ASSESSMENT OF HEALTH IMPLICATION ASSOCIATED WITH SNAILS AND SNAIL FARM SOILS IN WARRI AND SAPELE, DELTA STATE, NIGERIA.

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ABSTRACT

Microbiological analysis was carried out on snail visceral mass, snail effluent and soil samples from snail farms*Aspergillus and Penicilliumspp.*. The bacteria identified on the basis of morphological characteristics and biochemical tests were similar for all the samples. *Escherichia, Salmonella, Pseudomonas, Shigella and Bacillus spp.* were the bacteria isolated while the fungal isolates were *Mucor, Rhizopus, Aspergillus, Penicillium and Alternaria* spp. The microbial load ranged from $2.0x10^3$cfu/ml to $2.8x10^4$cfu/ml. *Escherichia, Staphylococcus*and *Mucor* spp. were isolated in all the samples while occurred least with a frequency of 12.25%. The antibiotic sensitivity test showed that *Klebsiella and Escherichia spp.* were highly resistant (100%) while the percentage resistance of the other organisms ranged from 40-60%.All the organisms were resistant to Chloramphenicol and Ampicillin. The total microbial counts, though lower than the specified standard limit of $1.0x10^5$cfu/ml may increase under unhygienic conditions which might result in health risks.

Keywords: Snails, Snail farm, Health, bacteria, fungi, antibiotics

INTRODUCTION

Snail is a common name for almost all members of the members of the Mollusca class ‘’Gastopod’’ that have coiled shell in the adult stage. They are active atnight and can live for several years and grow up to 20cm in length (Ekundayo and Fagade, 2005). Snail has been used for human food consumption and among the molluscan sea food that are widely distributed and consumed in Nigeria (Ezeama, 2004). The two primary areas of snail consumption in the world are West Africa and Western Europe. They are found extensively in the southern part of Nigeria and the entire West African coastal area, Central and South Africa where the weather is most favourable for their proliferation (Herbert and Kulbum, 2004). Many species of land snails are recognised in Nigeria but the popular species of economic interest are the West African Giant snails: *Achatinamarginata, Achatinaaclatina and Archatinafulica* (Adegbola, 1998). Declining population of *Archatinasp* has been reported and attributed to habitat loss through deforestation, over exploitation, indiscriminate harvesting, climatic change, high dependence on agricultural chemicals, lack of training on improved husbandry and diseases. (Ngenwi, 2010). Humans have used snails for food for many generations and despite this, most of the scientific works done on snails in West Africa have been from the point of view of animal parasitology where snails act as intermediate host of pathogenic nematodes (Wosu, 2003). Like most of other animals, the Giant African snails provide habitation to *infectious microorganisms and subsequent transmission of many disease producing microbes to man. (Adagbada et al.,* 2011). The association of animals and microbes has prompted many scientists to investigate the role of animals in spreading of pathogens to man and also provide some remedies in breaking the link in the chain of transmission of these pathogens to man. Bacterial infections leading to zoonotic diseases have been reported in snails. This can be due to production of toxins by bacteria such as *E. coli, Shigella, Staphylococcus*.There is a close association between the edible snails and microbes because their habitat is filth, sewage and manure and rotten materials. There is therefore a high level of snail interaction with pathogens from filth in which they live. The pathogens remain for further development and may finally be spread in the faeces and visceral fluid they produce (Fagbowo *et al*., 2006).

Giant African snail has been used in

the treatment of several ailments. Snail meat can be used to treat patients with whooping cough. The visceral fluid produced by the snails can also be used to cure hypertension, ulcer and asthma, kidney diseases, tuberculosis, anaemia (Odowu *et al.,* 2004). It can also be used for curing anaemia, high blood pressure, reduction of haemorrhoid and constipation (Alexander, 1997). It has also been reported to aid good voice maintenance and improve poor eyesight. Due to the belief of the tremendous medical property of snail, there is a growing interest for snail, hence the growth in snail farming.Heliculture or snail farming is the process of raising large snails specifically for human consumption and more recently to obtain shell for cosmetic use (Ngenwi, 2010). Adebayo *et al.* (2011) isolated *E. coli, Klebsiellaaerogenes, Staphylococcus aureus, Proteus spp, Citrobacter, Baccillus cereus, Streptococcus salivarus, Salmonellatyphi and Vibrio spp, Clasdosporium. Aspergillus, Fusariumoxysponin and Cryptococcus* from snails.Adagbada *et al.* (2011) also isolated *Salmonella spp, Shigellaspp, Aeromonas, Vibrio, Pseudomonas, Enterobacter, Klebsiella, Staphylococcusaureus and Yersiniaspp* and were found to show multipleresistance to some antibiotics. Studies have also shown that potential pathogens inhabit different organisms and tissues including the lungs, liver, kidney and stomach of clinically healthy African Giant land snails (Akpavie *et al.,* 2000).Some of these organisms have been reported to cause rosy egg dideases in the snails. This causes affected eggs to turn reddish brown and stop development of eggs (Adagbada*et al.*, 2011). *Pseudomonas* also causes intestinal infections which may spread rapidly among dense population of snails. *Aeromonashydrophila* isolated in some snails has been implicated in weak immune systems of infected persons,*Salmonella species* causes Salmonellosis and enteric fever while strains of *E. coli* have been known to cause acute gastoenteritis in infants. *Shigella* harboured by some snails causes dysentery and the fungus *Fusarium* parasitizes the eggs of *Achatinafulica*. To check the rate and occurrence of these diseases, this study is aimed at:

- Identifying disease causing microbes; snail farm and snails
- Comparing the impact of snail farming on the soil microbial load and composition
- Comparing the microbial flora of snail to those of the shell and visceral mass.

METHODOLOGY

Collection of samples

Samples of soils and snails were obtained from snail farms located in Warri and Sapele, Delta state, Nigeria. Control soil samples were collected from a distance of about 1km from the snail farms. The soil samples were collected with sterile auger into polythene bags. The bags were labelled appropriately and stored at 4^oC in the refrigerator for further analysis.

Preparation of samples

Soil samples

Soil sample of 1g was weighed each into test tubes containing 5ml distilled water. They were shaken, after which they were serially diluted.

Snail effluent

Two samples of *Achatinafulica* were collected from each of five farms. The snail shells were washed thoroughly with normal saline into a beaker to give the snail shell effluent.

Snail visceral mass

The snails were washed thoroughly in running tap water and surface was disinfected by rinsing the shell with 90% ethanol solution. The shell was broken and the meat removed with sterilized forceps. It was crushed in a mortar and about 5g placed in 10ml sterilized water. This was gently shaken on a rotatory orbital shaker for about 30mins. It was filtered and the filtrate used for serial dilution.

Determination of total microbial count

The samples were pour-plated on Nutrient agar, Salmonella-Shigella agar, and McConkey agar for the isolation of bacteria and Saboraud Dextrose agar for fungal isolations. The plates were incubated at 37^oc for 24hrs and 25^oc for 3days for bacterial and fungal isolates respectively. The plates were then observed for growth and number of distinct colonies formed on each plate was counted

and expressed as colony forming units.

Characterization of isolates

The bacterial isolates were characterized based on morphological and biochemical characteristics while the fungal isolates were macroscopically examined for colonial morphology, colour, pigment texture and surface appearance and microscopically examined onlactophenol blue for the sexual and asexual reproductive structures. The complete identification was done by comparing viewed characteristics with those of known taxa (Collins and Lyne, 2004).

Antibiotic susceptibility test

On newly prepared nutrient agar, a loopful of each isolated organism was inoculated uniformly to achieve a confluent growth. Using a clamp, the antibiotic disc was placed on the seeded agar plates at equal spaces under aseptic conditions. The plates were incubated at 37^{o}c for 24hrs. The zones of inhibitions around each antibiotic was measured in ml

Table 1: Characteristics of bacterial isolates

Isolate	Morphological characteristics	Gram reaction	Catalase	Coagulase	Haemolysis	Urease	Citrate	Indole	Oxidase	H_2S	Glucose	Lactose
Bacilluscereus	Rod	+	+	+	+	+	+	-	-	-	+	+
Staphylococcusaureus	Cocci	+	+	+	+	-	-	+	-	-	+	+
Escherichiacoli	Rod	-	+	-	+	-	-	+	-	-	+	+
Salmonella	Rod	-	+		+		+	-	-	+	+	-
Klebsiellapneumonae		+	+	+	+	-	-	-		+	+	+
Pseudomonas	Rod	-	+	+	+	+	-	-	+	-	-	-

Table 2: Colour and structural characteristics of fungal isolates

Fungi	Colour	Structure
Mucorspp	White	Cottony to fluffy dark grey with sporangia which are erect, branched, globose to spherical, multispored. Sporangia are without apophyses with well-developedcolumella.
Aspergillusniger	Black	Mostly consists of dense erect conidiophores which terminate in a vesicle covered with phaliades. Conidia are one-celled, smooth or rough-walled. They form dry chains.
Penicillumsp	Green	Consists of dense conidiophores which are hyaline and may be smooth or rough-walled. Phaliades are flask-shaped consisting of a cylindrical basal part and a distinct neck or lanceolate. Conidia are globose, ellipsoidal and cylindrical.
Alternaria	Dark green	Possess branched chains of multi-celled conidia (dictyoconidia) which are smooth-walled and produced from branched, elongated condiophores.
Rhizopus	Yellowish green	Sporangiospores are globose to ovoid, one-celled, hyaline to brown and striate. They possess stolons and pigmented rhizoids. Sporangiophores are formed singly or in groups from modes directly above the rhizoids. They are collumelate, multi-spored and generally globose sporangia.

Table 3: Occurrence of bacterial Isolates

Samples	*Bacillus*	*Staphylococcus*	*Salmonella*	*Escherichia*	*Klebsiella*	*Pseudomonas*
Snail $farm_a$	+	+	+	+	+	+
Snail $farm_b$	+	+	+	+	+	+
Snail $farm_c$	+	+	+	+	+	+
Control	-	+	-	+	-	-
Snail V_a	+	+	+	+	+	+
Snail V_b	+	+	+	+	+	+
Snail V_c	+	+	+	+	+	+
SS_a	+	+	+	+	+	+
SS_b	+	+	+	+	+	+
SS_c	+	+	+	+	+	+
% occurrence	90	100	90	100	90	90

Key: SF – Snail farm
SV – Snail visceral mass
SS – Snail shell effluent
(+) – Present, (-) - Absent

Table 4: Occurrence of fungal Isolates

Samples	*Mucor*	*Rhizopus*	*Aspergillus*	*Penicillum*	*Alternaria*	pH
SF_a	+	-	-	-	-	8.3
SF_b	+	+	-	-	+	8.5
SF_c	+	-	-	-	-	8.4
Control	+	+	+	+	-	6.8
SV_a	+	-	-	-	-	8.0
SV_b	+	-	-	-	-	7.8
SV_c	+	-	-	-	-	8.2
SS_a	+	-	-	-	-	7.9
SS_b	+	-	-	-	-	8.0
SS_c	+	-	-	-	+	8.1
% occurrence	100	20	10	10	20	

Key : SF – Snail farm
SV – Snail visceral mass
SS – Snail shell effluent

Table 5: Total bacterial and fungal counts (Cfu/g)

	Bacteria						*Fungi*				
Samples	*Bacillus*	*Staphylococcus*	*Salmonella*	*Escherichia*	*Shigella*	*Pseudomonas*	*Mucor*	*Rhizopus*	*Aspergillus*	*Penicillum*	*Alternaria*
SF_a	1.9	3.0	1.5	2.8	2.5	1.9	1.0	-	-	-	-
SF_b	2.5	1.9	2.6	1.9	1.5	1.5	1.0	1.0	-	-	1.0
SF_c	2.8	2.5	2.8	2.5	2.2	1.8	2.0	-	-	-	-
Control	-	1.1	-	1.5	-	-	1.0	-	1.0	2.0	-
SV_a	2.1	2.2	2.4	9.0	1.2	1.3	1.0	-	-	-	-
SV_b	1.8	2.0	3.0	1.3	2.0	2.0	1.0	-	-	-	-
SV_c	2.4	2.4	3.5	1.6	1.8	2.8	2.4	-	-	-	-
SS_a	2.0	1.8	2.1	1.1	2.0	2.3	2.0	-	-	-	-
SS_b	1.5	9.0	1.6	1.6	1.4	1.7	1.0	-	-	-	1.0
SS_c	1.8	2.5	1.8	2.5	1.8	1.6	2.0	4.0	-	-	1.0

Key: SF – Snail farm
SV – Snail visceral mass
SS – Snail shell effluent, (-) – Absent

Table 6: Antibiotic sensitivity test of the isolates

Organism	PEF	OFX	SPT	SX	CH	SP	CPX	AM	AU	CN	%R
Bacillus	R	S	R	R	R	R	S	R	S	S	60
Staphylococcus	S	S	R	R	R	S	R	R	S	S	50
Salmonella	R	S	S	R	R	S	S	S	S	R	40
Escheridia	R	R	R	R	R	R	R	R	R	R	100
Klebsiella	R	R	R	R	R	S	R	R	R	R	90
Psseudomonas	S	S	R	S	R	S	S	R	S	R	40
% Resistance of the isolates to each antibiotic	67	33	83	83	100	33	50	100	33	66	

PEF – Peflaxine
OFX – Ofloxacin
SPT – Streptomycin
SX – Septrin
CH – Chloramphenicol
SP – Sperfloxacin
CPX – Ciprofloxacin
AM – Ampicillin
AU – Augmentin
CN – Gentamycin
R – Resistance
S – Sensitive

and recorded.

RESULTS AND DISCUSSION

The results show that the snail farm soils, control and the snail harbour various bacterial and fungal species. The bacterial species isolated were *Salmonella*, *Bacillus*, *Pseudomonas, Staphylococcus*, *Klebsiella* and *Escherichiaspp* while the fungal isolates were *Mucormucedo*, *Rhizopusnigricans*, *Aspergillusniger*, *Penicillium* and *Alternaria* spp. (Tables 1 and 2). *Escherichiacoli* and Staphylococcus spp were detected in all the sample studied while *Staphylococcusspp* had the highest count which was obtained from farm A while the lowest was obtained in the control. All the bacterial isolates were found present in all the samples except the control where *Salmonella* and *Klebsiella* were not present.

Efunloye *et al.* (2011) reported that species of *Staphylococcus* inhabit the intestine of land snail. The snail farm soil and control had Staphylococcus present so the presence of S. aureus in the snail could have been from the soil. The occurrence of E. coli in the samples could also be indicative of faecal pollution (Olowe *et al.*, 2008) and also could be part of the normal flora of the snail. Molluscs in general have been reported to be potential carriers of Escherichia spp. (Spronston *et al.* , 2006). Gastropods have been reported to find mammalian faeces/manure an attractive food source (Speicer, 2011). This together with regular ingestion of contaminated soil demonstrates the potential to internal pathogen carriers.

Pseudomonas, Salmonella, Shigella and Bacillus spp were absent in the control but present in the other samples (snail farm soil, snail visceral mass and snail shell effluents). Microbes feed on decomposing materials such as rotten vegetables, snail droppings and decayed leaves (Adagbada *et al*., 2011). The ability of the organism to utilize snail droppings and decayed leaves could be responsible for their proliferation in snail farms hence constituting a potential route of microbial entry into snails.The microflora of the visceral mass could be from snails licking slime of infected, dead and rotten snails. Baptist *et al.* (2005) reported that soils contaminated with microbial species are possibly responsible for their presence in snails. The presence of these microbes in the ingested decomposing organic material could be responsible for their presence in snail farm and potential routes for microbial entry into snail (Neto *et al*., 2008).*Mucor* had 100% occurrence and *Alternaria* had 20% in the samples while *Rhizopus and Aspergillus and Penicillium* had 10%. All the organisms except Alternaria were present in the control. All the organisms isolated are among the fungi normally found in the soil. Their absence in the other samples associated with snail can be attributed to factors that have to do with the soil. Rhizopus and Alternariaspp present in snail farm and visceral mass

(Table 4) could be due to interactions between the snail and soil. Snails and their shells have been reported to be rich in elements such as oxygen, silicon, aluminium, copper iron, calcium and soils where snails are being farmed have been found to be high in these components especially calcium (Subba and Ghosh, 2001). The presence of Calcium makes the soil alkaline which could have resulted in the low growth of the fungal population. Soil factors such as fertility, light, pH, soil aeration and nature of soil influence microbial population and distribution in soil. Snail farming leads to an increase in the number and diversity of soil microbes. Pathogenic organisms such as *Vibrio, Salmonella, Shigella* and coliforms have been reported to be increased in soil as a result of snail farming. This increase is attributed to snail droppings, litter materials, decayed food materials, contaminated water, feeds and faecal materials which cause changes in the physico-chemical properties of the soil microbial community (Ekundayo and Fagade, 2005). The total microbial counts obtained within the study were within the specific standard counts of 1.5 x 10^5 cfu/ml for bacteria. The low counts can be attributed to the high concentration of some elements such as silicon, calcium, Magnesium, Iron, Aluminium which might have inhibited the growth of some organisms thus reducing the population. The physico-chemical properties of the soil could have been affected by the snail farming practice which in turn affects the microbial population and type of organisms present in the snail. (Ekundayo and Fayade, 2005).

The antibiotic sensitivity test showed that *Bacillus*, *Klebsiella*and *Escherichiacoli* had a resistance of 70%, 90% and 100% respectively to the antibiotics. All the organisms were resistant to Chloramphenicol and Ampicillin while *Streptomycin*, *Septrin*, *Peflaxine* and *Gentamycin* had 83%, 83%, 67% and 66% respectively. The results showed that there was high antibiotic resistance among the isolates. This might be due to the misuse and overuse of antibiotics in the feeding and care of the snails and farm and also the personnel in charge of the snail farm. High resistance of *Bacillus*, *Klebsiella* and *E. coli* to more than one or two drugs makes them multi-drug resistant (Wasly *et al.*, 2000). This creates a problem in the treatment of infections that might arise from these organisms. The resistance could also be as a result of chromosonal or plasmid DNA but the resistance to high levels of antibiotics has been ascribed in most instances to the presence of plasmids. (Adeleke *et al.,* 2003).

The health implication of presence of *E. coli*in snails and farms has been reported as indication of secondary pollution. *E. coli* is known to be associated with the GIT of warm blooded animals (Adebayo *et al.*, 2011). It is the causative agent of diseases such as diarrhoea, dysentery, gastroenteritis (Kumar *et al.*, 2005; Olowe *et al.*, 2008). Staphylococcus has been implicated in a number of chemical infections (Komolafe and Adegoke, 2008). *Aspergillusspp* has been reported to produce aflatoxin that are highly carcinogenic (Prescott *et al.*, 2005).

Snail farming brought about an increase in the number and diversity of the soil microbes. The increase in the pathogenic microorganisms (*Bacillus*, *Salmonella*, *Klebsiella*, *Escherichia* and *Pseudomonas*) may be attributable to the presence of decayed food materials, plant litter, fecal and decomposed dead snails available to the microorganisms as source of nutrients, thus enhancing their growth. The antibiotic sensitivity test revealed that most of these isolated pathogenic organisms are resistant to the known antibiotics. It is therefore expedient that farmers provide and maintain hygienic environment in snail farming to help prevent proliferation of these infectious agents. Personnels that frequent the snail farm should practice good hygiene and adequate cooking of the meat should be ensured in order to destroy or reduce the load of microorganisms that might be present.

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