



Isolation and Multiple Antibiotic Resistance Pattern of Enteric Bacteria Isolated from Faecal Samples of *Thryonomys swinderianus* in Sekona Osun State, Nigeria

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Authors' contributions

This work was carried out in collaboration among all authors. Author OMA designed the study, performed the statistical analysis and wrote the first draft of the manuscript, managed the literature searches. Author OF wrote the protocol read and managed the literature searches approved the final manuscript. Author CIO collected the samples and managed the analyses of the study, read and approved the final manuscript. Author AAO managed the analyses of the study, read and approved the final manuscript. Authors OAT and COO managed the literature searches read and approved the final manuscript. All authors read and approved the final manuscript.

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ABSTRACT

Thryonomys swinderianus (grasscutter) is a source of meat with high nutritional values, low in cholesterol and relatively high in protein therefore, it is a sort for in Nigeria and some other African countries. The aim of this study was to evaluate the potentials for zoonotic infections and for the presence of antibiotic resistant bacteria in the faecal samples of *Thryonomys swinderianus*. Fresh faecal samples were collected from seven (7) *Thryonomys swinderianus* at selling point in Sekona,

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Osun State, Nigeria. They were cultured on appropriate media and a total of 62 enteric bacterial isolates were recovered, they are:- *Escherichia coli* 22 (365.5 %), *Enterobacter cloacae*, 12 (19.4%), *Citrobacter koseri* 9 (14.5%), *Citrobacter freundii* 4 (6.6 %), *Klebsiella pneumoniae* 10 (16.1 %), *Klebsiella oxytoca* 1 (1.6 %) and *Serratia liquefaciens* 4 (6.6%) *Escherichia coli* O157:H7 were found to be 50% of the *Escherichia coli* present. Antibiotic susceptibility test was done using the Kirby-Bauer disc diffusion method. Using commercially available antibiotic disc. The percentage of multiple antibiotic resistance (MAR) for all the isolates were quite high for cefiazidime (100%), augumentin (99.1%), cefuroxime (97.1%), cerixime (85.02%), but relatively low for ciprofloxacin (3.4%), ofloxacin (1.82%), and all the isolates were susceptible to nitrofurantoin. All the enteric bacteria isolated from faecal samples of grass cutter were multiple antibiotic resistance bacteria and of great health concern. In conclusion *Thryonomys swinderianus* harbors different enteric bacteria which could cause several infections in humans, and nitrofurantoin can help the physician in the management of infections caused by the animals.

Keywords: *Thryonomys swinderianus*; Enteric bacteria; Multiple antibiotic resistance.

1. BACKGROUND

Thryonomys swinderianus (Grasscutter) is a wild herbivorous rodent found in the sub-Saharan region of Africa. It is the biggest after porcupine in the rodent class. They are considered delicacy, high prized source of protein and agricultural pest of cereals and other crops [1]. The nutritive value of its meat is among the important factors that influence the meat quality and consumer acceptability. The main components of meat quality are the protein and lipid contents. Meat fat contains several types of lipids, including triglycerides as the main components, phospholipids and cholesterol, with the phospholipids component being relatively constant compared to the triglycerides and cholesterol [2]. Compared to others such as rabbit meat, grasscutter meat is very low in cholesterol (48.5-53.4 mg/100 g, 135 mg/100 g fresh weight) and high in protein (18.1% v. 14-25%) [3]. Like rabbit meat, it has a very high mineral (e.g. iron, calcium and phosphorous) content compared to beef, mutton, and chevon, hence it is a highly sort for source of nutrition [4]. Enteric bacterial pathogens span several genera, including *Escherichia*, *Salmonella*, *Shigella*, *Yersinia*, *Vibrio*, and *Campylobacter*, they resides in the intestines of animals, these pathogens are closely associated with faecal contamination of foods and are major causes of gastrointestinal (GI) tract infections.

The faeces of Grasscutter like other warm blooded animals contain different types of bacteria, viruses, fungi and parasites which are pathogenic for humans. The infectious agents transmitted from animals to humans account for about 61% of infections which results to outbreaks of most novel pathogens worldwide

[5]. Zoonotic transmission can be influenced by many factors, which has to do with contact between animal reservoirs and the human population. Contact through direct consumption of contaminated meat and indirect contact through water and dust particles. Most gastro intestinal tract infections caused by bacteria cannot be effectively treated by current antibiotic therapies because the antibiotics are either ineffective, cause severe symbiosis of the intestinal microbiota, or trigger serious complications, such as septicaemia from antibiotic-induced endotoxin release [6].

2. MATERIALS AND METHODS

Faecal samples of grass cutter were collected from Sekona, Osun State, Nigeria between December 2018 and March 2019. A total of seven grass cutter were sampled. Faecal samples were collected with the use of sterile swab stick from each grass cutter immediately after captured or killed by the hunters. They were labelled and transported to the laboratory for immediate microbiological analysis. Isolation of the bacterial was done using streak method on MacConkey agar and incubated at 37°C for 24hrs. Identification and characterization of bacteria isolates were carried out using cultural, morphological methods and biochemical test according BERGEY's Manual of Systematic Bacteriology. The identity of the isolates were confirmed using Analytical Profile Index 20E Kit (API20E). *E. coli* isolates were cultured on Sorbitol Macconkey agar to identify *E. coli* O157:H7.

Antibiotic susceptibility tests were performed to determine the resistance patterns of the identified bacterial isolates to commonly used

antibiotics in Nigeria. Antibiotics discs used include; Gentamicin (GEN) 10µg, Cefiazidime (CAZ) 30µg, Ofloxacin (OFL) 5µg, Augumentin (AUG) 30µg, Nitrofurantion (NIT) 300µg, Ciprofloxacin (CPR) 5µg, Cerixime (CXM) 5µg, Cefuroxime (CRX) 30µg. This test was carried out by Kirby-Bauer's disc diffusion method on Mueller-Hinton agar. The inoculum were standardized to 0.5 Macfarlane standard. Plates were then incubated at 37°C for 24 hours, after which the diameter of zones of inhibition were measured and interpreted according to the Clinical Laboratory Standard Institute 2018 guidelines [7].

3. RESULTS

A total of seven faecal samples were collected from seven grass cutter in Sekona, Osun State Nigeria and 62 enteric bacteria isolates were recovered. They were;- *Escherichia coli* 22 (36.5 %), *Enterobacter cloacae*, 12 (19.4%), *Citrobacter koseri* 9 (14.5%), *Citrobacter freundii* 4 (6.6 %), *Klebsiella pneumoniae* 10 (16.1 %), *Klebsiella oxytoca* 1 (1.6 %) and *Serratia liquefaciens* 4 (6.6%) as shown in Table1. Out of the 22 *E. coli* isolates 11 (50%)

were *E. coli* 0157:H7. They showed pale colour on Sorbitol Macconkey agar.

Table 2 shows the Analytical Profile Index 20E Kit (API20E) identification profile of bacteria isolates. The of bacteria species identified were:- *Citrobacter koseri*, *Citrobacter freundii*, *Enterobacter cloacae*, *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *Serratia liquefaciens*.

Table 3 shows the antibiotics susceptibility test for the 62 isolates. All the isolates were sensitive to Nitrofurantion (NIT). Only *Citrobacter* spp. shows resistant to gentamicin (7.7 %), and only *E. coli* show resistance to Ofloxacin (9.1%) while *E. coli* (9.1%) and *Citrobacter* (7.7%) were resistant to Ciprofloxacin. In all, the percentage resistance was quite high for ceftazidime (100%), augmentin (99.1%), cefuroxime (97.1%), cefixime (85.02%), but relatively low for ciprofloxacin (3.4%), ofloxacin (1.82%), and gentamicin (1.5%) as depicted in Table 4. The isolates showed different resistance pattern to the antibiotic, all the isolates were resistant to two or more antibiotic used (Table 3).

Table 1. Occurrence and percentage frequency of enteric bacteria isolates from faecal samples of grass cutter

S/N	Isolates	Number of Isolate (n)	Percentage (%)
1	<i>Escherichia coli</i>	22	35.5
2	<i>Enterobacter cloacae</i>	12	19.4
3	<i>Citrobacter koseri</i>	9	14.5
4	<i>Citrobacter freundii</i>	4	6.5
5	<i>Klebsiella pneumoniae</i>	10	16.1
6	<i>Klebsiella oxytoca</i>	1	1.6
7	<i>Serratia liquefaciens</i>	4	6.5
	Total	62	100

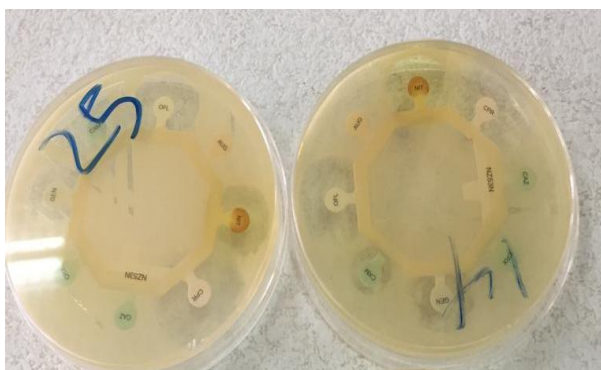


Fig. 1. Antibiotic susceptibility result for *Escherichia coli*

Table 2. Analytical profile index 20E Kit (API20E) Identification profile identity of bacteria isolates

Isolate Code	API Code	Isolate Identity
SE2	3355773	<i>Citrobacter koseri</i>
SE17	3605773	<i>Citrobacter freundii</i>
SE28	3304763	<i>Enterobacter cloacae</i>
SE15	7146573	<i>Escherichia coli</i>
SE60	5214773	<i>Klebsiella pneumoniae</i>
SE 34	4245773	<i>Klebsiella oxytoca</i>
SE 44	5621100	<i>Serratia liquefaciens</i>

Table 3. Antibiotic resistance of enteric bacteria isolates from faecal samples of grass cutter using Mueller-Hinton agar

Antibiotics	<i>Escherichia coli</i> (22)	<i>Enterobacter cloacae</i> (12)	<i>Citrobacter koseri</i> (9)	<i>Citrobacter freundii</i> (4)	<i>Klebsiella pneumoniae</i> (10)	<i>Klebsiella oxytoca</i> (1)	<i>Serratia</i> (4)
NIT	0	0	0	0	0	0	0
CPR	9.1	0	7.7	0	0	0	0
CAZ	100	100	100	100	100	100	100
CRX	95.5	100	100	100	100	0	100
GEN	0	0	7.7	0	0	0	0
CXM	68.2	100	53.9	15.4	80	100	100
OFL	9.1	0	0	0	0	0	0
AUG	95.5	100	100	100	100	100	100

Key: Gentamicin (GEN), Cefiazidime (CAZ), Ofloxacin (OFL), Augumentin (AUG), Nitrofurantion (NIT), Ciprofloxacin (CPR), Cerixime (CXM), Cefuroxime (CRX).

Table 4. Multiple Resistance patterns of Isolates from faecal samples of grass cutter

Isolates	Resistance Pattern	Frequency
<i>Escherichia coli</i> (22)	AUG, CAZ, CRX	1
	AUG, CAZ, CPR	6
	CAZ, CRX, CXM	1
	AUG, CAZ, CRX, CXM	1
	AUG, CAZ, CRX, CPR, CXM	12
	AUG, CAZ, CRX, CXM, GEN	1
<i>Enterobacter cloacae</i> (12)	AUG, CAZ, CRX, CXM	12
	AUG, CAZ	1
<i>Klebsiella</i> (10)	AUG, CAZ, CRX	1
	AUG, CAZ, CRX, CXM	8
	AUG, CAZ, CXM	1
<i>Klebsiella oxytoca</i> (1)	AUG, CAZ, CRX	1
<i>Citrobacter koseri</i> (11)	AUG, CAZ, CRX	1
	AUG, CAZ, CRX, CXM	6
	AUG, CAZ, CRX, CXM, GEN	1
	AUG, CAZ, CRX, CPR, CXM,	1
<i>Citrobacter freundii</i> (4)	AUG, CAZ, CRX	3
	AUG, CAZ, CRX, CXM	1
<i>Serratia</i> (4)	AUG, CAZ, CRX, CXM	4

Key: Gentamicin (GEN), Cefiazidime (CAZ), Ofloxacin (OFL), Augumentin (AUG), Nitrofurantion (NIT), Ciprofloxacin (CPR), Cerixime (CXM), Cefuroxime (CRX).

4. DISCUSSION

In this study, Seven (7) *Thryonomys swinderianus* were sampled from Sekona, Osun State Nigeria, and 62 enteric bacteria were isolated and identified. Five different bacteria species were identified and characterized. These animals are healthy carrier of these bacteria

species since they are not associated with any form of infection. *E. coli*, the most abundant facultative bacterial species in the normal microbiota of the large intestines of animals and humans, was the most occurrence isolated bacteria in this study this is in agreement with the study of Carlos where *E. coli* was found to be most abundant in bacteria isolates from wild

birds in Rio de Janeiro [8]. Also, *E. coli* was also found to be the most prevalent and wide spread among the different animal groups in the zoo according to the study of Tavakoli [9]. *E. coli* causes food borne diseases, which include diarrhoea and could as well leads to severe kidney problem through its direct or indirect consumption.

Eight different antibiotics were tested against the isolates and different resistant pattern were seen, and all the isolates were resistant to two or more antibiotics. The percentage resistance was quite high for cefiazidime, augumentin, cefuroxime, cefixime, ciprofloxacin, ofloxacin but all the isolates were susceptible to nitrofurantion. Although, study showed that bacteria isolates from animal faeces has resistance to nitrofurantion, although it was relatively low [10]. Also, there has been reports of antibiotic resistance in gram-negative bacteria in studies on wild life faecal samples [11,12].

The emergence of multidrug resistance strains poses a major threat to the patients globally. Several studies on wildlife populations observed a correlation between the level of antimicrobial resistance in bacteria and contact with human populations [13]. Since *Thryonomys swinderianus* does not have contact with human population and they have not been predisposed to antibiotics in any way because they are wild animals, the multiple antibiotic resistant seen in these isolates could be an indication that the resistance exist in nature and that the isolates has already develop resistance to available antibiotics. The use of pesticides and fertilizers by farmers is also a factor that can bring about resistance of the available antibiotics because grasscutters are herbivorous, and may consume plants that have been treated with fertilizers and pesticides which can bring about mutation in their genome or gene sequence. Due to this mutation, the isolate can develop resistance to the available antibiotics [11].

5. CONCLUSION

Grasscutter meat is highly accepted by all social classes in the urban and rural communities of the West African sub-region and a niche of Southern Africa serving as an important source of the highly priced animal protein because of its leanness and unique organoleptic properties. This animal (*Thryonomys swinderianus*) harbors different enteric bacteria of which *E. coli* is predominant. *E. coli* 0157:H7 has been

identified to be half of the *E. coli* population. The isolates are multidrug resistance therefore; proper cleaning of the grasscutter meat should be ensured before cooking, because these organisms can be transferred to man through consumption of contaminated and unclean food proper disposal of intestinal content should be ensured to avoid contamination of water and environment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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