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MULTI DRUG AND MULTIPLE ANTIBIOTICS INDEXES OF BACTERIA ISOLATED FROM BEANS PUDDING (MOI-MOI) SOLD IN ABRAKA, DELTA STATE, NIGERIA

***Idise, O. E, Odum, E. I. and Ajari, O. S.**

Department of Microbiology, Delta State University, Abraka, Nigeria

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ABSTRACT

The microbial quality of four forms of Moi-moi sold in Main and Small markets sold in Abraka, Delta State, Nigeria and the prevalence of multidrug resistant (MDR) and multi antibiotic resistance index (MARI) of bacterial isolates were studied using standard methods. The microbial load of the moi-moi ranged respectively from 5.5 – 6.0 and 4.7 – 5.18Log_{10}cfu/g for total aerobic and coliform counts. Seven organisms - four Gram positive – *Bacillus* sp, *Staphylococcus* sp, *Streptococcus* sp, *Micrococcus* sp and three Gram negative – *Escherichia* sp, *Pseudomonas* sp, *Klebsiella* sp were isolated. All the isolates were MDR as they were resistant to 4 – 7 and 4 – 8 of the antibiotics tested for positive and negative isolates respectively with corresponding MARI values of 0.4 - 0.7 and 0.4 - 0.8. Some of the isolates are reportedly pathogenic and their presence in the samples could pose great public health concern. Aside difficulty that would be encountered in treatment of diseases caused by the isolates, they could act as vehicles for the transmission of resistant strains in the environment. There is thus the need to properly re-heat the moi-moi prior to consumption as well as embark on public enlightenment on importance of good hygienic practice during preparation, storage and sale of the food.

Key words: Moi-Moi. MDR. MARI. Isolates. Pathogens. Resistance.

***corresponding author email**: emmaidise@yahoo.com. Phone: +2348136506553, +2348057592441

INTRODUCTION

Moi-moi is a traditional Nigerian steamed bean pudding made from a mixture of washed and peeled black-eyed beans, onions and fresh ground pepper. It could be cooked in bowls, banana leaves and aluminium foil and

commonly served at parties, wedding receptions and other occasions. It is a protein-rich staple food in Nigeria. Moi-moi could also be eaten alone or with bread as a snack, with rice as a meal or with 'ogi' for breakfast or supper. It can be taken with 'garri' in the afternoon. It fits into the foods classed as ready-to-eat (RTE) which can be described as the status of foods being ready for immediate consumption, without further processing [1]. A general observation of our society shows a social pattern characterized by increased mobility and less family and home activities which, more often than not, predisposes a great population of persons to depend on RTE foods. However, the mode of preparation and presentation for sale of such foods could expose them to microbial contamination from the environment prior to purchase by consumers. Ready to eat foods could be raw or cooked, hot or chilled and can be consumed without further heat treatment [2].

Safe food is a basic human right. However, such foods like Moi-moi are frequently contaminated with pathogens which cannot be detected organoleptically but can lead to disease and even death. This is further encouraged when they are presented during sales under conditions that can encourage the growth of such pathogens to attain infectious doses [3].

Microbial quality of food indicates the amount of microbial contaminations as well as quality of food preparation, storage and handling. The microbial count of prepared Moi-moi is therefore a key factor in assessing its quality and safety as it will reveal the level of hygiene adopted by the handlers in course of preparation and presentation for sale [4].

Antimicrobial resistance (AMR) and multi antibiotic resistance (MAR) are currently a great challenge worldwide as they result in decreased effectiveness of drugs [1, 5]. AMR bacteria and associated genes in foods such as Moi-moi abound, apparently due to increased abuse and misuse of antibiotics for human treatment and for animal production. Their presence in foods, including home cooked and road side foods has been well documented [1, 2, 5, 6, 7, 8, 9, 10, 11, 12, 13].

Food contamination with antimicrobial resistant (AMR) and multi antibiotics resistant (MAR) isolates pose a major threat to public health. Thus, this study was aimed at determining the microbial quality, as well as the prevalence of AMR and MAR bacteria in Moi-moi sold in Abraka, Delta State, Nigeria.

MATERIALS AND METHODS

Collection of Moi-moi samples

A total of sixteen (16) samples of moi-moi - four samples each of moi-moi containing Cray fish, full egg, half egg and moi-moi alone were purchased and stored in cellophane bags, previously sterilized by washing in 95% ethanol and air-dried, at eight per market, from the Main and Small markets in Abraka, Delta State, Nigeria. The samples were immediately transported to the Microbiology laboratory, Delta State University, Abraka for analysis.

Determination of bacterial load and isolation of bacteria

These were carried out in accordance with the procedures describe by [14] as follows: Homogenized each of the moi-moi samples with a mortar and pestle (previously washed and sterilized in a hot air oven (Gallenkamp, England) at 160^0C for 2h. serial dilution of one gram of each sample was carried out and aliquots of 1ml were inoculated on freshly prepared nutrient agar (NA) and MacConkey agar (MA), incubated at 37^oC for 24 and 36h respectively. Counts of colonies after incubation were recorded as microbial load of the samples. Pure cultures were obtained by inoculating discrete colonies on freshly prepared NA and MA and incubated at 37^oC for 24hrs and pure colonies were stored at 4^oC until needed.

Identification of bacterial isolates

The isolates were identified based on their morphology and biochemical reactions in accordance with procedures reported by [14].

Antibiotic sensitivity test

These were carries out using the disc-diffusion method as recommended by the Clinical Laboratory Institute Standards [15] as follows: Each bacterial isolate was cultured for 18h on NA and thereafter suspended in 2ml sterile normal saline. Turbidity was adjusted to match McFarland opacity standard No. 05 (equivalent to 1.5 x 10^8 bacterial densities). Bacterial suspensions of 0.1ml were dispensed on the surface of sterile Mueller-Hilton agar plates. A spreader, that was sterilized by dipping in 95% ethanol and allowed to air dry, was used to evenly spread the bacterial suspension in each plate. Each plate was allowed to air-dry for 5mins after which positive antibiotics disc - Tarivid (.10µg), Reflacin (10µg), Ciproflox (10µg), Augmentin (30µg), Gentamycin (10µg), Streptomycin (30µg), Ceporex (10µg), Nalidixic acid (30µg), Septrin (30µg) and Amplicin (30µg) and negative antibiotics disc - Ciproflox (10µg),Norfloxacin (10µg), Gentamycin (10µg), Amoxil (20µg), Streptomycin (10µg), Refampicin (20µg), Erythromycin (30µg), Chloramphenicol (30µg), Ampiclox (20µg) and Levofloxacin (20µg produced by Optum Laboratories, Lagos - were aseptically placed on the surface of the media of each isolate. The plates were then incubated for 18h at 37^0C. The zones of growth inhibition were measured using a caliper for each isolate. Classification was as per CLSI standards of $\leq$ 12mm (Resistant) and $\geq$ 13mm (Sensitive).

Identification of multidrug resistant (MDR) bacterial isolates: the number of antibiotics to which the isolate was resistant was recorded. This was used ti identify the MDR isolates which was taken as resistance to four or more tested antibiotics [16].

Calculation of Multiple Antibiotics Resistance Index (MARI): The following formula was used for the calculation:

$$A/B$$

Where A represents the number of antibiotics to which the bacterium is resistant and

B represents the total number of antibiotics tested [5].

Data analysis:

Microsoft excel 2010 was used to analyze the significance difference in parameters of the moi-moi samples.

RESULTS

There was no statistically significant difference in the total aerobic and coliform counts of the four moi-moi samples as f-crit (9.2766) was greater than f-cal (1.2795) at 95% confidence level.

The microbial load of the moi-moi samples are presented in Table 1. It was observed that the total aerobic counts were highest in moi-moi alone (MA) ($6\log_{10}$ Cfu/g) and decreased to moi-moi with half egg (MHE) ($5.6\log_{10}$Cfu/g), moi-moi with Cray fish (MCF) ($5.52\log_{10}$Cfu/g) and moi-moi with full egg (MFE) (5.5Log_{10}Cfu/g).

The identification of the bacterial isolates presented in Tables 2a and 2b revealed that a total of seven bacteria were isolated. All the seven were isolated from MCF (four Gram positive – *Bacillus* sp, *Staphylococcus* sp, *Streptococcus* sp, *Micrococcus* sp and three Gram negative – *Escherichia* sp, *Pseudomonas* sp, *Klebsiella* sp); five from MHE (three Gram positive - *Staphylococcus* sp, *Streptococcus* sp, *Micrococcus* sp and two Gram negative - *Pseudomonas* sp, *Klebsiella* sp); three from MA (two Gram Positive – *Streptococcus* sp, *Micrococcus* sp and one Gram negative - *Klebsiella* sp) and two from MFE (one Gram positive – *Bacillus* sp and one Gram negative - *Escherichia* sp).

Table 1: Microbial load (Log_{10}cfu/ml)

Sample	Total aerobic counts (TAC)	Coliform counts (CC)
MCF	5.52	5.08
MHE	5.60	5.00
MFE	5.50	4.70
MA	6.00	5.18

Key: MCF – Moi-moi with cray fish; MHE = Moi-moi with half egg; MFE = Moi-moi with full egg; MA = Moi-moi alone

Table 2a: Identification of bacterial isolates

Isolate	A	B	C	D	E	F	G
Source	MCF, MFE	MCF, MFE	MCF, MHE	MCF, MHE	MCF, MHE. MA	MCF, MHE. MA	MCF, MHE, MA
Shape	Rod	Rod	Rod	Cocci	Rod	Cocci	Cocci
Gram reaction	-	+	-	+	-	+	+
Aerobic growth	+	+	+	+	+	+	+
Anaerobic growth	+	+	+	+	-	-	+
Motility test	+	+	+	-	-	+	-
Endospore production	-	+	-	-	-	-	-
Citrate test	-	+	+	-	+	+	-
Indole test	+	-	-	-	-	-	-
Oxidase test	-	-	+	+	-	-	-
Catalase test	+	+	+	+	+	-	+
Lactose fermentation	+	-	-	+	-	+	-
Glucose fermentation	+	+	-	-	+	-	+
H_2S production	-	+	-	+	-	+	-
Acid production	+	+	+	-	+	+	-
Gas production	+	-	-	+	+	+	-
Organism identified	*Escherichia* sp	*Bacillus* sp	*Pseudomonas* sp	*Staphylococcus* sp	*Klebsiella* sp	*Streptococcus* sp	*Micrococcus* sp

Key: + = positive, - = negative, MCF – Moi-moi with cray fish; MHE = Moi-moi with half egg; MFE = Moi-moi with full egg; MA = Moi-moi alone

Table 2b: Bacterial isolates obtained from moi-moi samples

Sample	Isolates	Gram reaction	Number of isolates
MCF	*Escherichia* sp	-	
	Bacillus sp	+	
	Pseudomonas sp	-	
	Staphylococcus sp	+	
	Klebsiella sp	-	
	Streptococcus sp	+	
	Micrococcus sp	+	7
MHE	*Pseudomonas* sp	-	
	Staphylococcus sp	+	
	Klebsiella sp	-	
	Streptococcus sp	+	
	Micrococcus sp	+	5
MA	*Klebsiella* sp	-	
	Streptococcus sp	+	
	Micrococcus sp	+	3
MFE	*Escherichia* sp	-	
	Bacillus sp	+	2

Key: MCF = Moi-moi containing Cray fish, MFE = Moi-moi containing Full egg,
MHE = Moi-moi containing Half egg, MA = Moi-moi alone

The zones of inhibition of growth by tested antibiotics and the antibiotics sensitivity profile of the Gram positive isolates are respectively presented in Tables 3a and 3b while Tables 3c and 3d presents their multi drug resistance (MDR) and multiple antibiotics resistance index (MARI) respectively. It was observed that the zones of inhibition of Gram positive isolates ranged from 10 – 20mm; *Bacillus* sp, *Streptococcus* sp, *Staphylococcus* sp and *Micrococcus* sp were resistant to 5, 7, 4, 5 and sensitive to 5, 3, 6, 5 of the tested antibiotics in Tables 3a and 3b. in Tables 3c, all the Gram positive isolates were MDR. *Bacillus* sp was resistant to five - Norfloxacin, Amoxil, Streptomycin, Ampiclox, Levofloxacin; *Streptococcus* sp was resistant to seven - Norfloxacin, Gentamycin, Amoxil, Rifampicin, Erythromycin, Chloramphenicol, Streptomycin; *Staphylococcus* sp was resistant to four - Amoxil, Chloramphenicol, Streptomycin, Levofloxacin and *Micrococcus* sp was resistant to five - Gentamycin, Amoxil, Erythromycin, Chloramphenicol, Levofloxacin of the tested antibiotics. In Table 3d, MARI values of 0.5, 0.7, 0.4 and 0.7 were observed respectively for *Bacillus* sp, *Streptococcus* sp, *Staphylococcus* sp and *Micrococcus* sp.

Table 3a: Zones of inhibition of gram positive bacteria (mm)

Isolate	CPX	NB	CN	AML	RD	E	CH	S	APX	LEV
Bacillus sp	15	12	15	12	15	15	15	12	10	10
Streptococcus sp	15	10	10	12	12	0	10	10	16	15
Staphylococcus sp	15	15	15	10	15	20	10	10	15	10
Micrococcus sp	15	15	10	12	15	10	12	19	19	10

Key: CPX = Ciproflox (10 µg), NB = Norfloxacin (10 µg), CN = Gentamycin (10 µg), AML = sAmoxil (20 µg), RD = Rifampicin (20 µg), E = Erythromycin (30 µg), CH = Chloramphenico (30 µg), S = Streptomycin (10 µg), APX = Ampiclox (20µg) LEV = Levofloxacin (20 µg)

Table 3b: Antibiotics sensitivity profile of gram positive bacteria

Isolate	CPX	NB	CN	AML	RD	E	CH	S	APX	LEV	R	S
Bacillus sp	S	R	S	R	S	S	S	R	R	R	5	5
Streptococcus sp	S	R	R	R	R	R	R	R	S	S	7	3
Staphylococcus sp	S	S	S	R	S	S	R	R	S	R	4	6
Micrococcus sp	S	S	R	R	S	R	R	S	S	R	5	5

Key = Resistant (≤12mm), S = Sensitive (≥13mm)

Table 3c: Multi drug Resistance (MDR) of Gram positive isolates

Isolate	Antibiotics to which it was resistant	Number
Bacillus sp	Norfloxacin, Amoxil, Streptomycin, Ampiclox, Levofloxacin	5
Streptococcus sp	Norfloxacin, Gentamycin, Amoxil, Rifampicin, Erythromycin, Chloramphenicol, Streptomycin	7
Staphylococcus sp	Amoxil, Chloramphenicol, Streptomycin, Levofloxacin	4
Micrococcus sp	Gentamycin, Amoxil, Erythromycin, Chloramphenicol, Levofloxacin	5

Table 3d: Multi Antibiotics Resistance Index (MARI) of gram positive bacteria

Isolate	R	S	MARI
Bacillus sp	5	5	0.5
Streptococcus sp	7	3	0.7
Staphylococcus sp	4	6	0.4
Micrococcus sp	5	5	0.5

Key: MARI = Multiple Antibiotics Resistance Index

The zones of growth inhibition of Gram negative isolates by tested antibiotics and their antibiotics sensitivity profile are respectively presented in Tables 4a and 4b. Tables 4c and 4d respectively present their multi drug resistance (MDR) and multiple antibiotics resistance index (MARI). It was observed that the zones of inhibition of Gram negative isolates in Table 4a ranged from 10 – 20mm; *Escherichia* sp was resistant to 4 and sensitive to 6, *Pseudomonas* sp was resistant to 5 and sensitive to 5, and *Klebsiella* sp was resistant to 8 and sensitive to 2 of the tested antibiotics in Table 4b. This showed that all the Gram negative isolates wer MDR. In Table 4c, *Escherichia* sp was ressitant to four - Ciproflox, Rifampicin, Ampiclox, Erythromycin, *Pseudomonas* sp was resistant to five - Norfloxacin, Rifampicin, Ampiclox, Levofloxacin , Erythromycin and *Klebsiella* sp was resistant to eight - Amoxil, Norfloxacin, Gentamycin, Rifampicin, Ampiclox, Streptomycin, Levofloxacin, Erythromycin. The MARI values for Gram negative isolates presented in Table 4d were 0.4, 0.5 and 0.8 respectively for *Escherichia* sp, *Pseudomonas* sp and *Pseudomonas* sp.

Table 4a: Zones of inhibition of gram negative bacteria (mm)

Isolate	CPX	AML	NB	CN	RD	APX	S	LEV	CH	E
Escherichia sp	10	15	19	15	10	10	19	15	15	12
Pseudomonas sp	15	14	10	20	10	15	10	10	15	12
Klebsiella sp	13	12	10	10	10	10	10	10	16	12

Key: CPX = Ciproflox (10 µg), AML = Amoxil (20 µg), NB = Norfloxacin (10 µg), CN = Gentamycin (10 µg), RD = Rifampicin (20 µg), APX = Ampiclox (20 µg), S = Streptomycin (10 µg), LEV = Levofloxacin (20 µg), CH = Chloramphenicol (30 µg), E = Erythromycin (30 µg)

Table 4b: Antibiotics sensitivity profile of gram negative bacteria

Isolate	CPX	AML	NB	CN	RD	APX	S	LEV	CH	E	R	S
Escherichia sp	R	S	S	S	R	R	S	S	S	R	4	6
Pseudomonas sp	S	S	R	S	R	R	S	R	S	R	5	5
Klebsiella sp	S	R	R	R	R	R	R	R	S	R	8	2

Key = Resistant (≤12mm), S = Sensitive (≥13mm)

Table 4c: Multi drug Resistance (MDR) of Gram negative isolates

Isolate	Antibiotics to which it was resistant	Number
Escherichia sp	Ciproflox, Rifampicin, Ampiclox, Erythromycin	4
Pseudomonas sp	Norfloxacin, Rifampicin, Ampiclox, Levofloxacin , Erythromycin	5
Klebsiella sp	Amoxil, Norfloxacin, Gentamycin, Rifampicin, Ampiclox, Streptomycin, Levofloxacin, Erythromycin	8

Table 4d: Multi Antibiotics Resistance Index (MARI) of gram negative bacteria

Isolate	R	S	MARI
Escherichia sp	4	6	0.4
Pseudomonas sp	5	5	0.5
Klebsiella sp	8	2	0.8

Key: MARI = Multiple Antibiotics Resistance Index

DISCUSSION

The high microbial load that ranged from 5.5 – 6.0 Log_{10}cfu/g, observed in the moi-moi could have been due to the use of contaminated water and equipment in the preparation, handling, storage, presentation and preservation during sales. The results are in concordance with the findings of previous works by [2] in meat pie sold in Benin-City, Nigeria; [7] who reported same range in RTE rice in Benin-City, Nigeria; [8] who reported a range of 5.6 – 6.7 Log_{10}cfu/g in ready-to-eat (RTE) foods in Abeokuta, Nigeria; [9] in Onitsa – Owerri expressway, Nigeria; [10] in rice and moi-moi in Bali, Nigeria; (11) in India; [17] that had a range of 1.21 x 10^4 – 1.79 x 10^4cfu/g (4.08 – 4.25 Log_{10}cfu/g) in cooked rice in Lagos, Nigeria.

The seven isolates obtained in this study - four Gram positive – *Bacillus* sp, *Staphylococcus* sp, *Streptococcus* sp, *Micrococcus* sp and three Gram negative – *Escherichia* sp, *Pseudomonas* sp, *Klebsiella* sp - could have been due to poor hygienic practices of food handlers as they are normal flora of man as well as aforementioned reasons. The results agree with the reports of [18] in South Africa; [2] in meat sold in Benin-City, Nigeria; [7] in RTE rice in Benin-City, Nigeria; [8] in Abeokuta, Nigeria; [18] who reported these organisms in ready-to-eat food in South Africa; [9] who reported the organisms in foods sold along the Onitsa – Owerri expressway, Nigeria; [10] in RTE rice and moi-moi in Bali, Nigeria; [11];[1] from RTE foods in Ogun State, Nigeria.

The result that all the isolates were MDR may have been due to drug abuse and misuse often reportedly prevalent in cities where higher institutions are located. There is prevailing practices of self-medication, purchase of drugs across the counter without prescription and such inimical practices that permit development of resistant bacteria. The spread of such bacteria is often enhanced by plasmids rapidly across species boundaries. These isolates have been reported by previous workers – *Bacillus* sp, *Streptococcus* sp, *Micrococcus* sp, *Pseudomonas* sp and *Klebsiella* sp [1, 11]; *Staphylococcus* sp [6, 11, 18]; *Escherichia* sp [1, 5, 11, 12, 13,] - to be resistant to the tested antibiotics.

The observed MARI values that ranged from 0.4 – 0.7 for positive isolates and 0.4 – 0.8 for negative isolates conforms to the reports on MDR of the isolates [13]. These could equally be due to reasons provided above for the observed results on MDR of the isolates. The prevalence of MDR isolates in moi-moi is worrisome as the consumption rate is high and most of the isolates are reported pathogens. The prevailing situation could enable the food

to be a vehicle for the transmission of inimical effects. In the event of any outbreak of food-borne infection or intoxication, this could negate any form of treatment with the tested antibiotics, a situation that could lead to increased cost of treatment, further spread of the resistance to other organisms, even across species boundaries, longer period(s) of hospitalization and even death.

CONCLUSION

Moi-moi sold in Abraka, in the presentations studied, consists of high microbial load. All the isolates are multi drug resistant with high values of multiple antibiotics resistance indices. They therefore pose public health hazard to consumers as most of the isolates are reported pathogens. The moi-moi could therefore be vehicles for the transmission of resistant isolates in the event of any food-borne infections and/or intoxications.

RECOMMENDATIONS

There is the need for public enlightenment on the health hazards, good personal hygiene of food handlers and proper storage of the moi-moi during preparation and sales. Consumers should endeavor to re-heat the moi-moi properly, prior to consumption, to avert outbreak(s) of food-borne infection(s) and/ or intoxication(s).

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