okha, Santana and Oba market are unacceptable for human consumption. This could be attributed to *Escherichia coli* plate count of the RTE rice samples exceeded $>10^2$ CFU/g stipulated by Microbiological Guidelines for Food. Secondly, *Staphylococcus* plate count of the packaged rice samples is not below 10^3 CFU/g recommended for RTE foods. Other bacterial genera isolated from RTE rice samples include *Pseudomonas* sp., *Bacillus cereus* and *Klebsiella* sp. All the bacterial isolates demonstrated antibiotic resistance to most antibiotics they were exposed to which include ceftazidime, cefuroxime, cloxacillin, erythromycin and ceftriaxone. Multiple antibiotic resistance indices (MARI) of the bacterial isolates suggests that the bacteria strain emanated from an environment where a lot of antibiotics have been used indiscriminately. The ability of bacterial isolates from RTE rice wrapped with leaf and cellophane to produce biofilms and demonstrate hemolysis is an indication of their pathogenicity. It also suggest that the isolates could show increased resistance to commonly used antibiotics.

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