



## Antimicrobial Susceptibility Profile of Microorganisms Isolated from the Intestine and Body Parts of the African Giant Land Snail (*Achatina achatina*) Sold in Akure, Nigeria

A. K. Onifade<sup>1</sup> and E. A. Aiyenuro<sup>1\*</sup>

<sup>1</sup>Department of Microbiology, Federal University of Technology, P.M.B 704, Akure, Nigeria.

### Authors' contributions

This work was carried out in collaboration between authors OAK and AEA. Author OAK designed the study, managed the analyses of the study, wrote the protocol. Author AEA performed the statistical analysis and managed the literature searches. Authors OAK and AEA wrote the first draft of the manuscript. Author AEA performed the research work under close supervision of author OAK. Authors OAK and AEA read and approved the final manuscript.

### Article Information

DOI: 10.9734/JAMB/2018/40621

#### Editor(s):

(1) Pongsak Rattanachaikunsopon, Professor, Department of Biological Science, Faculty of Science, Ubon Ratchathani University, Thailand.

#### Reviewers:

(1) V. U. Olugbue, Akanu Ibiam Federal Polytechnic, Nigeria.

(2) Ron Bartzatt, University of Nebraska, USA.

Complete Peer review History: <http://www.sciencedomain.org/review-history/24036>

Original Research Article

Received 28<sup>th</sup> January 2018  
Accepted 3<sup>rd</sup> April 2018  
Published 7<sup>th</sup> April 2018

### ABSTRACT

**Aims:** To evaluate the antimicrobial susceptibility profile of microorganisms isolated from the intestine of African giant land snail (*Achatina achatina*) sold in Akure, Ondo state, Nigeria.

**Place and Duration of Study:** Snail samples were obtained from Ilara, Ogbese, Oja-Oba, Owena including Ecotourism and Wildlife department (EWM) of Federal University of Technology, Akure, Ondo state, between June and August, 2017. This research work was carried out at the Department of Microbiology laboratory, Federal University of Technology, Akure.

**Methodology:** A total of seventy-eight (78) snail samples were collected from different locations. The types and loads of fungi and bacteria in the body parts of the snail samples were determined. Identification and characterization of various bacterial isolates were based on Gram-staining technique and biochemical tests. The fungal isolates were identified by their morphological features

\*Corresponding author: E-mail: [aiyenuroademola@gmail.com](mailto:aiyenuroademola@gmail.com);

and lactophenol cotton blue staining procedure. Antimicrobial susceptibility profile of the isolates was evaluated using standard methods. *Klebsiella pneumoniae* being resistant to multiple antibiotics and as such subjected to plasmid analysis.

**Results:** *Escherichia coli* (17.1%), *Enterobacter* species (13.3%), *Klebsiella pneumoniae* (12.9%), *Proteus vulgaris* (15.7%), *Salmonella* species (15.7%) and *Staphylococcus aureus* (25.2%) were isolated from the body parts of the snails. While *Escherichia coli* (18.7%), *Enterobacter* species (7.32%), *Klebsiella pneumoniae* (14.63%), *Proteus vulgaris* (12.2%), *Pseudomonas aeruginosa* (11.38%), *Salmonella* species (13.82%), *Shigella* species (9.76%) and *Staphylococcus aureus* (12.2%) were isolated from the snail's intestine. The fungi isolated include: *Alternaria* species, *Aspergillus flavus*, *Aspergillus niger*, *Aspergillus oryzae*, *Fusarium* species, *Paecilomyces variotti*, *Penicillium marneffeii* and *Aspergillus oryzae*. Ofloxacin was the most effective antibiotic against the bacteria isolates, Ceftriaxone and Augmentin were the least effective on the isolates. Most of the fungal isolates were resistant to Griseofulvin. *Penicillium marneffeii* was resistant to Itraconazole, Ketonazole, Griseofulvin but sensitive to Terbinafine.

**Conclusion:** Findings from this study revealed that the African giant land Snail harbours bacterial and fungal pathogens, these pathogens have obvious public health implications.

**Keywords:** Bacteria; fungi; antibiotics; plasmid profile; African giant land snail.

## 1. INTRODUCTION

Snails are Gastropod mollusks, meaning they belong to the same group of Octopuses, which are part of the phylum Mollusca. At the same time, they are members of the class Gastropoda which include all snails and slugs. Mollusks are classified as invertebrate animals with a soft unsegmented body. The two prominent snail species found abundantly in this part of the world are the edible giant land snails: *Achatina achatina* and *Archatina marginata* [1]. The close contact of wild snails with soil; their uncontrolled contact with soil and their uncontrolled feeding pattern make the snail susceptible to microbial contamination [2]. The meat can be easily contaminated with pathogens and serve as a vehicle of transferring infectious agents to consumers. According to [3], there is a close association between snails and microorganisms because their habitat is made up of filth, sewage, manure rotten materials and poor latrine system which increase the microbial load of land snails. Despite rich nutritional values of snails, the involvement of the Molluscs in the transmission of infection mostly as secondary host for pathogens makes it necessary to study the microbiology of the resident snail [4].

African giant land 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 [5]. Due to the belief of the tremendous medical property

of snail, there is a growing interest in snail, hence the growth in snail farming [6]. Heliculture or snail farming is the process of raising large snails specifically for human consumption and more recently to obtain shell for cosmetic use [7].

The use of antimicrobials for any infection, real or feared, in any dose over a period of time, forces microbes to adapt or die (selective pressure), the surviving microbes which carry drug resistance genes may be transferred to other strains within their own genus and species and across them even to other unrelated species [8]. Antimicrobial agents are classified by their specific mode of action. These agents may interfere with cell wall synthesis, inhibit protein synthesis, interfere with nucleic acid synthesis or inhibit a metabolic pathway. Resistance to antimicrobial agents has become important in clinical management and control of many diseases and deserves scientific intervention to bring about some control measures [9]. This study therefore sought to bring out the microbial loads of the snail, *Achatina achatina*; their susceptibility patterns to different antibiotics and antifungal agents and to evaluate the plasmid profile of bacterial pathogens that showed multiple antibiotic resistance.

## 2. MATERIALS AND METHODS

### 2.1 Study Area Description

Akure is a city in south-western Nigeria, and is the largest city and capital of Ondo state. It is situated in the tropic rainforest zone in Nigeria. The city had a population of 484,798 as at the

2006 population census, with a total land area of 991 km<sup>2</sup>. Akure lies about 7°25' North of the equator and 5°19' East of meridian. The rural settlements under study for this research are: Ilara, Ogbese, Oja-Oba, Owena including Ecotourism and Wildlife department (EWM) of Federal University of Technology, Akure.

## 2.2 Sample Size and Collection of Samples

A total of seventy eight (78) samples of the African giant land snails (*Achatina achatina*) were collected from five locations in Akure, Ondo state. Snail samples were obtained very early in the morning; between June and August, 2017. This is due to the fact that the snails were obtained from the forests overnight and have not been exposed to any form of stress. They were collected in sterile plastic containers and conveyed to the laboratory within 2-3 hours for microbial analysis. Snail samples used for this study were much easier to obtain as the rainy season favoured their availability.

## 2.3 Sampling Procedure and Microbiological Analysis

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. Afterwards, the snails were de-shelled under an aseptic condition. The foots were separated from the mouthparts, the intestinal portions were removed using a dissecting kit, and the fluid squeezed into sterile test tubes as described by Ugoh et al. [2]. A sterile swab stick was used to swab body parts of the snails and streaked uniformly on the agar plates, the minced portion were serially diluted and inoculated on the prepared agar plates; this was done to evaluate the microbial loads in the intestine and other body parts of the snails.

## 2.4 Isolation, Identification and Characterization of Isolates

Culture media used include nutrient agar, MacConkey agar, Eosin methylene blue agar, *Salmonella Shigella* agar and potato dextrose agar, they were prepared according to the manufacturer's specifications. Glasswares including test tubes, conical flask, measuring cylinders, pipettes, beakers, syringes and bijou bottles were sterilized by autoclaving at 121°C for 15 minutes. Microorganisms were isolated via

the pour plate method; the bacterial plates were incubated at 37°C for 24 hours, colonies were counted and recorded in cfu/ml, discrete colonies were sub-cultured to obtain pure isolates and were characterized using morphological and biochemical tests. Bacterial isolates were identified with reference to Bergey's Manual of Determinative Bacteriology [10]. Fungal plates were incubated for 48-72 hours at 28°C, colonies were sub-cultured to obtain pure isolates, morphological features of discrete fungal colonies were observed, the colonies were stained with lactophenol cotton blue and viewed under the microscope and identified [10].

## 2.5 Antimicrobial Sensitivity Testing

Antimicrobial sensitivity tests were carried out to determine the resistance and susceptibility of bacteria and fungi to antibiotics and antifungal agents respectively. The antibiotics sensitivity test was carried out using Kirby Bauer's disc diffusion method. Antifungal sensitivity assay was carried out using the agar well diffusion method [10].

## 2.6 Plasmid Analysis

Plasmid curing was carried out in order to determine the location (plasmid-borne or chromosomal) of the drug resistance marker(s). The curing (elimination) of the resistant plasmids of the resistant isolates was done using sub inhibitory concentration of 10mg/ml of ethidium bromide as described by [11,12,13] with slight modifications.

## 2.7 Statistical Analysis of Data

Data obtained were subjected to analysis of variance and means were compared using Duncan's New Range Test (DNMRT) with the aid of SPSS software version 17 at  $p \leq 0.05$  level of significance [4].

## 3. RESULTS

### 3.1 Microbial Loads

The microbial loads of different parts of the snail's body were evaluated and compared to that of the snail's intestine which is the core of this study. The results (Table 2) revealed that *Escherichia coli* (17.1%), *Enterobacter* species (13.3%), *Klebsiella pneumoniae* (12.9%), *Proteus vulgaris* (15.7%), *Salmonella* species (15.7%) and *Staphylococcus aureus* (25.2%)

were present in the body parts of the snails. The following bacteria were isolated from the intestine of the snail and their percentage occurrence; *Escherichia coli* (18.7%), *Enterobacter* species (7.32%), *Klebsiella pneumoniae* (14.63%), *Proteus vulgaris* (12.2%), *Pseudomonas aeruginosa* (11.38%), *Salmonella* species (13.82%), *Shigella* species (9.76%) and *Staphylococcus aureus* (12.2%). The fungi isolated from the intestine include: *Alternaria* species (10.26%), *Aspergillus flavus* (12.82%), *Aspergillus niger* (23.07%), *Aspergillus oryzae* (12.82%), *Fusarium* species (15.38%), *Paecilomyces variotti* (17.95%), and *Penicillium marneffeii* (7.70%). *Aspergillus niger* was the most prevalent in all the locations used for the study.

### 3.2 Antimicrobial Sensitivity Results

#### 3.2.1 Antibiotic sensitivity results

The result of the antibiotic sensitivity test revealed that the most effective antibiotic against

the bacteria isolates was Ofloxacin. Ceftriaxone and Augmentin were the least effective on the isolates (Figs. 1 and 2).

#### 3.2.1.1 Plasmid profile

Two strains of *Klebsiella pneumoniae* being resistant to multiple antibiotics and as such subjected to plasmid analysis, the results revealed that *Klebsiella pneumoniae<sub>a</sub>* showed chromosomal resistance pattern while *Klebsiella pneumoniae<sub>b</sub>* was plasmid mediated (Plate 1).

#### 3.2.2 Antifungal sensitivity results

Among the antifungal drugs used for the antifungal sensitivity test, Terbinafine was the most effective being sensitive to majority of the fungal isolates. Most of the isolates were resistant to griseofulvin. *Penicillium marneffeii* was resistant to itraconazole, ketonazole, grieofulvin but sensitive to terbinafine (Fig. 3).

**Table 1. Biochemical and cultural characteristics of bacterial isolates**

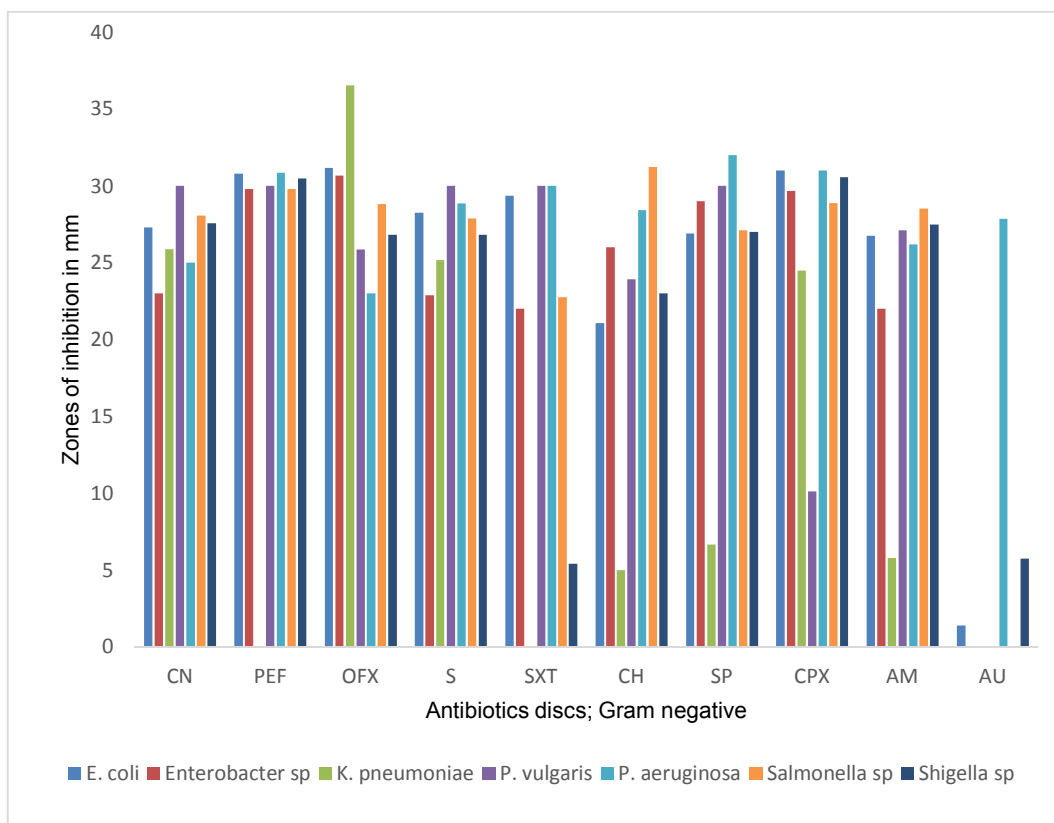
Ox	H <sub>2</sub> S	MR	VP	Ind	Mot	Lac	Man	Glu	Suc	Cit	Suspected organisms
-	-	+	-	+	+	+	+	GA	+	-	<i>Escherichia coli</i>
-	-	+	-	-	-	-	+	A	-	-	<i>Shigella</i> species
-	+	+	-	-	+	-	+	A	-	-	<i>Salmonella</i> species
-	-	+	+	-	-	+	+	+	+	+	<i>Staph. Aureus</i>
-	-	-	+	-	-	+	+	GA	+	+	<i>K.pneumonia</i>
+	-	-	+	-	+	-	-	A	-	+	<i>P. aeruginosa</i>
-	+	+	-	+	+	-	-	GA	+	+	<i>Proteus vulgaris</i>
-	-	-	+	-	+	+	+	GA	+	+	<i>Enterobacter</i> species

Keys: Ox- oxidase, H<sub>2</sub>S- hydrogen sulphide, MR- methyl red, VP- voges proskauer, Ind- indole, Mot- motility, Lac- lactose, Man- mannitol, Glu- glucose, Suc- sucrose, Cit- citrate

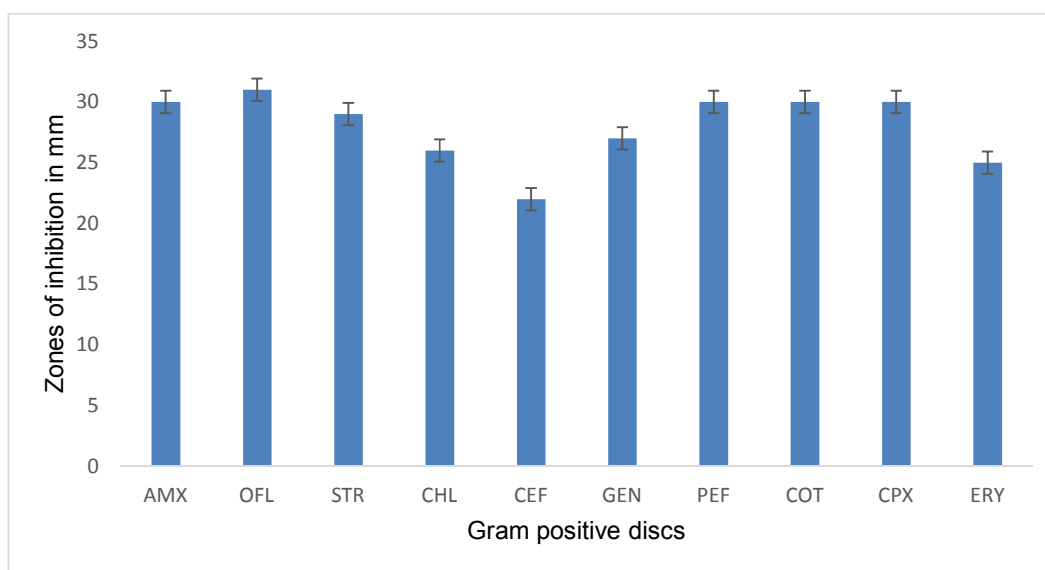
**Table 2. The distribution of bacterial isolates in the intestine and other parts of the snail samples**

Bacteria	Owenna		Ogbese		Ilara		Oja-Oba		FUTA/EWM	
	B.P	Int	B.P	Int	B.P	Int	B.P	Int	B.P	Int
<i>E. coli</i>	8	2	10	11	6	7	7	3	5	0
<i>Salmonella</i> sp	6	0	9	5	7	3	6	4	5	5
<i>Shigella</i> sp	0	1	0	6	0	3	0	2	0	0
<i>Staph. Aureus</i>	10	1	12	5	10	5	11	1	10	3
<i>Klebsiella</i> sp	5	4	6	8	6	3	5	3	5	0
<i>Pseudomonas</i> sp	0	1	0	6	0	4	0	2	0	1
<i>Enterobacter</i> sp	4	0	7	3	6	1	5	2	6	3
<i>Proteus vulgaris</i>	6	2	8	5	5	1	6	4	8	3

Keys: B.P - body parts; Int – intestine



**Fig. 1. Antibiotic sensitivity patterns of gram negative isolates**  
 Keys: CN = Gentamycin; PEF = Pefloxacin; OFX = Tarivid; S = Streptomycin; SXT = Septrin; CH = chloramphenicol; SP = Sparfloxacin; CPX = Ciprofloxacin; AU = Augmentin;

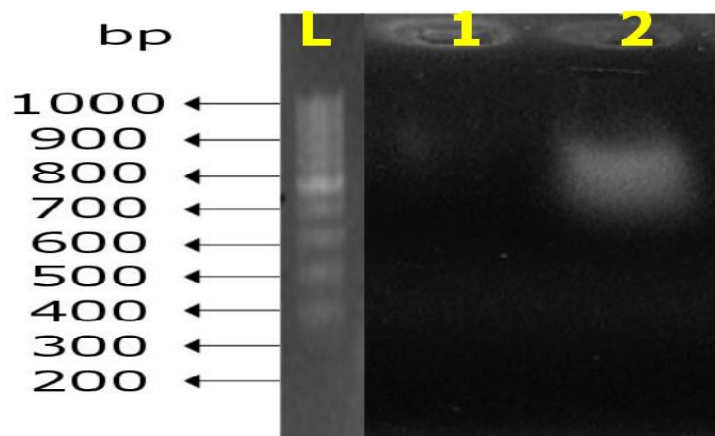


**Fig. 2. Antibiotic sensitivity patterns of Gram positive isolate (Staphylococcus aureus)**  
 Keys: AMX = Amoxycillin; OFL = Ofloxacin; STR = Streptomycin; CHL = Chloramphenicol; CEF = Ceftriaxone; GEN = Gentamycin; PEF = Pefloxacin; COT = Cotrimoxazole; CPX = Ciprofloxacin; RY = Erythromycin;

### 3.2.3 Plasmid analysis

The result obtained in this work agrees with that of Agbonlahor et al. [14] who recorded the occurrence of *Proteus* species (10.4%), *Escherichia coli* (5.7%), *Pseudomonas aeruginosa* (4.2%), *Salmonella* species (0.3%), *Yersinia* species (0.6%). Adagbada et al. [3]; Ugoh et al. [2] and Lucy Agnes et al. [4] also reported similar results. Most of these bacteria belong to the *Enterobacteriaceae* family, they are

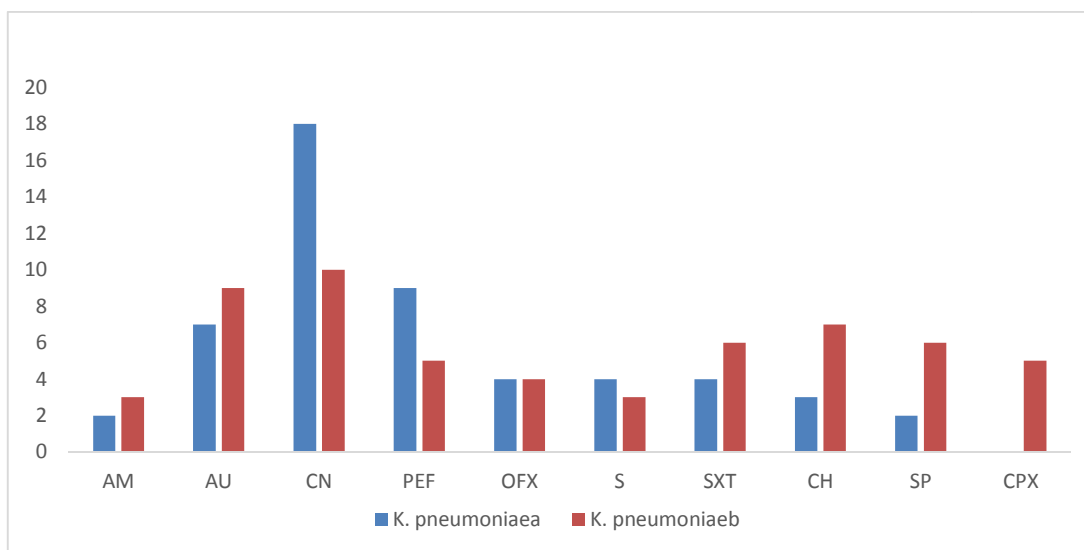
found in the intestinal tract of humans and animals. They are present in substantial amount in the human faeces, the Giant African land snails feeds on almost any kind of fruit or vegetables that have been contaminated with human and animal faeces in the forest. However, further studies need to be conducted on the chemical constituents of snail fluids by analytical experts in view of its high medicinal value to people of Ondo states.



**Plate 1. Agarose electrophoresis showing plasmid profile of *Klebsiella pneumoniae* isolated from the intestine of the giant African land snail**

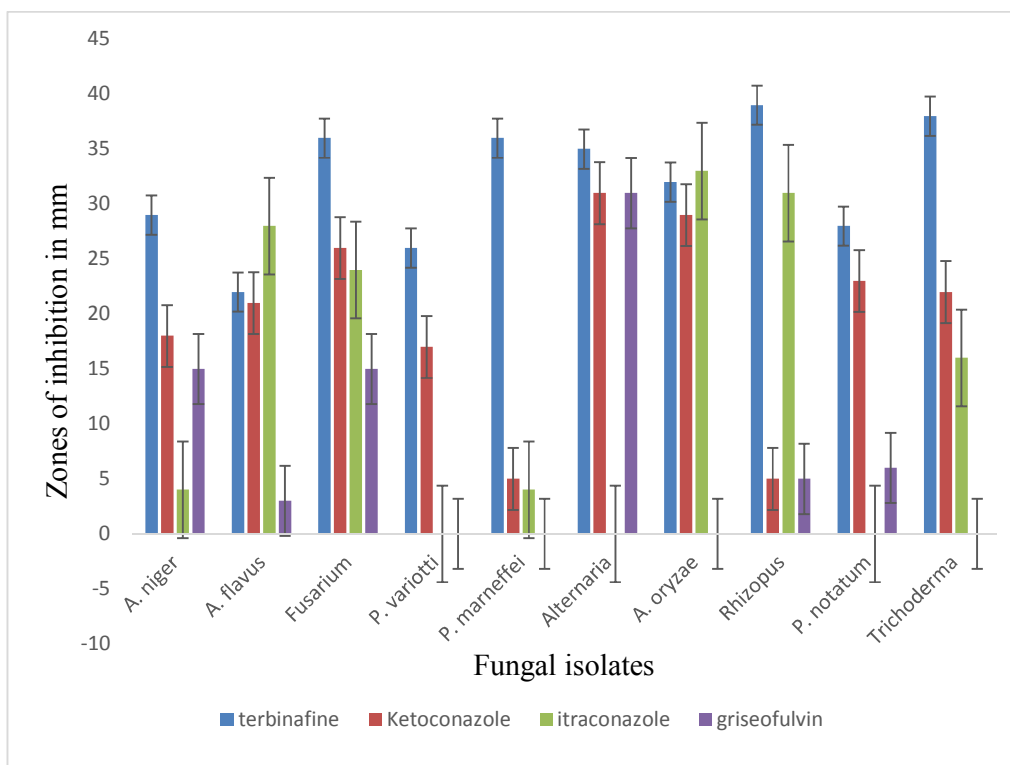
*Klebsiella pneumoniae<sub>a</sub>* showing chromosomal resistant pattern while *Klebsiella pneumoniae<sub>b</sub>* being plasmid mediated

Keys: L – ladder isolate1 -*Klebsiella pneumoniae<sub>a</sub>* isolate2 -*Klebsiella pneumoniae<sub>b</sub>*



**Fig. 3A. Antibiotics sensitivity results of the *K. pneumoniae* after plasmid curing**

Keys: CN = Gentamycin; PEF = Pefloxacin; OFX = Tarivid; S = Streptomycin; SXT = Septrin; CH =Chloramphenicol; SP = Sparfloxacin; CPX = Ciprofloxacin; AU = Augmentin;



**Fig. 3B. Antifungal susceptibility patterns of fungal isolates**

#### 4. CONCLUSIONS

The result demonstrates the presence of microorganisms in the Giant African land snail, *Achatina achatina*. Microorganisms isolated from the snails poses serious health hazards including gastrointestinal infections. The potency of antibiotic and antifungal agents in the effective treatment of these infections cannot be over emphasized. However, the increasing resistance to antibiotics by these organisms is worrisome.

#### ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the authors.

#### COMPETING INTERESTS

Authors have declared that no competing interests exist.

#### REFERENCES

1. Ajayi SS, Tewe SO, Milligan, JK. Influence of seasonality on aestivation and

behaviour of the forest African giant land snail, *Archachatina marginata* (Swaison). Bulletin of Annual Health Proc. 2009;28(6): 328.

2. Ugoh S, Chukwudi and Ugbenyo, Aisha John. 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. Research. 2013;5(9):56-59.
3. Adagbada AO, Orok AB, Adesida SA. The prevalence and antibiotic susceptibility of enteropathogens isolated from land snails commonly eaten in Cross River and Akwa Ibom states, South Southern Nigeria. Asian Journal of Pharmaceutical and Health Sciences. 2011;1(3):123-127.
4. Lucy Agnes N, Victoria A, Josephine NT, Daniel L, Isaac A. Evaluation of African giant snails (*Achatina* and *Archachatina*) obtained from markets (wild) and breeding farms. Academic journals. 2016;10(7):1.
5. Odowu OF, Ogunleye RJ, Tayo GN, Richard BAO. Medical values of snails. Journal of Microbiology and Antimicrobials. 2004;3(4):13-22.

6. Akpomie OO, Assessment of health implication associated with snails and snail farm soils in warri and sapele, Delta state, Nigeria. Nigerian Journal of Science and Environment. 2013;12(2):50-56.
7. Ngenwi AA, Mathew JM, Etchu KA, Oben FJ. Characteristics of snail farms and constraint increased product in Western Central Africa. African Journal of Environmental Science and Technology. 2010;4(5):274-278.
8. Komolafe O. Antibiotic resistance in bacteria - An emerging public health problem. Malawi Medical Journal. 2003; 15(2):63-67.
9. Soyegbe AO, Ajayi AO, Ngwenya E, Basson AK, Okoh AI. Vancomycin and oxacillin co-resistance of commensal staphylococci. Jundishapur Journal of Microbiology. 2014;7(4):12-14.
10. Fawole MO, OSO BA. Laboratory manual of microbiology. Ibadan: Spectrum Books; 2001.
11. Akortha EE, Filgona J. Transfer of gentamicin resistance genes among enterobacteriaceae isolated from the outpatients with urinary tract infections attending 3 hospitals in Mubi, Adamawa State. Scientific Research and Essay. 2009;4(8):745-752.
12. Sheikh AR, Afsheen A, Sadia K. Abdu W. Plasmid borne antibiotic resistance factors among indigenous Klebsiella. Pakistan Journal of Botany. 2003;35(2): 243-248.
13. Yah SC, Eghafona NO, Oranusi S, Abouo AM. Widespread plasmid resistance genes among proteus species in diabetic wounds of patients in the Ahmadu Bello University Teaching Hospital (ABUTH) Zaria. African Journal of Biotechnology. 2007;6(15): 1757-1762.
14. Agbonlahor DE, Imoyera PI, Igumboe EO, Akhabue EE, Ekundayo PA, Orhue DT, Obasuyi BO, Osuide OO. The bacteriology of edible giant Africa land snail (*Archachatina maginata*) commonly found in Southern Part of Nigeria. Journal of Medical Laboratory Science. 1994; 4(3):26-32.

© 2018 Onifade and Aiyenuro; This is an Open Access article distributed under the terms of the Creative Commons Attribution License (<http://creativecommons.org/licenses/by/4.0>), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Peer-review history:

The peer review history for this paper can be accessed here:  
<http://www.sciencedomain.org/review-history/24036>