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Bacterial Flora and Proximate Composition of Edible Frogs (*Ptychadena mascareniensis* and *Ptychadena pumilio)* from Some Locations in Rivers State, Nigeria

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Aim: To determine and compare the bacterial flora and proximate composition of edible frogs from some selected Local Government Areas (LGA) of Rivers State.
Place and Duration of Study: Live adult edible frogs were collected from six locations in six Local Government Areas in Rivers State which includes; Biara (E 7º29' 2.097'', N 4º78' 70.608'') in Gokana, Rumuodumaya (E 7º0' 57.16.908'', N 4º52' 40.398'') in Obio/Akpo, Umuikere Oyigbo (E 6º55' 50.606'', N 9º1' 13.831'') in Oyigbo, Diobu (E 6º59' 6.882'', N 4º48' 29.514'') in Port Harcourt, Igwuruta (E 4º55' 57.006'', N 7º1'13.692'') in Ikwerre and Akpajo (E 5º50' 67.306'', N 9º1' 43.112'') in Eleme Local Government Areas of Rivers State. This study was conducted from July 2017 to February 2018, covering both the wet and dry seasons.
Methodology: The microbiological analyses were carried out using standard microbiological methods. Conventional and molecular identification methods were used to identify the bacteria isolated. Proximate compositions of the two species of edible frogs were also determined using standard methods. Analysis of variance (ANOVA) was used to test for significant difference between the data obtained from the various locations using a computer based program SPSS version 22.

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Results: Results revealed that *Ptychadena pumilios* had high bacteria counts across the six Local Government areas. Counts of total heterotrophic bacteria for *Ptychadena pumilios* ranged from 1.0 $x10^8$cfu/g to 9.0 $x10^8$cfu/g, Total *Staphylococcus* count ranged from 1.0 $x10^5$cfu/g to 9.4 $x10^5$cfu/g, Total coliform counts ranged 1.0 $x10^5$cfu/g to 8.9 $x10^5$ cfu/g, Total Feacal Coliform counts ranged from 1.4 $x10^4$cfu/g to 9.0 $x10^4$cfu/g. while for *Ptychadena mascareniensis* Total heterotrophic bacterial counts ranged from 2.2 $x10^8$ to 4.5 $x10^7$cfu/g, Staphylococcal counts ranged from 1.8 $x10^5$cfu/g to 9.5 $x10^5$cfu/g, Total coliform counts ranged 1.0 $x10^5$cfu/g to 9.8 $x10^5$cfu/g, Total Feacal Coliform ranged from 1.2 $x10^4$cfu/g 9.8 $x10^4$cfu/g. Statistically there were significant differences in bacterial counts in the six studied LGAs. Statistically there were significant differences in the bacterial counts from the six studied locations. A total of 259 bacterial isolate belonging to the following genera: *Escherichia, Staphylococcus*, *Bacillus, Klebsiella,* and *Pseudomonas* were isolated during this study. The proportion of nutritional composition obtained in this study ranged as follow: carbohydrate from 1.82 to 2.24, crude fibre 2.28 to 3.00, ash 3.99 to 4.89%, lipid 11.90 to 12.32%, protein13.74 to14.00%, moisture 64.45 to 65.74%, for the species of the edible frogs tested.

Conclusion: The presence of *Escherichia coli* identified indicates faecal contamination and indicates the possible presence of potential pathogens. Proper processing and cooking of the frog meat will destroy these organisms, thereby preventing food borne infections. Improper handling and cooking may lead to food borne infections with greater effect observed in immunocompromised patents, the elderly and children. *Ptychadena mascareniensis* and *Ptychadena pumilio* have high protein contents therefore, they could be considered as an alternative source of protein despite the bacterial load.

Keywords: Bacterial flora; proximate composition; Ptychadena mascareniensis; Ptychadena pumilio.

1. INTRODUCTION

The importance of meat to humans cannot be overemphasized, as they are gotten from several sources, and it serves as the major source of nutrients and vitamins to the body. The high cost of meat and red meat related problems, have now attracted the focus of research to other sources of meat or alternatives which would help take care of the health challenges and which would be less costly and easy to consumption with little or no health risk [1]. Since meats contain essential classes of food such as, carbohydrate, proteins, fat, vitamins and minerals, they provide the nutritional requirements of man in the appropriate quantities [2]. The provision of these nutritional entities becomes a major problem in most developing countries such as Nigeria leading to under or malnutrition. In a view to reduce such menace in Nigeria some lesser known animals which can serve as food are studied for their nutritive and non-nutritive values for human consumption. One class of such known animals that could be considered for this purpose is the amphibian [3].

Their meat is becoming popular as a source of protein in many countries including Nigeria [3]. Frogs are now reared on large scale for both local consumption and for export in countries like; Malaysia, Brazil, Indonesia, Mexico, France and USA [4,5]. This meat is also a delicacy in parts of Rivers State where they are harvested from the forests and temporary ponds in water logged areas; the gut is removed and discarded while the rest of the animal is cooked [6]. The common species found in Rivers State is *Ptychadena* species which include *P. mascareniensis, P. oxyrhynchus, P. pumilio, P. bibroni, P. schubotzi and P. longirostris and the African bullfrog, H. occipitalis*, are consumed by the locals in Igwuruta, Rivers State [7]. In parts of Oyo State (Nigeria), similar species are also consumed: the gut is removed; the rest of the frog is pinned to sticks and smoked. These are then sold in their local markets for consumption [6]. The meat serves as food as well as a source of income or foreign exchange [3].

The Edible Frog (*Pelophylax esculentus)* is consumed worldwide by humans and other animals such as herons and related species and grass snakes, and is a largely aquatic species. Previous studies indicated that microbial community composition varies from one marine environment to another [8,9,10], but can be relatively consistent in similar marine environments separated by long distances [11,12,13]. Similarly, subseafloor sedimentary environments with different properties separated by a few tens of kilometres also have distinct communities [11,14,15].

Despite these differences over relatively short geographic distances, microbial community composition in individual deep-seawater masses can be relatively constant for thousands of kilometres [12]. And broadly, similar microbial communities inhabit similar subsea floor sedimentary environments separated by thousands of kilometres [11].

These observations are consistent with the old adage, 'Everything is everywhere but the environment selects in which microorganisms are considered to be ubiquitously dispersed because of their small size, large numbers and low extinction rates [16]. Hence, necessitate this research to determine and compares the bacterial diversities and proximate composition of edible frogs in six different locations in Rivers State, Nigeria. Proximate composition of food is the term applied to the proportion of moisture, fat, carbohydrate, protein, fibre and ash present in foods. From an industry standard proximate composition include five constituents; Ash, Moisture, Proteins, Fat and Carbohydrates. Analytically, four of the five constituents are obtained via chemical reactions and experiments. The fifth constituent, (carbohydrates) is a calculation based on the determination of the four others. Proximate should nearly always add up to 100%, any deviation from 100% displays the resolution of the chemical test that is, small variations in the way each test is performed, chemist to chemist will accumulate or overlap the compositional make-up [17].

Therefore, the aim of this study is to determine and compare the bacterial flora and proximate composition of edible frogs from some selected Local Government Areas in Rivers State which is used as meat.

2. MATERIALS AND METHODS

2.1 Sample Collection/Study Area

Live adult edible frogs were collected from six selected locations in six Local Government Areas of Rivers State such as Biara (E 7º29' 2.097'', N 4º78' 70.608'') in Gokana, Rumuodumaya (E 7º0' 57.16.908'', N 4º52' 40.398'') in Obio/Akpo, Umuikere Oyigbo (E 6º55' 50.606'', N 9º1' 13.831'') in Oyigbo, Diobu (E 6º59' 6.882'', N 4º48' 29.514'') in Port Harcourt City, Igwuruta (E 4º55' 57.006'', N 7º1' 13.692'') in Ikwerre and Akpajo (E 5º50' 67.306'', N 9º1' 43.112'') in Eleme Local Government Areas. The Samples were collected in sterile plastic containers and transported to the Microbiology Laboratory of Rivers State University with ice pack within 24 hours of collection.

2.1.1 Duration of study and sample size

The study was conducted during wet and dry seasons, starting from July 2017 to February 2018, a total of 192 samples were collected during the period.

2.2 Microbiological Analysis

2.2.1 Sample processing

The samples were processed for microbiological analyses by dissecting in order to isolate the enteric bacteria. One gram of the intestinal gut was homogenized in 9 ml of sterile normal saline after which the homogenized samples were serially diluted to 10^{-4} [6]. For isolation of the bacteria from the skin, ten (10) grams of the whole frog were submerged into 90 ml of sterile normal saline and shaken vigorously in order to dislodge the bacteria associated with it. Further 10 - fold serial dilutions were carried out by adding 1 ml of the initial dilution to 9.0 ml of appropriate diluents. Finally 0.1 ml of appropriate dilutions was inoculated on dried nutrient agar, Mannitol salt agar and MacConkey agar.

2.2.2 Isolation and enumeration of bacterial isolates

An aliquot (0.1 ml) of 10^{-3} to10^{-4} dilutions of each samples were inoculated on Nutrient agar (for total Heterotrophic bacteria), while an aliquot (0.1 ml) of 10^{-3} dilution on Mannitol and MacConkey agar for isolation and enumeration of Staphylococci species and enteric bacteria respectively, using the spread-plate technique as described by Prescott et al. [18]. The plates were inoculated in duplicates and incubated under aerobic condition at 37°C for 24 hours except for the MacConkey plates used for the isolation of faecal coliform that was incubated at 45°C for 24 hours. The numbers of colonies in each plate was counted and mean values calculated for duplicate dilutions, which was expressed as colony forming unit per gram (cfu/g) using the equation below;

(cfu/g) = (Number of Colonies / Dilution x Volume plated (0.1ml))

2.2.3 Maintenance of pure culture

Discrete bacterial colonies that grew on the respective media plates were subcultured using

streak plate method onto fresh medium and incubated for 24 hours at 37°C. The pure bacterial cultures were then maintained according the method as adopted by Amadi et al. (2014) using ten percent (v/v) glycerol suspension at -4°C.

2.2.4 Characterization and identification of isolates

The isolates were characterized based on their appearance on the culture media that is; shape, colour, wetness, dryness, etc, while identification of the characterized isolates was done via Biochemical tests such as Gram Reaction, Catalase, Oxidase, Motility, Citrate, Indole, MR/VP, Glucose, Lactose, Fructose, Manitol, Sucrose, Galactose according to Bergey's Manual of Determinative Bacteriology [19].

2.3 Proximate Analysis

The proximate composition of the two species of edible frogs was determined according to standard methods described by [20] which was carried out in duplicates. The parameters analysed includes: moisture content, crude protein, total available carbohydrate, ash, and averages taken.

2.3.1 Determination of moisture

The crucibles were cleaned and dried using the air oven for 10 minutes. They were kept in the desiccators to cool and weighed. The samples were thoroughly mixed and 5 g weighed into the crucibles. Crucibles plus content were placed in the oven at 103 ± 2°C overnight. The crucibles were then removed and reweighed after cooling. They were dried for another one hour to ensure constant weight. The moisture content was calculated using the formula below:

% Moisture= (Loss of weight of sample (g) / Weight of samples (g)) × 100

2.3.2 Determination of crude protein

This was done using the Micro-Kjehdhal method [20]. Half gram(0.5 g) of the flog sample was weighed into one quarter size filter paper, one tablet of catalyst was added followed by 10 ml concentrated sulphuric acid in a digestion flask in duplicates. The flasks were then placed in the heating unit inside the fuming cupboard and heated slowly until the sample boiled. The temperature was then increased until foaming ceased and the content of the flask completely liquefied. The digestion was done by boiling vigorously while agitating the flask until solution became completely clear. Digestion was terminated, samples cooled and weighed into a 100 ml flask with distilled water. Five millilitres (5 ml) of boric acid mixed. Indicator solution was transferred into a 100 ml conical flask placed at the end of the condenser of the micro kjehdhal distillation apparatus so that the adapter dipped into the liquid. Ten millilitres (10 ml) aliquot of sample was pipette into a micro kjehdhal flask for distillation. Ten millilitres (10 ml) of 45% Sodium hydroxide (90 in 200 ml) was poured carefully down the inclined neck of the solution. The flask was immediately attached to the splash head of the distillation apparatus. Steam was passed through alkaline liquid (i.e. NaOH + aliquot) slowly until it boiled. The liquid was trapped and distilled into 5 ml boric acid in the conical flask until 50 ml of the distillate was collected with a green colour and then titrated with 0.045N Sulphuric acid. The bank was prepared in the same way. Crude protein was calculated as follows:

$$\text{Nitrogen \%} = \frac{\text{Titre-Blank} \times \text{Normality of acid} \times 1.4}{\text{Weight of Sample} \quad 1}$$

Crude protein % = Total nitrogen (%) × 6.25

Where 6.25 = conversion factor.

2.3.3 Determination of fat

The Micro-Soxhlet extraction method was used [20]. Two-grams (2 g) of the dried samples used for the determination of moisture content were used for fat extraction. This was to make the fat more available for extraction. The samples were wrapped in a filter paper and held with the clip in the extraction unit in which a weighed flask containing 50 ml of petroleum ether (60-90°C) was attached while on the heating plate. The extractor was connected to a reflux condenser on a steam bath for 3 hours. The petroleum ether extract was evaporated to dryness at 100°C for 5 minutes. The flask were cooled in the dessicator and weighed. Extractable fat was calculated using the equation;

% Fat = {(Weight (g) of flask + fat – Weight of flask without fat / Weight (g) of sample before drying) × 100}

2.4 Determination of Ash (AOAC 1990)

Six crucibles were washed and placed in the oven for 5 minutes. The crucibles were removed,

cooled in the desiccators for one hour and weighed. 5 g of the sample was weighed into each crucible, placed on hot plate under a fume hood and temperature was slowly increased until smoking ceased and the samples became completely charred. The crucibles were placed inside the muffle furnace and ashed overnight at 550°C. The crucibles were removed from the furnace and placed in the desiccator for an hour [20]. When cooled to room temperature, each crucibles plus ash was weighed and weight of ash calculated as follows;

Weight of empty dish = A

Weight of dish + unashed sample = B

Weight of dish + ashed sample = C

{(C – A/B – A) × (100 / 1)}

Absorbance of dilute standards × Weight (g) of sample = 25 × absorbance of dilute sample

2.4.1 Determination of total available carbohydrate (TAC)

A gram of the sample was weighed and transferred into a graduated 100ml stoppered measuring cylinder. 10 ml of water was added and stirred with a glass rod to dispense the sample thoroughly. 13 ml of 52% perchloric acid reagent was added using a measuring cylinder and constantly stirred with a glass rod for 20 minutes. Samples were noticed to digest by forming slightly thick slurry. The glass rod was washed down with water and the content made up to 100 ml. it was mixed and filtered into a 250 ml graduated flask. The measuring cylinder was rinsed with water and transferred in to the graduated flask, made up to mark and thoroughly mixed. 10 ml of the extracted sample was diluted to 100 ml with water and 1 ml of the diluted filtrate pipette into a test tube. Blank and glucose standard in duplicates were prepared and anthrone reagent rapidly pipette in all tubes, stoppered and content thoroughly mixed. The tubes were placed in a boiling water bath for exactly 12 minutes after which they were cooled to room temperature. The solution was transferred to 1 cm glass cuvettes and the absorbance of the sample and standards read at 630nm against the reagent black using a spectrophotometer [21]. Total available Carbohydrate (TAC) as percent glucose was calculated using the equation;

TAC (as % glucose) = {25 × absorbance of dilute sample / Absorbance of dilute standards × Weight (g)}

3.5 Statistical Analysis

Statistical analysis was carried out on the data obtained during this study. Analysis of variance (ANOVA) was used to test for significance difference ($P \leq 0.05$) in the counts between the sampling locations and seasons. This was done using a computer based program SPSS version 22.

3. RESULTS AND DISCUSSION

Total heterotrophic bacterial, total coliform, faecal coliform and Staphylococcal counts from the two edible species of frog (*P. mascareniesis* and *P. pumilio*) were determined across the six selected Local Government Areas of Rivers State, namely Gokana, Eleme, Obio/Akpo, Ikwerre, Port Harcourt City and Oyigbo (Table 1 and 2). The counts were obtained during wet and dry seasons in all the selected L.G.As. The results obtained revealed that wet seasons had the highest microbial load across the sampling locations.

Total heterotrophic bacterial counts during dry season ranged from 2.5 $x10^7$ cfu/g (Ikwerre) to 5.2 $x10^7$ cfu/g (Port Harcourt), Staphylococcal counts 1.8x 10^5 cfu/g (Gokana) to 9.5 $x10^5$ cfu/g (Port Harcourt), Total coliform counts range from 1.6 $x10^5$ cfu/g (Oyigbo) to 9.7 $x10^5$ cfu/g (Eleme), faecal coliform counts ranged from 2.0 $x10^4$ cfu/g (Oyibgo) to 9.8 $x10^4$ cfu/g (Gokana) for *P.mascareniensis*. Total heterotrophic bacterial counts ranged from 2.2 $x10^7$ cfu/g (Port Harcourt) to 9.0 $x10^7$ cfu/g (Gokana), Staphylococcal counts 1.7 $x10^5$cfu/g (Ikwerri) to 9.4 $x10^5$cfu/g (Eleme), Total coliform counts range from 1.0 $x10^5$cfu/g (Port Harcount) to 8.4 $x10^5$cfu/g (Eleme), total faecal coliform count ranged from 4.8 $x10^4$ cfu/g (Gokana) to 9.0 $x10^4$ cfu/g (Obio/Akpo) for *P. pumilio.*

Total heterotrophic bacterial counts during wet season ranged from 2.2 $x10^8$ cfu/g (Port Harcourt) to 4.0 $x10^8$ cfu/g (Gokana), Staphylococcal counts 1.8x 10^5 cfu/g (Port Harcourt) to 9.0 $x10^5$ cfu/g (Oyigbo), Total coliform counts range from 1.0 $x10^5$ cfu/g (Eleme) to 9.2 $x10^5$ cfu/g (Ikwerre), faecal coliform counts ranged from 1.2 $x10^4$ cfu/g (Gokana) to 7.8 $x10^4$ cfu/g for *P. mascareniensis.*

Table 1. Mean bacterial counts of the samples during the dry season

LGA	THBC (cfu/g)		SC(cfu/g)		TCC(cfu/g)		TFC(cfu/g)	
	P. mascareniensis	***P. pumilio***	***P. mascareniensis***	***P. pumilio***	***P. mascareniensis***	***P. pumilio***	***P. mascareniensis***	***P. pumilio***
Gokana	4.0×10^{7}	9.0×10^{8}	1.8×10^{5}	3.8×10^{5}	2.8×10^{5}	8.0×10^{5}	9.8×10^{4}	4.8×10^{4}
Eleme	2.7×10^{7}	6.7×10^{7}	4.4×10^{5}	9.4×10^{5}	9.7×10^{5}	8.4×10^{5}	4.6×10^{4}	4.9×10^{4}
Obio/ Akpo	4.5×10^{7}	2.5×10^{7}	6.4×10^{5}	1.4×10^{5}	9.4×10^{5}	4.0×10^{5}	3.6×10^{4}	9.0×10^{4}
Ikwerre	2.5×10^{7}	8.9×10^{7}	3.7×10^{5}	1.7×10^{5}	8.5×10^{5}	5.5×10^{5}	7.4×10^{4}	6.4×10^{4}
Port Harcourt	5.2×10^{7}	2.2×10^{7}	9.5×10^{5}	4.0×10^{5}	9.1×10^{5}	1.0×10^{5}	5.9×10^{4}	5.0×10^{4}
Oyigbo	3.7×10^{7}	3.7×10^{7}	6.7×10^{5}	7.0×10^{5}	1.6×10^{5}	6.0×10^{5}	2.0×10^{4}	5.0×10^{4}

KEY: THBC= Total heterotrophic bacterial count, SC= Staphylococcal counts, TCC = Total coliform count, FC= Feacal Coliform

Table 2. Mean bacterial counts of the samples during the wet season

LGA	THBC (cfu/g)		SC(cfu/g)		TCC(cfu/g)		TFC(cfu/g)	
	P. mascareniensis	***P. pumilio***	***P. mascareniensis***	***P. pumilio***	***P. mascareniensis***	***P. pumilio***	***P. mascareniensis***	***P. pumilio***
Gokana	4.0×10^{8}	1.0×10^{8}	6.8×10^{5}	5.8×10^{5}	2.2×10^{5}	1.9×10^{5}	1.2×10^{4}	1.4×10^{4}
Eleme	2.8×10^{8}	8.7×10^{8}	8.4×10^{5}	8.8×10^{5}	1.0×10^{5}	1.1×10^{5}	5.4×10^{4}	6.5×10^{4}
Obio/Akpo	2.9×10^{8}	2.0×10^{8}	3.8×10^{5}	4.0×10^{5}	9.8×10^{5}	8.8×10^{5}	5.8×10^{4}	6.7×10^{4}
Ikwerre	4.4×10^{8}	4.4×10^{8}	3.0×10^{5}	2.5×10^{5}	9.2×10^{5}	8.9×10^{5}	7.7×10^{4}	8.8×10^{4}
Port Harcourt	2.2×10^{8}	1.2×10^{8}	1.8×10^{5}	1.0×10^{5}	1.6×10^{5}	1.7×10^{5}	7.8×10^{4}	8.8×10^{4}
Oyigbo	2.7×10^{8}	2.7×10^{8}	9.0×10^{5}	8.0×10^{5}	2.0×10^{5}	1.8×10^{5}	3.8×10^{4}	4.8×10^{4}

KEY: THBC= Total heterotrophic bacterial counts, SC= Staphylococcal counts, TCC = Total coliform counts, FC= Feacal Coliform

Table 3. Morphological and biochemical characteristics of the bacterial isolates

	Morphology					Biochemical							Sugar fermentation						Probable organism
S/N	Colour	Size	Texture	Gram RXN	Shape	Catalase	Oxidase	Motility	Citrate	Indole	MR	VP	Glucose	Lactose	Fructose	Manitol	Sucrose	Galactose	
1	Golden yellow	small	moist	+ve	cocci	-	-	+	+	-	+	-	+	-	+	+	-	-	*Staphylococcus sp*
2	Greenish	small	moist	-ve	rod	+	+	+	-	+	+	-	-	+	-	-	+	+	*Pseudomonas spp.*
3	metallic sheen	small	moist	-ve	rod	-	+	-	+	+	-	-	+	+	+	+	-	-	*E. coli*
4	Creamy	small	moist	+ve	rod	+	-	-	+	-	+	-	+	+	-	-	+	-	*Bacillus spp*
5	light pink	large	moist	-ve	rod	-	+	-	+	+	-	-	+	+	+	+	+	-	*Klebsiella sp*
6	Creamy	small	moist	+ve	rod	+	-	-	+	-	+	-	+	+	-	-	+	-	*Bacillus spp*
7	metallic sheen	small	moist	-ve	rod	-	+	+	-	+	-	+	-	+	+	-	-	-	*E. coli*
8	light pink	large	moist	-ve	rod	-	+	-	+	+	-	-	+	+	+	+	+	-	*Klebsiella sp*
9	metallic sheen	small	moist	-ve	rod	-	+	+	-	+	-	+	-	+	+	-	-	-	*E. coli*
10	Golden yellow	small	moist	+ve	cocci	-	-	+	+	-	+	-	+	-	+	+	-	-	*Staphylococcus sp*
11	metallic sheen	small	moist	-ve	rod	-	+	-	+	+	-	-	+	+	+	+	-	-	*E. coli*
12	light pink	large	moist	-ve	rod	-	+	-	+	+	-	-	+	+	+	+	+	-	*Klebsiella sp*
13	Greenish	small	moist	-ve	rod	+	-	+	-	+	+	-	-	+	-	-	+	+	*Pseudomonas spp.*

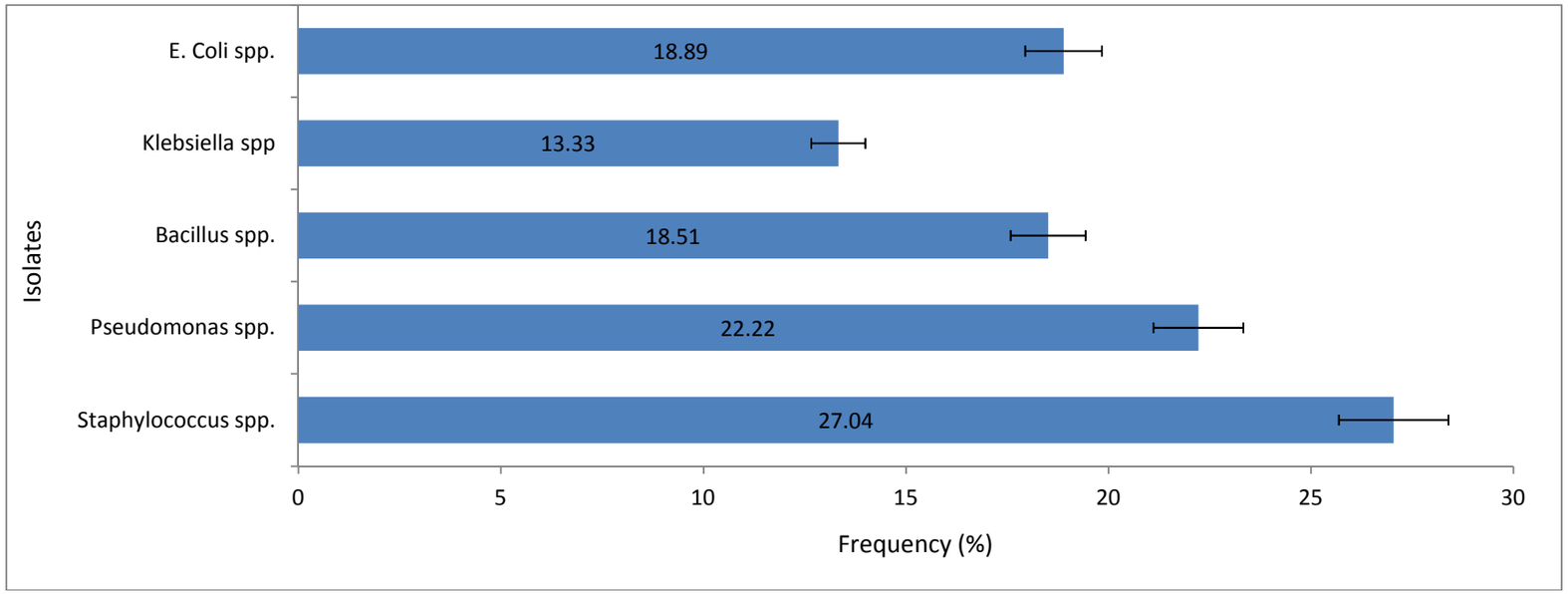

Fig. 1. Frequency of occurrence of the bacterial isolates across the sampling locations

Total heterotrophic bacterial counts ranged from 1.0 $x10^7$ cfu/g (Gokana) to 8.7 $x10^7$ cfu/g (Eleme), total Staphylococcal counts 1.0 $x10^5$ cfu/g (Port Harcourt) to 8.8 $x10^5$ cfu/g (Eleme), Total coliform count range from 1.1 $x10^5$ cfu/g (Eleme) to 8.9 $x10^5$cfu/g (Ikwerre), total faecal coliform counts ranged from 1.4 $x10^4$ cfu/g (Gokana) to 8.8 $x10^4$ cfu/g (Ikwerre and Port Harcourt) for P*. pumilio.* The results of bacteriological counts obtained in this study are similar to the counts reported by Douglas and Amuzie [6] though, in their research samples were collected from one location. A total of two hundred and fifty nine bacterial isolates belonging to the following genera; *Bacillus, Pseudomonas*, *Staphylococcus, Escherichia* coli, and *Klebsiella* were isolated in this study from the two edible species of frogs, across the six sampled locations during wet and dry seasons. The results showed that the microorganisms isolated from the frogs had a percentage rate of *27% for Staphylococcus spp, 22.22% for Pseudomonas spp, 18.51% for Bacillus, 18.89% Escherichia coli and 13.33% for Klebsiella spp.* percentage rate of the bacterial isolates from the two edible frogs across the six sampling locations (Table 3). The results revealed that, *Staphylococcus* spp. had the highest frequency of occurrence across the six selected locations, followed by *Pseudomonas* spp. while *Klebsiella spp.* had lowest frequency of occurrence (Fig. 1). The result obtained is in agreement with the report of Amadi et al. [22]. They carried out research on Microbiological flora and proximate composition of the large African cricket, and observed that *Staphylococcus* spp. had the highest frequency of occurrence. The high percentage occurrence may be due to poor handling, since this organism is a normal flora of the hand, the method of capturing the frogs and containers used in putting them.

3.1 Distribution of Bacterial Isolates in the Sampling Locations

The distribution of the bacterial isolates from the two edible frogs across the six sampling locations is shown in Fig. 1. The Figure revealed that, *Staphylococcus* spp. had the highest frequency followed by *Pseudomonas* spp. while *Klebsiella spp.* had the lowest frequency of occurrence.

Bacterial isolates obtained in this study from the two edible species of frogs were (259) two hundred and fifty nine bacterial isolates belonging to the genera *Bacillus* spp. *Pseudomonas* spp., *Staphylococci* spp*, Escherichia* coli, and *Klebsiella spp.* The distribution of the bacterial isolates from the two edible frogs across the six sampling Locations is shown in Table 3. The results revealed that, *Staphylococcus* spp. had the highest frequency of occurrence across the six selected sampled Local Government Area of Rivers State followed by *Pseudomonas* spp. while *Klebsiella spp.* had lowest frequency (Fig. 1). The result obtained is in agreement with report of food analysis [23].

The results of the proximate composition of two edible frogs tested in this study are presented in (Fig. 2). It revealed that Moisture content had the highest value followed by protein while carbohydrate had the lowest value in the two frogs. *P. manscareniensis* had high nutritional values than the *P. pumilio* species.

The nutritional values of two edible frogs were evaluated. The results of the proximate composition of the two edible frogs tested in this study are presented in Fig. 2. It revealed that moisture content had the highest value followed by protein while carbohydrate had the lowest value in the two frogs. *P. manscareniensis* had higher nutritional values than the *P. pumilio* species. The result obtained is in accordance with the report of Amadi and Kiin-Kabari [22] they research into Nutritional potentials of the larva of *Rhynchophorus phoenicis* and observe similar range of nutritional value obtained in this study. The proportion of nutritional composition obtained in this study ranged as follows: carbohydrate ranged from 1.82 to 2.24, crude fibre ranged from 2.28 to 3.00, ash ranged from 3.99 to 4.89%, lipid 11.90 to 12.32% and protein ranged from 13.74 to 14.00%, moisture ranged from 64.45 to 65.74%. The results of this study revealed that frogs harbour a lot of pathogens which could be as a result of their immediate environment. These pathogens are sometimes harmful to the frogs as well as the end consumer [24]. As the consumption of edible frogs' increases, the possibility of contracting zoonotic infections also increases. These microorganisms such as *Escherichia coli*, *Pseudomonas* spp, *Staphylococcus aureus*, *Bacillus* spp and *Klebsiella spp.* obtained from this study demonstrates that frogs can be potential sources of various infectious diseases. According to Lyhs [25] species of *Klebsiella* and *Escherichia coli*

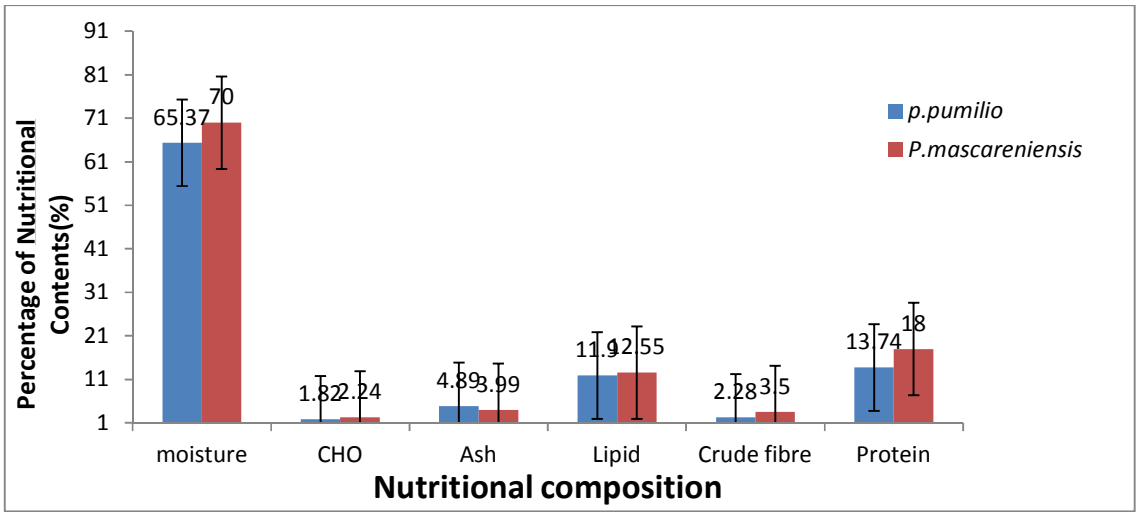

Fig. 2. Nutritional composition of the two edible frogs tested in this study

are enteric pathogens and are found in the frogs as a result of feacal contamination. These contaminations are from the immediate surroundings of the frogs, since some farmers and members of the communities around use the bush as toilet and defecation by other animals [6]. On the other hand, *Bacillus spp.* and *Staphylococcus aureus*, can be found in frogs products as a result of bacterial contamination during processing, storage or preparation for consumption. *Bacillus* sp is also a normal flora of the soil environment and has the ability to survive in the soil environment for a long time due to its ability to produce endospores [26]. *Pseudomonas* species are predominantly environmental isolates and could be part of the transient microflora in the body of the Frogs.

4. CONCLUSION

Most of the Bacteria identified in this study are known to be potential pathogens. The bacterial population in this study is higher during wet seasons than dry season when compared. The high bacteria counts could be as a result of the environmental factors associated with the habitant of the frog. There are significant differences in the bacterial counts across the sampled locations.

5. RECOMMENDATIONS

Ptychadena mascareniensis and *Ptychadena pumilio* both have high protein content therefore they could be considered as an alternative source of protein despite the bacterial load but it must be properly cooked or roasted before consumption.

CONSENT

It is not applicable.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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