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Isolation and Characterization of bacterial species associated with edible snails (*Achatina achatina)* sold in major markets within Abakaliki metropolis

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ABSTRACT

The objective of this study was to determine the prevalence of some bacterial species in edible snails (*Achatina achatina*) sold at various market outlets within Abakaliki metropolis. A total of 50 edible snails were randomly obtained at different locations in 5 major markets within Abakaliki metropolis. Results showed that 61 bacterial isolates which belonged to 6 bacterial species: *E. coli*, *Pseudomonas* spp, *Shigella* spp, *Enterobacter* spp, *Salmonella* spp and *Klebsiella* spp, were present in the snails. The suspected bacteria were identified and characterized using standard microbiology techniques. Results revealed that *Escherichia coli* had the highest prevalence frequency (45.9 %) followed by *Salmonella* spp (18 %), *Shigella* spp (13.1 %), *Pseudomonas* spp (11.5 %) and *Enterobacter* spp (6.6 %) being the least. The contamination of snails through sales in the open market without covering them and poor handling are several factors that contribute to snails being carriers of pathogenic bacteria. Therefore, it is imperative to ensure adequate care in its preparation so that their consumption will not cause serious epidemic threat.

Keywords: *Achatina achatina, Abakaliki metropolis*, bacterial species, edible snails.

INTRODUCTION

Snail is a common name which is applied to most of the members of the molluscan class of gastropods that have coiled shells in their adult stage. They include sea snails, land and fresh water snails but the general word "snail" applies more to land snails (Agbonlahor *et al*., 1994). They are found in a very wide range of environments, including ditches, land, deserts, and the abyssal depths of the sea. The two prominent snail species found abundantly in this part of the world are the edible giant land snails: *Achatina achatina* and *Archachatina 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 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). Nigeria witnessed an overwhelming growth of the aquaculture sector during the last two decades and ranked second in aquaculture production (Albequerque *et al*., 2009). While this growth is much appreciated in terms of food security, the health risk associated with the aquaculture produce is another important concern. Some years ago, increased attention is given to the possibility of cultured snail as vector of human pathogenic bacteria (Adagbada *et al*., 2010). Snail living in natural environment is known to harbour pathogenic *Enterobacteriaceae*. At least 40 genera have been identified in this family, including *Salmonella, Proteus, Serratia, Enterobacter, Citrobacter, Pseudomonas,* and *Klebsiella* (Fagbuaro *et*

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al., 2006). Some of the members of this family can live in the gut without causing health problems in individuals of good health, while others almost always cause signs of infection, including vomiting, diarrhoea, and related symptoms. In addition, most diseases in humans are caused by opportunistic enteric pathogens, which are prevalent in the rearing environment (Ebenso *et al*., 2011). Reports of the occurrence of pathogenic strains of *E. coli* from snails' sources and outbreaks of illness due to them were also increasing. Many cases of food poisoning are caused by infection with enteric bacteria, as are some more serious conditions, such as the plague (Fagbuaro *et al.,* 2006). People usually become infected with enteric bacteria as a result of poor hygiene and contact with people who have existing infections. There is a very close association between snails and microbes because of their habit which brings about the high level of microbial interaction with water snail. The close association between snails and microbes is because of their habit filth, sewage and rotten materials (Agbonlahor *et al.,* 1994). It is therefore not surprising that the high level of microbial interaction with snails, making them to become naturally contaminated with pathogen from the filth in which they live (Fagburo *et al.,* 2006). Hence, there is need to investigate the prevalence of enteric bacteria and other pathogenic bacteria in edible snails sold in Abakaliki metropolis, Ebonyi State.

Materials and Methods

Sample Collection:

A total of 50 edible snails were purchased randomly in different locations of major markets (Meat market, Nwofe market, Eke-Aba market and Iboko market) within Abakaliki metropolis and transported to department of Applied Biology, Ebonyi State University for identification before immediately transporting them to the laboratory unit of the department of Applied Microbiology, Ebonyi State University for bacteriological analysis

Isolation, identification and characterization of the bacterial isolates:

Isolation and identification of isolates from the snail samples were aseptically carried out using standard microbiology techniques as described by Chees brough, 2005. The following media were used for bacteria isolation: Nutrient agar, MacConkey agar, Eosine Methylene Blue (EMB) agar and Salmonella-Shigella agar (SSA). All the bacterial isolates were identified based on their colony morphology, Gram's reaction, catalase test, motility test and biochemical tests such as oxidase test, citrate test, indole test, Methyl red Voges-Proskauer (MRVP) test, urease test, hydrogen sulphide production test, and sugar fermentation test.

Snail Sample Processing:

Under aseptic condition, the outer shells of the snails were washed in running tap water using a nail brush, and rinsed with distilled water up to three times. 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 was separated from the mouthparts, the intestinal portions were removed using a dissecting kit, the snail was dissected longitudinally and the fluids were squeezed into sterile container. The fluids were homogenized with 10 ml of distilled water before labeling the sterile containers.

Culturing of Snail Samples:

Exactly 5 ml of snail fluid diluted with distilled water was transferred to each Petri-dish before pouring Nutrient Agar already prepared using pour plate method. The plates were allowed to gel/solidify, and then the plates were incubated at 37 ^{0}C for 24 hours before observing the growth.

RESULTS & DISCUSSION

A total of fifty (50) edible Snails (*Achatina achatina*) were bought from 5 major Markets within Abakaliki Metropolis. Ten snails were bought in each of the markets. Most of the bacteria isolated from the snails belong to the Entero-bacteriaceae family, which is found in the intestinal tract of humans and animals.

Table-1. Frequency of bacterial isolates associated with snail species (*Achatina achatina*) collected from 5 different markets in Abakaliki

Suspected bacteria	Meat Market (10)	Eke-aba Market (10)	Iboko Market (10)	Nwofe Market (10)	Ahia-ofu Market (10)	Total number of bacterial isolates
E. coli	5	4	6	5	8	28 (45.9 %)
Pseudomonas spp	1	0	2	0	4	7 (11.5 %)
Shigella spp	0	1	2	0	5	8 (13.1 %)
Enterobacter spp	1	1	0	0	2	4 (6.6 %)
Salmonella spp	2	2	3	0	4	11 (18 %)
Klebsiella spp	2	0	1	0	0	3 (4.9 %)
Total	11	8	14	5	23	61 (100 %)

Results showed that 61 bacterial isolates which belong to 6 bacterial species: *E. coli*, *Pseudomonas* spp, *Shigella* spp, *Enterobacter* spp, *Salmonella* spp and *Klebsiella* spp, were present in the snails (Table-1). Results revealed that *Escherichia coli* had the highest prevalence frequency (45.9 %) followed by *Salmonella* spp (18 %), *Shigella* spp (13.1 %), *Pseudomonas* spp (11.5 %) and *Enterobacter* spp (6.6 %) being the least (Table-1). This study agrees with the work of Agbonlahor *et al*. (2010) who recorded the occurrence of *Escherichia coli* (15.7 %), *Proteus* species (10.4 %), *Pseudomonas aeruginosa* (4.2 %), *Shigella* spp (0.3 %) and *Yersinia* spp (0.6 %) in snails. This showed that *Achatina achatina* snails collected from the 5 different markets (Meat market, Eke-aba market, Iboko market, Nwofe market and Ahia ofu market) might have obvious public health implications, especially gastro-intestinal infections when improperly cooked before consumption. All the bacterial species except *Shigella* spp were found in snail samples obtained from meat market. *Pseudomonas* spp was absent in the snail samples obtained from two markets (Eke-aba and Nwofe) while they were present in the other markets. The bacterial species were absent in the snails obtained from Nwofe market except *E. coli* (Table-1). Also, *Klebsiella* spp was absent in 3 markets (Eke-aba, Nwofe and Ahia-ofu). *Salmonella* spp was present in the snails obtained from all the markets except one (Nwofe market). *Enterobacter* spp was absent in Iboko and Nwofe markets. All the bacterial species except *Klebsiella* spp were found in the snail samples from Iboko market except *Enterobacter* (Table-1). The 6 bacterial species were present in the snail samples obtained from Ahia-ofu market (Table-1).

The prevalence of *Escherichia coli*, *Pseudomonas* spp, *Shigella* spp, *Enterobacter* spp, *Salmonella* spp and *Klebsiella* spp in the snails (*Achatina achatina*) obtained from the 5 market outlets will pose serious public health problem if consumed raw or undercooked. This result is in agreement with findings of Adagbada *et al*., 2011 who reported *Escherichia coli, Enterobacter* species, *Pseudomonas* species, *Klebsiella* species, *Shigella* species and *Aeromonas* spp from *Achatina achatina* in four markets within Cross River and Akwa Ibom states, Nigeria. *E. coli*, *Pseudomonas* spp, *Shigella* spp and *Salmonella* spp were more prevalent in the snail samples obtained from Ahia-ofu market while *Klebsiella* spp was more prevalent in Meat market. The least bacterial prevalence was majorly observed in the snail samples from Nwofe, Eke-aba and meat markets respectively. The contamination of snails with faecal material; feeding on decaying matter, faecal contamination of water, sales in the open market without covering them and poor handling are several factors that contribute to snails being carriers of *E.* coli, *Enterobacter* and other bacterial species (WHO, 2007). The pathogenic organisms isolated in this study pose a serious public health threat to the populace. The risk of food borne illness is on the increase and the need to provide effective ways of managing this condition is of immense significance. This is supported by FDA (2011) which reported that heat application to meat product at a temperature of 90 ^{0}C for 10 minutes is an effective way of eliminating pathogens from meat products.

CONCLUSION

Food-borne illnesses due to consumption of snails may occur when the mollusks that contain pathogenic microorganisms are consumed raw or improperly cooked. Microbiological assessment of edible snails (*Achatina achatina*) in this study showed that they haboured highly pathogenic bacteria of potential public health threat especially to consumers in areas where the demand for the snail meat is high. Therefore, it is imperative to ensure adequate care in its preparation so that their consumption will not cause serious epidemic threat. Also, more emphasis should be placed on strategies to reduce the contamination of edible snails (*Achatina achatina*), 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, strict adherence to Good Agricultural Practices (GAPs) and good management Practice by snail sellers.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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