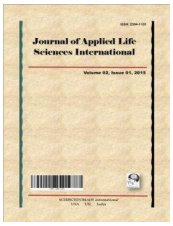

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Occurrence, Antibiotic Susceptibility Pattern and Physiological Studies of *Pseudomonas* Species Isolated from Ready to Eat Foods in Ibadan, Oyo State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Author FIF designed the study, wrote the protocol and the first draft of the manuscript. Author MD managed literature searches, analyses of the study and performed the statistical analysis. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: This study was conducted to determine the occurrence, antibiotic susceptibility pattern and physiological properties of *Pseudomonas species* isolated from ready to eat foods in some selected areas in Ibadan, Oyo state, Nigeria.
Study Design: Identification of *Pseudomonas* species in ready to eat foods, determination of its antibiotics sensitivity pattern and physiological properties.
Place and Duration of Study: All works were carried out in the Department of Microbiology, Faculty of Science, University of Ibadan, from January, 2015 – February, 2016.
Methodology: The *Pseudomonas* species were isolated using culture depended method and identified based on morphological and biochemical characteristics. Susceptibility of the microorganisms to antibiotic compounds was tested by employing the disc diffusion method and the physiological study was carried out using standard methods and the growth of microorganisms was measured using the spectrophotometer.

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Results: A total of 196 *Pseudomonas* species were isolated from *wara*, smoked shawa fish, (*Llisha africana*) Meat pie and Kununzaki and they were identified as *P. aeruginosa* (43), *P. putida* (61), *P. mendocina* (41), *P. alcaligenes* (2), *P. xiamenensis* (9), *P. fluorescens* (1), *P. fragi* (39). It was revealed that the highest total aerobic count (TAC) of $2.73\pm0.25x10^8$ $cfug^{-1}$ was observed in *wara* obtained from Bodija market while the corresponding *Pseudomonas* count (PC) of $4.85\pm2.74x10^7$ $cfug^{-1}$ was recorded in *wara* sample collected from Sawmill market. The lowest TAC of $1.27\pm2.12x10^5$ $cfug^{-1}$ was noted in smoked fish purchased from Bodija market while the corresponding PC of $2.80\pm2.04x10^3$ $cfug^{-1}$ was recorded in smoked fish purchased from Moniya market. The screening for antibiotic susceptibility showed that 93.5% of the *Pseudomonas* species was resistant to Aztreonam from *wara* purchased from Oritamerin market. Highest Multiple Antibiotic Resistance (MAR) index recorded was 0.7 by *P. xiamenensis* and the least was 0.1 shown by *P. aeruginosa, P. putida. P. mendocina*. The physiological studies showed that all the Pseudomonas species grew at temperature range of 30 -42°C. NaCl concentration range of 2-5% and pH range of 3-9 which infers that the eradication of this bacteria from the environment will be very difficult. In conclusion, the presence of high load of *Pseudomonas* species in ready to eat foods is an indication of high level of contamination and could pose a serious problem to public health especially in the area of antibiotic therapy.

Keywords: Pseudomonas; occurrence; ready to eat foods; antibiotics; physiological studies.

1. INTRODUCTION

Pseudomonas species are heterogeneous group of Gram negative bacteria which are aerobic, catalase positive rods, motile and non spore formers. They are included in the genus *Pseudomonas,* and under the class of Gamma proteobacteria [1]. They exhibit simple nutritional requirement which adapts them to various environments making them to be ubiquitous in nature. They can be isolated from various sources for example soil, fresh water, humans, cosmetics, herbal products, food and equipments. *Pseudomonas* species are opportunistic pathogens and cause enormous damage and serious economic loss especially in food industry when the storage conditions are not efficient. USDA Economic Research Service in 1997 reported that over ninety-six billion pounds worth of food was lost in the United States of America in 1995. They have been reported to be responsible for various infectious diseases for example gastrointestinal discomfort in humans [2]. There are documented information about their roles in spoilage of foods with high water activity such as meat, fish, vegetables and milk [3,4]. The concern in food safety procedure is based on the significant removal of health hazards which could emerge from consumption of un-hygienically produced food and food products by the consumers. According to FAO [5], and Kaorola et al. [6], health hazards arising from food could be microbiological, pesticide induced, indiscriminate and excessive use of food additives. Food spoilage of *Pseudomonas* origin exhibits symptoms such as unpleasant odours, tastes and texture as well as the deterioration of the food [7]. Apart from being a pathogenic and spoilage microorganism pseudomonas species possesses another special attribute which is the ability to transfer antibiotics resistance factors to other microorganisms in the different ecological niches they inhabit [8]. The possession of a flexible genetic make-up accords them the ability to transfer antibiotic resistance genes amongst themselves and the environment which might lead to difficulty during antibiotic therapy [9]. In this present work, efforts were made to investigate the occurrence, antibiotic susceptibility pattern and physiological behaviour of pseudomonas species isolated from ready to eat foods in other to prevent their consequences.

2. MATERIALS AND METHODS

2.1 Sample Collection

Four (4) food samples namely: *wara*, Kununzaki, smoked Shawa fish (*Llisha africana*) and Meatpie were collected from six (6) different markets in Ibadan namely: Bodija, Moniya, Oritamerin, Oje, Saw mill (Old Ife road) and Gege markets and transported under hygienic conditions to the postgraduate laboratory, University of Ibadan. Four (4) Vendors were chosen at random for each food product and a total of 96 samples collected at the end of the study.

2.2 Isolation and Identification

Ten grams of each food sample was weighed into 90 mL of sterile distilled water and homogenised. Serial dilution was carried out

according to the method described by Meynell and Meynell [10]. Aliquot of 0.1 mL from dilutions 10^{-2}, 10^{-4}, 10^{-6} was separately plated on plate count agar (PCA) (Oxiod, UK) and Cetrimide-Fucidin-Cephaloridine (CFC) supplemented *Pseudomonas* agar (Oxiod, UK) and incubated for 24 hours at 30°C. Representative colonies were selected and streaked repeatedly until pure colonies were obtained. Pure cultures were identified based on parameters such as gram reaction, biochemical tests and with reference to Bergey's manual of systematic bacteriology [11].

2.3 Antibiotic Susceptibility Testing

Susceptibility of *Pseudomonas* species to antimicrobial compounds was tested employing the disc diffusion method according to Clinical and Laboratory Standards Institute [12] guidelines. Isolates were inoculated in Normal saline and adjusted to 0.5 McFarland's standard before testing. A sterile swab stick that has been previously soaked in the culture suspension was used to seed Mueller-Hinton agar plates and seven different antibiotic sensitivity discs were placed at equal distance from each other on the plate and incubated for 24 hours at 37 °C. The antibiotics used included; aztreonam (ATM; 30 µg), ceftazidime (CAZ; 30 µg), ciprofloxacin (CIP; 5µg), colistin (CT; 10 µg), gentamicin (CN; 10 µg), imipenem (IPM; 10 µg), piperacillin (PRL; 100 µg) (Oxiod, UK). The zone of inhibition was measured in millimetres.

2.4 Physiological Study of Isolates

Growth of isolates at different temperatures ranges: Ten mL of Nutrient broth was prepared and dispensed into screw-capped bottles and sterilized. One mL of the culture suspension (1.0 $x10^{-5}$) was used to inoculate the broth and incubated at 30, 35, 37 and 42°C for 24 h. A spectrophotometer (CECIL 2031 model) set at 560 nm was used to measure the optical density and the un-inoculated tubes served as control.

Growth of isolates at different pH ranges: Ten mL of Nutrient broth was prepared and the pH adjusted using 0.1 phosphate buffer to 3.0, 5.0, 7.0, and 9.0, dispensed into series of screw-capped bottles and sterilized. One mL of the culture suspension was used to inoculate the broth and incubated at 30°C for 24 h. A spectrophotometer (CECIL 2031 model) set at 560nm was used to measure the optical density .Un-inoculated tubes served as control.

Growth of isolates at different concentrations of sodium chloride: Nutrient broth (10 mL) was prepared containing 2, 3, 4 and 5% (w/v) NaCl, dispensed into series of screw-capped bottles and sterilized. One mL of the culture suspension was used to inoculate the broth and incubated at 30°C for 24 h. A spectrophotometer (CECIL 2031 model) set at 560 nm was used to measure the optical density. Un-inoculated tubes served as control.

3. RESULTS AND DISCUSSION

The identification result of *Pseudomonas* species isolated from ready to eat foods is shown in Table 1. It was observed that the isolates were made up of *P. aeruginosa (13), P. putida (61), P. mendocina (41), P. alcaligenes (2), P. xiamenensis (9), P. fluoroscenes (1)* and *P. fragi (31) P. putida* had the highest number while *P. fluoroscenes* had the lowest. This observation is similar to the earlier findings of Amusa and Odunbakun [13] and Chukwu et al. [14]. In addition, other researchers such Olowafemi and Simisaye [15], Liao [16], Bello and Osho [17] had earlier reported the isolation and identification of *Pseudomonas* species from different samples especially food and food products The presence of *Pseudomonas* species in ready to eat foods might have emanated from soil and water environments this might probably lead to spoilage of the food resulting in huge economic loss and causation of gastroenteritis [16]. Other likely sources of contamination mentioned are food handlers, dirty utensils, working tables and trays used production [18].

Table 2 shows the total aerobic count (TAC) and Pseudomonas count (PC) obtained from ready-to-eat foods. The highest TAC of $2.73x10^{8}$±0.25 cfu/g was recorded in wara sample obtained from Bodija market, while the highest PC of 4.85×10^{7}±2.74 cfu/g was recorded in wara sample collected from sawmilll market. The lowest TAC of $1.27x10^{5}$±2.12 cfu/g was recorded in smoked fish obtained from Bodija market, while the lowest PC of $2.80x10^{3}$±2.04 cfu/g was recorded in smoked fish obtained from Moniya market. It was observed that the TAC recorded in all the ready-to-eat food samples were above the NAFDAC recommended standard. According to FAO [5], the safe microbial load in food and food products should not exceed 1.0×10^{6} cfu/g. The observation is in conformity with the reports of Adesiyun [19], Okonko et al. [20] Bello et al. [21] and Adesetan et al. [22]. It was equally observed that there is a

variation in members of *Pseudomonas* species isolated amongst the ready-to-eat food samples considered. Similar observation had been earlier reported by Wogu et al. [23]. High TAC and PC in food and-the presence of bacterial load of 7×10^4 in ready-to-eat food samples revealed a high level of contamination or under processing [18]. It is important to note that occurrence of pathogenic microorganisms such as *Pseudomonas* sp with enormous documented reports for traits such as antibiotics resistance is a threat to public health [18,24]. Microbial food contamination may arise from the way/manner these foods are covered by vendors and hawkers which exposed them to contamination through dust and aerosols [25]. Bello and Osho [17] reported that consumption of contaminated food by pathogenic microorganisms will cause food borne-illness because of the toxins they produce in food [26]. Chukwu et al. [14] reported that mobility and mortality of Nigerians is caused by significantly the consumption of contaminated food.

The Antibiotic Sensitivity Pattern and MAR index of *Pseudomonas* species is described in Table 3. The result obtained revealed that the organisms can be categorized based on 3 criteria namely: the source of ready to eat food, MAR index and response to antibiotics. Two species of P. *fragi* showed the same antibiotic reaction with the same MAR index of 0.3 while another 2 species of P. *fragi* showed different sensitivity patterns with the same MAR index of 0.3. However one species of P. *fragi* showed a similar MAR index of 0.3 but a distinct antibiotic reaction from the others observed. In addition 4 species of *P*. *fragi* showed different sensitivity patterns to the ones earlier observed with a MAR index of 0.0. Also, one species showed a different sensitivity pattern with a MAR index of 0.4. It was also observed that another species of *P. fragi* showed a different antibiotic sensitivity patterns to the ones earlier observed with a MAR index of 0.4. Similar trends were also seen in the other species of *Pseudomonas* isolated in this study.

The present study confirmed the earlier submission of Oladipo and Fajemilo [18] and Schoedez et al. [27], which reported the global susceptibility of ready-to-eat food to antibiotics resistant bacteria which constitute threat to antibiotics therapy and public health. The presence of antibiotic resistant bacteria in food might be caused by inappropriate, uncontrolled or indiscriminate use of antibiotics.

Bacteria are capable of resisting antibiotics by the production of inactivating or modifying enzymes, ability to change cell membrane, blocking movement of antibiotic and evading the site of antimicrobial activity [28].

The percentage phenotypic resistance pattern *of Pseudomonas* species from Ready to Eat Foods in Some Markets in Ibadan Municipality is shown in Table 4. All *Pseudomonas* species isolated were sensitive and resistant and showed intermediate responses to all the antibiotics used in the study. It was observed that the *Pseudomonas* species isolated from meat pie samples from Sawmill market showed the highest resistance of 75% to Aztreonam while *Pseudomonas* species isolated from Kununzaki samples from Sawmill and Gege market showed the highest resistance of 40% and 65% respectively to Aztreonam. In addition, *Pseudomonas* species obtained from *wara* samples showed the highest resistance of 93.5% to Aztreonam while the *Pseudomonas* species isolated from Gege market showed the highest resistance of 80% to Aztreonam.The *Pseudomonas* species obtained from the meatpie samples showed the highest sensitivity of 100% to Imipenem, Colistinsulphate and Ciprofloxacin while the *Pseudomonas* species isolated from Kununzaki from Sawmill and Gege market showed a highest sensitivity of 100% and 92% respectively to Imipenem and Gentamicin and Imipenem respectively. In addition, *wara* obtained from Orita merin market showed the highest sensitivity of 100% to Piperacillin and Imipenem while *Pseudomonas* species from smoked fish from Gege market showed 100% sensitivity to Imipenem, Colistinsulphate and Ciprofloxacin.

There are earlier reports about the efficacy and broad spectrum activity of these antibiotics [18] and it is suggested that they could be employed in the treatment of bacterial infection.

The physiological study of the *Pseudomonas* species isolated from the ready to eat foods is shown in Table 5. The result of the physiology study showed that the *Pseudomonas* species grow at the temperature range of 30-42°C, NaCl concentration of 2-5% and pH of 3-9. It was observed that the bacteria could grow at both acidic and basic range and at wide range of temperature and NaCl concentration. The implication of this is that it will be very difficult to eliminate *Pseudomonas* species from the environment.

Table 1. Gram and biochemical reactions of the isolates from ready to eat foods in some markets in Ibadan Municipality

Probable Identity	Gram rxn	Oxidase rxn	Catalase rxn	Motility	Starch hydrolysis	Citrate	Indole	Growth on MacConkey	H_2S reaction	Gelatin hydrolysis	Glucose	Sucrose	Xylose	Mannitol	Sorbitol	Arabinose	Fructose	Inositol	No of Isolates obtained
P. aeruginosa	-	+	+	+	-	+	-	+	-	-	+	-	+	-	-	-	-	-	43
P. putida	-	+	+	+	-	+	-	+	-	-	+	-	+	-	-	+	-	-	61
P. mendocina	-	+	+	+	-	+	-	+	-	-	+	-	+	-	-	+	+	-	41
P. alcaligenes	-	+	+	+	-	+	-	+	-	+	-	-	+	-	-	-	-	-	2
P. xiamenensis	-	+	+	+	-	+	-	+	+	-	-	+	+	-	-	+	-	-	9
P. fluorescens	-	+	+	+	-	+	-	+	-	+	+	+	+	-	+	+	+	-	1
P. fragi	-	+	+	+	-	+	-	+	-	-	+	+	+	-	-	+	+	-	39

Key: -rxn = Reaction

Table 2. Total aerobic count (TAC) and *Pseudomonas* count (PC) of the ready to eat foods from the selected markets in cfug^{-1}

Parameter		Sawmill	Oje	Oritamerin	Gege	Bodija	Moniya
Wara	TAC	$1.15x10^{8}±0.17x10^{8b}$	$4.43x10^{7}±0.86x10^{7a}$	$2.10x10^{8}±0.17x10^{8c}$	$2.95x10^{7}±0.32x10^{7a}$	$2.73x10^{8}±0.25x10^{8d}$	$2.51x10^{7}±0.18x10^{7a}$
	PC	$4.85x10^{7}±2.74x10^{7c}$	$1.80x10^{7}±4.42x10^{7ab}$	$3.18x10^{7}±0.80x10^{7ab}$	$2.50x10^{6}±0.86x10^{6a}$	$2.90x10^{7}±0.86x10^{7ab}$	$1.76x10^{6}±1.08x10^{6a}$
Meatpie	TAC	$3.94x10^{7}±1.68x10^{7b}$	$3.90x10^{7}±0.44x10^{7b}$	$3.18x10^{5}±4.24x10^{5a}$	$1.52x10^{5}±2.58x10^{5a}$	$2.92x10^{5}±3.63x10^{5a}$	$3.44x10^{3}±4.93x10^{a}$
	PC	$4.15x10^{5}±0.79x10^{5a}$	$4.29x10^{5}±0.17x10^{5a}$	$2.40x10^{5}±2.88x10^{5a}$	$1.14x10^{5}±1.96x10^{5a}$	$2.12x10^{5}±2.21x10^{5a}$	$3.10x10^{3}±4.35x10^{3a}$
Kunu	TAC	$1.83x10^{7}±1.07x10^{7a}$	$3.15x10^{7}±0.82x10^{7a}$	$3.97x10^{7}±1.99x10^{7a}$	$3.97x10^{7}±0.30x10^{7a}$	$3.32x10^{7}±0.36x10^{7a}$	$3.45x10^{7}±0.60x10^{7a}$
	PC	$3.33x10^{3}±0.42x10^{3a}$	$4.53x10^{3}±3.28x10^{3a}$	$2.29x10^{6}±3.06x10^{6a}$	$2.37x10^{6}±2.97x10^{6a}$	$1.79x10^{5}±0.80x10^{5a}$	$4.17x10^{3}±2.47x10^{3a}$
Smokefish	TAC	$1.80x10^{8}±0.20x10^{8b}$	$4.84x10^{6}±8.10x10^{6a}$	$4.18x10^{6}±3.27x10^{6a}$	$2.89x10^{6}±1.65x10^{6a}$	$1.27x10^{5}±2.12x10^{5a}$	$1.42x10^{7}±2.42x10^{7a}$
	PC	$9.56x10^{4}±8.01x10^{4a}$	$2.05x10^{5}±2.40x10^{5a}$	$3.75x10^{5}±2.27x10^{5a}$	$2.89x10^{5}±1.29x10^{5a}$	$1.90x10^{4}±2.69x10^{4a}$	$2.80x10^{3}±2.04x10^{3a}$

Mean values followed with different lower case letters are statistically significantly different at p <0.05
Key: TAC: Total aerobic count, PC: Pseudomonas count

Table 3. Antibiotic sensitivity pattern and MAR index of isolates obtained from the study

Probable identity	PRL	CAZ	ATM	IPM	CT	GN	CIP	MAR index	No of isolates obtained
P. fragi	R	S	S	S	S	R	S	0.3	2
P. fragi	S	R	R	S	S	S	S	0.3	2
P. fragi	R	S	R	S	S	S	S	0.3	1
P. fragi	S	S	R	S	S	S	S	0.0	4
P. fragi	S	R	R	S	R	S	S	0.4	1
P. fragi	S	R	R	S	S	S	R	0.4	1
P. mendocina	S	S	R	S	S	S	S	0.0	2
P. mendocina	S	S	S	S	S	S	R	0.0	1
P. mendocina	S	R	R	S	S	S	S	0.3	1
P. mendocina	S	S	R	S	R	S	S	0.3	1
P. mendocina	R	R	S	S	S	R	S	0.4	1
P. mendocina	S	R	R	S	R	S	S	0.4	1
P. mendocina	R	R	R	S	S	R	S	0.6	1
P. mendocina	R	R	R	S	S	S	S	0.4	1
P. mendocina	R	S	R	S	S	R	S	0.4	1
P. mendocina	S	R	R	R	S	R	S	0.6	1
P. aeruginosa	S	S	R	S	S	S	S	0.0	5
P. aeruginosa	R	R	R	S	S	S	S	0.4	2
P. putida	R	S	S	S	S	S	S	0.0	1
P. putida	S	S	S	S	R	S	S	0.0	1
P. putida	S	S	R	S	S	S	S	0.0	1
P. xiamenensis	R	R	R	S	R	R	S	0.7	1
P. xiamenensis	S	R	R	S	S	S	S	0.3	1
P. xiamenensis	S	S	R	S	S	S	S	0.0	1
P. xiamenensis	R	S	S	S	S	S	S	0.0	1
P. xiamenensis	R	S	S	S	S	S	S	0.0	1
P. fluorescens	S	S	R	S	S	R	S	0.3	1

Key: PRL=Piperacillin, CAZ=Ceftazidime, ATM=Aztreonam, IPM=Imipenem, CT=Colistinsulphate, GN=Gentamicin, CIP=Ciprofloxacin

Table 4. Percentage phenotypic resistance pattern *of Pseudomonas* species from ready to eat foods in some markets in Ibadan Municipality

Sample/ location	PRL (%)			CAZ (%)			ATM (%)			IPM (%)			CT (%)			GN (%)			CIP (%)		
Meat pie (Sawmill	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Market)	50	0	50	75	0	25	25	0	75	100	0	0	100	0	0	75	0	25	100	0	0
Kununzaki	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
(Sawmill Market)	80	0	20	80	0	20	37.4	22.6	40	100	0	0	80	0	20	100	0	0	73.	6.5	20
Wara (Oritamerin	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Market)	100	0	0	35	0	65	0	6.5	93.5	100	0	0	62	0	38	82	0	18	78.	3.2	18
Kununzaki (Gege	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
Market)	75	0	25	50	0	50	18.	16.1	65	92	0	8	85	0	15	65	0	35	90.	9.7	0
Smoked Fish	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**	**S**	**I**	**R**
(Gege Market)	20	0	80	70	0	30	63	7	30	100	0	0	100	0	0	67	3	30	100	0	0

Key: PRL=Piperacillin, CAZ=Ceftazidime, ATM=Aztreonam, IPM=Imipenem, CT=Colistinsulphate, GN=Gentamicin, CIP=Ciprofloxacin, S= Sensitive, I= Intermediate, R= Resistant

Table 5. Physiological study of isolates obtained from the study

Isolate	Temperature (OD)				NaCl Concentration (OD)				pH (OD)			
	30°C	**35°C**	**37°C**	**42°C**	**2%**	**3%**	**4%**	**5%**	**3**	**5**	**7**	**9**
P. aeruginosa	0.205	0.135	0.083	0.05	1.152	1.114	1.017	0.983	0.22	1.073	1.298	1.326
P. xiamenensis	0.51	0.365	0.342	0.077	0.474	0.406	0.36	0.183	0.156	0.892	0.976	1.138
P. mendocina	0.926	0.469	0.263	0.107	1.08	0.934	0.842	0.638	0.18	0.961	0.981	1.231
P. fluorescens	0.477	0.315	0.277	0.122	1.044	0.962	0.842	0.638	0.203	0.872	0.997	1.42
P. alcaligenes	0.43	0.35	0.21	0.15	1.01	0.792	0.635	0.5	0.15	0.92	0.97	1.21
P. fragi	1.9	0.321	0.317	0.312	1.017	0.508	0.428	0.414	0.123	0.998	1.559	1.625
P. putida	0.218	0.138	0.021	0.011	1.385	1.093	0.768	0.615	0.165	0.968	1.032	1.272

4. CONCLUSION AND RECOMMENDATION

The results of this study points out that ready to eat foods were found to contain *Pseudomonas* species which is higher than recommended level of NAFDAC which implies that these foods could be a problem to public health.

Also, the presence of antibiotic resistant *Pseudomonas* species in ready to eat foods shown in this study reveals that their presence in ready to eat foods could increase the spread of drug resistance genes to the normal flora of the human intestine.

Relevant agencies of government should enlighten and educate food handlers on the importance of good personal hygiene and good manufacturing practices, which are sure ways of reducing food-borne diseases/ illnesses.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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