https://dx.doi.org/10.4314/jpb.v19i3.1
Vol. 19 no. 3, pp. 128-135 (September 2022)
http://ajol.info/index.php/jpb

Journal of
PHARMACY AND
BIORESOURCES

Bacteriological evaluation of instant noodles stored under sub-optimal conditions from selected markets in Sagamu-Ibafo axis of Ogun State, Nigeria

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Received 21st February 2022; Accepted 19th June 2022

Abstract
This study evaluated 8 brands of 5 packets each of instant noodles for bacterial load, clinical status, and resistance of the isolates to conventional antibiotics, in addition to pH. These brands were cultured on plate count agar for bacterial enumeration while isolates were challenged on selective media for bacteria of clinical status. The antibiogram was determined using standard methods. The pH varied from acidic (4.92) as shown in GPJ 3 to closely neutral (6.94) as recorded in HWJ 2. TTJ1 had the lowest average bacteria count of 1.6×10^3 while BFJ5 with 6.3×10^5 counts was the highest. Prevalence of isolates of *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* varied in the ratio 40:30:30. Of the 40 samples, 8 of the 16 *Escherichia coli* were resistant to gentamicin, ciprofloxacin, and Augmentin, 7 of the 12 *Staphylococcus aureus* elicited resistance to ampicillin and cotrimoxazole while 8 of the *Pseudomonas aeruginosa* were resistant to meropenem, cotrimoxazole, Augmentin, and cephalexin. Presence of bacteria of clinical status, in counts that exceeded recommended safe limits for ready-to-eat noodles and higher numbers of resistant isolates to the antibiotics appropriated, could be attributed to product storage under sub-optimal conditions which portend a public health risk.

Keywords: *Bacteriological investigation; Instant noodles; Sagamu-Ibafo axis; Ogun State; Nigeria*

INTRODUCTION

Instant noodle is a type of staple food made from unleavened dough which is rolled, flattened, cut, molded, spiced and configured to various forms during processing. Noodles were first produced in Indonesia in 1972, exported to West Africa in 1988 and were also produced in 1995 for the first time in Nigeria by Indofood®. The three companies that produce instant noodles in Nigeria are in Ogun

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State, Port Harcourt and Kaduna with production rate of eight million packets of noodles daily.Noodles are versatile foods that can be quickly prepared and carried along to work or trips, picnics, schools, partying and festival centers. In Nigeria, consumption of instant noodles increased from 1.1 billion packets in 2009 to 1.44 billion packets in 2013, making Nigeria the highest consumer in Africa followed by Egypt that consumed 130 million packets in 2013[1].

Instant noodles are made with wheat flour of hard wheat (*Tritium aestivum*), that are low in fibre and protein contents, and also poor in essential amino acid; lysine. Instant noodles lack some nutritional components such as dietary fibre in appropriate proportion, therefore incorporation of lentil on noodles improves its fibre content and indirectly contributes to protein and mineral content[2].Noodle may be dried or refrigerated before cooking. Addition of alkaline salts can help strengthen the structure and hence improve the firmness of the final products [3]. In Nigeria, the leading brand of noodles is Indomie which is the most popular among the young and the adults, in eatery and roadside culinary chef shops and in schools. Other competing brands in the markets were made up of many brand names. Food characteristics such as taste, nutrition, convenience, safety, long shelf life, and reasonable price have made noodles popular. Quality factors obligatory for instant noodles are color, flavor, and texture, cooking quality, rehydration rates throughout final preparation, and the presence or absence of rancid taste after prolonged storage [4].

Instant noodles have become more widely adopted for everyday use and their storage has been facilitated by the introduction of dried noodles. Technological innovation has also been applied to enhance noodles quality and adaptation to meet up with consumer demands. Instant noodles comprise a fast growing sector of the pasta industry. This is because instant noodles are convenient, easy to cook, low cost and have relatively long shelf-life [5].

The presence of bacterial mediated food toxins cannot be underestimated in various noodles, which could be transited into the products from the raw materials, equipment, personnel, and packaging material and storage facilities and environment. Garci et.al. [6] study of ready-to-eat noodles showed a total of 70 samples of which 21 were reported to contain *Pseudomonas* sp. Air, soil and water pollution, biotic and abiotic factors contribute to the presence of bacteria pathogen in processing and processed noodles which can cause food poisoning [6]. The acceptable safe limit of bacteria in ready-to-eat food also, should not be underestimated which are determined by bacteria types and carriage loads in the foods, once the safety limits are exceeded, the food become a unwholesome which could endanger the health of the consumers. The acceptable limit of bacterial loads 10^3 per gram of ready to eat foods varies from one country to another and also, due to the presence of dioxin and other structurally hormone like compound that could be extracted from the glues used in binding noodles. It has aroused curiosity from many quarters [7].

Sequel to the observation that has become a common practice among children and even adults that eat instant noodles raw when on errand with it, before getting to their cooking hub, which could possibly cause gastrointestinal infection and intoxication. Therefore, this study was carried out to evaluate the microbial quality of some selected instant noodles purchased from road side culinary chefs and local shops in Ibafo-Sagamu markets, determine the pH and resistance of the isolates of bacteria from these selected products to conventional antibiotics with a view to advising consumers on the risk associated with eating raw instant noodle and other related parboiled foods stored under sub-optimal conditions.

EXPERIMENTAL METHODS

Collection of samples. Five packets each of eight different brands (40 samples) of instant noodles with varied batch numbers were purchased from road side culinary chefs and local markets within Sagamu-Ibafo axis of Ogun state and immediately transferred to the laboratory for bacteriological analysis.

Determination of pH. The pH of each noodles sample was determined by weighing four gram (4 g) into 15 mL of sterile distilled water. Handheld pH meter with microprocessor manufactured by HANNA Instruments was used to determine the pH.

Preparation of samples. Each packet of instant noodle was torn with a sterile scalpel and one gram of the sample was suspended in nutrient broth, thereafter incubated for 4 hours, swab sticks were dipped into each test-tube containing the turbid suspension of bacterial growth and were subculture onto nutrient agar for bacterial plate count, and were thereafter subculture on to Mannitol Salt Agar, Cetrimide Nutrient Agar Eosin Methylene Blue Agar media and the plates were incubated aerobically at 37^{o}C for 24 hours.

Enumeration of bacteria in noodles. One gram (1g) of each instant noodle was suspended into 9mL of sterile distilled water and six times (10^{-6}) serially diluted and pour plate technique was used to determine the microbial load in each sample. An appropriate dilution (0.1mL) was inoculated into sterile Petri-plates containing molten standard plate count agar medium and were left to solidify. Thereafter incubated at 37^{o}C for 24-48 hours. The preparations that were made up of varied batch number for each instant noodle sample were done in triplicate and the mean counts for the triplicates were recorded.

$$\text{cfu/g} = \frac{\text{level of dilution plated} \times \text{number of colonies counted}}{\text{volume plated}}$$

Isolation and identification of bacterial isolates. Samples from nutrient agar plates were each sub-cultured to various selective agar media; MacConkey Agar, Eosin Methylene Blue, Cetrimide Nutrient Agar and Mannitol Salt Agar and incubated at 37^{0}C for 24-48 hours for the isolation of bacteria. The morphological profiles of the colonies obtained were observed, Gram-stained and biochemically characterized; catalase, coagulase, methyl red, indole, oxidase, tests and fermentation of sugars was carried out for the isolation of bacterial isolates.

Determination of antibiogram. Antimicrobial susceptibility patterns of the isolates obtained to selected antibiotics were determined using the Kirby-Bauer modified agar diffusion method. *Staphylococcus aureus* strain ATCC 29213 was used as reference. A volume of 0.1mL of the overnight broth culture of each isolate was pipette into 9.9mL of sterile distilled water in test tubes. Standardization of the inoculums was performed by diluting the broth culture until turbidity matched the 0.5 McFarland standards. A sterile cotton swab was dipped into each of the standardized suspension, drained and used for inoculating 20ml of Sensitivity test agar (Oxoid, UK) on to 100-mm disposable plate (Sterlin, UK). The inoculated plates were air dried for 30 min, and antibiotic discs (Oxoid, UK) were placed on the agar using flamed forceps and were gently pressed down to ensure maximum contact. Discs containing the following antibiotics were used for the susceptibility testing; gentamicin (10 µg/mL), ciprofloxacin (30 µg/mL), ampicillin (30 µg/mL), meropenem (20 µg/mL), erythromycin (30 µg/mL), tetracycline (30 µg/mL), cotrimoxazole (30 µg/mL), cefuroxime (30 µg/mL), augmentin (30 µg/mL), vancomycin (25 µg/mL), ceftriaxone (30 µg/mL) and cephalexin (30 µg/mL). The plates were incubated aerobically at 37^{o}C for 24 hours before measuring the diameter of zones of growth inhibition. Sensitive, Intermediate and resistant strains

were marked S, I and R respectively and were analyzed as specified by CLSI guide.

Statistical analysis. Data collected from this study were statistically analyzed and bar graph plotted for mean, standard deviation and standard error with Graph Pad Prism Version 8.01.

RESULTS

Table 1 contains bacteria counts (cfu/g), Product Batch Number for each of the samples, frequency of isolates and the pH obtained from the selected instant noodles examined. The frequency of occurrence of the isolates obtained in prevalence ratios of 40:30:30 were shown in Table 2. The antibiogram of the different isolates obtained showed varied susceptibility and resistant patterns to the selected antibiotics of therapeutic values. Figure 1: Graphical analysis showing the mean value, standard deviation and standard error of the average bacterial population of each brand of instant noodles , every brands exceeded the $<10^4$cfu/g satisfactory safe limit threshold of bacterial counts for ready-to-eat food (Table 1) .

Figure 2 are some selected growth profiles of *Escherichia coli, Pseudomonas aeruginosa and Staphylococcus aureus* on eosin metyhylene blue agar medium, cetrimide nutrient agar medium and mannitol salt agar medium respectively, an evidence of microbial status of the examined instant noodles.

DISCUSSION

Five (5) packets of Eight(8) brands of instant noodles samples of varied batch numbers each were examined in this study; BFJ, GPJ, MMJ, HWJ, TTJ, RCJ, CCJ and HGJ. The instant noodles sampled, elicited aesthetic appeal in their packages, but were not properly stored as specified by the manufacturer, thus, the condition of storage of these items could play a significant role of speedy degradation of these products quality [8].

Environmental factors such as pH, available water, temperature, packaging materials and personnel do play significant roles in determining the types of microbes that colonize the end-products. The pH of the products examined in this study varied from slightly acidic (4.92) as shown in GPJ3 to closely neutral (6.94) as shown in HWJ 2 in Table 1. The significant pH shift could be as a result of mishandling and improper storage condition could trigger increase or limit microbial growth because pH homeostasis in a medium equilibrate and adjust acidophiles that release metabolites to initiate pH shift and such metabolite in response to pH shift could cause gastrointestinal discomfort [9]. The pH obtained from the products examined agrees with the study of Okafor and Omodamiro (2006) on assessment of chemical/phytotoxin and microbial contamination of pasta foods marketed in Nigeria[10]. Though, the proximate composition was not examined, elimination of specific ingredient and sufficient preservatives could also affect the pH and aid the speedy growth of microbial contaminants.

Microbial food safety is an increasing public health concern, and any food that exceeds the threshold of safety level should be regarded as metabolic poison. The microbial load obtained from the noodles varies from batch to batch and brand to brand. In addition, the number of colony forming unit per gram was higher in some but slightly lesser in some items; BFJ noodle sample had bacterial load that ranged between 1.7×10^5 and 3.5×10^5 in GPJ, $1.6 \times 10^3$and 4.2×10^5 in MMJ instant noodle, HWJ, TTJ, RCJ, CCJ and HMJ instant noodles had varied bacterial loads, though varied but are relatively closer to each other as shown in Table 1, which serves as an indication of significant microbial load per gram and an evidence of tilting towards spoilage.

Table 1: Bacteria Counts (cfu/g), Product Batch Number and the pH of selected instant noodles.

Samples	Batch Number	pH	Bacterial Population (cfu/g)	Bacteria identified
BFJ 1	851170222.2	6.28	3.0×10^4	*E. coli*
BF J2	63150123.1	6.90	3.4×10^4	*E. coli*
BFJ 3	222110555.5	6.71	$2.9. \times 10^3$	*E. coli*
BFJ 4	431730334.2	6.90	1.9×10^3	*E. coli*
BF J5	3314160112.3	6.31	6.3×10^5	*P.aeruginosa*
GPJ1	1416/601	5.32	$2.2. \times 10^5$	*S.aureus*
GPJ2	1414/004	5.57	1.7×10^5	*S.aureus*
GPJ3	1211/003	4.92	3.5×10^5	*S.aureus*
GPJ4	1610/001	4.97	2.9×10^5	*S.aureus*
GPJ5	1313/003	5.27	3.5×10^5	*S.aureus*
MMJ1	A2532121.2	5.62	4.2×10^5	*S.aureus*
MMJ2	C3214721.5	6.14	3.4×10^5	*S.aureus*
MMJ3	D1122312.2	5.84	4.3×10^5	*P.aeruginosa*
MMJ4	E2341521.3	6.21	1.6×10^3	*E. coli*
MMJ5	G1123124.2	5.26	3.5×10^5	*E. coli*
HWJ1	2521.002	5.92	1.9×10^5	*E. coli*
HWJ2	3307.001	6.94	3.8×10^5	*E. coli*
HWJ3	6703.008	6.71	2.4×10^5	*E. coli*
HWJ4	7103.005	6.30	2.7×10^5	*P.aeruginosa*
HWJ5	4705.003	6.84	2.9×10^5	*P.aeruginosa*
TTJ1	115/003	6.21	1.6×10^3	*P.aeruginosa*
TTJ2	2205/007	5.36	3.7×10^5	*P.aeruginosa*
TTJ3	1317/005	5.91	1.8×10^5	*P.aeruginosa*
TTJ4	1214/009	5.91	$1.9 \times `10^5$	*P.aeruginosa*
TTJ5	1505/003	6.78	3.2×10^3	*P.aeruginosa*
RCJ1	J5341532.2	5.49	2.9×10^5	*P.aeruginosa*
RCJ2	C2231432.1	5.58	2.8×10^5	*S. aureus*
RCJ3	A600322.3	5.95	1.8×10^5	*S. aureus*
RCJ4	B4305211.7	5.84	3.8×10^5	*S. aureus*
RCJ5	F1123421.5	6.22	2.3×10^5	*S. aureus*
CCJ1	1516/601	6.88	2.6×10^5	*E. coli*
CCJ2	3814/004	6.29	2.6×10^5	*E. coli*
CCJ3	2211/003	6.64	2.6×10^5	*E. coli*
CCJ4	4610/001	6.93	3.8×10^5	*P.aeruginosa*
CCJ5	5313/003	5.95	1.9×10^5	*S. aureus*
HMJ1	A0331600.01	6.71	3.2×10^5	*E. coli*
HMJ2	D145201.07	6.52	2.3×10^5	*E. coli*
HMJ3	M221203.08	6.37	3.6×10^5	*E. coli*
HMJ4	G123502.88	6.74	1.9×10^5	*E. coli*
HMJ5	M512204.07	6.69	2.4×10^5	*P.aeruginosa*

Table 2: Frequency of occurrence of isolates in the instant noodle samples examined.

Isolate identities	Number of occurrences	% Prevalence
E. coli	16	40
S. aureus	12	30
P.aeruginosa	12	30

$$\text{Prevalence} = \frac{\text{Number of occurrences}}{\text{Total number}} \times 100$$

Table 3: Antibiogram of the isolates from the samples of instant noodles

Antibiotics	*E.coli* (16)			*S.aureus* (12)			*P. aeruginosa* (12)		
	S	I	R	S	I	R	S	I	R
Gentamicin	6	2	8	2	-	10	5	-	7
Ciprofloxacin	8	-	8	3	1	8	7	2	3
Ampicillin	4	5	7	5	-	7	2	-	10
Meropenem	3	2	11	2	2	8	3	1	8
Erythromycin	5	4	7	2	1	9	2	-	10
Tetracycline	6	3	7	3	-	9	2	-	10
Cotrimoxazole	2	-	14	2	3	7	3	1	8
Cefuroxime	4	1	11	4	-	8	3	2	7
Augmentin	6	2	8	6	1	5	4	-	8
Vancomycin	6	-	10	3	2	7	4	2	6
Ceftriaxone	5	2	9	5	-	7	5	-	7
Cephalexin	5	2	9	5	1	6	4	-	8

S: sensitive; I: intermediate; R: resistance

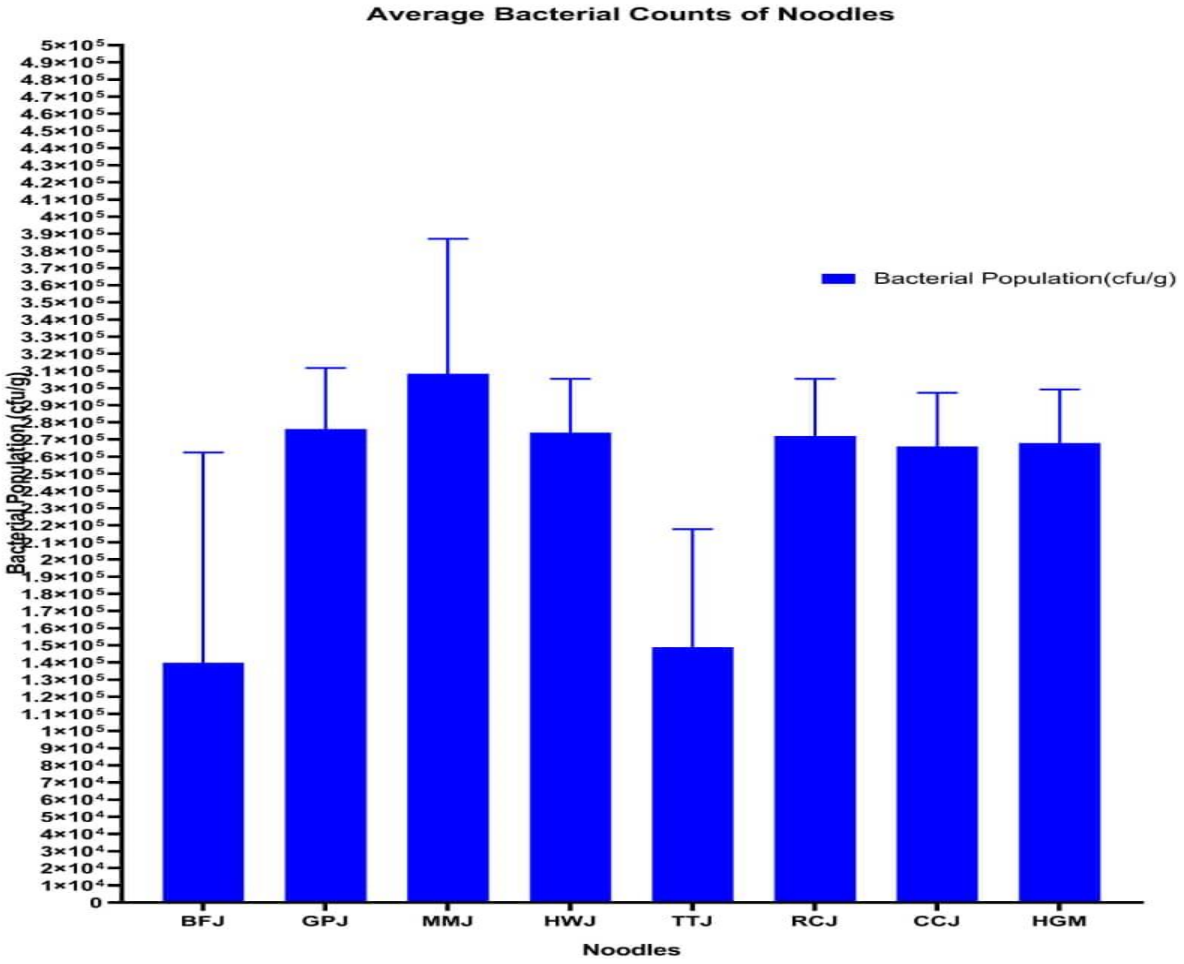

Figure 1: Graphical analysis showing the mean value, standard deviation and standard error of the average bacterial population of each brand of instant noodles , every brands exceeded the $<10^4$cfu/g satisfactory safe limit threshold of bacterial counts for ready-to-eat food as shown in Table 1.

A **B** **C**

Figure 2*:* A: *Escherichia coli,* **B:** *Pseudomonas aeruginosa and* C: *Staphylococcus aureus* isolates from instant noodles examined in the present study.

This could be attributed to adherence or non-adherence to production protocols adopted and efficacy of handling quality control rules and regulations as regarded each products(instant noodles), which corroborates the study of Akhigbemidu et al. [11] on the assessment of microbial qualities of noodles and the accompanying seasonings. Other factors such as personnel, storage temperature, equipment and inherent properties within and outside the noodle, and lack of compliance of the sampled noodles to the regulatory limit could contribute to microbial carriage load of the sample [12].

Of the total 40 isolates obtained from each sample products, 16 were made up of *Escherichia coli* while 12 each were obtained from *Staphylococcus aureus* and *Pseudomonas aeruginosa* respectively, there distribution varied and cut across batches and branded delineations. The resistance of selected isolates to conventional antibiotics tested based on their observable growth profiles on conventional culture media, were remarkable. Eight of the sixteen isolates of *Escherichia coli* were resistant to gentamicin, ciprofloxacin and augmentin while 9 of the isolates elicited resistance to ceftriaxone and cephalexin and 11 of the isolates were resistant to meropenem and cefuroxime respectively. *Staphylococcus aureus* elicited varied resistance in high values to the antibiotics used, 7 of the 12 isolates were resistant to ampicillin and cotrimoxazoles, 9 isolates resistant to erythromycin and tetracycline while 10 were resistant to gentamicin. The number of *Pseudomonas aeruginosa* that exhibited resistance to the antibiotics used in this study were in the ratios of 8 isolates to meropenem, cotrimoxazoles, augmentin, and cephalexin while 10 each of the 12 isolates were resistant to meropenem and tetracycline respectively. Remarkable number of resistance in comparison to intermediate and susceptibility values obtained in this study could be attributed to the sub-optimal storage these products were exposed, which enable the multiplication of these bacteria and other related spoilage factors, the resistance obtained could be transferred horizontally to other bacteria in the same family and beyond, portends a threat of unimaginable magnitude, that could result in therapeutic failure and economic loss[13].

Conclusion. Typically, the threshold level for observation of food spoilage by odour, taste, or sight is not reached until the spoilage microflora exceeds about $<10^4$cfu/g, which was the required satisfactory safe limit for ready-to-eat food[14]. All the noodle samples examined in this study exceeded the safe limit for ready-to-eat foods. Therefore, foods that are meant to be properly stored, cooked before consumption should not be eaten raw however tempting its aesthetic packaging [15] Public awareness should be done to sensitize peoples on the health risks that could results from eating uncooked instant noodles stored under sub-optimal conditions that were sold in local markets.

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