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Different processing additives is efficacious on microbial loads and antibiotics sensitivity pattern of giant African land snail (*Archachatina marginata*)

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Abstract

Snails are harvested for food in many parts of the world but are susceptible to environmental contaminations and pollutants due to the continuous ingesting of bacteria from the soil and the environment which they are found. Thus, the study has been designed to investigate the distribution and prevalence of micro-organisms in snails in humid tropics of Nigeria. Fifteen samples of Archachatina marginata were collected from Melege village, Ose local government area, Ondo State, Nigeria. The foot and head were analyzed microbiologically for bacterial loads before and after processing with five cleansing reagents: lime, alum, vinegar, salt and ash. Lime proved most effective reagent for decontaminating snail meats as it had the highest reduction of microbial load after processing (57.1%), followed by salt (44%) and the least was alum (20%). The mean microbial load on the head ranged between 9-22cfu/cm^2 before and 4-12cfu/cm^2 after processing, while foot ranged between 13-19cfu/cm^2 before and 8-14cfu/cm^2 after processing. Eight isolates belonging to nine genera including Micrococcus luteus [10(21.31%)] most predominant, followed by Escherichia coli [8(13.11%)], Proteus vulgaris [8(13.11%)], Klebsiella spp [6(16.39%)], Bacillus spp [6(9.84%)], Aeromonas spp [5(11.48%)], Streptococcus pyogenes [6(8.2%)], and least was Enterobacter spp [4(6.56%)] respectively. Results showed different pathogenic bacteria in snails. The presence of higher number of pathogenic Klebsiella spp and Escherichia coli among others, encountered in Archachatina marginata is an indication of public health hazard and also a warning signal for possible occurrence of food borne. The result showed lime as the most effective reagent in processing snail meat. The antibiotic susceptibility patterns of the bacterial isolates showed that all the bacteria isolated were susceptible to Gentamicin (GEN), only Proteus vulgaris and Bacillus spp were susceptible to Ceftazidime (CAZ) and Cloxacillin (CXC) respectively and all the were resistant to Ampicillin (AMP). The presence of these microorganisms showed that snail's samples harbor some potential pathogenic bacteria of medical importance and washing with some cleansing additives, especially lime is recommended for proper decontamination. The resistance of the organisms to some of the drugs portrayed the global increased concern over the continuous use of antibiotics in human and veterinary medicine and the resultant effects.

Keywords: *Archachatina marginata*, Cleansing reagents, Snail meats, Microbial load, Public health

Introduction

Micro-livestock such as the giant land snail has been domesticated to solve the problem of protein insufficiency (National Research Council, 1991) across the producing countries of the world. In some places, instead of rearing in captivity, some snails are harvested from the wild for food as the main source of animal protein, vitamins and minerals especially in coastal communities

of Nigeria and other parts of Africa (Ebenso and Ebenso, 2011). Although being a delicacy, snails are prone to environmental contaminations and pollutants due to continuous ingesting of bacteria from the moist soil and the environment in which they are found (Ebenso and Ologhobo, 2009 and Walker *et al.*, 2000). Based on this and if not properly processed, it will lead to various diseases transmitted to the consumers. For example, molluscs have been implicated as vehicles for human infections caused by *Escherichia coli* and *Enterococcus* spp and are used as hygiene indicators (Frahm and Obst, 2003; Mani-Lopez, 2012).

In order for the snail to be safe for human consumption, additives are chemicals that are added to food to improve it in some ways However, providing a safe nutritious diet in order to maintain health remain the major acceptable objective for food processing (Obatolu, 2016). There has been little information about the microbial loads of *A. marginata* and effectiveness of cleansing agents. The use of antibiotic in livestock and human medicine across the world was based on wanton misuse and abuse of antibiotics leading to the unusually high prevalence rate of antibiotic resistance (Delepierre *et. al.*, 2012; Adelowo, 2017). Therefore, the present study was focused on investigating the distribution and prevalence of micro-organisms in land snails.

Materials and methods

Collection of samples, sterilization of glassware and media preparation

Samples of snail species of *A. marginata* were obtained from Melege village market in Owo. All glassware used were sterilized in the oven for 170°C for 2hours and subsequently allowed to cool before used. All media used were sterilized at 121°Cfor 15 minutes in the autoclave. The media used were Plate Count Agar (PCA), Blood Agar, Mueller-Hinton Agar, Pepton water, MacConkey Agar, Simmon Agar and Triple Sugar Iron Agar (TSI).All the media were prepared according to Adewole *et al.* (2013).

Sample preparation, microbial analysis for isolation and identification

Three samples of snail were used for the five different processing reagents; lime, alum, vinegar, salt solution and ashes. The snails were washed with water to remove all surface contaminants. The snails were dissected and a part of about 1cm on the foot of each snail was swab and dropped into the prepared peptone water labeled body-before (BB) corresponding to the reagent to be used in processing the snail sample. For lime reagent: L^1FB signifying lime reagent, foot and before processing for the first snail. The other snails were processed by washing with the reagents and were also swab, done just like before processing the snails for both foot and body labeled as FA and BA respectively. The procedures above were performed for different reagents that were used respectively. The PCA was used for microbial counts. This was done by using sterile syringe to withdraw 1mL of each of the inoculated peptone waters which were dispensed in each of the sterile Petri dishes. About 20mL of the PCA was then poured and plated in each of the petri dishes. The plates were rotated gently to disperse inoculums in medium and allowed to gel and incubated in the incubator at 37°C for 24 hours. The growth of the colonies on the plates were observed and counted as reported by Adewole *et al*. (2013). The standard biochemical tests were conducted to identify bacterial isolates into species according to the description in Bergey's Manual of Systematic Bacteriology (Krieg and Holt, 1984; Cheesbrough, 2003) by

comparing their morphological and biochemical characteristics with standard reference organisms.

Sensitivity test

Mueller-Hinton agar plates were aseptically prepared along with commercially prepared antibiotic disc that was used. The bacteria isolates were tested using the following antibiotic agents to determine their susceptibility. Such as: ofloxacin 10µg, nitrofurantoin 200µg, gentamicin 10µg, augmentin 30µg, ciprofloxacin µg, penicillin 10µg, chloramphenicol 10µg, tetracycline 10µg, septrin 30µg and ampicillin 30µg were determined by using agar-disc diffusion method as described by Bauer *et al.* (1996).The inoculated plates containing the antibiotics were incubated at 37ºC for 24 hours, after which the diameter of zone of inhibition around each antibiotic disc were then measured to the nearest millimeter and interpreted according to the current sensitivity test of Clinical and Laboratory Standard Institute (2008).

Results

As shown in Table 1, the result indicated a high percentage reduction from processing the snail head with lime (57.1%), followed by salt (44%), vinegar (38.5%), ash (23.1%), and the least was alum with a percentage reduction of 20%. Also for the foot, lime was also had the highest percentage reduction of 34.7%, followed by salt (30.4%), alum (18.5%), ash (16.1%), and the least was vinegar (10%)

Table 1: Total bacterial count in cfu/cm ^{2} in the sampled snails

Reagents	Sample sites	Before	After	Difference	% Reduction
Lime	Head	22	6	16	57.1
	Foot	13	8	5	23.8
Alum	Head	18	12	6	20
	Foot	19	14	5	15.2
Vinegar	Head	9	4	5	38.5
	Foot	11	9	2	10.0
Salt	Head	18	7	11	44.0
	Foot	15	8	7	30.4
Ash	Head	16	10	6	23.1
	Foot	18	13	5	16.1

A total of 8 isolates comprising of 8 different genera of both gram negative and positive bacteria were isolated in this study.The isolates with the highest percentage of occurrence was *Micrococcus luteus* with 21.31% followed closely by *Klebsiella* spp and the least was from *Enterobacter* spp. with 6.56% as shown in Table 2.

Table 2: Frequency and % of occurrence of bacterial isolates

Isolates	*Frequency of occurrence*	*% of occurrence*
Micrococcus luteus	13	21.31%
Klebsiella spp	10	16.39%
Proteus vulgaris	8	13.11%
E. coli	8	13.11%
Aeromonas spp	7	11.48%
Bacillus spp	6	9.84%
Streptococcus pyogenes	5	8.20%
Enterobacter spp	4	6.56%
Total	**61**	**100%**

The resistant pattern of the various isolates revealed that *Micrococcus luteus* had the highest resistant of (13) to CAZ, CAC and AUG respectively, while *Klebsiella* spp. followed closely with (10) to CAZ, CXC, AUG and AMP respectively and the least resistant were from *Proteus* spp., *Enterobacter* spp., *Streptococcus pyogenes* and *E. coli* with (1) to CPR, OFL and ERY respectively and *Aeromonas* spp showed no resistance to NIT as shown in Table 3.

Table 3: Antibiotic susceptibility patterns of the bacterial isolates from the snail's samples

Isolates	CAZ	CXC	GEN	CPR	OFL	AUG	NIT	AMP	CTR	ERY	CLX	COT
E. Coli	0(8)	0(8)	5(3)	7(1)	6(2)	4(4)	0(8)	0(8)	NT	NT	NT	NT
Enterobacter spp	0(4)	0(4)	2(2)	3(1)	3(1)	0(4)	2(2)	0(4)	NT	NT	NT	NT
Klebsiella spp	0 (10)	0 (10)	7(3)	8(2)	8(2)	0 (10)	6 (4)	0 (10)	NT	NT	NT	NT
Aeromonas spp	0 (7)	0 (7)	4(3)	5(2)	5(2)	4(3)	7(0)	0 (7)	NT	NT	NT	NT
Proteus spp	6 (2)	0(8)	4(4)	7(1)	7(1)	0(8)	5(3)	0(8)	NT	NT	NT	NT
Bacillus spp	0 (6)	1(5)	4(2)	NT	NT	2(4)	NT	NT	0(6)	3(3)	0(6)	2(4)
Micrococcus luteus	0 (13)	0(13)	10 (3)	NT	NT	0(13)	NT	NT	0(13)	7(6)	6(7)	9(4)
Streptococcus pyogenes	0 (5)	0(5)	3(2)	NT	NT	0(5)	NT	NT	0(5)	4(1)	0(5)	3(2)

KEY: CAZ = Ceftazidime, **CXC**= Cloxacillin, **GEN**= Gentamicin, **CPR**= Ciprothoxacin, **OFL**= Oflaxacin, **AUG**= Augmentin, **NIT**= Nitrofurantoin, **AMP**= Ampicillin, **ERY**= Erythromycin, **CLX**= Cloxacillin, **COT**= Co-Trimoxazole, **NT=** Not sensitive. Figures in brackets represent the total number of resistance and outside figures the number of susceptibility of the isolates.

Discussions

Swabs from both the foot and the body of the giant African snail, before and after processing with a washing/decontaminating agent yielded marked growth of bacteria. The presence of these organisms could be attributed to the fact that snails are voracious feeders and eat almost all kinds of foods bringing them in contact to different kinds of surfaces. The high total viable counts recorded in this study showed the microbial diversity (difference in form and species) in this snail, environmental condition and season during collection of snails and unhygienic practice employed by the local dwellers of the area where snails are been collected. These showed an agreement with the work of Eze *et al.* (2006) where a higher number of bacteria was gotten after handling by the local market women compared to when it is yet to get to the market. On comparing the bacterial contamination between the foot and body before processing both with reagents, the result showed a little higher number in that of the foot. This could be an indication of environmental/surface contamination on which the snail craws agreeing with Ekundayo and Fagade (2005) who conducted a research on the microbial flora associated with the soil of edible land snail farms, and he was able to conclude that a considerable number of bacteria are commonly associated with this habitat. The difference in the bacterial load after washing with reagents may imply the efficacy of these reagents on the bacteria and this was obvious in the percentage reduction of the bacterial load. This is obviously established by the greatest reduction from snail washed with lime. That is, the higher the percentage reduction of a reagent, the more the efficient the reagent is for washing and decontaminating snail meats. Several additives such as sodium hydroxide and organic acids have been reported to be used in decontamination of food products such as beef, pork, and poultry products (Bauermeister *et al*., 2008; Mani-Lopez *et*

al., 2012).

Microorganisms isolated in this study have been earlier found in foods, environment and other places, and their pattern is similar to previous reports by Serrano *et al*. (2004). The results obtained by both Efiuvwevwere and Ezeama (2004) and Eze *et al*. (2006) showed similar pattern of bacterial isolates which is also in accordance with the bacteria gotten in this research work. The rate of occurrence of the bacteria isolated from the snail samples showed *Micrococcus luteus* to be the most abundant, followed by *Klebsiella spp, Proteus vulgaris*, *E.coli*, *Aeromona* spp, *Bacillus* spp, *Streptococcus pyogenes,* while *Enterobacter* spp was the least predominant.

Food-borne illnesses due to consumption of snails occur when the molluscs that contain pathogenic microorganisms are consumed raw or improperly cooked. The health implications of these pathogenic microorganisms with high count in this study cannot be overemphasized. *Escherichia coli* for instance can induce gastroenteritis (Olowe *et al*., 2008). Such hazards are more appropriate in some regions where the demand for the snail meat is high and the vendors in an attempt to meet with the demand usually undercook. The result here was similar to Serrano *et al*. (2004) report, which states that the presence of mesophilic aerobic bacteria, *Enterobacteriacea*e, *S. aureus* and coliforms among ready to eat snails. The presence of *Aeromonas* could lead to gastrointestinal disease as Clarence *et al.* (2009). *Klebsiella* may cause infections of the urinary and respiratory tract in humans.

The Antibiotic susceptibility patterns of the bacterial isolates show their sensitivity to different antibiotics. All of them were susceptible to Gentamicin (GEN), only *P. vulgaris* and *Bacillus spp*are susceptible to Ceftazidime (CAZ) and Cloxacillin (CXC) respectively and all are resistant to Ampicillin (AMP). This will help in treating any possible food borne diseases that resulted in the consumption of snail by the use of new line or newly produced antibiotics. Since some of the bacteria were showing resistance. The presence of bacteria resistance to some of the tested drugs with the snail's samples is in line with the reports of Adelowo (2009); Adelowo *et al.* (2014) and Walsh and Duffy (2012) that significant transient increase in antibiotics resistance in 16 poultry farms in Oyo and Osun States and streptomycin and tetracycline resistance bacteria in apple leaves and flowers in apple orchards in Switzerland respectively. The emergence of resistance by bacteria to some of the antibiotics evaluated is an indication of declining in clinical effectiveness of these drugs in Nigeria. It also revealed the trend in the resistance of microorganisms to the use of antibiotics in livestock and aquaculture production in Nigeria.

Conclusion

Microbial loads of snail coupled with sensitivity test in this study showed that the snail samples harboured quite a number of highly pathogenic bacteria of potential public health hazard to the dependents on snail's meat as a protein source. The difference in the bacterial load after washing with reagents showed the efficacy of these reagents on the bacteria and this is obviously showed in the percent reduction of the bacterial load.

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