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Impact of Various Slime Removal Treatment and Antibiogram of Bacterial Isolates from Snails (*Achatina fulica*) Harvested in Port Harcourt Metropolis

Deidei, H. B*., Amadi, L.O. and Aleruchi, O.
Department of Microbiology, Rivers State University,
Nkpolu-Oroworukwo, Port Harcourt, Rivers State, Nigeria.
***Corresponding Author:** *deideihope9@gmail.com*

ABSTRACT

Snail meat is an alternative source of protein to those from animal origin and highly consumed worldwide. Snails absorb a variety of substances, and harbors significant microbiota which can act as a medium for the spread of infectious agents to consumers. This study evaluates the effect of different slime removal agents (Alum, Vinegar and Noni leaf extract) on microbial load and antibiogram of bacteria from snail (*Achatina fulica*) in Port Harcourt using standard microbiological methods. Results showed mean range of total heterotrophic bacterial, total coliform count, faecal coliform count and total *Salmonella/Shigella* count for the control and wild snail were; 1.45±0.35 - 10.70±2.12CFU/g, 1.55±0.35-8.70±0.85, 2.15±0.49- 11.80±1.98, and 2.50±1.13-20.40±1.69CFU/g respectively. The wild snail sample recordeed the highest microbial load for all the bacteria groups. Alum recorded the highest bacteria removal and hence lowest count with mean ranges from 1.45±0.35 to 7.50±0.71 CFU/g; Vinegar ranging from 2.55±0.49 to 11.40±1.41 CFU/g and the least being Noni leaf extract 2.55±0.21 to 11.80±1.98CFU/g. Bacteria isolated were; *Bacillus, Escherichia, Micrococcus, Pseudomonas, Klebsiella, Staphylococcus, Salmonella,* and *Shigella*. The molecular characterization of isolates showed *Pseudomonas* sp had 83.4% relatedness to *Pseudomonas aeruginosa* (EU373426), *Bacillus* sp had 90.79% to *Bacillus cereus* (ON763803), *Staphylococcus* sp had 99.16% to *Staphylococcus warneri* (MK256311), *Aeromonas* sp had 73.1% to *Aeromonas hydrophila* (OR364740) and *Shigella* sp had 96.96% to *Shigella flexneri* (OK326507). Virulence results showed that *Bacillus* sp. was 66.7% biofilm positive and 33.3% haemolysis positive, *E. coli* was 66.7% and 66.7%, *Micrococcus* was 0% and 100%, *Pseudomonas* sp was 100% and 0%, *Klebsiella,* was 0% and 50%, *Staphylococcus* sp was 100% and 66.7%, *Salmonella* and *Shigella* sp were 0% and 100% positive for biofilm and haemolysis production respectively. Nitrofurantoin, Levofloxacin, Ofloxacin and Nalidixic acid were the most potent antibiotics and *Pseudomonas* and *Salmonella* were the most resistant isolates. The organisms isolated from this study were potential pathogens and have the ability to cause disease which may result in food poisoning. It is imperative to ensure adequate care in the preparation of snails so that their consumption will not cause serious epidemic threat.

Keywords: Snail (*Achatina fulica*), slime removal, vinegar, noni, alum, haemolysis, biofilm, virulence, antibiogram.

Introduction

In addition to the typical sources of protein, which are primarily meat and fish, snails or mollusks, are good providers of both protein and mineral elements. Snail meat is a nutrient-dense diet that is rich in protein, iron, calcium, and phosphorus, low in salt, fat, and cholesterol, a good source of iron and nearly full of all the amino acids required by humans (Akinnusi, 2002). The meat is rich in important fatty acids that are good for your health, like linoleic and linolenic acids. The largest snail in Africa that is known to exist is the African giant land snail, or *Archachatina marginata* (Olawoyin and Ogogo, 2006). Snails are highly productive and fertile. They reproduce sexually even though they are hermaphrodites (Akinnusi, 2004).

According to Omole and Kehinde (2005), snails are picky about their mate and will occasionally show no interest in mating with other members of their own species that live a significant distance away. Since there is a plenty of flora for them to eat, forests, farms, and gardens are the primary habitats for snails. The savannah, home to a diverse range of African terrestrial gastropods, and the tropical forest are the two most prevalent vegetation types in Africa, according to Raut and Barker (2002).

Snails are not a highly developed industry in Africa. According to Miegoue *et al.* (2019), a significant amount of snails eaten by the population are wild snails. The people of Africa value snail flesh highly. Its demand is growing daily since it provides a significant income stream for those residing in rural

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West and Central African rainforest and savanna-derived areas. During the rainy season, they are harvested from their native habitat, which includes farms, savannahs, tropical woods, and frequently gardens with an abundance of flora for food (Ngenwi *et al.*, 2010).

Mature snails are frequently gathered in both protected and unprotected regions in order to meet the growing demands of consumers (Cobbinah *et al.*, 2008; Fagbuaro, 2015; Ndah *et al.*, 2017; Miegoue *et al.*, 2019). It is linked to deforestation and unsound farming methods (agrochemical use, slash and burn, bush fires, etc.), which may cause the population of wild snails to decline.

Snails absorb harmful substances, and have significant populations of native bacteria, coliforms, and other microorganisms (ICMSF, 2005). Pathogens can easily contaminate snail meat, which can then act as a medium for the spread of infectious agents to consumers. According to Kirkan *et al.* (2006), fresh snail samples contained *L. monocytogenes*, which may have been contaminated by soil particles.

Therefore, despite the snail's high nutritional content, research on the microbiology of the resident snail is essential due to the mollusks' role in the spread of infection, mostly as secondary hosts for pathogens. Given that eating snails that have been harvested in the wild may cause bacterial infections, systematic snail farming will help address the issue of declining snail populations and provide meat that is generally healthier and less contaminated by microorganisms (Kirkan *et al.*, 2006).

Additives are chemicals put to food to make it better in some respects, so that the food stuff may be safely consumed by humans. Nonetheless, maintaining health through a safe and nutrient-rich diet continues to be the primary goal that can be justified by food processing (Obatolu, 2016).

Information regarding the microbiological loads and efficacy of cleaning chemicals on snails is scarce. Antibiotic resistance is extremely common because of the wanton misuse and abuse of antibiotics in livestock and human treatment around the globe (Delepierre, 2012).

It is therefore necessary to determine the antibiogram of the bacteria isolated from snail as to ascertain the antibiotics for use as a first line solution in the treatment of infections acquired from the consumption of inadequately processed snails.

Materials and Methods

Study Area

The area for the study was Port Harcourt. This is the capital of Rivers State, where the demand for snail meat is high in this metropolitan city. The cultured snails were bought from Elekahia Housing Estate for with coordinates of 4.8217^0N and 7.02601^0E in Port Harcourt Local Government Area whereas the wild snails were harvested from Rumukurushi Pipeline Farm in Obio-Akpor Local Government Area with coordinate (4.8584^0N and 7.0209^0E) all in Rivers State**.**

Sample Collection and Preparation

The snail specie used for this study was *Achatina fulica* because it is one of the most preferred choices by consumers in Port Harcourt Metropolis. A total of eight (8) snails were obtained for analysis. Upon arrival at the laboratory, under sterile conditions, the snails were washed extensively with distilled water to remove contaminants present at the surface of their shells and disinfected with ethanol 70% (v/v). The snails were aseptically shucked to obtain the meat using a long sterile metal. After the removal of the intestine and stomach, the edible part (i.e. the foot) was separately washed with each of the different slime removal agents (Alum, Vinegar and Noni leaf extract) and the control were not treated with any slime removal agents. For each sample, the fleshes obtained were cut into smaller pieces to obtain 25g which was used for microbiological analysis.

Enumeration Isolation of Bacteria

The snail samples obtained were aseptically placed in 9ml sterile normal saline. They were later subjected to ten-fold serial dilution using sterile normal saline. Serial dilution was carried out until a dilution of 10^{-4} was obtained. Then aliquots of 0.1 ml of the 10^{-1}, 10^{-2} and 10^{-3} dilutions were spread plated onto the surface of sterile solid Nutrient agar, *Salmonella Shigella* Agar (SSA), Eosin Methylene Blue (EMB) and MacConkey agar. Inoculation was done in duplicates and inoculated plates were incubated at 37°C for 24-48 hours. After incubation colonies were counted and used for enumeration of bacterial load while distinct colonies (based on size, colour, texture and shapes) were subcultured onto freshly prepared nutrient agar plates. The pure cultures were preserved refrigerated in sterile nutrient agar slant. These cultures were used for identification of the bacterial isolates (Cheesbrough, 2006).

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Identification of Bacterial Isolates

The bacterial isolates were subjected to Gram staining and microscopic examination, and the following biochemical tests: catalase, oxidase, coagulase, motility, citrate utilization, indole production, Methyl Red-Vogues Proskauer (MRVP), 7% salt (NaCl) tolerance, starch hydrolysis, and fermentation tests using glucose, lactose, mannitol, and xylose tests were carried out as described by Peekate (2022). Results obtained from the tests were keyed into the search dialogue of the online bio-database software "Advanced Bacterial Identification Software (ABIS)" available at https://www.tgw1916.net/bacteria_logare.html, to reveal the possible identity of the isolates.

Statistical Analysis

Statistical analysis of the antibiotic resistance data were expressed as percentages or frequency of the isolates. A two-way analysis of variance (ANOVA) without replication was used to determine the significant differences in the levels of resistance prevalence among the selected antibiotics between the isolates. A P-value of <0.05 was be considered at 95% level of significance to ascertain significant differences in the data that was obtained during the study. Where there is significant difference, Duncan Multiple Range Test (DMRT) was used to separate the means (Bewick *et al.*, 2004).

Results

Results of the mean microbial load of Snails from both sources using slime removal singly are presented in Table 1. Results before the treatment with slime removal showed the control for cultured snail ranged from 3.95±0.92-6.55±0.07 and the wild snail ranged from 8.70±0.85-20.40±1.69.

The treatment results showed that the mean range of the total heterotrophic bacterial, total coliform count, faecal coliform count, and total *Salmonella Shigella* count was; 1.45±0.35 - 10.70±2.12, 1.55±0.35-8.70±0.85, 2.15±0.49- 11.80±1.98, and 2.50±1.13-20.40±1.69 $\log_{10}$CFU/g, respectively.

Results of the mean microbial load of Snails from both sources with the combination of the different slime removal are presented in Table 2. Results showed that the mean range of the total heterotrophic bacterial, total coliform count, faecal coliform count, total *Salmonella/Shigella count* was; 1.9±0.2-27.9±0.5, 1.4±0.3- 7.6±0.7, 1.4±0.4-8.0±1.4 and 1.2±0.1-6.1±0.9 $\log_{10}$CFU/g, respectively. There were a highly significant difference ($p \leq 0.000$) in the total mean count for total heterotrophic bacteria, a highly significant difference ($p \leq 0.023$) in total coliform count, a highly significant difference ($p \leq 0.009$) in the Faecal coliform count and also a highly significant difference ($p \leq 0.002$) in the total *Salmonella Shigella* count.

Results of the prevalence of bacterial isolates across the samples are presented in Fig. 1. Results showed that *Bacillus* spp. were isolated from control wild (CNT-W), wild alum (W-AL), wild vinegar (W-V), wild noni (W-N), wild vinegar (W-V), wild noni (W-N), and control cultured (CNT-C), *E. coli*, control cultured (CNT-C), Cultured alum (C-AL), Cultured noni(C-N), and all samples, *Micrococcus* was isolated from control Cultured (CNT-C), C- Cultured alum (AL), and Cultured noni (C-N), *Pseudomonas*, control wild (CNT-W) and control cultured (CNT-C), *Klebsiella* sp. was isolated from Cultured vinegar (C-V), cultured noni (C-N), and all samples but cultured alum (C-AL), *Staphylococcus* spp, all but wild alum (W-AL), *Salmonella* sp. and *Shigella* sp., were isolated from all samples.

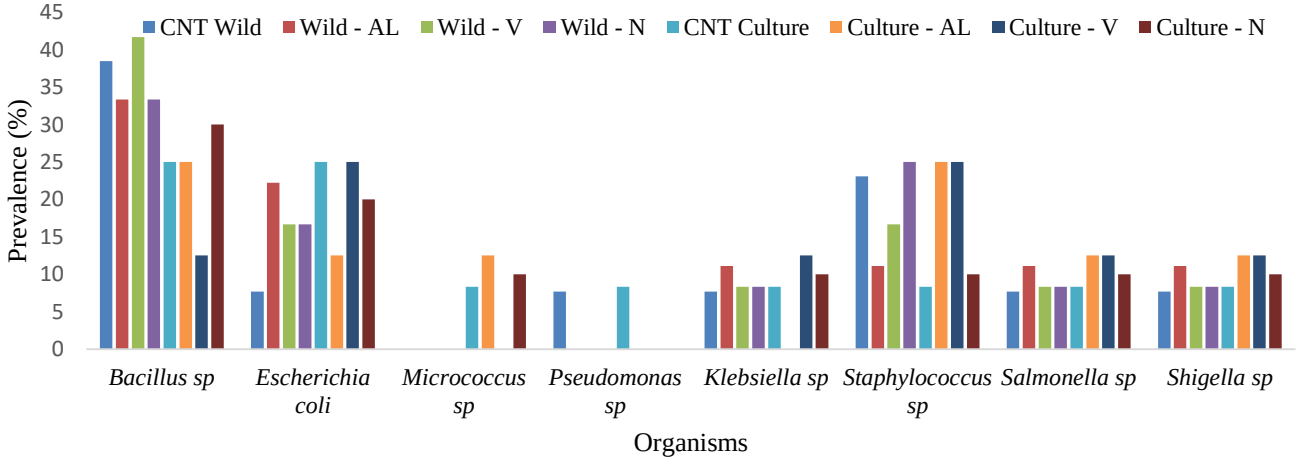

Fig. 1: Prevalence of the Bacteria in the Various Cultured Snail Samples

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Table 1: Mean Microbial Counts of Snails Subjected to Different Slime Removal Agents (Treatments)

Microbial group	Snail slime removing agent/Type of Snail/Microbial count								P value
	Control		Alum		Noni		Vinegar		
	Cultured	Wild	Cultured	Wild	Cultured	Wild	Cultured	Wild	
Total heterotrophic bacteria ($\times10^7$CFU/g)	3.95±0.92^{ab}	10.70±2.12^{ab}	1.45±0.35^{a}	2.85±1.06^{ab}	4.00±1.84^{ab}	6.75±1.91^{b}	2.70±1.98^{ab}	6.15±2.05^{b}	0.009
Total coliform ($\times10^5$ CFU/g)	5.95±0.21^{c}	8.70±0.85^{d}	1.90±0.28^{ab}	1.55±0.35^{ab}	2.55±0.21^{ab}	2.60±0.00^{b}	2.55±0.49^{ab}	6.05±0.35^{c}	0.000
Faecal coliform ($\times10^3$ CFU/g)	6.40±5.09^{abc}	10.00±1.13^{bc}	5.00±2.83^{ab}	2.15±0.49^{a}	2.65±0.21^{a}	11.80±1.98^{c}	7.80±2.55^{abc}	11.40±1.41^{c}	0.022
Total *Salmonella/ Shigella* ($\times10^3$ CFU/g)	6.55±0.07^{ab}	20.40±1.69^{f}	7.50±0.71^{cd}	2.50±1.13^{a}	4.15±0.21^{ab}	9.00±0.28^{de}	4.40±1.41^{ab}	10.00±1.13^{e}	0.000

*Means with similar superscript down the group showed no significant difference (p≤0.05)

Table 2: Mean Microbial Counts of Snails with Consortia of Slime Removal Agents

Microbial group	Snail slime removing agent/Type of Snail/Microbial count								P value
	Alum+Noni		Alum+Vinegar		Vinegar+Noni		Alum+Noni+Vinegar		
	Cultured	Wild	Cultured	Wild	Cultured	Wild	Cultured	Wild	
Total heterotrophic bacteria ($\times10^7$CFU/g)	2.1±0.9^{a}	27.9±0.5^{c}	1.9±0.2^{a}	13.8±0.3^{b}	2.7±0.1^{a}	14.0±1.7^{b}	4.6±3.9^{a}	1.9±1.2^{a}	0.000
Total coliform ($\times10^5$ CFU/g)	7.6±0.7^{c}	1.4±0.3^{a}	1.9±1.2^{a}	2.5±1.1^{ab}	5.5±2.1^{bc}	3.9±0.2^{ab}	3.5±2.1^{ab}	1.5±0.7^{a}	0.023
Faecal coliform ($\times10^3$ CFU/g)	2.6±0.4^{ab}	7.0±1.4^{a}	1.7±0.6^{ab}	8.0±1.4^{c}	7.0±1.4^{c}	1.4±0.4^{a}	5.0±2.8^{bc}	5.0±1.4^{bc}	0.009
Total *Salmonella/ Shigella* ($\times10^3$ CFU/g)	3.8±0.8^{bc}	1.2±0.1^{a}	4.9±0.6^{bcd}	3.0±1.4^{ab}	5.7±0.4^{cd}	6.1±0.9^{d}	8.3±0.9^{e}	5.0±1.4^{bcd}	0.002

*Means with similar superscript down the group showed no significant difference (p≤0.05)

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The percentage frequency of biofilm and haemolysis production ability of the identified bacterial isolates is presented in Table 3. Results revealed that 66.7% of *Bacillus* sp. was positive for biofilm production and 33.3% were positive for haemolysis, 66.7% of *E. coli* was positive for biofilm production and 66.7% were positive for haemolysis, *Micrococcus* had no biofilm production but 100% for haemolysis. *Pseudomonas* sp was 100%positive for biofilm production and 0% were positive for haemolysis, *Klebsiella*, was 0% positive for biofilm production and 50% were positive for haemolysis, *Staphylococcus* sp was 100%positive for biofilm production and 66.7% were positive for haemolysis, *Salmonella* and *Shigella* sp were 0% positive for biofilm production and 100% were positive for haemolysis.

Table 3: Percentage Frequency of Biofilm Production

Isolates	Biofilm	Haemolysis
Bacillus sp. (9)	6(66.7%)	3(33.3%)
E. coli (3)	2(66.7%)	2(66.7)
Micrococcus sp. (1)	0(0)	1(100%)
Pseudomonas sp. (1)	1(100)	0(0)
Klebsiella sp. (2)	0(0)	1(50%)
Staphylococcus sp. (3)	3(100)	2(66.7%)
Salmonella sp. (1)	0(0)	1(100)
Shigella sp. (1)	0(0)	1(100)

Keys: % = percentage

Results of the antibiotics susceptibility pattern of isolates are presented in Table 4. Results showed that *Pseudomonas* was (100%) resistant to Imipenem Cilastatin, Cefotaxime, Cefuroxime, Nalidixic acid, Cefexime, Gentamicin and *Pseudomonas* were 100% susceptible to Ampiclox, Ceftriaxome sulbactram, Levofloxacin, Nitrofuranterin, Ofloxacin, Amoxicillin clavulavate,

The result of antibiotic pattern of *Salmonella* sp revealed that greater number *Pseudomonas* sp showed 100% resistance to Imipenem Cilastatin, Cefotaxime, Ampiclox, Cefuroxime, Cefexime, Ofloxacin, Amoxicillin clavulavate, Gentamicin. *Pseudomonas* sp showed 100% susceptible to Nalidixic acid, Levofloxacin, Nitrofuranterin.

The result of antibiotic pattern of of *Klebsiella* revealed that *Klebsiella* sp showed 100% resistance to Imipenem Cilastatin, Cefuroxime and 50% to Cefotaxime, Ampiclox, Ceftriaxome sulbactram, Nalidixic acid, Cefexime and Cefotaxime. *Klebsiella* sp showed 100% susceptible to Levofloxacin, Nitrofuranterin, Ofloxacin and 50% to Ceftriaxome sulbactram, Cefotaxime and Gentamicin.

Results of the antibiotics susceptibility pattern of *Bacillus* sp showed that the isolates were 100% resistant to Ampiclox, Cefuroxime, Ceftriaxome sulbactram, Cefexime and Gentamicin was 66.7, 33.3, 66.7 and 33.3 while all the isolates were 100% susceptible to Nalidixic acid, Levofloxacin, Nitrofuranterin, Ofloxacin and gentamicin.

The result of antibiotic pattern of of *Staphylococcus* sp is presented in Table 8 revealed that *Staphylococcus* sp showed 66.7% resistance to Imipenem Cilastatin, Cefotaxime and Amoxicillin clavulavate, Ampiclox, Nitrofuranterin and Cefexime. *Staphylococcus* sp showed 100% susceptible to Ofloxacin and Gentamicin and Levofloxacin 66.7%.

The result of antibiotic pattern of *Micrococcus* sp revealed that *Micrococcus* sp showed 50% resistance to Imipenem Cilastatin, Cefotaxime, Ampiclox, Nitrofuranterin. *Micrococcus* sp showed 100% Cefuroxime, Ceftriaxome sulbactram, Nalidixic acid, Levofloxacin, Ofloxacin, Gentamicin and 50% Imipenem Cilastatin, Cefotaxime, Cefexime, Amoxicillin clavulavate

Results of the multiple antibiotics resistant indices of the bacterial isolates are presented in Table 5. Results showed that the isolates exhibited multi drug resistance with MAR index greater than 0.2.

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Table 4: Antibiogram Profile of Bacterial Isolates

Antibiotics/ Conc.	*Pseudomonas* sp			*Salmonella* sp			*Klebsiella* sp			*Bacillus* sp			*Staphylococcus* sp			*Micrococcus* sp		
	R n (%)	I n (%)	S n (%)	R n (%)	I n (%)	S n (%)	R n (%)	I n (%)	S n (%)	R n (%)	I n (%)	S n (%)	R n (%)	I n (%)	S n (%)	R n (%)	I n (%)	S n (%)
Imipenem Cilastatin (IMP) (10µg)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	1(11.1)	0(0.00)	8(88.9)	3(100)	0(0.00)	0(0.00)	1(50)	0(0.00)	1(50)
Cefotaxime (CTX) (25µg)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(50)	0(0.00)	1(50)	4(44.4)	2(33.3)	3(22.2)	3(100)	0(0.00)	0(0.00)	1(50)	0(0.00)	1(50)
Ampiclox (ACX) (10µg)	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)	1(50)	0(0.00)	1(50)	2(33.3)	3(22.2)	4(44.4)	2(66.7))	0(0.00)	1(33.3)	1(50)	0(0.00)	1(50)
Cefuroxime (CXM) (30µg)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	0(0.00)	3(33.3)	6(66.7)	1(33.3)	0(0.00)	2(66.7))	0(0.00)	0(0.00)	2(100)
Ceftriaxome sulbactram (CRO) (45µg)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	1(50)	0(0.00)	1(50)	0(0.00)	1(11.1)	8(88.9)	1(33.3)	0(0.00)	2(66.7))	0(0.00)	0(0.00)	2(100)
Nalidixic acid (NA) (30µg)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)	1(50)	0(0.00)	1(50)	0(0.00)	1(11.1)	8(88.9)	1(33.3)	1(33.3)	1(33.3)	0(0.00)	0(0.00)	2(100)
Levofloxacin (LBC) (5µg)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	1(11.1)	8(88.9)	0(0.00)	1(33.3)	2(66.7))	0(0.00)	0(0.00)	2(100)
Nitrofurantoin (NF) (30µg)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	2(100)	1(11.1)	2(22.2)	6(66.7)	2(66.7))	0(0.00)	1(33.3)	1(50)	0(0.00)	1(50)
Cefexime (ZEM) (5µg)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	1(50)	0(0.00)	1(50)	0(0.00)	2(22.2)	7(77.8)	2(66.7))	0(0.00)	1(33.3)	0(0.00)	0(0.00)	2(100)
Ofloxacin (OFX) (5µg)	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	9(100)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	2(100)
Amoxicillin clavulavate (AUG) (30µg)	0(0.00)	0(0.00)	1(100)	1(100)	0(0.00)	0(0.00)	2(100)	0(0.00)	0(0.00)	2(22.2)	0(0.00)	7(77.8)	3(100)	0(0.00)	0(0.00)	0(0.00)	1(50)	1(50)
Gentamicin (GN) (10µg)	1(100)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	1(50)	1(50)	0(0.00)	2(22.2)	7(77.8)	0(0.00)	0(0.00)	3(100)	0(0.00)	0(0.00)	2(100)

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Table 5: Multiple Antibiotic Resistance (MAR) Index of the Bacterial Isolates

Organism	MAR Index								
	0.00	**0.1**	**0.2**	**0.3**	**0.4**	**0.5**	**0.6**	**0.7**	**0.8**
E. coli	0(0.00)	0(0.00)	0(0.00)	2(66.7)	0(0.00)	1(33.3)	0(0.00)	0(0.00)	0(0.00)
Pseudomonas	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Salmonella	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(100)	0(0.00)
Klebsiella	0(0.00)	0(0.00)	0(0.00)	1(50)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(50)
Bacillus	4(44.44)	2(22.22)	2(22.22)	1(11.11)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Staphylococcus	0(0.00)	0(0.00)	0(0.00)	1(33.33)	1(33.33)	0(0.00)	0(0.00)	0(0.00)	1(33.33)
Micrococcus	0(0.00)	1(50)	0(0.00)	1(50)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)

Discussion

Snail meat is highly consumed worldwide and the report of the Food and Agriculture Organization revealed that in 2017, 18,331 tons of snail meats were consumed in the world (FAO, 2019). This present study has revealed the microbial load and antibiogram of snail meat treated with various slime removal agents. The microbial counts from both snail sources wild and cultured; showed that total heterotrophic bacterial had the highest counts followed by total coliform, faecal coliform and total *Salmonella/Shigella* count being the least. The findings showed a very high bacterial load. The microbial load of the wild snail sample was higher than the microbial loads of the cultured snail sample in the present study, when compared with the study of Adegoke *et al.* (2010), who had a lower microbial load ranging from 6.0 - 2.00 x 10^7 CFU/g. The high microbial load from this study could be attributed to environment factors and geographical location. There were high significant differences from various counts at ($P<0.05$).

The results for mean microbial counts using different slime removal agents for both wild and cultured snail, showed that the consortia of Alum, Noni and Vinegar (AL+N+V) had the highest bacterial removal and hence the lowest counts with mean ranges 1.5±0.7 to 8.3±0.9, Alum and Vinegar (AL+V) 1.7±0.6 to 13.8±0.3, vinegar and Noni (V+N) 1.5±0.4 to 14.0±1.7 and the least (AL+N) 1.4±0.3 to 27.9±0.5.

The high removal of microbial load using alum could be attributed to the potency of alum as an antimicrobial agent which had been visibly demonstrated over the years through the myriads of its beneficial activities. Undiluted white distilled vinegar has a strong effect against *Salmonella spp.* and *P. aeruginosa* at an exposure time of 30 s, but does not work well against *S. aureus* and *Escherichia coli* (Rutala *et al.*, 2013). Vinegar is mainly comprised of acetic acid, a weak organic acid, for which an antimicrobial effect is mainly delivered by its undissociated form, by passive diffusion through the cell wall of the bacteria. The resulting change of the internal pH is believed to have an inhibitory effect on the bacteria by releasing protons (Ricke, 2003). Acetic acid has already been used in the food industry to inhibit food pathogens. Various studies have shown a protective effect of acetic acid on various types of meat (Mani-López *et al.*, 2012).

The bacterial isolates linked to eight genera were identified to be *Bacillus, Escherichia, Micrococcus, Pseudomonas, Klebsiella, Staphylococcus, Salmonella,* and *Shigella* spp. The study revealed that the snails are home to a large variety of microorganisms. Numerous of these organisms have been identified as potential pathogens, meaning that consuming infected snail raw or incorrectly cooked could lead to food-borne diseases (Adegoke *et al.*, 2010).

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These bacteria were identified in studies conducted by Nwuzo *et al.* (2016). Similar isolates have also been found in *A. achatina* by Adagbada *et al*. (2011), who linked the presence of *Pseudomonas* and other soil organisms to the close association of snails with soil. Ebenso *et al.* (2012) isolated *Salmonella* sp, *Vibrio* sp, and *E. coli* from edible land snails. Efuntoye *et al*. (2011) and Cardoso *et al.* (2012) reported that Staphylococcus species resides in the gastro-intestinal tract of snails. The presence of *E. coli* may be attributed to faecal contamination. This is contrary to the study by Parlapani *et al.* (2014), which isolated *Salmonella* sp. in snails, indicating a contact with faecal matter. The snail may be a potential carrier of Escherichia sp. since gastropods find mammalian faeces an alluring source of nutrients (Speicer, 2001). One possible explanation for the existence of these organisms, like *Bacillus* sp., is that snails consume decomposing plant matter, which harbors and promotes the growth of microbes.

Bacillus had the highest number of prevalence followed by *Escherichia* and *Staphylococcus, Salmonella, Shigella* and the least prevalence was *pseudomonas* sp. this was in contrast with the study of Nwuzo *et al.* (2016) who *had Escherichia coli* to be the highest prevalence frequency followed by *Salmonella, Shigella, Pseudomonas and Enterobacter* being the least.

The prevalence of *Salmonella, Pseudomonas, Shigella, Enterobacter, Escherichia coli,* and *Klebsiella* in snails can be extremely dangerous to public health if eaten raw or undercooked. Adagbada *et al.* (2011) found that *Achatina achatina* was the source of *Escherichia coli, Enterobacter* species, *Pseudomonas* species, *Klebsiella* species, *Shigella* species, and *Aeromonas* spp. in four markets located in the Nigerian states of Cross River and Akwa Ibom. These findings are consistent with the present study.

Drug-resistant microorganisms are becoming a major health concern in developing countries. Some authors have presented evidence suggesting that regular use of antibiotics has not always resulted in bacterial isolates developing greater resistance (Kumar and Schweizer, 2005). Though they produce numerous types of resistance, Sharada and Ruban, (2010) state that the levels of bacterial resistance fluctuate with different isolates and with time. Depending on the isolate, the bacterial isolates' antibacterial activity differed.

Results of the antibiotics susceptibility pattern of *Bacillus* sp. Results showed that the isolates were 100% resistant to Ampiclox, Cefuroxime, Ceftriaxome sulbactram, Cefexime and Gentamicin was 66.7, 33.3, 66.7 and 33.3 while all the isolates were 100% susceptible to Nalidic acid, Levofloxacin, Nitrofuranterin, Ofloxacin and gentamicin.
Results of the antibiotics susceptibility pattern of isolates of *Pseudomonas* showed that *Pseudomonas* was (100%) resistant to Imipenem Cilastatin, Cefotaxime, Cefuroxime, Nalidic acid, Cefexime, Gentamicin and *Pseudomonas* were 100% susceptible to Ampiclox, Ceftriaxome sulbactram, Levofloxacin, Nitrofuranterin, Ofloxacin, Amoxicillin clavulavate,

The result of antibiotic pattern of of *Salmonella* sp revealed that greater number *Pseudomonas* sp showed 100% resistance to Imipenem Cilastatin, Cefotaxime, Ampiclox, Cefuroxime, Cefexime, Ofloxacin, Amoxicillin clavulavate, Gentamicin. *Pseudomonas* sp showed 100% susceptible to Nalidic acid, Levofloxacin, Nitrofuranterin.

The result of antibiotic pattern of of *Klebsiella* sp revealed that *Klebsiella* sp showed 100% resistance to Imipenem Cilastatin, Cefuroxime and 50% to Cefotaxime, Ampiclox, Ceftriaxome sulbactram, Nalidic acid, Cefexime and Cefotaxime. *Klebsiella* sp showed 100% susceptible to Levofloxacin, Nitrofuranterin, Ofloxacin and 50% to Ceftriaxome sulbactram, Cefotaxime and Gentamicin.

The result of antibiotic pattern of of *Staphylococcus* sp revealed that *Staphylococcus* sp showed 66.7% resistance to Imipenem Cilastatin, Cefotaxime and Amoxicillin clavulavate, Ampiclox, Nitrofuranterin and Cefexime. *Staphylococcus* sp showed 100% susceptible to Ofloxacin and Gentamicin and Levofloxacin 66.7%.

The result of antibiotic pattern of of *Micrococcus* sp revealed that *Micrococcus* sp showed 50% resistance to Imipenem Cilastatin, Cefotaxime, Ampiclox, Nitrofuranterin. *Micrococcus* sp showed 100% Cefuroxime, Ceftriaxome sulbactram, Nalidic acid, Levofloxacin, Ofloxacin, Gentamicin and 50% Imipenem Cilastatin, Cefotaxime, Cefexime, Amoxicillin clavulavate

According to Lou *et al.* (2011), food has a significant role in the effective transmission of antimicrobial resistance (AMR) factor to customer digestive tracts.

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AMR could therefore be transmitted to consumers, which makes the existence of these resistant isolates in the current investigation potentially problematic. With MAR values greater than 0.2, the bacterial isolates further demonstrated multidrug resistance. A threat to public health, multiple drug resistance is the term used to describe the antimicrobial resistance that certain microbes exhibit to numerous antimicrobial medicines (Magiorakos, 2014). Bacterial isolates may become more resistant to specific antibiotics if they are exposed to them on a regular basis (Obire *et al*., 2009). Additionally, bacteria that alter the antibiotics' target sites may develop resistance to medicines to which they were previously susceptible. If the antibiotics' target site is altered, it's possible that the bacteria won't respond to them in any recognized way. Other tactics that might result in a rise in antibiotic resistance include the production of enzymes that inactivate antibiotics, including beta lactamase, which breaks down the active component of antibiotics (Hamad, 2010).

In conclusion, this study examined Impact of Various Slime Removal Treatments and Antibiogram of Bacterial Isolates from Snails (*Achatina fulica*). According to the study it was revealed that, snails (*Achatina fulica*) harbored highly pathogenic bacteria of potential public health threat especially to consumers in areas where the demand for the snail meat is high. Results showed that the bacterial isolates belonged to eight genera: *Bacillus, Escherichia, Micrococcus, Pseudomonas, Klebsiella, Staphylococcus, Salmonella,* and *Shigella* spp. were identified from the different samples (wild and cultured).

The result for the various slime removal showed that the consortia of alum, noni and vinegar (AL+N+V) had the least microbial load and hence was the best in terms of reducing microbial loads on the snail sample. The antibiogram analysis showed varying degrees of antibiotic resistance and a high multi-drug resistance among the bacterial isolates. The Antibiogram also showed that Nitrofuranterin, Levofloxacin, Ofloxacin and Nalidic acid were the most potent antibiotics.

Emphasis should be placed on strategies to reduce the contamination of edible snails, especially where environmental conditions favour the abundance of many pathogens. Such strategies include; cooking snail meat properly before consumption, proper washing of the snail meat with hot water.

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