Studies on the isolation of enteropathogens associated with the intestines of Giant African land snails (*Achatina* and *Archachatina)* species sold in Gwagwalada, FCT, Abuja – Nigeria

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Abstract: Studies on the entero-pathogens associated with the intestines of Giant Africa land snail (*Achatina* and *Archachatina* species) was carried out. A total of 180 snail samples of three different species of Giant Africa Land Snail (GALS) (*Achatina achatina* (Giant Ghana tiger snail), *Achatina fulica* (East Africa land snail or garden snail) *and Archachatina marginata* (West Africa snail or big Black snail)) were obtained from Gwagwalada market , FCT Abuja were used. The snails were deshelled, homogenized in 10ml of distilled water and cultured on Nutrient agar, Mac Conkey agar, Salmonella-Shigella agar, and Eosine methylene blue (EMB) agar using spread plate method and incubated for 24 hours at 37^0C. The growth obtained on Mac Conkey agar was sub cultured on EMB to confirm *Escherichia coli* and biochemical tests on the isolates were carried out. Forty-five bacteria were isolated. They include *Salmonella* species and *Shigella* species both gave 3(6.6%) each. *Aeromonas* spp, *Vibrio* spp and *Pseudomonas* species gave 2(4.4%) each. *Enterobacter* and *Klebsiella* species are both 3(6.6%), *Staphylococcus aureus* gave 4(8.8%), *Proteus* spp 7(15.5%), *Escherichia coli* 8(17.7%) and *Yersinia* species 1(2.2%). Some of the bacterial isolates show resistance to most antibiotics tested and as such displayed multidrug resistance. Most of the bacteria isolated in this study have been shown to be involved in gastro-intestinal infections therefore, there is need for proper processing of snails before consumption.

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1. Introduction

Snail is a common name which is applied to most of the members of the molluscan class Gastropoda that have coiled shells in the adult stage. When the word is used in its most general sense, it includes sea snails, land snails and freshwater snails. The word snail without any qualifier is however more often applied to land snails than to those from the sea or freshwater. Snail-like animals that naturally lack a shell, or have only an internal shell, are often called slugs, and land species that have only a very small shell (that they cannot retract into) are called semi slugs (Fredericks, 2010).

The Giant African Land Snail or the " Giant Ghana Tiger snail"(*Achatina achatina*), the "Garden" or "foolish Snail" (*Achatina fulica*), and Margies or West Africa snail or "big Black snail" (*Archachatina marginata*) are large terrestrial snails that can reach up to 20cm (8 inches) in length and 10cm (4 inches) in maximum diameter. These snails are about the size of an average-sized adult fist. The brownish shell with darker brown vertical stripes covers at least half of the length of the snail (Ohlweiler *et al*., 2010).

The smaller of these snails (*Achatina fulica*) grow to be about 3-4 inches long (shell length), while the larger ones (*Achatina achatina*) can have a shell up to 10-11 inches long (in a snail this size the body stretched out would be about 14-15 inches). The average life span is about 5-6 years, but can live up to 10 years.

Land snail habitat ranges from the dense tropical high forest in southern Nigeria to the fringing riparian forests of the derived Guinea Savannah (Ajayi *et al*., 2009; Odaibo, 2007). From November to March each year, Nigerian snails aestivate because of the hot dry weather.

The two prominent snails species found abundantly in this part of the world are the edible giant land snails: *Achatina achatina* and *Archatina marginata* (Ajayi *et al*., 2009). They are found extensively in the Southern parts of Nigeria and the entire West African coastal area, central and South Africa, where the weather is most favourable for their proliferation (Herbert and Kilburn, 2004).

Snails are cold-blooded animals and therefore sensitive to changes in atmospheric humidity and temperature. When rainfalls the epiphragm breaks and very cold water stored inside before aestivation pours out of the aperture (Ajayi *et al*.,2009), and the snails emerge to eat the new plant growth and the soft soil (Ajayi *et al*.,2009; Odaibo, 2007). Snails thrive best on temperature of about 10-23°C (Albequerque *et al*., 2009). Thus, it is important to note that the organism can cause infections to man when the snail meat is not properly cooked and when the processing is not done under sanitized condition (Fagbuaro *et al*., 2006).

2.0 Materials and Methods

2.1. Collection of Samples

The GALS used for this study were purchased from Gwagwalada market, FCT, Abuja Nigeria and put in sterile plastic containers and conveyed to the laboratory for microbial analysis.

2.2 Procedure

One hundred and eighty snail samples were processed for this study. The outer shells of the snails were washed in running tap water using a nail brush, and rinsed in several changes of distilled water. The shells were then disinfected using cotton wool moistened in 70% alcohol.

After wards, the snails were then de-shelled under an aseptic condition. The foot were separated from the mouthparts, the intestinal portions were removed using a dissecting kit, and the fluid squeezed into sterile test tubes. The fluid was homogenised with 10ml of sterile water and inoculated on the already prepared Mac Conkey agar, Nutrient Agar, Salmonella-Shigella Agar and EMB agar Petri dishes using the streak plate method according to Barrow (1993). The plates were incubated for 24 hours at 37^0C. The bacterial colonies in the plates were sub cultured to obtain pure cultures of the bacterial isolates.

2.3 Sterilization and Preparation of Media

The Nutrient agar media used in this work was obtained from Himedia Laboratories pvt. Ltd. 23, Vadhani Ind. Est., LBS Marg, Mumbai-400086, India, the Salmonella-Shigella agar from Micromaster Thane (W) 400607, Maharashtra, India, Eosin Methylene Blue agar (Levine) from Oxoid Ltd. Basingstoke Hampshire England, Mac Conkey agar from Micromaster Thane (W) 400607, Maharashtra, India in the powdered form and reconstituted with distilled water according to the Manufacturers' instructions. They were sterilized by autoclaving at 121°C and 15p.s.i for 15 minutes and cooled to 45°C before dispensing into sterile Petri-dishes and left to gel.

Glassware including test tubes, conical flask, measuring cylinders, pipettes were sterilized by autoclaving at 121°C for 15 minutes.

2.4 Isolation of Pure Cultures

The bacterial colonies observed on the Petri dishes were sub cultured to obtain pure cultures. The pure cultures were put in agar slants and stored in the refrigerator at 4^0C until required for analyses.

2.5 Identification of Bacterial Isolates and Antibiotics Susceptibility Testing

The bacterial isolates identification was based on colony morphology, cultural characteristics and biochemical tests using the description of Doyle (2008) and Patel (2008), Harrigan and Mc Cance (2003) and Schaad (2005) confirmed according to Buchanan and Gibbons (2000). Antimicrobial susceptibility tests were carried out using the commercial antibiotics multidisc.

3.0 Results

The distribution of the bacterial isolates from the intestines of the three species of GAL snails is as presented below in table 1.

Table 1: Distribution of bacterial isolates from the intestine of the three species of GAL snail

Bacterial Isolates	**Species of Giant African Land snail**		
	A. Achatina	***A. Fulica,***	***A. marginata***
Aeromonas spp	1	1	0
Enterobacter spp	1	2	0
Escherichia coli	2	5	1
Klebsiella spp	0	2	1
Proteus mirabilis	2	4	1
Proteus vulgaris	2	4	1
Pseudomonas spp	0	2	0
Salmonella spp	1	1	1
Shigella spp	1	1	1
Staphylococcus aureus	1	2	1
*Vibr*o spp	0	1	1
Yersinia spp	0	0	1
X	**11 (24.4%)**	**25 (55.6%)**	**9 (20%)**

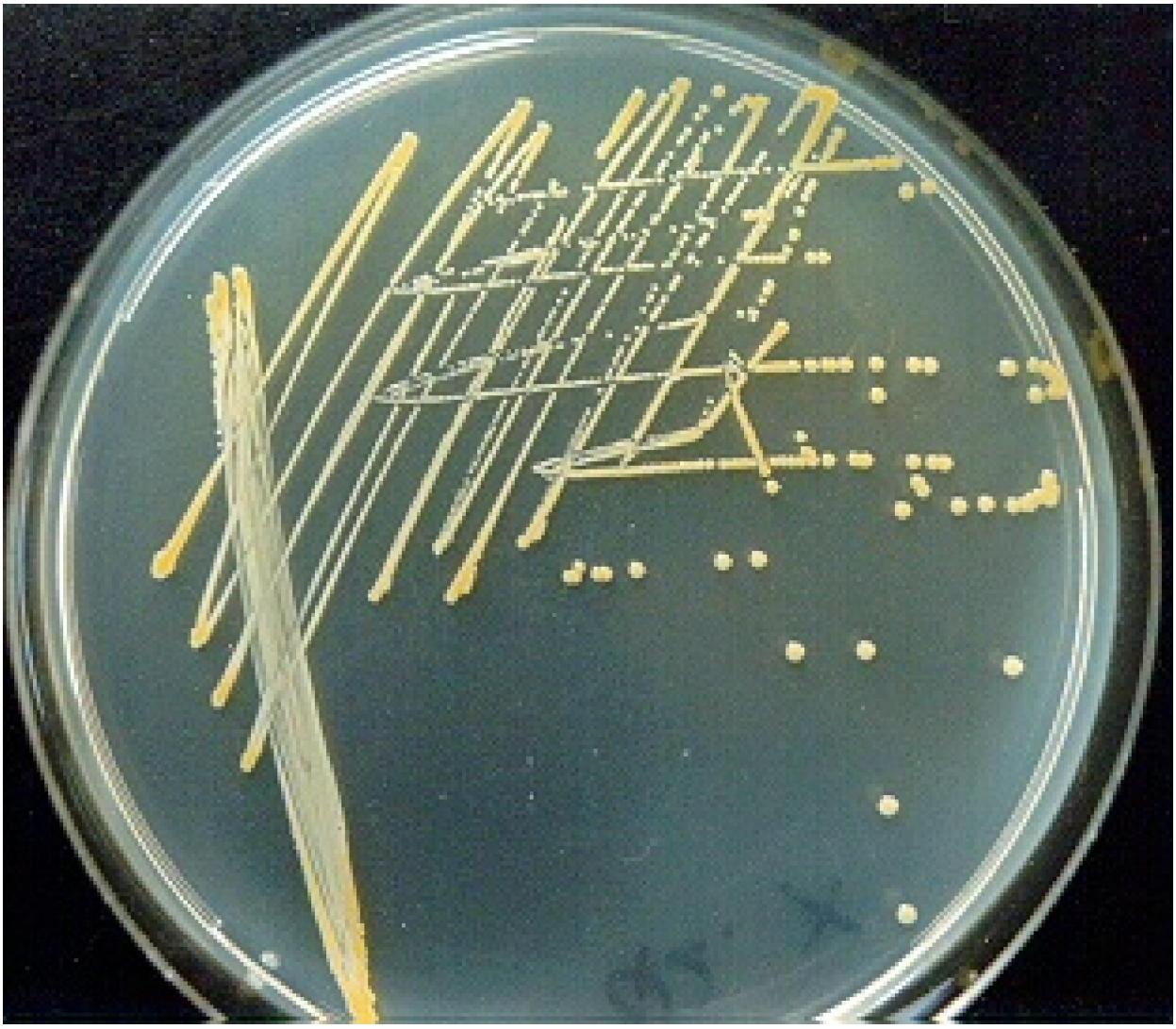

Plate 1: *Staphylococcus aureus* on Nutrient agar plate

Table 2: Frequency of isolation of bacteria from snail species (*Achatina achatina*, *Achatina fulica and Archachatina marginata*)

Bacterial Isolates	**Frequency and percentage of Isolation(%)**
Aeromonas spp	2(4.4)
Enterobacter spp	3(6.6)
Escherichia coli	8(17.7)
Klebsiella spp	3(6.6)
Proteus mirabilis	7(15.5)
Proteus vulgaris	7(15.5)
Pseudomonas spp	2(4.4)
Salmonella spp	3(6.6)
Shigella spp	3(6.6)
Staphylococcus aureus	4(8.8)
*Vibr*o spp	2(4.4)
Yersinia spp	1(2.2)
Total	**45 (100)**

Table 3: Antibiogram pertain of the Bacterial Isolates

Bacterial Isolates	Frequency	No and percentage of susceptibility tests (%)	No and percentage of antibiotics susceptible (%)	No and percentage of antibiotics resistant (%)
Aeromonas spp	2	22	12(2.4)	10(2.0)
Enterobacter	3	33	20(4.0)	13(2.6)
Escherichia coli	8	88	66(13.3)	22(4.4)
Klebsiella spp	3	33	15(3.0)	18(3.6)
Proteus mirabilis	7	77	33(6.7)	44(8.8)
Proteus vulgaris	7	77	16(3.2)	61(12.3)
Pseudomonas spp	2	22	9(1.8)	13(2.6)
Salmonella spp	3	11	26(5.3)	7(1.4)
Shigella spp	3	33	11(2.2)	22(4.4)
S. aureus	4	44	19(3.8)	25(5.1)
Vibrio spp	2	22	15(3.0)	7(1.4)
Yersinia spp	1	11	8(1.6)	3(0.6)
Total	**45**	**495**	**250(50.3)**	**134(49.2)**

4.0 Discussion

The results of this work revealed that snails harbour bacterial pathogens, which have obvious public health implications because they constitute health hazards especially gastro-intestinal infections. The bacterial isolates reported in this work is in agreement with the findings of Adagbada *et al*., 2011 who isolated *Staphylococcus auerus, Escherichia coli,* and *Aeromonas* spp, *vibro* spp, *pseudomonas* spp from *Achatina* species using four market in cross river and Akwa Ibom states Nigeria. It is also in agreement with the report of Adegoke *et al*., 2010 who isolated *Escherichia coli*, *Staphylococcus aureus* from different species of snails (*Achatina fulica*, *Limcolaria* species and *Helix pomatia)* gotten from Uyo, Akwa Ibom State Nigeria but differed in the isolation of *Bacillus subtilis, Lactobacillus* spp, , *Micrococcus luteus* and *Bacillus cerus*

The result obtained in this work agrees with that of Agbonlahor *et al.,*2010 who recorded the occurrence of *Proteus* spp (10.4%), *Escherichia coli* (5.7%), *Pseudomonas aeruginosa* (4.2%), *Salmonella* spp (0.3%), *Yersinia* spp (0.6%). Most of these bacteria belong to the Enterobacteriaceae family, which is found in the intestinal tract of humans and animals. They are known to be human faecal indicator organisms and as such, the Giant Africa land snails feed on materials that are probably contaminated with human and animal faeces.

In this study, the variable response shown by the bacterial isolates to antibiotics used in the tests is worrisome since antibiotics are never administered to snails, and antibiotic resistance was exhibited. This suggests probably that the contamination could come from human faecal matter and as such poses as serious health hazards.

In addition, since *Salmonella* species has been proven to survive in dried products, it will be unhealthy for consumers to eat snail meat that is not cooked first before drying.

Conclusion

The result demonstrates high enteric bacteria in giant land snails and the affinity land snails *Archachatina marginata* have to decaying organic matter deposits. Therefore, caution must be taken in the consumption of the giant land snails as it portents a serious health hazards.

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