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PREVALENCE OF ENTERIC BACTERIA ISOLATES FROM AQUARIUM SNAIL (*Ampullaria Spp.*) IN ABIA STATE, NIGERIA

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ABSTRACT: *The freshwater snail (Ampullaria spp.) was evaluated to determine the presence of enteric-pathogens commonly present. The fresh aquarium snail samples were collected from 5 different open markets where they were displayed for sale at Aba and Umuahia. They were processed in the veterinary laboratory of Michael Okpara University of Agriculture Umudike. Different bacterial ranging from salmonella, pseudomonas, Escherichia coli, Proteus, Shigella, Aeromonas, Enterobacter, Klebsiella and Staphylococcus were isolated. The presence of these pathogenic organism showed that Ama-ogbonna and Umungasi market recorded the highest isolate while New market, Ekeakpara and Umuahia central market recorded the least in that order: Escherichia coli, Proteus spp. and Salmonella spp. 30 (25.00%), 26(23.33) and 21(17.50%) recorded the most frequently isolated bacteria while Aeromonas and Staphylococcus spp. recorded the least frequently isolated bacteria 4(3.30%) and 4(3.30%). Due to the fact that these bacteria isolate present health related challenges on consumption of snail, there is the need for snails to be properly washed and cooked before eating.*

ORIGINAL ARTICLE

Key words: *Freshwater Snail, Bacterial Ranging, Cooking, Eating*

INTRODUCTION

The two prominent snail species found abundantly in the world are the edible giant land snail. Achatina achatina and Archachatina marginata (Ajayi et al., 1980). They are found majorly in southern parts of Nigeria, North African coast area, central and South Africa where the weather is most favourable for their proliferation (Herbert et al., 2001). It has been observed that edible snails obtained from swamps in North African coast for consumption in North America carry with them Salmonella species (Andrews et al., 1975). Snail meat is a delicacy in diets of people in Southern Nigeria (Ebenso and Ebenso, 2011). Mollusc has been reported to implication as vehicles for human infections caused by *E. coli.* The *E. coli* have been reported to have long-term survival in manure, soil and pasture (Fenlon et al., 2000). Agbonlahor et al. (1994) while investigating the bacteriology of edible African snails in the town of Ekpoma, Irrua, Iruekpen and Benin city all in Edo State, Nigeria isolated various Enterobacterceae organism thereby creating awareness on the possible public health risks that may result in the consumption of improperly processed snail meat. These organisms may remain in snails not as pathogens but as normal flora, but they can also cause diseases if eaten raw or improperly cooked. According to WHO (2009) estimates 200,000 deaths from food borne pathogens (especially *salmonella* and *E.coli*). There is a very close association between snails and microbes because of their habit filth, sewage and rotten materials. It is therefore not surprising the high level of microbial interaction with water snails, making them to become naturally contaminated with pathogens from filth in which they live (Fagburo et al., 2006). Significant numbers of aquarium snails are sold to the public and if carrying salmonella, these snails may present a public health risk similar to that presented by the aquarium turtles. It was reported that food safety and public health officials attribute a rise in the incidence of food borne illness to changes in demographics and consumer life style that affect the way food is prepared and stored (Collins, 1997). The objective of this study is to evaluate the snails for presence of enteric pathogens and inform the public the health implications associated with consumption of poorly cooked snail meat.

MATERIALS AND METHODS

A total of 120 samples of snails were purchased from 15 retail outlets in Ama-ogbonna market, Umungasi market, Umuahia central market, New market and Ekeakpara market all in Aba and Umuahia, Abia State. The snails were purchase from these markets and kept in a plastic container thereafter the samples were transported

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to the laboratory and processed immediately. The snail samples weighed from 4.5 to 64.2g, with mean weight of 12.2g. The outer shell of the snails were swabbed with sterile cotton wool swab sticks and then washed in running water using a thin brush, afterwards thoroughly washed with sterile water to remove all surface contaminants.

Processing and Culture

The shell was separated from the snail by careful dissection. The mouth and foot parts of the snail was used while the intestine was discarded. They were homogenized using mortal and pistle and diluted using sterile saline added to them. Following seriel dilution it was inoculated on selenite-F, nutrient agar and MarcConkey agar. This was incubated for 24hr at 37^{o}C. Cowan and steels method was used as prescribed Barrow (1993).

Identification and bacteria enumeration

Following incubation, the bacterial colonies obtained where sub-cultured for purity purposes. Various biochemical test including methyl red, mortility, indole, oxidase, catalase, voges-Proskauer, citrate, sugar fermentation and Gram reaction test was carried out for identification. The bacteria count of the snail was obtained by adding 9.0ml of peptone water to 1ml of each snail sample to obtain a 1:10 dilution 10^{-1} to $10^{-10,}$ 0.1ml of the 10^{5} dilution was spread on MacConkey agar and incubated for 24hr at 37^{o}C. The colonies present on the plate was counted and the total viable number was calculated using the dilution factor. Total viable count (cfu/gm)=colonies counted X reciprocal of dilution factor N X 10^{-5}.

RESULTS

Escherichia coli bacterial isolates was highest (30) while *Aeromonas spp* was the lowest (4) as represented above from various snail sample in Aba and Umuahia. The number of positive samples showed that conformed enterococci was 43 while the mean count was $6.8x10^{4}$. The fecal coliforms only presented 12 positive samples. The frequency of isolation of enteropathogenic bacteria shows that *Escherichia coli* has the highest frequency of isolation (25.00%) while *Aeromonas* and *Staphylococcus spp* has the least frequency of isolator (3.30%).

Table 1 - Sample types, range and location of snail

Sample type	Code range	Source
Redbase snail	M_{1-24}	New market
Brown snail	B_{25-49}	Umungasi market
Brown snail	B_{50-74}	Umuahia central market
Dark snail	D_{75-99}	Ekeakpara market
Dark snail	$D_{100-120}$	Ama-ogbonna market

A total of one hundred and twenty snail samples from different market; M – multi colour shell snail; B – Brown shell snail; = Dark shell snail

Table 2 - Bacterial isolates distribution of snail from various locations

ORGANISMS	Ama Ogbonna mkt	New Mkt	Umungasi Mkt	Umuahi Central Mkt	Ekeakpara Mkt
Escherichia coli	5+	3+	8+	4+	10+
Staphylococcus spp	-	2+	+	+	-
Aeromonas spp	-	2+	-	-	2+
Pseudomonas spp	6+	4+	3+	-	2+
Salmonella spp	7+	-	4+	3+	7+
Klebsiella spp	3+	+	4+	-	-
Enterobacter spp	2+	+	-	2+	+
Shigella spp	-	+	2+	-	+
Proteus spp	6+	12+	4+	6+	+

- = None; + = degree of presence; Mkt = market

Table 3 - Total count of most common bacteria in aquarium snails

Assay	No of samples tested	No of samples	Mean (cfu)	Range (cfu)
Fecal coliforms	54	12	$1.4x10^{5}$	$2.8x10^{3}$ – $4.5x10^{5}$
Confirmed enterococi	43	43	$6.8x10^{4}$	$2.6x10^{3}$ – $2.8x10^{5}$
Completed coliforms	54	54	$1.8x10^{8}$	$8.4x10^{3}$ – $3.0x10^{9}$

Table 4 - Frequency of bacteria isolation from snail (Ampullaria spp)

(30) 25.00%	*Escherichia coli*
(4) 3.30%	*Aeromonas spp*
(4) 3.30%	*Staphylococcus spp*
(15) 12.50%	*Pseudomonas spp*
(21) 17.50%	*Salmonella spp*
(6) 5.00%	*Enterobacter spp*
(5) 4.16%	*Shigella spp*
(26) 23.33%	*Proteus spp*
(7) 5.83%	*Klebsiella spp*
Total 100.00%	

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DISCUSSION

The results of this study shows that the bacteria load of enterococci present in snail is reasonably high, the bacteria flora in each of the snail sample range from 5-8 organisms/g. and are capable of causing health risk. An infective dose of up to $10^4 cfu^{-g}$ especially of salmonella is dangerous for humans when consumed via contaminated snail food, this is in agreement (Giaccone et al., 2012). It will be unhealthy for consumers to eat snail meat that is not properly cooked first and dried since it is known that *salmonella spp* survive in dry products this was supported (Urabe et al., 2008).

The study shows that *E. coli* presented the highest volume of enterobacteria organism present in snail. The high occurrence of *E. coli* was supported (Sprosten et al., 2006). These organisms of the family Enterobactericaea are found in the intestinal tracts of humans and animals in the soil and can be pathogenic to man. The results suggest that contamination of snail with fecal material, feeding of decaying matter, fecal contaminations of water, sell in the open market without covering them, poor handing are several factors that contribute to snail being carrier of enterobacteria organism, this was in agreement with (WHO, 2007). The result in Table 3 shows that enterococci count ($P<0.05$) range from $2.60\text{-}2.80x10^{-4} cfu^{-g}$ while the fecal coliform ranged from $2.8\text{-}4.5x10^4$ cfu^{-g}. These volumes are above the recommended $10^{-2} cfu^{-g}$ limits of HPA, (2009). The association of *pseudomonas spp* with aquarium snails may also have public health significance and this fact indicate that aquarium could be another source of nosocomial infections. These pathogenic organisms isolated from the five market visited have serious health implication to man. The risk of food borne illness is on the increase and the need to provide effective way of managing this condition is of immerse significance. This is supported by FDA (2011) which reported that heat application (90^oC for 10 minutes) is an effective way of eliminating pathogens from food.

CONCLUSION

Several pathogenic organisms were isolated from water snail, the methods presently being used for commercial production of water snails sold to the public need to be thoroughly examined to reduce the microbial load accumulated by the snails. Further studies need to be carried out to determine the best way necessary to eliminate these pathogenic microorganisms in snails.

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