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The Prevalence and Antibiotic Susceptibility Pattern of Entero-pathogens Isolated from Land Snails Commonly Eaten in Cross River and Akwa Ibom States, South-southern Nigeria

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ABSTRACT

A microbiological investigation was conducted on a total of 200 snail samples from 4 sampling sites including Watt market, Odukpani junction, Uyo market and Itam junction in Cross river and Akwa ibom states for the presence of enteric pathogens. 45 bacteria were isolated. The snails were deshelled, homogenized and cultured on Agar media and incubated for 24 hours. The isolates recovered include *Salmonella* species and *Shigella* spp 3(6.6%), *Aeromonas, Vibrio* and *Pseudomonas* spp 2 (4.4%) respectively, *Enterobacter* and *Klebsiella* spp 3 (6.6%), *Staphylococcus aureus* 4 (8.8%), *Proteus* spp 7 (15.5%), *Escherichia coli* 8 (17.7%) and *Yersinia* spp 1 (2.2%). The population of bacterial isolates showed a mean bacterial count of $4.2 \times 107 \pm 0.3$ c. f.u/g in the intestinal mass. The foot/head region had a mean bacterial count of $1.3 \times 10^7 \pm 0.25$ c. f.u. /g. The visceral fluid had the least mean bacterial count, $1.6 \times 10^2 \pm 0.04$ c. f.u./g. The bacterial isolates also showed multi-drug resistance to most antimicrobials. Because of the significance of these bacterial isolates, in that they contribute to gastro-intestinal infections there is need for proper processing of snails before eating.

INTRODUCTION

Most animals provide habitation to infectious micro-organisms and subsequent transmission of many diseases-producing microbes to man, this is because the basic language in the ecosystem is that of interaction for the transfer of energy through commensalism, symbiotic mutualism and parasitism. Such close association between animals and microbes has prompted many workers to investigate the role of animals in the dissemination of enteric pathogens.

Agbonlahor *et al*, [1], while investigating the bacteriology of edible African land snails in the town of Ekpoma, Irrua, Iruekpen, and Benin city all in Edo state, Nigeria; Umunede in Delta, Owerri in Imo state, Ore in Ondo state and Osogbo in Osun state, isolated *Escherichia coli, Proteus* species, *Plesiomonas* species, *Pseudomonas* species, *Bacillus* species, *Citrobacter* species, *Klebsiella* species, *Enterococcus* species, *Alcaligenes* species, *Aeromonas hydrophila, Salmonella* species, *Yersinia* species, *Flavobacterium* and *Staphylococcus aureus*. This investigation created 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.

The two prominent snail species found abundantly in this part of the world are the edible giant land snails: *Achatina achatina* and *Archachatina marginata* [2]. 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 [3]. There is a very close association between snails and microbes because their habitat is filth, sewage, manure rotten materials and poor latrine system, it is therefore not surprising, the high level of microbial interaction with land snails, making them to become naturally contaminated with pathogens from the filth in which they live. The pathogens thus remain in their bodies throughout their further development and may finely be spread in the faeces and visceral fluid they produce [4].

This study is aimed at creating awareness of the possible health risks that may result in the consumption of improperly cooked snail meat. It is intended to analyze the snails for the presence of enteric pathogens and evaluate the sensitivity of the pathogens to antibiotics.

MATERIALS AND METHODS

Disinfection & Pre-Analysis

200 snail samples were processed for this study; the weight of

each was taken and recorded. The outer shells of the snails were swabbed with sterile cotton wool swab sticks that were pre-moistened with sterile physiological saline. They were then washed in running water using a nail brush, afterwards thoroughly washed with sterile water. The shells were then disinfected using 70% alcohol.

Homogenization & culture

The snails were asceptically de-shelled and the visceral fluid collected into sterile test tubes. The intestinal portion was asceptically separated from the foot and mouth. These 2 separate portions were homogenized and sterile saline added to them. The minced portions were serially diluted, and inoculated on Selenite F, Alkaline peptone water, MacConkey Agar and Deoxycholate Citrate Agar for the primary isolation of enteric pathogens. They were incubated for 24 hours at 37^0c. Processing was done using the appropriate media and procedures according to Cowan and Steel [5].

Identification and antibiotic susceptibility testing

After 24 hours incubation, the bacterial colonies were subcultured for purity to obtain discrete colonies. Characterization was done using Gram's reaction test, citrate, oxidase, catalase, indole, motility, methyl red, and Voges-Proskauer and sugar utilization tests. The antimicrobial susceptibility test was done using the common antibiotics to determine the susceptibility of the isolates, the disc diffusion method is used for susceptibility testing as described by the Clinical and Laboratory Standards Institute CLSI [6].

Bacterial count

For total viable bacteria count for the visceral fluid, foot and mouth, and the intestinal mass of the snails,9.0ml of sterile peptone water was added to 1ml of each snail sample to obtain 1:10 dilution, this is a tenfold serial dilution 10^{-1} to 10^{-10}; 0.1ml of the 10^{-5} dilution was spread on MacConkey Agar. This was done for each site of the snails. It was then incubated at 37^oc for 24 hours. All colonies on the plates were counted and the total viable number was calculated using the dilution factor.

Total viable count (cfu/gm) = reciprocal of dilution factor × colonies counted N $\times 10^{-5}$

RESULTS

Table No.3: Distribution of bacterial isolates from snails collected from different sites

Bacterial Species	Sources Of Snails			
	Watt Market	Odukpani Junction	Uyo Market	Itam Junction
Aeromonas spp	+	-	-	+
Enterobacter spp	+	+	+	-
Escherichia coli	2+	2+	2+	2+
Klebsiella spp	+	+	+	-
Proteus mirabilis	2+	2+	+	2+
Proteus vulgaris	2+	2+	+	2+
Pseudomonas spp	+	+	-	-
Salmonella spp	+	-	+	+
Shigella spp	+	-	+	+
Staphylococcus aureus	+	+	+	+
Vibrio spp	-	+	+	-
Yersinia spp	-	-	+	-
X =	13 (29.0%)	11(24.4%)	11 (24.4%)	10(22.2%)

Formula

Class distribution / Total distribution x 100% = X

Table No.1: Number of samples, code numbers, source and mode of procurement

Code number	Sample	Source /Procurement
Y_1-Y_{24}	Yellow snail sample	Watt market in Calabar. By purchase
Y_{25}-Y_{50}	Yellow snail sample	Odukpani Junction. By purchase
Y_{51}-Y_{72}	Yellow snail sample	Uyo Market. By purchase
Y_{73}-Y_{96}	Yellow snail sample	Itam Junction. By purchase
B_{97}-B_{120}	Black snail sample	Watt Market. By purchase
B_{121}-B_{144}	Black snail sample	Odukpani Junction. By purchase
B_{145}-B_{176}	Black snail sample	Uyo Market. By purchase
B_{177}-B_{200}	Black snail sample	Itam Junction. By purchase

Y: Yellow snail sample
B: Black snail sample

Table No.2: Total count of bacteria/gm in each snail sample of different anatomical sites

Anatomical Site	Range (c.f.u./gm)	Mean (c.f.u./gm)
Outer shell	NE	-
Foot/head	1.8 x 105 – 3.0 x 108	1.3 x 107 ± 0.25
Intestinal mass	1.8 x 105 - 6.0 x 108	4.2 x 107 ± 0.3
Visceral fluid	1.0 x 102 – 2.0 x 104	1.6 x 102 ± 0.04

NE = Not Estimated

Table No.5: Biochemical identification and characterization of isolates from snails in uyo and calabar (gram negative rods only)

Possible organism	Oxidase	H2S	MR	VP	Indole	Motility	TCBS	Lactose	Mannitol	Glucose	Sucrose	Citrate	O/F
Esherichia coli	-	-	+	-	+	+	NT	+	+	GA	±	-	F
Vibrio cholerae	+	-	V	V	+	+	YC	-	+	A	+	±	O
Shigella dysenteriae	-	-	+	-	-	-	NT	-	±	A	-	-	F
Esherichia coli	-	-	+	-	+	+	NT	+	+	GA	±	-	F
Vibrio cholerae	+	-	V	V	+	+	YC	-	+	A	+	±	O
Vibrio cholerae	+	-	V	V	+	+	YC	-	+	A	+	±	O
V.parahemolyticus	+	-	V	V	+	+	BGC	-	+	A	-	±	O
Klebsiella spp	-	-	-	+	-	-	NT	+	+	GA	+	+	F
salmonella typhi	-	+	+	-	-	+	NT	-	+	A	-	-	F
S.typhi	-	+	+	-	-	+	NT	-	+	A	-	-	F
Esherichia coli	-	-	+	-	+	+	NT	+	+	GA	±	-	F
pseudomonas spp	+	-	-	+	-	+	NT	-	-	A	-	+	O
V.parahemolyticus	+	-	V	V	+	+	BGC	-	+	A	+	±	O
proteus mirabilis	-	+	+	-	+	+	NT	-	-	GA	±	+	F
proteus mirabilis	-	+	+	-	+	+	NT	-	-	GA	±	+	F
proteus vulgaris	-	+	+	-	+	+	NT	-	-	GA	+	+	F
proteus vulgaris	-	+	+	-	+	+	NT	-	-	GA	+	±	F
Shigella dysenteriae	-	-	+	-	-	-	NT	-	±	A	-	-	O
salmonella typhi	-	+	+	-	-	+	NT	-	+	A	-	-	O
Enterobacter spp	-	-	-	+	-	+	NT	+	+	GA	±	+	F
aeromonas spp	+	-	V	V	-	+	NT	-	+	A	+	±	O
yersinia spp	-	-	NT	NT	-	+	NT	-	+	NT	+	-	F

Key:
MR =Methyl Red V.P. = Voges Proskauer O/F = Oxidation – Fermentation
H2S = Hydrogen Sulphide production TSI = Triple Sugar Iron TCBS = Thiosulphate Citrate Bile Salt Agar
+ = Positive - = Negative V =Variable A = Acid G = Gas ALK = Alkaline
F = Fermentative O = Oxidative YC = Yellow colonies BGC = Blue- Green colonies

Table No.4: Frequency of isolation of bacterial species from snails in calabar and uyo

Bacterial Species	Frequency 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%)
Staph aureus	4 (8.8%)
Vibrio spp	2 (4.4%)
Yersinia spp	1 (2.2%)
Total:	45 (100%)

Table No.6: Names of antimicrobials, code and disc potency used in nthe test

Antimicrobial	Code	Disc potency
Ampicillin	AP	25µg
Chloramphenicol	CH	30 µg
Teteracycline	TET	30 µg
Gentamycin	CN	10 µg
Ofloxacin	OFX	10 µg
Streptomycin	ST	10 µg
Ceftazidime	CAZ	10 µg
Cotrimoxazole	SXT	25 µg
Rocephin	RX	10 µg
Nalidixic acid	NAL	10 µg
Amoxicillin	AXM	10 µg

Table No.7: Antimicrobial susceptibility pattern of bacterial isolates

Bacteria spp	N	AP	CH	TET	CN	OFR	ST	CAZ	SXT	RX	NAL	AXM	%S	%R
Aeromonas spp	2	S	S	R	S	S	R	S	R	S	R	R	16.6	10
Enterobacter	3	S	S	S	S	S	R	S	S	S	R	S	16.6	6.6
Escherichia coli	8	R	R	S	S	S	R	S	R	R	S	S	17.7	6.6
Klebsiella spp	3	R	R	S	S	S	R	S	S	R	S	S	I5.5	8.8
Proteus mirabilis	7	R	R	R	S	S	R	S	S	R	R	S	15.5	8.8
Proteus vulgaris	7	R	R	R	S	S	R	S	S	R	R	S	15.5	8.8
Pseudomonas spp	2	R	R	R	S	S	R	S	R	R	R	R	7.7	16.6
Salmonellas pp	3	R	R	R	S	S	S	NT	R	NT	NT	S	12.2	7.7
Shigella spp	3	R	S	S	S	S	R	NT	R	NT	NT	S	13.3	4.4
Staph aureus	4	R	R	R	S	S	S	S	R	S	R	R	13.3	11.1
Vibrio spp	2	S	S	R	S	S	R	S	S	NT	NT	NT	13.3	3.3
Yersinia spp	1	R	S	S	S	S	S	S	R	NT	S	NT	15.5	4.4
						Total	=	45					172.7%	97.1%
						X	=						14.39	8.09
						A.M	=						3.83	2.15
						H.M	=						1.61	3.10
						G.M.	=						14.10	5.99

Key:
S = sensitive, R = resistant, NT = Not tested, X = mean, AM = arithmetic mean, HM = harmonic mean, GM = geometric mean.
For S, zone diameter >3mm, for R, zone diameter < 3mm, n = no of strain tested

DISCUSSION

In this analysis, the bacterial flora in each of the snail sample exceeded 10-6 organisms/g. It was suggested that edibles with bacterial count of more than10-6/g are likely to constitute health hazard because the concentration of their metabolites will be enough to cause intoxication directly.

Also, this work has revealed that snails harbour bacterial pathogens, which has obvious public health implications because they contribute to gastrointestinal infections. Such organisms include Aeromonas spp (4.4%), Salmonella spp 3 (6.6%), Vibrio spp 2 (4.4%), Yersinia spp 1(2.2%), Pseudomonas spp 2 (4.4%) and many others, supporting the findings of Adegoke et al, (7) who isolated Bacillus subtilis, Staphylococcus aureus, Lactobacillus spp., Escherichia coli, Micrococcus luteus and Bacillus cereus from different species of snails (Achatina fulica, Limcolaria sp. and Helix pomatia) gotten from Uyo, Akwa Ibom state, Nigeria. In another study on land snails, Agbonlahor et al, (1), recorded the occurrence of Proteus spp (10.4%), Escherichia coli (5.7%), Pseudomonas aeruginosa (4.2%), Salmonella spp (0.3%), Yersinia enterocolitica (0.6%) and many others. These organisms of the family Enterobacteriaceae are found in the intestinal tract of humans and animals and in the soil and can be pathogenic to man. This result suggests contamination with faecal matter or through feeding on decayed matter since snails are omnivorous. Contamination of marketed foods and food products could be from faecal contamination or from the water used to wash them before they are marketed, it could also come from market condition since they are displayed on tables without being covered, houseflies perching on them freely thereby transferring micro organism from one source to another, bad consequences follow poor handling of food products [8].

In this study, the variable response shown by the bacterial isolates to antibiotics used in the test is worrisome since antibiotics are never administered to snails, and antibiotic resistance was exhibited.

Also since Salmonella spp has been proven to survive in dried products [9], it will be unhealthy for consumers to eat snail meat that is not cooked first before drying.

The general result of this analysis demonstrates high enteric bacterial load in land snails and it helped to determine the sanitary quality of Achatina marginata. However, further studies need to be conducted on the chemical constituents of snail fluid by analytical expert in view of its high medicinal value to the people of Akwa- Ibom and Cross River States.

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